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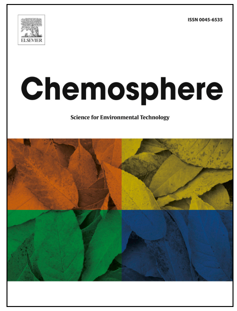

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Abstract

Soil pollution by metal(loid)s is one of the most significant problems in Europe. To remediate and potentially rehabilitate these contaminated sites, phytoremediation procedures are being put into place, often using amendments to help offset the extreme conditions of such soils. The aim of this study was to define the best amendment to use on the field. This was done by studying how the addition of three different amendments (biochar, compost and iron grit), alone or in combination, could affect: (i) soil physico-chemical properties, (ii) *Salix viminalis* growth, and (iii) metal(loid) stabilization. A 69 day-mesocosm study was thus set up using a former mine technosol, the three amendments applied alone or combined, and *S. viminalis* cuttings. The results showed that biochar and/or compost improved the soil fertility and the soil pore water characteristics, with reductions of acidity, metal(loid) mobility and toxicity, while iron grit amendment presented negative effects on such parameters. Such ameliorations allowed better plant growth and higher biomass production. In addition, stress indicators (leaf pigment content and root guaiacol peroxidase activity) showed a reduction in plant stress following biochar and/or compost application. Finally, among the different treatments, the use of compost or a biochar-compost combination showed better results in terms of improvement of soil conditions, increase in plant growth and reduced translocation of metal(loid)s towards upper parts, making these two treatments a valuable option for a field trial.

Keywords: biochar, compost, iron grit, metal(loid)s, Phytomanagement, *Salix viminalis*

Highlights

Biochar and compost amendments, alone or combined, improved soil fertility

The dose application of iron grit had negative effects on plant growth

Biochar and/or compost amendments improved the growth of *Salix viminalis*

Metal(loid)s were mainly accumulated in roots with low translocation to upper parts

Abbreviations

DOC = dissolved organic carbon

DW = dry weight

EC = electrical conductivity

GPOD = guaiacol peroxidase

OM = organic matter

SOM = soil organic matter

SPW = soil pore water

WHC = water holding capacity

1 Introduction

Soil pollution is an important issue in Europe, which has three million possibly polluted sites (Khalid et al. 2016). The majority of these sites (around 35%) are polluted by metal(loid)s (Panagos et al. 2013), which is why metal(loid)s are one of the eight major threats to European soils (Kidd et al. 2015). Metal(loid) soil pollution, coming from anthropogenic activities, *i.e* mining and smelting activities, agricultural use of fertilizers, industrial and commercial activities, transports (Khalid et al. 2016, Panagos et al. 2013), has adverse effects on both the environment (loss of biodiversity and ecosystemic functions) and human health (cancer, interference with enzyme activity) (Hughes 2002, Sharma and Agrawal 2005).

Consequently, metal(loid) polluted soils need to be remediated. One of the techniques used nowadays for soil rehabilitation is phytoremediation, which is the use of green plants and their associated microorganisms to lower the toxic effects of the environmental contaminants (Khalid et al. 2016). Phytoremediation has many advantages compared to conventional physical and chemical remediation techniques. It is a low cost and environmentally friendly technique that is well accepted by the public (Paz-Ferreiro et al. 2014). Furthermore, it restores the biodiversity and the soil functions. Different strategies exist in phytoremediation, depending on the goals and soil pollution type. However, phytostabilization is most suited for large highly contaminated areas needing rapid rehabilitation (Gomes et al. 2016). It relies on the introduction of plants which can stabilize pollutants at the root zone (roots and rhizosphere) by accumulation or precipitation, thus reducing contaminant mobility and bioavailability (Mahar et al. 2016). Moreover, the vegetation cover prevents wind erosion and water leaching.

Different plant species can be used in phytoremediation and the choice of the best species is a key parameter for successful phytoremediation. Willow trees are suitable candidates for phytostabilization (Chen et al. 2014), as they are fast growing plants and produce a large biomass associated to a deep and wide root system (Marmiroli et al. 2011). They can also tolerate metal(loid) stress and accumulate substantial amounts of metal(loid)s in their tissues. For instance, Kacálková et al. (2014) showed that different willow and poplar clones exhibited normal growth on a copper contaminated soil, while a study previously done by our team showed that *Salix viminalis* L. was able to survive, albeit with reduced growth, on a multi-contaminated mine site (Lebrun et al. 2017). Moreover, *S. viminalis* and *S. purpurea* were able to accumulate high amounts of As, Pb and Sb when grown on a multi-contaminated site (Bart et al. 2016).

However, the difficult conditions encountered in some polluted soils, such as extreme pH, low nutrient contents and high metal(loid) concentrations, make it difficult to establish a vegetation cover. Therefore, in such context, phytoremediation installation requires the application of amendments to improve soil physico-chemical properties, to allow plant growth (Galende et al. 2014). One amendment gathering attention for assisted phytoremediation in recent decades is biochar. It is a porous, carbon rich material obtained from the pyrolysis of biomass under low oxygen conditions (Barrow 2012, Paz-Ferreiro et al. 2014). It is characterized by an alkaline pH, a high cation exchange capacity, a large surface area and a high water holding capacity (WHC) (Ding et al. 2017, Lee et al. 2013, Paz-Ferreiro et al. 2014). Biochar is mainly made up of carbon (between 29 and 90 % (Cha et al. 2016)), hydrogen, oxygen, nitrogen and sulfur, as well as other trace elements (Tan et al. 2017). Such properties make biochar a good conditioner to improve the soil's physico-chemical properties, thus making plant growth possible (Rizwan et al. 2016). Moreover, its large surface area and high amounts of oxygen containing functional groups enable biochar to sorb metal(loid)s, reducing their solubility and mobility (Ding et al. 2017, Paz-Ferreiro et al. 2014). However, biochar's available nutrient levels are sometimes low (Fischer and Glaser 2012), which means they may need to be provided by another amendment, such as compost. Compost is the product of conversion and reclamation of organic materials by microbial degradation (Huang et al. 2016). It is rich in humus substances and microorganisms, and contains large amounts of plant nutrients (N, P, K, Ca, Mg, S) and other essential trace elements (Fischer and Glaser 2012). In addition to improving soil fertility (Alvarenga et al. 2013, Gil-Loaiza et al. 2016), compost has the potential to sorb metal(loid)s (Fischer and Glaser 2012, Huang et al. 2016). However, although biochar and compost soil application showed (i) improvements in soil conditions (Agegnehu et al .2016; Hmid et al. 2015), as well as (ii) soil cationic metals sorption (Hmid et al. 2015; Nie et al. 2018), they are less efficient in stabilizing metal anion such as arsenic, and can even increase its mobility sometimes, due to soil pH increase (Beesley et al. 2011). This can be problematic in multi-contaminated soils polluted by both cationic and anionic metal(loid)s. On the contrary, iron amendments, such as iron grit, have been shown to reduce As mobility and soil pore water concentrations efficiently (Kumpiene et al. 2006), due to As adsorption on iron hydroxides (Miretzkly and Cirelli 2010).

Therefore, in multi-contaminated soils, the best solution may be to combine different amendments. Indeed, Ruttens et al. (2006) showed that using compost in combination to cyclonic ashes and steel shots permitted to immobilize the metal(loid)s using different mechanisms and on different binding sites. We hypothesized that the association of two or three amendments with contrasting properties would lead to an increased improvement in soil conditions and plant growth compared to the application of single amendments. Indeed, through their diverse properties and positive effects on soil, the different amendments should complement each other. Consequently, the aim of this study was to evaluate the effects of adding three different amendments (biochar, compost and iron grit) to an As and Pb contaminated soil, and select an amendment or a combination of amendments showing the best results. The amendments were added to the soil either alone or combined, in order to study the effects on: (i) the soil physico-chemical properties, (ii) *S. viminalis* growth and metal(loid) compartmentation, and (iii) metal(loid) soil stabilization.

2 Material and Methods

2.1 Study site

A former silver-lead extraction mine site located in Pontgibaud was studied. The contaminated disbursed and crushed technosol covers 15 ha. This mine history, soil sampling and properties were described in previous works (Lebrun et al. 2018a, b). In addition, soil total CHNS contents were determined and gave the following results: C 0.14 ± 0.03 %, H 0.22 ± 0.00 %, N 0.09 ± 0.02 % and S 0.87 ± 0.14 %.

2.2 Amendments

Three amendments were applied to the mine contaminated soil: a commercial biochar, a commercial compost and industrial iron grit.

The first amendment was biochar, provided by La Carbonerie (Crissey, France). It was obtained by the slow pyrolysis of hardwood biomass (*Quercus sp.*, *Carpinus sp.* and *Fagus sp.*) at 500 °C (residence time: 3 h, heating rate: 2.5 $°C.min^{-1}$), followed by a sieving to obtain a particle size between 0.2 and 0.4 mm. This biochar had already been used in a previous study by Lebrun et al. (2018a), together with three other biochars obtained from the same feedstock but presenting different particle sizes (inf. 0.1 mm, 0.2 – 0.4 mm, 0.5 – 1 mm, 1 – 2.5 mm). Based on the results of this previous study, the 0.2 – 0.4 mm particle size biochar was selected as it showed a better ability to increase soil fertility, plant growth and metal(loid) stabilization than the coarser biochars. Moreover, compared to the finest biochar (inf. 0.1 mm), the 0.2 – 0.4 mm biochar particle size had a lower risk of leaching, making it more suitable for a possible field application. The biochar characteristics, and the methods used for the characterization are described in Table S1. The second amendment used was a commercial compost, made from manure of different animal origins (horse, cow, pig, chicken) and vegetable materials (compost KB, Scotts, France). The compost was applied directly, without sieving. Compost characteristics are described in Table S1. The last amendment used was iron grit, an industrial product used as an angular abrasive in the paint industry, made up of 97 % iron in addition to other mineral elements (C, Mn, Si, P and S) in low concentrations, and sieved to 0.5 mm. pH and EC were 7.64 ± 0.33 and 110 ± 36 µS.cm^{-1}, respectively (determined using the method described in Lebrun et al. 2018a).

