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Benefits of Corn-Cob Biochar to the Microbial and Enzymatic Activity of Soybean Plants Grown in Soils Contaminated with Heavy Metals

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Abstract: Synchronous effects of biochar on heavy metals stress, microbial activity and nodulation process in the soil are rarely addressed. This work studied the effects, under greenhouse conditions, of selected heavy metals Cd^{2+}, Pb^{2+} and Ni^{2+} on soybean plants grown in two different soils amended with biochar, and studied their effect on the microbial and enzymatic activity. As a result of the interference between heavy metals and biochar, biochar overcame heavy metal problems and maintained a microbial population of major groups (bacteria–fungi). There was an increase in the degree of resistance (*RS*) of the major microbial groups to heavy metals when biochar was added to the soil under study. Numbers of bacterial nodules significantly increased, particularly by using the higher rate of biochar compared to the control, either by adding biochar alone or by mixing it with the selected heavy metals. The arginase activity was increased by 25.5% and 37.1% in clay and sandy soil, respectively, compared to the control. For urease (UR), the activity was increased by 105% and 83.8% in clay and sandy soil, respectively, compared to the control. As a result, considerations of using biochar as a soil amendment should be first priority.

Keywords: corncob biochar; heavy metals; enzymatic activity; nodules; soil health

1. Introduction

There is increasing trepidation about soil pollution by heavy metals because of their harmfulness to plants, animals and human beings and their deficiency of biodegradability [1,2]. In agroecosystems, microorganisms play significant roles in nutrient cycling, organic matter decomposition and plant nutrient utilization [3,4]. Microbial properties, e.g., soil microbial biomass, diversity and activity of soil microbial groups, are commonly used as indicators of metal contamination, due to their high sensitivity to metal-induced stress [5] and rapid response to disturbances, their ecological relevance and capability to provide information on the integration of many environmental factors. Previous studies showed that heavy metal contamination has both long-term [6] and short-term [7] toxicity effects on soil microbial communities. Soil enzymes, as natural catalysts of many soil processes connected with decomposition of organic substances, participate in the processes of releasing and making minerals available to plant roots in the rhizosphere. Enzymatic activity is an early indicator of changes in the level of intensity of biological processes and the level of soil degradation and it is usually correlated with its physical and chemical properties [8,9]. Certain biochars have also been shown to change soil biological community composition and abundance [10]. The influence of biochars on soil enzyme activities, however, is still poorly documented.

One soil amendment that has received much consideration recently is biochar. Biochar is a carbon-rich product from burning biomass in the presence of little or an absence of

oxygen (pyrolysis), and its potential to improve soil fertility, mitigate climate change, decrease the bioavailability of a range of heavy metals and improve soil water relations has been recognized worldwide [11–14]. Biochars are produced from various types of lignocellulosic biomass feedstocks and other material such as poultry and turkey litter, swine, dairy and cattle manure and biosolids [15]. The chemical composition and performance characteristics of biochar depend on the feedstock source and the parameters involved in the pyrolysis process such as the highest temperature treatment (HTT), the residence time at which the feedstock is exposed to HTT and the rate of increase in temperature [16]. The pyrolysis parameters affect, e.g., the porosity, specific surface area, stability and the chemical composition of biochar. The first two properties are considered most important in terms of material applicability. Biochar application has been evaluated for a wide range of soils including sands, sandy loams and clay loams [17]. Biochar addition may improve crop productivity by retaining more water from rainfall in arid regions and reduce the frequency or rate of irrigation water in irrigated regions. Biochar addition strategies could therefore be a successful method for reducing water consumption while sustaining crop productivity.

Despite the apparent benefits of biochar towards a variety of soil chemical parameters, the effects of biochar on soil microorganisms have received much less attention [10,18–20]. However, the response of soil microorganisms to soil amendment with biochar may vary depending on soils and agricultural practices [21]. Only a few studies have focused on biochar's effects on N_2 fixation of leguminous crops [22,23]. As far as we know, little information is available on the effect of biochar on the nitrogen fixation process. Furthermore, the literature simply does not provide enough information on the effect of heavy metals on nodulation/nitrogen nutrition of most leguminous crops in soils amended with biochar.

Considerations of soil–biochar interactions, including the application of biochar to soil for improving agricultural productivity and food security, should be the first priority. The major impetus of the present investigation aimed to study the following: (I) Try to get a clear answer about how biochar affects the microbial population and enhances soil fertility. (II) Study the relationship between the biochar soil amendments and enzymes related to the nitrogen cycle in the absence or presence of heavy metals. Such information is required to have a secure and healthy agricultural product.

