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ACCEPTED MANUSCRIPT

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18
19 **Abstract**

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

36
37 **Keywords:** biochar, compost, iron grit, metal(loid)s, Phytomanagement, *Salix viminalis*

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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

75 bioavailability (Mahar et al. 2016). Moreover, the vegetation cover prevents wind erosion and water
76 leaching.

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

87 However, the difficult conditions encountered in some polluted soils, such as extreme pH, low nutrient
88 contents and high metal(loid) concentrations, make it difficult to establish a vegetation cover.
89 Therefore, in such context, phytoremediation installation requires the application of amendments to
90 improve soil physico-chemical properties, to allow plant growth (Galende et al. 2014). One
91 amendment gathering attention for assisted phytoremediation in recent decades is biochar. It is a
92 porous, carbon rich material obtained from the pyrolysis of biomass under low oxygen conditions
93 (Barrow 2012, Paz-Ferreiro et al. 2014). It is characterized by an alkaline pH, a high cation exchange
94 capacity, a large surface area and a high water holding capacity (WHC) (Ding et al. 2017, Lee et al.
95 2013, Paz-Ferreiro et al. 2014). Biochar is mainly made up of carbon (between 29 and 90 % (Cha et al.
96 2016)), hydrogen, oxygen, nitrogen and sulfur, as well as other trace elements (Tan et al. 2017). Such
97 properties make biochar a good conditioner to improve the soil's physico-chemical properties, thus
98 making plant growth possible (Rizwan et al. 2016). Moreover, its large surface area and high amounts
99 of oxygen containing functional groups enable biochar to sorb metal(loid)s, reducing their solubility
100 and mobility (Ding et al. 2017, Paz-Ferreiro et al. 2014). However, biochar's available nutrient levels
101 are sometimes low (Fischer and Glaser 2012), which means they may need to be provided by another
102 amendment, such as compost. Compost is the product of conversion and reclamation of organic
103 materials by microbial degradation (Huang et al. 2016). It is rich in humus substances and
104 microorganisms, and contains large amounts of plant nutrients (N, P, K, Ca, Mg, S) and other essential
105 trace elements (Fischer and Glaser 2012). In addition to improving soil fertility (Alvarenga et al. 2013,
106 Gil-Loaiza et al. 2016), compost has the potential to sorb metal(loid)s (Fischer and Glaser 2012,
107 Huang et al. 2016). However, although biochar and compost soil application showed (i) improvements
108 in soil conditions (Agegnehu et al. 2016; Hmid et al. 2015), as well as (ii) soil cationic metals sorption
109 (Hmid et al. 2015; Nie et al. 2018), they are less efficient in stabilizing metal anion such as arsenic,
110 and can even increase its mobility sometimes, due to soil pH increase (Beesley et al. 2011). This can
111 be problematic in multi-contaminated soils polluted by both cationic and anionic metal(loid)s. On the

112 contrary, iron amendments, such as iron grit, have been shown to reduce As mobility and soil pore
113 water concentrations efficiently (Kumpiene et al. 2006), due to As adsorption on iron hydroxides
114 (Miretzky and Cirelli 2010).

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

127

128 2 Material and Methods

129 2.1 Study site

130 A former silver-lead extraction mine site located in Pontgibaud was studied. The contaminated
131 disburged and crushed technosol covers 15 ha. This mine history, soil sampling and properties were
132 described in previous works (Lebrun et al. 2018a, b). In addition, soil total CHNS contents were
133 determined and gave the following results: C 0.14 ± 0.03 %, H 0.22 ± 0.00 %, N 0.09 ± 0.02 % and S
134 0.87 ± 0.14 %.

135

136 2.2 Amendments

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

139 The first amendment was biochar, provided by La Carbonerie (Crissey, France). It was obtained by the
140 slow pyrolysis of hardwood biomass (*Quercus sp.*, *Carpinus sp.* and *Fagus sp.*) at 500 °C (residence
141 time: 3 h, heating rate: 2.5 °C.min⁻¹), followed by a sieving to obtain a particle size between 0.2 and
142 0.4 mm. This biochar had already been used in a previous study by Lebrun et al. (2018a), together
143 with three other biochars obtained from the same feedstock but presenting different particle sizes (inf.
144 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 –
145 0.4 mm particle size biochar was selected as it showed a better ability to increase soil fertility, plant
146 growth and metal(loid) stabilization than the coarser biochars. Moreover, compared to the finest
147 biochar (inf. 0.1 mm), the 0.2 – 0.4 mm biochar particle size had a lower risk of leaching, making it
148 more suitable for a possible field application. The biochar characteristics, and the methods used for the

149 characterization are described in Table S1. The second amendment used was a commercial compost,
150 made from manure of different animal origins (horse, cow, pig, chicken) and vegetable materials
151 (compost KB, Scotts, France). The compost was applied directly, without sieving. Compost
152 characteristics are described in Table S1. The last amendment used was iron grit, an industrial product
153 used as an angular abrasive in the paint industry, made up of 97 % iron in addition to other mineral
154 elements (C, Mn, Si, P and S) in low concentrations, and sieved to 0.5 mm. pH and EC were $7.64 \pm$
155 0.33 and $110 \pm 36 \mu\text{S}\cdot\text{cm}^{-1}$, respectively (determined using the method described in Lebrun et al.
156 2018a).

