

Effect of biochar amendments on the growth and development of ‘Vera’ crisp lettuce in four soils contaminated with cadmium

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ABSTRACT

Cadmium is an extremely toxic heavy metal that affects agricultural lands, concentrations above 0.2 mg kg⁻¹ in leafy vegetables is restricted for human consumption. Biochar is a promising amendment for degraded soils with low fertility and high levels of heavy metals contamination, since it can reduce Cd²⁺ levels in vegetables. However, these reductions depend on the nature of the soil and biochar properties. This pot experiment in greenhouse conditions evaluated the effect of biochars amendments under Cd stress conditions on lettuce (*Lactuca sativa* L. var. *crispa* L.) plants grown in four soils: Ferralsol (FS), Andosol (AS), Umbrisol (US) and Technosol (TS). Six treatments were compared: Biochars doses at 3, 6, 9 and 12 t ha⁻¹, conventional fertilization (NPK) and control absolute. Biochars were obtained by slow pyrolysis from five agricultural residues: Palm empty fruit bunches (PEFBB), palm kernel (PKB), wood waste (WWB), coffee pulp (CPB) and rose stems (RSB). The CPB amendment resulted in a fresh weight plant increase of 238% to 323% in 3 to 12 t ha⁻¹ comparing with control treatment, while WWB induced a fresh weight decrease of 90.85%. The PKB amendment presented a 20% additional fresh weight in comparison to PEFBB in Ferralsol. These results indicate that biochar positively contributes to the water status. Application of RSB at 12 t ha⁻¹ resulted in a decrease of 50.61% in Cd concentration and a decrease of 37.4% in Cd concentration in CPB at a 12 t ha⁻¹, both results representing promising alternatives to remediate contaminated soils. The AS+CPB and US+RSB assays showed a significant negative correlation of leaf Cd concentration when increasing biochar doses were used indicating the mitigation of the phytotoxic effect of Cd in plants.

Key words: Biochar, cadmium phytoabsorption, cadmium stress, *Lactuca sativa*, slow pyrolysis, soil remediation.

INTRODUCTION

Cadmium (Cd²⁺) is a highly mobile and extremely toxic metal that affects agricultural lands specially in acid soils; its origin is linked to parent material (Cd concentrations of 0.01 to 2.6 mg kg⁻¹ have been reported in sedimentary rocks, 0.07 to 0.25 mg kg⁻¹ in igneous rocks and 0.11 to 1.0 mg kg⁻¹ in metamorphic rocks), and anthropic activities such as a municipal solid waste disposal, metal industry, phosphate fertilizers and mining (Kubier et al., 2019). Accumulation of this metal in plant biomass can promote the emergence of serious chronic diseases (itai-itai disease, lung cancer, gastrointestinal cancer, kidney damage) and damage to organs and tissues of organisms that consume it (Liu et al., 2018).

Biochar is a fine, C-rich compound with high amount of essential nutrients and high-water holding capacity. Numerous investigations have demonstrated biochar can contribute to improve nutrient availability, soil fertility and efficient water use, increase crop growth and yield and heavy metal sequestration (Agegnehu et al., 2017). Biochar is obtained by the

pyrolysis of organic materials at temperatures between 300 and 1000 °C under conditions of hypoxia or anoxia (Gao et al., 2020), such as animal waste and crop residues (ashes, bones, biomass waste and manure) (Glaser and Birk, 2012). The use of biochar as an amendment holds promise for remediating soils contaminated with heavy metals including Cd, specifically biochar obtained from sources such as bamboo, hardwood, wood, cotton stalks, eucalyptus, orchard prune residue, chicken manure, green waste, rice straw, and quail litter which have significantly reduce Cd bioavailability (Zhang et al., 2013). Nevertheless, in Cd polluted soils the use of biochar from oil palm residues, coffee pulp and rose stem, has not been reported in greenhouse experiments, which may represent an agroindustrial waste reuse option in agricultural fields where these materials are abundant.

In Cundinamarca, Colombia, Cd levels of 20.9 mg kg⁻¹ that affect specially cocoa crops in this region have been reported (Sandoval-Pineda et al., 2020). Some studies have reported immobilization and mitigation of Cd using biochar in neutral pH soils (Woldetsadik et al., 2016; Zheng et al., 2017). However, few studies have been carried out to mitigate Cd levels in contaminated tropical soils, but these few ones have shown promising results; for example, Ferralsols and Regosols amended with sugarcane straw showed potential to reduce Cd pollution in soils (Melo et al., 2016). In Colombia's case, investigations addressing heavy metals in agricultural production are even scarcer (Mahecha-Pulido et al., 2017) and no study has been carried out in Colombia to remediate Cd contamination in soils with biochars from agro-industrial waste.

Plant uptake of Cd represents a threat to food systems, significant decreased growth and development in lettuce has been reported when exposed to Cd²⁺ (Yazdi et al., 2019). Lettuce exhibits pronounced sensitivity to Cd²⁺ exposure due to its high phytoabsorption and translocation rates, which is usually displayed in noticeable symptoms; as a result, it is usually used as a bioindicator in studies of Cd²⁺ pollution (Tang et al., 2016). This research aims to evaluate the effect of biochar amendments under soils contaminated with Cd on 'Vera' lettuce plants (*Lactuca sativa* L. var. *crispa* L.) grown in four orders of tropical soils (Ferralsols, Andosols, Umbrisols and Technosols).

