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Effect of biochar amendments on the mobility and (bio) availability of As, Sb and Pb in a contaminated mine technosol

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Abstract

Reclaiming degraded and contaminated soils with biochar amendments has become increasingly popular. Biochar, a fine-grained and porous biocarbon produced by the slow pyrolysis of biomass under oxygen limited conditions is foreseen as a possible solution to reduce metal(loid)s availability and phytotoxicity and simultaneously to improve the soil quality. Mining activities are one of the main concern generating potentially toxic elements (PTE) such as metal(loid)s-rich wastes and technosols. The effects of biochar addition (2% and 5%) on a former mine technosol characterized by high levels of PTE were investigated in laboratory during short-term experiments where technosols were potted and cultivated with dwarf beans (*Phaseolus vulgaris* L.) as indicator plants. Soil and soil pore water (SPW) physico-chemical properties were determined together with total dissolved concentrations of As, Sb and Pb in SPW sampled by Rhizons and available concentrations as measured by DGT (Diffusive Gradients in Thin-Films). Additionally indicator plant biomass and PTE concentrations in their organs (roots, stem and leaves) were determined. The results of the present study indicate that addition of 2 and 5% biochar to contaminated technosols increased soil and SPW pH, electrical conductivity (EC) and soil water content (SWC), decreased the labile concentration of Pb while increasing the As and Sb solubility. Indicator plants

cultivated on biochar amended mine technosols showed a smaller biomass and higher PTE concentration levels in the various organs.

Keywords: Biochar, Metal (loid)s, Remediation, Mobility, (Bio-)availability, Technosol.

Abbreviations:

Soil water content (SWC), electrical conductivity (EC), total organic carbon (TOC), soil pore water (SPW), dissolved organic carbon (DOC), garden soil (G), technosol (PF), mixture of 50% garden soil and 50% technosol (PFG), cation exchange capacity (CEC), potential toxic elements (PTE), diffusive gradient in thin films (DGT), dry weight (DW), lead (Pb), antimony (Sb), arsenic (As).

1. Introduction

Reclaiming degraded and contaminated soils with potentially toxic elements (PTE) such as metal(loid)s in the context of post mining activities is now obvious to the community (Fellet et al., 2014). These anthropogenic activities have significantly changed soil PTE concentrations and availability with consequently hazardous effects on ecosystems and human health (Paz-Ferreiro et al., 2014). The recovery of PTE contaminated sites is notoriously difficult through conventional technologies whose effectiveness is highly variable and dependent on the contaminants, soil properties and site topography (Marques et al., 2009). In situ stabilization techniques aim at decreasing the labile PTE pool, reduce their bioavailability and/or toxicity to plants and animals and providing nutrient supply and simultaneously promoting other beneficial processes such as water retention by incorporating organic and mineral amendments (Komarek et al 2013; Hattab et al., 2014; Le Forestier et al., 2016). These techniques are able to enhance one or several processes such as metal adsorption through increased surface charge, formation of organic and inorganic metal complexes, sorption on Fe, Mn, and Al oxides, and precipitation (Ahmad et al., 2012; Komarek et al., 2013). Soil organic amendment (biosolids, sawdust, wood ash) shows potential to immobilize most PTE (Gadepalle et al., 2007). However, as the organic matter decomposes, the adsorbed PTE may be potentially released (van Herwijnen et al., 2007). Therefore the use of more refractory organic amendments such as biochar is having a huge interest for the potential stabilization of PTE (Fellet et al., 2011; Paz-Ferreiro et al., 2014). Biochar is a fine-grained and porous amendment produced by biomass pyrolysis under oxygen limited conditions (Sohi et al., 2009). It possesses several organic functional groups (such as carboxyl $-(C=O)OH$, hydroxyl $-OH$, amine groups, aromatic and anomeric $O-C-O$ carbons) on its surfaces charged negatively. However, during its oxidation in soil these functional groups could increase over time (Verheijen et al., 2010). The formation of functional groups and adsorption sites on surfaces and within pores of biochar could influence its cation exchange capacity (CEC)

(Liang et al., 2006) and consequently its capacity to form complexes with metal ions which allow decreasing negative PTE effects on ecosystems.

However, there is a lack of agreement over the influence of organic amendments such as biochars on PTE immobilization in contaminated soils (Beesley et al., 2010a, b). Moreover, to date the application of biochars to contaminated soils has not been systematically investigated to any great extent (Beesley and Marmiroli, 2011).

Therefore the main thrust of this work was to gain additional information on biochar effects on PTE contaminated mine technosols. The effects of two rates (2% and 5%) of biochar amendments on As, Sb and Pb mobility and availability and plant growth and phytoavailability were investigated with pot experiments where technosols were cultivated with *Phaseolus vulgaris* L. together with control garden soils and mixtures of garden soils and technosols.

2. Material and methods

2.1. Site description and experimental design

Our study focuses on a former gold mine, named La Petite Faye, located 60 km northeast (GPS coordinates: 01°34'23"E; 46°08'37"N) of Limoges (France, Massif Central). The mine is no longer in use since 1964, and its old mining activity has led to the accumulation of approximately 35,000 t of wastes contaminated by PTE such as As, Pb and Sb in a settling basin delimited by loamy dams on its sides (Wanat et al., 2014).

In this study three different types of soils were used: i) Technosol, soil collected from the top 20 cm of La Petite Faye contaminated site (named PF); ii) Garden soil, which correspond to a control soil collected between 5 to 25 cm deep in the park of Orleans University, France (named G); iii) a mixture of 50% Garden soil and 50% Technosol (V/V) (named PFG). These three soils were placed in 2 L pots and amended with biochar at 0%, 2% and 5% (w/w). Biochar was a commercial charcoal provided by VT Green Company (Saint Bonnet de Rochefort, France) and obtained from wood biomass through a slow pyrolysis process at a temperature of 500 °C. The main biochar physico-chemical properties and total PTE content are reported in Table 1.

For each treatment studied (PF, G, PFG), three replicates of 2 mm sieved soil were prepared and mixed with 0%, 2% or 5% of biochar. The potted soils were allowed to equilibrate for 5 days at field capacity using distilled water before introducing *Phaseolus vulgaris* L. germinated seeds.

2.2. Soils characterization

Soils characterization was performed before adding the germinated *Phaseolus vulgaris* seeds in the different tested conditions. The soil water content (SWC) was calculated according to Bart et al. (2015). Soil pH and Electrical Conductivity (EC) were measured in 1:2.5 soil/water suspensions using a glass electrode pH meter (Cyberscan 500, Eutech instruments, Landsmeer, The Netherlands). Total Organic Carbon (TOC) content was determined with a Shimadzu©, TOC 5000A carbon (Shimadzu corporation, Kyoto, Japan),

according to NF ISO 14235 procedure (SAS Laboratoire, Ardon). Total PTE concentrations (As, Pb, Sb) were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (ULTIMA 2, HORIBA, Labcompare, San Francisco, USA) according to Bart et al. (2015).

2.3. Soil pore water (SPW) characterization

At the end of the preliminary 5 days period and before adding the germinated *Phaseolus vulgaris* seeds, SPW was collected from 3 pots per treatment by using soil moisture samplers (Rhizon) (model MOM, Rhizosphere Research Products, Wageningen, The Netherlands). Rhizons were placed in pots under a 45° position and allowed to equilibrate for 24h under vacuum (Cattani et al., 2006) to collect the soil pore solution (=30 ml per pot). SPW collected by rhizons was directly used to measure pH, EC, and DOC (Dissolved Organic Carbon) concentration with a Shimadzu®, TOC 5000A carbon analyzer (Shimadzu corporation, Kyoto, Japan) as described by Hattab et al. (2015).

Total dissolved PTE (As, Pb and Sb) concentrations in SPW were determined directly by Inductively Coupled Plasma atomic emission spectroscopy (ICP-AES) (ULTIMA 2, HORIBA, Labcompare, San Francisco, USA), using the rhizon sampled solutions.

