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Ameliorative Effects of Biochar for Cadmium Stress on Bean (*Phaseolus vulgaris* L.) Growth

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Abstract: In order to investigate the changes in the morphological and biochemical characteristics of bean plants in response to biochar treatment under cadmium (Cd) stress, a pot experiment was conducted in a greenhouse. Bean plants were subjected to different amounts of Cd (0, 100, 150 and 200 mg kg^{-1}) and biochar applied at different doses (0, 2.5 and 5%). Under Cd stress, the growth and development of bean seedlings were remarkably inhibited, whereas the biochar treatment could effectively improve the heavy metal tolerance of bean seedlings. Cd stress caused an increase in the hydrogen peroxide (H_2O_2), malondialdehyde (MDA), proline and sucrose content, catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activity of leaves. However, biochar treatments reduced the CAT, POD and SOD activity of bean seedlings. Growing beans on Cd medium led to a significant reduction in plant nutrient element content. However, biochar amendment to the soil elevated the plant nutrient element content compared to untreated soil. Cd content of the bean seedlings increased with increasing Cd doses. There was a sharp decrease in available concentration for Cd with the addition of biochar. In conclusion, biochar incorporation into the soil can alleviate the adverse impacts of Cd stress on the growth of bean seedlings.

Keywords: heavy metal; growth; bean; physiology; biochar

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1. Introduction

Heavy metals are often associated with anthropogenic activities. Heavy metal pollution in agricultural areas has become an important issue, especially in developed countries [1]. They accumulate in the environment due to sewage sludge, rock phosphate, mining applications and industrial activities [2]. Heavy metals such as Cd have a toxic effect on plants and adversely affect their morphological, physiological and biochemical properties [3]. Compared to other heavy metals, Cd is more mobile in soil. It can endanger plant, animal and human health by accumulating both in the soil and plants [4].

Cadmium is known to be a toxic element that poses serious human health risks through the ingestion of contaminated agricultural produce if found in high concentrations in soil [5]. Contamination of agricultural soil with Cd has become a global concern because of its adverse effects on health and food safety. Increased heavy metal levels are an important stress factor for the environment and humans. Heavy metals can mix with water, be taken up by plants and released into the atmosphere as a gas, or be adsorbed by soil components such as clay or organic matter. These heavy metals, which are included in the food chain with plant and animal production, adversely affect human health [6]. Today, heavy metal toxicity and accumulation in plants is a serious environmental problem. Heavy metals such as Cd have been reported to affect plant growth and productivity in many agricultural products negatively. Moreover, it has been reported that cadmium increases ROS (Reactive Oxygen Species) and decreases photosynthetic activity in plants [3].

Biochar, which is obtained by heating (pyrolysis) biomass at high temperatures and in an oxygen-free environment, is considered an additive that can improve the physical, chemical and biological properties of the soil and increase the efficiency of plant production due to its properties such as resistance to degradation, high specific surface area and negative surface charge [7]. A wide variety of biomass sources are used in the production of biochar, such as wood and bark, agricultural waste, animal manure and other waste products [8]. Biochar is porous, has a large surface area (~500 m^2 g^{-1}), and has high water-holding and cation exchange capacities. It is rich in aromatic and humic substances [9]. Biochar holds six times its own weight in water in the soil, which makes it a good nutrient for the soil by enabling the plants to absorb elements such as phosphorus and nitrogen from the soil more easily [10]. In general, biochar has important properties such as improving soil properties, plant fertilizer and carbon storage in the soil. It also increases soil fertility by binding agricultural chemicals, reducing climate change (reducing CO_2 and CH_4 emissions), disposal of wastes that cause environmental pollution in waste management, and use in energy production [9].

On the other hand, research on the employment of biochar to remove pollutants from soil and wastewater has gained importance in recent years. All these factors have been influential in the development of the modern biochar industry in recent years. The porosity and surface functional groups of biochar have shown that it will be a suitable adsorbent for the removal of heavy metals and phenolic compounds in soil and water [11].

Biochar can be used to improve agriculture and the environment in a variety of ways, and its soil persistence and nutrient retention make it an ideal soil conditioner to increase crop yields [12]. Biochar can be used to remove heavy metals. It is considered eco-friendly, cheap and shows high removal efficiency [13]. Biochar is an effective organic resource that increases the buffer capacity of the soil and serves the enhancement of biodiversity. It is one of the main losses of improving soil health as well as causing the soil to be enriched physically, chemically and microbiologically. It has the capacity to enable sustainable plant production with the stabilization technique of soils, especially in Cd metal pollution.

Beans (*Phaseolus vulgaris* L.), belonging to the Leguminosae family, are the most produced species in the world. It is a very important crop for food consumption worldwide as a source of proteins and nutrients. Bean (*Phaseolus vulgaris* L.) is the most selective edible legume in terms of ecological conditions. Bean is a type of vegetable that is significantly affected by abiotic stress factors, as in other plants. Heavy metal stress is one of them and negatively influences growth causing yield and quality losses in crops [5].

Recently, the potential impact of biochar on the remediation of Cd-contaminated lands has attracted attention [14]. Biochar, as a soil amendment, can be employed as an adsorbent to remediate various heavy metals in soil [15]. For this purpose, this study aimed to evaluate the role of the biochar application rate in the alleviation of negative impacts of Cd on the growth, physiological and biochemical properties of common beans (*Phaseolus vulgaris* L.).

2. Materials and Methods

2.1. Experimental Material

The study was conducted at Atatürk University in Erzurum (39°54′ N; 41°15′ E), Turkey, in 2022, under greenhouse conditions where the average photosynthetically available radiation measured at noon ranged from 1098 to 1490 µmol m^{-2} s^{-1}. Bean (*Phaseolus vulgaris* L. Hınıs cv.) plants were maintained under natural light conditions, the average day/night temperatures of 27/20 °C and 65% relative humidity during the span of the experiment.

Bean (*Phaseolus vulgaris* L. Hınıs cv.) seeds were used as plant material. Biochar produced by using the Thermal Conversion Process (TCP™) was obtained from SYNPET Technologies (Istanbul, Turkey). The chemical properties of the biochar are presented in Table 1. The soil used in this study was sampled to a depth of 15 cm from agricultural fields in Erzurum province, Turkey (39°55′ N, 41°61′ E). The composition of the soil is given in Table 2.

Table 1. Chemical characteristics of biochar produced from urban wastes.

Properties	Unit	Analysis Results
pH	-	7.8
EC	dS m^{-1}	0.38
Total (humic + fulvic)	%	4.9
Organic Nitrogen	%	1.6
C	%	21.54
H	%	1.26
N	%	1.38
O	%	2.1
Pb	mg kg^{-1}	162
Cd	mg kg^{-1}	10
Cu	mg kg^{-1}	393
Ni	mg kg^{-1}	310
Zn	mg kg^{-1}	1187
Cr	mg kg^{-1}	449
Mn	mg kg^{-1}	549
K	mg kg^{-1}	10,290
P	mg kg^{-1}	22,980
Mg	mg kg^{-1}	7372
Ca	mg kg^{-1}	57,500
Fe	mg kg^{-1}	25,680

Table 2. Physical and chemical properties of the starting soil.