157

158 2.3 Experimental design

159 A mesocosm experiment was set up using the following seven treatments: (i) unamended Pontgibaud
160 technosol (P), (ii) Pontgibaud amended with 5% biochar (w/w) (PB), (iii) Pontgibaud amended with
161 5% compost (w/w) (PC), (iv) Pontgibaud amended with 1.5% iron grit (w/w) (PI), (v) Pontgibaud
162 amended with 5% biochar (w/w) and 5% compost (w/w) (PBC), (vi) Pontgibaud amended with 5%
163 biochar (w/w) and 1.5% iron grit (w/w) (PBI) and (vii) Pontgibaud amended with 5% biochar (w/w),
164 5% compost (w/w) and 1.5% iron grit (w/w) (PBCI). Amendment application doses were chosen
165 based on previous studies (Codling and Dao 2007; Feng et al. 2016; Lebrun et al. 2018a; Ruttens et al.
166 2006). These different mixtures were put into 2L plastic pots (1.8 kg) (n=19), and one non-rooted
167 cutting of *S. viminalis* L. was placed in 14 pots, while five pots were let unvegetated. The plants were
168 watered every two days and grown for 69 days in a greenhouse with the following conditions:
169 temperature $22 \pm 2 \text{ }^\circ\text{C}$, light intensity $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, photoperiod 16 h and irrigation was provided
170 every two days based on the water lost through evapotranspiration.

171

172 2.4 Substrate analyses

173 After the mixtures were prepared, the different substrates were analyzed for WHC, as described in
174 Lebrun et al. (2018a). In addition, three simple extractions were performed: the first one using aqua-
175 regia (3 mL HCl + 6 mL HNO₃) digestion, which corresponded to the pseudo-total As, Fe and Pb
176 concentrations. The second one determined the CaCl₂-extractable As, Fe and Pb fractions, while the
177 last one determined the NH₄NO₃-extractable fractions, both according to the method described in
178 Pueyo et al. (2004): 0.01 M CaCl₂ (1:10 solid:liquid ratio) or 1 M NH₄NO₃ (2:5 solid:liquid ratio).
179 After the different digestions, As, Fe and Pb concentrations were measured by Inductively Coupled
180 Plasma Atomic Emission Spectroscopy (ICP-AES) (ULTIMA 2, HORIBA, Labcompare, San
181 Francisco, USA). A calibration of the apparatus was done before each analysis sequence.
182 After plant growth (T69), soil samples were taken from non-vegetated pots (corresponding to bulk
183 soil) and vegetated pots (corresponding to rhizosphere soil) by shaking the roots into a plastic bag to
184 remove the soil attached to them. Soil organic matter (SOM) contents were determined on these

185 samples by the loss-on-ignition method at 600°C, using a muffle furnace (Nabertherm L9/11/C6, GS,
186 Geprüfte Siderheit, Germany).

187

188 2.5 Soil pore water (SPW) sampling and analysis

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

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

200

201 Finally, As, Fe and Pb concentrations in SPW were determined after acidification (83.3 µL HNO₃ in a
202 5 mL sample) by ICP-AES.

203

204 2.6 Plant analysis

205 2.6.1 Leaf measurements

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

210

211 2.6.2 Root analysis

212 At the end of the experiment time course, root guaiacol peroxidase (GPOD) activity was determined
213 on three plants per treatment as follows: for the enzyme extraction, 1 g of fresh root was crushed in
214 12.5 mL buffer (Tris-HCl 0.05 M, EDTA 0.01 M, MgCl₂ 0.003 M, pH 7.0) in an ice-cold mortar and
215 centrifuged twice (5000 x g, 15 min, 4 °C). The supernatant, corresponding to the enzyme extract, was
216 collected and stored at -20 °C until further analysis.

217 Guaiacol peroxidase activity measurement was adapted from Marchand et al. (2016). It was performed
218 in microplates by adding 150 µL phosphate buffer (0.05 M, pH 6.1), 60 µL H₂O₂ (1 %), 60 µL
219 guaiacol (80 mM) and 30 µL of enzyme extract to a microplate. The reaction was started by adding the

220 enzyme extract, and the increase in absorbance at 390 nm was assessed for 1 min and 30 sec. The
221 activity was calculated using $\epsilon = 26.6 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ and expressed as the quantity of guaiacol transformed
222 in one second. The total protein quantity of the enzyme extract was measured according to the
223 Bradford method (Bradford, 1976) using SAB as standard.

224

225 2.6.3 Organ dry weight and metal(loid) distributions

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

$$(ii) \text{ Metal(loid) quantity} = [\text{metal(loid)}]_{\text{organ}} \times \text{DW}_{\text{organ}}$$

230

231 2.7 Statistical analyses

232 The data were analyzed statistically using the R software version 3.1.2 (R Development Core Team,
233 2009). The treatments were compared two at a time by testing the normality and homoscedasticity,
234 using the Shapiro test and the Bartlett test respectively. Next, the means were compared using a
235 Student test for normal data or a Wilcox test for non-normal data. In addition, for the soil pore water
236 parameters, the time and plant effects were analyzed using an Anova test for normal data or a Kruskal
237 test for non-normal data, followed by a post-hoc test, TukeyHSD or Pairwise.wilcox, respectively. The
238 difference was considered significant at $p < 0.05$. Finally, Pearson correlation coefficients were
239 calculated between the different parameters, with the significant ones ($p < 0.05$) being cited in the
240 following section.

241

242 3 Results and Discussion

243

244 3.1 Substrate characteristics

245 3.1.1 Soil physico-chemical properties

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

255 the increase of soil organic matter content, as organic matter (OM) can absorb water and improve the
256 soil pore system, leading to a better soil structure retention (Giusquiani et al. 1995).
257 Moreover, the intensity of the increase in WHC with biochar and compost amendments (PB, PBC)
258 was lowered when iron-grit was added with the organic material (PBI and PBCI). Such observation
259 has not been previously described, but could be explained by the fact that iron grit is known for being
260 unable to retain water, contrary to biochar and compost.

