

Article

Jack Bean Development in Multimetal Contaminated Soil Amended with Coffee Waste-Derived Biochars

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Abstract: Coffee waste-derived biochar was found to immobilize heavy metals in contaminated soil, although there are few studies involving these materials. Given the large amount of waste generated in the coffee industry, this presents a relevant opportunity to contribute to the circular economy and environmental sustainability. Therefore, the objective of this study was to evaluate the effects of the application of biochars derived from coffee grounds and coffee parchment in the remediation of a Cd, Zn and Pb contaminated soil and at the development of jack beans (*Canavalia ensiformis*) in this area's revegetation. The biochars were pyrolyzed at 700 °C, and the treatments were: contaminated soil (CT); contaminated soil + calcium carbonate (CaCO₃); contaminated soil + 5% (weight (*w*)/weight (*w*)) coffee ground biochar and contaminated soil + 5% (*w/w*) coffee parchment biochar. These treatments were incubated for 90 days, followed by the cultivation of jack beans for 60 days. Soil samples, soil solution and plants were analyzed for nutrients and heavy metals. The addition of coffee grounds and coffee parchment biochars significantly reduced the contents of heavy metals in the soil compared to the Control (32.13 and 42.95%, respectively, for Zn; 26.28 and 33.06%, respectively, for Cd and 28.63 and 29.67%, respectively, for Pb), all of which had a superior performance than the CaCO₃ treatment. Thus, following the observed reduction in the soil soluble fraction of metals, its uptake by the plants was also reduced, especially limiting Cd and Pb accumulation in plant dry matter. In addition, coffee parchment biochar promoted a greater accumulation of nutrients in the shoots, i.e., for K and P (1450 and 21.5 mg pot⁻¹, respectively, dry matter basis) compared to the control (54.4 and 9.3 mg pot⁻¹, respectively). Therefore, coffee parchment biochar use in association with jack beans may represent a viable tool for the remediation of metal contamination concomitantly with revegetation of the contaminated area.



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1. Introduction

Soil has been considered a waste disposal medium over the years due to its resilience under adverse conditions. However, the belief that this assimilation is infinite has contributed to soil contamination and, consequently, to other associated negative environmental impacts. Among the contaminants, heavy metals have attracted attention in recent years and have become a global concern.

Heavy metals occur naturally in the soil and may have their concentrations increased by human activities, especially from the use of pesticides and fertilizers [1] and the chemical, mining and steel industries [2]. Among heavy metals, elements, such as Zn and Cu are part of biochemical reactions and are, therefore, essential to living organisms, although they can become toxic depending on their concentrations [3]. On the other hand metals, such as Cd and Pb, are among the main contaminants of soils and have no known function in organisms, representing high toxicity for plants and animals even at low concentrations [4].

The effects of heavy metals on humans depend on the level of contamination and exposure time and can cause kidney, bone, and central nervous system problems [5]. In relation to plants, inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress are some of the direct effects caused by these metals; indirect effects could involve a reduction in beneficial microorganisms in the soil due to heavy metals toxicity, which reduces the enzyme activities necessary for plant development. These effects consequently contribute to reduced productivity and economic impacts, as well as the risk of entering the food chain [6,7].

Metal availability in the soil depends on physical, chemical, or biological processes [8]. The main factors that govern the mobility of an element in soil are pH, redox potential, organic matter content, texture, mineral composition, and the chemical form of the element [9]. Understanding the mobility and bioavailability of these elements in the soil is essential for assessing risks [10] and for consequently developing a plan for remediation. An alternative for remediation processes is the use of in situ stabilization methods, which basically consist of immobilizing the metal in the soil by adding materials (inorganic or organic) [11]. By limiting the solubility and availability of these elements to living organisms, the impact on the environment is consequently reduced, allowing the development of plants [12].

There is a range of materials that are added to contaminated soils to immobilize heavy metals, and the use of biochar in this context has been widely reported in the literature. Biochar consists of a carbonaceous byproduct from the pyrolysis process, carried out under low or no oxygen supply and variable temperatures (350–1000 °C) [13]. The characteristics of biochars are highly dependent on the pyrolysis conditions. For example, when performed at higher temperatures (500 to 700 °C) [14], it results in a material with greater porosity and consequently greater specific surface [15]. The presence of high surface areas favors the retention of organic and inorganic contaminants, reducing their mobility in contaminated soils [16,17].

Biochars produced from coffee industry residues have shown efficiency in immobilizing heavy metals [18–20], which is positive since coffee is one of the most consumed beverages on the planet, and in its entire production chain, there is a large amount of waste. For example, it is estimated that for each ton of processed coffee beans, approximately 1.1 tons of waste are generated [21]. Among them is coffee parchment, which consists of a thin endocarp film that covers the bean and is obtained by wet pulping [22]. In the soluble coffee industry, for each ton of soluble coffee produced, for example, approximately 450 kg of grounds are generated [23]. This scenario illustrates the waste generation potential, which does not have a well-defined destination but could be used as biochar. However, there are few studies involving these residues for biochar production; thus, this approach can be an economical and environmentally viable alternative and can contribute to the circular economy.