Properties	Unit	Analysis Results
Sand	%	40.20
Silt	%	35.20
Clay	%	26.40
Cation-exchange capacity (CEC)	cmolc kg^{-1}	36.5
pH	-	7.40
EC	dS·m^{-1}	0.126
$CaCO_3$	%	2.80
Organic matter	%	1.06
NH_4-N	mg kg^{-1}	1.74
NO_3-N	mg kg^{-1}	0.86
P	mg kg^{-1}	3.20
K	cmolc kg^{-1}	2.48
Ca	cmolc kg^{-1}	18.42
Mg	cmolc kg^{-1}	2.25
Na	cmolc kg^{-1}	0.21
B	mg kg^{-1}	0.64
Cu	mg kg^{-1}	0.76
Fe	mg kg^{-1}	4.44
Zn	mg kg^{-1}	0.38
Mn	mg kg^{-1}	0.46

2.2. Experimental Set-Up

Biochar was weighed at the rate of 0%, 2.5% and 5% by soil weight and mixed thoroughly with soil before filling the pot. Three biochar doses (sandy loam soil in 2.5 L pots, 2.5% (*w*/*w*) biochar mixed thoroughly with the sandy loam soil prior to filling into 2.5 L pots, 5% (*w*/*w*) biochar mixed thoroughly with the sandy loam soil prior to filling into 2.5 L pots) were applied under different cadmium levels. For the control treatment, only soil was filled in the pots. For heavy metal stress treatments, cadmium ($CdSO_4 \cdot 8H_2O$)

was mixed with the medium at three different concentrations (0, 100, 150 and 200 mg kg^{-1}) and incubated for 3 weeks. Irrigation was done at intervals of 2–3 days. Three bean seeds were sown for each pod 21 days later. After emergence, one vigorous seedling was left for each pod. The treatments consisted of three different doses of biochar (0%, 2.5% and 5% w/w) and four different doses of Cd (0, 100, 150, 200 mg kg^{-1}) arranged in a completely randomized block design with three replications and four plants in each replicate.

2.2.1. Plant Analyses

Bean seedlings were harvested 40 days after sowing. In this study, morphological (plant height, plant stem diameter, leaf area, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight) and biochemical (mineral elements (N, P, K, Ca, Mg, S, Mn, Fe, Zn, B and Cd), H_2O_2, MDA, proline, sucrose, CAT, POD, SOD) parameters were investigated. During harvest, plants were cut from the soil level, and above-ground biomass (shoot) and roots were separated for measurements and analysis.

The leaf area was determined by a leaf area meter (CI-202 Portable Laser Leaf Area Meter by CID Bio-Science, Camas, WA, USA). Assays of MDA, H_2O_2 and antioxidant enzyme activity were performed by UV/Vis spectrophotometer according to Sarafi et al. [16].

For the determination of mineral nutrition content, the leaves of the bean cultivars were dried at 68 °C for 48 h and grounded. Mineral contents were analyzed by a coupled plasma spectrophotometer (Optima 2100 DV; Perkin-Elmer, Shelton, CT, USA) [17].

2.2.2. Statistical Analyses

Data obtained from the measurements were evaluated statistically using analysis of variance (ANOVA). For statistical analysis of data, the SPSS program was used. The differences among the means were compared using the Duncan multiple range test (DMRT) ($p < 0.05$).

3. Results

The variation analysis results are given in Table 3. According to the results, it was determined that the effects of biochar, heavy metal stress, and their interaction were significant. Under Cd stress, the growth and development of bean seedlings were remarkably inhibited, whereas the biochar treatment could effectively improve the heavy metal tolerance of bean seedlings. In fact, biochar incorporation into soil improved the growth properties of the bean seedlings under non-stressed conditions. A total of 200 mg kg^{-1} Cd stress led to a decrease in seedling height, stem diameter, leaf area, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight by 22.52%, 15.23%, 23.61%, 50.81%, 51.10%, 53.07% and 54.35%, respectively, compared to non-stressed control. However, 5% biochar treatment increased seedling height, stem diameter, leaf area, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight by 20.76%, 16.07%, 25.32%, 46.68%, 38.67%, 37.41% and 36.36%, respectively, compared to the control (Table 4).

Table 3. The effects of main factors and their interactions on morphological and physiological parameters (Two-way ANOVA).

Source of Variation	df	F	P	Source of Variation	df	F	P
Seedling height				H_2O_2			
Biochar	2	92,952	0.000	*Biochar*	2	197,256	0.000
Cadmium	3	57,454	0.000	*Cadmium*	3	506,930	0.000
Biochar × *Cadmium*	6	36,255	0.000	*Biochar* × *Cadmium*	6	194,987	0.000
Stem diameter				MDA			
Biochar	2	63,958	0.000	*Biochar*	2	846,670	0.000
Cadmium	3	13,222	0.000	*Cadmium*	3	441,557	0.000
Biochar × *Cadmium*	6	21,152	0.000	*Biochar* × *Cadmium*	6	341,080	0.000
Leaf area				Prolin			
Biochar	2	413,797	0.000	*Biochar*	2	99,903	0.000
Cadmium	3	249,781	0.000	*Cadmium*	3	44,808	0.000
Biochar × *Cadmium*	6	168,587	0.000	*Biochar* × *Cadmium*	6	40,358	0.000
Shoot fresh weight				Sucrose			
Biochar	2	673,353	0.000	*Biochar*	2	100,830	0.000
Cadmium	3	320,615	0.000	*Cadmium*	3	150,039	0.000
Biochar × *Cadmium*	6	223,516	0.000	*Biochar* × *Cadmium*	6	71,010	0.000
Shoot dry weight				CAT			
Biochar	2	236,191	0.000	*Biochar*	2	306,891	0.000
Cadmium	3	332,283	0.000	*Cadmium*	3	1,976,213	0.000
Biochar × *Cadmium*	6	147,006	0.000	*Biochar* × *Cadmium*	6	617,455	0.000
Root fresh weight				POD			
Biochar	2	208,588	0.000	*Biochar*	2	178,290	0.000
Cadmium	3	645,707	0.000	*Cadmium*	3	1073,937	0.000
Biochar × *Cadmium*	6	215,983	0.000	*Biochar* × *Cadmium*	6	339,953	0.000
Root dry weight				SOD			
Biochar	2	192,606	0.000	*Biochar*	2	246,309	0.000
Cadmium	3	637,786	0.000	*Cadmium*	3	819,616	0.000
Biochar × *Cadmium*	6	213,488	0.000	*Biochar* × *Cadmium*	6	289,177	0.000
N (%)				Mn			
Biochar	2	14,390	0.000	*Biochar*	2	165,724	0.000
Cadmium	3	15,107	0.000	*Cadmium*	3	124,677	0.000
Biochar × *Cadmium*	6	7134	0.000	*Biochar* × *Cadmium*	6	75,115	0.000
P				Fe			
Biochar	2	327,040	0.000	*Biochar*	2	76,797	0.000
Cadmium	3	211,438	0.000	*Cadmium*	3	32,367	0.000
Biochar x Cadmium	6	124,227	0.000	*Biochar* × *Cadmium*	6	26,847	0.000
K				Zn			
Biochar	2	109,336	0.000	*Biochar*	2	68,609	0.000
Cadmium	3	97,853	0.000	*Cadmium*	3	60,733	0.000
Biochar × *Cadmium*	6	50,368	0.000	*Biochar* × *Cadmium*	6	36,034	0.000
Ca				B			
Biochar	2	90,955	0.000	*Biochar*	2	67,570	0.000
Cadmium	3	70,744	0.000	*Cadmium*	3	89,851	0.000
Biochar × *Cadmium*	6	40,465	0.000	*Biochar* × *Cadmium*	6	43,090	0.000
Mg				Cd			
Biochar	2	88,446	0.000	*Biochar*	2	231,747	0.000
Cadmium	3	77,414	0.000	*Cadmium*	3	1,831,292	0.000
Biochar × *Cadmium*	6	40,905	0.000	*Biochar* × *Cadmium*	6	652,684	0.000
S							
Biochar	2	93,833	0.000				
Cadmium	3	44,787	0.000				
Biochar × *Cadmium*	6	34,816	0.000				

Table 4. Effects of biochar on growth properties of bean seedling under Cd pollution.