2.4. DGT measurements

The available concentrations of As, Pb and Sb in soils were determined at the end of the 5 days period using standard Chelex-100 and ferrihydrite cylindrical DGT (Diffusive gradient in thin films) units (DGT Research, Lancaster, UK) with an active surface area of 3.14 cm². The DGT devices consisted of three layers I) a Chelex 100 binding resin layer II) a polyacrylamide diffusion gel layer and III) filter membrane 0.45 µm filter membrane covering the diffusion gel (Zhang et al., 2001; Ernstberger et al., 2002). To determine the PTE concentrations in the soils, two DGT probes were manually inserted into tested soils. After a 24 h period of insertion, metals and metalloids accumulated respectively in the Chelex-100 and ferrihydrite resin-gel layer were extracted by immersion for 24 h in 1 ml of 5% HNO₃ for the Chelex resin gel and 1 ml of 1M HCl for the ferrihydrite resin gel. These solutions were further diluted 10 times before analysis. The total mass of metal (M) accumulated per unit area of the DGT probe over its deployment time (t) was calculated as previously reported in Hattab et al. (2014). Successively, the R values were calculated from the [PTE_{DGT}] divided by [PTE_{SPW}]. Comparison of C_{DGT} with the independently measured SPW concentration C_{sol} (both determined at the same soil moisture content) provides a ratio (R) that gives an indication of the extent of depletion of SPW concentrations at the DGT interface (Ernstberger et al., 2002).

2.5. Plant growth and analysis

Dwarf beans (*Phaseolus vulgaris* L.) seeds were pre-germinated before planting. In detail, seeds were disinfected with commercial bleach (15 min in 0.5 % sodium hypochlorite) then rinsed in sterilized water (3 times 5 min) and germinated in water saturated perlite for 72 hours at 28°C in dark. Two germinated seeds were then planted in 2 L plastic pots and grown for 15 days in a controlled growth chamber (temperature:

day 25 °C and night 20 °C; light period: day 13 h and night 11 h; light intensity: 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$); six pots were used for each treatment and corresponded to a total of 12 plants per tested soil. At the end of the growth period (15 days), plant growth was calculated by measuring the plant height (hypocotyl and epicotyl height). Additionally, at the same time, biomass allocations in the different organs (roots, stem and leaves) were determined by measuring dry weight (DW). PTE concentrations in the different organs of *Phaseolus vulgaris* L. were measured using the protocol described in Bart et al. (2015) by ICP-AES (ULTIMA 2, HORIBA, Labcompare, San Francisco, USA).

2.6. Statistical analysis

Results were analyzed with the SPSS statistical software package (SPSS, Chicago, IL, USA). Means are expressed with their standard error and were compared by ANOVA. In each case, the number of replicates (n) is indicated. Statistical test were considered significant at $P \leq 0.05$.

3. Results

The garden soil (G) was used as a reference soil (Control). Results for the different treatments were compared to G modality results.

3.1. Soil and SPW characteristics

3.1.1. Soil characteristics

Table 2 shows the physico-chemical characteristics of the soils studied.

Garden soil (G) and Technosol (PF) have the same SWC corresponding approximately to 43%. When adding 5% of biochar to control soil (G), SWC increased significantly by 40% and reached 59%. No significant effect was observed when adding 2% biochar to garden soil. Concerning technosol (PF), 2% biochar increased SWC by 13% whereas 5% biochar increased SWC by 38%. For the mixture (PFG) only 5% biochar induced SWC increase by 45%.

pH measured in PF soil was lower by 1 unit compared to G which values were 8.2. For the control garden soil, only when adding 5% biochar a 0.2 pH unit increase was observed. When adding biochar to PF at 2% and 5% we obtained a linear pH increase corresponding to 0.4 unit and 1.3 units respectively. For PFG soil, only 2% biochar induced a slight pH increase of 0.2 unit.

EC measured for G ($164 \mu\text{S cm}^{-1}$) was 5 times higher than the one measured in PF ($33 \mu\text{S cm}^{-1}$). Addition of 5% biochar to G induced a 15% increased of EC, whereas 2% biochar addition had no significant effect. For PF and PFG, biochar addition increased EC. For PF this increase was of $15 \mu\text{S cm}^{-1}$ when adding 2% biochar and $53 \mu\text{S cm}^{-1}$ when adding 5% biochar. Biochar addition to PFG soil induced a linear EC augmentation corresponding approximately of $10 \mu\text{S cm}^{-1}$ per biochar percent. For PFG 5% the EC value was $170 \mu\text{S cm}^{-1}$.

In PF, total organic carbon (TOC) content (0.47%) was 8 times lower than for G (3.67%). Biochar amendment to G increased TOC by 2 times, only when adding 5% biochar. For PF, biochar incorporation caused a linear TOC content increase corresponding to 0.9 % per biochar percent. For PFG, 2% biochar did not affect significantly TOC content whereas 5% biochar enhanced 3.4 times TOC value which reached the value of 7.82%.

Concentrations of As, Pb and Sb in G, PF and PFG soils were measured only in non-biochar amended soils since no PTE were present in the biochar (Table 1). Total PTE concentrations in the garden soil G were 42.8, 30.1 and 4.9 mg·kg⁻¹ for As, Pb and Sb respectively. For the PF soil, the concentrations of As, Pb and Sb were respectively 24, 11 and 10 times higher (1028, 321 and 51 mg·kg⁻¹ respectively) than those measured for the control soil (G). For the treatment corresponding to the mixture of 50% technosol to 50% garden soil (PFG) (V/V), we obtained 574, 187 and 40 mg·kg⁻¹ for As, Pb and Sb respectively.

3.1.2. SPW characteristics

Table 3 shows the physico-chemical characteristics of the SPW collected before adding the *Phaseolus vulgaris* L. germinated seeds in the different soils tested. The pH of PF SPW was 5.9 ± 0.05 , whereas the garden soil (G) and the mixture between PF and G (PFG) pH were significantly more basic with values of 6.9 and 7.4 respectively. The biochar addition in G and PF soil increased significantly the SPW pH. Indeed for the 2% biochar addition, the increase corresponded to an 0.24 and 1 unit increase for G and PF conditions respectively, whereas for the 5% biochar addition this increase was more important and corresponded to 0.52 and 1.5 unit for G and PF conditions respectively. With regards to PF and G mixture (PFG), no strong difference was observed when adding biochar; the average pH for these conditions was approximately of 7.5. Garden soil (G) showed an EC 3 times higher than that measured in PF soil (902 and 275 $\mu\text{S cm}^{-1}$ for G and PF respectively). The addition of 50% of G soil to the PF soil significantly increased the EC of the PF soil to a level close to that measured in the G soil ($847 \pm 36 \mu\text{S cm}^{-1}$ compared to $902 \pm 21 \mu\text{S cm}^{-1}$ for PFG and G respectively). Two and 5% biochar addition to PF soil induced a 1.5 and a 1.8 times SPW EC increase respectively. When adding 2% biochar to G and PFG soil, no significant increase of EC was observed, whereas a 5% biochar addition induced a 13% and 15% increase respectively. The addition of 2% and 5% biochar did not affect DOC concentration in all treatments tested.

The addition of biochar to G soil did not impact SPW PTE concentration. As, Pb and Sb concentrations in G SPW were 0.07, 0.02 and 0.02 mg L⁻¹ respectively. When comparing PTE concentrations in PF and G, As, Pb and Sb concentrations were 224, 4.5 and 28.5 times higher respectively. Two and 5% biochar addition decreased the Pb concentration in PF SPW by 33% and 70% respectively ($0.06 \pm 0.01 \text{ mg L}^{-1}$, $0.02 \pm 0.01 \text{ mg L}^{-1}$) whereas Pb concentration in PFG SPW increase by 41% and 24% respectively ($0.06 \pm 0.01 \text{ mg L}^{-1}$, $0.05 \pm 0.01 \text{ mg L}^{-1}$). In the opposite, biochar addition in PF induced an As SPW concentration increase by 33% and 70% for 2 and 5% biochar respectively. For biochar-amended PFG soil, only 5% biochar induced a 55% As SPW concentration increase, compared to PFG. Two and 5% biochar amendment to PF induced a 2 times Sb concentration increase in SPW (1 mg L^{-1}). For PFG soil, only a 5%

biochar addition increased Sb concentration in SPW (0.21 mg L⁻¹ and 0.26 mg L⁻¹ for PFG and PFG5% respectively).

