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Use of Biochar Derived from Soybean Stover, Wood Bark, and Rice Straw for Lead Immobilization in Polluted Soil by Maize Crops

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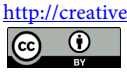

Abstract

Heavy metals are a growing global problem that is released into the environment by both natural and human sources, including Lead (Pb). Lead is the most prevalent heavy metal contaminant in the environment and causes poisoning that not only limits plant growth but also creates health problems for animals and human beings. High Pb concentrations cause plants to produce less bloom and negatively impact the growth of cereal crops, such as rice, wheat, and maize. Various methods have been adopted to minimise the impact of these metals on nutrients that lead to health issues. In the current research, to lessen the harmful impacts of deadly heavy metals, organic treatments such as compost, manure, and biochar are used. The effects of applying varying rates of biochar (BH) from wood bark, rice straw, and soybean stubble will be evaluated on soil contaminated with Pb. The under-studied groups like RSB + WBB, RSB + SSB, and WBB + SSB resulted in the tallest plants, the highest dry weights of roots and shoots, and the highest quality output. When compared to the control group, we found that the use of RSB, WBB, and SSB significantly affected the attributes of plant growth characteristics, including plant height, shoot dry weight, and root dry weight in maize plants. However, outcomes were significantly more meaningful in the treatment options of RSB + SSB, WBB + SSB, and RSBB + WBB + SSB. The results suggested that immobilizing agents, including biochar derived from wood bark, rice straw, and soybean stubble, may

immobilize lead (Pb) in soil. This would limit the amount of lead that can be recovered using DTPA and suggest a lower level of Pb immobilization for maize crops.

Subject Areas

Environmental Sciences

Keywords

Lead, Biochar, PH, RSB + SSB, WBB + SSB, RSBB + WBB + SSB

1. Introduction

Heavy metals (HMs) from industries and subsequent collection in crops and soil have turned into a global issue for both natural and human well-being [1]. Pollution due to HMs in soil medium is one of the emerging concerns worldwide. The chronic impact of heavy metals, especially the higher concentration impact of lead, is increasing in agricultural land. Pb-contaminated soil usually exists in urban and semi-urban areas because of Industrialization [2]. Both natural and human sources can discharge the HMs contaminants into the environment and they can end up in the air, soil, or water. Pb pollution is a complicated environmental problem also, and its contamination is, for the most part, brought about by different anthropogenic exercises like mining, refining, and horticulture exercises like pesticides and fertilizers, paints and preservative substances [3]. Heavy metals cause the crucial immobilization process, which reduces the toxicity and mobility of organic contaminants that can be eliminated. Immobilization, with regards to HM treatment, can be characterized as the most common way of utilizing a substance response to hold something. The immobilization strategies depend on the straightforward rule that a reduction in the centralization of the metal's versatile structures [4]. The key benefits of immobilization are minimum site disturbance, simplicity, and quickness, comparatively less expensive than alternative corrective measures, addition of nutrients to the polluted site is very acceptable to the general public, effective against a wide range of inorganic pollutants, and less likely to spread contamination. Because it requires less work and energy, this technique is very popular today [5].

Organic treatments such as biochar, manure, and compost are the most competent, suitable, and economically beneficial methods to decrease the damaging effects of lethal heavy metals. Some remediation approaches have been used for the treatment of trace metal polluted sites, which are phytoremediation and physical treatments. Some organic soil additives, such as biochar, farmyard manure, and poultry manure, have been acknowledged at the same time as HMs immobilizing agents because of their capacity to restrict HMs in various ways [6]. Biochar has garnered significant attention for its potential to enhance plant growth and

remediate heavy metal-contaminated soils, including those contaminated with Pb. Its unique properties contribute to its effectiveness in immobilizing lead and promoting plant health. The physical adsorption mechanism is crucial in preventing lead uptake by plant roots. Additionally, biochar's hydrophobic nature can reduce the leaching of lead from the soil into groundwater and can improve soil physical properties by increasing its aggregation and porosity. This enhanced soil structure promotes better water infiltration, aeration, and root growth. Additionally, biochar can help to improve soil fertility by retaining essential nutrients, such as nitrogen and phosphorus [7]. By immobilizing lead and improving soil conditions, biochar indirectly benefits plant growth. Reduced lead uptake by plants minimizes the toxic effects of lead on plant physiology, including inhibition of photosynthesis, nutrient deficiencies, and oxidative stress. Moreover, biochar's ability to improve soil structure and nutrient availability can enhance plant root development and overall vigor. The mechanisms by which biochar immobilizes lead in soil are complex and multifaceted. Physical adsorption, chemical complexation, and cation exchange are the primary processes involved. Additionally, biochar can indirectly influence lead immobilization by promoting the growth of beneficial soil microorganisms that can participate in lead immobilization processes [8].

Immobilization with different amendments is a low-cost and environment-friendly technique. By applying organic treatments, such as organic matter for stabilizing or immobilizing metal, the chemical, biological, and physical properties of soil responded favourably [9]. Recently, there has been a rise in interest in using biochar to immobilize HMs in soils. In addition to enhancing soil quality and considerably lowering crop uptake of HMs, biochar can immobilize HMs in polluted soils [10]. However, based on the kind of biochar used, the soil characteristics and species of HMs increased. HMs mobilization in the soil may also happen by following biochar application [11]. The long-term effects and ecological implications of biochar amendments in soils are complex and depend on various factors, including soil properties, climate, biochar type, and application rate. While biochar can offer benefits such as improved soil structure, and contaminant immobilization, it is crucial to consider potential impacts on nutrient cycling, microbial communities, and biodiversity [12].

Maize, due to its better output potential, ease of handling following harvest, and quick growing period, currently holds third place in Pakistan behind wheat and rice in terms of crop husbandry. Present-day study and development primarily focus on crop residue, including rice straw, wheat straw, corn stalk, cotton stalk, and grass [13], as well as forest waste, including pine waste and palm waste. Pb poisoning has been linked to histological changes in leaves, thinning of the leaf blade tips, narrowing of the xylem arteries, and magnification of the xylem and phloem in the vascular bundles, according to studies on soybeans [14]. Therefore, the study has been planned to determine how to immobilize the Pb from lead-contaminated soil, to evaluate the efficiency of plant-derived biochar from soybean Stover, wood bark, and rice straw on lead immobilization, how lead affects the nutritional value

of maize, and finally analyzing the optimal application rate of biochar for the immobilization of Pb in polluted soil and maize crop growth.