2.3 Experimental design

A mesocosm experiment was set up using the following seven treatments: (i) unamended Pontgibaud technosol (P), (ii) Pontgibaud amended with 5% biochar (w/w) (PB), (iii) Pontgibaud amended with 5% compost (w/w) (PC), (iv) Pontgibaud amended with 1.5% iron grit (w/w) (PI), (v) Pontgibaud amended with 5% biochar (w/w) and 5% compost (w/w) (PBC), (vi) Pontgibaud amended with 5% biochar (w/w) and 1.5% iron grit (w/w) (PBI) and (vii) Pontgibaud amended with 5% biochar (w/w), 5% compost (w/w) and 1.5% iron grit (w/w) (PBCI). Amendment application doses were chosen based on previous studies (Codling and Dao 2007; Feng et al. 2016; Lebrun et al. 2018a; Ruttens et al. 2006). These different mixtures were put into 2L plastic pots (1.8 kg) (n=19), and one non-rooted cutting of *S. viminalis* L. was placed in 14 pots, while five pots were let unvegetated. The plants were watered every two days and grown for 69 days in a greenhouse with the following conditions: temperature 22 ± 2 °C, light intensity 800 µmol.m^{-2}.s^{-1}, photoperiod 16 h and irrigation was provided every two days based on the water lost through evapotranspiration.

2.4 Substrate analyses

After the mixtures were prepared, the different substrates were analyzed for WHC, as described in Lebrun et al. (2018a). In addition, three simple extractions were performed: the first one using aqua-regia (3 mL HCl + 6 mL HNO_3) digestion, which corresponded to the pseudo-total As, Fe and Pb concentrations. The second one determined the $CaCl_2$-extractable As, Fe and Pb fractions, while the last one determined the NH_4NO_3-extractable fractions, both according to the method described in Pueyo et al. (2004): 0.01 M $CaCl_2$ (1:10 solid:liquid ratio) or 1 M NH_4NO_3 (2:5 solid:liquid ratio). After the different digestions, As, Fe and Pb concentrations were measured by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (ULTIMA 2, HORIBA, Labcompare, San Francisco, USA). A calibration of the apparatus was done before each analysis sequence.

After plant growth (T69), soil samples were taken from non-vegetated pots (corresponding to bulk soil) and vegetated pots (corresponding to rhizosphere soil) by shaking the roots into a plastic bag to remove the soil attached to them. Soil organic matter (SOM) contents were determined on these samples by the loss-on-ignition method at 600°C, using a muffle furnace (Nabertherm L9/11/C6, GS, Geprufte Siderheit, Germany).

2.5 Soil pore water (SPW) sampling and analysis

SPWs were sampled twice during the experiment: before the introduction of cuttings, corresponding to T0, and before plant harvest, corresponding to T67. Sampling was performed using soil moisture samplers (Rhizon) (model MOM, Rhizosphere Research Products, Wageningen, The Netherlands), as described in Lebrun et al. (2017). SPW samples (20 mL) were used directly to measure pH (pHmeter, FE20/EL20, Mettler-Toledo AG2007), and EC (conductimeter SOLEA, Tacussel electronique, Lyon, France), and to realize a toxicity test (Qu et al. 2013). This test was performed on microplates using *Photobacterium phosphoreum*: 50 µL of SPW sample was mixed with 50 µL of phosphate buffer (100 mM, pH 7.0) and 50 µL of bacterial suspension (A_{600} = 1). The microplate was covered and incubated at room temperature for 30 min, the luminescence was measured in a luminometer (PolarStar Omega, BMG Labtech). Relative luminescence was calculated using Pontgibaud treatment (P) at T0 as the reference, and using the following equation:

$$\text{(i) Relative luminescence} = \frac{\text{luminescence of the sample}}{\text{luminescence of the reference}}$$

Finally, As, Fe and Pb concentrations in SPW were determined after acidification (83.3 µL HNO_3 in a 5 mL sample) by ICP-AES.

2.6 Plant analysis

2.6.1 Leaf measurements

After 69 days of growth, leaf pigment (chlorophyll, flavonoids and anthocyanins) contents were measured using a mobile sensor clip (DUALEX SCIENTIFIC^{+TM} FORCE A) (Cerovic et al. 2012) on the three last mature leaves. At harvest time and for each plant, all leaves were photographed, and the images were used to determine total leaf surface area using ImageJ software.

2.6.2 Root analysis

At the end of the experiment time course, root guaiacol peroxidase (GPOD) activity was determined on three plants per treatment as follows: for the enzyme extraction, 1 g of fresh root was crushed in 12.5 mL buffer (Tris-HCl 0.05 M, EDTA 0.01 M, $MgCl_2$ 0.003 M, pH 7.0) in an ice-cold mortar and centrifuged twice (5000 x g, 15 min, 4 °C). The supernatant, corresponding to the enzyme extract, was collected and stored at -20 °C until further analysis.

Guaiacol peroxidase activity measurement was adapted from Marchand et al. (2016). It was performed in microplates by adding 150 µL phosphate buffer (0.05 M, pH 6.1), 60 µL H_2O_2 (1 %), 60 µL guaiacol (80 mM) and 30 µL of enzyme extract to a microplate. The reaction was started by adding the enzyme extract, and the increase in absorbance at 390 nm was assessed for 1 min and 30 sec. The activity was calculated using $\varepsilon = 26.6\ mM^{-1}.cm^{-1}$ and expressed as the quantity of guaiacol transformed in one second. The total protein quantity of the enzyme extract was measured according to the Bradford method (Bradford, 1976) using SAB as standard.

2.6.3 Organ dry weight and metal(loid) distributions

At the end of the experiment, newly formed organs (leaves, stems and roots) were harvested separately and dried at 60 °C for 72 h to determine their dry weight (DW). Metal(loid) (As, Fe and Pb) concentrations were then determined by ICP-AES, according to Bart et al. (2016). Finally, total metal(loid) organ contents were calculated using the following equation:

$$\text{(ii) Metal(loid) quantity} = [metal(loid)]organ\ x\ DWorgan$$

2.7 Statistical analyses

The data were analyzed statistically using the R software version 3.1.2 (R Development Core Team, 2009). The treatments were compared two at a time by testing the normality and homoscedasticity, using the Shapiro test and the Bartlett test respectively. Next, the means were compared using a Student test for normal data or a Wilcox test for non-normal data. In addition, for the soil pore water parameters, the time and plant effects were analyzed using an Anova test for normal data or a Kruskal test for non-normal data, followed by a post-hoc test, TukeyHSD or Pairwise.wilcox, respectively. The difference was considered significant at $p < 0.05$. Finally, Pearson correlation coefficients were calculated between the different parameters, with the significant ones ($p < 0.05$) being cited in the following section.

3 Results and Discussion

3.1 Substrate characteristics

3.1.1 Soil physico-chemical properties

The unamended Pontgibaud technosol presented a low WHC of about 29.80 % (Table 1). Apart from for the iron treatment alone (PI), which led to a slight but significant decrease of the soil WHC, all the other amendment treatments improved the soil WHC. Biochar and compost amendments increased soil WHC, with their combination (PBC) leading to the highest WHC increase, which was also observed by Liu et al. (2012). Such results could be due to (i) the high WHC of the amendments (Akhtar et al. 2014), which values in our experiment were 183 % and 299 % for biochar and compost respectively, this can be explained by the biochar porous structure, hydrophobicity and high surface area (Molnar et al. 2016), and (ii) compost improvement of the soil structure by increasing the micro- and macro-porosity (Celik et al. 2014). Furthermore, soil WHC variations can also been explained by the increase of soil organic matter content, as organic matter (OM) can absorb water and improve the soil pore system, leading to a better soil structure retention (Giusquiani et al. 1995).

Moreover, the intensity of the increase in WHC with biochar and compost amendments (PB, PBC) was lowered when iron-grit was added with the organic material (PBI and PBCI). Such observation has not been previously described, but could be explained by the fact that iron grit is known for being unable to retain water, contrary to biochar and compost.

SOM content (Table 1) is an important parameter for soil quality, as it can improve biological, physical and chemical soil conditions (Fischer and Glaser 2012). It was determined at the end of the experiment, in both bulk and rhizosphere soils, and followed the same trend in both cases. SOM of P0% substrates was 2.60 % in bulk soil and 2.51 % in rhizosphere soil and increased with all the amendments. The lowest increase was observed with the iron amendment alone (2.97 % in bulk and 2.94 % in rhizosphere soil), while the highest was found with the combination biochar-compost (7.28 % in bulk and 8.31 % in the rhizosphere) and biochar-compost-iron (7.84 % in bulk and 7.81 % in the rhizosphere). Previous studies showed an increase in SOM after biochar or compost amendment, and attributed this to amendment composition, as biochar is mainly made up of organic fractions (Janus et al. 2015), and compost contains large amounts of OM (22 %). In addition, such improvement can be due to the fact that the diverse amendments, biochar, compost and iron grit, application could have increase the microorganism diversity and activity, thus inducing a transformation of the organic matter. Furthermore, it should be noted that SOM content did not differ between bulk and rhizosphere soils, indicating that plant growth had no effect on SOM content.

