Effects of biochar amendment on root traits and contaminant availability of maize plants in a copper and arsenic impacted soil

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Abstract

Background and aims

Biochar has been proposed as a tool to enhance phytostabilisation of contaminated soils but little data are available to illustrate the direct effect on roots in contaminated soils. This work aimed to investigate specific root traits and to assess the effect of biochar amendment on contaminant availability.

Methods

Amendment with two different types of biochar, pine woodchip and olive tree pruning, was assessed in a rhizobox experiment with maize planted in a soil contaminated with significant levels of copper and arsenic.

Results

Amendment was found to significantly improve root traits compared to the control soil, particularly root mass density and root length density. Copper uptake to plants and ammonium sulphate extractable copper was significantly less in the biochar amended soils. Arsenic uptake and extractability varied with type of biochar used but was not considered to be the limiting factor affecting root and shoot development.

Conclusions

Root establishment in contaminated soils can be enhanced by biochar amendment but choice of biochar is key to maximising soil improvement and controlling contaminant availability.

Keywords: Rhizobox; phytostabilisation; biochar; contaminated soil; availability

Introduction

Restoring degraded soils using low impact, cost-effective remediation techniques has been increasingly investigated over the last few decades, given the extremely high number of contaminated sites generated in the wake of anthropogenic activities and the expense involved in remediating these sites (Onwubuya et al. 2009). Phytoremediation in the form of phytostabilisation is one low impact remediation option which aims to stabilise soil structure and reduce negative contaminant effects simultaneously (Kidd et al. 2009).

The use of degraded sites for biomass crop generation is a proposed solution for deriving commercial benefit from a phytoremediation approach (Atkinson et al. 2008). Maize (*Zea mays*) is one potential crop choice due to its quick growth cycle and high biomass production. It has been previously used to investigate contaminant impact on plant health and growth (Lin et al. 2008) in addition to in studies assessing its potential

as a biomass crop grown in contaminated soil (Witters et al. 2012a; Witters et al. 2012b).

Successful phytoremediation relies on good plant development in the form of healthy root structure and high root and shoot biomass in conjunction with minimal root to shoot translocation of contaminants to minimise transfer across the food chain (Karami et al. 2011; Wenzel 2009). However, plant establishment on a contaminated site can be problematic and the use of amendments, particularly organic materials, can enhance biomass yield and improve plant health (Clemente et al. 2012). The use of biochar as an amendment for re-establishing plant growth in contaminated environments (Beesley et al. 2011; Karami et al. 2011) is one potentially cost effective approach, particularly if waste-derived biochars are used, but field derived data are scarce mainly due to concerns over increased mobility of some contaminants, particularly arsenic (Beesley et al. 2013; Beesley et al. 2010). The literature available for biochar amendment of uncontaminated/agricultural sites have highlighted the positive effects of biochar amendment on root growth (Lehmann et al. 2011; Prendergast-Miller et al. 2011; Prendergast-Miller et al. 2014) but this has not yet been fully studied in the context of contaminated soils. A wider knowledge of the effects of biochar amendment on root growth in contaminated systems is essential in addition to a better understanding of contaminant behaviour patterns before confident field scale application.

Based on the hypothesis that biochar amendment alters root growth and available contaminant pools in polluted soils, the objectives of this work were to: investigate root morphology and architecture in a contaminated soil amended with biochar using a rhizobox approach and; to assess the effect of biochar amendment on available/mobile contaminant pools in the soils and on measured plant uptake.

Materials and methods

Experiment set up

Soil was collected from the topsoil (0-15 cm) of the vicinity of the tailings dump of the disused copper mine El Fernandito in Garganta de los Montes (40°55'3.14"N; 3°40'23.36"W), near Madrid, Spain, sieved to 2mm, and air dried. The soil had a sandy loam texture (54% sand, 39% silt and 7% clay), pH of 6.8, low organic matter content (1.08%) and high total As and Cu concentrations (74 and 404 mg kg^{-1}, respectively). The soil also contained enhanced levels of Zn and Mn (260 and 606 mg kg^{-1}, respectively).

Two biochars, derived from the slow pyrolysis of pine woodchip (PB) and olive tree pruning (OB), were used to amend the contaminated soil and were lightly crushed and sieved to 0.5 to 2mm. Biochars were produced in a pilot plant at 450 °C with a residence time in the reactor of approximately 15 minutes. Biochar samples were produced by the University of León (Natural Resources Institute, Spain) in the framework of the project "Proyecto Biocar: Estudio del Biocarbón como Sumidero de Carbono" (IPT-440000-2010-8). The biochars differed greatly in their properties as shown in Table 2.1.

Maize seeds were washed and pre-germinated before planting. They were washed by sonicating in 10% sodium hypochlorite for 30 minutes and then in deionised water for 30 minutes. They were then placed on tissue paper moistened with deionised water and several drops of calcium sulphate (1.5 mM) and incubated at 28°C for 72 hours for germination.

The pre-germinated maize seeds were grown in rhizoboxes (25 cm x 10 cm x 1 cm) for 21 days in a controlled growth chamber (temperature day 25°C (night 20°C); relative humidity day 40% (night 60%); hours of light day 13 hours (night 11 hours); light intensity 520µmol m^{-2} s^{-1}). 300g of the contaminated mine soil was amended with 3% pine woodchip or olive tree pruning biochar (thoroughly mixed). This treatment approach is equivalent to a field application rate of approximately 50t/ha, based on an assumption of 1600-1700t soil/ha. The control treatment was not amended with char.