2. Materials and Methods

2.1. Source and Type of Soils

Two soil types with different characteristics were used. Clay soil was collected from the Experimental Farm of the Faculty of Agriculture, Minia University, El-Minia, Egypt. Newly reclaimed sandy soil was obtained from Shousha zone, Agricultural Research Center, Minia University. For greenhouse experiments, each soil from the top 15 cm layer was air dried, thoroughly mixed and passed through a 2-mm sieve. The physical and chemical analyses of the two soils used are presented in Table 1 in accordance with Page et al. [24]. Microbial biomass C (C_{mic}) was determined as described by Vance et al. [25]. Microbial biomass N (N_{mic}) was determined as described by Horwath et al. [26].

2.2. Source and Type of Biochar

The biochar was produced from corncob by slow pyrolysis (30 min, 600 °C). Biochar in its loose condition was air dried and finely ground to pass through a 2-mm sieve before being used for the pot experiment. The actual quantities of biochar applied to the pot experiment were equivalent to the application rates of 0 t ha^{-1} (control), 20 t ha^{-1} (B1) and 40 t ha^{-1} (B2), respectively (Figure 1). Sub-samples of the dried, ground and sieved soil and biochar were used to determine some physiochemical properties. Most of the methods employed are of the standard methods used by the methods of soil analysis [24]. Some important characteristics of the investigated corn biochar are given in Table 2.

Table 1. Physical and chemical properties of the soils investigated.

Texture Grade	Clay Soil	Sandy Soil
(Field capacity) F.C %	38.26	13.96
(Permanent wilting point) PWP %	15.11	3.46
(Water holding capacity) WHC %	47.66	18.22
Available water (F.C–PWP) %	23.15	10.50
Available water (WHC–PWP) %	32.55	14.76
Bulk Density (BD) g cm^{-3}	1.18	1.63
Particle Density g cm^{-3}	2.26	2.61
pH (1:2.5 H_2O)	7.7(7.4) [a]	8.5(8.3) [a]
CEC (cmol(+) kg^{-1} soil)	37.87	3.66
EC (dS m^{-1} at 25 °C)	1.22	2.58
(Soil organic carbon) S.O.C g kg^{-1}	16.8	4.1
Organic matter g kg^{-1}	28.34 [b]	7.28 [b]
C/N ratio	20.99	17.75
Total N g kg^{-1}	1.41	0.41
Total P g kg^{-1}	1.16	0.29
Total K g kg^{-1}	12.88	2.7
(Microbial biomass C) C_{mic}	61.45	34.67
(Microbial biomass N) N_{mic}	18.32	6.65
C_{mic}: N_{mic}	3.35	5.21
Mineral N (mg kg^{-1})	78.24	22.43

Total heavy metals content (mg kg^{-1}) [c]	Cd	Ni	Pb	Cd	Ni	Pb
	0.55	43.7	31.4	3.9	44.3	52.7

[a] Numbers in parentheses are pH values obtained for soil by Ca Cl$_2$ ratio of 1:2.5. [b] Organic matter determined by loss on ignition. [c] Akagi and Nishimura [27].
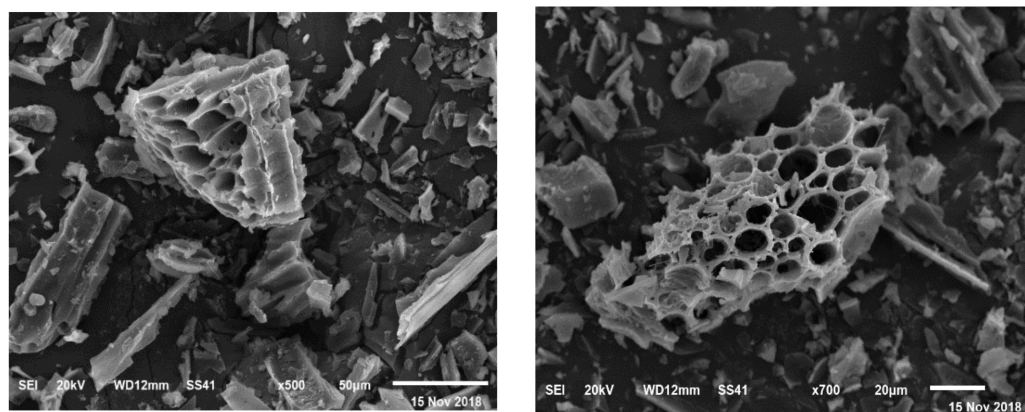
Figure 1. Scanning electron micrograph of biochar derived from corncob used in the greenhouse experiments (Photo: Samir A. Haddad).