The effectiveness of the immobilization of heavy metals in the soil by the addition of biochar can be verified by measuring the development of plants in this medium. The choice of species must take into account its ability to develop in environments contaminated by metals, as well as its production of biomass, to enable the determination of metals in tissues and, therefore, evaluate the effects provided by the addition of biochar to contaminated soil. In this context, the jack bean (*Canavalia ensiformis*) has been reported in the literature as a resistant species with phytoextractor potential [24–26]. Jack bean is a shrub legume widely used as a green cover in tropical and subtropical regions around the world, adapting to almost all types of soil [27], with production between 20 to 40 tons of green mass and 4 to 8 tons of dry mass per hectare in a 120-day cycle [28]. These characteristics make it interesting to cultivate this species concomitantly with the addition of biochar to soils contaminated by heavy metals, as it provides benefits to the soil, such as the addition of organic matter and protection against erosive processes [29,30], in addition to its remedial potential.

Therefore, the objective of this work was to evaluate the effect of coffee grounds and coffee parchment biochar in the remediation of Cd, Zn and Pb contaminated soil and at the development of jack bean in the area revegetation. In view of this objective, this study starts from the hypothesis that both biochars will reduce the availability of metals in the soil, favoring the development of plants.

2. Materials and Methods

The biochars were obtained from the slow pyrolysis of coffee grounds and coffee parchment biomass at 700 °C. The complete physicochemical characterization and the detailed description of the analyses performed on the biochars can be verified in our previous studies [20,31]. Some of the main attributes of coffee grounds (GR) and coffee parchment (PCM) biochars are shown in Table 1.

Table 1. Main attributes of the coffee grounds and coffee parchment biochars.

Attribute	GR	PCM
Total Carbon (C) (%)	62.3	69.3
Organic carbon (OC) %	31.9	38.5
pH (water 1:10)	9.5	9.6
Phosphorus (P) (g kg ⁻¹)	1.6	1.5
Potassium (K) (g kg ⁻¹)	2.7	55
Calcium (Ca) (g kg ⁻¹)	40	4.6
Cation exchange capacity (CEC) (mmol _c kg ⁻¹)	34.4	275.8
Specific surface area (SSA) (m ² g ⁻¹)	45	32

The experiment was carried out in a greenhouse with samples of a loamy loam soil multicontaminated with heavy metals from a steel mill area where Zn processing was carried out, located in the municipality of Três Marias (Minas Gerais), Brazil (Coordinates: 18°12'21" S latitude and 45°14'31" W longitude). Soil samples were collected in the 0–20 cm layer, air dried and passed through a 2 mm sieve for chemical (fertility) and physical characterization (Table 2). The OC content was obtained by wet oxidation and determined by colorimetry [32]. The pH was determined in calcium chloride (CaCl₂) (0.01 mol L⁻¹) and H + Al by SMP buffer [33]; the levels of P, Ca, Mg and K were determined by an ion exchange resin [34]. The S content was extracted by calcium phosphate and determined in a spectrophotometer [35]; the B content was determined in hot water [36]. The phytoavailable levels of potentially toxic metals were extracted by DTPA at pH 7.3 [37] and determined in an inductively coupled plasma atomic emission spectrometer (ICP OES). The determination of granulometry, from which the texture of the soil was determined, was performed using the pipette method using dispersants: sodium hexametaphosphate and water [38].

Table 2. Initial characterization of the multicontaminated soil.

OC	pH	H + Al	P	Ca	Mg	K	S	B
g dm ⁻³	(CaCl ₂)	mmol _c dm ⁻³	mg dm ⁻³		mmol _c dm ⁻³		--- mg dm ⁻³ ---	
11	5.9	20	45	44	16	4	22	0.1
Zn	Cu	Mn	Ni	Cd	Pb	Sand	Silt	Clay
----- mg dm ⁻³ -----				----- % -----				
1901	46	200.6	0.1	13.1	383	24.3	50.4	25.3

The experimental design consisted of a completely randomized design, with four treatments and four replications. The treatments were contaminated soil (control); contaminated soil in which the pH was corrected with calcium carbonate (CaCO₃) to 7.0; contaminated soil + 5% (*w/w*) coffee ground biochar (GR) and contaminated soil + 5% (*w/w*) coffee parchment biochar (PCM).

Considering that biochars produced at higher temperatures (700 °C) tend to have an alkaline pH [39] and that pH is one of the main factors that influence the immobilization of metals [40], the treatment with raising the soil pH to 7 aimed to compare the effect of raising the pH on the availability of metals compared to treatments with biochars. To obtain this treatment, samples of the multicontaminated soil were mixed with a dose of CaCO₃ and previously incubated for 15 days with a humidity equivalent to 60% of the soil water-holding capacity (WHC). The dose of CaCO₃ used was determined in previous experiments. After this period, the experiment continued, in which, for each treatment, 3 kg of contaminated soil samples were added to pots (4 L) and, for treatments with biochar, doses equivalent to 5% (*w/w*) were added and homogenized. The treatments were incubated for 90 days in a greenhouse at room temperature and humidity maintained at 60% of the WHC, whose control was performed gravimetrically. There was no additional fertilization.

After incubation, soil samples were collected at each treatment and analyzed to determine the soil fertility parameters as described above. Then, 12 jack bean seeds were sown in each pot, and Rhizon-type soil solution samplers were installed. The soil solution was collected in three periods during cultivation, and 24 h before each collection, the pots were saturated with water, corresponding to 100% of the WHC.