261
262 SOM content (Table 1) is an important parameter for soil quality, as it can improve biological,
263 physical and chemical soil conditions (Fischer and Glaser 2012). It was determined at the end of the
264 experiment, in both bulk and rhizosphere soils, and followed the same trend in both cases. SOM of
265 P0% substrates was 2.60 % in bulk soil and 2.51 % in rhizosphere soil and increased with all the
266 amendments. The lowest increase was observed with the iron amendment alone (2.97 % in bulk and
267 2.94 % in rhizosphere soil), while the highest was found with the combination biochar-compost (7.28
268 % in bulk and 8.31 % in the rhizosphere) and biochar-compost-iron (7.84 % in bulk and 7.81 % in the
269 rhizosphere). Previous studies showed an increase in SOM after biochar or compost amendment, and
270 attributed this to amendment composition, as biochar is mainly made up of organic fractions (Janus et
271 al. 2015), and compost contains large amounts of OM (22 %). In addition, such improvement can be
272 due to the fact that the diverse amendments, biochar, compost and iron grit, application could have
273 increase the microorganism diversity and activity, thus inducing a transformation of the organic
274 matter. Furthermore, it should be noted that SOM content did not differ between bulk and rhizosphere
275 soils, indicating that plant growth had no effect on SOM content.

276
277 3.1.2 Soil metal(loid) concentrations and availabilities

278 Non-amended Pontgibaud technosol presented high pseudo-total concentrations of As (1.06 g.kg^{-1}), Fe
279 (6.32 g.kg^{-1}) and Pb (23.39 g.kg^{-1}) (Table 2). Decreases of pseudo-total As, Pb and Fe concentrations
280 were observed when applying biochar and/or compost, which can be ascribed to a dilution effect
281 caused by the addition of the amendments. Whereas, iron amendment application increased pseudo-
282 total Fe concentrations, as observed by Galdames et al. (2017).

283
284 Pseudo-total metal(loid) concentrations provide information about the total levels of the contaminants
285 present in the soil. However, parts of these contaminants are bound to fractions, such as oxides and
286 OM, and therefore are not immediately mobilizable and bioavailable for living organisms present in
287 soil (Tessier et al. 1979). However, single extractions performed using salts, *i.e.* CaCl_2 representing the
288 mobilizable fraction, and NH_4NO_3 corresponding to the exchangeable fraction, give information about
289 the quantity of metal(loid)s present in the soil that can potentially be mobilized and thus present a risk
290 for the environment (Gupta et al. 1996).

291 CaCl₂- and NH₄NO₃-extractable As, Fe and Pb concentrations were much lower than pseudo-total
292 concentrations, for all three metal(loid)s in all treatments (Table 2). This could be related to the
293 previous aging of the studied soil (P), which decreases metal(loid) mobility (Montiel-Rozas et al.
294 2015). Indeed, in their study, Lu et al. (2009) showed that Cu mobility decreased with aging and
295 attributed this to a Cu diffusion and sequestration towards the soil micro- and meso-pores.
296 Regarding amendment effects on extractable fractions, decreases in metal(loid) concentrations were
297 observed in several cases (Table 2). More specifically, compared to P0%, extractable As decreased
298 with treatments containing biochar or iron, extractable Fe decreased with the biochar treatments while
299 extractable Pb decreased with all amendments except iron alone, with the highest decrease observed
300 with compost alone. Moreover, NH₄NO₃ extractions were more sensitive to the amendments than
301 CaCl₂ extractions. One of the explanations for such decreases of extractable metal(loid)s could be an
302 adsorption and complexation of metal cations onto the amendment's surface (Lu et al. 2017), as well as
303 the pH increase induced by amendment application (Zheng et al. 2013) for the positively charged
304 metals (Pb and Fe). Indeed, an increase in soil pH also increases the number of negative charges in the
305 soil, which promotes electrostatic interactions with positively charged metals, and thus decreases their
306 mobility (Lu et al. 2017). Such soil pH influence on metalCaCl₂- and NH₄NO₃-extractable
307 concentrations was reported in Table S2. Significant correlations between SPW pH (at T0) and CaCl₂-
308 or NH₄NO₃-extractable values were: -0.44 for Fe-CaCl₂-extractable concentrations (p-value < 0.01), -
309 0.76 for Pb-CaCl₂-extractable concentrations (p-value < 0.001), -0.42 for Fe-NH₄NO₃-extractable
310 concentrations (p-value < 0.05) and -0.85 for Pb-NH₄NO₃-extractable concentrations (p-value <
311 0.001).

312
313

314 3.2 Soil pore water (SPW) physico-chemical properties

315 3.2.1 SPW pH

316 At the beginning of the experiment (T0), Pontgibaud SPW presented an acidic pH of 3.74 (Table 3).
317 All the amendments, whether alone or in combination, increased SPW pH (Table 3). The highest pH
318 rise was observed for the PBC treatment (pH 7.30), while the amendments with biochar (PB) and
319 biochar-compost-iron (PBCI) led to lower pH increases (with no significant difference between them),
320 with pH values of 6.73 and 6.68, respectively. PBI (pH 5.66) and PC (pH 5.53) conditions showed
321 similar SPW pH increases, while iron amendment alone induced the lowest increase (pH 4.38) of all
322 treatments. After 67 days, SPW of the P0% substrate was still acid, in both unvegetated (pH 3.66) and
323 vegetated pots (pH 3.78). SPW pH was higher in pots containing amendments, compared to P0%,
324 although the order of increase differed between unvegetated and vegetated conditions. In unvegetated
325 pots, the highest SPW pH increases compared to T0 were observed for the PBC treatment (pH 7.93),
326 followed by PB (pH 7.43), PC (pH 7.06), PBC (pH 5.72), PBI (pH 3.98) and PI (pH 3.69). In
327 vegetated pots, PBC treatment again showed the highest SPW pH rise (pH 7.83) then SPW pH

328 decreased in the order PB (pH 7.47), PC (pH 7.03) and PBCI (pH 6.37). After 67 days of plant growth,
329 pots amended with iron and biochar-iron did not significantly differ from P0% treatment in terms of
330 SPW pH. Such results were consistent with previous researches. Biochar addition increases SPW pH
331 by a liming effect due to its alkaline nature (pH 8.98) (Houben et al. 2013, Lebrun et al. 2017) and
332 induced by several mechanisms: (i) the dissolution of metal oxides, hydroxides and carbonates
333 (Houben and Sonnet 2015) and (ii) the presence of COO^- and O^- functional groups on the biochar
334 surface that can bind to H^+ (Houben and Sonnet 2015). Regarding compost, the SPW pH increase can
335 be attributed to: (i) the pH of the compost itself (pH 7.53) (Montiel-Rozas et al. 2015), (ii) a proton
336 consumption (Madejon et al. 2014) and (iii) the addition of soluble cations such as Ca, Mg and K
337 (Fischer and Glaser 2012, Liu et al. 2012). Finally, after iron incorporation to soil, Fe^0 can corrode,
338 consuming protons, which increases pH (Qiao et al. 2018).