2. Material and Methods

The "Evergreen Nursery, Faisalabad, Pakistan" plant store sold the experimental soil. The pH of the soil was measured using the McLean technique, which involves making a suspension of the soil and deionized water (1:1), shaking it for about an hour, and then measuring the result on a pH metre that has been calibrated (model WTW7110, Weilheim, Germany). Similarly, soil organic matter, soil texture, and cation exchange capacity (CEC) were all measured using the Walkley-Black, Gee and Bauder, Jackson, and Rhoades techniques, respectively. The techniques of Watanabe and Olsen, Richards and Allison, and Moodie, were used to determine total phosphorus, exchangeable potassium, and calcium carbonate. Table 1 lists the characteristics of the test soil.

Table 1. Physiochemical characteristics of experimental soil.

Characteristics	Units	Amount
Clay	%	29.7 ± 1.07
Silt	%	27 ± 0.97
Sand	%	40.3 ± 0.80
Organic matter content (OMC)	%	0.84 ± 0.03
Bicarbonate (HCO_3)	%	0.17 ± 0.01
pH	-	8.4 ± 0.30
Cation exchange capacity (CEC)	$cmolc \cdot kg^{-1}$	29.2 ± 1.06
Electrical conductivity (EC)	$DS \cdot m^{-1}$	3.8 ± 0.14
Content of calcium carbonate ($CaCO_3$)	%	2.9 ± 0.11
Phosphorus (P)	$mg \cdot kg^{-1}$	8.3 ± 0.30
Potassium (K)	$mg \cdot kg^{-1}$	81 ± 2.94
Nitrogen (N)	$mg \cdot kg^{-1}$	174 ± 6.31
Total Pb	$mg \cdot kg^{-1}$	1000 ± 36.2
Diethylenetriaminepentaacetic acid (DTPA)-extractable Pb	$mg \cdot kg^{-1}$	6.1 ± 0.22

Biochar was produced through the pyrolysis of biomass. The preparation process can vary widely depending on factors such as the biomass used, heating temperature, residence time, and heating atmosphere. Characterization techniques such as surface area measurement are used to assess the properties of biochar that influence its ability to immobilize lead. These properties include surface area, functional groups, cation exchange capacity, pH, particle size, and production conditions. These factors are essential for optimizing biochar production and application for effective lead immobilization and soil remediation.

2.1. Using a Hydrometer to Determine the Soil Texture

A hydrometer was used to measure the soil texture [15]. Hot plate, beakers, determining containers (1000 ml), electrical balance (NAPCO, JA-410), drying oven, hydrometer with Bouyoucos scale in g/L, cup receptacle, and dirt dispersion stirrer (operating at high speed) were some of the key equipment utilised in this operation. Reagents like alcohol and a dispersion solution (10 g of sodium carbonate and 40 g of sodium hexametaphosphate were combined with 1 litre of deionized water) were required. Then, deionized water was added to the combination to fill the volume in a 1 La hydrometer jar or calibrated cylinder. 60 cc of dispersing solution was added to the IL hydrometer jar. De-ionized water was used to dilute the mixture. After completely blending the suspension, a reading was obtained using a hydrometer, R. Again, we used a temperature range of 2˚C to 20˚C to take blank measurements. A unique paddle was used in the hydrometer jar to mix the suspension, and once the paddle was removed, the hydrometer was placed right into the suspension. The entire procedure was carried out to ascertain the silt and clay concentration. After withdrawing the paddle for 40 seconds, the froth was dipped into one drop of alcohol and the hydrometer's reading (Rsc) was obtained. After that, the suspension was mixed in a hydrometer jar to determine the clay concentration. We removed the paddle and did not stir the mixture after that. At 60˚C, silt was represented by a downward line, sand by an upward line, and clay by a horizontal line, all with percentages from the USDA textural triangle.

2.2. Determination of OM in Soil and Soil pH

SOM was calculated by Jackson's Walkley-Black technique in 1962 [16]. In this technique, the following chemicals were used: diphenylamine indicator $(C_6H_5)_2NH$, 0.5 M ferrous ammonium sulphate solution, potassium dichromate $(K_2Cr_2O_7)$-1 N, and 98% concentrated orthophosphoric acid (H_3PO_4). A 500 ml beaker was filled with a 1 g sample of air-dried soil. A 20 ml solution of H_2SO_4 concentration was added using a dispenser, and a 10 ml solution of 1 N potassium dichromate $(K_2Cr_2O_7)$ was added using a pipette. After mixing the suspension, the beaker was left for 0.5 hours. Then, by the dispenser, H_3PO_4 (10 ml) and 200 ml of pure water were added, and the mixture was allowed to cool. A diphenylamine indicator (10 - 15 drops) was put into a beaker and maintained on a magnetic stirrer with a Teflon-coated stirring bar. With 0.5 M ammonium sulphate, the solution sample was titrated.

Volumetric flasks, glass beakers, glass rods, electrodes, cylinders, pH metres (model WTW 7110, Weilheim, Germany), and plastic wash bottles are among the equipment needed to calculate soil pits. De-ionized water 4.0 pH buffer solution and 7.0 pH buffer solution are the reagents used to calculate pH. A glass beaker (100 ml) was collected, and 10g of dirt from the experimental soil was placed inside. 25 ml of deionized water was added to a volumetric flask by a ratio of dirt to solution: 1:2.5. To properly mix the fluid, a glass rod was utilised which was then left for 30 minutes while being stirred every 10 minutes. The suspension was left

in situ for two hours to allow for the solids to settle. Following the calibration of the pH metre, the pH of each batch of sample suspensions was measured. This was accomplished by utilising pH buffer solutions and a standard process. Every 30 seconds, an electrode was dipped 3 cm deep into the suspension, and the reading was recorded on a piece of paper, up to one decimal. After washing, the electrode was cleaned and sufficiently dried using tissue paper to dry it. With a pH of 8.2, our test soil possessed alkaline characteristics (Table 1).

2.3. Cation Exchange Capacity (CEC)

To test soil cation exchange capacity, a centrifuge tube (40 ml) was collected and filled with a 10 g air-dried soil sample. The tubes were filled with a 33 ml solution of sodium acetate trihydrate and stoppers before being shaken for around 4 - 5 minutes. The samples were left for centrifugation at 3000 revolutions per minute (RPM) until the supernatant solution was cleared. The supernatants were thoroughly decanted and disposed of according to protocol. Four times this surgery was carried out. The same process was then performed after adding 33 ml of 95% pure ethanol to the samples. The supernatant solution was removed after each 33 ml wash with 95 per cent ethanol of the sample. Three times, samples were rinsed with 33 cc of 95 per cent ethanol. Electric conductivity (EC) in the supernatant (400 S/cm) was measured. To determine the amount of absorbed sodium from the sample, 33 ml of ammonium acetate was collected, shaken for 5 minutes, and then centrifuged at 3000 rpm until the clear supernatant solution was obtained. Decanted supernatant liquids were added to an empty 100 ml flask along with ammonium acetate (IN). Shaking the mixture was left to ensure proper mixing.