The jack bean plants were thinned out six days after germination (DAG), leaving five plants per pot, at which time the first soil solution was collected. For this, a 60 mL syringe was attached to the sampler, and a vacuum was applied, waiting for the volume to be filled. The sampler had a porous plate at the tip (0.10 µm) so that the resulting sampled solution was ready for analysis. In the soil solutions, the following parameters were determined: pH and cations [potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), cadmium (Cd) and lead (Pb)] by ICP OES; anions (fluoride, chloride, nitrate, nitrite, phosphate and sulfate) by modular HPLC ion chromatography (Metrohm) and dissolved organic carbon (COD) in a TOC-L elemental analyzer (Shimadzu). These attributes were used to evaluate the chemical speciation using Visual MINTEQ software (Version 3.0, Sweden). After 30 days of germination, the second collection of the solution was carried out, and at 60 days, the third collection was carried out, and the plants were harvested.

After harvesting, the shoots and roots were separated. Soil samples were also collected. The shoots of the treatments were analyzed in terms of leaf area using the li-3100c Area Meter equipment, and a sample was collected for analysis by scanning electron microscopy using a Jeol—JSM 5800LV microscope, whose sampling and analysis procedure was described previously [24]. In sequence, the shoots were washed with water followed by a 1% HCl solution and distilled water. The roots were separated from the soil by sieving and washed in distilled water. Then, the samples of shoots and roots were kept in an oven at 65 °C with forced air circulation until reaching constant weight and, after drying, were weighed and ground in a Wiley mill. The ground samples (2 mm) were then subjected to digestion with 2 mL of hydrogen peroxide (H₂O₂) 30% + 3 mL of concentrated nitric acid (HNO₃) in a microwave (CEM/MARS 5 XPRESS model) according to the EPA3051a method [41] for the determination of macro- and micronutrients and potentially toxic elements in shoots and micronutrients and potentially toxic elements in roots. Soil samples were chemically characterized as described above.

The data obtained in this study were submitted to an analysis of variance ($p < 0.05$), and for those with significant treatment effects, the means were compared by Tukey's test ($p < 0.05$) using Sisvar software. v. 5.4 (Build 80) (Lavras—MG, Brazil).

3. Results and Discussion

Soil chemical composition varied as a function of sampling time and between treatments (Table 2). The highest pH values were observed in the PCM treatment, both before planting (pH 7.1) and after cutting the plants (pH 6.8). An increase in soil pH after biochar addition to soil is well reported in the literature, which occurs mainly due the presence

of hydroxides and carbonates in the biochar chemical matrix [4,12]. In general, with the exception of the PCM treatment, the other treatments resulted in pH values within the range considered adequate (6.5) [42], as increases in pH contribute to the reduction in the availability of heavy metals, micronutrients or not, through precipitation, limiting the absorption and development of plants [43].

After harvesting, a significant increase in OC was observed in the soil samples for all treatments evaluated (approximately 0.1%). This increase was related to the crops used since leguminous crops, such as jack bean, are used as cover crops and play an important role in increasing OC in the soil [44]. Jack bean roots constitute the light fraction of OC added to the soil, and when decomposed, they contribute to the increase in labile C [28], which in turn is accounted for as soil OC, explaining the increase observed after growing the plants. The addition of OC to the soil contributes to the activity of microorganisms and, consequently, to the cycling of nutrients and soil fertility. Biochars did not induce a significant effect on the OC content due to the determination methodology used, in which the wet oxidation by dichromate of organic matter mainly extracts the easily oxidizable fraction of OC.

Biochars, in turn, are mostly composed of aromatic carbon that has a high stability to decomposition [45] and, therefore, they are not accounted for in this determination, although the labile fraction of C can contribute to the increase in OC in the soil depending on the applied dose [46]. In this context, an increase in the OC content was verified in soil that received sugarcane bagasse biochar [47].

Among the nutrients that increased in the soil samples after the incubation period, we highlight the K content ($21.1 \text{ mmolc dm}^{-3}$) in the PCM treatment compared to the CT ($3.4 \text{ mmolc dm}^{-3}$) and Ca in the CaCO_3 and GR treatments (74.8 and $77.5 \text{ mmolc dm}^{-3}$, respectively) compared to $55.8 \text{ mmolc dm}^{-3}$ in the CT. This increase in Ca and K contents in the soil is related to the biochars chemical composition, with high values of K for PCM, and Ca for GR (Table 1). An increase in P and K content in soil is reported for an incubation study with maize and wheat straw biochars [48]. The presence of K and Ca in coffee waste-derived biochars results in soil fertility improvement; this has also been reported by other authors [49,50].