339 After 67 days, for treatments PB, PC, PBC, a pH increase was observed compared to SPW pH values
340 measured at T0, with no difference between vegetated and non-vegetated conditions. This can be
341 explained by the oxidation of biochar after its incorporation into soil, which induces the formation of
342 oxygen-containing functional groups and increases the number of negative charges on the biochar
343 surface. Both functional groups and negative charges can bind H^+ ions and therefore increase SPW pH
344 (Cheng et al. 2008). On the contrary, SPW pH of the PI and PBI treatments decreased with time,
345 showing a higher decrease with plant growth for the PI treatment (pH 4.00 in T67-*Salix* compared to
346 pH 3.69 in T67+*Salix*), and no plant effect for PBI SPW pH at T67. When iron is added to the soil, as
347 is the case with biochar, it oxidizes with time, which causes a soil acidification, as one mole of iron
348 oxidized led to the formation of two moles of H^+ and thus to a pH decrease (Miretzky and Cirelli
349 2010).

350

351 3.2.2 SPW electrical conductivity (EC)

352 SPW EC of P0% treatment was low at T0 ($222 \mu\text{S}\cdot\text{cm}^{-1}$) (Table 3) and increased with all amendments,
353 except for iron alone (Table 3). The highest SPW EC increase was observed with biochar-compost (x
354 9.8), followed by compost alone (x 8.5), biochar-compost-iron (x 7.9), biochar alone (x 4.8) and
355 biochar-iron (x 3.3), which induced the lowest increase. After 67 days, P0% treatment presented a
356 SPW EC of $547 \mu\text{S}\cdot\text{cm}^{-1}$ in non-vegetated pots and $622 \mu\text{S}\cdot\text{cm}^{-1}$ in pots with *S. viminalis* plants. The
357 order of SPW EC increase with the different amendments, alone or combined, differed between non-
358 vegetated pots and the vegetated conditions. In detail, at the end of all treatments, in non-vegetated
359 substrates, the highest rise was observed for biochar-iron ($2367 \mu\text{S}\cdot\text{cm}^{-1}$), biochar-compost-iron (2302
360 $\mu\text{S}\cdot\text{cm}^{-1}$), and biochar ($2261 \mu\text{S}\cdot\text{cm}^{-1}$) treatments, with no significant difference between those three
361 conditions. The addition of biochar and compost combined induced a 3.5-fold increase, while the
362 addition of iron and compost alone increased SPW EC by 3.1 and 2.5-fold, respectively. In vegetated
363 pots, at the same date, SPW EC increase followed the order PBCI ($3109 \mu\text{S}\cdot\text{cm}^{-1}$), PBC ($3058 \mu\text{S}\cdot\text{cm}^{-1}$),
364 PC ($2685 \mu\text{S}\cdot\text{cm}^{-1}$), PB ($2685 \mu\text{S}\cdot\text{cm}^{-1}$), PBI ($2490 \mu\text{S}\cdot\text{cm}^{-1}$) and PI ($1665 \mu\text{S}\cdot\text{cm}^{-1}$). The evolution

365 of SPW EC with time showed that it increased in all conditions except PBC. In PBCI and PC
366 substrates, the increase was only significant in vegetated pots; in P, PI and PBI treatments, there was
367 no significant difference between the vegetated and non-vegetated conditions, while the SPW EC rise
368 in PB was higher with plants compared to the non-vegetated pots. Biochar can raise SPW EC through:
369 (i) ash accretion (Nigussie et al. 2012) and (ii) dissolution of salts and nutrient leaching due to pH
370 increase (Janus et al. 2015, Lomaglio et al. 2016). Compost effect on SPW EC can be attributed to: (i)
371 the elevated compost EC ($3419.67 \mu\text{S}\cdot\text{cm}^{-1}$) (Rossini-Oliva et al. 2017) and probably, as described in
372 Eigenberg et al. (2002) (ii) the mineralization of N contained in compost. Finally, the application of
373 iron alone did not increase SPW EC at T0 ($261 \mu\text{S}\cdot\text{cm}^{-1}$). However, SPW EC in PI increased with
374 time ($1720 \mu\text{S}\cdot\text{cm}^{-1}$ in non-vegetated pots and $1665 \mu\text{S}\cdot\text{cm}^{-1}$ with plants), At the end of the experiment,
375 SPW EC in PI substrates was higher than in P0%. This can be explained by the iron oxidation.

376

377 3.2.3 SPW metal(loid) concentrations

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

395

396 SPW Fe concentration was low in P0% at T0 ($0.05 \text{ mg}\cdot\text{L}^{-1}$) (Table 3) and only increased with iron
397 amendments, alone or combined with biochar and compost. The Fe concentration increase was higher
398 when iron was applied alone to P0% ($20.06 \text{ mg}\cdot\text{L}^{-1}$) compared to PBI ($2.70 \text{ mg}\cdot\text{L}^{-1}$) and PBCI (0.10
399 $\text{mg}\cdot\text{L}^{-1}$) (Table 3). This increase is explained by the addition of Fe to SPW, which is released by iron
400 grit. At the end of the experiment, Fe SPW concentrations in P were $0.04 \text{ mg}\cdot\text{L}^{-1}$ and $1.13 \text{ mg}\cdot\text{L}^{-1}$ in
401 vegetated and non-vegetated pots, respectively. Iron grit amendment increased SPW Fe