3.1.2 Soil metal(loid) concentrations and availabilities

Non-amended Pontgibaud technosol presented high pseudo-total concentrations of As (1.06 $g.kg^{-1}$), Fe (6.32 $g.kg^{-1}$) and Pb (23.39 $g.kg^{-1}$) (Table 2). Decreases of pseudo-total As, Pb and Fe concentrations were observed when applying biochar and/or compost, which can be ascribed to a dilution effect caused by the addition of the amendments. Whereas, iron amendment application increased pseudo-total Fe concentrations, as observed by Galdames et al. (2017).

Pseudo-total metal(loid) concentrations provide information about the total levels of the contaminants present in the soil. However, parts of these contaminants are bound to fractions, such as oxides and OM, and therefore are not immediately mobilizable and bioavailable for living organisms present in soil (Tessier et al. 1979). However, single extractions performed using salts, *i.e* $CaCl_2$ representing the mobilizable fraction, and NH_4NO_3 corresponding to the exchangeable fraction, give information about the quantity of metal(loid)s present in the soil that can potentially be mobilized and thus present a risk for the environment (Gupta et al. 1996).

$CaCl_2$- and NH_4NO_3-extractable As, Fe and Pb concentrations were much lower than pseudo-total concentrations, for all three metal(loid)s in all treatments (Table 2). This could be related to the previous aging of the studied soil (P), which decreases metal(loid) mobility (Montiel-Rozas et al. 2015). Indeed, in their study, Lu et al. (2009) showed that Cu mobility decreased with aging and attributed this to a Cu diffusion and sequestration towards the soil micro- and meso-pores.

Regarding amendment effects on extractable fractions, decreases in metal(loid) concentrations were observed in several cases (Table 2). More specifically, compared to P0%, extractable As decreased with treatments containing biochar or iron, extractable Fe decreased with the biochar treatments while extractable Pb decreased with all amendments except iron alone, with the highest decrease observed with compost alone. Moreover, NH_4NO_3 extractions were more sensitive to the amendments than $CaCl_2$ extractions. One of the explanations for such decreases of extractable metal(loid)s could be an adsorption and complexion of metal cations onto the amendment's surface (Lu et al. 2017), as well as the pH increase induced by amendment application (Zheng et al. 2013) for the positively charged metals (Pb and Fe). Indeed, an increase in soil pH also increases the number of negative charges in the soil, which promotes electrostatic interactions with positively charged metals, and thus decreases their mobility (Lu et al. 2017). Such soil pH influence on metal$CaCl_2$- and NH_4NO_3-extractable concentrations was reported in Table S2. Significant correlations between SPW pH (at T0) and $CaCl_2$- or NH_4NO_3-extractable values were: -0.44 for Fe-$CaCl_2$-extractable concentrations (p-value < 0.01), -0.76 for Pb-$CaCl_2$-extractable concentrations (p-value < 0.001), -0.42 for Fe-NH_4NO_3-extractable concentrations (p-value < 0.05) and -0.85 for Pb-NH_4NO_3-extractable concentrations (p-value < 0.001).

3.2 Soil pore water (SPW) physico-chemical properties

3.2.1 SPW pH

At the beginning of the experiment (T0), Pontgibaud SPW presented an acidic pH of 3.74 (Table 3). All the amendments, whether alone or in combination, increased SPW pH (Table 3). The highest pH rise was observed for the PBC treatment (pH 7.30), while the amendments with biochar (PB) and biochar-compost-iron (PBCI) led to lower pH increases (with no significant difference between them), with pH values of 6.73 and 6.68, respectively. PBI (pH 5.66) and PC (pH 5.53) conditions showed similar SPW pH increases, while iron amendment alone induced the lowest increase (pH 4.38) of all treatments. After 67 days, SPW of the P0% substrate was still acid, in both unvegetated (pH 3.66) and vegetated pots (pH 3.78). SPW pH was higher in pots containing amendments, compared to P0%, although the order of increase differed between unvegetated and vegetated conditions. In unvegetated pots, the highest SPW pH increases compared to T0 were observed for the PBC treatment (pH 7.93), followed by PB (pH 7.43), PC (pH 7.06), PBC (pH 5.72), PBI (pH 3.98) and PI (pH 3.69). In vegetated pots, PBC treatment again showed the highest SPW pH rise (pH 7.83) then SPW pH decreased in the order PB (pH 7.47), PC (pH 7.03) and PBCI (pH 6.37). After 67 days of plant growth, pots amended with iron and biochar-iron did not significantly differ from P0% treatment in terms of SPW pH. Such results were consistent with previous researches. Biochar addition increases SPW pH by a liming effect due to its alkaline nature (pH 8.98) (Houben et al. 2013, Lebrun et al. 2017) and induced by several mechanisms: (i) the dissolution of metal oxides, hydroxides and carbonates (Houben and Sonnet 2015) and (ii) the presence of COO^- and O^- functional groups on the biochar surface that can bind to H^+ (Houben and Sonnet 2015). Regarding compost, the SPW pH increase can be attributed to: (i) the pH of the compost itself (pH 7.53) (Montiel-Rozas et al. 2015), (ii) a proton consumption (Madejon et al. 2014) and (iii) the addition of soluble cations such as Ca, Mg and K (Fischer and Glaser 2012, Liu et al. 2012). Finally, after iron incorporation to soil, Fe^0 can corrode, consuming protons, which increases pH (Qiao et al. 2018).

After 67 days, for treatments PB, PC, PBC, a pH increase was observed compared to SPW pH values measured at T0, with no difference between vegetated and non-vegetated conditions. This can be explained by the oxidation of biochar after its incorporation into soil, which induces the formation of oxygen-containing functional groups and increases the number of negative charges on the biochar surface. Both functional groups and negative charges can bind H^+ ions and therefore increase SPW pH (Cheng et al. 2008). On the contrary, SPW pH of the PI and PBI treatments decreased with time, showing a higher decrease with plant growth for the PI treatment (pH 4.00 in T67-*Salix* compared to pH 3.69 in T67+*Salix*), and no plant effect for PBI SPW pH at T67. When iron is added to the soil, as is the case with biochar, it oxidizes with time, which causes a soil acidification, as one mole of iron oxidized led to the formation of two moles of H^+ and thus to a pH decrease (Miretzky and Cirelli 2010).

3.2.2 SPW electrical conductivity (EC)

SPW EC of P0% treatment was low at T0 (222 $\mu S.cm^{-1}$) (Table 3) and increased with all amendments, except for iron alone (Table 3). The highest SPW EC increase was observed with biochar-compost (x 9.8), followed by compost alone (x 8.5), biochar-compost-iron (x 7.9), biochar alone (x 4.8) and biochar-iron (x 3.3), which induced the lowest increase. After 67 days, P0% treatment presented a SPW EC of 547 $\mu S.cm^{-1}$ in non-vegetated pots and 622 $\mu S.cm^{-1}$ in pots with *S. viminalis* plants. The order of SPW EC increase with the different amendments, alone or combined, differed between non-vegetated pots and the vegetated conditions. In detail, at the end of all treatments, in non-vegetated substrates, the highest rise was observed for biochar-iron (2367 $\mu S.cm^{-1}$), biochar-compost-iron (2302 $\mu S.cm^{-1}$), and biochar (2261 $\mu S.cm^{-1}$) treatments, with no significant difference between those three conditions. The addition of biochar and compost combined induced a 3.5-fold increase, while the addition of iron and compost alone increased SPW EC by 3.1 and 2.5-fold, respectively. In vegetated pots, at the same date, SPW EC increase followed the order PBCI (3109 $\mu S.cm^{-1}$), PBC (3058 $\mu S.cm^{-1}$), PC (2685 $\mu S.cm^{-1}$), PB (2685 $\mu S.cm^{-1}$), PBI (2490 $\mu S.cm^{-1}$) and PI (1665 $\mu S.cm^{-1}$). The evolution of SPW EC with time showed that it increased in all conditions except PBC. In PBCI and PC substrates, the increase was only significant in vegetated pots; in P, PI and PBI treatments, there was no significant difference between the vegetated and non-vegetated conditions, while the SPW EC rise in PB was higher with plants compared to the non-vegetated pots. Biochar can raise SPW EC through: (i) ash accretion (Nigussie et al. 2012) and (ii) dissolution of salts and nutrient leaching due to pH increase (Janus et al. 2015, Lomaglio et al. 2016). Compost effect on SPW EC can be attributed to: (i) the elevated compost EC (3419.67 $\mu S.cm^{-1}$) (Rossini-Oliva et al. 2017) and probably, as described in Eigenberg et al. (2002) (ii) the mineralization of N contained in compost. Finally, the application of iron alone did not increase SPW EC at T0 (261 $\mu S.cm^{-1}$). Howerver, SPW EC in PI increased with time (1720 $\mu S.cm^{-1}$ in non-vegetated pots and 1665 $\mu S.cm^{-1}$ with plants), At the end of the experiment, SPW EC in PI substrates was higher than in P0%. This can be explained by the iron oxidation.

