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Synergetic contribution of sugarcane bagasse and derived biochar: Insights into cadmium stress tolerance of maize (*Zea mays* L.) with physiochemical exploration

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Abstract

Cadmium (Cd) and other heavy metals are significant micropollutants originating from excessive industrial activities, inappropriate fertilizer use, and atmospheric deposition. The availability and movement of Cd can be minimized through adsorption using potential adsorbents like sugarcane bagasse (SB) and sugarcane bagasse-derived biochar (SB-BC). It has been reported that organic amendments such as SB and SB-BC affect the bioavailability of heavy metals. A field study assessed the impact of SB and SB-BC on the physiological and biochemical properties of maize plants grown in Cd-contaminated soil. Compared with High Stress Cadmium (HS-Cd), in No Stress Cadmium (NS-Cd), the combined application of 1% SB and 1% SB-BC displayed maximum response in plant physiological and biochemical properties; improved the performance of IRGA traits, chlorophyll content (CHL), relative water content (RWC) get increased as leaf chlorophyll (52%), RWC (29%), A (11%), E (57%), Gs (41%) and Ci (24%)a marked decrease in shoot (15%) and root (27%) Cd concentration, enhanced antioxidant enzymatic and non-enzymatic response: Up-regulated the superoxidase (SOD) by 34%, peroxidase (POD) by 44%, catalase (CAT) by 29%, ascorbate peroxidase (APX) by 22%, and total phenolics (TP) by 55%, ascorbic acid (ASA) by 33%, glutathione (GSH) by 34%, glutathione reductase (GR) by 19%; the decreased lipid peroxidation and membrane damage: rebated the level of H_2O_2 *(55%),* O_2 *(43%), content which alleviated the malondialdehyde (MDA) content by 46% and electrolyte leakage (EL) by 53% in maize plant; aggravated the profiling of compatible solutes: 18% proline content (PC), 43% soluble sugars (SS), 31% soluble proteins (SP), and 26% glycine betaine (GB) accumulation amplified, relative to their respective treatments of control and LS-Cd and HS-Cd groups. The combined application of SB and SB-BC (each at 1%) can be an eco-friendly and cost-effective approach to stabilize the Cd within the contaminated soils.*

Keywords: Biochar; cadmium; adsorption; antioxidants; abiotic stress; sugarcane bagasse; Cd stabilization; food security; micropollutants

Introduction

Various agricultural and industrial activities such as use of sewage sludge (Riaz *et al.*, 2022), metal smelting, and mining can pollute the soils with micropollutants (heavy metals) that have caught the attention globally (Madhav *et al.*, 2024). To increase per capita income, the farmers' community uses an inappropriate amount of fertilizers and pesticides (Qadir *et al.*, 2022). Cadmium (Cd) is a primary soil contaminant especially in agricultural fields that might cause serious food security and human health problems due to its greater toxicity potential as well as persistent and bio-accumulative nature (Gong *et al.*, 2021). It is reported that Cd-contamination is responsible for causing significantly deleterious consequences on physiological, morphological, and biochemical activities (Zulfiqar *et al.*, 2022). Cd is a persistent toxic heavy metal that prevails extensively, specifically within the agricultural soils hence adversely affecting both, the cropping agro-ecosystem and human health by entering the food web (Zulfiqar *et al.*, 2022). Cadmium also induces oxidative stress to plants by producing reactive oxygen species (ROS) such as hydroxyl radical (OH^-), superoxide anions (O^{2-}) and hydrogen peroxide (H_2O_2) eventually contributing to electrolyte leakage and membrane rupture (Cuypers *et al.*, 2023).

Sugarcane bagasse (SB) contains some of the natural and decomposable chemical elements that are required for plant

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growth and have properties which help in pollutants removal/immobilization (Zafeer *et al.,* 2024). Being eco-friendly, economically feasible and having minimum pollutant indexes, SB has been the major focus of the researchers of material science. Due to the addition of various materials during sugarcane production, SB, bye-product of sugar production have variable properties and has capability to be effectively used as bio sorbent when added to Cd-contaminated soils. The other way of proper usage of the SB is the production of biochar (BC) for incorporation into the agricultural soil. Biochar is prepared through pyrolysis (thermal decomposition) in the absence of oxygen. Nowadays, biochar is considered one of the most effective organic amendments for the multiple advantages to the soil as well as to the crop (Mousavi *et al*., 2023). Biochar has been proven to be significantly more beneficial to enhance the soil organic carbon storage for longer time and comparatively less prone to biochemical decomposition rather than un-charred organic materials (Lehmann *et al*., 2021).

Biochar is a high-carbon, spongy and permeable solid material that can be produced by the biomass pyrolysis in an oxygen-deprived environment (Sivaranjanee *et al.,* 2023). High pH and cation exchange capacity (CEC), porous texture and bulky surface area along with active functional groups are the key properties of BC (Singh *et al.,* 2023). It is reported in previous literature that BC has significantly increased the soil productivity, nutrient content, soil carbon sequestration potential as well as mitigated greenhouse gases emissions (Elkhlifi *et al.,* 2023). Moreover, Amin *et al.* (2023) also reported the effectiveness of BC in decreasing the heavy metals bioavailability within the soil profile. The persistent nature of BC can be owed to the formation of the aromatic carbon by aliphatic carbon (C) chains and hence it stays in the soil for thousands of years (Liu *et al.,* 2023). On the other hand, the inconsistent fraction of the BC that is present within the fixed planes of C can easily be decomposed by the action of soil microbes, oxidation or weathering processes ultimately decreasing the biochar proportion within the soil as reported by Xie *et al.* (2024). Latest researches as documented by Ghazouani *et al.* (2023) have revealed that various environmental factors can significantly alter the intrinsic properties of BC with the passage of time once it is incorporated into the soil. The BC uses various mechanisms such as cation exchange, precipitation, cation-π interaction, electrostatic attraction, and surface complex formation for the immobilization of heavy metals within the water or soil medium (Wang *et al.,* 2024). However, Wang and Hou (2024) reported that the interaction of BC with dissolved organic matter, microorganisms and minerals due to the ageing in the field soils can induce considerable modification in acidity and O^- containing functional group of BC. Consequently, adversely affects the functioning of BC application in field as well as disturbing its potential of heavy metals adsorption. Murtaza *et al.* (2024) reported that artificial ageing caused a significant improvement in the O^-comprising functional groups content of most BCs.

The extent of toxicity posed by heavy metals on the crops is mainly determined by their bioavailable fraction in soil

Table 1: Physicochemical properties of the experimental soil and biochar

Property	**Soil**	**Biochar**	**Sugarcane Bagasse**
pH	8.09	9.47	6.45
EC (dS m^{-1})	2.61	1.64	0.62
Surface area (m^2 g^{-1})	-	97.3	4.30
Sand (%)	53.1	-	-
Silt (%)	24.2	-	-
Clay (%)	22.7	-	-
Soil Texture	Sandy clay loam	-	-
SOM (%)	0.83	-	-
CEC ($cmol_c$ kg^{-1})	5.49	-	-
N (%)	0.108	3.88	1.44
Carbon contents (%)	-	39	35
Ash contents (%)	-	21	18
Volatile content (%)	-	23	19
Total P (%)	0.088	0.41	0.22
DTPA-Extractable heavy metals (mg kg^{-1})			
Zn	39.7	11.9	-
Cd	0.11	n.d.	-
Total heavy metals (mg kg^{-1})			
Cd	3.2	n.d.	-

specifically in the root area as stated by Amin *et al.* (2023). Use of any organic or inorganic amendments can effectively decrease the bioavailable fraction of heavy metals that can ultimately reduce the heavy metal uptake and accumulation through roots (Liu *et al.*, 2022). The coexistence of various concentrations and kinds of heavy metals further worsens the polluted environment; hence there surges a dire need to remediate the contaminated soils by the use of different approaches for heavy metals removal. Providentially, BC has emerged as a promising, cost-effective and eco-friendly adsorbent for the immobilization of heavy metals (Rathnayake *et al.*, 2021). Another research carried out by Lu *et al.* (2018) concludes that biochar remarkably reduced concentration and the bioavailability of Cd in Cd-polluted soil. It transforms Cd from extractable form to strongly bound form and hence decreases the Cd bioavailability.

Nevertheless, the role of biochar in stabilizing the micropollutants specially the heavy metals has been widely documented by several researchers in the past studies but there are some limitations/gaps that needed special attention. Previous studies have focused on the narrow adsorbents, and have not explored the combined effect of sugarcane bagasse and its derived biochar. Also, there is lack of studies examining the combined effects of organic amendments on both heavy metal stabilization and plant health. So, the current research trial was planned to fill these research gaps by demonstrating the combined effectiveness of sugarcane bagasse and its derived biochar on maize growth in Cd contaminated soil.

This research plan was based on the hypothesis that sugarcane bagasse (SB), and its biochar (SB-BC) or both may be helpful to overcome the Cd contamination in the maize crop.