2.4. Electrical Conductivity (EC)

Richards, 1954 published a technique for measuring electrical conductivity in USDA Handbook 60. [16] acknowledged this approach for calculating the EC of soil. The reagent used to measure electrochemical conductivity (EC) was potassium chloride (KCI) (0.01 N), and the equipment used to measure EC was a vacuum filtration system and a conductivity bridge. A sample of experimental soil was obtained and placed in a 100 ml glass beaker. Using a graduated cylinder, 50 ml of sterilised water was added to the beaker. After properly mixing the solution with a glass rod, it was left for 30 minutes. Every 10 minutes, the suspension was kept stirring. Upon passing an hour, the suspension was disturbed. The bottom of the Buchner funnel was used to molten Whatman 42 filter paper, which was then affixed to cover all the holes. Following the activation of the vacuum pump and opening of the suction, suspension was introduced to the Buchner funnel. Filtration was performed before the dirt on the Buchner funnel began to break. The electric conductivity meter the documented manual technique was used to calibrate (BANTE, DDS-12DW, and Chicago, USA).

2.5. Calcium Carbonate ($CaCO_3$)

The method to test $CaCO_3$ in soil that [17] defined was employed in this investigation.

The equipment utilised in this technique includes a volumetric pipette, burette, hot plate, and Erlenmeyer flask. 95 per cent ethanol (C_2H_5OH), sodium hydroxide (NaOH) solution, and hydrochloric acid (HCI) solution were used to quantify the total $CaCO_3$ in the soil. In addition to an indicator (methyl orange), sodium carbonate (Na_2CO_3) and phenolphthalein indicator reagents were utilised. A 250 ml Erlenmeyer flask was filled with a soil sample that had been sieved (0.15 mm) and dried by air. A volumetric pipette was used to measure 10 ml of HCI (1 N) and add it to the flask. After swirling the flask, it was filled with 50 - 500 ml of deionized water, a graduated cylinder, and 2 - 3 drops of phenolphthalein. The flask was then left overnight. The titration was carried out using NaOH (IM) solution while the flask was being swirled. The reading-R was obtained after the solution had been adjusted until a bright pink colour began to show. NaOH (1 N) and HCI (IN) solutions were employed in standardisation to estimate $CaCO_3$. Using a pipette, 10 ml of Na_2CO_3 (1 N) was added to a 100-millilitre Erlenmeyer flask along with two drops of the indicator fluorescent methyl orange. Countermeasure for HCI (IN) was made by titrating with a burette. After titration, the solution turns dark orange. You may calculate the HCL normalcy by using the formula NHCL = 10 × N Na_2CO_3/V HCL.

2.6. Potassium and Phosphorus

The method put out by [18] to determine the total phosphorus in the soil was also used in this experiment. Spectrophotometers block digesters, and vortex tube stirrers were among the equipment utilized. By modifying a few techniques, some reagents like ammonium heptamolybdate, ammonium vanadate in nitric acid, per-chloric acid ($HClO_4$), and a stock solution were created. The soil digestion process described below was used: A calibrated digestion tube (250 ml) was collected and filled with a 2.15 g sample of air-dried 0.15 mm soil. After that, the soil sample was well blended with 30 ml of $HClO_4$ (60%) and pumice boiling granules. After the research specimens were ready, they were put into absorption pipes, which were heated to a gentle temperature of 100˚C on a block digester rack. Up until the prepared samples digest and fumes or white acid develop, gradually increase the temperature to 180˚C. Small amounts of per-chloric acid were used to clean the sides of certain digesting tubes. The event persisted for 15 to 20 minutes until the insoluble material changed into white sand. After the digesting process, the following samples were left to be collected. Using a pipette, 10 ml of ammonium vanadomolybdate and 5 ml of the digested sample were put into a volumetric flask. For dilution, deionized water was then added. The standard curve was then drawn using the procedures below: Using a pipette, a 5 ml sample of the 2 - 10 ppm standard solution was obtained and put through the same process as the measured samples that had been digested. Using 410 nm wavelengths, the absorbance of standards, blanks, and samples was measured after every 10 minutes. Standard stock solution and IN Ammonium acetate (NH_4OAc) solution were employed as reagents and were made according to standard procedures. A 5 g soil

sample that had been air-dried and sieved through 2 mm was put into a centrifuge tube. The mixing tube was covered with a clean rubber stopper, 33 ml of NH_4OAc solution was added, and it was shaken vigorously for five minutes using a mechanical shaker.

2.7. Observing the Chemical Properties of Soil Including DTPA-Extractable Pb

The DTPA-extraction technique provided by [19] was employed in this investigation. AAS model "280FS, Agilent Technologies, Santa Clara, CA, USA" and a reciprocal, mechanical shaker was among the equipment utilised in this experiment. DTPA extraction solution was made using the following conventional procedure: 1.97 g of DTPA and 1.1 g of $CaCl_2$ were dissolved in a beaker. Deionized water was used to dissolve the substance, and then it was placed into a 1 L volume. Triethanolamine (TEA) was weighed at 14.92 g and placed in a beaker before being moved to a 1 L flask and filled to 900 ml with deionized water. The pH of the HCL (6 M) solution was adjusted to 7.3, and the volume of the solution was increased to 1 L. TEA (0.1 M), CaCl2 (0.1 M), and DTPA (0.005 M) were all present in the produced DTPA solution.

2.8. Determination of Pb DTPA Extracts on AAS

Nickel content in DTPA extracts was measured using an AAS with a slit burner head (10 cm) for air-acetylene flame. Hollow cathode lamps made by PyeUnicam (England) were used as sources of spectrum radiation from the elements being studied. AAS was operated by the manual's explicit instructions. After using several appropriate cations standards, a calibration curve was created. The needed Pb was then measured in extracts using the relevant lamp provided by AAS. Ni was determined based on the calibration curve.

The Environmental Science & Engineering Department's laboratories at Government College University in Faisalabad, Pakistan, supported the studies on soil and chemical processes. The university's botany department was picked to carry out various operations (determination of ROS and antioxidants) and plant-related methodologies. We set up and conducted pot tests at the university's botanical garden. In the labs of the Department of Environmental Science and Engineering at the University, a variety of tools and machinery were utilised to quantify Ni in plant and soil samples as shown in Figure 1.

Figure 1. Plant and soil samples in the university garden.

2.9. Harvesting of Maize Plants

Using an inch scale, the height of the plants about the significant physical characteristics of each treatment were determined. The plants were then trimmed from above ground by cutting the stems with scissors. After being harvested, plants were placed in an ice-filled bucket to provide a chilly atmosphere, and they were then labelled with the appropriate labels for the various treatments. Fresh roots and leaves were separated from plants in specific proportions to analyse the levels of ROS, biochemical remarks, and enzymatic activity. The plant branches were repeatedly washed with tap water to eliminate dirt particles that were stuck to them.