The micronutrient and potentially toxic element contents in the soil samples also varied significantly in relation to the collections performed and between the treatments evaluated (Table 3). Regarding the evaluation before planting, there was a significant reduction in the average phytoavailable levels of Zn, Cd, and Pb in the CaCO_3 (19.55; 10.81 and 10.2%, respectively), GR (32.13; 26.28 and 28.63%, respectively) and PCM (42.95; 33.06 and 29.67%, respectively) treatments in relation to the CT, and for Cd and Pb, there was no difference between the GR and PCM treatments. As verified for Cd, Zn, and Pb, the addition of biochars and the reduction in the mobility of heavy metals have been discussed by other authors. A reduction was observed in the available levels of Cd, Cu, Zn, and Pb by 25.8, 97.3, 62.2, and 97.9%, respectively, in fine-textured soil that received 5% rice straw biochar pyrolyzed at $500 \text{ }^\circ\text{C}$ [51]. Similar results were observed after 90 days of incubation with 5% rice straw biochar obtained at $550 \text{ }^\circ\text{C}$, with reductions of 52.4, 60.3, and 68.7% in the extractable levels of Cd, Zn, and Pb, respectively, in the soil [52]. The authors also highlighted that the increase in pH was a determining factor for obtaining these results based on the effect of precipitation. However, in this study, biochars differed in CaCO_3 content, indicating that the immobilization of metals did not occur only by the pH effect but by other mechanisms, such as complexation [53]. This is true especially in the GR treatment, in which the pH did not differ significantly from the treatment with CaCO_3 before and after planting.

Table 3. Fertility attributes, nutrients and potentially toxic elements evaluated in soil samples before planting and after jack bean plant harvesting.

Trat	Collect	OC	pH	P	K	Ca	Mg	H + Al
		g dm ⁻³	-	mg dm ⁻³		----- mmolc dm ⁻³ -----		
CT	Before planting	11.4 a (B)	6.1 c (A)	76.3 b (A)	3.4 c (A)	55.8 b (A)	22.9 a (A)	16.4 a (A)
CaCO ₃		11.0 ab (B)	6.5 b (A)	74.3 b (A)	3.5 bc A	74.8 a (A)	19.2 b (A)	12.0 b (B)
GR		10.5 b (B)	6.6 b (A)	87.4 a (A)	4.3 b A	77.5 a (B)	18.9 b (A)	11.2 b (B)
PCM		10.7 ab (B)	7.1 a (A)	86.6 a (A)	21.1 ab	42.5 c (A)	14.8 c (A)	7.5 c (B)
VC (%)		3.49	0.68	2.56	1.14	3.03	4.43	3.36
CT	After harvest	12.6 a (A)	5.8 c (B)	62.0 b (B)	3.0 b (A)	49.7 c (B)	19.3 a (B)	17.8 a (A)
CaCO ₃		12.5 a (A)	6.3 b (B)	67.0 ab (B)	1.7 c (B)	71.0 b (A)	18.3 a (A)	14.5 b (A)
GR		12.6 a (A)	6.3 b (B)	73.0 a (B)	2.3 bc (B)	90.7 a (A)	18.3 a (A)	13.0 b (A)
PCM		11.6 b (A)	6.8 a (B)	73.8 a (B)	22.0 a (A)	48.8 c (A)	16.0 b (A)	9.5 c (A)
VC (%)		3.39	2.42	7.16	7.8	8.9	5.61	10.06
		B	Cu	Fe	Mn	Zn	C d	P b
		----- mg dm ⁻³ -----						
CT	Before planting	0.15 a (B)	45.62 a (A)	36.41 a (B)	202.06 a (A)	1930.82 a (A)	13.83 a (A)	383.93 a (A)
CaCO ₃		0.15 a (B)	40.44 a (A)	30.73 a (A)	162.70 b (A)	1553.33 b (A)	12.33 b (A)	344.77 b (A)
GR		0.29 a (B)	32.55 b (A)	27.05 a (A)	133.82 c (A)	1310.44 c (A)	10.19 c (A)	274.01 c (B)
PCM		0.78 a (A)	27.13 b (A)	24.12 a (A)	115.88 d (A)	1101.56 d (A)	9.26 c (A)	270.03 c (A)
VC(%)		10.58	7.91	21.64	2.25	5.12	4.88	4.33
CT	After harvest	0.3 b (A)	45.2 a (A)	45.17 a (A)	181.12 a (B)	1882.80 a (A)	13.61 a (A)	378.12 a (A)
CaCO ₃		0.3 b (A)	41.02 ab (A)	31.38 b (A)	157.21 b (A)	1570.24 b (A)	12.53 b (A)	338.01 b (A)
GR		0.4 ab (A)	36.24 b (A)	34.31 b (A)	140.38 c (A)	1390.00 c (A)	10.61 c (A)	294.56 c (A)
PCM		0.6 a (B)	28.88 c (A)	17.67 c (A)	119.40 d (A)	1198.79 d (A)	9.46 d (A)	285.57 c (A)
VC (%)		25.7	7.36	11.4	3.7	5.47	4.39	3.81

Trat = Treatment. CT = Control, GR = Coffee grounds biochar, PCM = Coffee parchment biochar. Numbers followed by the same letter are not different ($p \leq 0.05$) ($n = 4$). Small letters compare treatments in each collection, and capital letters in the parentheses compare the collections for each treatment. VC (%) = variation coefficient.

