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Biochar Affects Heavy Metal Uptake in Plants through Interactions in the Rhizosphere

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Abstract: Heavy metals in soil pose a constant risk for animals and humans when entering their food chains, and limited means are available to reduce plant accumulation from more or less polluted soils. Biochar, which is made by pyrolysis of organic residues and sees increasing use as a soil amendment to mitigate anthropogenic C emissions and improve agronomic soil properties, has also been shown to reduce plant availability of heavy metals in soils. The cause for the reduction of metal uptake in plants when grown in soils enriched with biochar has generally been researched in terms of increased pH and alkalinity, while other potential mechanisms have been less studied. We conducted a pot experiment with barley using three soils differing in metal content and amended or not with 2% biochar made from *Miscanthus x giganteus*, and assessed plant contents and changes in bioavailability in bulk and rhizosphere soil by measuring extractability in acetic acid or ammonium nitrate. In spite of negligible pH changes upon biochar amendment, the results showed that biochar reduced extractability of Cu, Pb and Zn, but not of Cd. Rhizosphere soil contained more easily extractable Cu, Pb and Zn than bulk soil, while for Cd it did not. Generally, reduced plant uptake due to biochar was reflected in the amounts of metals extractable with ammonium nitrate, but not acetic acid.

Keywords: rhizosphere; heavy metal; bioavailability; plant; uptake

1. Introduction

Mining, smelting, land applications of sewage sludge and other human activities have led to widespread metal contamination of soils. Heavy metals such as Cd, Cu, Pb and Zn often coexist in contaminated soils and their mobility and bioavailability is of global concern due to uptake in plants and increasing human exposure through food [1]. Using lime or alkaline organic materials as soil amendments can increase soil pH and thereby reduce bioavailability of heavy metals [2,3]. A wide range of studies have also highlighted biochar as a soil amendment that can reduce metal uptake in plants [4–6], in addition to its beneficial effects through enhancing soil C levels and improving related soil properties [7,8]. The beneficial effects of biochar on metal availability in soil are partly due to its capacity to increase soil pH, and partly due to its surface properties and capacity for sorption of ions and fixation of heavy metals [9]. Thus, significant amounts of heavy metals/metalloids, such as As, Cd, Cu, Ni, Pb and Zn have been shown to be sorbed by a wide range of biochar products, which were produced from a variety of feedstock such as wood, grass, husks, manure and broiler litter, and charring conditions including medium to high temperature pyrolysis and low temperature hydrothermal liquefaction [10–13]. Liming is far less complicated and less expensive than amendments with biochar, so the reasons for using biochar should, apart from general soil improvement, be related to additional immobilization of metals. To study such additional effects, low alkalinity biochars are

particularly useful [14]. When assessing bioavailability of metals, studies measuring plant uptake provide the most reliable data. However, as such studies are time-consuming and expensive, various extraction methods can provide relatively good estimates, or at least distinguish between efficient and less efficient means to affect bioavailability in comparative studies. Numerous extraction methods have been proposed, and some have been shown to be fair predictors of metal availability in soil [15,16]. The rhizosphere is the first point of contact between soil and a root system. Still, responses to soil amendments with biochar have largely focused on above-ground biomass and crop yields [17]. Root system responses to biochar will be affected by changes in soil properties like pH, bulk density, water holding capacity, metal and metalloid availability and changes in soil microbial activity [18]. Rhizosphere soil is modified partly through the action of root exudates and is thus potentially a hotspot for altered mobility of heavy metals, as compared to bulk soil [19]. Root exudates frequently acidify the rhizosphere through excretion of H+ ions and organic acids, a mechanism that mobilizes and enhances uptake of several plant nutrients [20], including trace elements [21]. In addition, heavy metals are affected by the chemical and biological processes occurring in the rhizosphere [22], resulting in depletion or enrichment according to the sorption properties and soil diffusion rates of different the metals [23]. A better mechanistic understanding by biochar effects on metal availability, including the respective contribution of pH and sorption components, is necessary to devise successful remediation strategies.

The objective of the present research is to characterize the availability of Cd, Cu, Pb and Zn in soils amended or not with biochar, focusing on differences between rhizosphere and non-rhizosphere (bulk) soil. Our approach featured measurements of extractable metals and pH in rhizosphere and bulk soil, and related these data to true bioavailability measured as metal uptake in plants.