MATERIALS AND METHODS

Soils

The pots experiments were conducted under greenhouse conditions using soils sampled from the first 30 cm in four Colombian agricultural production systems: The Andosol soil (AS) comes from a coffee plantation located in Chaparral (3°49'50.5'' N, 75°34'01.7'' W; 1882 m a.s.l.), Tolima; Ferralsol soil (FS) comes from an oil palm plantation located in San Carlos de Guaroa (3°49'10.56'' N, 73°22'17'' W; 269 m a.s.l.), Meta; Technosol soil (TS) comes from a grazing field of bovine cattle at the campus of National University of Colombia, Cundinamarca (4°38'17.2'' N, 74°05'19.8'' W, 2556 m a.s.l.), Bogotá; and Umbrisol soil (US) comes from a commercial rose greenhouse in the municipality of Madrid (4°44'38.12'' N, 74°15'11.768'' W; 2558 m a.s.l.), Cundinamarca. The soils of the study were identified according to the FAO classification system. The properties of the soils utilized in the experiments vary, among other, in terms of texture, organic C contents, effective cation exchange capacity and nutrient contents (Table 1).

Table 1. Physicochemical properties of soils used in biochar experiments.

Texture	Ferralsol (FS) Loamy sandy	Andosol (AS) Loamy sandy	Technosol (TS) Clayey	Umbrisol (US) Silty clay loam
Bulk density, g cm ⁻³	1.29	0.90	1.3	1.0
Sand (> 0.05 mm), %	52.90	18.60	5.0	68.0
Loam (0.05-0.002 mm), %	24.70	15.40	12.0	8.0
Clay (< 0.002 mm), %	15.70	66.00	83.0	24.0
pH	4.70	4.83	5.8	6.0
C organic, %	2.69	5.13	11.5	7.7
N, %	-	0.44	1.0	Nd
Ca, cmol ₍₊₎ kg ⁻¹	1.40	5.81	20.9	6.9
Mg, cmol ₍₊₎ kg ⁻¹	0.62	1.60	4.3	3.7
K, cmol ₍₊₎ kg ⁻¹	0.25	1.20	3.6	2.1
Na, cmol ₍₊₎ kg ⁻¹	0.06	0.03	0.1	-
Al, cmol ₍₊₎ kg ⁻¹	1.97	0.29	-	0.0
ECEC, cmol ₍₊₎ kg ⁻¹	4.30	8.92	29.0	12.71
P, mg kg ⁻¹	27.80	7.59	94.6	50.0

ECEC: Effective cation exchange capacity.

Biochar production and characterization

Biomass from agro-industrial residues generated by crops nearby to soil sampling locations, corresponds to: Palm empty fruit bunches (PEFBB), palm kernel (PKB), tree pruning (WWB), coffee pulp (CPB) and rose stems (RSB). These materials were subject of a slow pyrolysis process in a rotary kiln (7 m long and 0.6 m diameter), from an initial temperature of 200 °C with a heating rate of 5.5 °C s⁻¹ until 450 °C and a residence time of 45 min; each resulting biochar was sieved to obtain particles smaller than 2 mm prior to its use as agricultural amendments; physicochemical properties of each biochar are presented in Table 2.

Plant material and pots establishment

The experiment was carried out in the greenhouse facilities at Faculty of Agricultural Sciences, National University of Colombia, Bogotá (4°38'11.89" N, 74°05'17.65" W; 2656 m a.s.l.), with a mean temperature of 19.2 °C. Five pot experiments were established using a completely randomized design (CRD). The soils were collected from locations where agro-industry waste was produced and utilized for biochar production according to Table 3. Pots were filled with 2 kg air-dried soil samples that were spiked with 10 mg CdCl₂ kg⁻¹ (for a final Cd soil concentration of 5 mg CdCl₂ kg⁻¹) and left to react during 1 wk. Thus, four biochar doses (3, 6, 9 and 12 t ha⁻¹), fertilization treatment (NPK) were added to pots and an absolute control treatment for each experiment without addition of amendment or fertilizer. A week later, 'Vera' lettuce (*Lactuca sativa* L. var. *crispa* L.) seedlings (~ 30 d of emergence and four true leaves) were transplanted to the individual pots. A drip irrigation system (uniformity coefficient of 89.3%) was used to supply approximately 384 mL per plant each week.

Variables

The number and length of leaves were measured every 15 d. Canopy of the plants was estimated using photographs taken 60 cm above the growing media's surface, according to the methodology used by Gheshm and Brown (2018) and processed with the ImageJ software (U.S. National Institutes of Health, Bethesda, Maryland, USA). Relative chlorophyll content was obtained with a chlorophyll content meter (CCM-200, ADC BioScientific, Hoddesdon, UK). Stomatal conductance was measured on the fifth leaf from 08:00 to 11:00 h, utilizing a SC-1 sheet porometer (Decagon Devices,

Table 2. Physicochemical properties of biochars used in experiments.

Biomass	PKB	PEFBB	RSB	WWB	CPB
pH	7.62	10.19	10.4	8.57	9.75
EC, dS m ⁻¹	0.63	0.56	1.66	0.1	0.39
CEC, cmol(+) kg ⁻¹	3.05	28.34	39.13	16.14	41.13
C:N ratio	50.10	26.28	22.06	64.68	17.11
Water holding capacity, %	7.5	85.83	77.50	26.25	45.00
Porosity, %	52.46	80.42	81.65	73.54	73.37

PKB: Palm kernel biochar; PEFBB: palm empty fruit bunches; RSB: rose stem biochar; WWB: wood waste biochar; CPB: coffee pulp biochar; EC: electrical conductivity; CEC: cation exchange capacity.

Table 3. Description of the five experiments conducted utilizing four different soil types and five biochars used as an amendment.