3.2.3 SPW metal(loid) concentrations

At the beginning of the experiment (T0) SPW As concentrations were below detection limit in five of the seven treatments, *i.e* P0%, PB, PI, PBI and PBCI (Table 3). However, in two treatments using compost (PC and PBC), SPW As concentrations showed a slight but significantly higher value compared to P, with values of 0.06 $mg.L^{-1}$ and 0.02 $mg.L^{-1}$, respectively (Table 3). At the end of the experiment, in non-vegetated pots, SPW As concentration in P0% was 0.01 $mg.L^{-1}$, and only increased when P was amended with compost and biochar + compost, showing a 77-fold and 20-fold increase, respectively. Finally, in vegetated pots at T67, PB SPW presented a lower As concentration, while SPW As concentrations for PI, PBC and PC showed a 1.3-fold, 4.7-fold and 18-fold increase, respectively, compared to Pontgibaud $[As]_{SPW}$ (0.03 $mg.L^{-1}$). Regarding the evolution of SPW As concentrations with time, an increase was observed in all treatments, except for PBCI, with no difference between vegetated and non-vegetated conditions. Similar SPW As concentration increases have been observed in previous studies (Clemente et al. 2010, Lebrun et al. 2018b) and could be attributed to the ability of compost to release dissolved organic compounds which can interact with As and dissolve it into the soil solution (Lebrun et al. 2018b). Another explanation for the change in SPW As concentration in response to amendments could be SPW pH. Indeed, Beesley et al. (2013) showed that As concentration increase in SPW was due to a pH rise which favored phosphorus in the competition with As for binding sites.

SPW Fe concentration was low in P0% at T0 (0.05 $mg.L^{-1}$) (Table 3) and only increased with iron amendments, alone or combined with biochar and compost. The Fe concentration increase was higher when iron was applied alone to P0% (20.06 $mg.L^{-1}$) compared to PBI (2.70 $mg.L^{-1}$) and PBCI (0.10 $mg.L^{-1}$) (Table 3). This increase is explained by the addition of Fe to SPW, which is released by iron grit. At the end of the experiment, Fe SPW concentrations in P were 0.04 $mg.L^{-1}$ and 1.13 $mg.L^{-1}$ in vegetated and non-vegetated pots, respectively. Iron grit amendment increased SPW Fe concentrations, although it was only significant for PI and PBI in non-vegetated pots. Finally, in unvegetated pots, the addition of biochar-compost decreased SPW Fe concentration to non-detectable levels, and in pots with plants, biochar addition, and to a lesser extent the biochar-compost amendment, decreased SPW Fe concentrations. This decrease is consistent with the ability of biochar to immobilize metallic cations through the formation of metal hydroxides, carbonate and phosphate precipitates, and by electrostatic interaction (Park et al. 2011), which can also explain the smaller increase in SPW Fe concentrations at T0 in PBI and PBCI compared to PI. Moreover, as Fe is a cation, it tends to be immobilized with increasing pH, which was observed with the biochar amendment. This hypothesis was confirmed by the significantly low negative correlations between SPW pH and SPW Fe concentrations at T0 ($r = -0.44$, p-value < 0.001), T67-*Salix* ($r = -0.4$, p-value < 0.05) and T67+*Salix* ($r = -0.33$, p-value < 0.05) (Table S2). Finally, in six out of the seven treatments, *i.e* P, PB, PC, PI, PBI and PBCI, SPW Fe concentrations increased with time. There was a significant difference between vegetated and non-vegetated pots for PB, which showed higher SPW Fe concentrations in non-vegetated pots compared to the vegetated ones. In PI and PBI treatments, there was no significant difference between vegetated and non-vegetated pots whereas in P0%, PC, and PBCI treatments, the SPW Fe concentration increase was only significant for vegetated pots.

At T0, Pb concentration was very high in Pontgibaud SPW, 14.96 mg.L^{-1} on average (Table 3). Iron application to P led to a 50 % decrease in SPW Pb concentrations while in treatments PBI, PC and PB, SPW Pb concentrations were 89 %, 90 % and 96 % lower compared to P, respectively, with no significant difference between the three treatments (Table 3). Finally, a 98 % and a 99 % decrease in SPW Pb concentrations was observed with biochar-compost and biochar-compost-iron amendments, respectively, compared to P0%. At T67, SPW Pb concentration was 8.10 mg.L^{-1} in non-vegetated pots and 7.26 mg.L^{-1} in vegetated pots. Concentrations were decreased with all the different amendments. In non-vegetated pots, the lowest Pb concentration decrease was observed when applying biochar-iron (79 % decrease) and iron alone (77 % decrease), with no significant difference between those two treatments. Biochar-compost-iron and biochar amendments also showed drops in SPW Pb concentration, by 91 % and 93 %, respectively. Finally, the application of compost alone showed a higher Pb concentration decrease (99 %) than the application of both biochar and compost (98 %). The pattern for this decrease in the vegetated pots was different from that observed in non-vegetated conditions. Indeed, in the vegetated pots, the lowest decrease was observed with the iron amendment alone (69 %), followed by biochar-iron (77 %), compost alone (90 %), biochar alone (91 %), biochar-compost-iron (92 %) and finally the combination of biochar and compost led to the highest SPW Pb concentration decrease (97 %). No significant change in SPW Pb concentration was observed between T0 and T67 (with or without plants) in PB, PBC and PBI treatments. In P0% and PI substrates, SPW Pb concentrations were lower at T67 compared to T0, with no difference between unvegetated and vegetated conditions, while SPW Pb concentration decrease was only observed in non-vegetated pots for PC treatment, compared to T0. Finally, a slight increase in SPW Pb concentration with time was observed for the PBCI treatment. The capacity of the three different amendments, biochar, compost and iron grit, to decrease SPW Pb concentrations has been shown in previous studies. Indeed, Lomaglio et al. (2016) explained the 70 % decrease observed in SPW Pb concentration following a pinewood biochar addition to a mining soil by the precipitation of Pb on phosphates and carbonates present in the biochar. Moreover, Pb can sorb onto biochar surfaces (Park et al. 2011) through interactions with oxygen functional groups (Jiang et al. 2012). Concerning compost, in their review, Huang et al. (2016) showed that it potentially had a biosorbent role on the metal(loid)s due to humic substances which contain many organic functional groups. For iron grit, when incorporated into the soil, its oxidation formed iron oxides, which have the capacity to sorb Pb (Houben et al. 2012). Finally, like iron, Pb is a metallic cation in solution; therefore, at pH > 6 it can form hydroxide precipitates and complexes with OM, which decrease its mobility and solubility (Oustriere et al. 2017). The important role of pH on Pb mobility and thus SPW Pb concentrations was corroborated by the negative correlations between SPW pH and Pb SPW concentrations at T0 ($r = -0.87$, p-value < 0.001), T67-*Salix* ($r = -0.63$, p-value < 0.001) and T67+*Salix* ($r = -0.64$, p-value < 0.001) (Table S2).

3.2.4 SPW toxicity

SPW metal(loid) concentrations give information on the total amount of metal(loid)s present in the soil solution, and which are therefore susceptible to being leached into underground water. However, some of these metal(loid)s can be complexed with dissolved organic carbon (DOC) for instance, which can diminish their plant uptake potential and lower their toxicity towards the different organisms present in the environment. Therefore, the SPW toxicity needs to be evaluated. To assess the SPW toxicity, a toxicity test using *P. phosphoreum* was performed. SPW relative luminescence was calculated and compared to a reference (P0% at T0) considered as 1. A relative luminescence above 1 showed a lower toxicity compared to the reference, while a value below 1 showed a higher toxicity. At the beginning of the experiment (T0), no change of SPW toxicity was observed in any amended treatments compared to P (Table S3). This could be due to the short time between the mixing of the substrates and the SPW sampling. After 67 days, in both non-vegetated and vegetated pots, SPW toxicity decreased compared to P with all amendments, except with iron alone (0.80 for T67-*Salix*). Such results were consistent with the capacity of biochar to immobilize metal(loid)s (Koltowski et al. 2017), and to reduce the genotoxicity of contaminated soils (Rees et al. 2017). In 2014, Beesley et al. showed that compost induced the formation of DOC-metal(loid) complexes, reducing the free metal(loid) contents by 50 % in SPW. Moreover, the results showed that even though compost application increased As SPW concentrations, it reduced the overall SPW toxicity, showing that As mobilization from the soil could have happened through complexion with DOC, thus preventing its toxicity towards *P. phosphoreum*, and probably towards plants too. Finally, the increase in SPW toxicity observed with the application of iron alone can be explained by the toxicity of iron itself.

Regarding the evolution of SPW toxicity with time, no change was observed for PI whereas the toxicity decreased after 67 days compared to T0 for P0%, PBI and PBCI, with no plant effect. Finally, the reduction in SPW toxicity for PB, PC and PBC compared to T0 was higher with plant growth.

3.3 Plant growth measurements

3.3.1 Leaf surface area

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Plants grown on unamended P0% soil presented a total leaf area of 151.80 $cm^2.plant^{-1}$ (Table 4). The amendment with iron alone decreased the total leaf area by 2.3-fold, while the biochar-iron combination amendment had no effect. The plants grown on the other four treatments, *i.e* PB, PC, PBC and PBCI, presented a higher total leaf area of 377.64 $cm^2.plant^{-1}$, 425.99 $cm^2.plant^{-1}$, 393.00 $cm^2.plant^{-1}$ and 298.24 $cm^2.plant^{-1}$, respectively, compared to P. Similar results have been observed using organic waste amendment on a sandy loam (Sebastiani et al. 2004) and two different biochars (woodchip biochar and olive tree pruning biochar) on a disused Cu mine (Brennan et al. 2014).

The increase in total leaf area observed with biochar and compost amendments, alone or combined, can be explained by the improvement in the soil physico-chemical characteristics. Indeed, as shown in the previous sections, biochar and compost amendments induced: (i) an increase in soil WHC and SOM (Table 1), (ii) an increase in SPW pH and EC (Table 3), (iii) a decrease in SPW metal(loid) concentrations (Table 3) and (iv) a toxicity reduction (Table S3). The decrease observed with the iron grit amendment could be due to the toxic effect of Fe, as shown in the *P. phosphoreum* toxicity test (Table S3).