3.2. Soil exposure intensity of PTE (DGT)

As, Pb and Sb in control soil (G) were not detected in the three conditions analyzed, G, G2% and G5%.

PF amended with 5% biochar provided the highest concentration of available As compared with PF and PF 2%. The available concentrations of As measured in the PF 5% were 1.5 fold higher than PF soil. Addition of 2% and 5% biochar to PF decreased the A_{SR} values by 1.2 fold compared to the PF.

The 2% and 5% biochar addition to PF soil decreased significantly the available concentration of Pb measured by the DGT by 3 fold and 2 fold respectively compared to PF without biochar (Fig 1). The R values calculated from the [Pb_{DGT}] divided by [Pb_{SPW}] measured in the PF5% was 2 and 5 fold higher than that measured in the PF0% and PF2% respectively Fig 2. The Pb_R values in the three types of soil with and without biochar was as follows: PF2% < PF < PF5%.

Sb concentration in PF5% was highest compared with PF and PF 2%. The available concentrations of Sb measured in the PF 5% were 2 fold higher than that measured in the PF soil. PF2% and 5%, showed a Sb_R enhance of 0.8 fold compared to the PF. Addition of biochar to PFG soil had not any significant effect on the availability of As, Pb and Sb compared to the PFG; excepted on the availability of As measured in the PFG soil treated with 5% biochar.

3.3. Effect of biochar on plant growth (height and dry weight)

Differences in plant growth parameters were recorded in Fig. 3 according to soil composition and biochar amendment after 15 days of treatment. In the case of PF treatments even when adding biochar (PF, PF2%, PF5%), no growth was observed in the roots and in the aerial parts (all *Phaseolus vulgaris* L. germinated seeds died from the beginning of the experiment). Consequently, data corresponding to plant analysis for these treatments are missing in all the paper.

Under control conditions (G), biochar did not affect hypocotyl growth, whereas epicotyl growth was stimulated approximately by 40 % in both biochar treatments (Fig. 3(a)). Concerning PFG treatment, height was at least 2 times less important than G. Indeed, both hypocotyl and epicotyl development were negatively affected by biochar addition. Five % biochar produced a 2 times stem growth decreased when comparing PFG5% to PFG.

At the end of the experiment (day 15), biomass productions (FW and DW) were recorded in roots, leaves and stem (hypocotyl +epicotyl) for all treatments (Fig. 3(b-c)). As observed for height, FW and DW were positively affected by biochar addition in G treatments. Whereas for PFG treatments, FW and DW decreased for both biochar addition. The addition of 50% of G to PF allowed *Phaseolus vulgaris* L. to grow even if the biomass production was less important than G, approximately 2 times. For PFG treatments, biochar additions decreased leaves and stem biomass. Indeed, for 5% biochar, leaves and stem biomass

productions (FW and DW) were significantly affected by 75% and 50% for leaves and stem respectively, compared to PFG. For roots, biomass productions were always higher in G compared to PFG. However, biochar addition did not significantly affect roots biomass.

3.4. PTE concentration in indicator plant organs

No As was detected in stems of plant grown on G soil even when biochar was added. Moreover, biochar did not affect As concentration in both roots and leaves (Fig. 4). Lastly, for G and whatever biochar amendment rate, As concentrations in roots were 10 times higher compared to leaves. As concentrations in roots for PFG, PFG2% and PFG5% were approximately 600 mg kg^{-1} and corresponded to 20 times the value obtained for roots of G treatments. In leaves of *Phaseolus vulgaris* L. plants grown in PFG soil supplemented with biochar, the As concentrations were in between 5 and 9 mg kg^{-1} and did not display difference according to biochar addition. Finally, when adding biochar to PFG, As concentrations in stems increased significantly (5 times) compared to PFG and represented 9 times the values obtained in leaves.

In figure 5, no Pb was detected in the organs of plant grown on G, G2% and G5%. For PFG treatments, no Pb was detected in stem. In roots Pb concentrations were not significantly affected by biochar addition and were in between 16 and 21 mg kg^{-1} . Lastly, biochar 2% induced a 5 times decrease Pb concentration in leaves (16 mg kg^{-1} for PFG versus 3 mg kg^{-1} for PFG2%). No Pb was detected in leaves of PFG5% treated-plants.

For G biochar-amended soils, Sb concentrations measured in roots were similar (approximately 4 mg kg^{-1}) (Fig. 6). In stem, for all conditions (G, G2% and G5%), Sb concentrations were approximately 1.5 mg kg^{-1} . In the same conditions, Sb concentrations in leaves were not significantly affected by biochar and were included in between 2.4 and 4.7 mg kg^{-1} . For plant grown on PFG biochar-amended soils, no Sb concentration difference was observed in roots, the average of Sb concentration in this organ was ca. 40 mg kg^{-1} . In leaves of PFG treated plants, biochar addition induced a 2 times Sb decrease for both biochar concentration tested. On the opposite, biochar addition to PFG induced a significant Sb increase in stems (5.9 mg kg^{-1} , 31.6 mg kg^{-1} and 16.4 mg kg^{-1} for PFG, PFG2% and PFG5% respectively).

4. Discussion

4.1. Soil and SPW characteristics

The experiment reveals that soil biochar amendment (2% and 5%) have several contrasted effects. The addition of 5% biochar induced a 40% increase in SWC when added to the control soil G. The same behavior has been observed for PFG, with an increase of SWC by 43%, while for PF both biochar concentrations increased SWC significantly by 20 and 23% for PF2% and PF5%, respectively. Agegnehu et al. (2015) reported that application of 0.1 % biochar (obtained from willow wood) to a weathered soil increased SWC by 28%, the same results were also observed in other studies (Troy et al., 2014). These studies reported an increase of the water capacity due to high adsorption capacity and porous structure of biochar that allow holding water in the soil (Streubel et al., 2011). Our results showed also a pH significant increase in G5%,

PF5%, PF2% and PFG2% due to the high pH of the biochar itself (pH 8.2), as confirmed by Rees et al. (2015) using biochar obtained from wood on a smelter soil at a concentration of 5%. Chintala et al. (2014) demonstrated that over an increase of pH, biochar modifies also soil EC. The pH increase, with a concurrent decrease in the mobility of cationic metals in soils, is due to reduced competition between H^+ ions and metal ions for cation exchange sites either directly on the surface of biochar or as a general liming effect on the soil matrix. Addition of biochar into soil caused an increase in EC due to enhance of nutrient (cations and anion) leaching into the soil solution (Chan et al., 2008). TOC analysis showed a huge increase in presence of biochar, for all three soil (G, PF and PFG) in the same way as observed by Méndez et al. (2012) who found an increase of TOC values by 2 and 3 fold when adding 4 or 8% of biochar (from sewage sludge) in an agricultural soil. Biochar is known from a long time as an amendment to supply a range of agronomic benefits (Smith, 1980; Lin et al., 2012). These modifications of soil characteristics are due to the high carbon content of biochar, to its ability to retain water and to modify soil pH (Thies and Rillig, 2009).

As observed for all soils, SPW pH increased after biochar addition in all treatments. Such results were already observed by Puga et al. (2015a) when contaminated soils of a zinc mining area were amended by 1.5%, 3% or 5% of biochar produced from sugar cane straw. They found that there was a slight but significant ($p < 0.05$) increase in the SPW pH (about 0.3 units) when adding 5% biochar. Beesley et al. (2013) demonstrated similar results using a biochar obtained by orchard prune on soil impacted by mining activities in Mina Mónica (Madrid, Spain) known to have high concentrations of As. They found that in the case of biochar additions, SPW pH was significantly increased above that of the contaminated and control soils. In our study we did not observe any increase in DOC amount even if we observed a TOC increase. Kloss et al. (2014) showed a DOC decrease when adding 3% biochar (woodchip) to an agricultural soil. This effect is associated to the biomass and technics used to produce biochar, like heating rate, holding time and temperature. These parameters influence significantly biochar structural and chemical characteristics (Lin et al., 2012). Rostad et al. (2010) reported that biochars produced from cellulose, lignin, pine and switchgrass under pyrolysis temperatures ranging from 250°C to 900°C contained dissolved organic carbon, which was highest in the low temperature biochars and correlated with the higher aliphatic content and higher acid functional groups of these low temperature produced biochars. In conclusion, in our study the presence of biochar modified soil chemical (e.g. pH, CEC and EC) (Liang et al., 2006) and physical properties (e.g. soil water retention, hydraulic conductivity) (Ouyang et al., 2013).