After cultivation and harvesting, the concentration of macronutrients in the soil samples was generally reduced in relation to the values before planting, which is consistent with plant uptake. For heavy metals, no reduction in content was observed after cultivation in relation to the post incubation values, indicating that the elements were not available for absorption, although the observed contents were extracted by a DTPA solution that simulates phytoavailable levels. The availability reduction in heavy metals in the soil due to the use of biochar is strongly supported by the literature. For example, a reduction of 85 and 63% for Cr and Cd contents in a contaminated soil due sugar-cane bagasse biochar application is reported [54]. In another study [55], an application of 5% tobacco stalk biochar significantly decreased the DTPA-extractable Cd and Pb by 10.4 and 13.6%, respectively. In this work, the results for the soluble fraction corroborate these results (Table 4), in which it was verified that only Zn remained readily available in the three collections, which is explained by its high initial content in the soil sample (Table 2). The other elements (Pb, Cu, Fe and Mn) were below the quantification limit (0.05, 0.05, 0.1, and 0.1 mg L⁻¹, respectively) during the entire period.

Table 4. Attributes analyzed in the soil solution for the treatments evaluated.

Trat	Collect	pH	DOC	Cations				
				K ⁺	Ca ²⁺	Mg ²⁺	Zn ²⁺	Cd ²⁺
----- mg L ⁻¹ -----								
CT	1 ^a (6 days)	6.7 c (A)	12.5 bc (A)	20.7 b (A)	159.6 c (A)	97.8 b (A)	50.1 a (A)	0.2 a
CaCO ₃		7.1 b (B)	22.2 b (A)	23.9 b (A)	391.2 a (A)	112.5 a (A)	8.9 b (A)	0.1 b
GR		7.3 b (B)	11.6 c (A)	32.6 b (A)	218.1 b (A)	85.3 c (A)	6.2 c (A)	<0.01
PCM		8.6 a (AB)	83.0 a (B)	1094 a (A)	28.6 d (A)	10.1 d (A)	0.2 d (A)	<0.01
VC (%)		0.97	4.9	15.2	22.61	11.86	6.7	14.81
CT	2 ^a (30 days)	6.5 c (A)	8.7 b (A)	8.0 b (A)	30.7 b (B)	13.5 a (B)	4.4 a (C)	<0.01
CaCO ₃		7.4 b (A)	15.3 b (A)	9.6 b (A)	82.2 a (B)	15.5 a (A)	1.5 b (C)	<0.01
GR		7.4 b (AB)	8.5 b (A)	6.2 b (A)	49.4 ab (B)	12.3 ab (A)	1.4 b (B)	<0.01
PCM		8.7 a (A)	109.1 a (A)	354.2 a (B)	5.6 b (A)	2.6 b (A)	0.3 b (A)	<0.01
VC (%)		1.31	23.7	35.02	8.73	18.38	16.17	-
CT	3 ^a (60 days)	6.6 c (AB)	10.5 b (A)	7.3 a (A)	34.6 ab (B)	18.1 a (B)	7.3 a (B)	<0.01
CaCO ₃		7.4 b (A)	12.8 b (A)	4.3 a (A)	26.4 ab (C)	6.9 b (A)	2.7 b (B)	<0.01
GR		7.5 b (A)	8.6 b (A)	6.1 a (A)	58.4 a (B)	19.5 a (A)	0.9 c (B)	<0.01
PCM		8.5 a (B)	38.3 a (C)	14.3 a (C)	5.2 b (A)	2.2 b (A)	0.5 c (A)	<0.01
VC (%)		2.36	24.59	16.33	13.92	15.31	11.55	-

Trat = Treatment. CT = Control, GR = Coffee grounds biochar, and PCM = Coffee parchment biochar. Numbers followed by the same letter are not different ($p \leq 0.05$) ($n = 4$). Small letters compare treatments in each collection, and capital letters in the parentheses compare the collections for each treatment. < = Lower than the equipment quantification limit. VC (%) = variation coefficient.

For the biochars, there was a significant reduction in Zn in relation to the CT in the soil solution, and in the PCM, this rate was 99.6%, which was directly proportional to the increase in pH. In an experiment that evaluated the effect of wood biochar pyrolyzed at 500 °C on the mobility of metals in soil from mining areas, a significant reduction was observed in the content of Zn and Pb in the soil solution, with doses of 2% and 5% biochar [56]. In addition, in the PCM treatment, Zn in solution remained associated with DOC, and the values reached close to 100% in the three collections (Figure 1), which is also related to the higher DOC content in this treatment. An increase in DOC content in the soil was also verified in a field experiment after 2 years with the application of sugarcane bagasse biochar [57].

Regarding the CaCO₃ and GR treatments, there was no significant difference in relation to pH; however, there was a significant reduction in the Zn content in the first and third collections (8.9 and 7.2 mg L⁻¹ and 2.7 and 0.9 mg L⁻¹ in the CaCO₃ and GR treatments, respectively), which once again clearly illustrates that the immobilization of metals did not occur only by the pH effect but by the sorption mechanisms of biochars [20]. In the GR treatment, the fraction of Zn complexed to DOC increased along the collections, reaching a value close to 25%. In the other treatments, the highest percentage of this element was in the form of free Zn²⁺ in solution. For Cd, only in the first collection of the solution performed in the CT and CaCO₃ treatments was soluble content obtained (Table 4) and mainly in the free form Cd²⁺ (Figure 1), which is the predominant species of this metal in solution up to pH 8 [58] and could be absorbed by plants. For treatments with biochar, soluble Cd was not observed in any of the collections. A reduction in Cd, Pb and Zn (29, 55 and 79%, respectively) in the soluble fraction in mining soil by the effect of wood biochar produced at 700 °C was also reported [59].