3.3.2 Organ dry weight (DW)

Plants presented a low growth on P0% soil, with leaf, stem and root DWs of 0.57 g, 0.17 g and 0.23 g, respectively (Fig. 1). Plant growth was increased with the different amendments, except when iron and biochar-iron were applied. Indeed, on the PI substrate, only root DW (0.39 g) was higher compared to P, while on PBI, roots presented a lower DW (0.16 g) compared to P roots. For all three organs (leaves, stem and roots), the highest increase was observed for PC and PBC, with DW increases of 3.3-fold, 6.8-fold and 4.4-fold for PC and 3-fold, 6.1-fold and 4.3-fold for PBC, respectively. When grown on the PB treatment, plants presented a leaf, stem and root DW of 1.48 g, 0.81 g and 0.65 g, respectively, which were not significantly different to the organ DW of plants grown on PBCI (leaves 1.42 g, stem 0.79 g and roots 1.02 g). Such improvements can be attributed to an improvement in the soil conditions (Abbas et al. 2018), as shown in the previous sections: (i) a supply or increase of availability of nutrients (Kołtowski et al. 2017), (ii) a supply of organic matter (Walter and Bernal 2008) (Table 1) and (ii) a decrease in metal(loid) SPW concentrations and extractable fractions (Park et al. 2011, Zheng et al. 2013) (Table 2, Table 3). Regarding the reduction of plant growth induced by the iron grit amendment, as well as for leaf parameters, this could be explained by the iron toxicity.

3.4 Plant metal(loid) accumulation and repartition

3.4.1 Metal(loid) concentrations

Leaf As concentration in plants grown on P0% was 0.90 $mg.kg^{-1}$ (Fig 2A) and only increased when the iron amendment was applied alone, causing a 7-fold rise in As concentrations. Stem As concentrations were low in all conditions and not affected by the different amendments. Finally, root As concentration was 818.74 $mg.kg^{-1}$ in P plants and decreased with biochar, biochar-compost and biochar-compost-iron amendments, with As concentrations of 613.17 $mg.kg^{-1}$, 606.87 $mg.kg^{-1}$ and 604.46 $mg.kg^{-1}$, respectively. This decrease can be explained by a dilution effect (Abbas et al. 2018). Indeed, root DW was higher in these treatments compared to P0%. Finally, it can be noted that even though compost application increased As concentrations in SPW, it induced low root As concentrations, associated to a low translocation towards aboveground parts, which could indicate that the solubilized form of As was not, or was poorly, available for plant translocation in the plant tissue.

Leaf, stem and root Fe concentrations were 90.10 $mg.kg^{-1}$, 29.37 $mg.kg^{-1}$ and 4983.92 $mg.kg^{-1}$, respectively in plants grown on P (Fig 2C). Leaf, stem and root Fe concentrations increased when the iron amendment was applied, alone or combined with biochar and compost. This can be attributed to Fe supply by iron grit, as shown in the pseudo-total and SPW concentration measurements. The amendment of P by biochar and/or compost decreased Fe concentrations in leaves and roots, which could be due to their ability to sorb metal cations in soil (Hmid et al. 2015) as shown by the soil simple extraction results and SPW concentrations (Table 2, Table 3).

Organ Pb concentrations in plants grown on unamended P0% were 54.68 $mg.kg^{-1}$ in leaves, 80.65 $mg.kg^{-1}$ in stem and 11784.31 $mg.kg^{-1}$ in roots (Fig. 2E). The application of iron, alone or combined with biochar, increased Pb concentrations in leaves, stem and roots. More specifically, Pb concentrations were 3 times higher in PI and PBI leaves, 2.7 times higher in PBI stems and 1.6 times higher in PI roots, compared to Pb concentrations in P plants. This increase in Pb concentration was surprising, as simple extractions ($CaCl_2$ and NH_4NO_3) and SPW measurements showed a decrease in Pb concentrations with the iron grit amendment (Table 2, Table 3). However, this increase could be related to the fact that organ DW was low and thus the metal(loid)s were more concentrated. Finally, the application of biochar and/or compost only led to a decrease in Pb concentrations in roots but did not affect leaf and stem Pb concentrations. Indeed, root Pb concentrations were 8518.46 $mg.kg^{-1}$ for PB, 6260.11 $mg.kg^{-1}$ for PC and 7054.65 $mg.kg^{-1}$ for PBC. Such a decrease can be explained by the immobilization of Pb by biochar and compost as well as a dilution effect induced by a higher root DW (Park et al. 2011).

Finally, it can be noted that for the three metal(loid)s, As, Fe and Pb, concentrations were much higher in roots compared to leaves and stems. A higher accumulation of metal(loid)s in the roots, associated with a low translocation towards upper parts, has been observed in previous studies (Beesley et al. 2013, Puckett et al. 2012). Roots are in direct contact with the soil contaminants, which makes them the primary adsorption and absorption site for metal ions (Wang et al. 2014). Following their entry into the roots, metal(loid)s can bind to ion exchangeable sites on cell walls (Wang et al. 2014) and could be blocked by the Casparian strip (Wang et al. 2014). Such root to aerial part restriction transport will protect the photosynthetic and metabolic plant apparatus (Borisev et al. 2008). These mechanisms have been demonstrated to be protective evolutive traits (Chen et al. 2014)

3.4.2 Metal(loid) quantities

Metal(loid) quantities were calculated based on organ DW and organ metal(loid) concentrations. The results showed that leaf, stem and root As quantities in P0% plants were 0.51 µg, 0.21 µg and 157.21 µg, respectively (Fig. 2B). The only amendment effect on organ As quantity was an increase observed in roots of plants grown on PC (x 4.6), PBC (x 3.8) and PBCI (x 4.3).

Leaf Fe quantity in P0% plants was 50.17 µg and increased in the order PBC (103.30 µg), PC (106.34 µg), PBI (167.50 µg) and PI (186.26 µg), while in stems, Fe quantity was 6.16 µg in P0% plants and increased with the three amendments using iron (Fig. 2D). Finally, roots of plants grown on P contained 918.06 µg of Fe, this Fe quantity increased in the order PBCI (21832.49 µg), PI (8464.03 µg), PBI (5674.11 µg), PC (3694.88 µg) and PBC (3515.90 µg).

Pb quantity in leaves of P0% plants was low (31.76 µg) (Fig. 2F) and increased with biochar-compost (78.92 µg) and biochar-compost-iron amendments (72.83 µg). Regarding the stems, all amendments, except iron alone, increased Pb quantity in the order PBC (70.12 µg), PC (59.22 µg), PB (45.87 µg), PBI (36.40 µg) and PBCI (33.44 µg) compared to P (16.14 µg). Finally, roots of P plants presented 2486.14 µg of Pb, and the four treatments PC, PI, PBC and PBCI induced an increase in Pb accumulation corresponding to a final Pb quantity of 6622.08 µg, 6405.50 µg, 6609.28 µg and 11210.80 µg, respectively.

Such data showed that the quantities of metal(loid)s extracted by the aerial parts of *Salix viminalis* from the soil after 69 days of growth were low, showing that the *S. viminalis* species would be more suitable for phytostabilization strategies over phytoextraction in this case.

3.5 Stress indicators

3.5.1 Leaf pigments

Leaves of plants grown on Pontgibaud technosol presented 25.88 $\mu g.cm^{-2}$ of chlorophyll (Fig. 3A). Iron amendment alone did not affect chlorophyll content, while biochar-iron application induced a slight decrease in leaf chlorophyll content, down to 22.23 $\mu g.cm^{-2}$. The other four amendment treatments decreased leaf chlorophyll content, with a higher decrease observed with biochar and/or compost. Indeed, leaf chlorophyll contents were 15.79 $\mu g.cm^{-2}$, 15.65 $\mu g.cm^{-2}$ and 15.87 $\mu g.cm^{-2}$ in leaves of plants grown on PB, PC and PBC substrates, respectively. This chlorophyll content decrease had been observed by Sorrenti et al. (2016) who attributed it to (i) a pH rise, which reduced Fe transport to plant cells and (ii) a sorption or precipitation of Fe on biochar surface. However, it should be noted that such a decrease in leaf chlorophyll content could be due to a dilution effect, as plants presented a higher total surface area with biochar and compost amendments. This was supported by the negative correlation between the leaf chlorophyll content and the total leaf surface area ($r = -0.56$, p-value < 0.001) (Table S2). Indeed, when considering the chlorophyll content and the total surface area to calculate the average chlorophyll content per plant, a different trend was observed. The lowest chlorophyll content was found in PI plants (1578.23 µg), followed by PBI plants (2705.61 µg), P plants (3928.58 µg), PBCI plants (5272.88 µg), PB plants (5962.94 µg), PBC plants (6236.91 µg) and PC plants (6666.74 µg). This increase in total chlorophyll content induced by compost and biochar could be due to the reduction of the metal(loid) toxicity (Abbas et al. 2018).

Leaf flavonoid levels in P0% plants were 0.39 $\mu g.cm^{-2}$ and were not affected by biochar and iron amendments alone or the combination of the three amendments (PBCI), whereas it decreased with biochar-iron associated application (0.30 $\mu g.cm^{-2}$) (Fig. 3B). The addition of compost, alone or in combination with biochar, increased flavonoid content in leaves to similar levels, 0.49 $\mu g.cm^{-2}$ and 0.47 $\mu g.cm^{-2}$, respectively.