402 concentrations, although it was only significant for PI and PBI in non-vegetated pots. Finally, in
403 unvegetated pots, the addition of biochar-compost decreased SPW Fe concentration to non-detectable
404 levels, and in pots with plants, biochar addition, and to a lesser extent the biochar-compost
405 amendment, decreased SPW Fe concentrations. This decrease is consistent with the ability of biochar
406 to immobilize metallic cations through the formation of metal hydroxides, carbonate and phosphate
407 precipitates, and by electrostatic interaction (Park et al. 2011), which can also explain the smaller
408 increase in SPW Fe concentrations at T0 in PBI and PBCI compared to PI. Moreover, as Fe is a cation,
409 it tends to be immobilized with increasing pH, which was observed with the biochar amendment. This
410 hypothesis was confirmed by the significantly low negative correlations between SPW pH and SPW
411 Fe concentrations at T0 ($r = -0.44$, p -value < 0.001), T67-*Salix* ($r = -0.4$, p -value < 0.05) and
412 T67+*Salix* ($r = -0.33$, p -value < 0.05) (Table S2). Finally, in six out of the seven treatments, *i.e* P, PB,
413 PC, PI, PBI and PBCI, SPW Fe concentrations increased with time. There was a significant difference
414 between vegetated and non-vegetated pots for PB, which showed higher SPW Fe concentrations in
415 non-vegetated pots compared to the vegetated ones. In PI and PBI treatments, there was no significant
416 difference between vegetated and non-vegetated pots whereas in P0%, PC, and PBCI treatments, the
417 SPW Fe concentration increase was only significant for vegetated pots.

418
419 At T0, Pb concentration was very high in Pontgibaud SPW, 14.96 mg.L^{-1} on average (Table 3). Iron
420 application to P led to a 50 % decrease in SPW Pb concentrations while in treatments PBI, PC and PB,
421 SPW Pb concentrations were 89 %, 90 % and 96 % lower compared to P, respectively, with no
422 significant difference between the three treatments (Table 3). Finally, a 98 % and a 99 % decrease in
423 SPW Pb concentrations was observed with biochar-compost and biochar-compost-iron amendments,
424 respectively, compared to P0%. At T67, SPW Pb concentration was 8.10 mg.L^{-1} in non-vegetated pots
425 and 7.26 mg.L^{-1} in vegetated pots. Concentrations were decreased with all the different amendments.
426 In non-vegetated pots, the lowest Pb concentration decrease was observed when applying biochar-iron
427 (79 % decrease) and iron alone (77 % decrease), with no significant difference between those two
428 treatments. Biochar-compost-iron and biochar amendments also showed drops in SPW Pb
429 concentration, by 91 % and 93 %, respectively. Finally, the application of compost alone showed a
430 higher Pb concentration decrease (99 %) than the application of both biochar and compost (98 %). The
431 pattern for this decrease in the vegetated pots was different from that observed in non-vegetated
432 conditions. Indeed, in the vegetated pots, the lowest decrease was observed with the iron amendment
433 alone (69 %), followed by biochar-iron (77 %), compost alone (90 %), biochar alone (91 %), biochar-
434 compost-iron (92 %) and finally the combination of biochar and compost led to the highest SPW Pb
435 concentration decrease (97 %). No significant change in SPW Pb concentration was observed between
436 T0 and T67 (with or without plants) in PB, PBC and PBI treatments. In P0% and PI substrates, SPW
437 Pb concentrations were lower at T67 compared to T0, with no difference between unvegetated and
438 vegetated conditions, while SPW Pb concentration decrease was only observed in non-vegetated pots

439 for PC treatment, compared to T0. Finally, a slight increase in SPW Pb concentration with time was
440 observed for the PBCI treatment. The capacity of the three different amendments, biochar, compost
441 and iron grit, to decrease SPW Pb concentrations has been shown in previous studies. Indeed,
442 Lomaglio et al. (2016) explained the 70 % decrease observed in SPW Pb concentration following a
443 pinewood biochar addition to a mining soil by the precipitation of Pb on phosphates and carbonates
444 present in the biochar. Moreover, Pb can sorb onto biochar surfaces (Park et al. 2011) through
445 interactions with oxygen functional groups (Jiang et al. 2012). Concerning compost, in their review,
446 Huang et al. (2016) showed that it potentially had a biosorbent role on the metal(loid)s due to humic
447 substances which contain many organic functional groups. For iron grit, when incorporated into the
448 soil, its oxidation formed iron oxides, which have the capacity to sorb Pb (Houben et al. 2012).
449 Finally, like iron, Pb is a metallic cation in solution; therefore, at $\text{pH} > 6$ it can form hydroxide
450 precipitates and complexes with OM, which decrease its mobility and solubility (Oustriere et al.
451 2017). The important role of pH on Pb mobility and thus SPW Pb concentrations was corroborated by
452 the negative correlations between SPW pH and Pb SPW concentrations at T0 ($r = -0.87$, $p\text{-value} <$
453 0.001), T67-*Salix* ($r = -0.63$, $p\text{-value} < 0.001$) and T67+*Salix* ($r = -0.64$, $p\text{-value} < 0.001$) (Table S2).

454

455 3.2.4 SPW toxicity

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

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

479

480 3.3 Plant growth measurements

481 3.3.1 Leaf surface area

482 .

483 Plants grown on unamended P0% soil presented a total leaf area of 151.80 cm².plant⁻¹ (Table 4). The
484 amendment with iron alone decreased the total leaf area by 2.3-fold, while the biochar-iron
485 combination amendment had no effect. The plants grown on the other four treatments, *i.e* PB, PC,
486 PBC and PBCI, presented a higher total leaf area of 377.64 cm².plant⁻¹, 425.99 cm².plant⁻¹, 393.00
487 cm².plant⁻¹ and 298.24 cm².plant⁻¹, respectively, compared to P. Similar results have been observed
488 using organic waste amendment on a sandy loam (Sebastiani et al. 2004) and two different biochars
489 (woodchip biochar and olive tree pruning biochar) on a disused Cu mine (Brennan et al. 2014).