Experiment: Soil type + Biochar				
AS+CPB	FS+PKB	FS+PEFBB	TS+WWB	US+RSB
0 t ha ⁻¹ CPB without Cd ⁺²	0 t ha ⁻¹ PKB without Cd ⁺²	0 t ha ⁻¹ PEFBB without Cd ⁺²	0 t ha ⁻¹ WWB without Cd ⁺²	0 t ha ⁻¹ RSB without Cd ⁺²
3 t ha ⁻¹ CPB	3 t ha ⁻¹ PKB	3 t ha ⁻¹ PEFBB	3 t ha ⁻¹ WWB	3 t ha ⁻¹ RSB
6 t ha ⁻¹ CPB	6 t ha ⁻¹ PKB	6 t ha ⁻¹ PEFBB	6 t ha ⁻¹ WWB	6 t ha ⁻¹ RSB
9 t ha ⁻¹ CPB	9 t ha ⁻¹ PKB	9 t ha ⁻¹ PEFBB	9 t ha ⁻¹ WWB	9 t ha ⁻¹ RSB
12 t ha ⁻¹ CPB	12 t ha ⁻¹ PKB	12 t ha ⁻¹ PEFBB	12 t ha ⁻¹ WWB	12 t ha ⁻¹ RSB
Conventional fertilization	Conventional fertilization	Conventional fertilization	Conventional fertilization	Conventional fertilization

AS: Andosol; FS: Ferralsol; TS: Technosol; US: Umbrisol; CPB: coffee pulp biochar; PKB: palm kernel biochar; PEFBB: palm empty fruit bunches; WWB: wood waste biochar; RSB: rose stem biochar.

Pullman, Washington, USA). Finally, whole plants were harvested at 60 d after transplant (dat) for root length, fresh weight and dry weight measurement; leaf weight ratio (LWR) was determined with the following formula:

$$\text{LWR} = (\text{Dry weight leaves} / \text{Total dry weight}) \times 100 \quad (1)$$

Total Cd content

Total Cd content was measured in foliar tissues of plants that reached the commercial harvest point (> 28 leaves and > 60 g) using the acid digestion method, using an atomic absorption spectrophotometer (UNICAM 969 AA) set at a wavelength of 228.8 nm.

Experimental design and statistical analysis

Five completely randomized design (CRD) experiments were established for each soil with biochar amendments (Table 3). Six treatments were evaluated, consisting of four different biochar doses (3, 6, 9 and 12 t ha⁻¹), a conventional fertilization (NPK) control and an absolute control with four replicates per treatment, for 24 experimental units per experiment and a total of 120 pots evaluated. Data that did not fulfill statistical normality assumptions was transformed with logarithmic transformation and box cox transformation. Data was subjected to ANOVA and multiple Tukey comparisons test (p value < 0.05) in search of differences between the treatments using the statistical RStudio software (RStudio, Boston, Massachusetts, USA).

RESULTS AND DISCUSSION

Effect on lettuce growth in the AS+CPB experiment

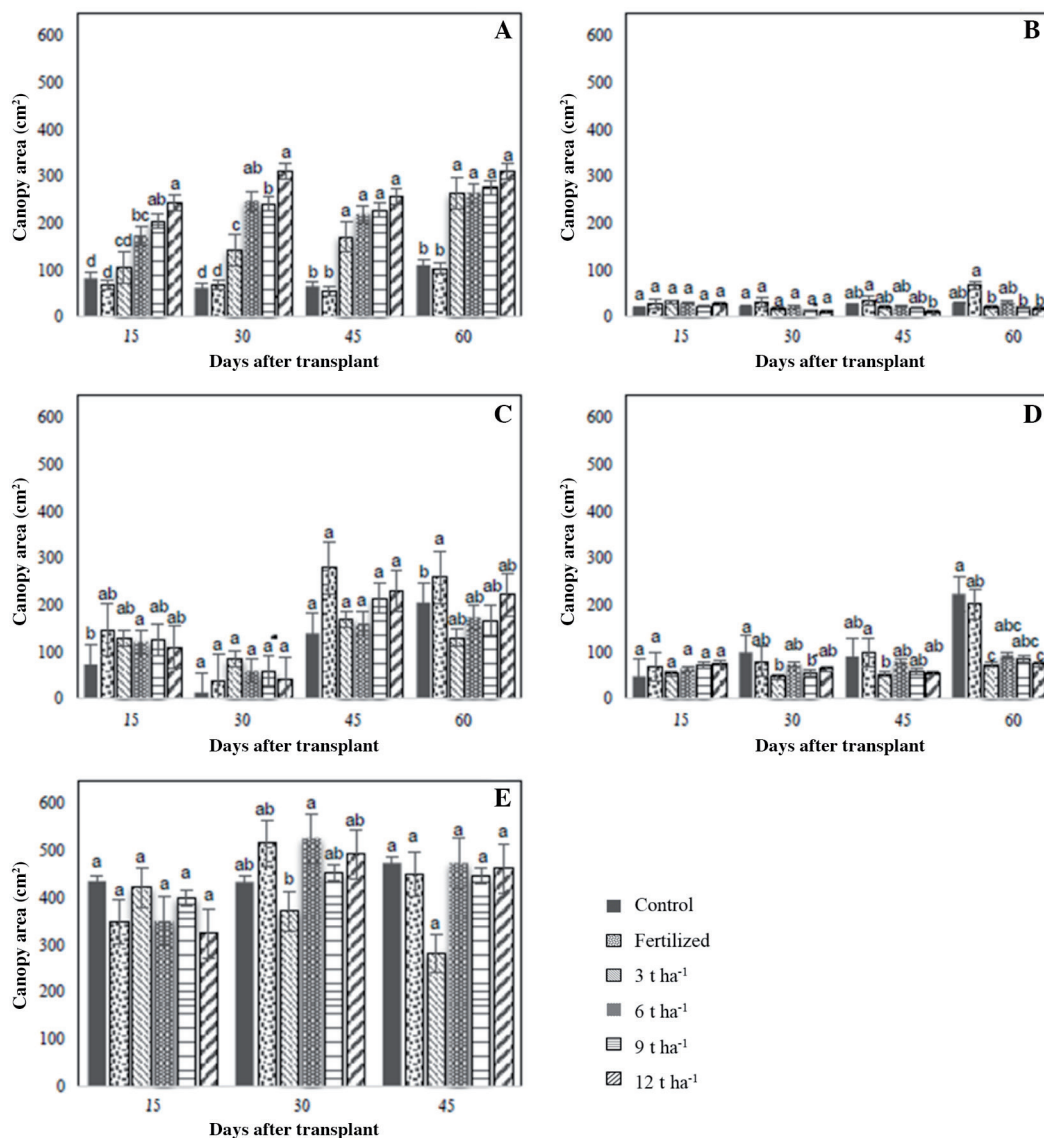
ANOVA results indicate that the AS+CPB experiment values of canopy area (Figure 1), root length, dry and fresh weights of root and leaves of lettuce plants (Table 4) are significantly different ($P < 0.05$) in lettuce plants grown in coffee pulp biochar vs. the absolute control. The biochar amendment increased the fresh weight 238% at 3 t ha⁻¹ to 323% at 12 t ha⁻¹ with respect to NPK fertilization and control with similar increments. A previous study has reported that coffee biochar contains N, P, Fe, Ca and K, and has the capacity to retain P and Mg ions, both characteristics which significantly contribute to the nutrition of plants grown in such amendment; in addition, other properties observed in this type of biochar are desirable for soil amendments, such as a basic pH (9.9), water holding capacity (4.5% to 35.0%), electrical conductivity (EC) less than 2 dS m⁻¹, and a 28 C:N ratio (Tangmankongworakoon, 2019). Our results showed similar values as it can be seen in Table 2. Also, we observed a high cation exchange capacity (CEC, 41.13 cmol₍₊₎ kg⁻¹) in CPB which plausibly changed edaphic conditions of AS and may have improved plant growth and possibly allowed other cations reduce Cd⁺² uptake.