Cd (mg kg $^{-1}$)	BC (%)	Seedling Height (cm)	Stem Diameter (mm)	Leaf Area (cm^2 plant^{-1})	Shoot Fresh Weight (g plant^{-1})	Shoot Dry Weight (g plant^{-1})	Root Fresh Weight (g plant^{-1})	Root Dry Weight (g plant^{-1})
0	0	22.96 ± 0.88 ^{cd**}	3.02 ± 0.08 ^{df**}	294.16 ± 1.01 ^{b**}	9.31 ± 0.32 ^{d**}	2.27 ± 0.11 ^{b**}	14.19 ± 0.22 ^{c**}	1.38 ± 0.49 ^{b*}
	2.5	30.25 ± 1.27 ^{a}	3.03 ± 0.12 ^{df}	310.56 ± 1.09 ^{a}	12.85 ± 0.33 ^{a}	2.59 ± 0.07 ^{a}	15.59 ± 0.23 ^{b}	1.68 ± 0.05 ^{a}
	5	25.66 ± 0.59 ^{b}	3.23 ± 0.04 ^{bc}	311.42 ± 2.57 ^{a}	13.11 ± 0.17 ^{a}	2.56 ± 0.09 ^{a}	16.32 ± 0.40 ^{a}	1.74 ± 0.02 ^{a}
100	0	22.13 ± 0.32 ^{de}	2.87 ± 0.07 ^{fg}	240.70 ± 7.17 ^{e}	7.34 ± 0.26 ^{f}	2.05 ± 0.09 ^{c}	8.78 ± 0.13 ^{f}	1.10 ± 0.01 ^{e}
	2.5	25.20 ± 0.47 ^{b}	3.19 ± 0.15 ^{cd}	266.91 ± 3.39 ^{d}	10.39 ± 0.41 ^{c}	2.26 ± 0.03 ^{b}	10.65 ± 0.39 ^{e}	1.21 ± 0.04 ^{d}
	5	24.30 ± 1.21 ^{bc}	3.41 ± 0.06 ^{a}	263.02 ± 1.77 ^{d}	11.62 ± 0.25 ^{b}	2.31 ± 0.03 ^{b}	11.87 ± 0.25 ^{d}	1.28 ± 0.01 ^{c}
150	0	20.79 ± 0.19 ^{e}	2.78 ± 0.07 ^{g}	236.41 ± 2.89 ^{e}	5.59 ± 0.31 ^{g}	1.15 ± 0.04 ^{f}	7.85 ± 0.22 ^{g}	0.95 ± 0.04 ^{f}
	2.5	25.12 ± 0.89 ^{b}	3.37 ± 0.12 ^{ab}	284.53 ± 1.08 ^{c}	11.39 ± 0.29 ^{b}	2.19 ± 0.05 ^{b}	9.40 ± 0.42 ^{f}	1.13 ± 0.02 ^{e}
	5	25.70 ± 0.51 ^{b}	2.99 ± 0.05 ^{ef}	307.90 ±1.61 ^{a}	8.55 ± 0.42 ^{e}	1.91 ± 0.07 ^{d}	10.54 ± 0.48 ^{e}	1.14 ± 0.03 ^{e}
200	0	17.79 ± 1.56 ^{f}	2.56 ± 0.09 ^{h}	224.71 ± 4.12 ^{f}	4.58 ± 0.19 ^{h}	1.11 ± 0.05 ^{f}	6.66 ± 0.47 ^{h}	0.63 ± 0.07 ^{g}
	2.5	22.12 ± 0.38 ^{de}	3.08 ± 0.07 ^{ce}	264.16 ± 4.40 ^{d}	9.17 ± 0.41 ^{d}	1.76 ± 0.08 ^{e}	8.85 ± 0.12 ^{f}	0.97 ± 0.03 ^{f}
	5	22.45 ± 1.19 ^{d}	3.05 ± 0.12 ^{de}	300.89 ± 8.78 ^{b}	8.59 ± 0.23 ^{e}	1.81 ± 0.06 ^{de}	10.64 ± 0.60 ^{e}	0.99 ± 0.03 f
Cd		**	**	**	**	**	**	**
BC		**	**	**	**	**	**	**
Cd × BC		**	**	**	**	**	*	**

Each value is the mean of three replicates ± SD. Data followed by a different letter in column were significantly different according to the DMRT **: $p < 0.01$, *: $p < 0,05$. Cd: Cadmium, BC: biochar.

Cd stress caused an increase in the H_2O_2, MDA, proline and sucrose content, and CAT, POD and SOD activity of leaves of bean seedlings. Under 200 mg kg^{-1} Cd stress, the H_2O_2, MDA, proline and sucrose content, and CAT, POD and SOD activity of leaves were increased in bean seedlings by 64.65%, 64.32%, 60.00%, 52.61%, 83.01%, 86.42% and 87.44%, respectively, and the exogenous biochar dramatically decreased the H_2O_2, MDA, proline and sucrose content, CAT, POD and SOD activity of bean seedlings under Cd stress conditions (Table 4). In this study, 5% biochar treatment increased the H_2O_2, MDA, proline, sucrose, CAT, POD and SOD content of leaves of bean seedlings under 200 mg kg^{-1} Cd stress by 33.49%, 51.78%, 60.00%, 30.33%, 25.13%, 24.10% and 42.85%, respectively, compared to the control (Table 5).

Table 5. Effects of biochar on MDA, H_2O_2, prolin and sucrose content, and antioxidant enzyme activity of bean seedling under Cd pollution.

Cd (mg kg^{-1})	BC (%)	H_2O_2 (mmol kg^{-1})	MDA (mmol kg^{-1})	Prolin (mmol kg^{-1})	Sucrose (%)	CAT (Eu g leaf^{-1})	POD (Eu g leaf^{-1})	SOD (Eu g leaf^{-1})
0	0	13.34 ± 0.42 ^{f**}	9.44 ± 0.43 ^{g**}	0.08 ± 0.003 ^{fg**}	1.00 ± 0.04 ^{e**}	55.48 ± 3.33 ^{f**}	3261.34 ± 72.78 ^{h**}	215.21 ± 20.68 ^{h**}
	2.5	12.66 ± 0.24 ^{f}	8.75 ± 0.46 ^{g}	0.07± 0.012 ^{g}	1.07 ± 0.08 ^{e}	51.16 ± 2.79 ^{f}	3017.83 ± 197.21 ^{h}	199.95 ± 4.95 ^{h}
	5	13.72 ± 0.26 ^{f}	8.80 ± 0.21 ^{g}	0.08 ± 0.009 ^{g}	1.08 ± 0.06 ^{e}	52.15 ± 5.48 ^{f}	2904.41 ± 274.68 ^{h}	192.30 ± 20.11 ^{h}
100	0	27.08 ± 0.33 ^{c}	16.78 ± 0.18 ^{c}	0.13 ± 0.003 ^{c}	1.75 ± 0.08 ^{c}	266.12 ± 9.44 ^{b}	19,934.21 ± 1514.75 ^{c}	1148.29 ± 101.59 ^{c}
	2.5	22.99 ± 0.53 ^{e}	12.14 ± 0.67 ^{de}	0.10 ± 0.010 ^{df}	1.41 ± 0.04 ^{d}	208.92 ± 7.46 ^{e}	17,072.32 ± 208.42 ^{de}	930.55 ± 18.78 ^{ef}
	5	23.56 ± 0.52 ^{de}	10.81 ± 0.35 ^{f}	0.11 ± 0.02 ^{d}	1.39 ± 0.02 ^{d}	226.35 ± 4.14 ^{d}	14,787.33 ± 393.00 ^{g}	796.61 ± 70.50 ^{g}
150	0	32.58 ± 1.96 ^{b}	23.18 ± 0.69 ^{b}	0.16 ± 0.009 ^{b}	1.89 ± 0.12 ^{b}	332.41 ± 8.85 ^{a}	22,747.22 ± 790.88 ^{b}	1315.58 ± 19.58 ^{b}
	2.5	23.93 ± 1.23 ^{de}	12.72 ± 0.35 ^{d}	0.10 ± 0.002 ^{df}	1.49 ± 0.07 ^{d}	233.06 ± 6.38 ^{cd}	15,280.85 ± 413.03 ^{fg}	878.58 ± 16.52 ^{fg}
	5	23.35 ± 0.53 ^{e}	11.74 ± 0.52 ^{e}	0.11 ± 0.011 ^{d}	1.43 ± 0.06 ^{d}	229.15 ± 10.33 ^{d}	15,241.88 ± 594.41 ^{fg}	831.18 ± 49.94 ^{g}
200	0	37.74 ±1.25 ^{a}	26.46 ± 1.08 ^{a}	0.20 ± 0.01 ^{a}	2.11 ± 0.07 ^{a}	326.55 ± 10.17 ^{a}	24,008.68 ± 375.06 ^{a}	1713.20 ± 46.44 ^{a}
	2.5	24.08 ± 1.20 ^{de}	12.98 ± 0.44 ^{d}	0.11 ± 0.005 ^{de}	1.40 ± 0.06 ^{d}	233.22 ± 6.38 ^{cd}	16,174.63 ± 1123.17 ^{ef}	1038.65 ± 56.88 ^{d}
	5	25.10 ± 0.88 ^{d}	12.76 ± 0.18 ^{d}	0.08 ± 0.011 ^{eg}	1.47 ± 0.06 ^{d}	244.48 ± 5.04 ^{c}	18,222.07 ± 812.48 ^{d}	979.15 ± 42.40 ^{de}
Cd		**	**	**	**	**	**	**
BC		**	**	**	**	**	**	**
Cd × BC		**	**	**	**	**	**	**