2.10. Measurement of the Contents of MDA, H_2O_2, and O_2^- in Maize Leaves

HO, contents and MDA in leaves were calculated using conventional techniques. Leaf extract was prepared from a fresh leaf sample (500 mg) using 5 ml of TCA (0.1%). After 15 min at 10,000 g, a 2.5 ml supernatant was obtained from the produced extract. TCA (20%) and TBA (0.5%) in a specific amount of 1 ml were mixed into the supernatant. The mixed liquid was heated to 95 degrees before chilling for 30 minutes in an ice bath. Then, absorbance at 600 nm and 532 nm were measured using a spectrophotometer. 500 mg of fresh leaf was extracted from 5 ml of K-P buffer to create a supernatant, which was then spun at 10,000 ×g for 15 minutes. 5 ml of TCA (0.1%) and M KI (1 m) and K-P buffer (10 mM) buffers were combined to create an aliquot.

2.11. Determination of CAT, SOD, POD, and APX Activities in Maize Leaves

To assess antioxidant enzymes in leaves, such as CAT and SOD, certain common techniques were modified. To measure SOD in leaves, a reaction mixture including 1 ml of enzyme extract, 50 mM sodium phosphate buffer with a pH of 7.8, 100 mM EDTA, and 10 mM pyrogallol was produced. A spectrophotometer operating at 420 nm was employed to measure the activity of SOD enzymes in a reactive mixture [20]. A supernatant reaction was created while CAT enzymes were being determined by combining enzyme extract (2 ml), 10 mM H2O, and potassium phosphate buffer (50 mM with pH at 7).

2.12. Lead Concentrations in Plant Parts as Well as Nutrients in the Leaves of Maize

For digestion, dried roots and shoots (each weighing 0.5 g) were mixed with HNO_3 and perchloric acid ($HClO_4$) at a ratio of 2:1 (v/v) [21]. Ni concentration in plant roots and shoots was assessed using plant digests on ICP-MS. The concentration of Mn, Zn, Fe, and Cu digested elements was also analysed using ICP-MS similarly. Values of the translocation factor (TF) and the bioconcentration factor of Pb in shoots (1 and 2, respectively) were quantified.

2.13. Bacterial Carbon in Post-Harvest Soil

After plan hoarding, [22] measured the amount of microorganisms present in the soil using established procedures. 3 g of fresh soil was obtained and serially diluted aqueous extracts from it. On beef extract-peptone, the extracts were then distributed to check for germs. Following the incubation, microbe counts were estimated over 2 - 5 days under the proper circumstances.

All scaling and experiments were carried out in triplicates to ensure the practical use of the study in the field.

3. Results

3.1. Plant Height, Shoot Dry Weight, and Root Dry Weight of Maize

The height of the maize plant ranged from 43.833 to 62.033 cm·pot^{-1} across all treatments. All treatments led to a significant increase in maize plant height compared to control. Significantly the highest plant height was recorded in treatment RSB + WBB + SBB compared to all other treatments. The treatment WBB resulted in significantly lower plant height compared to RSB, SSB, RSB + WBB, RSB + SSB, WBB + SSB and RSB + WBB + SSB. Moreover, significantly the lowest plant height was recorded in the control as compared to all treatments. The RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB and RSB + WBB + SBB treatments resulted in increases of 46.567, 45.633, 50.700, 54.233, 50.633, 47.667, and 62.033 of maize plant height, respectively, compared to control in **Figure 2(a)**. In every treatment, the shoot dry weight (DW) of the maize plant varied between 0.3967 and 0.5300 g^{-1}·pot. Remarkably, as compared to all other treatments, the minimum shoot dry weight was seen in the RSB + WBB + SSB treatment. It was shown that the SSB and RSB + SSB treatments were substantially comparable to one another. The lowest value was observed between the control and treatment groups (RSB + WBB + SSB). Notably, RSB is the highest shoot dry weight that was measured in comparison to the control. Furthermore, the control group had a considerably lower shoot dryness than all other treatment groups. Compared to the control, the shoot dry weight (DW) of the maize plant decreased by 0.4133, 0.4433, 0.4533, 0.4567, 0.4600, 0.4800, and 0.5300% for the RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB, and RSB + WBB + SSB treatments, respectively in **Figure 2(b)**. The root dry weight (DW) of the maize plant varied across all treatments, ranging from 0.3133 to 0.4800 g^{-1}·pot. The results showed a substantial similarity between the SSB and RSB treatments in comparison. The treatment SSB and RSB minimum values were substantially similar to the control. Notably, as compared to the control, the greatest root dry weight reported is RSB + WBB + SSB. Notably, when comparing RSB + WBB + SSB to all other treatments, the greatest root dry weight was seen in this combination. The shoot dry weight (DW) of the maize plant decreased by 0.4133, 0.4433, 0.4533, 0.4567, 0.4600, 0.4800, and 0.5300%, respectively, in response to the RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB, and RSB + WBB + SSB treatments, in comparison to the control in **Figure 2(c)**.

3.2. Malondialdehyde (MDA) and Hydrogen Peroxide (H_2O_2) Contents in Maize Leaves

The content of MDA in maize plants ranged from 3.7667 to 5.9000 $\mu mol^{-1} \cdot g \cdot FW$ across all treatments. Significantly the highest MDA content was found in the control treatment among all treatments. Following the control treatment, significantly higher MDA content was observed in the WBB treatment as compared to the remaining treatments. Accordingly, significantly higher MDA content was observed in treatments RSB treatment compared to the remaining treatments. Furthermore, significantly lower MDA content was noted in the treatment SSB compared to all the aforementioned treatments. Moreover, significantly the lowest MDA content was recorded in treatment RSB + WBB + SSB among all treatments. The treatments RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB and RSB + WBB + SSB resulted in decreases of 4.7667, 4.8333, 4.6667, 4.4000, 4.3000, 4.0333 and 4.7667 in maize plants MDA content, respectively, compared to the control in **Figure 2(d)**.

The hydrogen peroxide (H_2O_2) content in maize plants ranged from 30.667 to 41.333 $\mu mol^{-1} \cdot g \cdot FW$ in all treatments. Significantly the highest H_2O_2 content was noticed in control treatment among all treatments. After that, significantly higher H_2O_2 content was noted in RBS treatment compared to the remaining treatments. Accordingly, significantly higher H_2O_2 content was observed in treatment SSB compared to the rest of remaining treatments. Treatment WBB + SSB resulted in significantly lower H_2O_2 content in relative to aforementioned treatments. Moreover, significantly the lowest H_2O_2 content was recorded in treatment RSB + WBB + SSB among all treatments. The treatments RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB resulted in reduction by 37.667, 36.667, 36.333, 35.333, 34.333 and 30.667 in maize plants H_2O_2 content, respectively, compared to the control in **Figure 2(e)**.