All three treatment scenarios were conducted with five replicates and each replicate was planted with two seeds. A fine cloth was inserted into the bottom of the rhizobox to allow watering by capillary action, maintaining constant moisture content within the boxes. The rhizoboxes were covered with foil to exclude light and angled at 65° to encourage the roots to grow on the rhizobox/soil interface (Marschner and Römheld 1983).

A column experiment set up to run in parallel to the rhizoboxes explored the differences in leaching patterns between washed and unwashed olive tree pruning biochar. Washing consisted of sonication in ethanol for two hours before rinsing three times with deionised water and air drying. 700 g of soil only or 700 g of a soil and 3% char mix was packed into Perspex columns (cylinders 40 cm long x 5 cm diameter). Each treatment was performed in duplicate columns. A peristaltic pump was set to run deionised water through the system, collecting eight fractions on an accumulated volume basis. In total, 13500 ml of leachate was collected over 14 days and this volume simulated about 9 years of rainfall at the site from which the soil was removed.

Harvesting

After 21 days, the maize plants were harvested. Shoots were cut 1 cm above the soil surface, rinsed with deionised water, dried with tissue paper. Roots were sonicated twice in deionised water, rinsed and dried with tissue paper. All plant material was weighed for total fresh weight. Of the two plants per treatment replicate, one plant was used for enzymatic analyses (nitrate reductase in the shoots and acid phosphatase in the roots), and the second plant was used to determine concentrations of potentially toxic elements in the shoots after being dried at 60°C for 72 hours. The fresh roots of the second plant were stained with 5% Giemza Blue solution and kept at 4°C in a ziplock bag in deionised water until root characteristics analysis was carried out using WinRhizo® software.

Leaf area was determined by scanning (HP Photosmart C4280) and processing the images in GIMP 2 software. Root length, root surface area, diameter and diameter classes were determined by WinRhizo software following root preparation as described in the previous paragraph. Roots were scanned after being placed carefully on a transparent tray in 2-3mm of water (Himmelbauer et al. 2004).

Soil and biochar analysis

Soil particle size distribution was determined using standard method ISO 11277:2009 (ISO 2009). pH and electrical conductivity (EC) of the soil and biochar samples were determined in the water extract 1:5 (w/v) and 1:10 (w/v) respectively after stirring the mixture mechanically for 2 hours. Organic matter content (OM) was determined by loss on ignition at 550^{o}C for soil and following the TMECC method for biochar (TMECC 2002).

Biochar C and N contents were determined using an elemental analyser (LECO CHN-600). The water-soluble organic C (WSC), water-soluble inorganic C (WSIC) and water-soluble N (WSN) were determined using an automatic analyser for liquid samples (TOC-V CSN+TNM-1 Analyser, Shimadzu). Total P, K, Ca, Mg, Fe, Mn, Zn and Cu were determined after dry ash sample digestion using method 04.12-C (TMECC 2002). Total and water soluble (1:10 w/v) components were analysed as follows: P was determined colorimetrically (Murphy and Riley 1962); K by atomic emission spectroscopy; Ca, Mg, Fe, Mn, Zn and Cu by atomic absorption spectrophotometry and; As by atomic fluorescence spectroscopy (Millennium Excalibur, PS Analytical).

The liming value of biochar was measured as calcium carbonate equivalency according to the 04.08-A method (TMECC 2002). The biochar bulk density was estimated by weighing 10 mL of milled sample. The CO_2 adsorption method (273 K) using a Micromeritics ASAP 2020 instrument was performed to determine the surface area of the biochar samples. All biochar samples were degassed under vacuum at 200^{o}C for 8

hours prior to analysis. Cation exchange capacity (CEC) was measured by a modified ammonium-acetate compulsory displacement method (Gaskin et al. 2008). The germination index (GI) was determined using cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa* L.) (Zucconi et al. 1981).

Plant enzyme analysis and soil and plant tissue analysis of potentially toxic elements (PTEs)

Samples of 0.25 g fresh shoot material and 0.25 g fresh root material were extracted for nitrate reductase activity (Ruiz et al. 1999) and acid phosphatase activity respectively (Barrett-Lennard and Greenway 1982).

Total and extractable As concentrations in the treatments were determined by atomic fluorescence spectroscopy (Millennium Excalibur, PS Analytical) and Cu, Zn and Mn was determined by atomic absorption spectroscopy (AA800, Perkin Elmer) following autoclaving (Lozano-Rodriguez et al. 1995) and ammonium sulphate extraction (Vázquez et al. 2008) respectively. For total soil concentrations, 0.5 g of soil was transferred into 50 ml autoclave bottles to which 6 ml of MilliQ water, 6 ml of 65% HNO_3 and 4 ml of 33% H_2O_2 were added. The autoclave was set at a pressure of 1.5 kg cm^{-2} (125°C) for 30 minutes, samples were left to cool, then filtered and made up to 50 ml. Total plant concentrations (shoot tissue) were determined by weighing 0.1 g dried shoot tissue into 20 ml autoclave bottles to which 2 ml of MilliQ water, 1.5 ml of 65% HNO_3 and 1 ml of 33% H_2O_2 were added and made up to 5 ml once autoclaved, cooled and filtered. Extractable PTEs in the soils were determined by extracting 1.5 g of soil with 15 ml of $(NH_4)_2SO_4$ 0.1M in 50 ml tubes and shaking for four hours at 180 rpm. The samples were then filtered and 0.1 ml of HNO_3 was added.

Column leachate analysis

Column leachate fractions were analysed for pH, EC and DOC. Nitrate, chloride, phosphate and sulphate were analysed by ion chromatography (Dionex). As and Cu in the leachate were determined as described in the previous section.