Leaf anthocyanin content was low for P0% plants (0.04 a.u) (Fig. 3C) and increased with all amendment applications in the order PI (0.06 a.u), PBI (0.08 a.u), PB (0.10 a.u), PC (0.10 a.u), PBCI (0.11 a.u) and PBC (0.11 a.u).

These pigment (chlorophyll, anthocyanin and flavonoid) content increases could be explained by the reduction in metal(loid) toxicity, as shown by the soil and SPW analysis (Table 2, Table 3, Table S3). Indeed, it is known that metal(loid)s can limit the biosynthesis of pigments (Akula and Ravishankar 2011) through: (i) lipid peroxidation, which alters the membrane permeability and chloroplast ultrastructure (MacFarlane and Burchett 2001), and (ii) competition with essential elements required for pigment synthesis (Bajguz 2011).

3.5.2 Root guaiacol peroxidase (GPOD) activity

Guaiacol peroxidase is considered as a biomarker for metal(loid) stress in plants. Indeed, as demonstrated by Marchand et al. (2016), its activity presents a positive dose effect relationship with increasing metal(loid) exposure.

In this study, roots of P0% plants presented a guaiacol peroxidase (GPOD) activity of 43.63 $mol.L^{-1}.sec^{-1}.mg^{-1}$ protein (Fig. 4). The application of iron and biochar-iron to P had no effect on root GPOD activity, while the other treatments decreased its activity. More precisely, root GPOD activities were 34.66 $mol.L^{-1}.sec^{-1}.mg^{-1}$ protein, 25.68 $mol.L^{-1}.sec^{-1}.mg^{-1}$ protein, 23.08 $mol.L^{-1}.sec^{-1}.mg^{-1}$ protein and 29.05 $mol.L^{-1}.sec^{-1}.mg^{-1}$ protein in PB, PC, PBC and PBCI treatments, respectively. This decrease in root GPOD activity can indicate a lower metal(loid) stress, which can be linked to the soil immobilization of metal(loid)s, inducing a decrease in metal(loid) levels in the plants and thus a reduction in cell stress. Consequently, defense mechanisms against this stress decreased (Hmid et al. 2015). This explanation was attested by the correlation between the root GPOD activity and the root metal(loid) quantities (As: r = - 0.48, p-value < 0.01; Pb: r = - 0.43, p-value < 0.05) (Table S2). Moreover, Marchand et al. (2016) observed a negative relationship between the root GPOD activity and the root DW of *P. australis*, which was also observed in this case (r = - 0.47, p-value < 0.01) (Table S2).

4 Conclusion

A phytoremediation experiment was performed in mesocosm using an As and Pb contaminated technosol complemented with three different amendments, biochar, compost and iron, alone or combined, and using *S. viminalis* as the phytoremediator plant. The results showed that the amendment of Pontgibaud technosol improved the soil and SPW physico-chemical properties, such as increasing the pH, and drastically decreasing the availability of soil Pb. Such ameliorations allowed a better plant growth and reduced the stress applied to plants. However, the iron amendment showed toxic effects, probably due to a high dose application. Therefore, the initial hypothesis that combining amendments would lead to better results than adding a single amendment is only partly true. Indeed, the biochar-compost association tended to show better results than biochar or compost alone, however, associating iron with biochar induced toxic effects compared to biochar alone and even compared to non-amended Pontgibaud. In addition, the metal(loid) accumulations were higher in roots compared to aboveground parts, allowing plant leaf metabolism protection. Finally, the higher plant growth associated to a low metal(loid) translocation towards harvestable tissues observed with compost and biochar-compost amendments showed that these two treatments are good options to be used in an assisted phytostabilization process with *S. viminalis* plants. Indeed, in the field, the establishment of a vegetation cover, made possible by the addition of amendments, can reduce the metal(loid) percolation as well as the wind erosion, preventing the spread of contamination to the environment. In addition, the use of biochar in combination to compost would allow a longer time of effect without need to re-applied the amendments. Moreover, both biochar and compost have a positive environmental impact, and even though their production from waste products is still expensive due to the recent market opening, their economic and environmental costs are still more efficient than the physical and chemical remediation techniques used during previous decades. However, this needs to be assessed in field trial to verify such assumptions.

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Table 1. Soil water holding capacity (WHC, %) and soil organic matter content (SOM, %) of Pontgibaud technosol (P) alone (P0%) and amended with 5% biochar (B), 5% compost (C) or 1.5% iron grit (I), alone or combined. WHC was determined at the beginning of the experiment (T0), while SOM was determined on the bulk and the rhizospheric soils after 69 days of *Salix viminalis* growth. Different letters by column indicate significant difference ($p < 0.05$) (n= 3-5 ± SE). Plant effect was determined for each treatment between the bulk and the rhizosphere soil (ns = non-significant)

	WHC (%)	SOM (%)		
		Bulk soil	Rhizospheric soil	Plant effect
P0%	29.80 ± 0.47 **b**	2.60 ± 0.07 **a**	2.51 ± 0.03 **a**	**ns**
PB	40.11 ± 0.48 **e**	6.37 ± 0.24 **c**	6.82 ± 0.11 **d**	**ns**
PC	32.75 ± 0.49 **c**	3.76 ± 0.24 **b**	3.89 ± 0.15 **c**	**ns**
PI	28.11 ± 0.38 **a**	2.97 ± 0.05 **b**	2.94 ± 0.02 **b**	**ns**
PBC	44.55 ± 2.06 **f**	7.28 ± 0.30 **cd**	8.31 ± 0.20 **e**	**ns**
PBI	36.83 ± 0.26 **d**	6.64 ± 0.08 **c**	6.52 ± 0.16 **d**	**ns**
PBCI	40.61 ± 0.54 **ef**	7.84 ± 0.26 **d**	7.81 ± 0.20 **e**	**ns**

Table 2. As, Fe and Pb soil pseudo-total concentrations (g.kg^{-1} soil), $CaCl_2$-extractable and NH_4NO_3-extractable concentrations (mg.kg^{-1} soil) determined at the beginning of the experiment on Pontgibaud technosol (P) alone (P0%) and amended with 5% biochar (B), 5% compost (C) or 1.5% iron grit (I), alone or combined. Different letters by column indicate significant difference ($p < 0.05$) (n= 3-5 ± SE).

	Pseudo-total concentrations			$CaCl_2$-extractable concentrations			NH_4NO_3-extractable concentrations		
	[As](g.kg^{-1} soil)	[Fe](g.kg^{-1} soil)	[Pb](mg.kg^{-1} soil)	[As](mg.kg^{-1} soil)	[Fe](mg.kg^{-1} soil)	[Pb](mg.kg^{-1} soil)	[As](mg.kg^{-1} soil)	[Fe](mg.kg^{-1} soil)	[Pb](mg.kg^{-1} soil)
P0%	1.06 ± 0.02 **b**	6.32 ± 0.11 **b**	23.39 ± 1.02 **c**	1.13 ± 0.42 **cd**	3.70 ± 1.73 **de**	503.19 ± 50.43 **d**	0.24 ± 0.03 **e**	0.16 ± 0.06 **c**	348.09 ± 7.56 **e**
PB	1.00 ± 0.02 **ab**	5.96 ± 0.10 **ab**	18.93 ± 0.55 **b**	0.45 ± 0.07 **b**	0.67 ± 0.32 **bc**	294.13 ± 13.05 **c**	0.03 ± 0.00 **a**	0.02 ± 0.02 **ab**	83.91 ± 4.65 **c**
PC	0.91 ± 0.04 **a**	5.41 ± 0.21 **a**	18.88 ± 0.67 **b**	1.15 ± 0.10 **d**	1.16 ± 0.34 **cd**	22.23 ± 6.19 **a**	0.30 ± 0.05 **e**	0.18 ± 0.20 **bc**	5.50 ± 1.63 **a**
PI	1.01 ± 0.05 **ab**	23.25 ± 4.34 **ab**	18.42 ± 1.42 **abc**	0.69 ± 0.07 **c**	18.28 ± 3.79 **e**	506.54 ± 12.82 **d**	0.17 ± 0.01 **d**	16.14 ± 2.89 **d**	359.39 ± 5.62 **e**
PBC	0.84 ± 0.04 **a**	4.97 ± 0.30 **a**	16.42 ± 0.30 **a**	0.53 ± 0.09 **bc**	1.11 ± 0.31 **bc**	28.11 ± 6.52 **a**	0.05 ± 0.01 **ab**	0.00 ± 0.00 **ab**	10.47 ± 2.88 **ab**
PBI	0.99 ± 0.02 **a**	20.09 ± 2.41 **ab**	18.90 ± 0.44 **b**	0.47 ± 0.13 **abc**	11.40 ± 7.70 **e**	302.57 ± 18.32 **c**	0.08 ± 0.01 **c**	0.05 ± 0.06 **bc**	118.81 ± 5.12 **d**
PBCI	0.85 ± 0.06 **ab**	14.41 ± 2.66 **ab**	17.79 ± 2.41 **abc**	0.18 ± 0.02 **a**	0.00 ± 0.00 **a**	33.36 ± 2.05 **b**	0.06 ± 0.01 **bc**	0.00 ± 0.00 **ab**	18.83 ± 6.74 **b**

Table 3. Soil pore water physico-chemical characteristics determined at the beginning (T0) and at the end of the experiment, in non-vegetated (T67-*Salix*) and vegetated pots (T67+*Salix*) on Pontgibaud technosol (P) alone (P0%) and amended with 5% biochar (B), 5% compost (C) or 1.5% iron grit (I), alone (P0%) or combined. EC = electrical conductivity (µS.cm^{-1}). Capital letters indicate significant difference between the 7 treatments for each time, while minuscule letters indicate the difference between T0, T67-*Salix* and T67+*Salix* for each treatment ($p < 0.05$) (n = 5-12 ± SE).