Scan Electron Microscope (JEOL Model JSM-5910 SEM) at 20 kV imaging at different magnification levels was used (Figure 1) for the determination of structure and surface morphology of corncob biochar sample.

Soil and biochar total nitrogen were determined with a Shimadzu TOC-TN analyzer (Shimadzu Corp., Kyoto, Japan).

Table 2. Selected physiochemical characteristics of the studied corn biochar.

Biochar Property			
Bulk Density g/cm^3		0.26	
WHC %		58.7	
pH (1: 2.5 H$_2$O)		6.31	
EC (dS m^{-1} at 25 °C)		0.651	
CEC (cmol(+) kg^{-1} soil)		34.4	
Ash %		11.5	
Total organic carbon g kg^{-1}		562	
Total N g kg^{-1}		16.8	
C/N ratio		33.2	
Total P g kg^{-1}		3.2	
N/P ratio		5.27	
Total K mg kg^{-1}		480	
Total Ca mg kg^{-1}		650	
Total Mg mg kg^{-1}		40.6	
Total heavy metals (mg kg^{-1}) Dry matter	Cd	Ni	Pb
	1.42	6.41	3.56

2.3. Heavy Metals

Three heavy metals representing those commonly found in fertilizers and industrial wastes in Egypt were used in this study [28,29]. The salts of the heavy metals were cadmium sulfate (CdSO$_4$·8H$_2$O), lead acetate [Pb(CH$_3$COO)$_2$], and nickel sulfate (NiSO$_4$·6H$_2$O).

2.4. Greenhouse Experiments

The aim was to study the effect of the selected heavy metals on the microbial and enzyme activity of one of the most predominant leguminous crops representing food, feed and forage legumes (Soybean (*Glycine max*) (Giza 35)). Two randomized block design pot experiments with the two soils × three heavy metals (one rate 250 mg kg^{-1} soil) × two biochar rates 20 t ha^{-1} (B1), and 40 t ha^{-1} (B2), respectively were conducted under greenhouse conditions. In each experiment, 3.5 kg of soil was placed in plastic pots (30 cm diameter), treated with one liter of deionized water containing one heavy metal to make its concentration in soil 250 mg kg^{-1} soil, and the moisture content was adjusted to 60% of the water holding capacity by using deionized water.

The plants were grown (five seeds pot^{-1}, which were thinned to three plants after 10 day) for 90 days. Before planting, the seeds were treated with specific Bradyrhizobium inoculants containing a minimum of 3 × 10^9 viable cells mL^{-1}, supplied by Agriculture Genetic Engineering Research Institute (Cairo, EGY), by using a sucrose solution (200 g in 900 mL of deionized water) to aid the adhesion of the inoculants to the seeds and promote N$_2$ fixation. The soil moisture level of all pots was kept at ca. 60% of the field capacity during plant growth by randomly weighing the pots and adding deionized water as needed. The cultivation process was carried out during the 2018 season and the following treatments were conducted for clay and sandy soil (Table 3)

Plants were harvested at different stages of the plant growth, including the early flowering stage and the podding stage by cutting the shoots (stems and leaves) at the soil surface, the roots and seeds were then separated from the soil and the number of nodules per plant was counted.

Table 3. The cultivation process and treatments for clay and sandy soil.

Crop	Soils	
	Clay	**Sandy**
	Treatments	
soybean	Control (clay soil only)	Control (sandy soil only)
	Cd	Cd
	Ni	Ni
	Pb	Pb
soybean	Biochar 1 (B1)	Biochar 1 (B1)
	B1 Cd	B1 Cd
	B1 Ni	B1 Ni
	B1 Pb	B1 Pb
soybean	Biochar 2 (B2)	Biochar 2 (B2)
	B2 Cd	B2 Cd
	B2 Ni	B2 Ni
	B2 Pb	B2 Pb

2.5. Total Counts of Bacteria and Fungi

To evaluate the effects of the selected heavy metals on microbial population in soils amended or not amended with two different rates of biochar, 10 g of the soil rhizosphere was added to 95 mL of sterilized water, the solution was shaken for 5 min, then diluted (10^{-1} to 10^{-6}) and then the resulting solutions were plated directly onto the surface of a nutrient agar medium for bacteria, Martin [30] medium for fungi. After incubation at 25 or 30 °C for 10 days, the colony forming units were counted (CFU).

2.6. The Resistance Index (RS)

The soil resistance index (*RS*) was determined according to the counts of bacteria and fungi that resisted soil contamination with heavy metals using the following equation by Orwin and Wardle [31].

$$RS\,(t0) = 1 - \left[\frac{2|D0|}{C0 + |D0|} \right] \tag{1}$$

where *D*0 is the difference between the control sample (*C*0) and the contaminated soil samples (P_0). The *RS* values fall between −1 and +1 with a value of +1 showing the disturbance has no effect (high resistance), and lower values showing stronger adverse effect (less resistance).