Data analysis

Statistical analyses were carried out on SPSS. Data were checked to fit the hypothesis of normality and homoscedasticity. All data were normally distributed and were tested with a one-way ANOVA. Tukey's test was used as post-hoc for mean comparisons of the homoscedastic data. Games-Howell's test was used for the comparisons of non-homoscedastic data.

Results

Effect of biochar on shoot and root traits

Biochar amendment had a positive effect on most of the measured plant characteristics (Figs. 2.1-2.3). Biochar amendment significantly affected fresh shoot/root ratio (Fig. 2.1), which for both pine woodchip (PB) and olive tree pruning biochar (OB) amendments was greater than the control. In the PB treatment, the higher shoot/root ratio was due to an increase in shoot biomass while root biomass did not significantly differ compared to the control.. When amended with OB, both root and shoot biomass increased significantly compared to the control, in addition to the increase in the observed shoot/root ratio.

Leaf surface area significantly increased ($p<0.05$) in both biochar amended soils compared to the control. The same pattern was observed for root length and root surface area (both at $p<0.01$) (Fig. 2.2). By classifying the different root diameters into percentage composition (Fig. 2.3), root diameters <0.4mm seem predominant in the

biochar amended soils (>50%) compared to less than 30% in the control while root diameters of >1mm represent less than 5% in the char amended soils compared to more than 25% in the control. These results suggest that both PB and OB promoted root growth and production of fine roots. Likewise, both OB and PB amendment led to significantly higher root length density ($p<0.01$) compared to the control. Specific root length was significantly higher in the PB amended soil compared to the control ($p<0.05$), but this was not the case in the OB amended soil. In terms of root morphology, root length: root volume ratios were similar across treatments, while root tissue density was significantly lower in the PB treatment compared to the control at 0.1% versus 0.5% ($p<0.05$). Biochar amendment had a generally beneficial effect on plant resource allocation below ground, but this was not the case for all parameters. OB amended soils had a significantly higher root mass density than both PB amended soils and the control soils ($p<0.01$). Root length ratio was significantly greater in the PB treatment than both the control and the OB treatment ($p<0.05$). Root weight ratio was significantly lower in the PB amended soils compared to the control ($p<0.05$), suggesting PB amendment enabled the plant to allocate more resources to above ground (Table 2.2).

Effect of biochar on PTE extractability and plant uptake

The different biochars behaved very differently with regards to PTE extractability in the soils (Fig. 2.4). There was no significant difference in arsenic extractability between the control and PB amended soil, while OB amended soils had significantly higher extractable arsenic (at $p<0.05$). In contrast, both biochar amendments significantly decreased copper extractability compared to the control ($p<0.01$). Zinc extractability significantly decreased across treatments, in the order Control>PB>OB ($p<0.05$) while no significant differences were observed for Mn extractability (Fig. 2.5).

The patterns observed in PTE shoot uptake were slightly different (Fig. 2.6-2.7). Arsenic uptake in the shoots differed significantly between the two biochar treatments, with PB

treatments had significantly less shoot arsenic than OB treatments ($p<0.05$), although neither amendment differed significantly to the control. On the other hand, there was significantly less copper in the plant shoots from both the biochar treatments compared to the control ($p<0.01$). Shoot Zn and Mn concentrations were significantly reduced in the OB treatment compared to the control ($p<0.05$), but not in the PB treatment compared to the control.

Plant enzymatic activities as affected by biochar application

A significant downward trend was observed for nitrate reductase activity in plant shoots for OB amended soils compared to the control, while no differences were observed between the control and PB amendment. No significant differences were observed in acid phosphatase activity in the roots across treatments (Fig. 2.8).

Arsenic, copper and anion leaching after biochar application

Whereas As leaching rates were constant in the control soils, the olive tree pruning biochar significantly increased arsenic concentrations in the percolate water (Fig. 2.9). Although the washed biochar (WBC) reduced arsenic leaching from the column compared to the unwashed biochar (BC), the leachable portion of arsenic is very similar between the washed and unwashed chars (Table 2.3).

Dissolved organic carbon increased across treatments as highlighted by the cumulative totals: 1.5 mg kg^{-1} (+/- 0.02 SE) in the soil columns, 220.6 mg kg^{-1} in the unwashed biochar columns and 95.7 mg kg^{-1} (+/- 15.9 SE) in the washed biochar columns. Chloride leaching decreased with OB amendment while nitrate leaching significantly increased. Most phosphate fractions were below detection limits while there was no observed effect on sulphate concentrations (Table 2.4).

Discussion

Effect of biochar amendment on contaminant mobility

Results from the current study suggest that biochar amendment promotes root growth, increases available pools of arsenic for plant uptake while decreasing those of copper and zinc. The variable effect on arsenic availability in the soil according to char type (As in OB > As in PB) may be explained by the OB having a more available arsenic content as a result of its more alkaline pH and relatively high carbonate and soluble P contents compared to PB (Table 2.1). Additional soil and plant interactions over the course of the experiment may also have contributed to the observed data. As others have also suggested, char type needs to be chosen on a site specific basis (Beesley et al. 2011). Increased arsenic availability has also been observed in other studies in soil only and soil and plant systems (Beesley et al. 2010; Karami et al. 2011). The arsenic content in the olive tree pruning biochar itself and the increased pH caused by OB amendment (Table 2.1) may also be contributing to the increased arsenic release observed in the columns (Fig. 2.9). The leachable pool of copper observed in the unamended soil columns was doubled in the washed biochar and soil columns and tripled in the unwashed biochar and soil columns (Table 2.3). This is likely related to the enhanced concentrations of dissolved organic carbon (DOC) with biochar addition. Comparing the patterns observed for arsenic and copper in the soil control column, Cu is more easily leachable while arsenic appears to be poorly mobile. The data from the biochar amended columns illustrate that OB mobilises As to a greater extent than Cu. One previous study suggested that biochar amendment triggered a higher leaching capacity for As but not for Cu (Beesley and Marmiroli 2011). There are several possible explanations for this, the most likely being due to differences in biochar feedstock properties although differences in experimental design between the present study and the cited study may also be a contributing factor in the patterns observed. Nonetheless, although column studies are useful to get an idea of contaminant leaching capacity, they

do not fully represent a field scenario where plant interactions also have an effect on contaminant leaching.