3.3. Superoxide Dismutase (SOD), Peroxidase (POD), Carotenoids, Ascorbate Peroxide (APX), and Catalase (CAT) Activities in Maize

Across all treatments, maize plants' superoxide dismutase (SOD) activity was shown to range from 146.00 to 165.00 $U \cdot min^{-1} \cdot mg^{-1}$ proteins. Notably, out of all the treatments, treatment RSB + WBB + SSB had the greatest SOD activity. SOD activity was substantially increased after treatment WBB + SSB than after the other treatments. In addition, treatment RSB + SSB showed a much increased SOD concentration than the other treatments. Furthermore, compared to all other treatments, treatment RSB showed noticeably decreased SOD activity, and the control treatment produced noticeably the lowest SOD activity. In comparison to the control, the treatments RSB, WBB, SSB, RSB + WBB, RSB + SSB, and WBB + SSB increased the SOD activity of maize plants by 150.33, 149.00, 150.66, 152.00, 155.66, 158.00, and 165.00, respectively in **Figure 2(f)**.

Across all treatments, maize plant peroxidase (POD) activity was found to range

from 40.40 to 64.067 U·min^{-1}·mg^{-1} proteins. Notably, of all the treatments, treatment RSB + WBB + SSB had the greatest POD activity. POD activity was substantially greater with treatment WBB + SSB compared with the other treatments. In addition, treatment RSB + SSB showed a much larger POD content than the other treatments. Furthermore, compared to all other treatments, treatment RSB showed noticeably reduced POD activity, and the control treatment produced noticeably the lowest POD activity. The treatments RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB resulted in enhancement by 42.300, 43.500, 44.867, 55.067, 55.500, 57.500, 59.167 and 64.067 in maize plants POD activity, respectively, compared to the control in **Figure 2(g)**.

Carotenoid (CART) activity in maize plants was observed in the range of 6.6333 to 9.9333 mg^{-1}·g·FW across all treatments. Significantly the highest CART activity was determined in treatment RSB + WBB + SSB among all treatments. Treatment WBB + SSB resulted in significantly higher CART activity than rest of remaining treatments. Furthermore, significantly higher CART content was observed in treatment RSB + SSB compared to remaining treatments. Moreover, significantly lower CART activity was noted in treatment RSB and significantly the lowest CART activity resulted in control treatment, in comparison to all other treatments. The treatments RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB resulted in enhancement by 8.2333, 8.5667, 8.6667, 8.9667, 9.233, 9.4333 and 9.9333 in maize plants CART activity, respectively, compared to the control in **Figure 2(h)**.

Ascorbate peroxide (APX) activity in maize plants varied from 4.43333 to 6.13333 Unit·μmol·min^{-1}·mg^{-1} for all treatments. The RSB + WBB + SBB therapy showed notably the greatest APX activity across all treatments. The APX activity of treatment WBB + SSB was substantially greater than that of the other treatments. In addition, treatment RSB + SSB showed a much larger APX content than the other treatments. Furthermore, compared to all other treatments, treatment RSB showed noticeably decreased APX activity, and the control treatment produced noticeably the lowest APX activity. The APX activity in maize plants increased by 4.5000, 4.6000, 4.73333, 5.5000, 5.76667, 5.8333, and 6.13333 in the treatments RSB, WBB, SBB, RSB + WBB, RSB + SSB, WBB + WBB, and RSB + WBB + SBB, respectively, in comparison to the control 1.9 (see **Figure 2(i)**).

Catalase (CAT) activity in maize plants varied from 95.000 to 10,733 Unit·Umin^{-1}·mg^{-1} for all treatments. The RSB + WBB + SBB therapy showed noticeably the greatest CAT activity of all the treatments. Similar in their action, treatments WBB + SSB and RSB + SSB produced noticeably greater CAT activity than the others treatments. Treatment RSB + WBB showed a much greater CAT concentration than the other treatments. Furthermore, compared to all other treatments, treatment RSB showed noticeably reduced CAT activity, and the control treatment produced noticeably the lowest CAT activity. Compared to control, the treatments RSB, WBB, SBB, RSB + WBB, RSB + SSB WBB + WBB, and RSB + WBB + SBB increased CAT activity in maize plants by 96.3, 96.767, 99.567, 104.0,

105.67, 105.67, and 107.3, respectively in Figure 2(j).

3.4. Dehydroascorbate Reductase (DHAR) Content in Maize

In all treatments, the content in maize plants varied from 79.333 to 103.33 nkat·mg^{-1} protein FW. The RSB + WBB + SBB therapy showed notably the greatest DHAR activity of all the regimens. The DHAR activity of treatments WBB + SSB was much greater than that of the other treatments. In addition, treatment RSB + WBB showed a noticeably larger DHAR content than the other treatments. Furthermore, compared to all other treatments, treatment RSB showed noticeably reduced DHAR activity, and the control treatment produced noticeably the lowest DHAR activity. The study found that the DHAR content of maize plants was lower under the treatments RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB and RSBB + WBB + SSB respectively than under the control group by 85.667, 89.000, 90.667, 92.000, 95.000, 97.667, and 103.33 in Figure 3(a).

3.5. Lead Concentration in the Shoot and the Root of Maize

The concentration of lead (PB) in the maize plant shoot varied throughout the treatments, ranging from 0.1767 to 0.3433 mg^{-1}·kg·DW. Remarkably, in contrast to all other treatments, the lowest shoot lead concentration was seen in the RSB + WBB + SSB therapy. WBB + SSB treatments were shown to be substantially greater than the others in comparison. The lowest value was observed between the control and treatment groups (RSB + WBB + SSB). Remarkably, the highest lead concentration shot that was seen was RSB as compared to the control. Furthermore, the control group had the lowest lead content in the shot when compared to the other treatment groups. The concentration of shoot lead in maize plants was shown to be lower in the RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB, and RSB + WBB + SSB treatments than in the control group by 0.2733, 0.2567, 0.2467, 0.2333, 0.2200, 0.2100, and 0.1767, respectively in Figure 3(b).

In all treatments, the lead (PB) content in the roots of the maize plant varied from 17.33 to 26.667 mg^{-1}·kg·DW. Remarkably, as compared to all other treatments, the lowest root lead concentration was seen in the RSB + WBB + SSB therapy. WBB + SSB treatments were shown to be substantially greater than the others in comparison. The lowest value was observed between the control and treatment groups (RSB + WBB + SSB). Notably, RSB is the greatest lead concentration root that was seen when compared to the control. Furthermore, across all the treatments, the control group had the lowest lead content in the root. The root lead concentration of the maize plant decreased by 24.333, 24.000, 23.700, 21.667, 20.000, 19.133, and 17.733 in the case of the RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB, and RSB + WBB + SSB treatments, in comparison to the control in Figure 3(c).