2. Materials and Methods

A pot trial was set up using a full factorial design with three soils, two levels of biochar addition (none or 2% *w/w*) and three replicates. Two polluted soils were collected from a field site at different distances from a copper smelter located in the Lower Silesia region, Poland. Sixty years of copper smelter operation and atmospheric deposition of heavy metals onto near-by arable soil had caused high concentrations of Cd, Cu, Pb and Zn [24,25]. Highly polluted (HP) soil was collected from a field 0.3 km from the smelter (51°18′60.1″ N, 16°10′55.8″ W) and weakly polluted soil (WP) was collected 2 km from the smelter (51°19′55.9″ N, 16°10′31.8″ W). Soil with similar properties (pH, particle size distribution, C, N and clay content) was chosen as an unpolluted soil and collected at Dal, Viken County, SE Norway (60°14′39.2″ N, 11°11′0.60″ E), where the experiment was conducted. All soils were Cutanic Luvisols (FAO-WRB 2007 soil classification), containing about 75% of silt, <20% of clay and <5% of sand, having a pH in distilled water from 6.16 in control soil, 7.35 in highly polluted soil to 7.53 in weakly polluted soil. As all soils were cultivated, high pH values can be explained by application of calcium fertilizers, as Polish and Norwegian soils have low pH and liming is a standard procedure before cultivation. Another potential explanation for the high pH of HP and WP soils is the input of CaO and MgO from the copper smelter. In this area, intensive soil alkalization is observed due to alkaline dust emission from the smelter [26]. Basic characteristics of biochar and soil, as well as total concentrations of tested metals in both substrates are presented in Table 1. The biochar used was produced from *Miscanthus* in a Pyreg pyrolysis oven at 500–700 °C and characterized regarding pH, C, H and N content (using a CHN-1000, LECO, St. Joseph, MI, USA). The content of heavy metals was measured after digestion in concentrated HNO_3 using microwave-heated pressurized vials (Milestone Ultraclave III, Sorisole, Italy) followed by elemental analysis by inductively coupled plasma-mass spectroscopy 8800 ICP-MS triple Quad (Agilent Technologies, Santa Clara, CA, USA) [27].

Table 1. Composition of *Miscanthus* biochar used for soil amendment and soils used in the pot experiment.

	Biochar	Unpolluted Soil	Weakly Polluted Soil	Highly Polluted Soil
pH	9.16	6.16	7.53	7.35
Total C (%)	79.3	0.91	1.05	1.11
Total H (%)	0.47	-	-	-
Total N (%)	1.05	0.09	0.11	0.12
Total P (g/kg)	2.43	-	-	-
Cd (mg/kg)	0.05	0.15	1.25	1.91
Cu (mg/kg)	15.0	3.20	200	451
Pb (mg/kg)	0.62	11.1	118	211
Zn (mg/kg)	67.1	26.7	67.2	134

2.1. Polluted Soils and Experiment

The three soils were collected from the top 0–25 cm after plowing in October 2012, sieved to <2 mm and air dried. Biochar (2% *w*/*w*) was then mixed thoroughly into the soils and filled into triplicate freely draining (bottom holes covered with cotton gaze) 1 L polyethylene pots. Soil of each pot was fertilized individually with 69 mg/kg N [as a 50/50 mol ratio of NH_4NO_3 and $Ca(NO_3)_2$ $4H_2O$], 12 mg/kg P (as Na_2HPO_4 $2H_2O$), 90 mg/kg K (as K_2SO_4) and 22 mg/kg Mg (as $MgSO_4$ $7H_2O$) and watered to 70% of the soils individual water holding capacities. They were then sown with 10 seeds of barley (*Hordeum vulgare* L.,) before placing them in a temperature-controlled growth chamber (21 ± 1 °C; 16 h/8 h light/dark cycle providing 350 µmol/m/s photosynthetically active radiation). The number of seedlings was thinned to 5 per pot 4 days after germination and plants watered daily until they were harvested 4 weeks after germination (Figure 1). Shoots and roots were harvested separately, washed with Milli-Q water, oven dried (80 °C to constant weight) and dry weights recorded. Plant materials were analyzed for Cd, Cu, Pb and Zn after wet digestion with boiling 1 M perchloric acid (8 h). Samples of rhizosphere soil (soil adhering to roots after gentle crushing and shaking, constituting 2–5% of the total soil of the pot) and bulk soil were collected, dried at 60 °C for 48 h and weighed. Bioavailable fractions of heavy metals were extracted either with 1M NH_4NO_3 at pH 4.9 for 2 h (extracting readily exchangeable metal forms) or with 0.11 M acetic acid (CH_3COOH) at pH 2.9 for 16 h, according to the first step of the a sequential extraction procedure of the Standards, Measurements and Testing Program of the European Union (SM&T—formerly BCR) [28], extracting exchangeable, water and acid soluble (e.g., carbonates) metal forms.