Effect of biochar amendment on nutrient availability

Nitrate reductase is the enzyme responsible for reducing nitrate to nitrite. Lower activity here indicates less availability of nitrate to the plant shoots for conversion, which may have been caused by sorption of nitrate to the biochar particles (Jones et al. 2012). Biochar localises nitrate in the rhizosphere of biochar amended soils, resulting in less nitrate uptake by plants (Prendergast-Miller et al. 2011). Nonetheless, N dynamics are highly complex and a number of factors may be at play for the results presented (Clough et al. 2013).

In the case of the phosphatase enzyme, the reduced activity may be due to the increased uptake of arsenic observed in the plants and therefore reduced phosphate uptake, considering As is a well reported P analogue (Meharg and MacNair 1992; Moreno Jimenez et al 2008). Overall, the reduced enzymatic activity may be due to nutrient, enzyme or substrate sorption to the biochar (Lehmann et al. 2011). Variability in soil enzyme activity in the presence of char has been reported elsewhere (Bailey et al. 2011; Jones et al. 2012; Lehmann et al. 2011) although no specific data are available for plant enzymes in biochar amended soils.

The availability of other nutrients (e.g. K, Mg, Ca) in each treatment was not determined, thus the possibility that the addition of K or other nutrients due to biochar amendment (see biochar properties in Table 2.1) may have contributed to the improved plant growth cannot be ruled out. Although this aspect may be a potentially confounding factor in the results presented, this potential nutrient addition from biochar amendment would play a more significant role in agricultural soils or in a longer term experiment. Further studies elucidating interactions between nutrients and contaminants in

contaminated soils with respect to plant growth are required before making any conclusions on this matter.

Root response to biochar amendment in contaminated systems

Contaminant availability tends to be the principal limiting factor affecting plant growth in contaminated soils. The nutrient limitation commonly found in mine soils is another important factor. However, given the significant reductions in copper availability with biochar amendment (both in terms of extractability and actual uptake) and the corresponding improvements in root development with biochar amendment, our results suggest that excess copper was limiting plant establishment and survival in the presented study. The less consistent behaviour of the other contaminants compared to copper lend credence to this theory.

This study suggests that biochar has no clear detrimental effect on root establishment and, by reducing copper availability significantly in both char treatments, a net positive effect was observed, particularly with regards to root mass density and root length density. Another study found that biochar effects on root traits in agricultural soils were not as indicative of root behaviour as quantifying rhizosheath development and biochar particles in the rhizosphere (Prendergast-Miller et al. 2013). However, in contaminated soils, root traits appear to be useful indicators of root responses to biochar amendment compared to unamended contaminated controls, with significant differences observed across the majority of indicators. There is a scarcity of data investigating specific root responses to biochar amendment in contaminated systems, apart from some qualitative assessment (Beesley et al. 2013) and further studies are needed in order to fully evaluate the effects on a range of plants and in a range of contaminated soils.

Implications for phytomanagement of mine soils

These results suggest that biochar addition to contaminated mine soils may enhance plant cover by improving root development and promoting higher biomass both above and below ground. Not only are these soils affected by contamination, they tend to have poor physical properties and low nutrient and carbon statuses which can make plant establishment difficult. It appears that biochar amendment reduces soil toxicity to plants growing under these difficult conditions, at least in early stages of plant establishment, and may play a role in limiting contaminant dispersion. In terms of improving soil health, other studies have highlighted the beneficial effects of biochar addition to poor soils, for example, improved water holding capacity and cation exchange capacity (Busscher et al. 2010; Carter et al. 2013; Revell et al. 2012; Sukartono et al. 2011). If the trends observed in this study can be further demonstrated under field conditions, biochar will become a valuable yet affordable tool in the phytomanagement of degraded soils.

Conclusions

Root establishment in contaminated soils can be enhanced by biochar amendment but choice of biochar is key to maximising soil improvement, controlling contaminant availability to plants and controlling contaminant mobility overall.

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Table 1 Main characteristics (on a dry weight basis) of the two biochars (PB: pine woodchip biochar and OB: olive tree pruning biochar). *Data provided by J.A. Albuquerque.

Parameters	**PB**	**OB**
Bulk density (g cm^{-3})	0.63	0.36
Liming equivalence (g $CaCO_3$ kg^{-1})	7.4	80.1
pH[a]	7.52	9.34
Electrical conductivity[a] (µS cm^{-1})	256	2430
Organic matter (g kg^{-1})	981.9	900.3
C (g kg^{-1})	837.1	755.2
N (g kg^{-1})	3.6	11.0
P (mg kg^{-1})	148	1464
K (mg kg^{-1})	1708	9159
Ca (mg kg^{-1})	3316	28524
Mg (mg kg^{-1})	574	2088
Fe (mg kg^{-1})	200	496
Mn (mg kg^{-1})	153	50
Zn (mg kg^{-1})	42	24
Cu (mg kg^{-1})	134	114
As (mg kg^{-1})	1.7	6.1
Specific surface area (m^2 g^{-1})	288	265
Germination index (lettuce, %)	92	100
Germination index (cress, %)	117	84
Cation exchange capacity (cmol kg^{-1})	12.6	36.6
Water-soluble fractions		
Water-soluble organic C (WSC, mg kg^{-1})	920	1527
Water-soluble inorganic C (mg kg^{-1})	122	1020
Water-soluble N (WSN, mg kg^{-1})	10	19
WSC/WSN	90	82
Water-soluble P (mg kg^{-1})	6	17
Water-soluble K (mg kg^{-1})	256	2546

[a]water extract 1:10 (w/v).