3.6. DTPA Extractable Lead Concentration

In all treatments, the maize plant's DTPA extractable Lead (PB) content varied

from 31.200 to 44.867 mg^{-1}·kg of soil. Among all the treatments, RSB + WBB + SSB was shown to have the lowest minimum DTPA extractable lead concentration. WBB + SSB treatments were shown to be substantially greater than the others in comparison. The lowest value observed between the control and treatment groups (RSB + WBB + SSB). Remarkably, when comparing the maximal DTPA extractable lead concentration to the control, RSB was detected. Furthermore, the control group had the lowest DTPA extractable lead levels across all treatments. Relative to control, the DTPA extractable lead content of maize plants was reduced by 41.867, 38.967, 36.533, 35.100, 34.467, 33.300, and 31.200 in response to the RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB and RSBB + WBB + SSB, respectively in **Figure 3(d)**.

3.7. Fiber, Starch, Protein, Chlorophyll-a, and Chlorophyll-b Contents in Maize

In all treatments, the percentage of fiber in maize plants varied from 0.9567 to 1.6200%. The RSB + WBB + SBB therapy showed noticeably the greatest fiber activity of all the treatments. The fiber activity of the WBB + SSB therapy was much greater than that of the other treatments. In addition, treatment RSB + WBB showed a noticeably greater fiber content than the other treatments. Furthermore, compared to all other treatments, treatment RSB showed noticeably reduced fiber activity, and the control treatment produced noticeably the lowest fiber activity. In comparison to the control, the fiber content of maize plants was reduced by 1.0567, 1.1367, 1.1667, 1.2433, 1.3300, 1.4167, and 1.6200 in the treatments RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB and RSBB + WBB + SSB, respectively in **Figure 3(e)**.

In all the treatments, the starch content of the maize plants varied between 46.000 and 58.000%. Significantly, the RSB + WBB + SBB therapy showed the greatest starch activity of all the treatments. The starch activity of treatments WBB + SSB was much greater than that of the other treatments. In addition, treatment RSB + WBB showed a noticeably greater starch content than the other treatments. Furthermore, compared to all other treatments, treatment RSB showed noticeably decreased starch activity, and the control treatment produced noticeably the lowest starch activity. The starch content of maize plants was reduced by 48.333, 50.000, 51.333, 53.333, 54.333, 56.333, and 58.000% as compared to the control group under the treatments RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB and RSBB + WBB + SSB, respectively in **Figure 3(f)**.

In every treatment, the percentage of protein in maize plants varied between 6.900 and 13.900%. Among all treatments, the RSB + WBB + SBB therapy showed noticeably the highest level of protein activity. Protein activity was much greater in the WBB + SSB treatments than in the other treatments. In addition, treatment RSB + WBB showed a noticeably larger protein content than the other treatments. Furthermore, compared to all other treatments, treatment RSB showed noticeably decreased protein activity, and the control treatment produced noticeably the lowest

protein activity. The protein content of maize plants was reduced by 7.9333, 9.1000, 10.067, 10.433, 11.333, 12.167, and 13.900% as a result of the treatments RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB and RSBB + WBB + SSB, in comparison to the control respectively in **Figure 3(g)**.

In all treatments, maize plants' CHL-a concentration varied from 1.3000 to 2.1000 mg^{-1}·g·FW. The RSB + WBB + SBB therapy had the greatest CHL-a activity of all the treatments, by a wide margin. The CHL-a activity of treatments WBB + SSB was much greater than that of the other treatments. Additionally, compared to the other treatments, treatment RSB + WBB showed a noticeably greater CHL-a concentration. Additionally, in compared to all other treatments, treatment RSB showed noticeably reduced CHL-a activity, while control treatment produced much lower CHL-a activity. When compared to the control, the CHL-a level of maize plants was reduced by 1.4000, 1.4667, 1.5000, 1.6667, 1.7000, 1.9000, and 2.1000% for the treatments RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB and RSBB + WBB + SSB, respectively in **Figure 3(h)**.

The proportion of CHL-b in maize plants ranged from 1.7000 to 2.1000 across all treatments. Among all regimens, the RSB + WBB + SBB treatment had noticeably the highest CHL-a activity. Compared to the other treatments, CHL-b activity was significantly higher in the WBB + SSB treatments. Moreover, compared to the other treatments, treatment RSB + WBB had a notably higher CHL-b level. Additionally, treatment RSB generated clearly the lowest CHL-b activity when compared to all other treatments, while treatment control produced noticeably the highest CHL-b activity. In response to the treatments RSB, WBB, SSB, RSB + WBB, RSB + SSB, WBB + SSB and RSBB + WBB + SSB, the CHL-b level of maize plants was lowered by 1.4000, 1.4667, 1.5000, 1.6667, 1.7000, 1.9000, and 2.1000% compared to the control in **Figure 3(i)**.

4. Discussion

The effects of biochar (BH) derived from soybean Stover, wood bark, and rice straw at different rates applied to a Pb polluted soil had died by growing maize plants in a completely randomized designed pot experiment influence of these soil amendments on stabilization of Pb in soil. Pb uptake by plant structures and maize biomass production and physiological characteristics were investigated in our study with a few exceptions, we discovered that using RSB, WBB and SSB (either alone or at combined rates) considerably increased maize development and productivity compared with the control treatment. Comparatively, as compared to the control, the RSB + WBB, RSB + SSB, and WBB + SSB treatments yielded the tallest plants, the maximum roots and shoots dry weights and the maximum yield quality, previous research has found that growing maize plants in Pb-polluted soil reduced their dry biomass and growth parameters [23]. This is because lead pollution in the soil can have a detrimental impact on maize plants by interfering with nutrient uptake, disrupting metabolic processes, inducing oxidative stress, inhibiting root growth, and reducing photosynthesis. These