Figure 1. Pot experiment showing differences in barley (*Hordeum vulgare* L.) growth between soils and treatments 4 weeks after germination, from left to right: highly polluted soil (control), highly polluted soil with 2% (*w*/*v*) biochar, weakly polluted soil (control), weakly polluted soil with 2% (*w*/*v*) biochar, unpolluted soil (control), unpolluted soil with 2% (*w*/*v*) biochar. Note that only one of the triplicates of each treatment is shown.

2.2. Plant Translocation Factor Calculation

Translocation factors (TF), defined as a shoot to root ratio of metal concentrations, were calculated as follows:

$$TF = [Metal]_{shoot}/[Metal]_{root}$$

Such ratios illustrate metal translocation properties and are useful in evaluating the impact of remediation efforts or risk reduction for edible plants like barley [29].

2.3. Statistics

Data were subjected to two-way analyses of variance and differences between means compared using Tukey's test for multiple post hoc comparisons.

3. Results

3.1. Plant Growth and Metal Uptake

The addition of 2% biochar to soil did not increase plant biomass compared to unamended control treatments for non-polluted and weakly polluted soil (Table 2). In highly polluted soil, plants grown without biochar grew very poorly and only reached a biomass of 8–10% of plants in the corresponding biochar amended treatment. Plants growing in the weakly polluted soil reached 85% of the shoot biomass in the non-polluted soil, whereas corresponding growth of plants in the biochar-amended highly polluted soil was 67% of that in the non-polluted soil.

Table 2. Biomass of shoots and roots of barley grown in three differently polluted soils amended or not with biochar (2% by weight).

Soil	Treatment	Shoots			Root		
		(g/pot)		SEM	(g/pot)		SEM
Unpolluted soil	Control	1.63	a	0.05	0.56	a	0.03
	Biochar	1.76	a	0.12	0.52	a	0.08
Weakly polluted soil	Control	1.39	ab	0.08	0.39	b	0.03
	Biochar	1.34	b	0.13	0.29	c	0.04
Highly polluted soil	Control	0.09	d	0.01	0.02	d	0.01
	Biochar	1.16	c	0.12	0.25	c	0.02

Values (means and SEM, $n = 3$) for either shoots or roots followed by the same letter are not significantly different ($p < 0.05$, Tukey's test).

The metal content of plants increased with increasing degrees of metal pollution in the soils. The highest concentrations of metals in barley were found when plants were growing on highly polluted soil, and for this soil the biochar amendment reduced the metal concentration in shoots by 82% for Cd, 66% for Cu, 91% for Pb and 53% for Zn, compared to plants growing on the same soil without biochar amendment (Table 3). There were no significant differences in shoot metal concentrations between plants grown on biochar-amended and non-amended soil for the two soils with lower metal content. For the weakly polluted soil, root Cu concentrations were lower in plants grown on amended compared to non-amended soil, while there were no significant differences for the other metals. Roots of plants grown on non-amended highly polluted soil were too small to allow analyses in two out of three replicates.

Table 3. Metal concentrations (mg/kg) in roots and shoots of barley grown in three differently polluted soils amended or not with biochar (2% by weight).

Metal	Plant Part	Unpolluted Soil						Weakly Polluted						Highly Polluted					
		Control			Biochar			Control			Biochar			Control			Biochar		
				SEM			SEM			SEM			SEM			SEM			SEM
Cd	Root	3.18 *	a	0.35	3.16	a	0.37	3.51	a	0.34	4.15	a	0.60	19.3		$n = 1$ **	4.87	b	0.71
	Shoot	1.27	b	0.18	1.15	b	0.12	1.07	b	0.13	1.10	b	0.12	7.17	a	1.14	1.30	c	0.09
Cu	Root	27.3	a	3.00	23.1	a	3.19	132	b	3.29	190	a	21.7	155		$n = 1$	443	a	57.9
	Shoot	7.50	b	0.35	5.67	b	0.54	14.3	c	0.82	12.0	c	0.71	38.3	a	4.08	13.2	b	0.82
Pb	Root	23.7	a	4.05	19.4	a	4.06	32.5	a	13.4	24.4	ab	4.70	206		$n = 1$	35.4	b	2.22
	Shoot	17.0	ab	2.12	9.17	b	1.47	8.17	b	1.63	6.00	b	2.55	73.3	a	10.8	6.67	c	1.08
Zn	Root	41.9	ab	0.10	64.3	a	20.1	39.0	a	3.71	37.9	a	7.24	93.3		$n = 1$	77.3	a	11.6
	Shoot	28.5	b	2.45	28.5	b	0.61	37.3	a	1.95	34.0	a	2.83	105	a	26.7	49.3	b	2.35

* Mean values ($n = 3$) followed by the same letters are not significantly different ($p < 0.05$, Tukey's test), ** The three replicates were pooled due to very low plant mass in the control treatment.