Each value is the mean of three replicates ± SD. Data followed by a different letter in column were significantly different according to the DMRT **: $p < 0.01$. Cd: Cadmium, BC: biochar.

There were significant interactions between Cd treatments and biochar application rates on N, P, K, Ca, Mg, S, Mn, Fe, Zn, B and Cd content of the bean seedlings (Figure 1). Growing bean under 200 mg kg^{-1} Cd stress medium led to a significant reduction for N, P, K, Ca, Mg, S, Mn, Fe, Zn and B by 31.33%, 42.11%, 28.16%, 34.09%, 43.75%, 33.33%, 42.01%, 26.90%, 32.90% and 51.77% content, respectively. However, biochar amendment to the soil increased N, P, K, Ca, Mg, S, Mn, Fe, Zn, B and Cd content at 28.47%, 42.11%, 36.75%, 33.33%, 35.71%, 50.00%, 51.35%, 38.44%, 48.21%, 42.33% and 87.76%, respectively, compared to untreated soil. The Cd content of the plant increased with the increase in Cd application dose. The highest Cd content was obtained from 200 mg kg^{-1} Cd application doses without BC application. Notably, 5% biochar is more effective in decreasing uptake than the other biochar concentration (Figure 1).

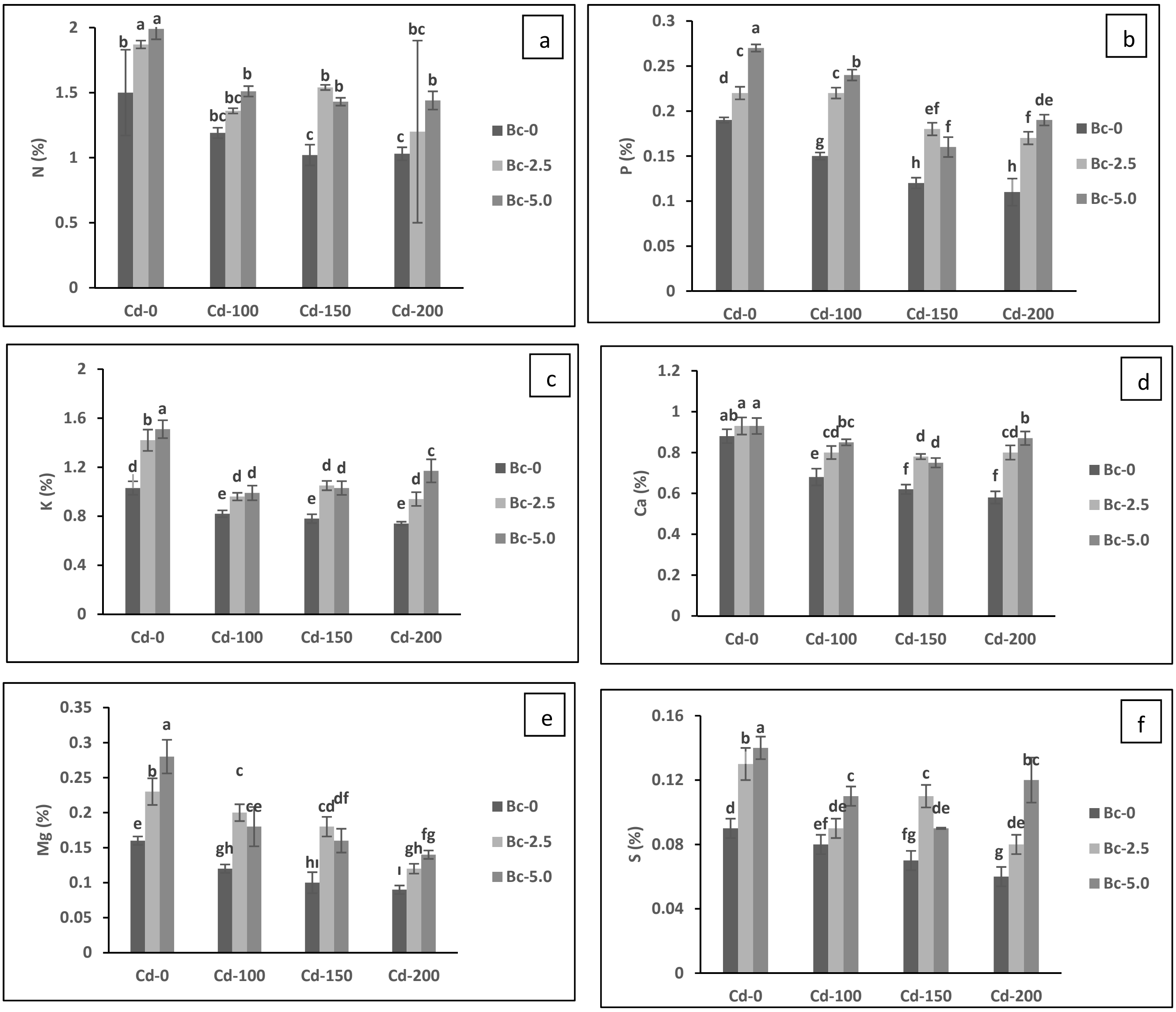

Figure 1. *Cont.*

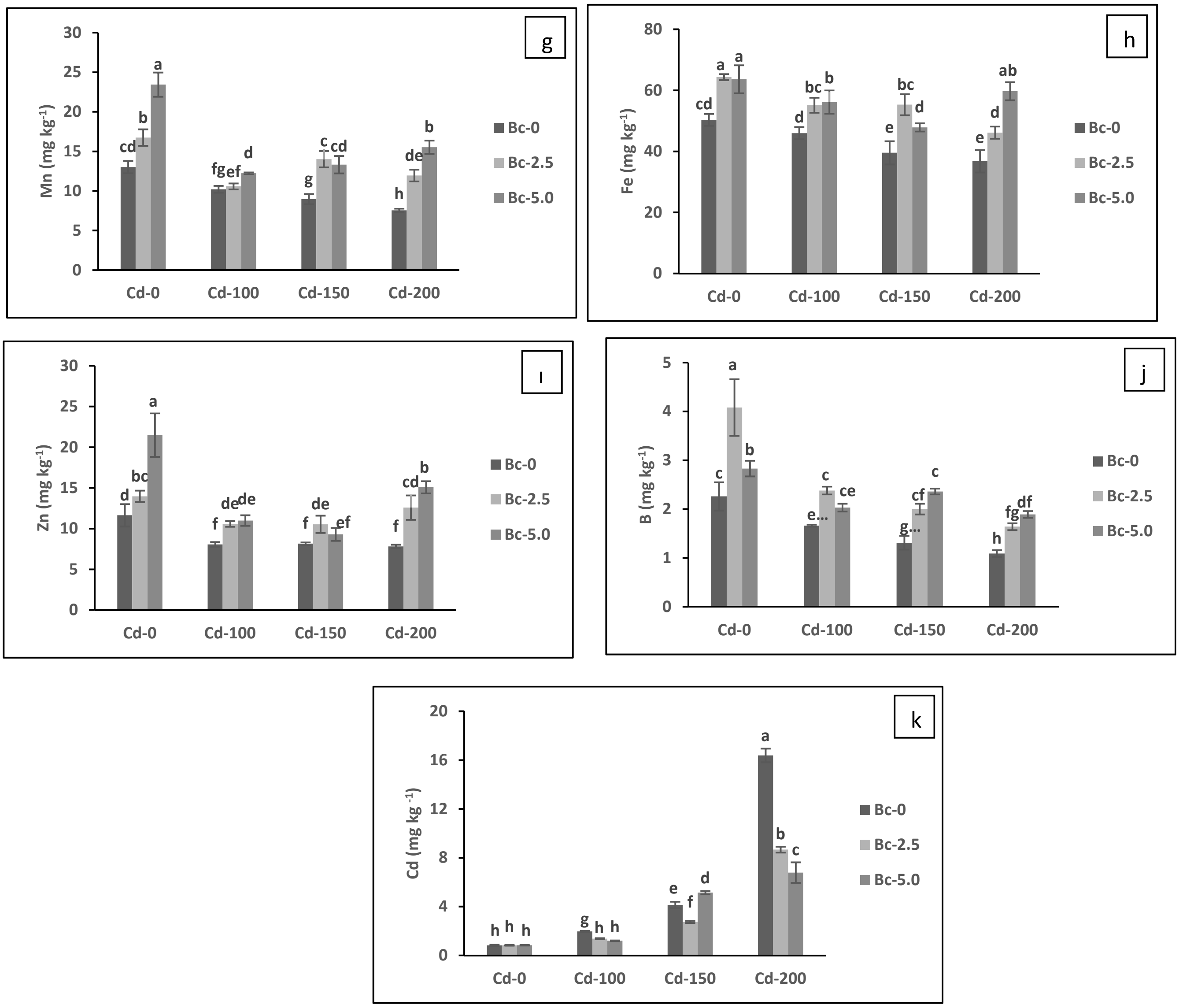

Figure 1. Effects of biochar on bean seedling N (**a**), P (**b**), K (**c**), Ca (**d**), Mg (**e**), S (**f**), Mn (**g**), Fe (**h**), Zn (**i**), B (**j**) and Cd (**k**) content under Cd pollution. Data followed by a different letter on bars were significantly different according to the DMRT ($p < 0.05$) Cd: Cadmium, BC: biochar.

4. Discussion

Heavy metals can cause serious dangers for living organisms, even at very low concentrations, since they do not have a biodegradable structure such as other pollutants. Heavy metals such as Cd have attracted the attention of many researchers not only because of their phytotoxicity but also because they can be absorbed into plant bodies and thus enter the food chain. The findings of the present study indicated that excess Cd caused a significant reduction in the growth of the bean. This lower growth may be due to Cd being toxic to plants [18]. However, biochar incorporation statistically significantly reduced bioinhibition of the growth of bean seedlings compared to non-treatment (Table 3). The findings of the study pointed out an increase in the growth of bean seedlings with applied biochar. Yousaf et al. [19] reported that the addition of biochar to the soil had a positive effect on plant growth because it reduced the bioavailability of cadmium in the soil. Biochar is rich in aromatic and humic substances, so it improves the physical, biochemical and biological properties of soils [9].

The negative effects on respiration, carbohydrate metabolism, chlorophyll formation, Calvin cycle, plant nutrient uptake and biosynthesis of DNA, RNA and other proteins are effective in the decrease of growth in plants grown under Cd toxicity. Since Cd is a phytotoxic element for soil organisms and plants [20,21], plant growth has been adversely affected, and a continuous decrease in the amount of dry matter has been observed. Cadmium toxicity decreased in the yield of different crops; at 30% for maize (*Zea mays* L.) [22], at 28.9% for chickpea (*Cicer arietinum*) [23] and at 37.0% for mungbean (*Vigna radiata*) [24].