2.7. Enzymatic Activity

Arginine activity (AR) was determined as described by Alef and Kleiner [32]. In this method, 2 g of soil was placed in a small flask (d = 2.2 cm, h = 4.4 cm), closed with a cotton plug and incubated at 30 °C for 1 h; 0.5 mL of an arginine solution (0.2% in water) was added dropwise. At specified times (up to 6 h), flasks were removed and stored at −20 °C. After thawing, the samples were immediately mixed with 8 mL 2 M KC1 under stirring for 15 min. After centrifugation 0.5 mL of the supernatant was mixed with 1.5 mL 2 M KCl, 1 mL 2% sodium phenolate, 0.5 mL 0.005% sodium nitroprusside and 0.5 mL sodium hypochlorite (0.005 M NaOCl in 0.125 M NaOH) incubated at 30 °C for 30 min; the extinction was measured at 630 nm.

Enzyme activity of urease (UR) in soil samples was assayed as described by Tabatabai [33] using urea as the substrate. Urease activity in the soil was determined by spectrophotometry at 578 nm. All enzyme activities were expressed as products per unit of dry soil mass and incubation time.

2.8. Media Used

1. Nutrient Agar: Beef extract (3 g), yeast extract (2 g), peptone (5 g), agar (15 g), distilled water of 1000 mL and pH (7.0).

    Modified Czapek–Dox agar medium: Sucrose (30 g), $NaNO_3$ (3 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), KCl (0.5 g), $FeSO_4 \cdot 4H_2O$ (0.01 g), K_2HPO_4 (1 g), agar (12 g), tap water of 1000 mL and pH 7.2.

2. Martin's medium for fungi: Glucose (10 g), peptone (5 g), KH_2PO_4 (1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), agar (20 g), distilled water of 1000 mL and rose bengal 1 part in 30,000 parts of medium.

    Streptomycin solution 30 $\mu g \ mL^{-1}$ medium. The rose bengal was added before, and the streptomycin after sterilization just before plating.

2.9. Statistical Analysis

Data were subjected to the analysis of variance (ANOVA). Means were compared using the Least Significant Difference (LSD) parameters using the general linear models' procedure of SAS system [34] for the combined experiments.

3. Results and Discussion

For convenience and clarity, the results will be discussed under subheadings corresponding to the processes studied.

3.1. Effects of Selected Heavy Metals on Microbial Activity in Soils Amended with Biochar

Heavy metal pollution of soil is known to adversely affect microbial activities at excessive concentrations. However, the response of soil microbial population to added heavy metal and metal combinations is poorly understood. Biochar is gaining attention as a potential soil amendment to remediate and regenerate the contaminated soils. The simultaneous effects of biochar on metal mobility, microbial abundance, bacterial diversity and carbon storage in the soil are scarcely addressed.

3.2. Fungal/Bacterial (F/B) Ratio

Biochar's effects on soil microbial abundance and community structure are key for understanding the biogeochemical cycling of nutrients and organic matter turnover but are poorly understood. In this study, the fungal/bacteria (F/B) ratio in the contaminated soils were determined with the application of biochar as a soil amendment.

Data presented in Tables 4 and 5 showed that, the F/B ratio significantly increased when increasing the biochar rates (B1, B2) after 60 days of cultivation and decreased significantly with the addition of heavy metals without biochar (control). For this experiment case, the response ratios of soil F/B were positively interrelated with biochar rates and study duration (Tables 4 and 5). Numerous studies had distinguished that the high application rates of biochar ($\geq$5%) in the long-term condition significantly increased soil F/B ratios with preferential motivation of soil fungi [35,36].

Table 4. Fungal/Bacterial (F/B) ratio in the rhizosphere of soybean plants grown in biochar amended soils (clay) alone or in combinations with selected heavy metals.