Calculated TF showed that metals taken up by barley were largely retained in roots, as shown by general TF values <1 (Table 4). Biochar application consistently reduced TF of plants grown in all three soils. Biochar application significantly decreased Zn and Cu transfer to shoots in barley grown on HP soil, but had no effect on Cd and Pb translocation in barley. On less polluted soils, no significant effect on TF was observed.

Table 4. Translocation factor (TF) calculated for barley grown in three differently polluted soils amended or not with biochar (2% by weight).

Soil	Treatment	TF			
		Cd	Cu	Pb	Zn
Unpolluted soil	Control	0.41a *	0.28a	0.74a	0.68a
	Biochar	0.37a	0.25a	0.52a	0.54a
Weakly polluted soil	Control	0.31a	0.11a	0.33a	0.97a
	Biochar	0.27a	0.06b	0.25a	0.97a
Highly polluted soil	Control	0.31a	0.29a	0.44b	1.88b
	Biochar	0.28a	0.03b	0.19b	0.54b

* Mean values (n = 3) followed by the same letter are not significantly different (Tukey's test, $p < 0.05$).

3.2. *Effects on Soil*

Biochar amendments did not affect soil pH (Table 5), except for the highly polluted soil where it caused a statistically significant increase of 0.1–0.2 units. In this soil, the pH also increased slightly in the rhizosphere compared to bulk soil (0.1 unit). For the two other soils, rhizosphere soil was slightly more acidic than bulk soil (0.15–0.3 units).

Table 5. Soil $pH_{(d\text{-}water)}$ measured in rhizosphere soil and bulk soil after 4 weeks growth of barley on three differently polluted soils.

Soil	Sampling	Control			Biochar		
				SEM			SEM
Unpolluted soil	Bulk soil	6.16	ab	0.11	6.36	a	0.14
	Rhizosphere soil	5.99	b	0.03	6.06	b	0.05
Weakly polluted soil	Bulk soil	7.88	a	0.03	7.93	a	0.12
	Rhizosphere soil	7.56	ab	0.04	7.68	b	0.20
Highly polluted soil	Bulk soil	7.52	a	0.03	7.63	a	0.03
	Rhizosphere soil	7.35	b	0.08	7.53	a	0.04

Within a soil, values (means and SEM, n = 3) followed by the same letter are not significantly different (Tukey's test, $p < 0.05$).

The biochar amendment significantly decreased the concentrations of NH_4NO_3-extractable Cu, Pb and Zn in the highly polluted soil, but no such decrease was seen when comparing metal concentrations in CH_3COOH-extracts (Table 6). In the weakly polluted soil, biochar reduced NH_4NO_3-extractable Cu, and Cd in rhizosphere soil. Rhizosphere soil frequently contained more Cu, Pb and Zn than bulk soil, and biochar tended to counteract this increase. For Cd, the tendency was opposite, and we observed lower extractable concentrations in the rhizosphere compared to bulk soil.

Table 6. Metal concentrations (mg/kg and SEM values) in extracts from rhizosphere soil and bulk soil from a pot experiment with barley, and the effects of biochar (2% by weight).