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

497

498 3.3.2 Organ dry weight (DW)

499 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,
500 respectively (Fig. 1). Plant growth was increased with the different amendments, except when iron and
501 biochar-iron were applied. Indeed, on the PI substrate, only root DW (0.39 g) was higher compared to
502 P, while on PBI, roots presented a lower DW (0.16 g) compared to P roots. For all three organs
503 (leaves, stem and roots), the highest increase was observed for PC and PBC, with DW increases of
504 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
505 grown on the PB treatment, plants presented a leaf, stem and root DW of 1.48 g, 0.81 g and 0.65 g,
506 respectively, which were not significantly different to the organ DW of plants grown on PBCI (leaves
507 1.42 g, stem 0.79 g and roots 1.02 g). Such improvements can be attributed to an improvement in the
508 soil conditions (Abbas et al. 2018), as shown in the previous sections: (i) a supply or increase of
509 availability of nutrients (Kołtowski et al. 2017), (ii) a supply of organic matter (Walter and Bernal
510 2008) (Table 1) and (ii) a decrease in metal(loid) SPW concentrations and extractable fractions (Park
511 et al. 2011, Zheng et al. 2013) (Table 2, Table 3). Regarding the reduction of plant growth induced by
512 the iron grit amendment, as well as for leaf parameters, this could be explained by the iron toxicity.

513

514 3.4 Plant metal(loid) accumulation and repartition

515 3.4.1 Metal(loid) concentrations

516 Leaf As concentration in plants grown on P0% was 0.90 mg.kg^{-1} (Fig 2A) and only increased when the
517 iron amendment was applied alone, causing a 7-fold rise in As concentrations. Stem As concentrations
518 were low in all conditions and not affected by the different amendments. Finally, root As
519 concentration was $818.74 \text{ mg.kg}^{-1}$ in P plants and decreased with biochar, biochar-compost and
520 biochar-compost-iron amendments, with As concentrations of $613.17 \text{ mg.kg}^{-1}$, $606.87 \text{ mg.kg}^{-1}$ and
521 $604.46 \text{ mg.kg}^{-1}$, respectively. This decrease can be explained by a dilution effect (Abbas et al. 2018).
522 Indeed, root DW was higher in these treatments compared to P0%. Finally, it can be noted that even
523 though compost application increased As concentrations in SPW, it induced low root As
524 concentrations, associated to a low translocation towards aboveground parts, which could indicate that
525 the solubilized form of As was not, or was poorly, available for plant translocation in the plant tissue.

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

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

546 Finally, it can be noted that for the three metal(loid)s, As, Fe and Pb, concentrations were much higher
547 in roots compared to leaves and stems. A higher accumulation of metal(loid)s in the roots, associated
548 with a low translocation towards upper parts, has been observed in previous studies (Beesley et al.
549 2013, Puckett et al. 2012). Roots are in direct contact with the soil contaminants, which makes them

550 the primary adsorption and absorption site for metal ions (Wang et al. 2014). Following their entry
551 into the roots, metal(loid)s can bind to ion exchangeable sites on cell walls (Wang et al. 2014) and
552 could be blocked by the Casparian strip (Wang et al. 2014). Such root to aerial part restriction
553 transport will protect the photosynthetic and metabolic plant apparatus (Borisev et al. 2008). These
554 mechanisms have been demonstrated to be protective evolutive traits (Chen et al. 2014)

555

556 3.4.2 Metal(loid) quantities

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

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

566 Pb quantity in leaves of P0% plants was low (31.76 μg) (Fig. 2F) and increased with biochar-compost
567 (78.92 μg) and biochar-compost-iron amendments (72.83 μg). Regarding the stems, all amendments,
568 except iron alone, increased Pb quantity in the order PBC (70.12 μg), PC (59.22 μg), PB (45.87 μg),
569 PBI (36.40 μg) and PBCI (33.44 μg) compared to P (16.14 μg). Finally, roots of P plants presented
570 2486.14 μg of Pb, and the four treatments PC, PI, PBC and PBCI induced an increase in Pb
571 accumulation corresponding to a final Pb quantity of 6622.08 μg , 6405.50 μg , 6609.28 μg and
572 11210.80 μg , respectively.

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

576

577 3.5 Stress indicators

578 3.5.1 Leaf pigments

579 Leaves of plants grown on Pontgibaud technosol presented 25.88 $\mu\text{g}\cdot\text{cm}^{-2}$ of chlorophyll (Fig. 3A).
580 Iron amendment alone did not affect chlorophyll content, while biochar-iron application induced a
581 slight decrease in leaf chlorophyll content, down to 22.23 $\mu\text{g}\cdot\text{cm}^{-2}$. The other four amendment
582 treatments decreased leaf chlorophyll content, with a higher decrease observed with biochar and/or
583 compost. Indeed, leaf chlorophyll contents were 15.79 $\mu\text{g}\cdot\text{cm}^{-2}$, 15.65 $\mu\text{g}\cdot\text{cm}^{-2}$ and 15.87 $\mu\text{g}\cdot\text{cm}^{-2}$ in
584 leaves of plants grown on PB, PC and PBC substrates, respectively. This chlorophyll content decrease
585 had been observed by Sorrenti et al. (2016) who attributed it to (i) a pH rise, which reduced Fe
586 transport to plant cells and (ii) a sorption or precipitation of Fe on biochar surface. However, it should

587 be noted that such a decrease in leaf chlorophyll content could be due to a dilution effect, as plants
588 presented a higher total surface area with biochar and compost amendments. This was supported by
589 the negative correlation between the leaf chlorophyll content and the total leaf surface area ($r = -0.56$,
590 $p\text{-value} < 0.001$) (Table S2). Indeed, when considering the chlorophyll content and the total surface
591 area to calculate the average chlorophyll content per plant, a different trend was observed. The lowest
592 chlorophyll content was found in PI plants (1578.23 μg), followed by PBI plants (2705.61 μg), P
593 plants (3928.58 μg), PBCI plants (5272.88 μg), PB plants (5962.94 μg), PBC plants (6236.91 μg) and
594 PC plants (6666.74 μg). This increase in total chlorophyll content induced by compost and biochar
595 could be due to the reduction of the metal(loid) toxicity (Abbas et al. 2018).