Treatment	Clay Soil											
	Days after Plant Emergence											
	15			30			60			90		
	F	B	F/B	F	B	F/B	F	B	F/B	F	B	F/B
Control	17	26	0.65	25	32.6	0.77	110	125	0.88	60	75	0.80
Cd	7	9	0.78	8	10	0.80	5	6	0.83	3	4	0.75
Ni	6	7	0.86	10	12	0.83	6	9	0.67	4	8	0.50
Pb	5	6	0.83	18	24	0.75	11	17	0.65	9	13	0.69
B1	30	35	0.86	105	61.3	1.71	180	140	1.29	90	90	1.00
B1 Cd	18	20	0.90	39	32	1.22	109	95	1.15	80	70	1.14
B1 Ni	20	24	0.83	43	38	1.13	114	102	1.12	86	82	1.05
B1 Pb	13	18	0.72	39	30	1.30	89	85	1.05	70	68	1.03
B2	42	50	0.84	160	85	1.88	210	186	1.13	170	130	1.31
B2 Cd	30	35	0.86	54	49	1.10	140	130	1.08	103	98	1.05
B2 Ni	31	37	0.84	58	53	1.09	145	132	1.10	109	89	1.22
B2 Pb	28	33	0.85	49	48	1.02	139	120	1.16	95	87	1.09
L.S.D at 0.05	16.97	10.92	0.13	35.76	19.20	0.21	42.98	26.37	0.20	27.14	40.63	0.16
L.S.D at 0.01	23.08	14.40	0.18	48.47	26.02	0.29	58.24	35.74	0.27	36.78	55.07	0.21

Table 5. Fungal/Bacterial (F/B) ratio in the rhizosphere of soybean plants grown in biochar amended soils (sandy) alone or in combination with selected heavy metals.

Treatment	Sandy Soil											
	Days after Plant Emergence											
	15			30			60			90		
	F	B	F/B	F	B	F/B	F	B	F/B	F	B	F/B
Control	12	14	0.86	20	25	0.80	105	115	0.91	52	65	0.80
Cd	4	6	0.67	8	11	0.73	3	5	0.60	0	3	-
Ni	5	7	0.71	14	15	0.93	9	12	0.75	3	9	0.33
Pb	4	5	0.80	9	17	0.53	8	9	0.89	4	5	0.80
B1	25	25	1.00	63	50	1.26	180	133	1.35	83	80	1.04
B1 Cd	18	14	1.29	39	22	1.77	96	83	1.16	72	70	1.03
B1 Ni	20	16	1.25	30	28	1.07	98	93	1.05	73	74	0.99
B1 Pb	16	12	1.33	24	21	1.14	79	72	1.10	68	70	0.97
B2	38	32	1.19	50	70	0.71	194	163	1.19	112	102	1.10
B2 Cd	27	23	1.17	45	39	1.15	109	103	1.06	93	90	1.03
B2 Ni	29	24	1.21	48	42	1.14	115	111	1.04	95	92	1.03
B2 Pb	24	20	1.20	40	39	1.03	106	108	0.98	87	78	1.12
L.S.D at 0.05	12.61	9.89	0.50	11.05	20.08	0.15	31.12	17.47	0.19	28.83	28.82	0.22
L.S.D at 0.01	17.08	13.41	0.68	14.97	27.21	0.20	42.17	23.67	0.26	38.01	39.05	0.29

L.S.D.—Least Significance Difference.

The F/B ratio was often used to assess the sustainability of agriculture systems [37] with important benefits including a more efficient crop nutrient uptake mediated by mycorrhizal fungi. The F/B ratio decreased with an increasing mineral N application rate, mainly due to suppression of fungal growth. The present result supports earlier studies of de Vries et al. [37] who showed that the application of mineral N fertilization reduced the F/B ratio, whereas the addition of organic amendments into soils increased the F/B ratio because of increasing fungal growth. This contrasts with results obtained by Gomez

et al. [38], who reported significantly lower F/B ratio in four soils (two sandy loam, clayey and clay loam) amended with fast pyrolysis biochar after 12 months of incubation.

Compared to bacteria, fungi can assimilate C sources more efficiently. Under high biochar loads, hyphae grow into biochar pores using more degradable and stable C sources [10].

3.3. Resistance Index (RS)

The soil resistance indicator (*RS*) is an effective measure of microbial responses to environmental stress [31] and is an effective indicator to measure the relative ability of the soil to continue functioning under stress conditions, as might happen through heavy metal stress. In the present study, a significant effect of the selected heavy metals on bacteria and fungi was demonstrated and verified by the decreasing values of the *RS*. The values of the resistance index (*RS*) for selected soil microorganisms were positive throughout the experiment. However, they differed depending on the type of heavy metal applied, the experimental time and the soil texture (Tables 6 and 7). Lower values indicate a stronger influence of pollution with heavy metals on the balance (lower resistance). Higher *RS* values of bacteria and fungi were noted in clay soil (more resistance to heavy metal toxicity) than sandy soil. The *RS* in sandy soil decreased to a lesser extent. Pb and Cd caused stronger disturbances of soil microorganisms than Ni in the same soils. Sensitivity of soil microorganisms to soil pollution by heavy metals was also shown by Wyszkowska et al. [39].