			Unpolluted Soil				Weakly Polluted				Highly Polluted			
Element	**Extractant**	**Sampling**	**Control**		**Biochar**		**Control**		**Biochar**		**Control**		**Biochar**	
Cd	CH_3COOH	Rhizosphere	0.09 a	0.16	0.04 b	0	0.67 b	0.16	0.40 b	0	1.20 a	0	1.20 a	0
		Bulk soil	0.09 a	0.16	0.093 a	0.16	1.07 a	0.16	1.07 a	0.16	1.60 b	0	1.60 b	0
	NH_4NO_3	Rhizosphere	0.025 a	0	0.025 a	0	0.50 a	0	0.25 b	0	0.33 a	0.10	0.42 a	0.10
		Bulk soil	0.025 a	0	0.033 a	0.10	0.42 ab	0.10	0.33 ab	0.10	0.33 a	0.10	0.58 a	0.10
Cu	CH_3COOH	Rhizosphere	2.93 a	0.33	2.67 ab	0.43	29.60 a	1.30	28.50 a	0.16	158.3 a	9.50	135.60 b	0.90
		Bulk soil	2.13 b	0.16	2.27 ab	0.16	27.10 a	1.07	26.90 a	1.93	134.8 b	1.30	127.70 b	10.2
	NH_4NO_3	Rhizosphere	1.67 a	0.10	0.75 b	0.31	3.33 a	0.20	1.42 bc	0.62	18.50 a	0.64	5.33 b	3.01
		Bulk soil	0.50 b	0.18	0.67 b	0.27	2.50 ab	0.64	1.17 c	0.10	10.08 b	1.64	6.50 b	0.94
Pb	CH_3COOH	Rhizosphere	ND *		ND		2.53 a	1.84	ND		11.60 a	1.02	12.10 a	3.42
		Bulk soil	ND		ND		3.33 a	2.57	ND		5.33 b	0.91	6.40 b	0.75
	NH_4NO_3	Rhizosphere	0.92 ab	0.10	1.83 a	0.51	2.08 a	0.51	0.83 a	0.10	1.83 a	0.37	0.58 b	0.20
		Bulk soil	0.25 b	0	1.50 a	0.64	2.33 a	0.82	1.67 a	0.91	1.75 a	0.47	0.58 b	0.10
Zn	CH_3COOH	Rhizosphere	4.00 b	0	5.20 a	0	20.90 a	0.16	18.70 ab	0.82	84.50 a	5.52	84.80 a	3.21
		Bulk soil	4.13 b	0.43	5.20 a	0.49	19.70 ab	1.66	18.00 b	0.75	86.00 a	2.21	80.40 a	6.73
	NH_4NO_3	Rhizosphere	1.58 a	0.82	1.92 a	0.27	2.08 a	0.91	1.08 ab	0.10	2.83 a	0.10	2.33 b	0.10
		Bulk soil	2.08 a	0.27	1.42 a	0.41	0.75 b	0.35	0.67 b	0.10	1.75 c	0.10	1.42 d	0.10

* ND: Not detected. Mean metal concentration and SEM ($n = 3$). For each soil, element and extractant, values followed by the same letter are not significantly different (Tukey's test, $p < 0.05$).

4. Discussion

Biochar has mainly been given attention in recent years due to its capacity for long-term storage of C in soil, and for the beneficial effects it has on soil properties that are important in agriculture [8]. An increasing number of reports have focused on aspects of mitigation of pollution for both metals/metalloids [30–32] and organic pollutants [33–35]. Relatively few studies have however addressed the potential reduction in bioavailability of metals to plants in a complementary way that accounts for both biological and chemical availability [31,36,37]. The present results confirm that biochar amendments can have profound effects on plant uptake in polluted soil, and that the changes that biochar mediates in soil are also apparent when estimating bioavailability by non-stringent soil extraction. A novel finding is that biochar results in a reduced bioavailability of the four metals studied, and that this reduction occurred at a pH which similar or very close to that of the bulk soil. Our results also represent a rare case showing that biochar can reduce extractability and plant uptake of metals while changes in soil pH remain negligible. By contrast, most reported attempts to sequester heavy metals in soil using biochar are accompanied by increases soil pH, which then accounts for at least part of the reduced metal uptake in plants [38–41]. Reducing heavy metal uptake without substantially increasing pH is important, as increases in soil pH beyond 7–8 lead to reduced availability of several plant nutrients, and may thus hamper plant growth, whether plants are used for sequestration, remediation or production.

Another novel finding in our study is the demonstration of Cu, Pb and Zn accumulation in rhizosphere soil in the presence and absence of biochar, and the finding that Cd behave differently from the three other metals we studied. Root-induced changes of rhizosphere pH play a major role in the bioavailability of the many pH dependent nutrients, but also potentially toxic metals [42]. It has been shown that when acidifying their rhizosphere, plants increase the solubility of toxic compounds [43,44], as observed in our study. Amongst the processes that are responsible for the differentiation of the rhizosphere relative to the bulk soil, the exudation of C-compounds [45] has been much studied for its major impact on soil biogeochemistry. Apart from mobilization mediated by acids, metal accumulation in rhizosphere soil has also been observed, and partly attributed to immobilization by root mucilage [46]. Carboxylic acids remain the most investigated component of root exudates due to their potential effect on metal bioavailability via complexation processes. Amongst the range of carboxylates exuded into the rhizosphere, malate, citrate and oxalate seem to have the most pronounced effects on metal complexation [47]. The effects of biochar on plant heavy metal uptake are generally dependent on soil pH [48], but may be inconsistent and vary for different metals. In our study, Cd seemed to be the most pH independent element, in agreement with e.g., Chen et al. [49] who described that biochar application resulted in large reductions of plant Cd concentrations (32–40%) in both acid, neutral, and alkaline soils.