Even if PTE mobility in a soil and SPW is able to change during time, depending on several factor such as pH, water content, mineral and organic content (Bolan et al., 2014), pH is one of critical parameter influencing the transfer of PTE in liquid phase from the soil solid phase. In detail, for metal cations (e.g. Cd and Pb) the mobility increase in acidic conditions and decrease in basic condition, whereas for anions (e.g. As and Sb) the behavior is exactly the opposite (Bolan et al., 2014).

As concentration in SPW of contaminated soils (PF and PFG) increased when biochar was added. These data are in agreement with Beesley et al. (2013) who found that after one week of biochar application, As concentration in soil pore water significantly increased (~9 fold). This As concentration increase in the

SPW was due to the pH values increase correlated to soil biochar addition. Zheng et al. (2012) demonstrated that when using a biochar obtained by rice on a mine soil there was a subsequent increase of As in SPW. It is widely reported that As activity in the soil solution is controlled by reactions of retention and release along the surfaces of Fe, Mn, and Al oxides and hydroxides (de Brouwere et al., 2004; Moreno-Jiménez et al., 2012a). Smith et al. (2002) and Stachowicz et al. (2008) determined that the presence of some cations (Ca^{2+} , Na^+ and Mg^{2+}) cause an increase in the As retention. Such other results were explained by sorption and desorption mechanisms associated to competition between As and P for binding sites. P is known to be an analog of As (Moreno-Jimenez et al., 2012b), consequently P increases As mobilization (Sadiq, 1997) since it competes with both arsenate and arsenite for binding sites at Fe-oxide surfaces in soils (Waltham and Eick, 2002). Beesley et al. (2013) reported that phosphorous concentrations measured in the SPW of mining soils treated with orchard prune biochar were more than 14 times greater than the one measured in the untreated contaminated soil. Further, as the pH increases, the number of positively charged species on mineral matrix decreases, which lowers the sorption capacity of negatively charged like As oxy-anions (Wilson et al., 2010). Biochar's physical structure may also influence the behavior of As. Klitzke and Lang (2009) explained that soil pH increase induced mobilization of humic acids which compete with As sorption. Additionally, a rise in pH often results in mobilization of arsenic in the soil (Beesley et al., 2010b; Moreno- Jiménez et al., 2012a).

In our experiment, the biochar addition showed a notable ability to reduce Pb solubility in SPW. One of the mechanisms suggested to explain this opposite behavior compared to As is the precipitation of metal(loid)s phosphates/carbonates which blocks Pb in the soil matrix. This mechanism has been proved to be efficient for Cd, Pb and Zn soil immobilization. These fairly stable metal-P compounds makes P application as an attractive technology for managing metal(loid)-contaminated soils in addition to biochar amendment (Bolan et al., 2014).

For Sb, the biochar addition to soil, increase the soil pH, which induced humic acids mobilization. Such mobilization could displace Sb from organic/inorganic binding sites (Klitzke and Lang, 2009) and made then more availability. Ahmad et al. (2014) found that biochar application to a contaminated soil increased Sb solubility and consequently its concentration in the SPW, which can be explain by the ability of the alkaline conditions ($\text{pH} \approx 8$) generated by the presence of biochar added to desorb Sb from the particles of soil (Johnson et al., 2005).

Several studies have also highlighted beneficial effects of biochar on the environment, such as adsorption of metal(loid)s and reduction of their toxicity, climate change mitigation (reduction of CO_2 release) and production of bioenergy (Atkinson et al., 2010; Kookana, 2010), making it a potential sustainable tool to improve soil quality and fertility (Lucchini et al., 2014).

4.2. PTE concentration in soil exposure intensity of PTE (DGT)

PF soil treated with 5% biochar provided the highest concentration of soluble and available As and Sb compared with PF and PF 2%. In general, we can notice that the addition of G to PF led to decrease the available concentration of As, Pb and Sb compared to the contaminated soil PFG 0%. In PFG soils, the

concentration of available As_{DGT} was negatively related with the phytoavailable concentration of As measure in plants. Mench et al. (2003), demonstrated that the addition of organic amendments to a contaminated gold mine spoil increased the As mobility. Moreover, they demonstrated also that the influences of organic amendments on the mobility, availability, and phytoavailability of As in such soil are not correlated.

Concerning Sb, a good relation was observed between mobile and phytoavailable concentrations in the stems and in the leaves of plants grown on PFG amended with 0%, 2% or 5% biochar. Such relation was not observed in roots. Namgay et al. (2010) investigated the effect of 3 rates of biochar (0%, 0.5% or 1.5%) on the availability of As, Cd, Cu, Pb, and Zn in a sandy soil. They showed that sorption of trace elements occurred in the following order: $Pb > Cu > Cd > Zn > As$. These results showed that biochar application can significantly reduce the availability of trace elements such as Pb and As to plants and suggest that biochar application may have potential for the remediation of soils contaminated by PTE. The pH may also influence As availability. Generally, soil $pH > 7$ increases the availability of anions such as arsenate and arsenite, while soil $pH < 7$ may favor the retention of anionic As due to an abundance of positive charges that neutralize the negative exchange sites in soil (Moreno-Jiménez et al., 2012b). Beesley et al. (2013) reported interesting results, finding that As concentration can increase in SPW after biochar addition, but transfer to the plant could be reduced. This would imply that at least some biochar could pose no risk of increasing PTE in plants and hence are safe in terms of food chain transfer, but leaching of As to nearby waters must be considered. Iron (hydr)oxides (Spuller et al., 2007), Al oxides and humic acids (Tighe et al., 2005) are reported to be, as with As, important sorbents for Sb in soil.

Our results showed that in PFG soils the presence of 2% or 5% biochar decreased significantly the concentrations of phytoavailable Pb. This decrease was not correlated with Pb concentrations in SPW. Moreover, no relation was observed between the available (DGT) and phytoavailable concentrations of Pb measured in plants grown on PFG soils. The lower availability of Pb can be explained by the presence of exchange sites on the surface of the biochar. These exchange sites are indeed responsible for the retention of PTE and their lower bioavailability (Fellet et al., 2014; Puga et al., 2015a). Shaheen and Rinklebe (2015) reported that the addition of organic materials such as biochar might reduce the Pb bioavailability by forming stable complexes with humic substances. These findings indicate that the biochar addition decreases the mobile fractions of Pb by transforming them to the less accessible organic fraction resulting in lower metal bioavailability to rapeseed plants. Sb was found to be strongly sorbed to amorphous Fe oxide and to hematite at $pH < 7$ (Tighe et al., 2005). In contrast to these mineral sorbents, the sorption of Sb to humic acids strongly decreases with increasing pH, which confirms our results (Tighe et al., 2005). The authors attributed an important role to humic acids retention of Sb in soils as high organic matter content inasmuch, as said before, could displace Sb from organic/inorganic binding sites. Based on these findings, one may presume that addition of biochar to the soil lead to an increase in dissolved and available Sb concentrations following a pH increase when organic matter is the main binding partner in soil (Klitzke and Lang, 2009). The R values calculated in our experience can be explained by the low concentrations of available As, Pb, and Sb measured (between 0 and 10 $\mu\text{g/l}$ for Pb and Sb, and between 0 and 450 $\mu\text{g/l}$ for As). Consequently,

the ratio between the available concentrations of PTE versus the mobile concentrations of PTE was very low (Vandenhove et al., 2007). Our results showed that the application of 2% or 5% of biochar increased the depletion of soil pore water concentrations at the DGT interface (Conesa et al., 2010). Hattab et al. (2014), demonstrated that when organic amendments (compost) was added to a copper contaminated soil, it reduced the available concentrations of PTE and as a result reduced also the R values in all the tested soils.