596 Leaf flavonoid levels in P0% plants were 0.39 $\mu\text{g}\cdot\text{cm}^{-2}$ and were not affected by biochar and iron
597 amendments alone or the combination of the three amendments (PBCI), whereas it decreased with
598 biochar-iron associated application (0.30 $\mu\text{g}\cdot\text{cm}^{-2}$) (Fig. 3B). The addition of compost, alone or in
599 combination with biochar, increased flavonoid content in leaves to similar levels, 0.49 $\mu\text{g}\cdot\text{cm}^{-2}$ and
600 0.47 $\mu\text{g}\cdot\text{cm}^{-2}$, respectively.

601 Leaf anthocyanin content was low for P0% plants (0.04 a.u) (Fig. 3C) and increased with all
602 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
603 (0.11 a.u) and PBC (0.11 a.u).

604 These pigment (chlorophyll, anthocyanin and flavonoid) content increases could be explained by the
605 reduction in metal(loid) toxicity, as shown by the soil and SPW analysis (Table 2, Table 3, Table S3).
606 Indeed, it is known that metal(loid)s can limit the biosynthesis of pigments (Akula and Ravishankar
607 2011) through: (i) lipid peroxidation, which alters the membrane permeability and chloroplast
608 ultrastructure (MacFarlane and Burchett 2001), and (ii) competition with essential elements required
609 for pigment synthesis (Bajguz 2011).

610

611 3.5.2 Root guaiacol peroxidase (GPOD) activity

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

615 In this study, roots of P0% plants presented a guaiacol peroxidase (GPOD) activity of 43.63 $\text{mol}\cdot\text{L}^{-1}\cdot\text{sec}^{-1}\cdot\text{mg}^{-1}$
616 protein (Fig. 4). The application of iron and biochar-iron to P had no effect on root GPOD
617 activity, while the other treatments decreased its activity. More precisely, root GPOD activities were
618 34.66 $\text{mol}\cdot\text{L}^{-1}\cdot\text{sec}^{-1}\cdot\text{mg}^{-1}$ protein, 25.68 $\text{mol}\cdot\text{L}^{-1}\cdot\text{sec}^{-1}\cdot\text{mg}^{-1}$ protein, 23.08 $\text{mol}\cdot\text{L}^{-1}\cdot\text{sec}^{-1}\cdot\text{mg}^{-1}$ protein and
619 29.05 $\text{mol}\cdot\text{L}^{-1}\cdot\text{sec}^{-1}\cdot\text{mg}^{-1}$ protein in PB, PC, PBC and PBCI treatments, respectively. This decrease in
620 root GPOD activity can indicate a lower metal(loid) stress, which can be linked to the soil
621 immobilization of metal(loid)s, inducing a decrease in metal(loid) levels in the plants and thus a
622 reduction in cell stress. Consequently, defense mechanisms against this stress decreased (Hmid et al.
623 2015). This explanation was attested by the correlation between the root GPOD activity and the root

624 metal(loid) quantities (As: $r = - 0.48$, $p\text{-value} < 0.01$; Pb: $r = - 0.43$, $p\text{-value} < 0.05$) (Table S2).
625 Moreover, Marchand et al. (2016) observed a negative relationship between the root GPOD activity
626 and the root DW of *P. australis*, which was also observed in this case ($r = - 0.47$, $p\text{-value} < 0.01$)
627 (Table S2).

628

629 4 Conclusion

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

653

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657

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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 ($p < 0.05$) ($n = 3-5 \pm SE$). Plant effect was determined for each treatment between the bulk and the rhizosphere soil (ns = non-significant)

	WHC (%)	SOM (%)		Plant effect
		Bulk soil	Rhizospheric soil	
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 ($p < 0.05$) ($n = 3-5 \pm \text{SE}$).

	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 ($\mu\text{S}\cdot\text{cm}^{-1}$). **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 ($p < 0.05$) ($n = 5-12 \pm \text{SE}$).

		pH		EC ($\mu\text{S}\cdot\text{cm}^{-1}$)		[As] ($\text{mg}\cdot\text{L}^{-1}$)		[Fe] ($\text{mg}\cdot\text{L}^{-1}$)		[Pb] ($\text{mg}\cdot\text{L}^{-1}$)	
			Time effect		Time effect		Time effect		Time effect		Time effect
P0%	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
	T67- <i>Salix</i>	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+ <i>Salix</i>	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
PB	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
	T67- <i>Salix</i>	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+ <i>Salix</i>	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
PC	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
	T67- <i>Salix</i>	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+ <i>Salix</i>	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
PI	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
	T67- <i>Salix</i>	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+ <i>Salix</i>	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
PBC	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
	T67- <i>Salix</i>	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+ <i>Salix</i>	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
PBI	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
	T67- <i>Salix</i>	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+ <i>Salix</i>	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
PBCI	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
	T67- <i>Salix</i>	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+ <i>Salix</i>	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 ($\text{cm}^2\cdot\text{plant}^{-1}$) 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 ($p < 0.05$) ($n = 14 \pm \text{SE}$).