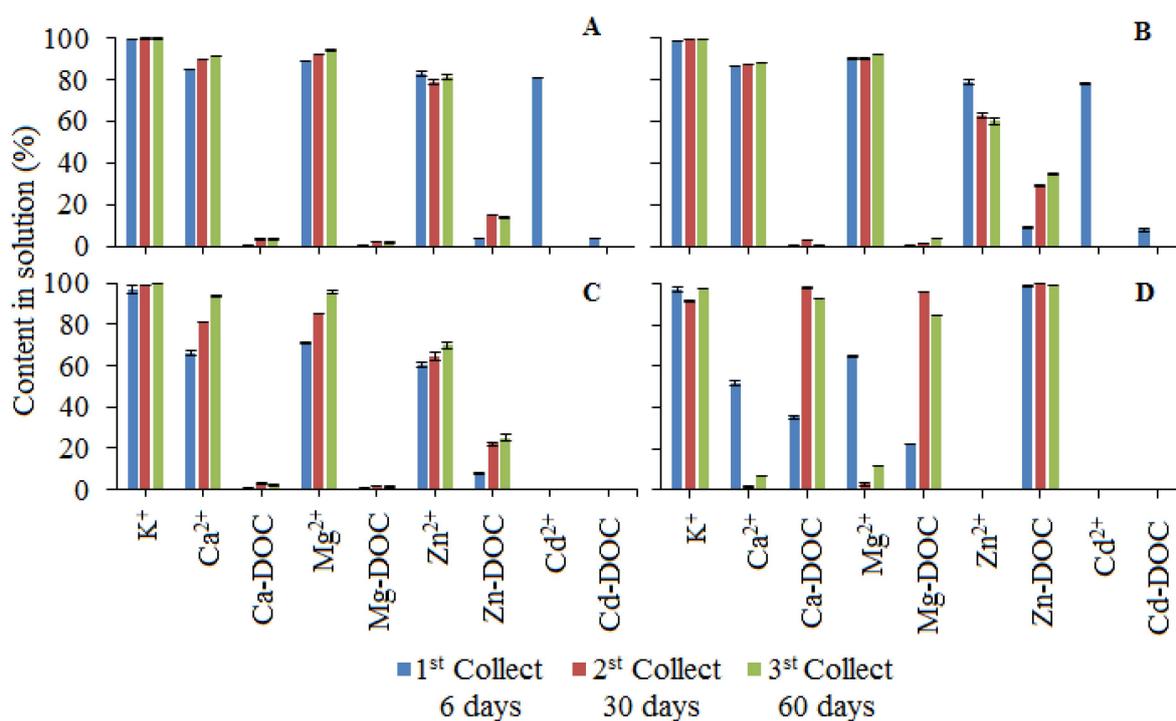


Figure 1. Distribution of the main cationic species and compounds obtained in the soil solution collected in the CT (A), CaCO₃ (B), GR (C) and PCM (D) treatments during 60 days of jack bean cultivation. CT = Control, GR = Coffee grounds biochar, and PCM = Coffee parchment biochar. Error bars indicate de standard deviation (n = 4).

The content reduction of heavy metals in the soil samples and the greater availability of nutrients in the treatments with biochar previously reported contributed to the greater development of the jack bean plants in relation to the CT (Figure 2), verified by the dry mass and leaf area (Figure 3). The lower development of the plants in the CT treatment compared to the other treatments was probably related to the oxidative stress provided by the toxicity of the metals, in which there was a release of reactive oxygen species, such as superoxide (O₂⁻) and hydrogen peroxide (H₂O₂), which are highly toxic and aggressive to plant metabolism, limiting their development [60].



Figure 2. Jack bean plants in the CT, CaCO₃, GR and PCM treatments at harvest day. CT = Control, GR = Coffee grounds biochar, and PCM = Coffee parchment biochar.