Materials and Methods

This field experiment was conducted at the farm area of the Institute of Soil and Environmental Sciences (ISES), University of Agriculture Faisalabad (UAF) (31°42'94" N, 73°07'50" E), Pakistan. Soil preliminary physico-chemical properties such as pH, EC, soluble cations and anions, SOM, and metal contents were determined according to standard procedures (Sparks, 1996) (Table 1). Both the organic amendments (SB and SB-BC) as well as Cd in the form of $CdCl_2$ were added in the field one month before sowing in the plots, sized 1 m ×1 m. The Biochar was produced through a slow pyrolysis process at 450 °C temperature for duration of 3 hours in the Agro-climatology laboratory of Department of Agronomy, UAF. Sugarcane bagasse obtained from the Shakar Ganj Mills Jhang (31°13'59"N 72°20'8"E) was used as feedstock material. Level of organic amendment (1%) screened out from pilot experiments and two levels of Cd were used, i.e. 0, 6 mg kg^{-1} (NS-Cd and HS-Cd, respectively). Both the organic amendments (SB and SB-BC) as well as Cd in the form of $CdCl_2$ were added in the field one month before the sowing. Organic amendments were applied at 1% and three levels of Cd contamination were tested, i.e. 0, 3, 6 mg kg^{-1} (NS-Cd, LS-Cd, and HS-Cd, respectively) making up a total of 36 treatments.

Post-harvest soil samples were collected to determine the initial physico-chemical properties and Cd concentration. Agronomic parameters (biological and grain yield), physiological parameters (stomatal conductance, transpiration rate, photosynthetic rate, chlorophyll content, and turgidity), ionic analysis and biochemical analyses (enzymatic analyses) were also done. Crop was harvested at complete maturity.

Estimation of growth parameters

Different growth parameters such as cob length, cob width, and plant height were measured by using scale. 1000 grain weight and number of grain/cob were counted manually by randomly selecting 5 cobs per plant and averaging the values. Shoot and root fresh weights were taken by using weighing balance. Following the preparation, the samples from root and shoot were initially subjected to first air-dry and later on oven drying at a temperature of 65 °C until reaching a constant weight within an involuntary air driven oven (Eyela WFO-600ND, Tokyo Rikakikai, Tokyo, Japan). The dry weight of samples from both, root and shoot was noted once a constant weight was achieved.

Estimation of physiological parameters

For the electrolyte leakage (EL) assessment, fresh leaves were submerged in the sterilized water at a temperature of 4 °C for almost 2 hours right after pressing as prescribed by Arvin and Donnelly (2008). The initial conductivity value was noted down by the use of a conductivity meter model DDS-307 (Leici Corporation, China) and was denoted by L1. Subsequently, the microsome was subjected to boiling at 100 °C in a water-bath for almost 20 minutes and later on cooling down to room temperature. Shortly thereafter that, the second conductivity value was recorded and tagged as L2. The relative electrical conductivity was calculated using the given formula (Eq. 1).

$$\text{Electrolyte leakage (\%)} = \frac{L1}{L2} \times 100 \qquad \text{Eq. 1}$$

Relative Water Content (RWC) was calculated as per Ahanger *et al.* (2018) along with minor changes. Briefly, fresh weight (FW) was noted by weighing the fresh leaves initially, then soaked into Petri dishes containing sterilized water in order to gain turgidity for 24 h, and subsequently their turgid weight (TW) was measured. The dry weight (DW) was after oven-drying. The following formula was employed in calculation of the RWC (Eq. 2).

$$RWC\ (\%) = \mathrm{FW} + \frac{\mathrm{DW}}{\mathrm{TW}} - DW \times 100 \qquad \text{Eq. 2}$$

Fresh leaves were homogenized in 80% acetone by the use of ice-chilled mortar and pestle to determine the total chlorophyll content as per Cock *et al*. (1976). Once the process of extraction was completed, the adjustment of the volume was done by using cold acetone up to 10 mL right after the filtration of homogenate. Optical densities were noted with the help of a UV-1800 spectrophotometer (Shimazdu, Kyoto Japan) at different wavelengths (645, and 663 nm). The following equations were used for the calculation of chlorophyll contents (Eq. 3 & Eq. 4)

$$\text{Chl a } (\text{mg } g^{-1}\text{FW}) = \frac{[12.7 \times \text{A663} - 2.69 \times \text{A645}] \times \text{V}}{1000 \times \text{W}} \qquad \text{Eq. 3}$$

$$\text{Chl b } (\text{mg } g^{-1}\text{FW}) = \frac{[22.9 \times \text{A645} - 4.68 \times \text{A663}] \times \text{V}}{1000 \times \text{W}} \qquad \text{Eq. 4}$$

Wherein, D645 and D663 represent the chlorophyll contents absorbance at above mentioned wavelengths correspondingly and fresh weight of leaves (g) is denoted by W. Photosynthesis-apparatus Li-6400 (LI-COR Inc., Lincoln, NE, USA) was employed intended for determination of transpiration rate, stomatal conductance, and the photosynthetic efficiency within the fully extended leaf. That subsequently helped maintaining both, the photosynthetic photon flux density (PPFD) and CO_2 concentration at 1000 m^{-2} s^{-1} and 400 µmol CO_2 mol^{-1}, respectively.

Analysis of enzymatic and non-enzymatic antioxidant enzymes

First of all, the harvested seedlings were subjected to freezing in liquid nitrogen by keeping at -80 ºC in order to determine the additional traits of osmotic and antioxidants content, oxidative stress, and secondary metabolites accumulation. All these spectrophotometric analyses were done using a HITACHI spectrophotometer (UV-3900, Hitachi High-Technologies Corporation, Tokyo Japan) device. Plant leaves weighing 0.5 g were crushed and powdered in a pre-chilled mortar and pestle, in addition to 5 mL of 0.1 *M* K_2SO_4 as buffer solution (having pH 7.8) and subsequently the paste was subjected to centrifugation at 4 °C for 20 mins. Later on, the filtrate was collected in order to estimate any further antioxidant enzymatic parameters. The superoxide dismutase (SOD) activity was determined on the basis of the proficiency for the inhibition in the nitro blue tetrazolim (NBT) decline through superoxide anion generated by riboflavin system using light intensity of 4000 W at 25 °C (Giannopolitis and Ries, 1977). Peroxidase (POD) activity was determined as per Bianco and Defez (2009), with the help of guaiacol ($C_7H_8O_2$) as an electron donor. The conversion rate of H_2O_2 into H_2O and O_2 molecules was observed and noted to determine the catalase (CAT) activity (Bianco and Defez, 2009). Glutathione reductase (GR) activity was determined using the protocol by Palma *et al*. (2018) whereas the NADPH glutathione-dependent oxidation was recorded at a wavelength of 340 nm for duration of 2 minutes. Ascorbate peroxidase (APX) activity was estimated using the protocol by Fan *et al*. (2020) by recording the H_2O_2-dependent ascorbate-oxidation at a wavelength of 290 nm for 3 minutes. Fresh leaves were crushed in 6% tricholoroacetic acid for the determination of ascorbate (AsA) contents, and subsequently the filterate was completely blended with thiourea (10%) and di-nitrophenyl-hydrazine (2%). Later, the samples were incubated in the water-tub for 15 minutes followed by cooling and adding about 80% of 5 mL sulfuric acid (H_2SO_4). The optical density was observed at 530 nm (Ahanger *et al*., 2018). The reduced glutathione (GSH) level was assessed as per Ahanger *et al*. (2018) procedure.

Summarizing soluble sugars (SS) and compatible solutes

To determine the proline content, fresh leaves along with sulphosalicyclic acid (2%) were subjected to manual grinding by the use of pestle and mortar, and then centrifuged at 3000 rpm for 10 minutes. Afterwards, filtrate (2 mL) was collected and poured into ninhydrin reagent (2 mL) and glacial acetic acid and subsequently placed for incubation at 100 °C for 1 hour. The reaction was stopped by transferring the sample to the ice bath and following the separation of proline using toluene whereas absorbance was noted at 520 nm.

Soluble sugars were determined through the Ahanger *et al*. (2018) method. 1000 mg of dried, crushed samples were standardized in ethanol (80%) followed by the centrifugation of the saturated sample at 500 rpm for 10 min. One (1) mL of supernatant solution was thoroughly mixed with 4 mL of anthrone (0.2%). Meanwhile the optical density was recorded at 620 nm.

The Bradford method (Bradford,1976) was followed to determine the soluble proteins by taking BSA as standard and recording the absorbance at 595 nm. In order to determine glycine butaine, 500 mg dried crushed sample was poured into 20 mL of distilled water and samples were subjected to overnight shaking at 25 °C. After taking the supernatant, filterate (0.5 mL) was mixed thoroughly with potassium Iodide (0.2 mL), dissipating the periodide crystals in the 1,2-dichloroethane and subsequently set aside for almost 3 h. Further calculations were done on the basis of standard curve while noting down the optical density at a wavelength 365 nm. In order to determine the total phenolic contents in 10 mL of acetone (80%), 0.5g of leaf tissue was taken by following the Folin-Ciocalteu protocol and eventually recording the absorbance at 765 nm.