Although, the DGT technique is a promising tool compared to sequential extraction techniques to assess the available PTE concentrations in a soil, in our case the presence of some singular characteristics such as high heavy metal concentrations, low pH, could limit its application (Conesa et al., 2010); additionally, it may miss aspects related to biochemical processes in the rhizosphere, spatial heterogeneity in metal concentrations and preferential growth of roots (Moradi et al. 2009).

4.3. Effect of biochar on plant growth

The significant amelioration in *Phaseolus vulgaris* L. yield (epicotyl height, dry weight) for aboveground organs grown in G could be associated to an increase of nutrient availability and plant uptakes enhanced by biochar application (Asai et al., 2009; Uzoma et al., 2011). It is known that biochar ameliorate agricultural soil characteristics (Smith et al., 2010), by allowing a better nutrient availability to plant through an increase of energy source which permit a better growth of microorganisms responsible of soil fertility (Smith et al., 2010). For the roots, we did not record any modification of the biomass produced in such conditions. The same results were observed by Puga et al. (2015a) when growing 4 weeks *Phaseolus vulgaris* L. plants on a sugarcane straw biochar amended soil. Biochar is known to increase macronutrient solubility as K^+ , Ca^{2+} , Mg^{2+} , N (nitrate, nitrite and ammonium), P (phosphates) (Laird et al., 2010; Taghizadeh-Toosi et al., 2012) and to allow soil carbon storage (Yin Chan and Xu, 2009).

In the case of PF no *Phaseolus vulgaris* L. growth was observed with or without biochar amendment, this complete growth inhibition was probably correlated with the highest As SPW concentration measured for these treatments.

Conversely for the PFG treatment we observed an opposite effect compared with G which demonstrated a beneficial biochar growth effect. In contaminated soils, plants showed a reduction in development and biomass production after biochar addition as consequence of PTE leached in the SPW. In fact, although plants require certain metal(loid)s for their growth, excessive amounts of these elements can become toxic to plants leading to a decline in plant growth, which sometimes results in the plant death (Schaller and Diez, 1991). PTE can have a direct toxic effect due to the PTE structure but they can also induce damage to cell structures due to induction of oxidative stresses (Jadia and Fulekar, 2009); finally they can have an indirect toxic effect due a decrease of essential nutrients ions absorption by plants through ion competition (Taiz and Zeiger, 2002). Moreover since As, Pb and Sb are not required for plant metabolisms they do not play a beneficial role in plant growth, (Beesley et al., 2013; Caporale et al., 2013).

4.4. PTE concentration in indicator plant organs

To understand the negative effect of biochar amendment on *Phaseolus vulgaris* L. growth we measured the PTE repartition in plant.

Only in the case of As good correlation was observed between dissolved As concentrations in SPW and the As accumulation in *Phaseolus vulgaris* L. plant; no correlation was observed with DGT-measured As concentrations. Song et al. (2004) also observed a good relationship between the Cu contents in the shoots of both *Silene vulgaris* and *Elsholtzia splendens* and the Cu concentrations in soil pore water, whilst the correlation was less with Cu_{DGT} . It is widely reported that PTE phytoavailability in soil is controlled by complex interactions governed by many geochemical factors including pH, redox conditions, mineralogy, microbial conditions, organic and inorganic ligands and competing cation interactions (Shahid et al., 2012). Thus only a low PTE fraction is readily available for plant (Pourrut et al., 2013). In our case, the analysis of PTE plants content showed that biochar addition induced a different PTE accumulation in *Phaseolus vulgaris* L. organs collected from plants grown on PFG amended soils (Fig. 4, 5, 6). The As concentration in the *Phaseolus vulgaris* L. organs showed the highest content in roots, followed by stem and leaves. Such results have been also observed in tomatoes by Carbonell-Barrachina et al. (1997) who found that the 83.2% adsorbed As remained in the root system, 16.8% in the stems organs and only 7.3% reached the leaves. Similar results have been observed for rice (Rahman et al., 2007) and wheat (Pigna et al., 2009). This has been explained as a survival mechanism of As sensitive plants to limit the As translocation from roots to shoots and/or fruiting bodies (Caporale et al., 2013). It is known that plants can absorb arsenic in the two inorganic forms (As^V and As^{III}) through aquaporins and phosphate transporters. Physiological and electrophysiological studies have shown that As^V is an analog of P and share the same transport pathway in plants, corresponding to phosphate transporter 1 family, most of which are strongly expressed in roots (Zhao et al., 2009) this could explain the highest As levels in these organs associated to a possible As adsorption on root surfaces. In plants, As^V could be converted in As^{III} by the arsenate reductase and then a neutral As $(OH)_3$ form is used to transport As through aquaporins channels in order to be sequestered and neutralized into the vacuole (Bleeker et al., 2006; Ellis et al., 2006; Tripathi et al., 2007), although it can also be transported via the xylem to other plant tissues. Movement through the xylem is controlled by the transpiration stream flow, but is also influenced by membrane transport proteins (Zhao et al. 2009). In the present study, the As increased mobility in soil due to biochar amendment allow an higher As translocation and accumulation in stem, as confirmed also by Zheng et al. (2012).

In the literature, the root system has been demonstrated to represent a very effective barrier for Pb uptake and translocation (Gupta et al., 2013). As observed for As, the greatest Pb accumulation was measured in roots suggesting the majority of Pb remaining localized in root system and only a small fraction is mobile and reached leaves (Pourrut et al., 2013). In plants, more than 90% of Pb exist in insoluble forms (Wierzbiicka et al., 2007) and is mainly linked to the cell walls components, pectins, hemicellulose, cellulose or lignin (Jiang and Liu, 2010; Krzeslowska, 2011). They can be also associated to the middle lamella of the cell wall (Jarvis and Leung, 2001) or linked to the plasma membrane (Seregin et al., 2004). It has been also demonstrated that Pb can be precipitated as an insoluble form in the tissue intercellular space (Jarvis and

Leung, 2001). Therefore the lowest mobility of Pb into the plant, in comparison with other metals, could be associated to its strong cell wall affinity (Seregin et al., 2004; Wierzbicka et al., 2007). In PFG, biochar amendment reduced Pb leaves accumulation this decrease was proportional to biochar soil concentration. Such obtained results are in agreement with Houben et al. (2013), Zheng et al. (2012) and Puga et al. (2015b) which demonstrated the biochar effects on Pb phytoavailability and consequently its uptake by plant. Therefore, the plants grown in the presence of biochar have accumulated mainly Pb in roots avoiding its translocation to stems and leaves where it should have produced cellular damages.

In contrast to the Pb results, we found a Sb leaves accumulation even if its concentration decreased according to the increase of biochar amendment. In *Phaseolus vulgaris* L., stems may play a barrier function to Sb translocation into leaves. Tschan et al. (2009) showed that the roots are the main Sb target but more often, several plant species accumulates Sb in the basal leaves and stem. They suggested that Sb reaches the plant upper part through the apoplastic pathway by passing an incompletely sealed or damage Casparian strip. As confirmed by Ren et al. (2014), cell wall can be considered as the first barrier against Sb entering cells where it was predominantly accumulated by its interaction with wall constituents, such as lignin and cellulose.

5. Conclusions

In the present work, the effect of biochar addition on the mobility, (phyto)availability and soil exposure intensity of three PTE, As, Pb and Sb, largely present in mine technosols, were evaluated in contaminated technosol amended with garden soil and biochar. In detail, the analysis was performed comparing the PTEs level in soil solution and *Phaseolus vulgaris* L. plants grown on uncontaminated and contaminated soils treated with a biochar rate of 0%, 2% and 5% (w/w).

Results showed that the 2% and 5% biochar applied rates had significant effect on PTE mobility and phytoavailability. PF which present the highest PTE total concentration did not allow plant growth. The biochar addition at 2% and 5% to this soil induced an increase in pH and EC and a correlate enhancing of Sb and As mobility and phytoavailability; on the opposite Pb content were reduced by the biochar amendments.