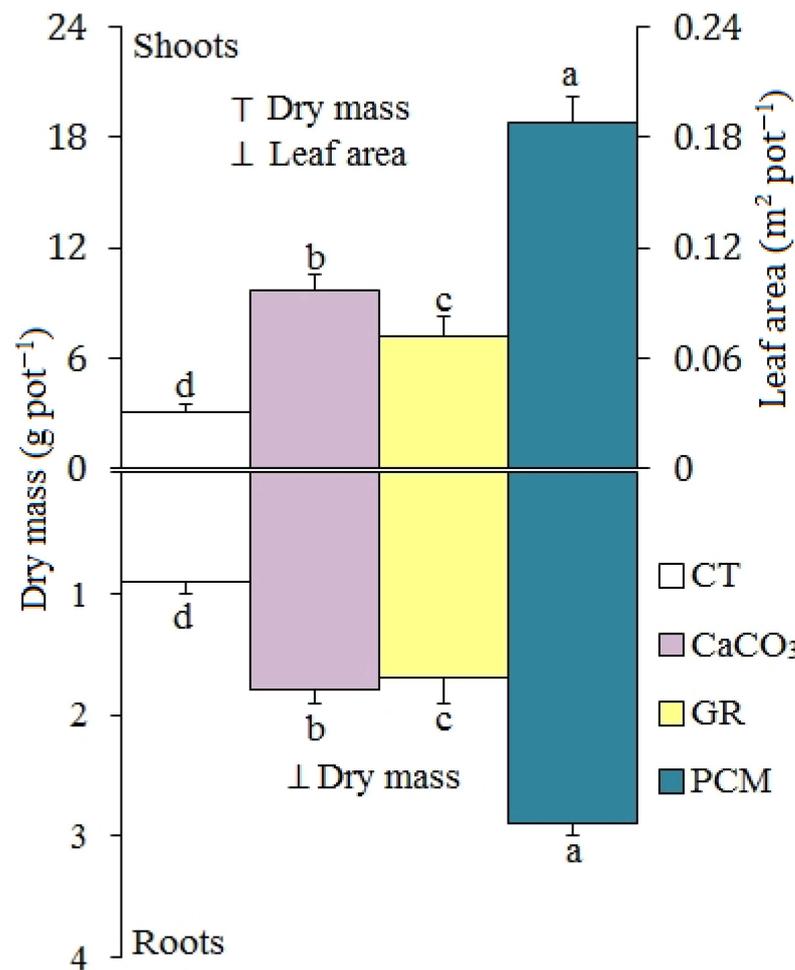


Figure 3. Jack bean dry mass and leaf area obtained in the treatments. CT = Control, GR = Coffee grounds biochar, and PCM = Coffee parchment biochar. Columns with the same letter are not different ($p \leq 0.05$). Error bars indicate de standard deviation ($n = 4$).

In this work, the PCM treatment provided the highest value of dry mass for both shoots and roots (18.8 and 2.92 g, respectively), while the lowest values were observed in the CT (3.13 and 0.92 g, respectively). It is noteworthy that the greater development of plants in the PCM was due to the improvement in soil fertility, corroborated by the increase in the levels of P and K and reduction in H + Al, as well as the reduction in the content of the metals Zn, Cd and Pb in the samples of soil (Table 3) and, consequently, the phytotoxicity.

The CaCO₃ treatment provided higher dry mass in the shoots in relation to the GR treatment, but there was no difference between the values obtained in the roots. Metal toxicity may have contributed to the alterations in the transport vessels observed in the CT (Figure 4A), whose internal structures indicated less homogeneity. A reduction in the number of conductive bundles and a thickening of the central vein of willow leaves grown in soil with the addition of zinc tailings is reported [61].

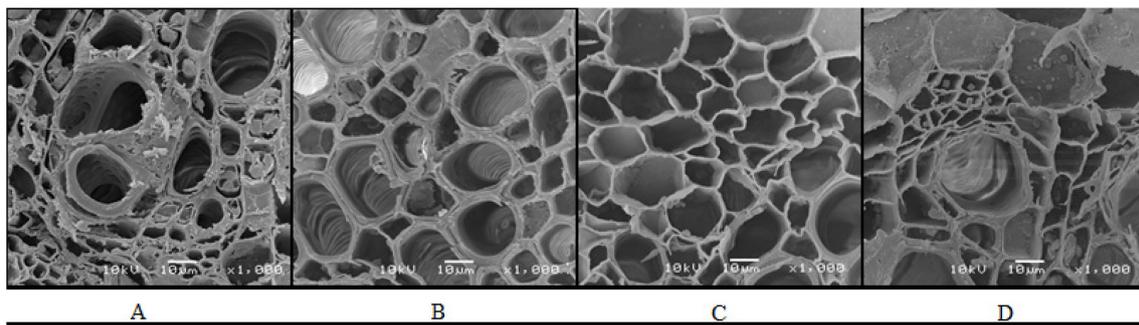


Figure 4. Images zoomed at 1000× of the inner interveinal structure of the leaves in the CT (A), CaCO₃ (B), GR (C), and PCM (D) treatments. CT = Control, GR = Coffee grounds biochar, and PCM = Coffee parchment biochar.

The effect of treatments and the difference in the development of plants grown in soil multicontaminated by heavy metals were reflected in the levels accumulated in the shoots and roots (Table 5), corroborating the levels previously observed in soil samples and in solution collections. Among the macronutrients, for example, the highest content of K accumulated in the shoots was obtained in the PCM treatment (1450 mg pot⁻¹), while in the CaCO₃ treatments, Ca and Mg had the highest levels (273.4 and 48 mg pot⁻¹, respectively), which is consistent since CaCO₃ is a source of Ca and was incubated before planting.

Table 5. Nutrient contents and potentially toxic elements accumulated in the shoots and roots of the plants as a function of the treatments evaluated.