Previously, Lima et al. (2018) found that the fresh weight of corn plants was similar between treatments of coffee husk biochar and conventional fertilization in sandy soils. According to the authors these results are related to the high specific surface of the biochar that allows water, N and P to be retained, a tendency to increase the pH, and to the presence of ashes that provide nutrients such as K; thus, the greater plant biomass obtained in this experiment may suggest that the use of CPB and its properties in AS positively influenced some soil characteristics improving plant nutrition and general growing conditions.

Effect on lettuce growth in FS+PKB and FS+PEFBB experiments

Plants grown in soil using palm empty fruit bunches (PEFBB) and palm kernel (PKB) biochars, obtained from agro-industrial waste over the Ferralsol, did not achieve adequate growth as they did not reach commercial point; we associated these poor results due to the soil properties such as low fertility, low organic matter, low pH value and exchange acidity present in Ferralsol soils (Table 1); this might have affected the biochar amendment results; however, in FS+PEFBB experiment significantly higher values of growth variables were observed when compared to those of FS+PKB, resulting in 20% more fresh weight (Table 4). These results are possibly due to the difference in pH of the materials (Table 2); while PEFBB has a basic pH of 10.19, PKB has a pH close to neutrality of 7.62, allowing PEFBB to correct the natural acidity of the FS. Additionally, PEFBB has three times more CEC than PKB, generating better fertility for plants in FS.

Figure 1. Effect of different biochars on means canopy area of ‘Vera’ crisp lettuce cultivated in four different soil types.



Different letters on bars correspond to the same response variable, indicate significant differences between canopy according to Tukey test ($P < 0.05$). Vertical bars correspond to standard error $n = 24$ per experiment.

A: AS+CPB; B: FS+PKB; C: FS+PEFB; D: TS+WWB; E: US+RSB; AS: Andosol; FS: Ferralsol; TS: Technosol; US: Umbrisol; CPB: coffee pulp biochar; PKB: palm kernel biochar; PEFBB: palm empty fruit bunches; WWB: wood waste biochar; RSB: rose stem biochar.

All doses of biochar utilized in FS+PEFB and FS+PKB experiments resulted in higher values of length, fresh and dry weight of root, and LWR than the absolute control, but not when compared to conventional fertilization treatment (Table 4). Although these biochars have desirable properties (Table 2), plants did not achieve adequate growth, but the results suggest that they can potentially reduce the use of chemical synthetic fertilizers. Previous research has demonstrated that it is possible to optimize plant growth results with compost-biochar and fertilizer-biochar mixes. Enhanced growth has been observed in plants when palm kernel shell biochar amendments mixed with fertilizers were used (for oil palm seedlings growth at the nursery) (Radin et al., 2018). The authors indicated this might be due to increases of pH by 0.59; greater content nutrients such as C, N, Ca, Mg, K; improvement in CEC; C:N ratio (from 9.7 to 17.5); and enhanced water holding capacity (from 16.7% to 17.5%). Likewise, biochar from empty fruit bunch has been found to have significant N and K contents, although the release is lower compared to conventional fertilizers (Dominguez et al., 2020).

Table 4. Effect of different biochars on means of growth variables measured at harvest in four different soils for different biochars and fertilizer treatments.