Malondialdehyde (MDA), O-2, and hydrogen peroxide (H_2O_2) content

For the determination of MDA, fresh leaves were crushed and standardized in 0.1% trichloroacetic acid and then subjected to centrifugation following the method of Heath and Packer (1968). The filtrate was methodically homogenized in 0.5% thiobarbituric acid at a temperature of 95 °C for almost 30 minutes. Finally, the absorbance was recorded at wavelength ranging from 532-600 nm followed

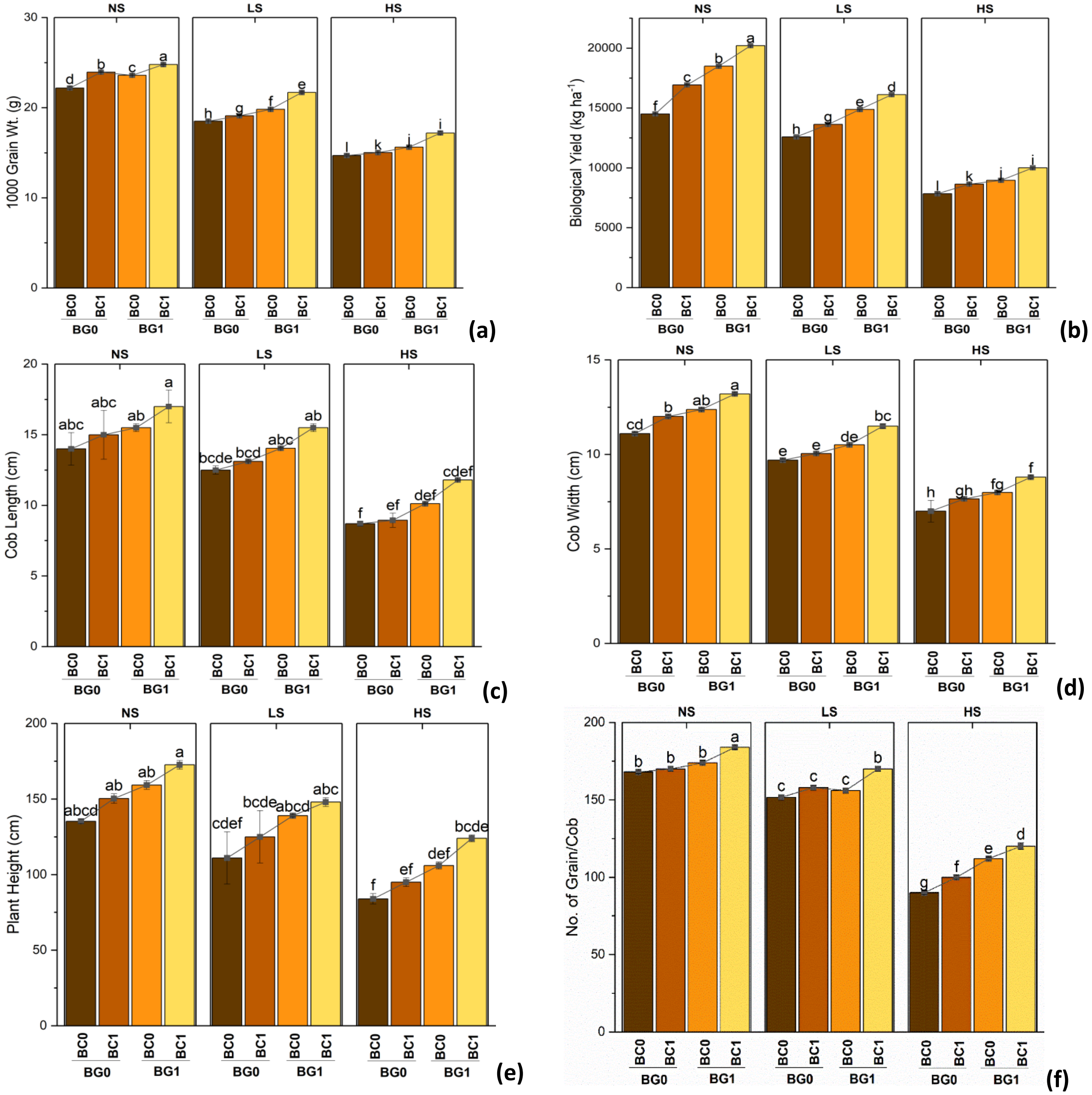

Figure 3.1: Effect of SB and SB-BC on (a) 1000 grain weight, (b) biological yield, (c) cob length, (d) cob width, (e) plant height and (f) Numbers of grains per cob of maize grown in Cd-contaminated soil (average of three replications)

by extinction coefficient (ε) of 155 mM^{-1} cm^{-1} for further computation. Hydrogen peroxide (H_2O_2) was calculated following the method of potassium iodide (KI). To measure the H_2O_2, approximately 100 mg fresh leaves were treated in 5 mL trichloroacetic acid (0.1%) and subsequently subjected to centrifugation at a rate of 10,000 rpm for nearly 10 minutes. Followed by mixing the 500 µL of filtrate in the potassium phosphate buffer of the same quantity with pH 7.0 and finally addition of 1 mL KI into the solution was done. The absorbance was noted at a wavelength of 390 nm right after homogenizing the solution (Elkelish *et al.*, 2021). Protocol by Chen *et al*. (2022) was used for the quantification of O_2 content and reading the absorbance at 530 nm.

Statistical analyses

Statistical analysis was executed by the use of SPSS (1BM SPSS, USA) and Origin (v. 9; Origin Lab Corp., MA, USA) was used for plotting the graphs. A two-way analysis of variance (ANOVA; SPSS statistics 22.0) was performed at significance less than p=0.05 to determine impacts of SB and SB-BC on the response of soil variables.

Results

Plant growth and yield traits

Sugarcane bagasse and its derived biochar had the notable impact on the physio-chemical as well as physiological attributes of the maize crop. In current research trial, it was examined that as compared to the control treatment, all plants in both groups; No stress and High Stress Cd (NS-Cd and HS-Cd) combined with the maximum dose of SB and SB-BC were healthier, looked greener, and showed better tolerance against Cd stress. The elevation of plant height, biological yield, cob length, cob width, number of grains per cob, and 1000-grain weight was more pronounced with the concentration of soil applications of SB and SB-BC, both individually and in combination (SB, SB-BC). Conversely, these parameters exhibited a decline with an escalation in Cd stress in HS-Cd group. Soil application with SB and SB-BC showed better plant growth without Cd stress (NS-Cd group) as compared to the high Cd stress (HS-Cd group).

In the NS-Cd group, a maximum of 21% increment in height, 34% in biological yield, 29% in cob length, 28% in cob width, 9.5% in no. of grains per cob, and 11% in the number of grains per row were examined with combine application of 1% SB and SB-BC compared to control (Figure 3.1).

Plant height, biological yield, cob length, cob width, no. of grains per cob, and 1000 grains weight in HS-Cd group, increased by 34, 26, 46, 15, 43 and 17% with combine addition of SB and SB-BC at 1% over its control treatments, respectively (Figure 3.1). The increase in plant growth parameters was minimum in the controls of each group, in which no SB and SB-BC were applied.

Plant shoot biomass, root biomass and cadmium accumulation indices

Relative to the control, all plants at each contamination level with the combined incorporation of SB and SB-BC (1% of each) exhibited an increase in both, root and shoot fresh (FW) and dry weight (DW) while decrease in both root and shoot Cd concentrations. Root fresh weight (RFW), root dry weight (RDW), shoot fresh weight (SFW), and shoot dry weight (SDW) significantly increased by soil applications of SB and SB-BC in combination and decrease in HS-Cd group. Likewise, there was a decline in shoot and root Cd fractions by the combined application of SB and SB-BC that resulted in better plant growth parameters without Cd stress (NS-Cd group) compared to LS-Cd and HS-Cd groups (Figure 3.2 e and f).

Percent increase measured in shoot and root weight; shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight were as 34%, 29%, 22%, and 28%, respectively, over control remained the highest with a combined application of 1% SB and SB-BC in the NS-Cd group. Shoot and root Cd were decreased by 25% and 31%, respectively, under the combined application of 1% SB and SB-BC relative to the control treatment (Figure 3.2 a-d).

Combined use of 1% SB and SB-BC in HS-Cd significantly enhanced the SFW, SDW, RFW, and RDW of both: shoots and roots (41, 26, 33, and 19%, respectively), relative to control treatments. Contrarily, a marked decrease in shoot (15%) and root (27%) Cd concentration was observed with combined addition of SB and SB-BC at 1% over its control treatments, respectively (Figure 3.2 a-d). The increase in the SFW, SDW, RFW, and RDW and decrease in shoot and root Cd concentration was lowest in controls of both groups, in which no SB and SB-BC were applied.