Biochar application to PFG soils induced a slight increase in pH and EC only at 5% rate probably due to alkaline pH and high EC values; for that, only in PFG5% an increase in As phytoavailability was observed. However, *Phaseolus vulgaris* L. plants grown on these soils, besides As content increase, showed a decrease in Pb and Sb content, especially in their leaves.

In conclusion, biochar soil amendments can reduce Pb mobility and/or bioavailability, however Sb and As mobility were increased. These results suggest mainly a negative effect of biochar on As soil stabilization which probably is responsible of the dwarf *Phaseolus vulgaris* L. biomass decrease.

However, to have a complete picture about biochar role in metal(loid)s soil stabilization, further experiments should include long term biochar field application on different kinds of soils, levels, and types/dose of metal(loid)s contamination.

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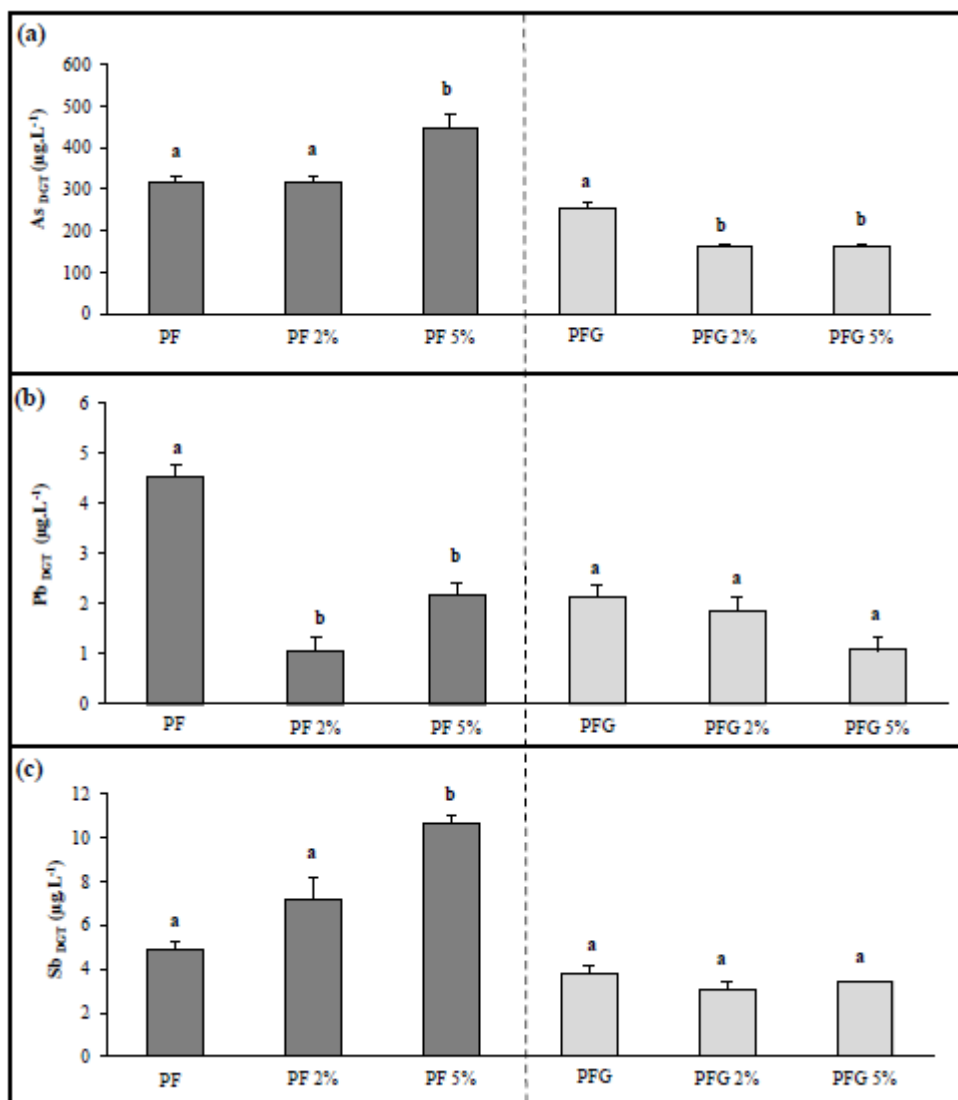
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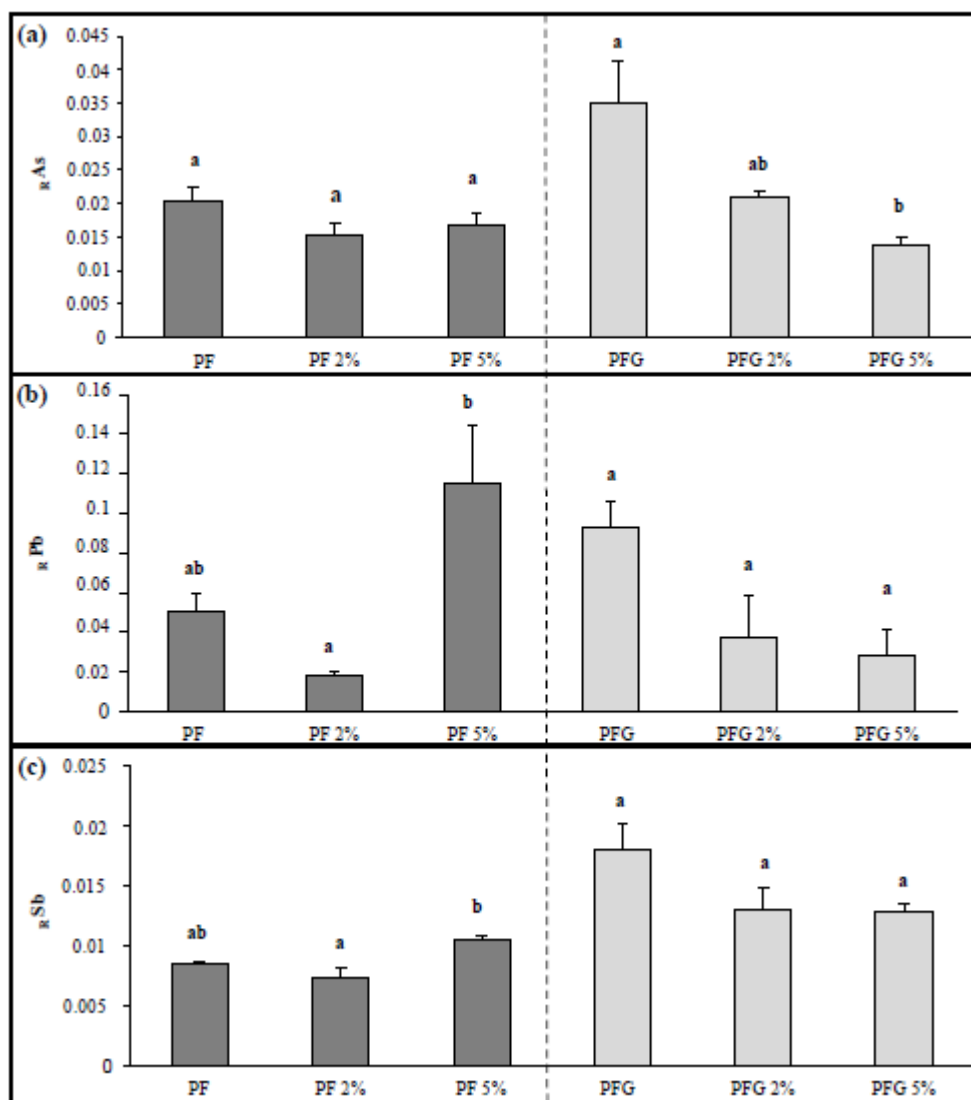
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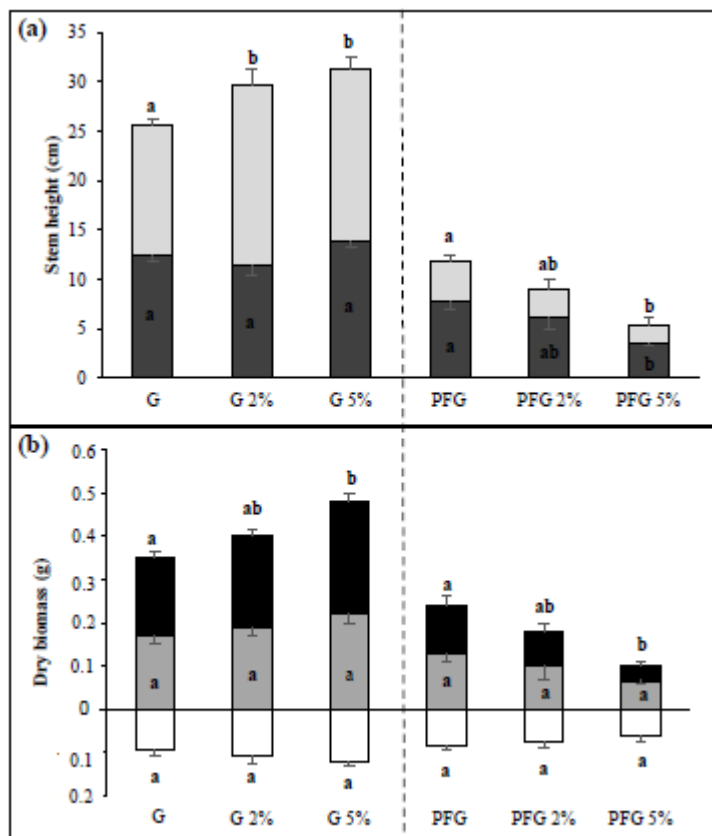
Values are mean \pm SE (n=3). Different letters on bar graphs indicate a significant difference ($p < 0.05$).