Trat	Plant part	K	P					Mg
			-----mg pot ⁻¹ -----					
CT	Shoot	54.41 c	9.25 c	14.42 b	15.09 c	6.5 c		
CaCO ₃		216.68 b	11.82 b c	64.3 a	273.43 a	48.0 a		
GR		188.39 b	14.38 b	61.62 a	146.13 b	33.23 b		
PCM		1450.91 a	21.52 a	62.45 a	151.28 b	29.85 b		
VC (%)		11.39	13.53	8.27	7.66	8.45		
		B	Mn	Fe	Cu	Zn	Cd	Pb
		-----mg pot ⁻¹ -----						
CT	Shoot	0.03 d (A)	0.07 d (A)	0.15 c (B)	<0.01	3.89 c (A)	0.01 c (A)	0.01 a (B)
CaCO ₃		0.13 c (A)	0.44 b (A)	0.46 b (B)	<0.01	7.11 b (A)	0.07 b (B)	0.01 a (B)
GR		0.25 b (A)	0.19 c (A)	0.34 b (A)	<0.01	8.0 a b (A)	0.06 b (A)	0.01 a (B)
PCM		0.57 a (A)	1.01 a (A)	0.89 a (A)	<0.01	8.81 a (A)	0.11 a (A)	0.02 a (B)
VC (%)		10.17	16.78	10.67	-	7.79	10.62	40.57
CT	Root	0.01 a (A)	0.01 a (A)	0.34 b (A)	0.04 c	3.35 b (A)	0.01 c (A)	0.06 c (A)
CaCO ₃		0.02 a (B)	0.04 a (B)	0.73 a (A)	0.05 b	5.8 a (B)	0.06 a (B)	0.11 b (A)
GR		0.03 a (B)	0.03 a (B)	0.02 c (B)	0.06 ab	3.58 b (B)	0.04 b (B)	0.13 b (A)
PCM		0.03 a (B)	0.08 a (B)	0.03 c (B)	0.06 a	5.07 a (B)	0.05 a (B)	0.20 a (A)
VC (%)		14.89	12.5	35.75	14.36	12.62	25.13	13.82

Trat = Treatment. CT = Control, GR = Coffee grounds biochar, and PCM = Coffee parchment biochar. Numbers followed by the same letter are not different ($p \leq 0.05$) ($n = 4$). Small letters compare the treatments in each plant part (shoot or root). Capital letters in the parentheses the elemental contents between shoots and roots for each treatment. < = Lower than the equipment quantification limit. VC (%) = variation coefficient.

The accumulated content of micronutrients and potentially toxic elements in the shoots and roots varied according to the treatment (Table 5). The highest accumulated values of B, Mn and Fe in the shoots were observed in the PCM treatment (0.57, 1.01 and 0.89 mg pot⁻¹, respectively), which is explained by the higher values of dry mass. Regarding Cu, it was not possible to detect amounts accumulated in the leaves; however, significant levels could be observed in the roots, with the lowest value observed in the CT.

Zn, as a potentially toxic metal, was present in greater quantity in the original samples of the contaminated soil (Table 2), which consequently contributed to its greater absorption by jack bean plants compared to the other microelements and the accumulated content (Table 5) in the roots and shoots. For shoots, treatments with biochar resulted in the highest accumulated values as a function of phytoavailable contents (8.81 and 8.0 mg pot⁻¹ in the PCM and GR treatments, respectively) compared to the CT (3.90 mg pot⁻¹). Similar results were observed for the roots, in which there was a lower value in the CT treatment in relation to the PCM. An accumulation of Cd and Pb in sunflower plants in contaminated soil that received 5% lychee biochar obtained at 500 °C was observed [62]. On the other hand, significant reductions in Zn, Cd, and Pb uptake by cabbage plants due to the addition of limestone and biochar in contaminated soil is reported [63].

For Cd, the PCM treatment provided the highest accumulation in the shoots in relation to the other treatments, with the same result observed for Pb in the roots. The highest accumulations verified in the PCM treatment were again related to the higher dry mass obtained for this treatment in relation to the other treatments, especially when compared to the CT. The tendency to accumulate metals in the roots is related to plant defense mechanisms and where they are complexed or stored in the vacuole, protecting the leaves and maintaining the active metabolism of the plants [64]. Even so, there is a greater translocation of Cd and Zn to the aerial part of the plants compared to Pb, which is consistent with the mobility of Zn and Cd inside the plant and the strong retention of Pb in the roots [65]. Similar results as a function of the translocation index (TI) calculated for Zn, Cd and Pb in cultivated jack bean plants is reported in a study [24], in which the authors observed values of 74, 37 and 90%, respectively. It is important to mention that even with the accumulation of Cd and Zn observed in the biochars, consequently provided by the higher dry masses, the absorbed values were much lower than the original soil contamination level. Nevertheless, although phytoextraction was not the main mechanism for reducing soil contamination, the use of biochars simultaneously with the cultivation of jack bean plants contributed to the improvement of soil quality compared to the initial conditions and the treatments without biochar, evidencing the potential of this species for the revegetation of areas contaminated by metals.

4. Conclusions

The results of this study confirmed the initial hypothesis, wherein the addition of coffee grounds and coffee parchment biochars significantly reduced the available heavy metal content in the soil compared to the Control (32.13 and 42.95%, respectively, for Zn; 26.28 and 33.06%, respectively, for Cd and 28.63 and 29.67%, respectively, for Pb). In addition, both biochars were superior compared to the treatment with CaCO₃ in heavy metal reduction, concluding that immobilization did not occur only by the pH effect but by other mechanisms, such as complexation.

The treatments with biochar also contributed to an improvement in soil fertility and the development of jack bean plants, especially coffee parchment biochar, in which the plants showed higher dry mass and accumulation of macronutrients.

The association between coffee parchment biochar and jack bean is a viable alternative for the remediation of soils contaminated by heavy metals, as it simultaneously favors metal immobilization and revegetation of the contaminated area, contributing to the improvement of soil quality.

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