Effect on the enzymatic antioxidant's activities

Soil application of SB and SB-BC significantly influenced the antioxidant enzymes (Such as APX, SOD, CAT, and POD) to different Cd stress as compared to the control treatment. In NS-Cd, SB and SB-BC treatments together resulted in a significant increase of 29%, 68%, 36% and 80% in SOD, POD, CAT and APX, respectively, as compared to the control treatment (Figure 3.3). Linear correlation was observed between activities of antioxidant enzymes and the dosage of SB and SB-BC in both stress

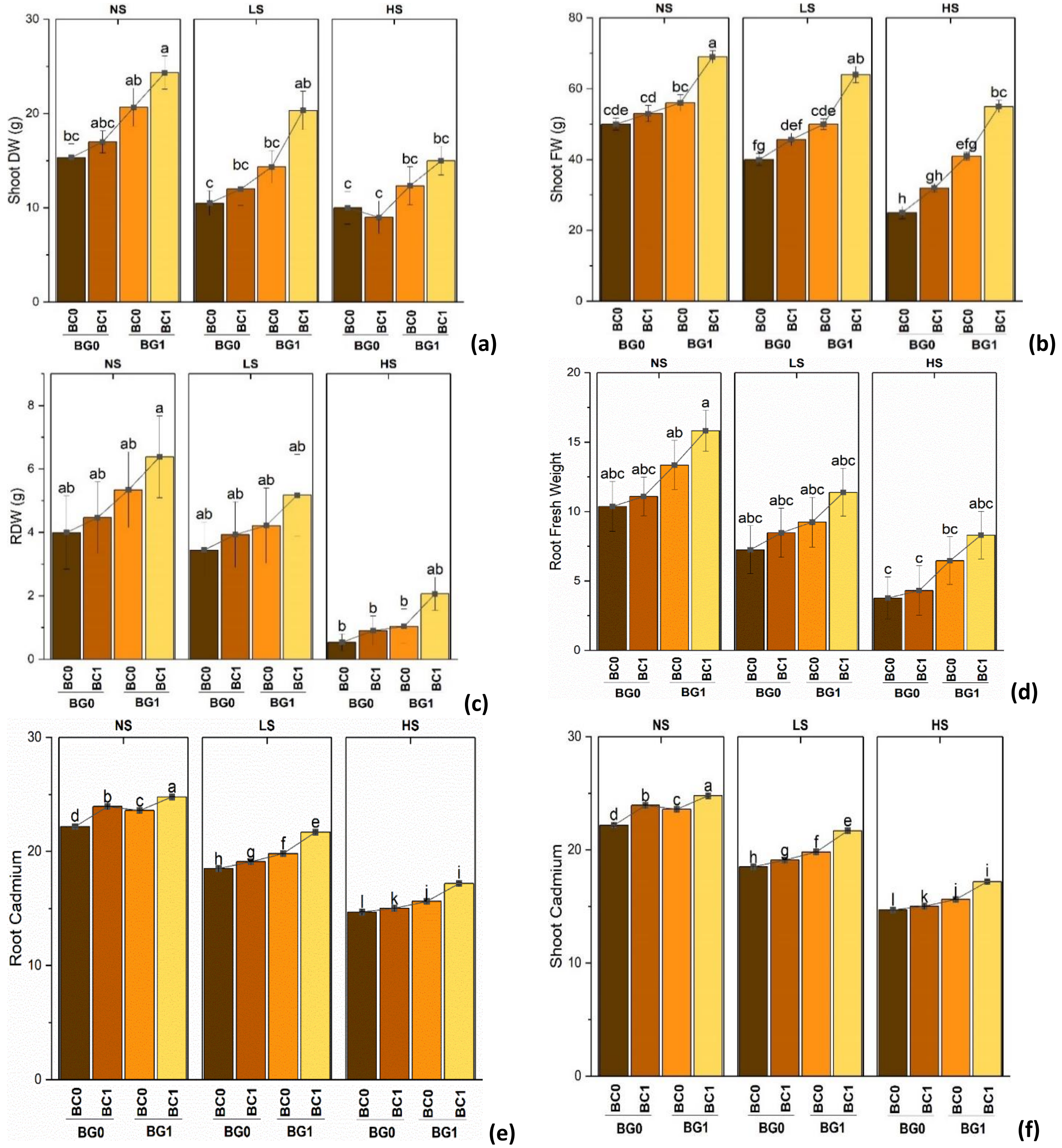

Figure 3.2: Effect of SB and SB-BC on (a) shoot dry weight, (b) shoot fresh weight (SFW), (c) root dry weight (RDW), (d) root fresh weight (RFW), (e) root cadmium (mg kg^{-1}) and (f) shoot cadmium (mg kg^{-1}) of maize grown in Cd-contaminated soil (average of three replications)

groups. Considerably, all the antioxidant enzymes exhibited amplified activities by the combined addition of 1% SB and SB-BC in both groups relative to control. Likewise, HS-Cd SB and SB-BC treatment together exhibited a prominent rise in SOD (38%), POD (85%), CAT (58%) and APX (74%) relative to control (Figure 3.3).

the rate of 2% exhibited notable increase in TP, AsA, GSH, and GR (27%, 39%, 15%, and 17%, respectively) as compared to the control treatment. Among all groups, the SB and SB-BC addition at 1% jointly provoked the non-enzymatic content generation (TP, AsA, GSH, and GR) in NS-Cd, which was followed by HS-Cd groups, compared with their respective control treatments (Figure 3.4).

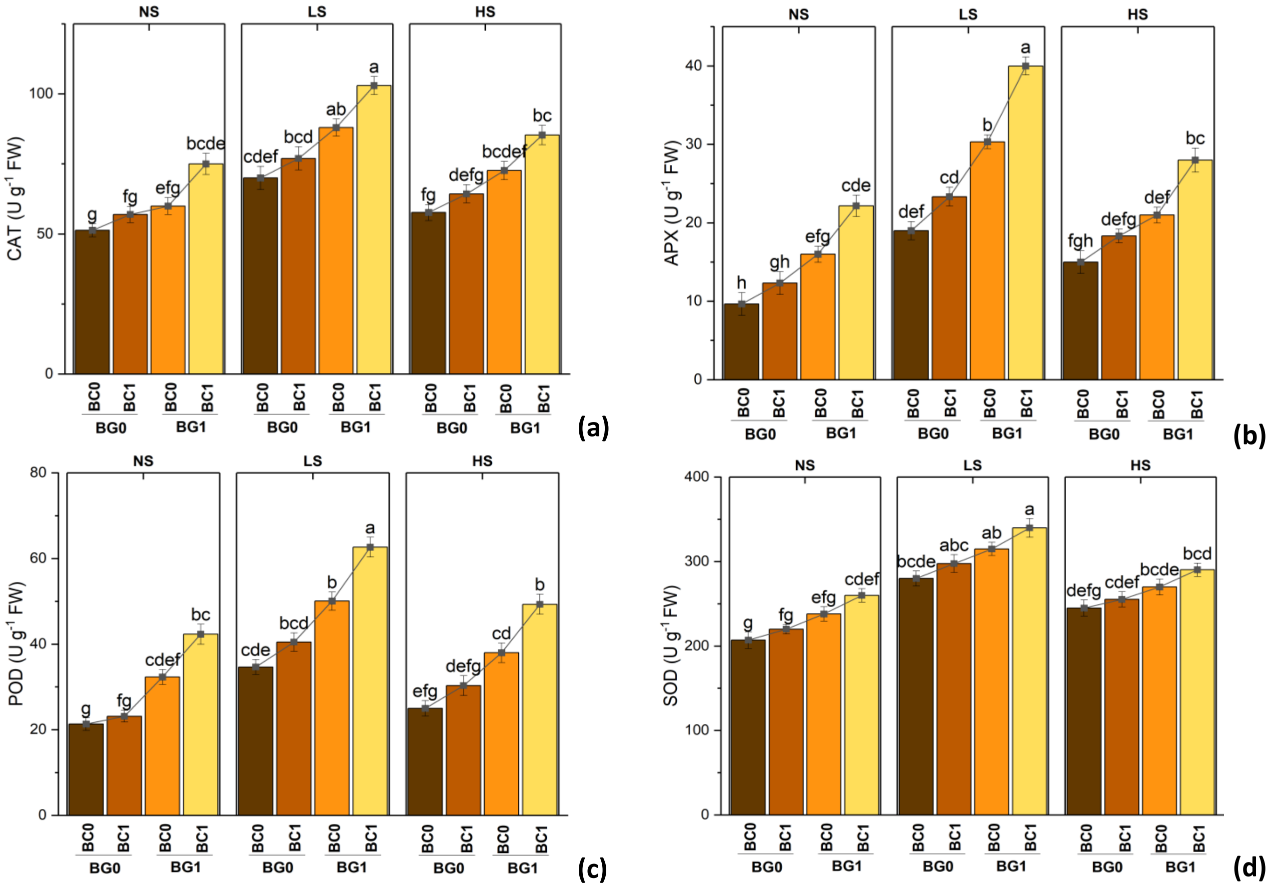

Figure 3.3: Effect of soil applied SB and SB-BC on (a) catalase (CAT), (b) ascorbate peroxidase (APX), (c) peroxidase (POD) and (d) superoxide dismutase (SOD) activity of maize grown in Cd-contaminated soil (average of three replications)

Effect on total phenolics (TP), ascorbate (ASA), and reduced glutathione (GR)

The antioxidant response, as indicated by TP, AsA, GSH, and GR was considerably augmented by SB and SB-BC in both stress groups (NS-Cd, HS-Cd). Within HS-Cd, 1% SB and SB-BC treatment resulted in proliferation of 1.1, 0.5, 0.25, and 0.7 -folds in TP, AsA, GSH, GR, respectively. In both groups, the non-enzymatic antioxidant proficiency was increased with the use of SB and SB-BC at the rate of 1% over control. In NS-Cd group, combined SB and SB-BC addition at

Effect on leaf chlorophyll (CHL), relative water content (RWC), and gaseous exchange attributes

A significant increase in the leaf chlorophyll contents (CHL), RWC, and gaseous exchange attributes was observed by exogenic application of SB and SB-BC under no stress of Cd stress (NS-Cd) that was followed by HS-Cd. Comparaed to the control treatment of NS-Cd and HS-Cd, there was a significant decrease in leaf chlorophyll (29% and 35%) and RWC (29% and 25%), respectively (Figure 3.5). The maximum decrease in photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), and interior CO_2 concentrations (Ci) was 24%, 21%, 11%, and 52% in HS-Cd as compared to NS-Cd control (Figure 3A-D). SB and SB-BC application with the same concentrations in HS-Cd plant exhibited highest decline of leaf chlorophyll (52%), RWC (29%), A (11%), E (57%), Gs (41%) and Ci (24%) as compared to their relative controls (Figure 3.5).