Table 6. Resistance index (*RS*) of bacteria to Cd, Ni and Pb in rhizosphere of soybean plants grown in biochar amended soils.

Treatment	Clay Soil				Sandy Soil			
	Days after Plant Emergence							
	15	30	60	90	15	30	60	90
Cd	0.21	0.18	0.02	0.03	0.27	0.28	0.02	0.02
Ni	0.16	0.23	0.04	0.05	0.33	0.43	0.06	0.07
Pb	0.13	0.15	0.07	0.09	0.22	0.51	0.04	0.04
B1 Cd	0.40	0.35	0.51	0.64	0.39	0.28	0.45	0.78
B1 Ni	0.52	0.45	0.57	0.84	0.47	0.39	0.54	0.86
B1 Pb	0.34	0.32	0.44	0.61	0.36	0.27	0.37	0.78
B2 Cd	0.54	0.40	0.54	0.60	0.56	0.39	0.46	0.79
B2 Ni	0.59	0.52	0.55	0.52	0.60	0.43	0.52	0.82
B2 Pb	0.49	0.39	0.48	0.50	0.45	0.39	0.50	0.61

Table 7. Resistance index (*RS*) of fungi to Cd, Ni and Pb in rhizosphere of soybean plants grown in biochar amended soils.

Treatment	Clay Soil				Sandy Soil			
	Days after Plant Emergence							
	15	30	60	90	15	30	60	90
Cd	0.26	0.18	0.02	0.03	0.20	0.25	0.01	0.00
Ni	0.21	0.23	0.04	0.03	0.26	0.54	0.05	0.03
Pb	0.17	0.15	0.07	0.08	0.20	0.29	0.04	0.04
B1 Cd	0.43	0.35	0.51	0.80	0.56	0.45	0.36	0.77
B1 Ni	0.50	0.45	0.57	0.91	0.67	0.31	0.36	0.78
B1 Pb	0.28	0.32	0.44	0.64	0.47	0.26	0.28	0.69
B2 Cd	0.56	0.40	0.54	0.44	0.55	0.39	0.39	0.71
B2 Ni	0.58	0.52	0.55	0.47	0.62	0.43	0.42	0.74
B2 Pb	0.50	0.39	0.48	0.39	0.46	0.33	0.38	0.64

All the results of this study indicated that *RS* for the investigated microbial groups differed depending on heavy metal type and soil texture. In the present study, increasing biochar rates caused a significant increase in soil resistance (Tables 6 and 7). Higher *RS* values were noticed in clay soil than sandy soil and revealed that clay soil was more resistant to heavy metal toxicity. The *RS* values in both sandy and clay soil decreased to zero when heavy metals were applied at 250 mg kg^{-1} soil after 90 days of cultivation. Lead (Pb) caused stronger soil disturbance than Cd and Ni in both studied sandy and clay soils.

Effect of Selected Heavy Metals on Nodulation and enzyme activities of Soybean Plants Grown in Biochar Amended Soils.

3.4. Effect on Nodulation

Heavy metals in soil are naturally found but may be enhanced by anthropogenic activities such as mining and agricultural practices. Bio-accumulation of heavy metals in the food chain, following their uptake to plants, can increase the ecotoxicological risks associated with remediation of contaminated soils using plants. In the current experiment corncob biochar was applied to a heavy metal contaminated soil at 20 t ha^{-1} (B1) and 40 t ha^{-1} (B2). Soybean ((*Glycine max*) (Giza 35)) was grown in pots containing soil and biochar mixtures, and control pots without biochar.