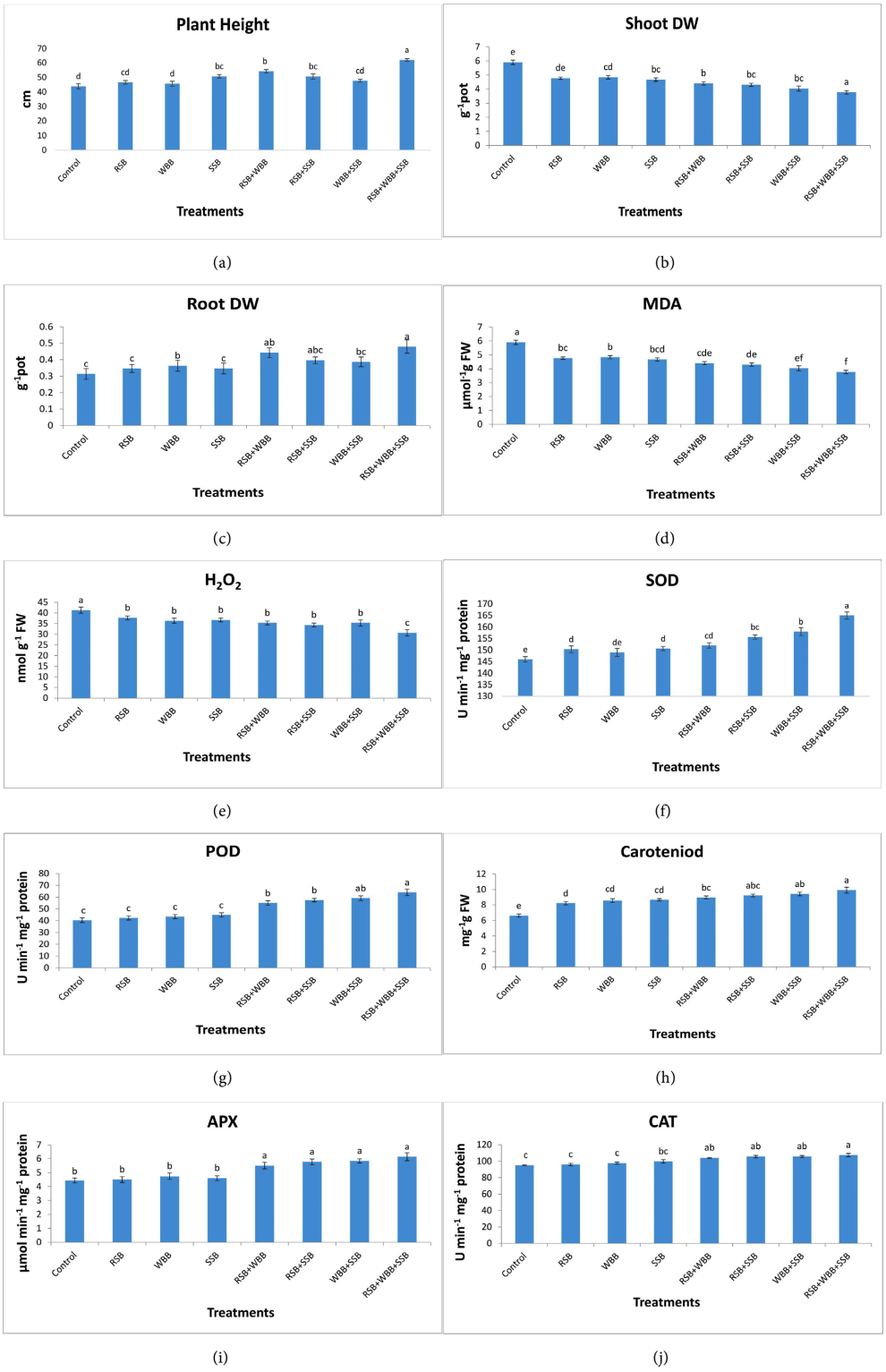

Figure 2. (a) Shows the height of the maize plant when compared with control; (b) Illustrates the shoot dry weight of maize plant; (c) Depicts the root dry weight of maize plant; (d) Exhibit the malondialdehyde content in maize leaves; (e) Shows the hydrogen peroxide (H_2O_2) content in maize leaves; (f) Exhibit the superoxide dismutase (SOD) activity in maize; (g) Illustrates the peroxidase (POD) activity in maize; (h) Shows the carotenoids activity in Maize; (i) Exhibit the ascorbate peroxide (APX) activity in maize; (j) Shows the catalase (CAT) activity in maize.