Metal availability in the rhizosphere may vary considerably, depending on soil characteristics like pH, organic C and clay content, but it may also vary depending on plant species and plant nutrition. Plant nutrient uptake may result in acidification or alkalinization, depending, e.g., on P availability and N-fertilization. The latter may show striking differences between acidifying fertilizers like urea and ammonium salts, while nitrate-based fertilizers cause a weak alkalinization. These effects are accentuated in rhizosphere soil, as they are driven by ion exchange during root uptake. Due to the tubular nature of roots, exudates like ions, carboxylates and mucilage emanating from roots are rapidly diluted and buffered or consumed as the distance from the root surface increases. Thus, the extension of the rhizosphere is limited, and our ability to assess, e.g., the true concentration of metals therein depends on the width of rhizosphere that is sampled. Our sampling method is rather crude and allows for both the inclusion of aggregates that may be wider than the extension of the rhizosphere, and exclusion of particles and parts of aggregates belonging to a zone close to roots which then is then analyzed as part of the bulk soil. This may explain the rather small differences we observed in pH and metal concentrations between rhizosphere soil and bulk soil. More elaborate rhizosphere studies featuring mesh-based interfaces between roots and rhizosphere soil [50], permit a

more precise sampling and a higher resolution of gradients forming in the rhizosphere. In such systems, the importance of plant characteristics (e.g., amount and lengths of root hairs) and soil properties (e.g., clay content and aggregation) may also be assessed.

Weak extractants like organic acids or salts seem to be better predictors for metal availability in soil than stronger extractants like EDTA, even when soils are amended with biochar [51,52], particularly for elements like Zn, Ni and Cu, and in soils with pH below 7. In our experiment, acetic acid extracted between 2 and 10 times more metals from soil than NH_4NO_3. Again, the weakest extractant most readily distinguished biochar-treated and non-treated soil, and between rhizosphere and bulk soil. The extraction of metals is mainly caused by desorption of cations, while the formation of colloids and soluble metal-organic complexes, which are hardly taken up by plants, is strongly suppressed due to the high ionic strength of the 1M NH_4NO_3 solution [28]. This can make NH_4NO_3 extractions more precise for plant uptake predictions in near neutral to alkaline soils.

While the extractable concentrations of metals in the rhizosphere give a momentary picture of metal availability to plants, the cumulative uptake of metals over the entire growth period is better reflected by the metal concentration in the plants. This uptake is distributed between roots and shoots, and as is commonly found, roots retain a large proportion of absorbed metals and translocate limited amounts to shoots. Low translocation ratios may partly stem from root surface adsorption of metals, contributing to an extension of the extraradical immobilization observed in rhizosphere soil [42]. Reichman and Parker [53] described that release of phytosiderophores (PS) by roots is an important factor influencing the availability of trace elements in rhizosphere, suggesting that this mechanism may protect plants from heavy metal toxicity, reducing element transfer to plant tissues [54]. Gupta and Singh [55] described that Graminaceous species synthesize and secrete Fe-chelating siderophores, the non-proteinaceous amino acids belonging to the mugineic acid family, that dissolve sparingly soluble Fe compounds in the rhizosphere. As biochar can be an important donor of Fe oxides, the presence of Fe from biochar can be an important factor inducing PS secretion by roots, reducing heavy metals uptake in tested soils.

5. Conclusions

Our results show, for the first time, that biochar may reduce the availability of Cu, Zn and Pb and Cd in rhizosphere soil, and thereby, contribute to reduced metal uptake in plants. In general, biochar application to soil increases soil pH, decreasing the bioavailability of heavy metals. Our set-up avoided this pH effect, and demonstrated a root-induced acidification of the rhizosphere that also occurred in biochar-amended soil, and that affected metal mobility as measured by soil extractions. Heavy metal uptake by plants was reduced mainly in highly polluted soil, indicating that soil amendments with biochar are particularly useful in cases where plant growth is severely limited by high bioavailability of heavy metals.

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