		pH		EC (µS.cm^{-1})		[As] (mg.L^{-1})		[Fe] (mg.L^{-1})		[Pb] (mg.L^{-1})	
			Time effect		Time effect		Time effect		Time effect		Time effect
	T0	3.74 ± 0.04 **A**	a	222 ± 12 **A**	a	0.00 ± 0.00 **A**	a	0.05 ± 0.04 **A**	a	14.96 ± 0.57 **D**	b
P0%	T67-*Salix*	3.66 ± 0.03 **A**	a	547 ± 43 **A**	b	0.01 ± 0.01 **A**	b	0.04 ± 0.02 **A**	a	8.10 ± 0.53 **E**	a
	T67+*Salix*	3.78 ± 0.06 **A**	a	622 ± 39 **A**	b	0.03 ± 0.01 **B**	b	1.13 ± 0.52 **CF**	b	7.26 ± 0.31 **E**	a
	T0	6.73 ± 0.09 **D**	a	1061 ± 62 **C**	a	0.00 ± 0.00 **A**	a	0.00 ± 0.00 **A**	a	0.53 ± 0.09 **B**	a
PB	T67-*Salix*	7.43 ± 0.01 **D**	b	2261 ± 107 **D**	b	0.03 ± 0.02 **AB**	b	0.13 ± 0.06 **A**	b	0.70 ± 0.06 **C**	a
	T67+*Salix*	7.47 ± 0.04 **C**	b	2685 ± 52 **BCD**	c	0.00 ± 0.00 **AC**	b	0.08 ± 0.04 **AB**	b	0.65 ± 0.04 **B**	a
	T0	5.53 ± 0.16 **C**	a	1890 ± 122 **DE**	a	0.06 ± 0.01 **C**	a	0.00 ± 0.00 **A**	a	1.49 ± 0.26 **C**	b
PC	T67-*Salix*	7.06 ± 0.10 **C**	b	1374 ± 131 **B**	b	0.77 ± 0.26 **C**	b	0.10 ± 0.10 **AB**	ab	0.09 ± 0.02 **A**	a
	T67+*Salix*	7.03 ± 0.03 **B**	b	2685 ± 211 **BCD**	b	0.54 ± 0.07 **D**	b	0.34 ± 0.20 **BC**	b	0.72 ± 0.70 **CDF**	ab
	T0	4.38 ± 0.05 **B**	c	261 ± 20 **A**	a	0.00 ± 0.00 **A**	a	20.06 ± 1.42 **D**	a	7.73 ± 0.36 **C**	b
PI	T67-*Salix*	4.00 ± 0.17 **B**	b	1720 ± 471 **BC**	b	0.02 ± 0.01 **A**	b	213.02 ± 124.88 **C**	b	1.85 ± 0.18 **D**	a
	T67+*Salix*	3.69 ± 0.07 **A**	a	1665 ± 490 **B**	b	0.04 ± 0.03 **AC**	b	265.25 ± 158.98 **F**	b	2.27 ± 0.33 **D**	a
	T0	7.30 ± 0.06 **E**	a	2172 ± 90 **E**	a	0.02 ± 0.01 **B**	a	0.27 ± 0.28 **A**	a	0.22 ± 0.07 **A**	a
PBC	T67-*Salix*	7.93 ± 0.05 **E**	b	1940 ± 151 **C**	a	0.20 ± 0.09 **BC**	b	0.00 ± 0.00 **B**	a	0.17 ± 0.02 **B**	a
	T67+*Salix*	7.83 ± 0.03 **D**	b	3858 ± 602 **C**	a	0.14 ± 0.05 **C**	b	0.01 ± 0.02 **AB**	a	0.20 ± 0.05 **A**	a
	T0	5.66 ± 0.13 **C**	b	730 ± 51 **B**	a	0.00 ± 0.00 **A**	a	2.70 ± 0.61 **C**	a	1.61 ± 0.38 **C**	a
PBI	T67-*Salix*	3.98 ± 0.21 **B**	a	2367 ± 238 **D**	b	0.02 ± 0.01 **A**	b	99.12 ± 39.36 **C**	b	1.66 ± 0.30 **D**	a
	T67+*Salix*	3.83 ± 0.13 **A**	a	2490 ± 229 **BC**	b	0.04 ± 0.01 **AB**	b	73.32 ± 19.43 **EF**	b	1.69 ± 0.14 **CD**	a
	T0	6.68 ± 0.08 **D**	a	1763 ± 47 **D**	a	0.00 ± 0.00 **A**	a	0.10 ± 0.04 **B**	a	0.15 ± 0.06 **A**	a
PBCI	T67-*Salix*	5.72 ± 0.59 **C**	a	2302 ± 261 **D**	ab	0.00 ± 0.00 **A**	a	19.07 ± 8.41 **A**	ab	0.77 ± 0.15 **C**	b
	T67+*Salix*	6.37 ± 0.44 **B**	a	3109 ± 250 **CD**	b	0.01 ± 0.01 **AB**	a	20.71 ± 11.37 **F**	b	0.60 ± 0.16 **ABF**	b

Table 4. *Salix viminalis* leaf total area ($cm^2.plant^{-1}$) determined after 69 days of growth on Pontgibaud technosol (P) alone (P0%) and amended with 5% biochar (B), 5% compost (C) or 1.5% iron grit (I), alone or combined. Letters indicate significant difference ($p < 0.05$) ($n = 14 \pm SE$).

	Total leaf area ($cm^2.plant^{-1}$)
P0%	151.80 ± 36.87 **b**
PB	377.64 ± 20.88 **cd**
PC	425.99 ± 21.94 **d**
PI	66.34 ± 8.74 **a**
PBC	393.00 ± 60.55 **cd**
PBI	121.71 ± 31.86 **ab**
PBCI	298.24 ± 35.65 **c**

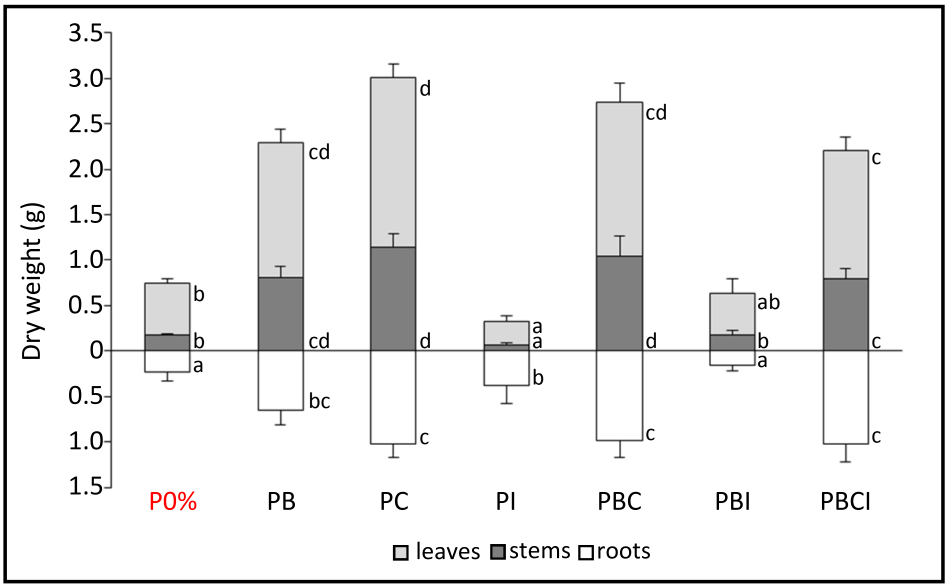

Fig 1. *Salix viminalis* organ (leaves, stems, roots) dry weight (g) determined after 69 days of growth on Pontgibaud technosol (P) alone (P0%) and amended with 5% biochar (B), 5% compost (C) or 1.5% iron grit (I), alone or combined. Letters indicate significant difference between treatments for each plant organ ($p < 0.05$) ($n = 5\text{-}8 \pm SE$).