Table 2 Root architecture (1); root morphology (2); plant resource allocation below ground (3). Letters denote statistical significance, p < 0.05*, <0.01**, <0.001***, n.s. not significant. PB: pine woodchip biochar and OB: olive tree pruning biochar.

			Control	PB	OB	Sig
1	root length density	root length/ soil volume mg cm^{-3}	0.447 (0.036) a	1.36 (0.162) b	1.289 (0.233) b	**
	specific root length	root length/root biomass cm mg^{-1}	0.106 (0.015) a	0.313 (0.08) b	0.141 (0.025) a	**
2	root length: root volume ratio	root length/root volume cm cm^{-3}	453.3 (35.9)	475.3 (72.2)	510.0 (38.9)	n.s.
	root tissue density	root biomass/root volume mg cm^{-3}	4523.0 (521.2) b	1682.9 (257.1) a	4412.1 (1217.7) b	*
3	root mass density	root biomass/ soil volume mg cm^{-3}	4.424 (0.431) a	5.106 (1.294) a	9.173 (0.499) b	**
	root weight ratio	root biomass/plant biomass mg mg^{-1}	0.619 (0.028) b	0.484 (0.055) a	0.526 (0.018) ab	**
	root length ratio	root length/plant biomass cm mg^{-1}	0.065 (0.009) a	0.143 (0.029) b	0.074 (0.013) a	**

Table 3 Curve parameters after fitting As and Cu leaching data to volume of leachate (after Fig. 7). In the hyperbolic curve, the term *a* is associated to the maximum cumulative leaching of As/Cu (in mg kg^{-1}) and *b* is associated to the volume (in L) needed to leach half of the maximum leaching. Statistical significance is indicated by ***p<0.001. WBC: washed olive tree pruning biochar and BC: unwashed olive tree pruning biochar.

As				
	Linear curve: $y=ax+y_0$			
	a	y_0	Adj. R^2	Sig.
Soil	$4.1 \cdot 10^{-6}$	0.0086	0.66	***
	Hyperbola: $y=ax/(b+x)$			
	a	b	Adj. R^2	Sig.
Soil+WBC	4.7	12.8	0.99	***
Soil+BC	4.5	7.5	0.99	***
Cu				
	Hyperbola: $y=ax/(b+x)$			
	a	B	Adj. R^2	Sig.
Soil	0.29	2.0	0.96	***
Soil+WBC	0.48	1.8	0.98	***
Soil+BC	0.91	1.5	0.97	***

Table 4 Cumulative total of 8 column leachate fractions (SE n=2) expressed as mg kg-1 dry weight soil

	Control (soil only)	3% washed OB	3% unwashed OB
NO3	113 (70)	386 (115)	1189 (59.6)
PO4	2.73 (2.27)	2.98 (1)	1.5 (0.71)
SO4	31 (6)	20.2 (5.08)	32.32 (4.12)
Cl	532 (347)	74.5 (5.3)	61.4 (12.1)

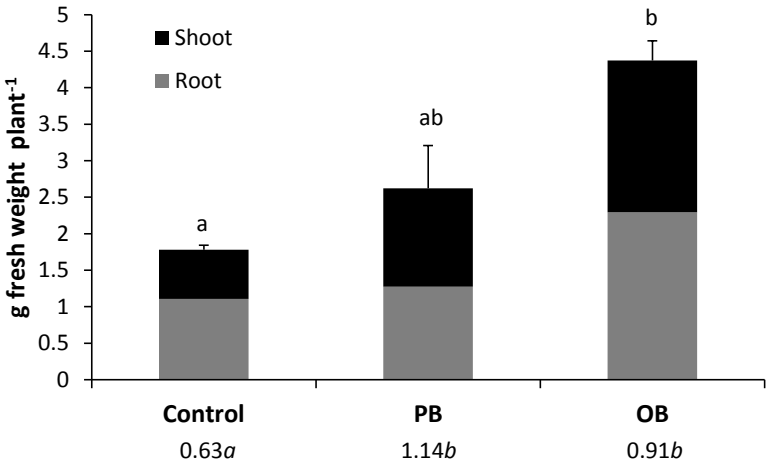

Fig. 1 Plant biomass (g of fresh weight per maize plant) in the mine soil with different biochars (PB: pine woodchip biochar and OB: olive tree pruning biochar). Mean ± SE (*n*=4-5). The shoot: root ratio was calculated and shown on the bottom of the *x* axis. Different letters indicate statistical differences between groups at p<0.05.