Soil	Biochar	Treatment	Root length	Leaf fresh weight	Root fresh weight	Leaf dry weight	Root dry weight	Leaf weight ratio
		t ha ⁻¹	mm	g	g	g	g	
AS	CPB	Control	11.23bc	12.88c	1.72d	5.79d	0.12c	0.86ab
		Fertilized	8.50c	12.84c	2.44d	6.00d	0.32c	0.84b
		3	15.38a	44.38b	5.60c	8.71c	0.73b	0.89a
		6	15.05a	49.04ab	9.83ab	10.05bc	1.49a	0.83b
		9	12.75ab	44.42b	9.08b	10.41ab	1.93a	0.83b
FS	PKB	12	12.83ab	55.07a	12.05a	11.92a	2.12a	0.82b
		Control	5.25a	0.57c	0.57a	0.11a	0.08a	0.52b
		Fertilized	10.23a	4.45ab	1.49a	0.78a	0.29a	0.78ab
		3	6.15a	1.46bc	0.84a	0.22a	0.12a	0.60ab
		6	5.57a	3.80ab	0.73a	0.21a	0.12a	0.78ab
FS	PEFBB	9	6.88a	2.52bc	1.01a	0.26a	0.13a	0.65ab
		12	2.06a	5.73a	0.72a	0.32a	0.18a	0.89a
		Control	3.40c	2.39a	0.67c	0.39a	0.12b	0.76ab
		Fertilized	4.45bc	4.99a	0.80c	0.48a	0.12b	0.85b
		3	6.00abc	8.15a	6.22a	1.09a	1.07a	0.55a
TS	WWB	6	7.78a	6.08a	2.09b	1.01a	0.64a	0.61b
		9	7.33ab	5.64a	2.42b	0.76a	0.31a	0.61b
		12	7.30ab	6.75a	2.58b	0.87a	0.33a	0.73b
		Control	5.30a	10.93a	2.83a	2.86a	0.90a	0.79ab
		Fertilized	5.45a	9.32ab	1.59ab	2.29a	0.48ab	0.84ab
US	RSB	3	6.83a	4.73b	2.46a	0.77b	0.82a	0.64b
		6	7.08a	7.04ab	1.79ab	1.15b	0.57ab	0.80ab
		9	6.23a	6.06ab	0.73b	1.09b	0.21b	0.88a
		12	7.25a	5.11b	2.04a	0.99b	0.40ab	0.73ab
		Control	-	76.96a	83.74a	1.49a	0.03b	0.56a
		Fertilized	-	165.10a	103.46a	1.53a	0.70ab	0.38a
		3	-	103.83a	61.06a	1.43a	0.40ab	0.44a
		6	-	192.57a	108.03a	1.69a	0.92ab	0.37a
		9	-	194.33a	87.19a	1.60a	0.79ab	0.36a
		12	-	153.33a	98.92a	1.60a	1.24a	0.36a

Distinct letters in the column indicate significant differences among values in each experiment (soil+biochar) according to Tukey's test ($P \leq 0.05$).

AS: Andosol; FS: Ferralsol; TS: Technosol; US: Umbrisol; CPB: coffee pulp biochar; PKB: palm kernel biochar; PEFBB: palm empty fruit bunches; WWB: wood waste biochar; RSB: rose stem biochar.

Effect on lettuce growth in the US+RSB experiment

Nonsignificant differences in canopy area (Figure 1), fresh and dry weight in leaves and roots of RSB treatment were found when compared to the control and fertilization treatment (Table 4). Nevertheless, after harvesting at 45 dat, we observed an increase in fresh weight of 152.51% at 9 t ha⁻¹ with respect to the control. No previous reports have been found in the literature about RSB or ornamental biochar plants effects on soil fertility or plant growth. However, increases in root length and dry weights in corn have been reported by Daza (2014) using compost elaborated with rose stems and roots (which contains high organic matter, Ca²⁺ and Mg²⁺ contents). Rose residues compost contains high organic matter percentages (42% to 51%), C:N ratio less than 20, pH between 5.5 and 8.0, EC less than 3 dS m⁻¹, and CEC between 97 and 151 cmol₍₊₎ kg⁻¹ (Idrovo-Novillo et al., 2018). The RSB proprieties such as a pH (10.4), CEC (39.13 cmol₍₊₎ kg⁻¹), C:N ratio (22.06), and water holding capacity (77.5%) may have positively influenced the soil conditions generating a better plant growth. Nevertheless, the results showed an optimal application up to 9 t ha⁻¹ probably due to a high EC 1.66 dS m⁻¹ of RSB; thus, we suggest a cautious use of this amendment.