Cadmium in the plant; reduces nitrate assimilation [25], causes closure of stomas [26], reduces the rate of photosynthesis and chlorophyll synthesis [27], changes their metabolism and inactivates enzymes [26]. It causes a decrease in chlorophyll synthesis [28], an increase in lipid peroxidation, and inhibition of N and S metabolism [29]. It was determined that the opening of stomata, respiration and photosynthesis activities decreased in plants exposed to Cd toxicity [30]. Stobort et al. [31] reported that chloroplast metabolism was impaired, and the activities of enzymes that played an important role in CO_2 fixation were inhibited because Cd reduced chlorophyll biosynthesis.

The negative effect of Cd applications on the growth of bean seedlings was partially eliminated with increasing amounts of biochar. In the conditions where Cd is not applied, the amount of the above-ground part and root dry matter of the bean seedlings has increased continuously with biochar (Table 3). The increase in the amount of dry matter created by biochar, even in the presence of Cd, can be explained by the fact that biochar provides an important adsorbent feature due to its porous and void structure, negatively charged surfaces and functional groups such as carboxyl, hydroxyl, phenoxyl and carbonyl [32]. Thanks to this feature of biochar, the adsorption and immobilization of biochar, especially in the reclamation of soils contaminated with heavy metals, the usefulness and mobility of these heavy metals decreases, and the negative effects of heavy metals are prevented [33,34].

Cd stress led to ROS accumulation in various crops, enhancing the malondialdehyde (MDA) contents [35–37]. Trovato et al. [38] pointed out that proline is involved in response to environmental stress conditions. Cd stress increased H_2O_2, MDA, proline and sucrose content and antioxidant activity of bean (*Phaseolus vulgaris* L.) seedlings. A total of 3 µM Cd was applied to the bean plant for 48 h, and it was observed that the expansion of the leaf cells was inhibited, and the cell wall elasticity decreased [39]. It has been reported that Cd causes oxidative damage in plants by increasing free oxygen radicals or reducing the amount of enzymatic or non-enzymatic antioxidants under Cd toxicity [40,41]. The excess Cd has been reported to lead to genotoxicity in various crops [42].

Correa et al. [43] applied 0, 6.25, 12.5 and 50 mg kg^{-1} Cd to the soil in their greenhouse experiment to determine the effect of Cd on the development, fresh weight, germination rate and antioxidative enzyme activity of lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea*) and oat (*Avena sativa*) plants. At the end of the experiment, it was determined that the effects of Cd applications on plant growth and biomass in lettuce, cabbage and oat plants had a negative effect, while the enzyme activities (catalase, peroxidase, superoxide dismutase and glutathione reductase) increased significantly compared to the control. Similarly, Yildirim et al. [44] showed that Cd contamination increased H_2O_2, MDA, proline and sucrose content and antioxidant activity of garden cress (*Lepidium sativum*). In the study, we observed biochar incorporation decreased H_2O_2, MDA, proline and sucrose content and antioxidant activity of bean seedlings. This may be due to the fact that biochar applications reduce the negative effect of Cd stress. In addition, biochar has strong interaction mechanisms with active surfaces, including ionic and covalent bonds [45]. With these properties, they bind heavy metals to their functional groups [34,46–48] and prevent the toxic effects of heavy metals.