Few nodules on the roots of soybean plants in soil without biochar were found. On the other hand, such nodules were observed in plants grown in soils with biochar even in combination with the selected heavy metals whether in the flowering or pudding stage. The number of nodules was higher in clay soil than the sandy one. Pb was the most deleterious metal and had the worst effect on the nodulation process followed by Cd and Ni. Adding biochar as a soil amendment had a clear and positive effect on increasing the number of nodules (Figure 2) in both clay and sandy soil (Table 8). Accordingly, biochar was used as an inoculant carrier to enhance the rhizobium survival rate and the soybean nodulation rate [40]. Moreover, this indicates that the biochar provided conditions for symbiosis with N$_2$ fixing bacteria, which is beneficial from the viewpoint of soil remediation in areas contaminated by heavy metals since it stimulates revegetation by the introduction of nitrogen through symbiotic fixation. This finding completely matched with [41], who stated that such limitations are a major barrier to the remediation of contaminated soils, even when organic amendments are added. Another factor was the difference between the plant roots. In the higher rates of biochar, plants showed more developed and distributed roots (Figure 3). Phytoremediation combined with the addition of biochar to soil could enhance soil biological activity because biochar amendment promotes root growth [42].
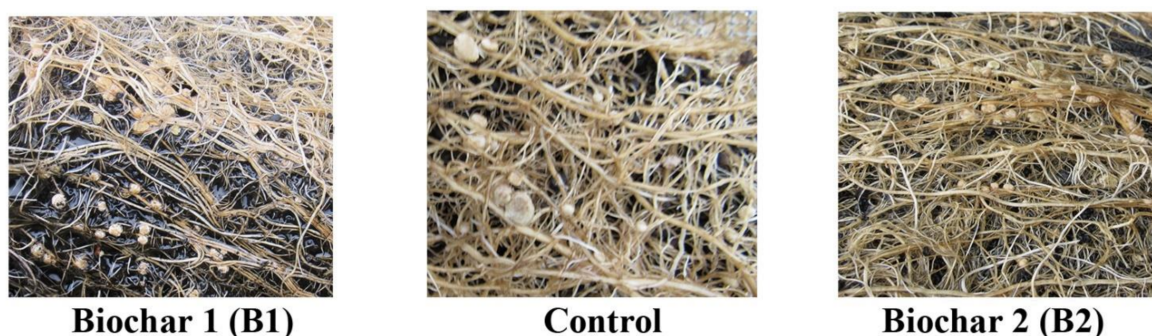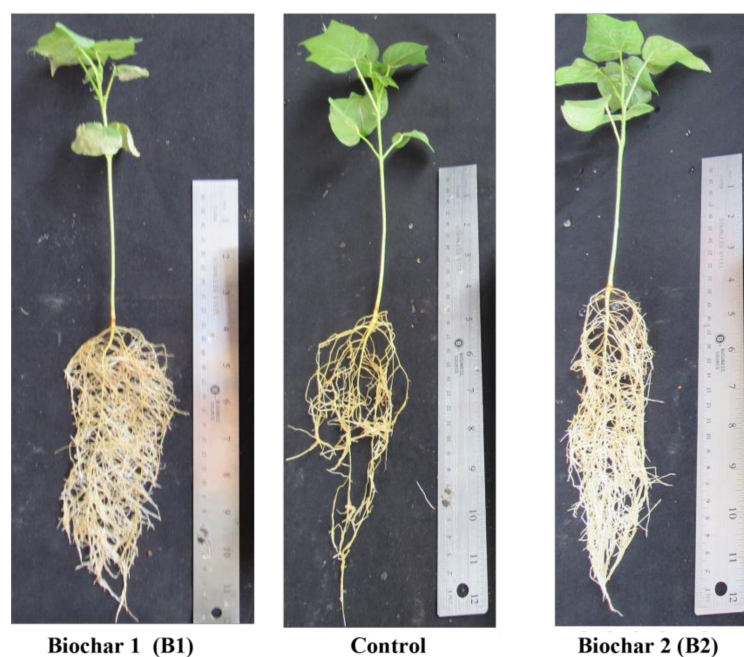

Figure 2. Nodules of soybean plants grown in soils amended or not amended with biochar (Photos: Samir A. Haddad).

Table 8. Effect of Cd, Ni and Pb on the number of nodules of soybean plants grown in biochar amended soils.

Treatment	Clay Soil		Sandy Soil	
	Number of Nodules			
	Flower Stage	Pudding Stage	Flower Stage	Pudding Stage
Control	19	14	10	8
Cd	9	5	0	0
Ni	10	4	6	4
Pb	7	5	6.5	5
B1	32	30	18	19
B1 Cd	18	16	8	16
B1 Ni	21	18	11	18
B1 Pb	17	13	9	14
B2	38	47	26	40
B2 Cd	20	27	11	22
B2 Ni	26	28	15	24
B2 Pb	19	25	12	21
L.S.D at 0.05	12.38	15.12	10.93	14.45
L.S.D at 0.01	16.78	20.49	14.82	19.57

Biochar 1 (B1)   Control   Biochar 2 (B2)

Figure 3. Root distribution of soybean plants grown in soils amended or not amended with biochar (Photos: Samir A. Haddad).

3.5. Effect of Biochar on Enzyme Activities

It is well established that enzyme activity is one of the most important indicators to monitor the effect of soil management, agricultural practices or contamination concerning soil health [43]. Enzyme activity also reflects the capacity to self-purify the soil contamination indirectly [44]. Thus, after using biochar as a soil amendment with two rates separately or in combination with selected heavy metals, the arginase and urease activities of soil samples were measured in the present study, and the results are shown in Table 9.