Fig. 1: Labile metal(loid)s concentrations (a=As, b=Pb and c= Sb) determined by DGT. PF = technosol; PF 2%= PF with 2% biochar; PF 5%= PF with 5% biochar; PFG= 50% technosol+50% garden soil; PFG 2%= PFG with 2% biochar; PFG 5%= PFG with 5% biochar.



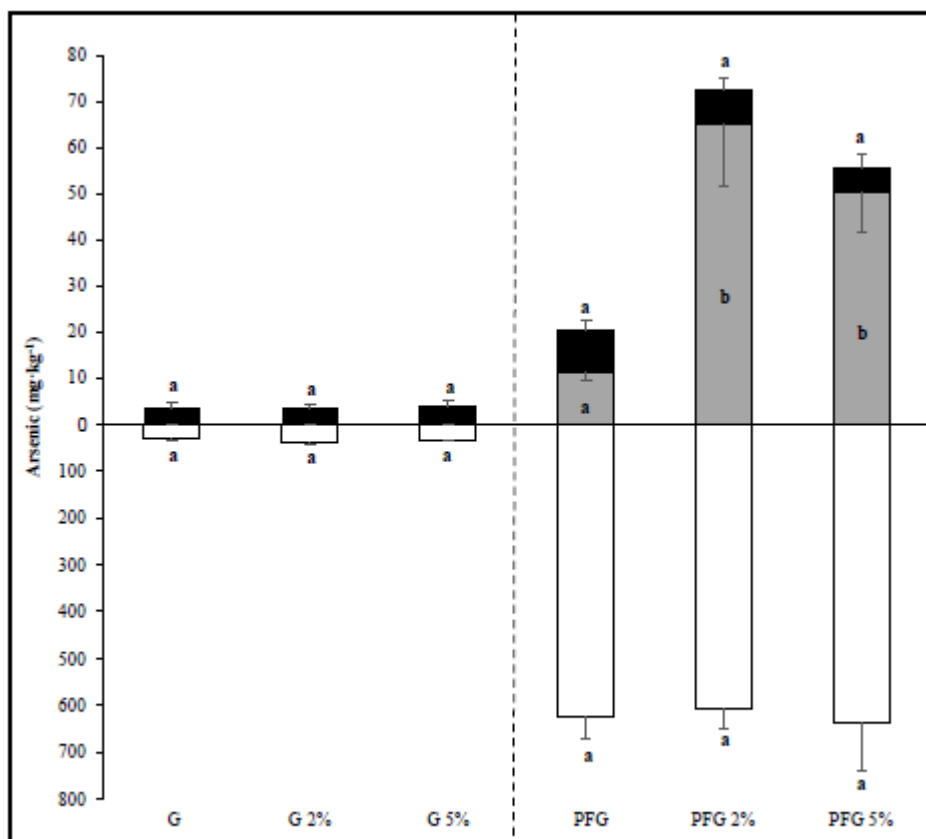
Values are mean \pm SE (n=3). Different letters on bar graphs indicate a significant difference ($p < 0.05$).

Fig. 2: R values of metal(loid)s (a =As, b= Pb, c= Sb) in soils determined as DGT calculations divided by total dissolved SPW concentrations (CDGT/CSPW). PF = technosol; PF 2%= PF with 2% biochar; PF 5%= PF with 5% biochar; PFG= 50% technosol+50% garden soil; PFG 2%= PFG with 2% biochar; PFG 5%= PFG with 5% biochar.



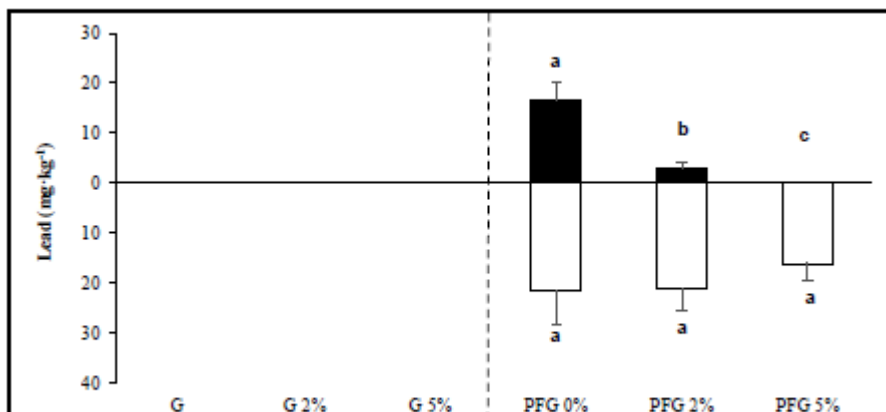
Values are mean \pm SE (n=12). Different letters on bar graphs indicate a significant difference (p<0.05).

Fig. 3: Plant indicator measurement after 15 days of growth, (a) stem height (hypocotyl, epicotyl), (b) dry biomass of the different organs (roots, stems, leaves). G= garden soil; G 2%= G with 2% biochar; G 5%= G with 5% biochar; PFG= 50% technosol+50% garden soil; PFG 2%= PFG with 2% biochar; PFG 5%= PFG with 5% biochar.