Different applications have been used to prevent Cd toxicity, and it has been reported that biochar applications give good results in increasing plant tolerance against heavy metal stress. It has been noted that the toxicity effect on crops such as soybean (*Glycine max*), corn (*Zea mays* L.), rice (*Oryza sativa*) and spinach (*Spinacia oleracea*) is reduced, and the yield increased with the application of biochar in heavy metal polluted soils [48]. The biochar amendment into soil has been shown to affect the physical, chemical and biological processes of the soil. It has a positive effect on the microbiological activity in the soil and increases the efficiency of mycorrhizae by providing nutrients in particular [49,50].

Biochar application significantly reduced the Cd concentration of the spinach plant aerial parts and roots. Mckenna et al. [51], Naik et al. [52] and Alia et al. [53] determined that the Cd concentrations of the spinach plant increased depending on the Cd doses applied in their greenhouse experiments. Similar results with bean plant [39], five different pepper (*Capsicum annuum*) varieties [54], two different rice varieties [55], lettuce, cabbage and oat plants [43], and lettuce [56] and tomato (*Solanum lycopersicum*) plants [57] have also been reported in previous experiments.

The most effective factor in reducing the amount of Cd available to plants from biochar is due to the binding of Cd on high cation exchange surfaces [58]. It has been stated that N, P, K and other plant nutrients, which are found at high levels in biochar, compete with Cd in the pellet and prevent the decrease of Cd concentration and its transport to the plant [59]. An obvious positive feature of biochar is that it provides nutrients directly to the plants, prevents the nutrient elements in the soil from being washed away, and indirectly improves the soil quality, increasing the nutrient utilization of the plants and, accordingly, increasing the efficiency of fertilizer use. It has been determined that the presence of Cd in the plant growth medium has negative effects on the intake of plant nutrients necessary for the development of plants. Higher uptake and mobility of Cd by plants significantly reduce Fe intake, causing chlorosis formation and nutrient deficiency by limiting K and Ca intake [60–62]. Inal et al. [63] reported that biochar applications increased the development of corn and bean plants, and caused an increase in N, P, K, Ca, Fe, Zn, Cu and Mn concentrations in bean and corn plants. Biochar holds six times its own weight in water in the soil, which makes it a good nutrient for the soil by enabling the plants to absorb elements such as phosphorus and nitrogen from the soil more easily [10].

Activated black carbon and humic substances can immobilize heavy metals such as Cd in the soil and inhibit their availability to plants [64–66]. Biochar can be employed as fertilizer or soil amendment because of its highly porous carbon structure. It can improve the chemical, physical and biological structure of the soil for plant cultivation. The physical characteristics of agriculture soil are positively and directly affected by biochar as a soil conditioner [67]. The porosity and surface functional groups of biochar have shown that it will be a suitable adsorbent for the removal of heavy metals and phenolic compounds in soil and water [11].

The reducing/inhibiting effect of biochar on the uptake of Cd is due to its functional groups. In addition to its alkaline character, biochar retains Cd in the soil significantly and prevents its uptake by plants, thanks to its high ion exchange capacity and functional groups [6]. In addition, biochar has strong interaction mechanisms, including ionic and covalent bonds on the inner sphere surfaces [45] and functional groups that heavy metals can bind to biochar [34,68]. With these properties, biochar prevents the decrease of their usefulness in the soil solution and thus their uptake by plants by forming/fixing bonds with heavy metals, especially Cd. Furthermore, the precipitation of biochar with heavy metals can be an important factor in preventing the uptake of heavy metals [69].

5. Conclusions

The findings of the study indicated that biochar amendment of the Cd-contaminated soil mitigated the negative impacts of Cd stress on bean plants. In conclusion, biochar incorporation into the soil can alleviate the adverse impacts of Cd stress on the growth of bean seedlings. Biochar application increased the mineral element uptake, whereas it reduced Cd content under Cd stress conditions. It has the capacity to enable sustainable plant production with the stabilization technique of soils, especially in Cd metal pollution.

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References

1. Wani, W.; Masoodi, K.Z.; Zaid, A. Engineering plants for heavy metal stress tolerance. *Rend. Fis. Acc. Lincei* **2018**, *29*, 709–723. [CrossRef]
2. Gallego, S.M.; Pena, L.B.; Barcia, R.A.; Azpilicueta, C.E.; Iannone, M.F.; Rosales, E.P.; Zawoznik, M.S.; Groppa, M.D.; Benavides, M.P. Unravelling cadmium toxicity and tolerance in plants: Insight into regulatory mechanisms. *Environ. Exp. Bot.* **2012**, *83*, 33–46. [CrossRef]
3. Tuver, G.Y.; Ekinci, M.; Yildirim, E. Morphological, physiological and biochemical responses to combined cadmium and drought stress in radish (*Raphanus sativus* L.). *Rend. Fis. Acc. Lincei* **2022**, *33*, 419–429. [CrossRef]
4. Rizwan, M.; Ali, S.; Abbas, T.; Zia-ur-Rehman, M.; Hannan, F.; Keller, C.; Al-Wabel, M.I.; Ok, Y.S. Cadmium minimization in wheat: A critical review. *Ecotoxicol. Env. Saf.* **2016**, *130*, 43–53. [CrossRef]
5. Alengebawy, A.; Abdelkhalek, S.T.; Qureshi, S.R.; Wang, M. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics* **2021**, *9*, 42. [CrossRef]
6. Zhang, J.; Tan, Z.; Huang, Q. Study on principles and mechanisms of new biochar passivation of cadmium in soil. *Biochar* **2021**, *3*, 161–173. [CrossRef]
7. Zhang, H.; Yu, X.; Jin, Z.; Zheng, W.; Zhai, B.; Li, Z. Improving grain yield and water use efficiency of winter wheat through a combination of manure and chemical nitrogen fertilizer on the Loess plateau, China. *J. Soil Sci. Plant Nutr.* **2017**, *17*, 461–474. [CrossRef]
8. Upadhyay, K.P.; George, D.; Swift, R.S.; Galea, V. The influence of biochar on growth of lettuce and potato. *J. Integr. Agric.* **2014**, *13*, 541–546. [CrossRef]
9. Lorenz, K.; Lal, R. Biochar Application to Soil for Climate Change Mitigation by Soil Organic Carbon Sequestration. *J. Plant Nutr. Soil Sci.* **2014**, *177*, 651–670. [CrossRef]
10. Glaser, B.; Wiedner, K.; Seelig, S. Biochar Organic Fertilizers from Natural Resources as Substitute for Mineral Fertilizers. *Agron. Sustain. Dev.* **2014**, *35*, 667–678. [CrossRef]
11. Akgul, G. Biochar: Production and Applications. Selcuk Univ. *J. Eng. Sci. Tech.* **2017**, *5*, 485–499.
12. Lehmann, J.; Joseph, S. Biochar for Environmental Management: Science, Technology and Implementation. Routledge: London, UK, 2015.
13. Shaheen, S.M.; El-Naggar, A.; Wang, J.; Hassan, N.E.; Niazi, N.K.; Wang, H.; Tsang, D.C.; Ok, Y.S.; Bolan, N.; Rinklebe, J. Biochar as an (Im) mobilizing agent for the potentially toxic elements in contaminated soils. In *Biochar from Biomass and Waste*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 255–274. [CrossRef]
14. O'Connor, D.; Peng, T.; Zhang, J.; Tsang, D.C.W.; Alessi, D.S.; Shen, Z.; Bolan, N.S.; Hou, D. Biochar application for the remediation of heavy metal polluted land: A review of in situ field trials. *Sci. Total Environ.* **2018**, *619*, 815–826. [CrossRef]
15. Mohan, D.; Sarswat, A.; Ok, Y.S. Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent-a critical review. *Bioresour Technol.* **2014**, *160*, 191–202. [CrossRef]
16. Sarafi, E.; Siomos, A.; Tsouvaltzis, P.; Chatzissavvidis, C.; Therios, I. Boron and maturity effects on biochemical parameters and antioxidant activity of pepper (*Capsicum annuum* L.) cultivars. *Turk. J. Agric. For.* **2018**, *42*, 237–247. [CrossRef]
17. Helrich, K. *Official Methods of Analysis of the Association of Official Analytical Chemists*; Association of Official Analytical Chemists: Washington, DC, USA, 1990.
18. Khan, Z.S.; Rizwan, M.; Hafeez, M.; Ali, S.; Javed, M.R.; Adrees, M. The accumulation of cadmium in wheat (*Triticum aestivum*) as influenced by zinc oxide nanoparticles and soil moisture conditions. *Environ. Sci. Pollut. Res.* **2019**, *26*, 19859–19870. [CrossRef]
19. Yousaf, B.; Liu, G.; Wang, R.; Rehman, M.Z.; Rizwan, M.S.; Imtiaz, M.; Murtaza, G.; Shakoor, A. Investigating the potential influence of biochar and traditional organic amendments on the bioavailability and transfer of Cd in the soil plant system. *Environ. Earth Sci.* **2016**, *75*, 374. [CrossRef]
20. Gussarson, M.; Asp, H.; Adalststeinsson, S.; Jensen, P. Enhancement of cadmium effects on growth and nutrient composition of birch (*Betula pendula*) by buthionine sulfoximine (BSO). *J. Exp. Bot.* **1996**, *47*, 211–215. [CrossRef]
21. Pereira, B.F.F.; Rozane, D.E.; Araujo, S.R.; Barth, G.; Queiroz, R.J.B.; Nogueira, T.A.R.; Moraes, M.F.; Cabral, C.P.; Boaretto, A.E.; Malavolta, E. Cadmium availability and accumulation by lettuce and rice. *Rev. Bras. Ciênc Solo.* **2011**, *35*, 645–654. [CrossRef]
22. Dresler, S.; Wójcik, M.; Bednarek, W.; Hanaka, A.; Tukiendorf, A. The effect of silicon on maize growth under cadmium stress. *Russ. J. Plant Physiol.* **2015**, *62*, 86–92. [CrossRef]
23. Hasan, S.A.; Hayat, S.; Ali, B.; Ahmad, A. Homobrassinolide protects chickpea (*Cicer arietinum*) from cadmium toxicity by stimulating antioxidants. *Environ. Pollut.* **2008**, *151*, 60–66. [CrossRef]