Table 9. Effect of Cd, Ni and Pb on arginase (AR) and urease (UR) activity of soybean plants grown in biochar amended soils.

Treatments	Clay Soil		Sandy Soil	
	Arginase (μg NH$_4$-N g^{-1} h^{-1})	Urease (μmol NH$_3$-N g^{-1} h^{-1})	Arginase (μg NH$_4$-N g^{-1} h^{-1})	Urase (μmol NH$_3$-N g^{-1} h^{-1})
Control	43	7.6	35	6.8
Cd	26	5.4	16	3.9
Ni	28	6.3	18	4.3
Pb	22	4.1	12	2.4
B1	49	12.5	43	10.2
B1 Cd	38	6.3	26	6.4
B1 Ni	40	8.8	30	5.9
B1 Pb	30	5.9	20	4.8
B2	54	15.6	48	12.5
B2 Cd	41	11.5	31	8.4
B2 Ni	46	13.2	35	9.6
B2 Pb	39	9.8	28	5.3
L.S.D at 0.05	3.57	2.66	3.95	1.68
L.S.D at 0.01	4.84	3.61	5.35	2.28

Urease plays an important role in the transformation of soil nitrogen. In our study, after 50 days from transplanting, the activity of arginase and urease was significantly ($p < 0.01$ or $p < 0.05$) increased with the low or high rate addition of corncob biochar (B1 or B2), and the B2 treatment was more effective than the B1. The arginase activity was increased by 25.5 and 37.1% in clay and sandy soil, respectively, compared to the control. For urase, the activity was increased by 105% and 83.8% in clay and sandy soil, respectively, compared to the control. This completely matches with the results obtained by Akmal et al. [22], who observed increases in enzymatic activity in greenhouse tomatoes following soil amendment with biochar, including alkaline phosphatase, urease and dehydrogenase, which led to increases in plant N, P and content. Jain et al. [45] reported an increase in the activity of enzyme (β-glucosidase) after 45 and 90 days. Biochar, through its sorption of organic and inorganic molecules, or blocking reactive places, inhibits the activity of some enzymes [10,46]. The study by Niemi et al. [47] showed that biochar had a poor effect on enzyme activity (arylsulfatase, α-glucosidase, β-glucosidase, β-xylosidase, cellobiosidase, β-N-acetyl-D-glucosaminidase, phoshodiesterase, phosphomonoesterase, alanine aminopeptidase and leucine aminopeptidase). The enzymatic activities considerably depend on organic matter, pH and the content of organic and mineral nutrients, which is related to the soil type [48].

Wang et al. [49] showed that reduction in the availability of heavy metals enhanced the growth of soil microorganisms and therefore resulted in an increase in enzyme activity per gram of soil. In this study, compared to the control, the arginase and urase activity was significantly increased for all biochar treatments, regardless of whether they were added separately or in combination with the selected heavy metals (Cd, Ni and Pb). Moreover, the addition of biochar may change the heavy metal stress level in the soil, which is another factor that could influence enzyme activity.

After the addition of biochar, it can immobilize and fix the metal cations in contaminated soil through adsorption and complexation, leading to a decrease in the bioavailability of heavy metal in contaminated soil. Meanwhile, biochar has proved to be an effective optimized soil property. Ippolito et al. [50] observed that the addition of biochar was able to subsequently reduce Cu, Cd and Zn bioavailability in soil. However, the responses of different enzymes to heavy metals have been shown to vary [51]. Based on arginase and urease activity, we can conclude that the application of biochar, in particular, corncob biochar, during the crop cultivation improved soil microbial activity and soil quality.

4. Conclusions

Microbial populations sharply decreased with the addition of the selected heavy metals in both clay and sandy soils not amended with biochar. On the other hand, on adding those heavy metals plus biochar as a soil amendment with two different rates, the amount of bacteria and fungi significantly increased in comparison with controls. the response ratios of soil F/B were positively correlated with biochar rates and growth duration. The values of the resistance index (*RS*) for selected soil microorganisms were positive throughout the experiment. However, they differed depending on the type of heavy metal applied, experimental time and soil texture. The number of bacterial nodules increased significantly, especially by using the higher rate of biochar compared to the control, either by adding biochar alone or mixed with the selected heavy metals. By adding biochar, it was possible to overcome the inhibition effect of heavy metals on the enzymatic activity (arginase and urease) under study. Adding biochar added to agricultural soils; one should consider its high variety, particularly in terms of enzyme activity. It remains a challenge for future enzyme activity studies in the soil to get a better understanding of biochar's impact on the soil environment, especially enzymatic activity.

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