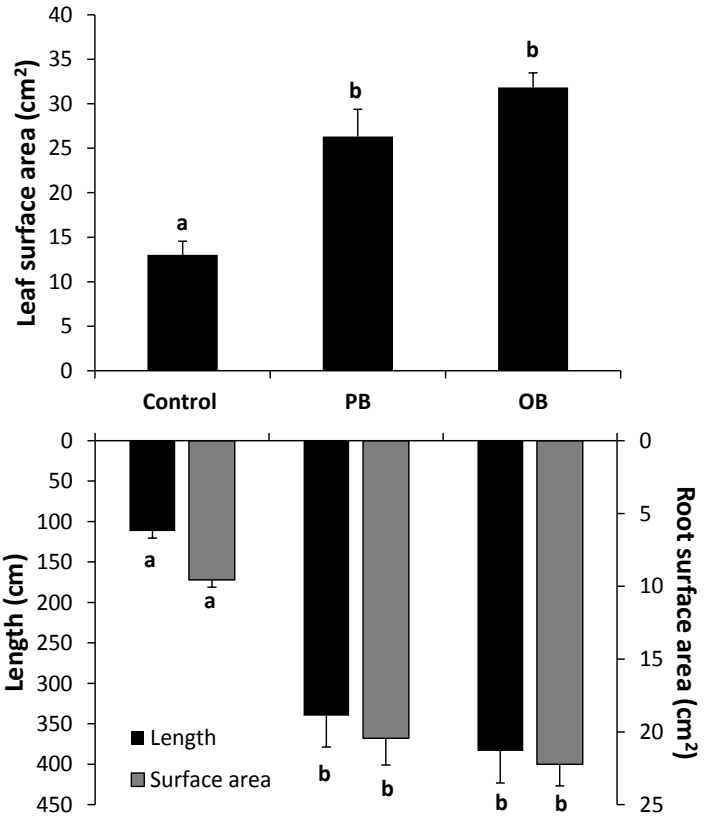

Fig. 2 Plant morphology as affected by biochar application to a mine soil: leaf surface area (top) and root length and root surface area (bottom). Mean ± SE (*n*=4-5). Different letters mean statistical differences between groups at p<0.05. PB: pine woodchip biochar and OB: olive tree pruning biochar.

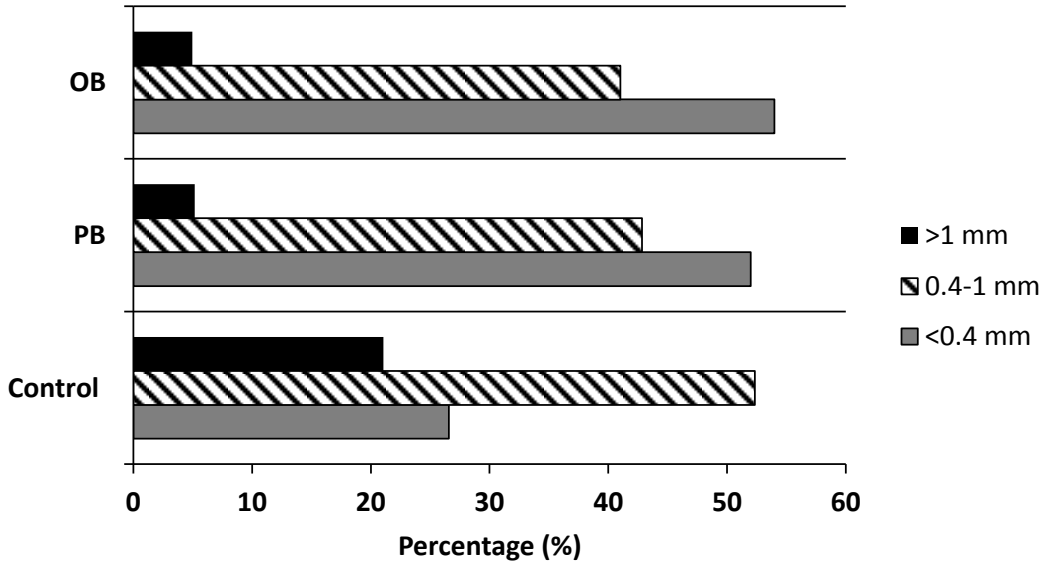

Fig. 3 Root diameter classes as affected by biochar application to a mine soil. Mean ± SE (*n*=4-5), where absent, error bars fall within symbols. PB: pine woodchip biochar and OB: olive tree pruning biochar.

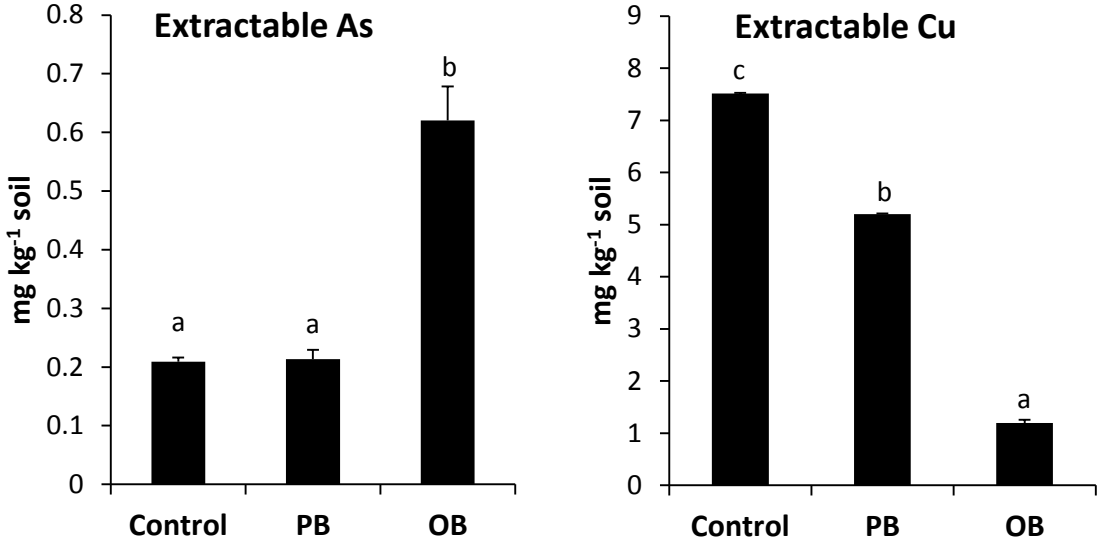

Fig. 4 Ammonium sulphate-extractable As and Cu in a mine soil with different biochar treatment (PB: pine woodchip biochar and OB: olive tree pruning biochar). Mean ± SE (*n*=5). Different letters signify statistical differences between treatments at $p<0.05$.