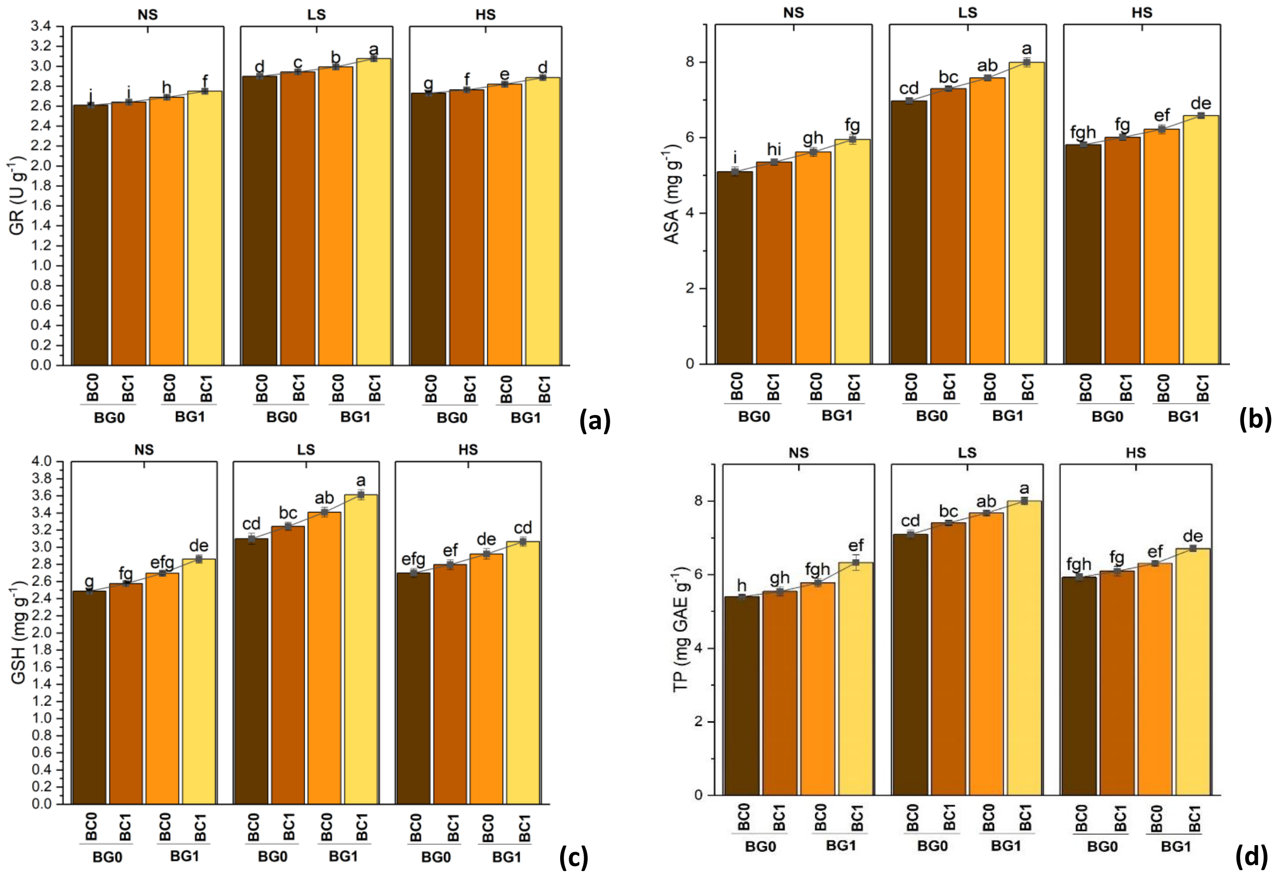

Figure 3.4: Effect of soil-applied SB and SB-BC on (a) glutathione reductase (GR), (b) ascorbic acid (AsA), (c) glutathione (GSH), and (d) total phenolics (TP) content of maize grown in Cd-contaminated soil (average of three replications)

Effect on membrane stability

There was a significant decrease in the production of ROS in plants with the application of SB and SB-BC over control treatment and consequently a significant reduction in lipid peroxidation and EL under-Cd stress was observed. However, the individual treatment from HS-Cd group exhibited an increase in H_2O_2 (0.9-fold) and O_2^- (0.11-fold) activity (Figure 3.6). Also, MDA (0.21-fold) and EL (0.35-fold) content was amplified in the HS-Cd group over NS-Cd control (Figure 3.6). In HS-Cd treated plants, maximum decline in H_2O_2 (70%) and O_2^- (1%) generation (Figure 3.6 c) was noticed at 2% SB and SB-BC boosted the MDA content (29%) and EL (39%) as compared relative to the control treatment (Figure 3.6).

Effect on osmolyte concentration

The combined application of SB and SB-BC significantly boosted the levels of proline (PC), total soluble protein (TSP), total soluble sugars (TSS), and glycine betaine (GB) in HS-Cd, followed by the NS-Cd group. In an individual treatment of HS-Cd, the amounts of GB, PC, TSP, and TSS were greater than NS-Cd by 48%, 37%, 12%, and 24% over NS-Cd control, respectively (Figure 3.7). However, the combined application of SB and SB-BC enhanced the osmolyte levels in both groups relative to the control treatment. Similarly, the concentrations of PC (11%), GB (39%), TSP (37%), and TSS (24%) increased in HS-Cd, after treatment with SB and SB-BC relative to control (Figure 3.7).

Principle components analysis (PCA)

The maize crop responses credited to various soil-

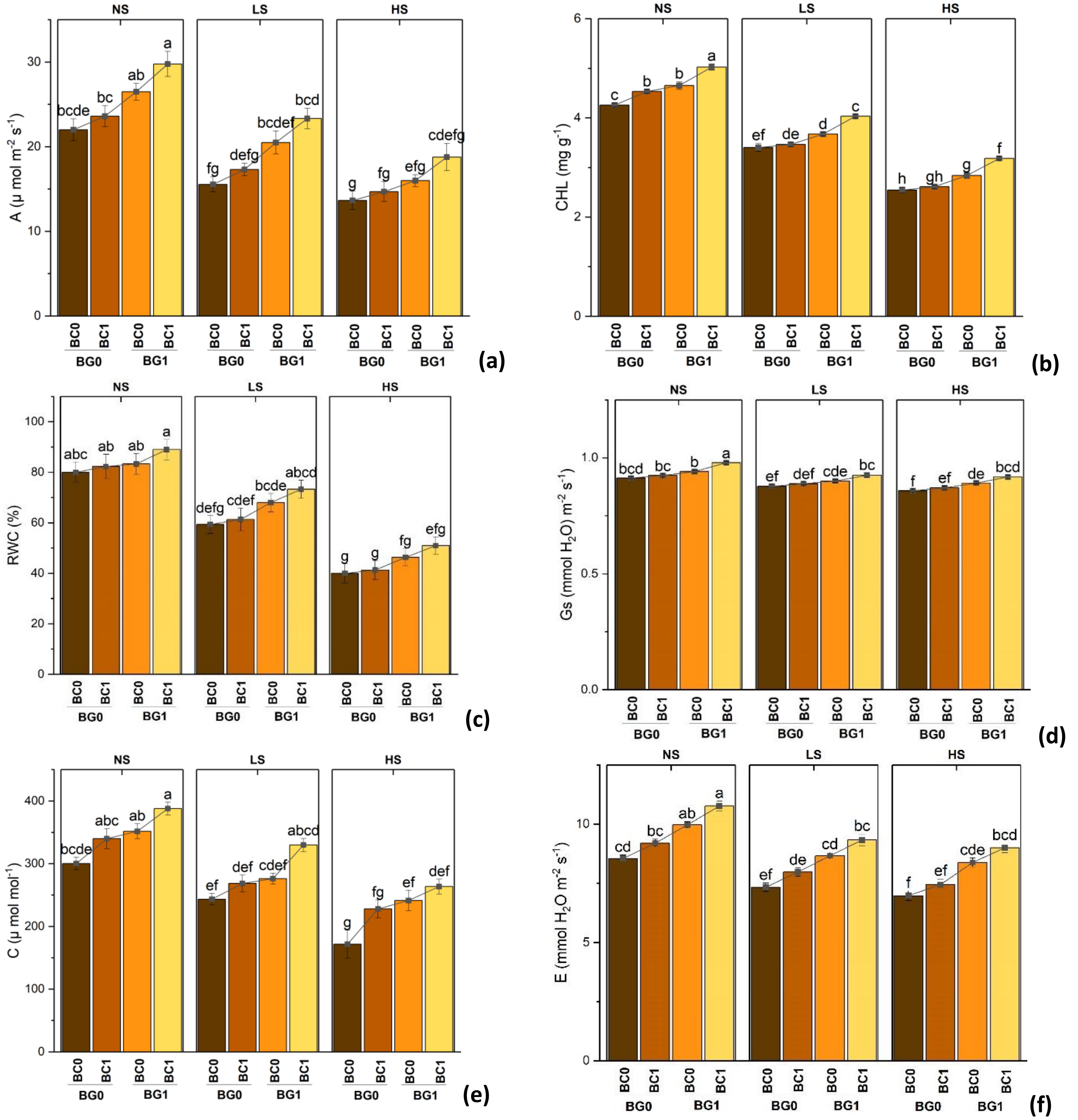

Figure 3.5: Effect of SB and SB-BC on (a) Photosynthesis (A), (b) Chlorophyll (CHL), (c) relative water content (RWC), (d) stomatal conductance (Gs), (e) CO_2 concentration (Ci) and (f) transpiration rate (E) of maize grown in Cd-contaminated soil (average of three replications)