Effect on lettuce growth in the TS+WWB experiment

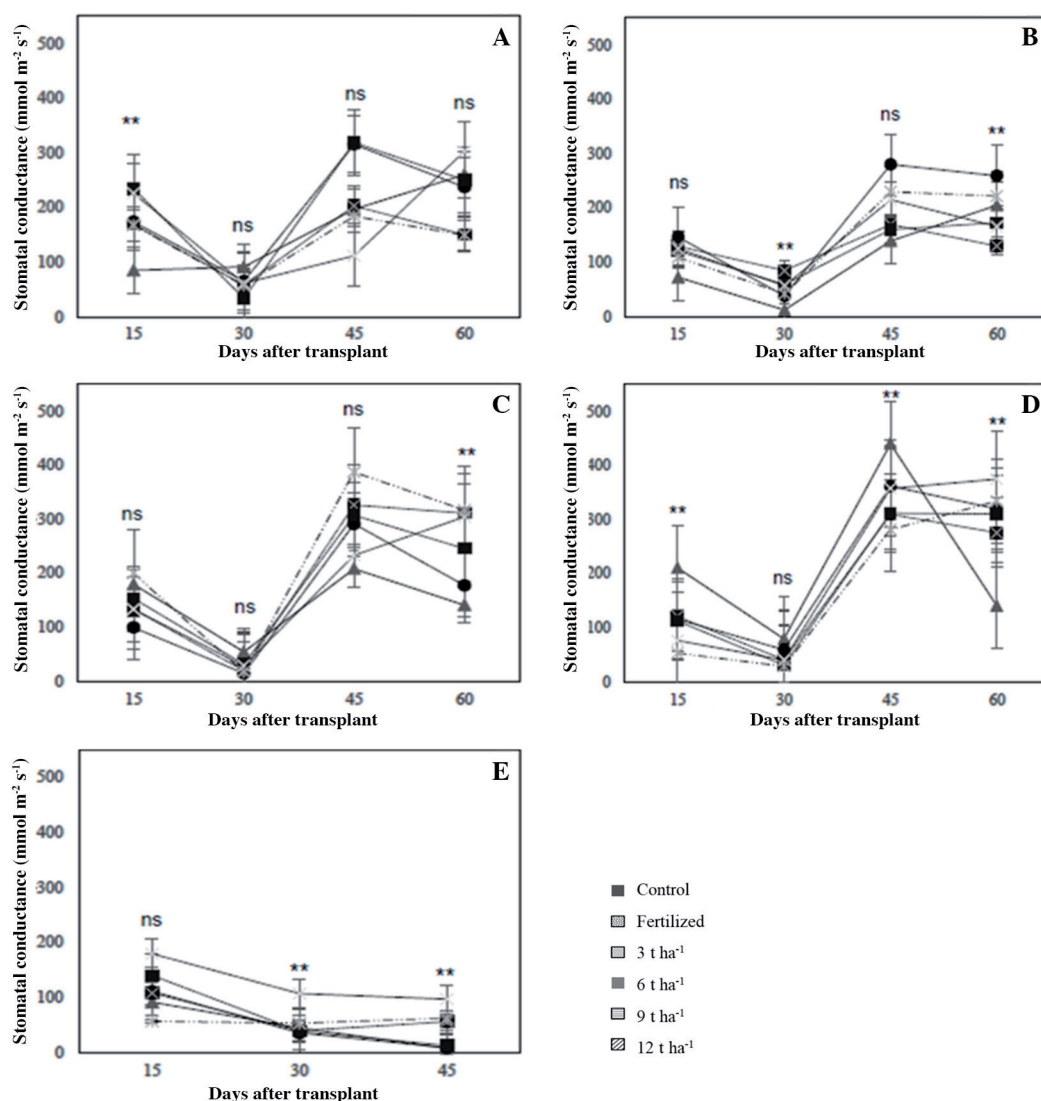
Plants grown on TS+WWB showed significantly lower length, canopy area, leaf dry and fresh weight; a decrease in fresh weight of ~ 90% was observed with respect to control and fertilized treatment (Table 4). These results may be associated with a high ratio C:N (64.68) of WWB which typically induce an inefficient use of N; according to Borchard et al. (2014) that reported similar negative results in corn crop yields using beech wood biochar (*Fagus sylvatica*) that was related to nutritional imbalances and N immobilization. In concordance, olive pruning biochar promoted a decrease in leaves weight

and inflorescence in broccoli, a result that was related to the decrease in available N (Garcia-Ibañez et al., 2020). Although these results show immediate negative effects by wood residues biochars, wood waste biochars from urban areas exhibit pH values of 9.6, EC of 0.6 dS m⁻¹, fulvic acids and high surface area which are suitable to remediate agricultural soils contaminated with heavy metals (Simón et al., 2018). In addition, have shown that simultaneous application of fertilizer and biochar (olive tree pruning) have a positive effect on broccoli growth and development (Garcia-Ibañez et al., 2020). This combination improved N, P and K available levels in soil and favored plant growth (Rafael et al., 2019).

Effects of biochar on lettuce plant physiology

No differences in stomatal conductance were found for AS+CPB, FS+PKB and TS+WWB, while for FS+PEFBB the average value of this variable was significantly higher with respect to the control and fertilization treatment; the stomatal conductance for US+RSB was lower for biochar treatments (Figure 2). Higher stomatal conductivity values have been observed in lettuce in a high-water availability soil which allows greater uptake of nutrients and higher photosynthetic efficiency productivity (Majid et al., 2021). On the contrary, a decrease in stomatal conductance has been found under water stress conditions (Galieni et al., 2015); thus it is possible that AS+CPB, FS+PKB and TS+WWB experiments did

Figure 2. Effect of different biochars on stomatal conductance of ‘Vera’ crisp lettuce cultivated in four different soil types.