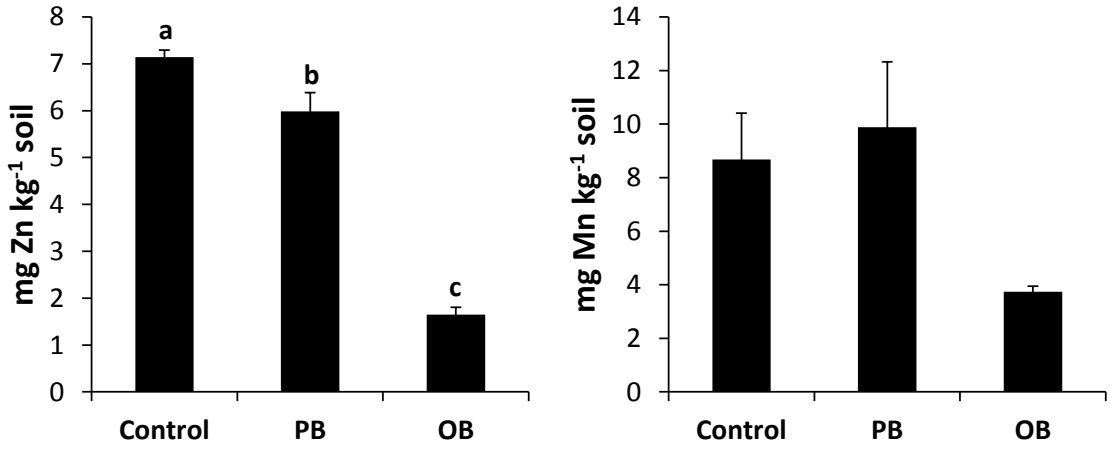

Fig. 5 Ammonium sulfate-extractable Zn and Mn in a mine soil with different biochar treatment (PB: pine woodchip biochar and OB: olive-tree pruning biochar). Mean ± SE (*n*=5). Different letters signify statistical differences between treatments at p<0.05.

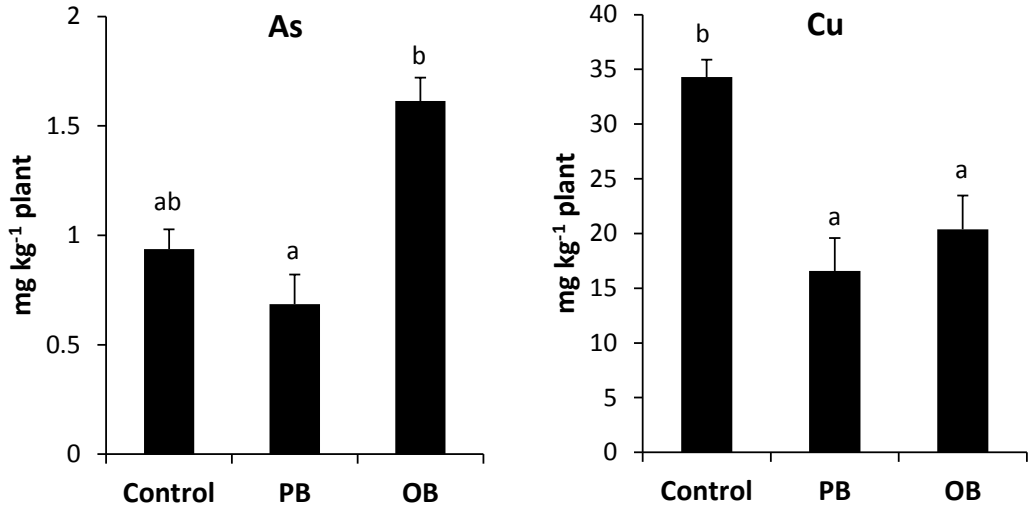

Fig. 6 Arsenic and copper concentration in shoots of maize plants growing on a mine soils with different biochar treatment (PB: pine woodchip biochar and OB: olive tree pruning biochar). Mean ± SE (*n*=4-5). Different letters mean statistical differences between groups at p<0.05.