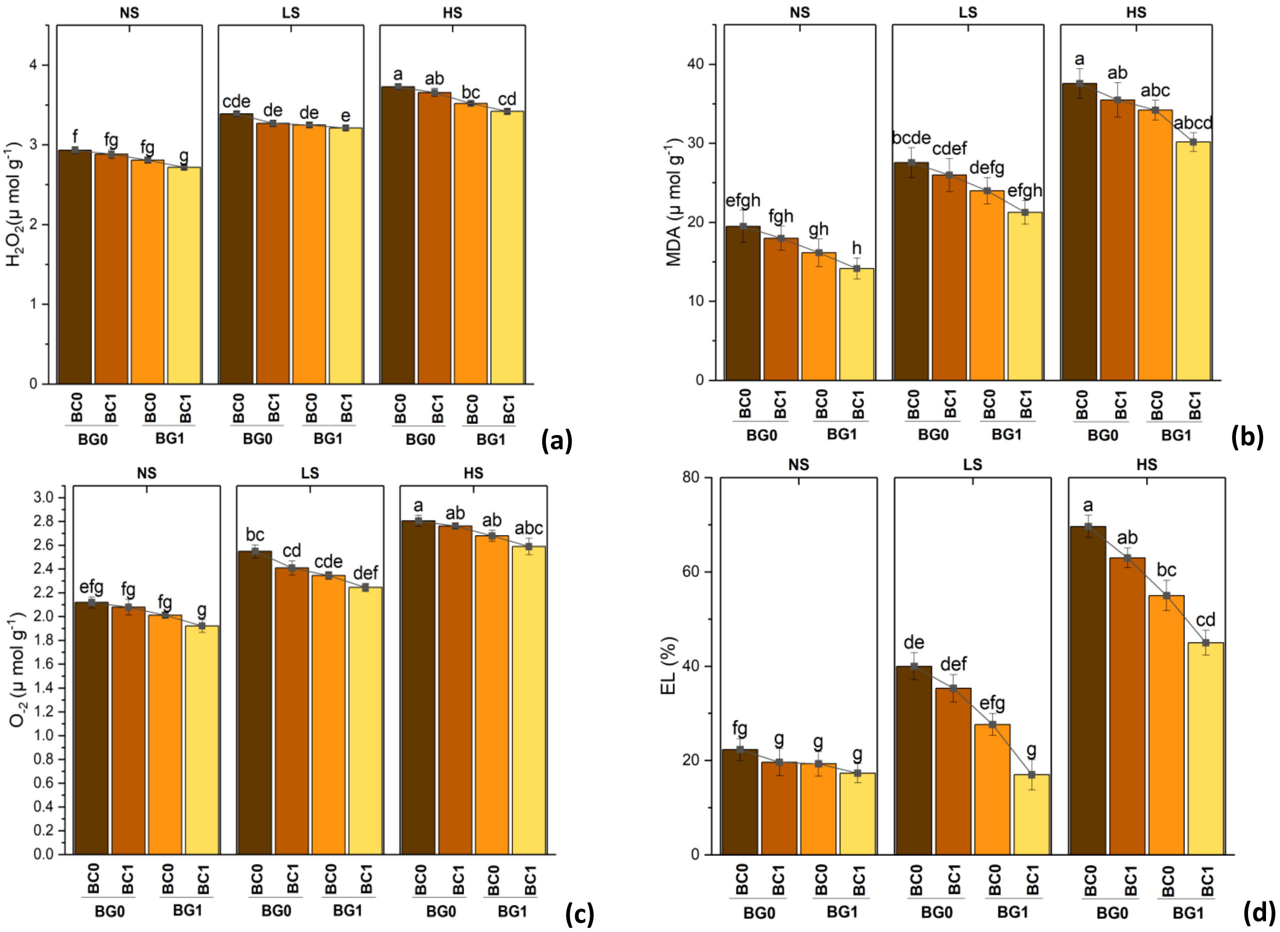

Figure 3.6: Effect of soil-applied SB and SB-BC on (a) hydrogen peroxide (H_2O_2), (b) malondialdehyde (MDA), singlet oxygen (O_2^-) and (d) electrolyte leakage (EL) of Maize grown in Cd-contaminated soil (average of three replications)

applied SB and SB-BC concentrations under Cd stress were shown in PCA (Figure 3.8). The first two components PC1 and PC2 being the primary contributors, held accountable for 89.9 % and 6.7 % correspondingly in overall dataset variation, as compared to rest of the database components. All the components were precisely distributed in first two components of this principle coordinate analysis. A strong separation was noticed between cadmium in root, shoot and bioavailable compared to other parameters. Moreover, these parameters distribution elucidated that combined metals stress to plants were mostly characterized to reduced plant morphological and physicochemical responses.

Parameters details as; RL = Root length; SL = Shoot length; PA = Projection area; SA = Surface area; RV = Root volume; SW = Shoot weight; RW = Root weight; B-Cd = Bioavailable Cd; S-Cd = Shoot Cd; R-Cd = Root Cd; B-Zn = Bioavailable Zn; S-Zn = Shoot Zn; R-Zn = Root Zn; Pn = Photosynthesis rate; E = Transpiration rate; gs = Stomatal conductance; Ci = Carbon dioxide concentration; Chl = Total chlorophyll contents; RWC = Relative water contents; Fv/Fm = maximum quantum yield of PSII; SOD = superoxide dismutase; POD = peroxidase; CAT = catalase; APX = peroxidase; H_2O_2 = hydrogen peroxide; EL = electrolyte leakage, and (e) O_2^-; superoxide contents.

Correlation among plant growth and morpho-physiological parameters

The stomatal conductance (Gs), leaf photosynthetic rate (A), intracellular concentration of CO_2 (Ci), and transpiration rate (E) are directly correlated to the overall plant performance under heavy metals stress and negatively associated to the oxidative stress (H_2O_2, O^{-2}) and membrane stability (MDA, EL) after soil applied SB-BC application under Cd stress. A direct positive correlation of IRGA parameters (such as A, E, Gs and Ci) with plant growth parameters (including height (PH) and biomass (PB), CHL, RWC and Fv/Fm) was observed by the SB and SB-BC application in Cd stressed soils. As there is a positive correlation between PH and PB under metal stress, they also exhibited remarkably strong positive correlation with RWC, total chlorophylls and photosystem II activity (Fv/Fm). There was a negative correlation between plant morpho-physiological attributes (such as CHL, PH, PB, RWC and Fv/Fm) and oxidative stress (H_2O_2, O^{-2}) and membrane stability indices (EL, MDA). Conversely, the chlorophyll content was positively correlated with RWC, Fv/Fm and other IRGA analysis. Heavy metal (Cd) stress led to the intensified generation of both, ROS (H_2O_2, O^{-2}) as well as of lipid peroxidation indicated by MDA, EL, demonstrating significant positive correlation between them (Figure 3.9).