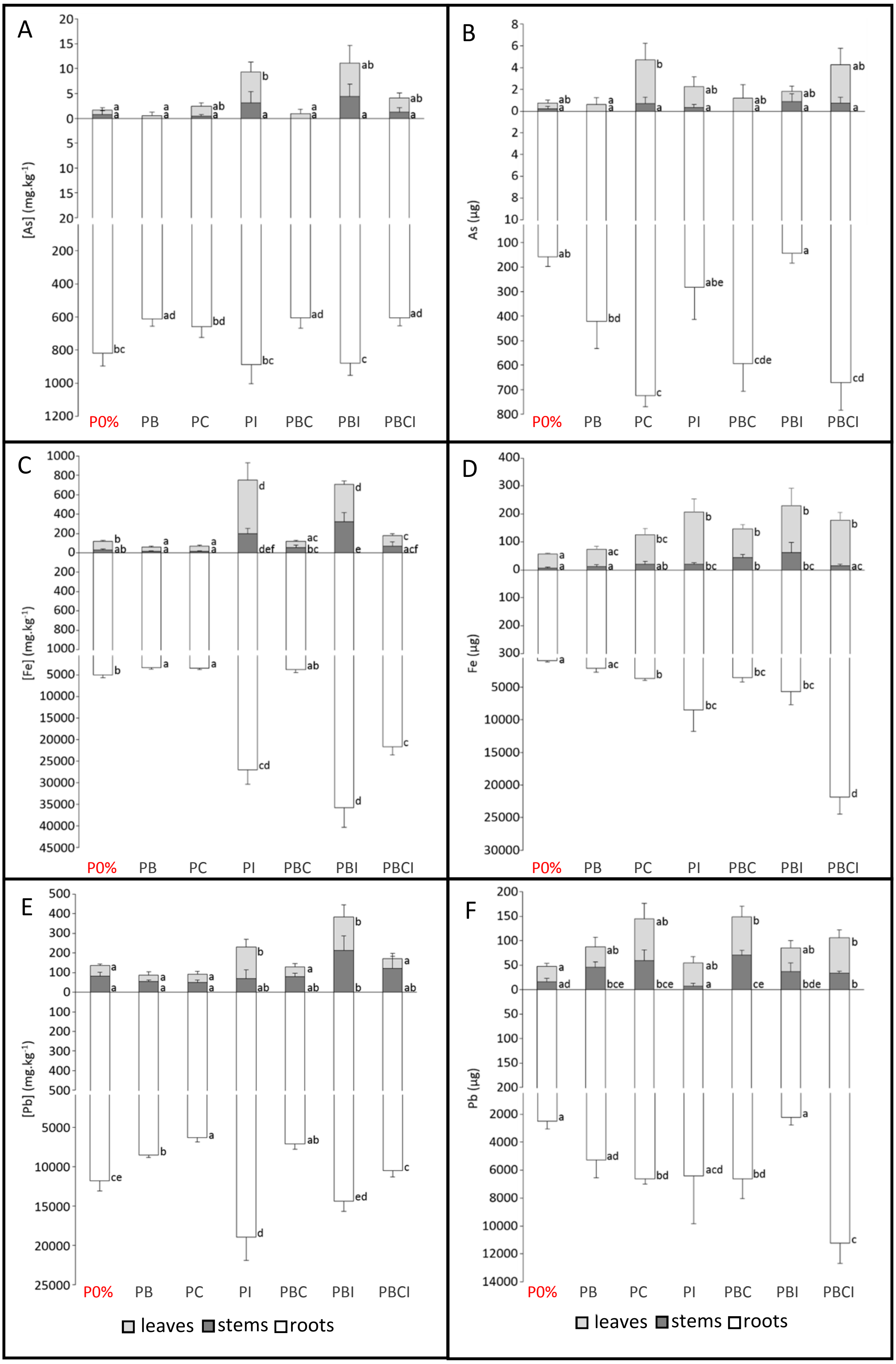

Fig 2. *Salix viminalis* organ (leaves, stems, roots) metal(loid) (As (A, B), Fe (C, D), Pb (E, F)) concentrations ($mg.kg^{-1}$) (A, C, E) and quantities (µg) (B, D, F) determined after 69 days of growth on Pontgibaud technosol (P) alone (P0%) and amended with 5% biochar (B), 5% compost (C) or 1.5% iron grit (I), alone or combined. Letters indicate significant difference between treatments for each plant organ ($p < 0.05$) ($n = 5\text{-}8 \pm SE$).

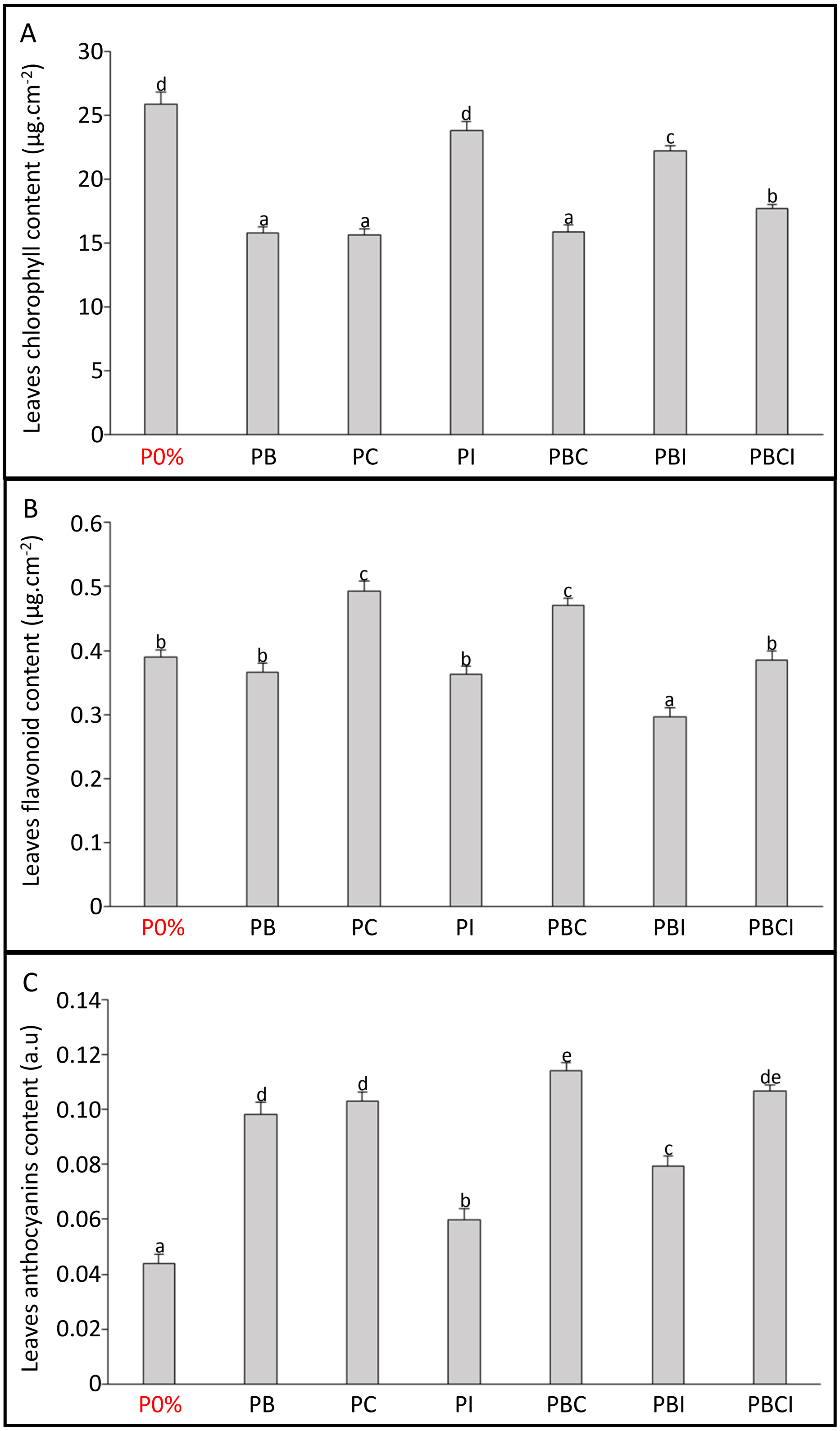

Fig 3. *Salix viminalis* leaf pigment contents (chlorophyll (A), flavonoids (B) and anthocyanins (C)) determined after 69 days of growth on Pontgibaud technosol (P) alone (P0%) and amended with 5% biochar (B), 5% compost (C) or 1.5% iron grit (I), alone or combined. Letters indicate significant difference ($p < 0.05$) ($n = 14 \pm SE$).

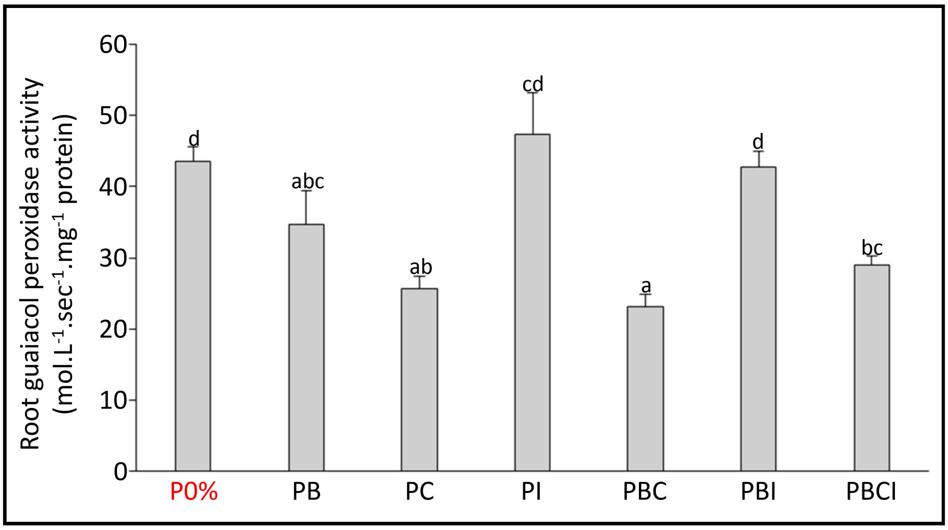

Fig 4. *Salix viminalis* root guaiacol peroxidase activity (mol.L^{-1}.sec^{-1}.mg^{-1} protein) determined after 69 days of growth on Pontgibaud technosol (P) alone (P0%) and amended with 5% biochar (B), 5% compost (C) or 1.5% iron grit (I), alone or combined. Letters indicate significant difference ($p < 0.05$) ($n = 3 \pm SE$).

Highlights

Biochar and compost amendments, alone or combined, improved soil fertility

The dose application of iron grit had negative effects on plant growth

Biochar and/or compost amendments improved the growth of *Salix viminalis*

Metal(loid)s were mainly accumulated in roots with low translocation to upper parts