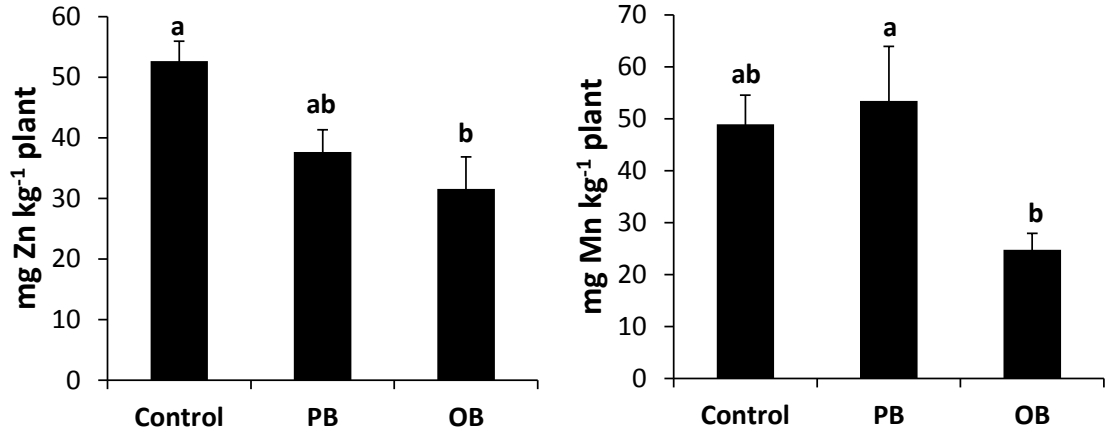

Fig. 7 Zinc and manganese concentration in shoots of maize plants growing on a mine soils with different biochar treatment (PB: pine woodchip biochar and OB: olive-tree pruning biochar). Mean ± SE (*n*=4-5). Different letters mean statistical differences between groups at p<0.05.

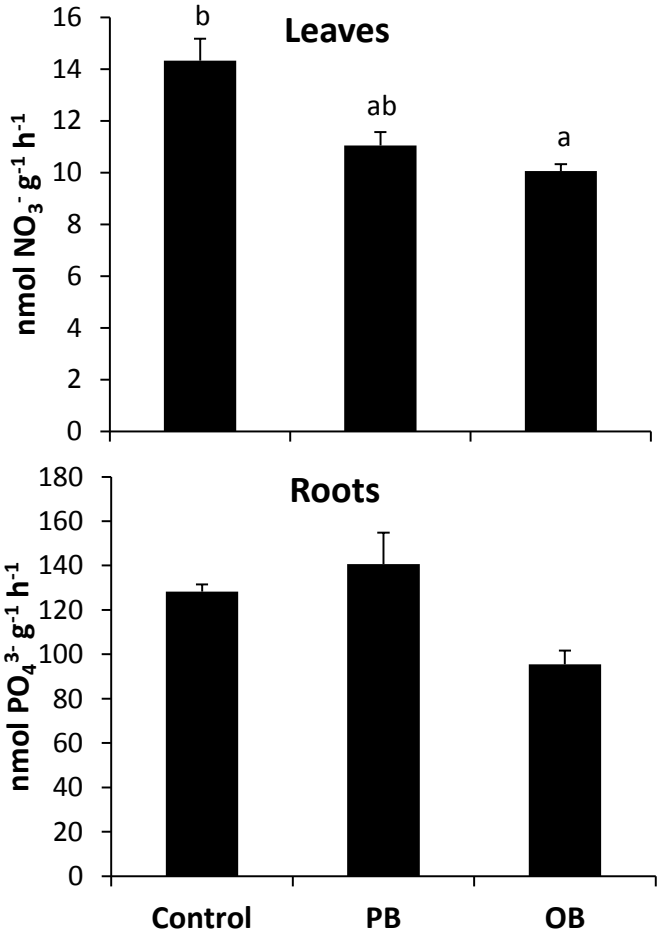

Fig. 8 Nitrate reductase activity in leaves and acid phosphatase activity in roots of maize growing on a mine soils with different biochar treatment (PB: pine woodchip biochar and OB: olive tree pruning biochar). Mean ± SE (*n*=3-5). Different letters signify statistical differences between groups at p<0.05.

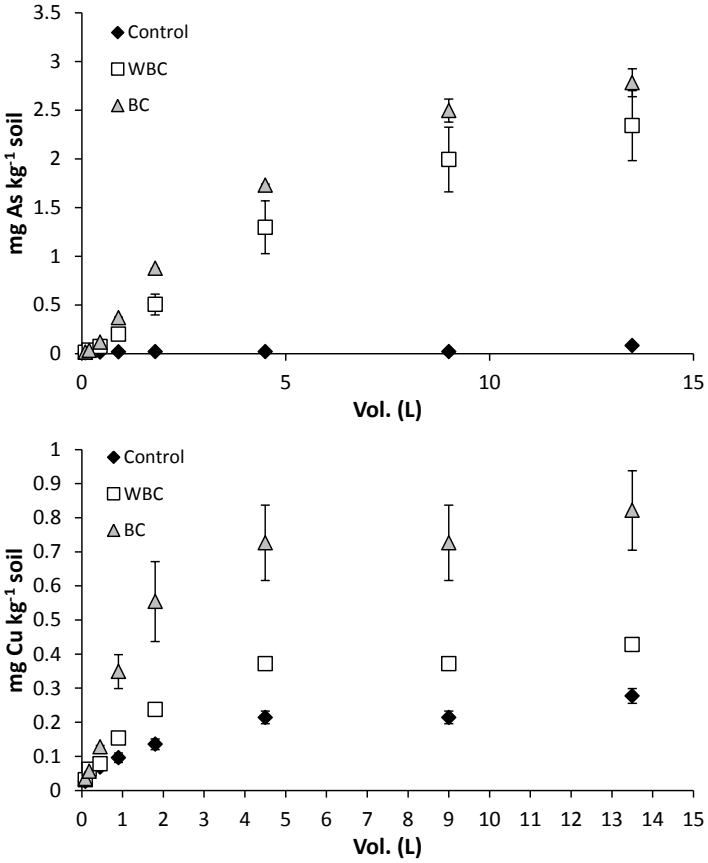

Fig. 9 Leaching of As (top) and Cu (bottom) in mg per kg of soil (on a dry weight basis) as evaluated by a leaching column experiment using washed (WBC) and unwashed (BC) olive tree pruning biochar over 14 days. Mean ± SE (*n*=2).