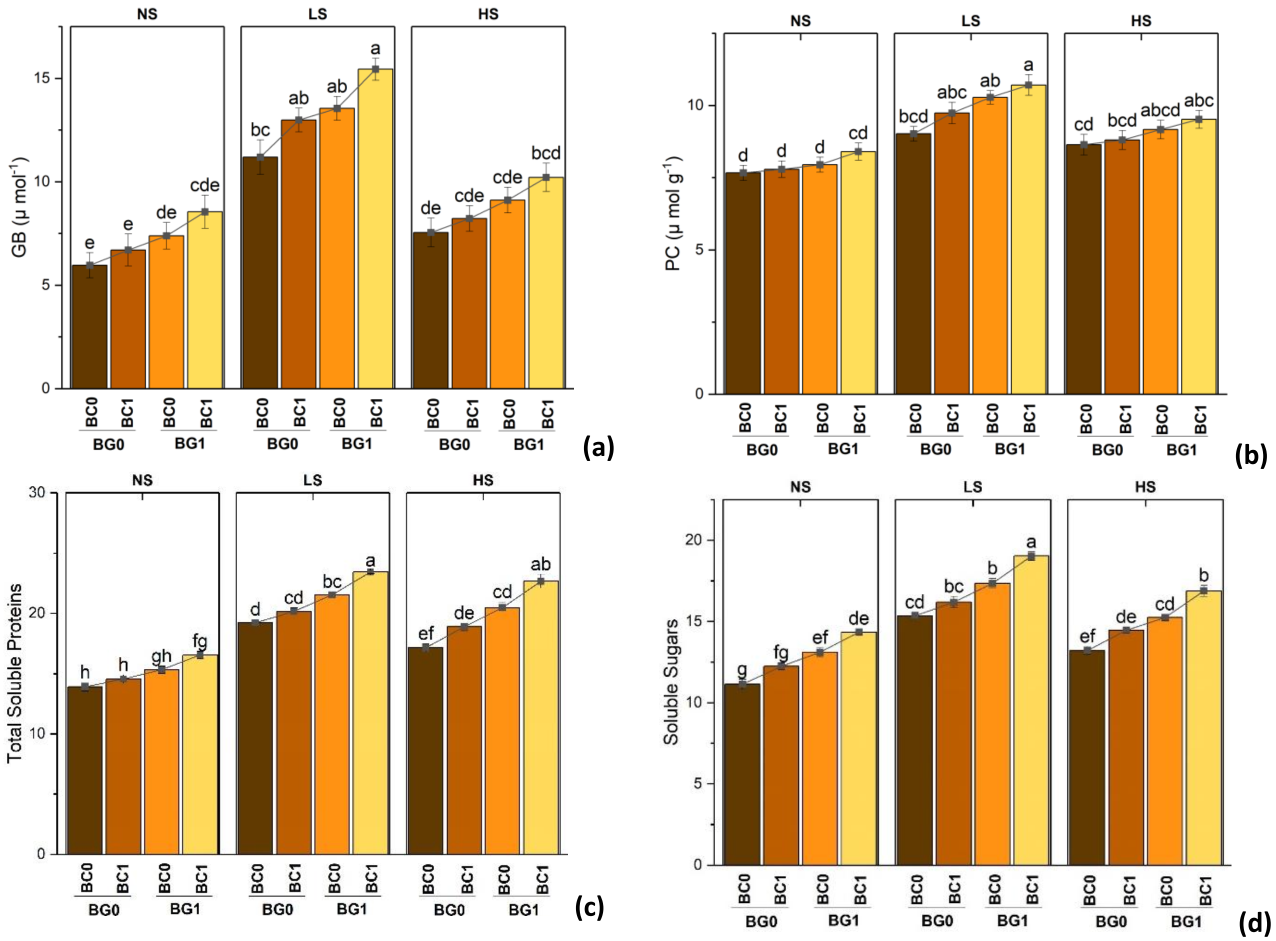

Figure 3.7: Effect of soil-applied SB and SB-BC on (a) glycine betaine (GB), (b) proline content (PC), (c) soluble protein (SP) and (d) soluble sugars (SS) of Maize grown in Cd-contaminated soil (average of three replications)

Correlations are displayed by specific colours (positive to negative); different colours and circle size are proportional to the correlation coefficient. RL = Root length; SL = Shoot length; PA = Projection area; SA = Surface area; RV = Root volume; SW = Shoot weight; RW = Root weight; B-Cd = Bioavailable Cd; S-Cd = Shoot Cd; R-Cd = Root Cd; B-Zn = Bioavailable Zn; S-Zn = Shoot Zn; R-Zn = Root Zn; Pn = Photosynthesis rate; E = Transpiration rate; gs = Stomatal conductance; Ci = Carbon dioxide concentration; Chl = Total chlorophyll contents; RWC = Relative water contents; Fv/Fm = maximum quantum yield of PSII; SOD = superoxide dismutase; POD = peroxidase; CAT = catalase; APX = peroxidase; CA = carbonic anhydrase; RA = rubisco activity TBARS = thiobarbituric acid reactive substances; LOX = lipoxygenase; H_2O_2 = hydrogen peroxide; EL = electrolyte leakage, and (e) O_2^-; superoxide contents.

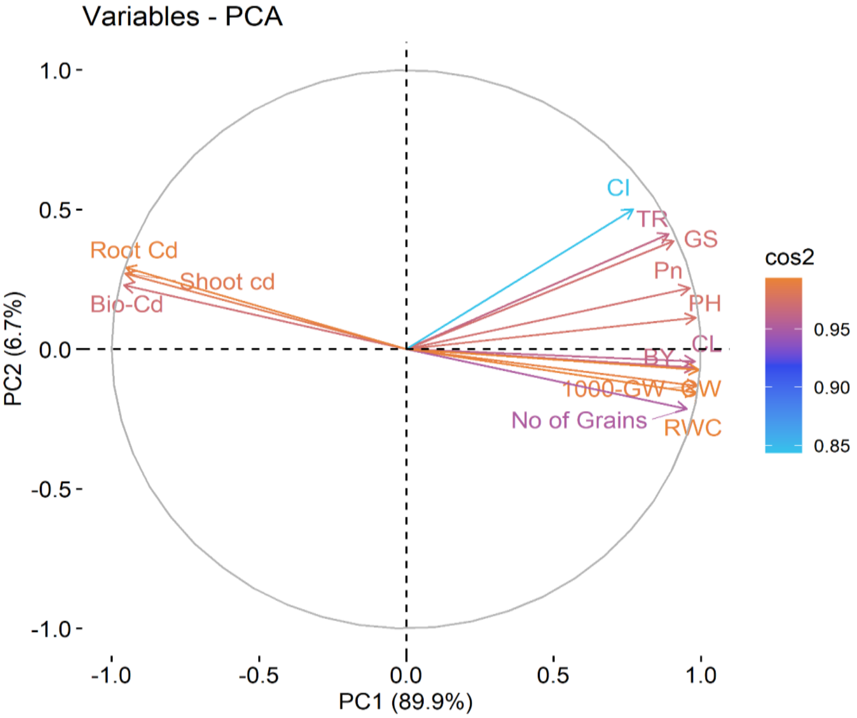

Figure 3.8: Principle component analysis (PCA) based on distribution of different parameters of SB-BC treated maize plants grown in Cd-contaminated soil

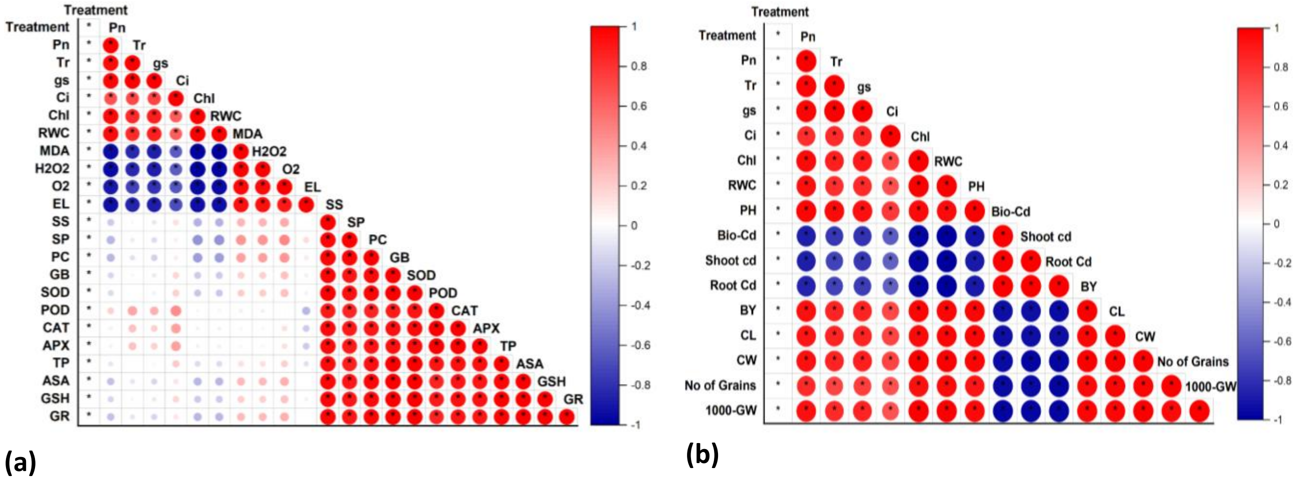

Figure 3.9: Capitals letters represents pearson's correlation matrix of; A) of plant morphological variables with plant physiological activities; b) among chemical properties, plant lipid peroxidation and biochemical traits in response to soil applied SB-BC under Cd stress

Discussion

This research was conducted to assess the comparative potential of sugarcane bagasse (SB) and sugarcane bagasse derived biochar (SB-BC) on maize growth in cadmium (Cd) contaminated soil. Feedstock for SB-BC was collected from Shakkar Ganj Mills Limited, Jhang and its pyrolysis was done in the Agro-climatology Laboratory of Department of Agronomy UAF, at temperature 450˚C for the duration of 3 hours.

In current study, the plot yield and dry biomass were significantly improved with an increased dose of biochar. It was found that by the application of BC, the Cd concentration in shoots and roots of maize was decreased as well as in the soil and these findings are in line with studies reported by Haider *et al*. (2022). Generally, there was a variation in response of maize to the different levels of SB and SB-BC, Cd concentrations and growth conditions (Fellet *et al.*, 2014). This increment in plant biomass can be attributed to the comparatively higher nutrients and micronutrients content of the biochar (Hou *et al.*, 2022). Additionally, SB-BC may also enhance plant biomass by refining physical, chemical and biological characteristics of soil (Hamidzadeh *et al.*, 2023). In current study, the combined application of SB and SB-BC (1%) considerably enhanced the fresh and dry weight, plant height, cholorophyll content, transpiration rate, photosynthetic and transpiration rate, relative water content and stomatal conductance. Counter wise, a significant decrease in soluble sugars (SS), electrolyte leakage (EL), antioxidants activity including APX, SOD, POD and GR, and proline content as well as Cd concentration was observed.