24. Wahid, A.; Ghani, A. Varietal differences in mungbean (*Vigna radiata*) for growth, yield, toxicity symptoms and cadmium accumulation. *Ann. Appl. Biol.* **2008**, *152*, 59–69. [CrossRef]
25. Gouia, H.; Ghobal, M.H.; Meyer, C. Effects of cadmium on activity of nitrate reductase and on other enzymes of the nitrate assimilation pathway in bean. *Plant Physiol. Biochem.* **2000**, *38*, 629–638. [CrossRef]
26. Sheoran, I.S.; Agarwal, N.; Singh, R. Effect of cadmium and nickel on in vivo carbon dioxide exchange rate of pigeon pea (*Cajanus cajan* L.). *Plant Soil* **1990**, *129*, 243–249. [CrossRef]
27. Sandalio, L.M.; Dalurzo, H.C.; Gomes, M.; Romero-Puertas, M.C.; Rio, L.A. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *J. Exp. Bot.* **2001**, *52*, 2115–2126. [CrossRef]
28. Zengin, F.K.; Munzuroglu, O. Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. *Acta Biol. Cracov. Bot.* **2005**, *47*, 157–164.
29. Márquez-García, B.; Pérez-López, R.; Ruíz-Chancho, M.J.; López-Sánchez, J.F.; Rubio, R.; Abreu, M.M.; Nieto, J.M.; Córdoba, F. Arsenic speciation in soils and Erica andevalensis Cabezudo & Rivera and Erica australis L. from São Domingos Mine area, Portugal. *J. Geochem. Explor.* **2012**, *119*, 51–59.
30. Sanita di Toppi, L.; Gabbrielli, R. Response to cadmium in higher plants. *Environ. Exp. Bot.* **1999**, *41*, 105–130. [CrossRef]
31. Stobort, A.K.; Griffiths, W.T.; Ameen-Burhari, I.; Sherwood, R.P. The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. *Physiol. Plant.* **1985**, *63*, 293–298. [CrossRef]
32. Zhao, B.; Nartey, O.D. Characterization and evaluation of biochars derived from agricultural waste biomasses from Gansu, China. In Proceedings of the World Congress on Advances in Civil, Environmental, and Materials Research, Busan, Republic of Korea, 30 April 2014.
33. Chen, X.; Chen, G.; Chen, L.; Chen, Y.J.; Lehmann, M.B.; McBride, A.G. Adsorption of copper and zinc by biochars produced from pyrolysis of hardwood and corn straw in aqueous solution. *Bioresour. Technol.* **2011**, *102*, 8877–8884. [CrossRef]
34. Regmi, P.; Moscoso, J.L.G.; Kumar, S.; Cao, X.; Mao, J.; Schafran, G. Removal of copper and cadmium from aqueous solution using switchgrass biochar produced via hydrothermal carbonization process. *J. Environ. Manag.* **2012**, *109*, 61–69. [CrossRef]
35. Xu, D.; Chen, Z.; Sun, K.; Yan, D.; Kang, M.; Zhao, Y. Effect of cadmium on the physiological parameters and the subcellular cadmium localization in the po- tato (*Solanum tuberosum* L.). *Ecotoxicol, Environ. Saf.* **2013**, *97*, 147–153. [CrossRef] [PubMed]
36. Zhao, S.; Ma, Q.; Xu, X.; Li, G.; Hao, L. Tomato jasmonic acid-deficient mutant spr2 seedling response to cadmium stress. *J. Plant Growth Regul.* **2016**, *35*, 603–610. [CrossRef]
37. Rizwan, M.; Ali, S.; Abbas, T.; Rehman, M.; Al-Wabel, M.I. Residual impact of biochar on cadmium uptake by rice (*Oryza sativa* L.) grown in Cd-contaminated soil. *Arab. J. Geosci.* **2018**, *11*, 630. [CrossRef]
38. Trovato, M.; Mattioli, R.; Costantino, P. Multiple roles of proline in plant stress tolerance and development. *Rend. Fis. Acc. Lincei* **2008**, *19*, 325–346. [CrossRef]
39. Poschenrieder, C.; Gunsé, B.; Barceló, J. Influence of cadmium on water relations, stomatal resistance and abscisic acid content in expanding bean leaves. *Plant Physiol.* **1989**, *4*, 1365–1371. [CrossRef] [PubMed]
40. Somasekharaiah, B.V.; Padmaja, K.; Prasad, A.R.K. Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): Involvement of lipid peroxidase in chlorophyll degradation. *Physiol. Plant.* **1992**, *85*, 85–89. [CrossRef]
41. Dogan, M. Effect of cadmium, chromium, and lead on micropropagation and physio-biochemical parameters of *Bacopa monnieri* (L.) Wettst. cultured in vitro. *Rend. Fis. Acc. Lincei* **2019**, *30*, 351–366. [CrossRef]
42. Gichner, T.; Patkova, Z.; Szakova, J.; Znidar, I.; Mukherjee, A. DNA damage in potato plants induced by cadmium, ethyl methanesulphonate and grays. *Environ. Exp. Bot.* **2008**, *62*, 113–119. [CrossRef]
43. Correa, A.X.R.; Rorig, L.R.; Verdinelli, M.A.; Cotelle, S.; Ferard, J.F.; Radetski, C.M. Cadmium phytotoxicity: Quantitative sensitivity relationships between classical endpoints and antioxidative enzyme biomarkers. *Sci. Total Environ.* **2006**, *357*, 120–127. [CrossRef]
44. Yildirim, E.; Ekinci, M.; Turan, M.; Ağar, G.; Dursun, A.; Kul, R.; Alim, Z.; Argin, S. Humic fulvic acid mitigated Cd adverse effects on plant growth, physiology and biochemical properties of garden cress. *Sci. Rep.* **2021**, *11*, 8040. [CrossRef]
45. Liang, F.; Li, G.; Lin, Q.; Zhao, X. Crop yield and soil properties in the first 3 years after biochar application to a calcareous soil. *J. Integr. Agric.* **2014**, *13*, 525–532. [CrossRef]
46. Cao, X.; Ma, L.; Gao, B.; Harris, W. Dairy-manure derived biochar effectively sorbs lead and atrazine. *Environ. Sci. Technol.* **2009**, *43*, 3285–3291. [CrossRef]
47. Liu, J.; Qu, W.; Kadiiska, M.B. Role of oxidative stress in cadmium toxicity and carcinogenesis. *Toxicol. Appl. Pharmacol.* **2009**, *238*, 209–214. [CrossRef]
48. Southavong, S.; Preston, T.R.; Man, N.V. Effect of soil amender (biochar or charcoal) and biodigester effluent on growth of water spinach (*Ipomoea aquatica*). *Livest Res. Rural. Dev.* **2012**, *24*, 2. Available online: https://www.researchgate.net/publication/286980798 (accessed on 16 November 2022).
49. Warnock, D.D.; Lehmann, J.; Kuyper, T.W.; Rillig, M.C. Mycorrhizal responses to biochar in soil—Concepts and mechanisms. *Plant Soil* **2007**, *300*, 9–20. [CrossRef]
50. Steiner, C.; Das, K.C.; Garcia, M.; Förster, B.; Zech, W. Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic ferralsol. *Pedobiol. Int. J. Soil Biol.* **2008**, *51*, 359–366. [CrossRef]
51. McKenna, I.M.; Chaney, R.L.; Williams, F.M. The effects of cadmium and zinc interactions on the accumulation and tissue distribution of zinc and cadmium in lettuce and spinach. *Environ. Pollut.* **1993**, *79*, 113–120. [CrossRef] [PubMed]