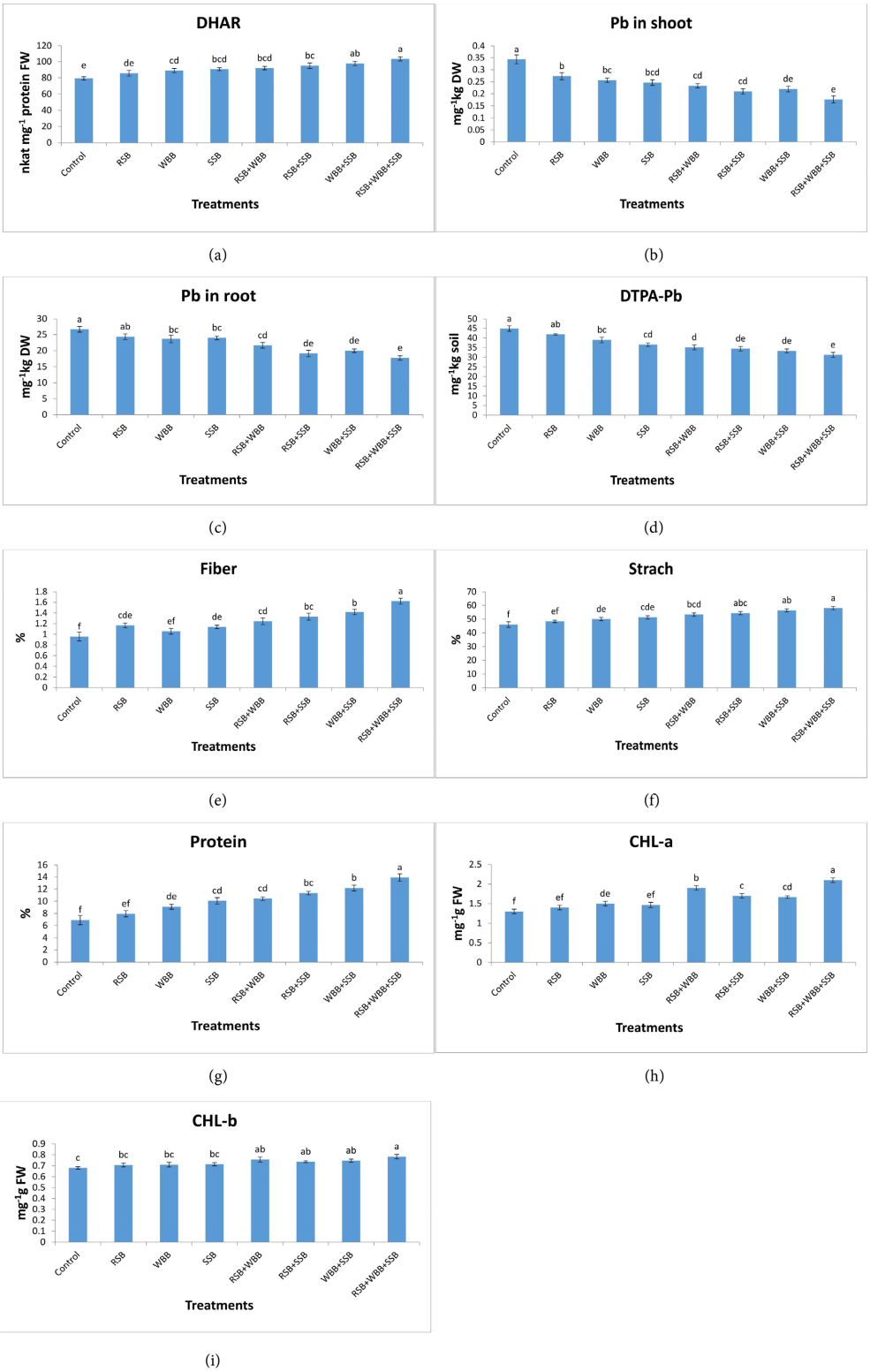

Figure 3. (a) Shows the dehydroascorbate reductase (DHAR) content in maize; (b) Shows the lead concentration in the shoot of maize; (c) Illustrates the lead concentration in the root of maize; (d) Shows the DTPA extractable lead concentration; (e) Shows the fiber content in maize; (f) Shows the starch content in maize; (g) Exhibit the protein content in maize; (h) Shows the chlorophyll-a content in maize; (i) Shows the chlorophyll-b content in maize.

combined effects can result in reduced dry biomass, stunted growth, and overall plant decline [24].

In addition, BH on maize crops boosted maize productivity in our study when compared to the control. Our results appeared to be highly supported by earlier research that showed an enhancement in the development and physiological characteristics of *Zea mays* L. and mustard seedlings on heavy metal-contaminated soil [25]. Furthermore, the decrease in Pb toxicity to maize crops may be related to Pb sorption on a wide surface region of soybean Stover, wood bark, and rice straw within soil involving different mechanisms such as precipitating action, exchange of ions and surface complexation [10]. Therefore, biochar can be a valuable tool for remediating heavy metal-contaminated soils and promoting plant growth. By immobilizing heavy metals, improving soil physical properties, and enhancing plant physiological processes, biochar can help to restore soil health and productivity [26].

Moreover, we discovered that the utilization of RSB, WBB, and SSB significantly influenced the attributes of plant growth characteristics such as plant height, shoot dry weight and root dry weight in maize plants as compared to the control treatment, however, it was observed that the outcomes were significantly more meaningful in RSB + SSB, WBB + SSB and RSBB + WBB + SSB, treatment options (**Figures 3(a)-(c)**). Previously, it has been observed that exposing plants to larger quantities of Pb reduced the growth and physiological properties of maize plants [27]. In comparison to the control treatment, we found a significant increase in the composition of photosynthetic parameters (chlorophyll a, and chlorophyll b) and a substantial reduction in maize plants cultivated in treatment methods without adding amendments with sets of combinations. These effects, however, were the most evident in the combined blends of RSB + SSB, WBB + SSB and RSBB + WBB + SSB (**Figure 3(h)** and **Figure 3(i)**). Our findings correspond with a previous study where plants that were exposed to increased Pb levels resulted in decreased photosynthetic properties of maize [28]. The experimental data of our study are consistent with earlier research in which BH treatment in Pb-stressed soil increased the values of biochemical components in quinoa [29], sunflower and rice [30]. Lead can damage chloroplasts and compete with essential nutrients, such as calcium, phosphorus, and iron, for uptake by plant roots. This competition can lead to nutrient deficiencies that hinder plant growth and development. High levels of lead can damage root cells, reducing their ability to absorb water and nutrients [31]. Furthermore, lead can inhibit the activity of various enzymes involved in plant metabolism, such as those involved in photosynthesis and respiration. This can disrupt essential physiological processes and reduce plant growth. Lead can interfere with the production and signaling of plant hormones, such as auxins and gibberellins, which play crucial roles in growth and development. It can induce the production of ROS as well, which can damage cellular components, including DNA, proteins, and lipids [32].

In this work, wing amendments of WBB, RSB, SSB and their combinations were

shown to increase stress resistance in heavy metal-affected maize crops. The above finding backs up the claim that organic amendments can induce stress resistance in various plants. In our experiment, all treatments, with some exceptions, increased the efficiency of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in maize plants, compared to the control treatment. Furthermore, the most prominent results were seen in the WBB + RSB, RSB + SSB, WBB + SSB and RSBB + WBB + SSB treatments (**Figure 3(f)**, **Figure 3(g)**, and **Figure 3(i)**). According to previous studies, Pb stress to plants has been observed to have significantly enhanced phytotoxicity while suppressing the function of antioxidant defence systems in maize plants [33], wheat and finger millet. Numerous cellular mechanisms in plants grown with metal stress conditions are disturbed due to excessive ROS production through the destruction of oxidant enzymes, oxidative damage, and biomolecules [34]. Our results are consistent with previous studies, where it was found that RSB, WBB, and SSB, reduced Pb oxidative stress while increasing antioxidant potential in maize. Following harvesting, we observed that Pb contents in maize crops, shoots, and roots, as well as plant-available Pb (DTPA-extractable Pb), were considerably lower throughout most treatments compared with the control group. However, application of RSBB + WBB + SSB showed the greatest reduction in Pb content in maize plants shoots (**Figure 3(b)**), roots (**Figure 3(c)**) as well as DTPA extractable Pb (**Figure 3(d)**). In comparison to the control, RSBB + WBB + SSB drastically treatment decreased Pb concentration levels in shoots by 36%, roots by 22%, and DTPA extractable Pb by 23% in maize plants, suggesting that RSB + WBB, RSB + SSB, WBB + SSB combined treatments successfully increased the treatment's capacity to reduce Pb concentrations.

5. Conclusion

Our study suggested that immobilizing agents, including biochar made from soybean stubble, wood bark, and rice straw, may immobilize Pb in soil, reducing the quantity of lead that can be extracted with DTPA and indicating a lower level of Pb phytoavailability for maize crops. Every treatment significantly decreased the Pb buildup in the roots and shoots of the maize plant as compared to the control. Therefore, considerable maize plant growth, dry biomass, and grain production were attained under Pb stress conditions. The following sequence of decrease in plant-accessible Pb was observed in the treatments: RSB + WBB > RSB + SSB > WBB + SSB. Additionally, the application of biochar to maize improved its antioxidant defence system against stress caused by lead and decreased the amount of reactive oxygen species. Nonetheless, given the outcomes of the current study, it is advised that more research be conducted to validate these findings using realistic and appropriate biochar concentrations in field settings.

Authors' Contributions

Hamza Khaliq: Literature review, sampling, experiment design, experimental work, and result analyses. Ahmed Abdullah Baeroom: Experiment design, methodology,

and resources. Muhammad Hasnain: Experiment design and resources. Anam Saira: Methodology and result analyses. Anam Aftab: Methodology and result analyses. Faiza Shahzad: Methodology. Asad Ullah: Resources.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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