The present study shows significant decrease in both root and shoot Cd concentration with application of SB-BC and similar results have been reported in the literature previously (Rassaei, 2023). Various mechanisms such as co-precipitation, precipitation, adsorption, and complexation may contribute to the decreased concentration of extractable metals. The addition of biochar having greater pH, larger content of carbon, phosphorus and minerals (Zn, Si) might facilitate these mechanisms as reported by Peng *et al*. (2023). It is expected that SB and SB-BC application induced higher pH that might have increased the sorption or precipitation of metal as the pH directly influences the surface charge properties ultimately modifying the adsorption or precipitations of heavy metals.

The greater surface area and functional groups associated with biochar might be helpful in increasing Cd sorption/adsorption. The phyto-availability of heavy metal was decreased by the Cd adsorption on the surface of biochar. The decreased up-take and bioavailability of heavy metal to the roots and shoots of the crop might also be attributed to the decline in available fraction of heavy metal due to the addition of biochar. von Gunten (2024) reported that increase in plant biomass could be directly linked to the decrease in heavy metal bioavailability in biochar-amended treatment as compared to the control treatment chiefly due to dilution effect. Sugarcane bagasse biochar possess high Si-content and it may decrease the Cd translocation either by elevating Si-induced apoplastic binding in crop roots or by interaction of metal with silica in both, endodermis and root pericycle as reported by Dinh *et al*. (2024). Biochar has significant potential for immobilization of Cd mainly by two mechanisms; 1) the presence of multiple functional group on the surface of biochar makes it suitable for complex formation with Cd that can ultimately limit the bioavailability of Cd to the crop and 2) by exchanging the absorbed cations such as Ca, Na, Mg, and K present on the surface of biochar with Cd as documented by Wang *et al*. (2024). Another mechanism involved in reduced Cd availability by biochar may be the precipitation of Cd with carbonates ($MgCO_3$ such as $CdCO_{3)}$ or phosphates (Cd_3 $(PO_4)^{2}$)) as reported by Meng *et al*. (2023). The remarkable properties of biochar such as greater surface area and pH, higher cation exchange capacity, porous structure, and presence of multiple functional groups makes it an effective substance to reduce the heavy metals mobility within the soil profile (Burachevskaya *et al*., 2023). Previously, it has been reported that many researchers employed higher dosages of biochar in order to reduce the heavy metal uptake or accumulation in the plants/crops (Qian *et al.*, 2024). The single application of biochar, as compared to the compost, has significantly a greater aptitude of improving soil properties as well as plant growth and immobilizing Cd subsequently reduced its bioavailability and phyto-toxicity, specifically it has great potential of reducing extractable heavy metals including Cd as reported by Ghorbani *et al*. (2023). Hence, higher levels of biochar along with other organic amendments may serve as a promising approach for reduction of heavy metals accumulation by crops.

It was found that the chlorophyll contents, photosynthetic rate, relative water content, stomatal conductance, and internal CO_2 concentrations were decreased amid Cd stress exposure. It was previously documented by Pandey *et al*. (2021) that Cd may cause damage to δ-aminolevulinic acid dehydratase (ALAD) that can ultimately diminish the photosynthetic pigments. The major reasons behind this reduced photosynthetic pigmentation and chlorophyll contents is attributed to the ability of Cd to interfere with photosynthetic electron transport, enzymes of Calvin cycle and thylakoid membrane (Baruah *et al.*, 2023). Also, another reason may be the unnecessary ROS production that triggers the oxidative stress and photosynthetic capacity of plants and consequently led to the negative activity of fixing carbon (Liu *et al.*, 2022). Additionally, the breakdown of cell membrane and electrolyte leakage (Srivastava *et al.*, 2021), both were directly correlated to the excessive ROS production in response to Cd stress. As the ROS are generated as by-product of plant cell metabolism and these may contain free radicals. An excessive amount of ROS are generated due to disturbance in plant cell homeostasis as a result of Cd exposure (Ali *et al.*, 2022).

It was also found that combined applications of SB and SB-BC enhanced the soil enzymatic activities in maximum aged and incubated soils as compared to the control treatment and these findings are in line with results reported by Lebrun *et al*. (2023). The direct and indirect influences of heavy metal concentration on the soil enzymatic activities have been reported in numerous studies (Rassaei, 2023). Cui *et al*. (2021) reported a strong positive correlation among plant enzymatic activities and soil pH. Our findings are also in accordance with the results reported by Cui *et al*. (2021) attributing the alteration in soil enzymatic activities to direct rise in the soil pH.

Membrane stability is one of the crucial properties needed for the efficient plant survival in both, biotic or abiotic stress as its injury might cause cellular expiry (Paes *et al*., 2022). Any cell damage or membrane instability is indicated by Malondialdehyde (MDA). Exposure to any kind of stress leads to the excessive MDS production and lipids peroxidation that ultimately pose serious threat to plant health (Morales *et al.*, 2022). In this study, Cd stress caused a significant amplification of MDA and EL generation in maize crop grown in Cd-contaminated soil. Membrane molecules are in-stabilized due to increased accretion of reactive species as a result of Cd toxicity. Plant cell can resist to the modifications caused by heavy metals stress by the action of proline and lipid peroxidation through prevention of damage caused by free redicals. It is reported that heavy metal toxicity can considerably enhance the proline and MDA content in crops (Ma *et al*., 2020). The accumulation of proline within the plant cell might have various objectives such as defense mechanism, osmoregulation, protein synthesis and maintenance, as well as detoxification of harmful free radicals. In another study conducted by Singh *et al*. (2022), an increased quantity of proline content was found that could be a potential mechanism of plant to survive the Cd toxicity.

Current study shows that application of sugarcane bagasse-derived biochar could be used as potential remediation strategy to combat cadmium contamination in the field soils. Hence, it proves to be a prospective sustainable amendment for Cd-contaminated for agricultural soils. SB-BC has a key role in improving soil health as well as decreasing the heavy metal uptake by the crop that ultimately makes it a probable cost-effective option for farmers, especially for those in developing areas like Pakistan. However, the current findings are limited to the crop type i.e., maize, and more extensive research is required to have better understanding of long-term impacts of continuous biochar application in field conditions as well as any potential environmental and economic risks. Consequently, the widespread adoption of SB-BC as an organic amendment will be based on incapacitating such challenges by additional studies and field trials.

Conclusion

The role of sugarcane bagasse (SB) and sugarcane bagasse derived biochar (SB-BC) on decreasing Cd accumulation in maize grown in Cd-contaminated field was assessed. It was concluded that high Cd stress (HS-Cd) caused significant alteration in the physiological and biochemical characteristics, plant growth and biomass mainly due to the Cd accumulation in both, shoots and roots. The extent of Cd fractions and its toxic effects were more evident and prevalent in HS-Cd group as compared to the No-stress group (NS-Cd). NS-Cd group exhibited the improvement in terms of growth. It was also concluded that the combined application (1%) of SB and SB-BC considerably improved the fresh and dry weight, plant height, chlorophyll content, stomatal conductance, photosynthetic rate, and relative water content compared to their individual application. On the other hand, there was a significant decline in the proline content, soluble sugars, electrolyte leakage, and antioxidant activity (including AsA, SOD, POD, and GR) and Cd concentration in the plant roots and shoots. As the BC comprises a considerable amount of nutrients and micronutrients that can help enhancing soil fertility; hence, we can conclude that the combined application of SB and SB-BC could serve as a potential approach to remediate the contaminated agricultural soils. Conclusively, the SB and SB-BC that was prepared by pyrolyzing the feedstock (SB) at a temperature of 350 °C and applied at rate of 1% has efficiently enhanced soil fertility status, decreasing the bioavailability of Cd and improving maize growth in Cd-contaminated soils.

Future recommendations

In order to assess the broader applicability of SB-BC, it is recommended that more studies should be conducted in future in diverse environmental conditions, crops, and modification of SB-BC with different reagents. Furthermore, research studies with longer duration are suggested to be conducted for monitoring the long-term impact of repeated SB-BC application on both, soil health and plant growth. Moreover, the soil nutrients, biochar degradation, metal bioavailability can also be assessed with the help of studies including longer ageing duration as compared to the current study. some systematic studies should also be carried out in order to explore the SB-BC and Cd interaction at molecular level. Additionally, the evaluation of economic viability and practical barriers to the application of higher amounts of biochar application will be critical for the widespread implementation of SB-BC in agriculture.

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Author Contribution

Sobia Riaz has planned and conducted the research, collected, prepared and analyzed the samples and performed statistical data analysis and written the manuscript. Ghulam Murtaza has supervised the whole research. Muhammad Saqib has helped in writing and critically reviewing the manuscript. Mansoor Hameed has helped in interpreting the statistical findings of the research.

Conflict of Interest

The authors have no affiliations with or involvement in any organization or entity with any financial interest.

Data Availability

This is the original data presented in this manuscript.

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