**Significant at P < 0.05; ns: nonsignificant.

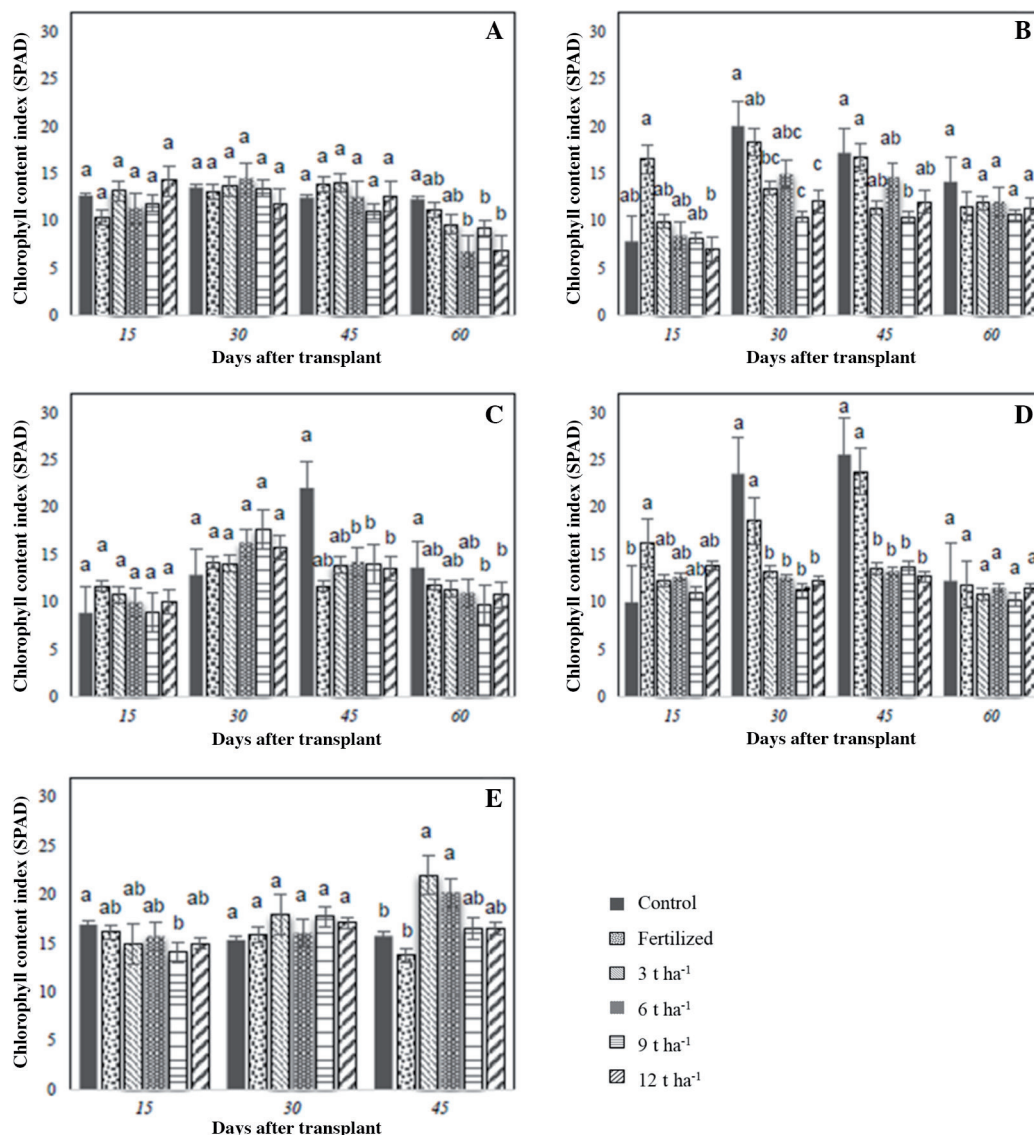
A: AS+CPB; B: FS+PKB; C: FS+PEFBB; D: TS+WWB; E: US+RSB; AS: Andosol; FS: Ferralsol; TS: Technosol; US: Umbrisol; CPB: coffee pulp biochar; PKB: palm kernel biochar; PEFBB: palm empty fruit bunches; WWB: wood waste biochar; RSB: rose stem biochar.

not provide adequate water availability in the soil which is in accordance with the results of the water holding capacity of these biochars as can be seen in Table 2.

It has been reported that biochar increases water holding capacity in different soil types (Günel et al., 2018) although the adequate combination between soil type and biochar still remains unclear (Somerville et al., 2019). Our results suggest that PKB is associated with a lower water holding capacity or Cd stress, so lettuce would take less water or close stomates to prevent Cd uptake, generating water stress in the plant. On the other hand, RSB amendment improve the water condition or decrease the availability of Cd, allowing a better water uptake by plant. Previously, in soils with heavy metal stress a decrease in stomatal conductance has been found using coconut husk biochar and orange shell biochar, something usually associated with a high-water holding capacity that improves water absorption in the plant (Silva et al., 2019).

The chlorophyll index values (Figures 3a-3d) were lower for AS+CPB, FS+PKB, FS+PEFBB and TS+WWB experiments with respect to the controls and decreased inversely with biochar doses. Previously it has been reported

Figure 3. Effect of different biochars on chlorophyll content index (SPAD) of ‘Vera’ crisp lettuce cultivated in four different soil types.



Different letters on bars correspond to the same response variable, indicate significant differences between stomatal conductance according to Tukey test ($P < 0.05$). Vertical bars correspond to standard error $n = 24$ per experiment.

A: AS+CPB; B: FS+PKB; C: FS+PEFBB; D: TS+WWB; E: US+RSB; AS: Andosol; FS: Ferralsol; TS: Technosol; US: Umbrisol; CPB: coffee pulp biochar; PKB: palm kernel biochar; PEFBB: palm empty fruit bunches; WWB: wood waste biochar; RSB: rose stem biochar.

that some biochars caused detriments in N and Mg concentrations in soils or induced water stress which may lead to chlorophyll reductions in plants (Safahani Langeroodi et al., 2019). In contrast, in US+RSB experiment chlorophyll index (Figure 3e) showed an increment specially at 3 and 6 t ha⁻¹, which agrees with the results of Gale and Thomas (2019), who found that application of wood shipping pallets biochar doses to soil increases or maintains chlorophyll levels unaffected.