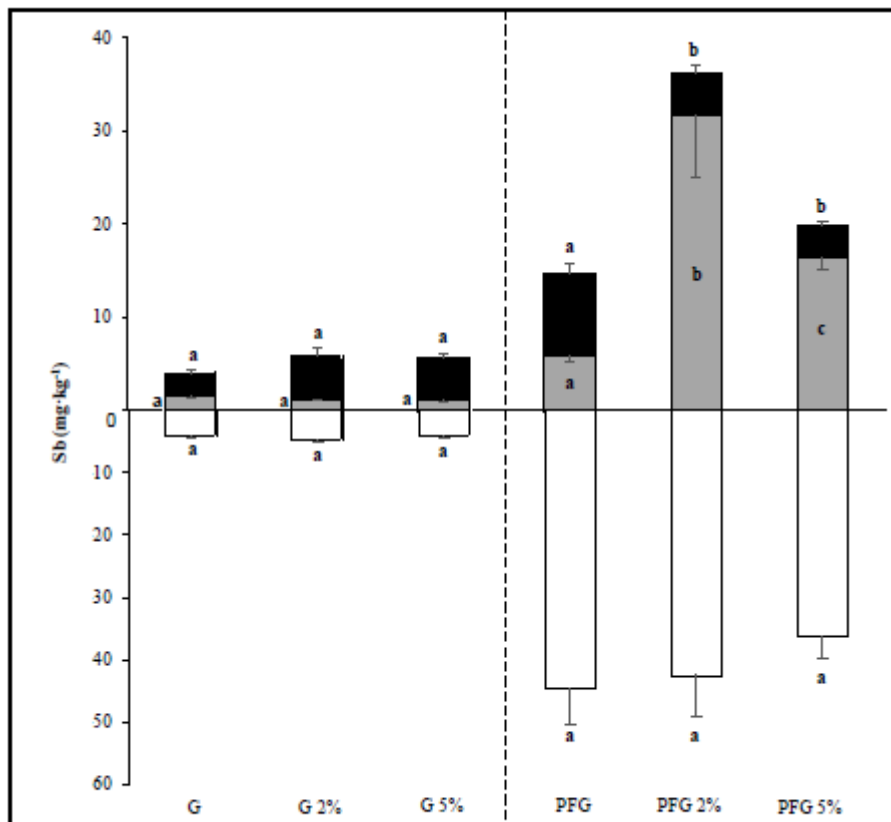
Values are mean \pm SE (n=12). Different letters on bar graphs indicate a significant difference ($p < 0.05$).

Fig. 4: Arsenic concentration in different organs (root, stems, leaves) of beans after 15 days of growth in different soil. G= garden soil; G 2%= G with 2% biochar; G 5%= G with 5% biochar; PFG= 50% technosol+50% garden soil; PFG 2%= PFG with 2% biochar; PFG 5%= PFG soil with 5% biochar.



Values are mean \pm SE (n=12). Different letters on bar graphs indicate a significant difference ($p < 0.05$).

Fig. 5: Lead concentration in different organs (root, stems, leaves) of beans plant after 15 days of growth in different soil. G= garden soil; G 2%= garden soil with 2% biochar; G 5%= garden soil with 5% biochar; PFG= 50% technosol+50% garden soil; PFG 2%= PFG with 2% biochar; PFG 5%= PFG with 5% biochar.



Values are mean \pm SE (n=12). Different letters on bar graphs indicate a significant difference ($p < 0.05$).

Fig. 6: Antimony concentration in different organs (root, stems, leaves) of beans plant after 15 days of growth in different soil. G= garden soil; G 2%= garden soil with 2% biochar; G 5%= garden soil with 5% biochar; PFG= 50% technosol+50% garden soil; PFG 2%= PFG with 2% biochar; PFG 5%= PFG with 5% biochar.

Table 1: Main Biochar physico-chemical properties (VTGreen)

Physico-chemical Parameter	Value
pH	8.2
EC (mS/cm)	9
Density	0.125
Water-insoluble (%)	85.2
Total exchange capacity (meq/kg)	46
Total porosity (%)	96
Water retention capacity (%) v/v	85
Retention capacity for air (%) v/v	11
Major elements	
Total Nitrogen (%)	<0.20
Total Organic Carbon (%)	73.7
P ₂ O ₅ (%)	<0.07
K ₂ O (%)	0.14
CaO (%)	0.36
MgO (%)	0.10
Na ₂ O (%)	<0.03
Sulfur (%)	<0.10
Trace elements	
As (mg/Kg)	<0.50
Cd (mg/Kg)	0.050
Cr (mg/Kg)	16.5
Co (mg/Kg)	0.54
Hg (mg/kg)	0.004
Mo (mg/Kg)	0.62
Ni (mg/Kg)	11.1
Pb (mg/Kg)	2.36
Se (mg/Kg)	<1

Table 2: Soil physico-chemical characteristics (soil water content, pH, EC) and PTE concentrations in garden soil (G), technosol (PF) and in the mixture prepared by mixing 50% of technosol and 50% of garden soil (PFG). Tested soils were amended with 0%, 2% or 5% biochar.

	G	G 2%	G 5%	PF	PF 2%	PF 5%	PFG	PFG 2%	PFG 5%
Soil									
SWC (%)	42.42 ± 0.73 a	44.1 ± 0.42 a	59.18 ± 1.3 b	43.47 ± 0.18 a	49.02 ± 0.29 b	59.75 ± 1.1 c	36.84 ± 0.41 a	41.07 ± 0.81 a	53.58 ± 2.05 b
pH	8.2 ± 0.048 a	8.13 ± 0.08 a	8.43 ± 0.067 b	7.15 ± 0.11 a	7.53 ± 0.045 b	8.42 ± 0.063 c	8.05 ± 0.054 a	8.3 ± 0.051 b	8.21 ± 0.067 ab
EC (µS cm ⁻¹)	163.86 ± 3.62 a	158.16 ± 2.77 a	187.66 ± 2.53 b	33.4 ± 0.43 a	48.4 ± 1.43 b	85.6 ± 1.33 c	141 ± 1.23 a	150 ± 1.2 b	170 ± 5.98 c
TOC (%)	3.67 ± 0.93	4.90 ± 1.01 a	6.98 ± 1.25 b	0.47 ± 0.01 a	2.15 ± 0.31 b	4.89 ± 0.95 c	2.31 ± 0.87 a	3.83 ± 1.05 a	7.82 ± 1.98 b
As (mg·kg ⁻¹)	42.76 ± 6.41			1027.88 ± 226.2			573 ± 136.2		
Pb (mg·kg ⁻¹)	30.14 ± 4.52			321.07 ± 19.82			186.86 ± 19.02		
Sb (mg·kg ⁻¹)	4.93 ± 0.74			51.52 ± 1.25			40.56 ± 11.14		

Table 3: Soil pore water (SPW) physico-chemical characteristics (pH, EC and DOC) and PTE concentrations determined in garden soil (G), technosol (PF) and in the mixture prepared by mixing 50% of technosol and 50% of garden soil (PFG). Tested soils were amended with 0%, 2% or 5% biochar.

	G	G 2%	G 5%	PF	PF 2%	PF 5%	PFG	PFG 2%	PFG 5%
pH	6.87 0.065a	± 7.11 0.017 b	± 7.39 0.039 c	5.94 0.043 a	± 7.03 0.027 b	± 7.42 ± 0.02 c	7.44 0.02 a	± 7.49 0.013 a	± 7.57 0.014 ab
EC (µS cm ⁻¹)	902.5 21.45 a	± 978 25.14 ab	± 1019 25.52 b	274.63 ± 5.1 a	404 19 b	± 489 ± 24 c	846.7 35.7 a	± 859.7 21.2 a	± 973 ± 27 b
DOC (mg L ⁻¹)	31.44 L- 1.65 a	± 31.51 1.02 a	± 35.41 1.23 a	21.60 ± 2.63 a	21.80 3.44 a	± 27.37 ± 3.90 a	32.77 3.00 a	± 34.33 1.15 a	± 32.26 2.11 a
As (mg L ⁻¹)	0.07 0.01 a	± 0.07 0.01	± 0.04 a 0.00 a	15.70 ± 0.97 a	21.00 0.92 b	± 26.80 ± 0.57 c	7.68 1.11 a	± 7.59 0.06 a	± 11.90 a 0.62 b
Pb (mg L ⁻¹)	0.02 0.00 a	± 0.02 0.00	± 0.03 a 0.00 a	0.09 0.01 a	± 0.06 0.01 b	± 0.02 ± 0.01 c	0.02 0.00 a	± 0.06 0.01 b	± 0.05 0.01 ab

Sb	(mg	0.02	± 0.02	± 0.02	±	0.57	0.98	± 1.01	0.21	± 0.23	± 0.26	±	
L-1)		0.00	a 0.00	a 0.00	a	±0.01	a 0.02	b ±	0.00	a	0.00	a 0.01	b
								0.01					b

Letters indicate a significant difference ($p < 0.05$).

Highlights

- Biochar treatments improve the soil quality and reduce mobility and (phyto)availability of Pb in soil pore water.
- Biochar treatments increase plant biomass production and influence metal uptake.
- Biochar decrease the labile concentration of Pb while increasing the As and Sb solubility