52. Naik, S.; Pandit, T.; Patra, P.; Das, D. Effects of graded levels of cadmium on spinach and cabbage grown in an inceptisol. *Commun Soil Sci. Plant Anal.* **2013**, *44*, 1629–1642. [CrossRef]
53. Alia, N.; Sardar, K.; Said, M.; Salma, K.; Sadia, A.; Sadaf, S.; Toqeer, A.; Miklas, S. Toxicity and bioaccumulation of heavy metals in spinach (*Spinacia oleracea*) grown in a controlled environment. *Int. J. Environ. Res. Public Health* **2015**, *12*, 7400–7416. [CrossRef]
54. Leon, A.M.; Palma, J.M.; Corpas, F.J.; Gomez, M.; Romero-Puertas, M.C.; Chatterjee, R.; Mateos, M.; Rio, L.A.; Sandalio, L.M. Antioxidant enzymes in cultivars of pepper plants with different sensitivity to candium. *Plant Physiol. Biochem.* **2002**, *40*, 813–820. [CrossRef]
55. Hassan, M.J.; Shao, G.; Zhang, G. Influence of cadmium toxicity on growth and antioxidant enzyme activity in rice cultivars with different grain cadmium accumulation. *J. Plant Nutr.* **2005**, *28*, 1259–1270. [CrossRef]
56. Dias, M.C.; Monteiro, C.; Moutinho-Pereira, J.; Correia, C.; Gonçalves, B.; Santos, C. Cadmium toxicity affects photosynthesis and plant growth at different levels. *Acta Physiol. Plant.* **2013**, *35*, 1281–1289. [CrossRef]
57. Lopez-Millian, A.F.; Sagardoy, R.; Solanas, M.; Abadia, A.; Abadia, J. Cadmium toxicity in tomato (*Lycopersicon esculentum* Mill.) plants grown in hydroponics. *Environ. Exp. Bot.* **2009**, *65*, 376–385. [CrossRef]
58. Bian, R.; Joseph, S.; Cui, L.; Pan, G.; Li, L.; Liu, X.; Zhang, A.; Rutlidge, H.; Wong, S.; Chia, C.; et al. A three-year experiment confirms continuous immobilization of cadmium and lead in contaminated paddy field with biochar amendment. *J. Hazard Mater* **2014**, *272*, 121–128. [CrossRef]
59. Zhang, Z.Y.; Jun, M.; Shu, D.; Chen, W.F. Effect of biochar on relieving cadmium stress and reducing accumulation in super japonica rice. *J. Int. Agric.* **2014**, *13*, 547–553. [CrossRef]
60. Larbi, A.; Morales, F.; Abadia, A.; Gogorcena, Y.; Lucena, J.J.; Abadia, J. Effects of Cd and Pb in sugar beet plants grown in nutrient solution: Induced Fe deficiency and growth inhibition. *Funct. Plant Biol.* **2002**, *29*, 1453–1464. [CrossRef]
61. Dong, J.; Wu, F.B.; Zhang, G.P. Influence of cadmium on antioxidant capacity and four microelement concentrations in tomato seedlings (*Lycopersicon esculentum*). *Chemosphere* **2006**, *64*, 1659–1666. [CrossRef]
62. Genchi, G.; Sinicropi, M.S.; Lauria, G.; Carocci, A.; Catalano, A. The effects of cadmium toxicity. *Int. J. Environ. Res. Public Health* **2020**, *17*, 3782. [CrossRef]
63. Inal, A.; Gunes, A.; Sahin, O.; Taskin, M.; Kaya, E. Impacts of biochar and processed poultry manure, applied to a calcareous soil, on the growth of bean and maize. *Soil Use Manag.* **2015**, *31*, 106–113. [CrossRef]
64. Radziemska, M.; Gusiatin, Z.M.; Cydzik-Kwiatkowska, A.; Cerdà, A.; Pecina, V.; Bes´, A.; Datta, R.; Majewski, G.; Mazur, Z.; Dziecioł, J.; et al. Insight into metal immobilization and microbial community structure in soil from a steel disposal dump that was phytostabilized with composted, pyrolyzed or gasified wastes. *Chemosphere* **2021**, *272*, 129576. [CrossRef]
65. Sultan, H.; Ahmed, N.; Mubashir, M.; Danish, S. Chemical production of acidified activated carbon and its influences on soil fertility comparative to thermo-pyrolyzed biochar. *Sci. Rep.* **2020**, *10*, 595. [CrossRef]
66. Zafar-ul-Hye, M.; Tahzeeb-ul-Hassan, M.; Abid, M.; Fahad, S.; Brtnicky, M.; Dokulilova, T.; Datta, R.; Danish, S. Potential role of compost mixed biochar with rhizobacteria in mitigating lead toxicity in spinach. *Sci Rep.* **2020**, *10*, 12159. [CrossRef]
67. Zafar-ul-Hye, M.; Danish, S.; Abbas, M.; Ahmad, M.; Munir, T.M. ACC deaminase producing PGPR *Bacillus amyloliquefaciens* and *agrobacterium fabrum* along with biochar improve wheat productivity under drought stress. *Agronomy* **2019**, *9*, 343. [CrossRef]
68. Mohammed, B.A.; Ellis, N.; Kim, C.S.; Bi, X.; Chen, W.H. Engineered biochars from catalytic microwave pyrolysis for reducing heavy metals phytotoxicity and increasing plant growth. *Chemosphere* **2021**, *271*, 129–808. [CrossRef]
69. Tong, X.; Li, J.; Yuan, J.; Xu, R. Adsorption of Cu(II) by biochars generated from three crop straws. *Chem. Eng. J.* **2011**, *172*, 828–834. [CrossRef]