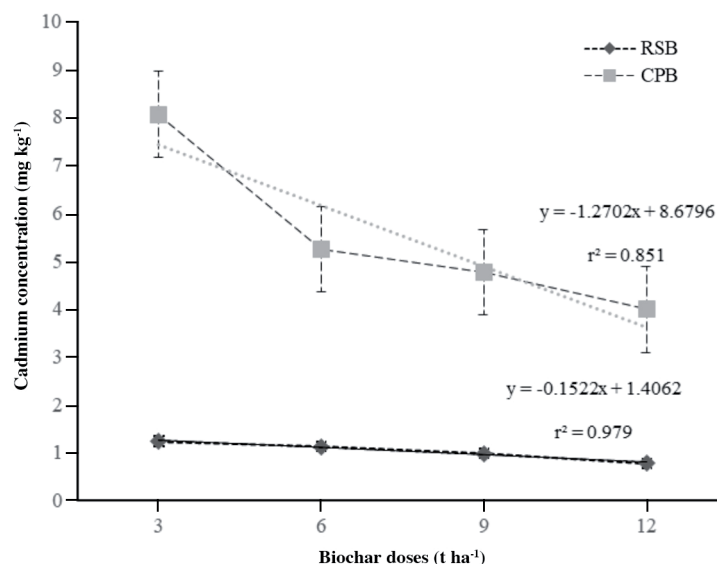
Phytotoxic effects of Cd on lettuce plants

In AS+CPB and US+RSB experiments, the use of biochars mitigated Cd stress conditions and plants reached commercial harvest point satisfactorily. For CPB we observed a high negative correlation ($r^2 = 0.85$) between the concentration of Cd²⁺ in plant tissues and the biochar doses, generating a reduction of 50.61% of Cd in leaves under a dose of 12 t ha⁻¹ in Andosol. Likewise, a high negative correlation was found between RSB doses and Cd concentration in the leaves ($r^2 = 0.97$), generating a reduction in the concentration of Cd of 37.34% under a dose of 12 t ha⁻¹ in Umbrisol (Figure 4). It is possible that this reduction in Cd uptake by plants is related to the high porosity of these biochars (Table 2) as this property has been reported to allow a high adsorption of Cd in biochar's surface (Fu et al., 2021). Although Cd²⁺ concentrations in both experiments are above the levels allowed by the European Union regulation 188 of 2014 for leafy vegetables (0.2 mg kg⁻¹), these biochars have potential to improve agricultural products quality by decreasing Cd concentrations, and can contribute to the solutions for less contaminated soils.

Biochars that presented higher values of pH (Table 2) resulted in better results in terms of growth (Figure 1). Similarly results were found by Zheng et al. (2017) using rice straw biochar in lettuce plants. These authors suggest that Cd²⁺ reduction in leaves is correlated to pH increase which promotes Cd²⁺ precipitation as oxy-hydroxides, compounds not available for plants (Simón et al., 2018). The low Cd phytoabsorption was explained by three levels: 1) Organic ligands of organic matter influence the sorption, bioavailability and toxicity of Cd²⁺; 2) clay minerals, carbonates, or Fe-hydrated oxides and Mn can precipitate Cd²⁺ as carbonate hydroxide; and 3) specific surface area of biochar interacts with soil particles and its ability to bind with trace metals (Li et al., 2020).

In FS+PEFB, FS+PKB and TS+WWB experiments the use of biochars did not mitigate Cd stress conditions and consequently plants did not reach commercial harvest point. Heavy metals cause disturbance of balance between reactive oxygen species (ROS) and defense mechanism of antioxidants resulting in oxidative stress and ultimately generate irreversible damage to plant cells and cell membranes and adversely effects on the uptake of water and nutrients and causes reduction in plant growth (Samet, 2020). In our experiments we found a shorter length, smaller leaf area and

Figure 4. Cadmium concentrations in leaves tissue in experiments with harvest point (AS+CPB and US+RSB). Linear correlation between biochar doses amendments and Cd concentration in leaves.



Vertical bars correspond to standard error n = 16 per experiment.
AS+CPB: Andosol+Coffee pulp biochar; US+RSB: Umbrisol+Rose stem biochar.

weight, reduced number of leaves and lower chlorophyll content in accordance with the results of Loi et al. (2018), who reported similar effects on lettuce growth at concentrations between 5 and 100 mg kg⁻¹ Cd²⁺; this heavy metal penetrates into roots via apoplast and symplast and is translocated to the shoots (Tang et al., 2020). This could cause competition between Cd and available nutrients for binding sites in proteins, thus affecting chlorophyll synthesis, metabolic processes and cellular stability, and consequently generates oxidative stress and lipid peroxidation (Irfan et al., 2013; Samet, 2020).

CONCLUSIONS

The effects of the biochars on growth of 'Vera' lettuce plants varied among soils. Coffee pulp biochar (CPB) on Andosol (AS) promoted the growth and development on lettuce plants vs. control treatments and fertilization. Rose stem biochar (RSB) in Umbrisol (US) generated a slight increase in fresh weight as shown up to a dose of 9 t ha⁻¹. Conversely, the use of palm kernel biochar (PKB) and palm empty fruit bunches (PEFBB) as amendment in Ferralsol (FS) did not promote growth and development of lettuce plants. Wood waste biochar (WWB) in the Technosol (TS) negatively affected the growth and development of lettuce plants.

Under the experiments US + RSB and AS + RSB lettuces reached the optimal size for marketing and exceeded the stress conditions by Cd. In the case of lettuces grown in US+RSB a lower phytoabsorption of Cd in leaves was observed at doses of 12 t ha⁻¹ which represented a reduction of 37.34% in Cd concentration with respect to control. Similarly, lettuces planted in AS+CPB at doses of 12 t ha⁻¹ CPB reduced Cd concentrations by 50.61%. When compared to control, which is due to a possible adsorption or retention of Cd by CPB and RSB. However, additional experimentation on dosage should be done to evaluate the degree of reduction of Cd of these materials in order to attain values under the maximum permitted limits for human consumption.

Biochars from palm residues and wood waste should be studied more widely; we recommend to carry out research with compost or fertilizer mixes to improve the agronomic performance of PKB and WWB given that there is evidence that other biochars in mixes can optimize soil and plant conditions unlike the sole biochar application. In addition, research about additional properties of these biochars should be expanded in order to understand the effects it generates on the soil ecosystem and how these materials interact with Cd²⁺ to prevent plant uptake.

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