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

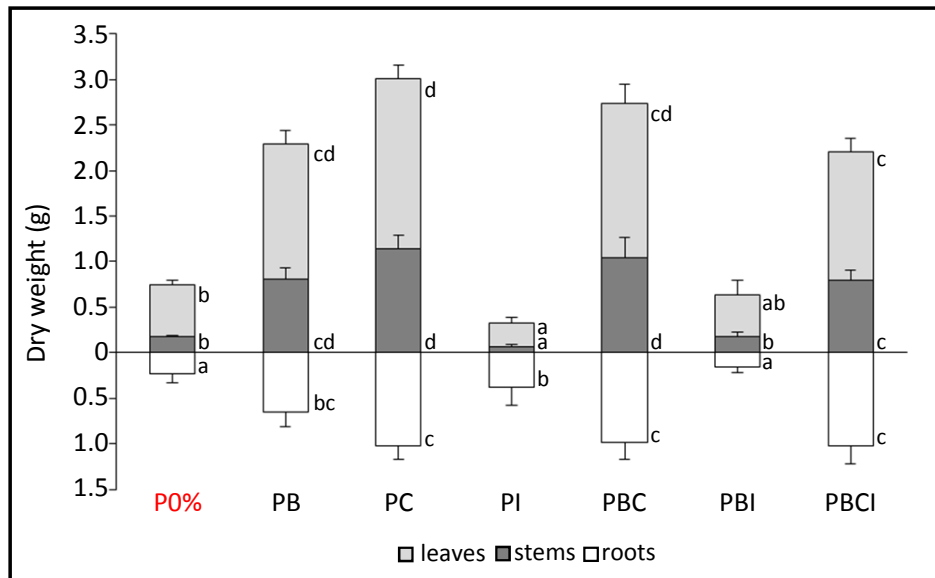


Fig 1. *Salix viminalis* organ (leaves, stems, roots) dry weight (g) determined after 69 days of growth on Pontgibaud technosol (P) alone (P0%) and amended with 5% biochar (B), 5% compost (C) or 1.5% iron grit (I), alone or combined. Letters indicate significant difference between treatments for each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

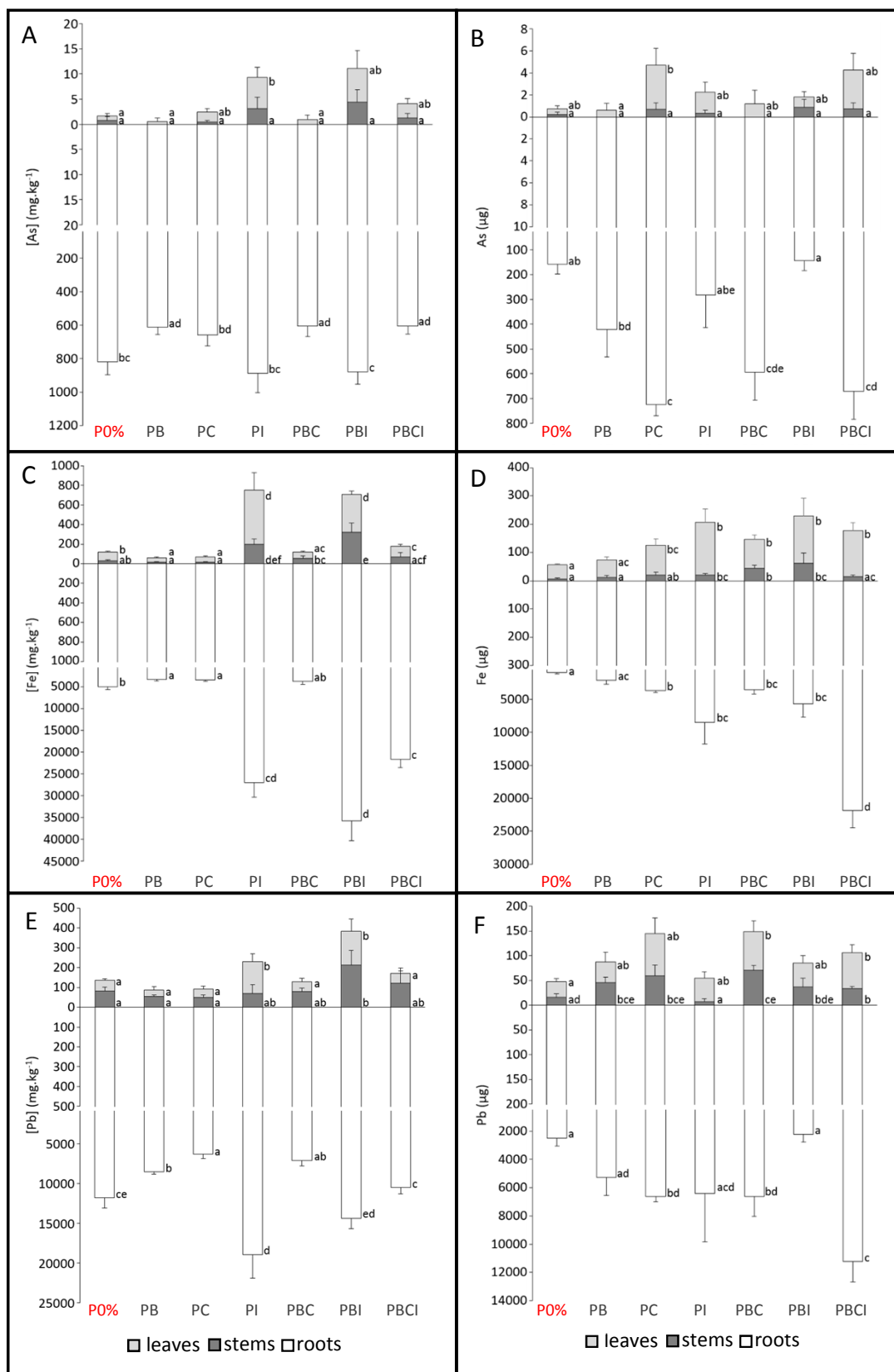


Fig 2. *Salix viminalis* organ (leaves, stems, roots) metal(loid) (As (A, B), Fe (C, D), Pb (E, F)) concentrations (mg.kg⁻¹) (A, C, E) and quantities (µg) (B, D, F) determined after 69 days of growth on Pontgibaud technosol (P) alone (P0%) and amended with 5% biochar (B), 5% compost (C) or 1.5% iron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

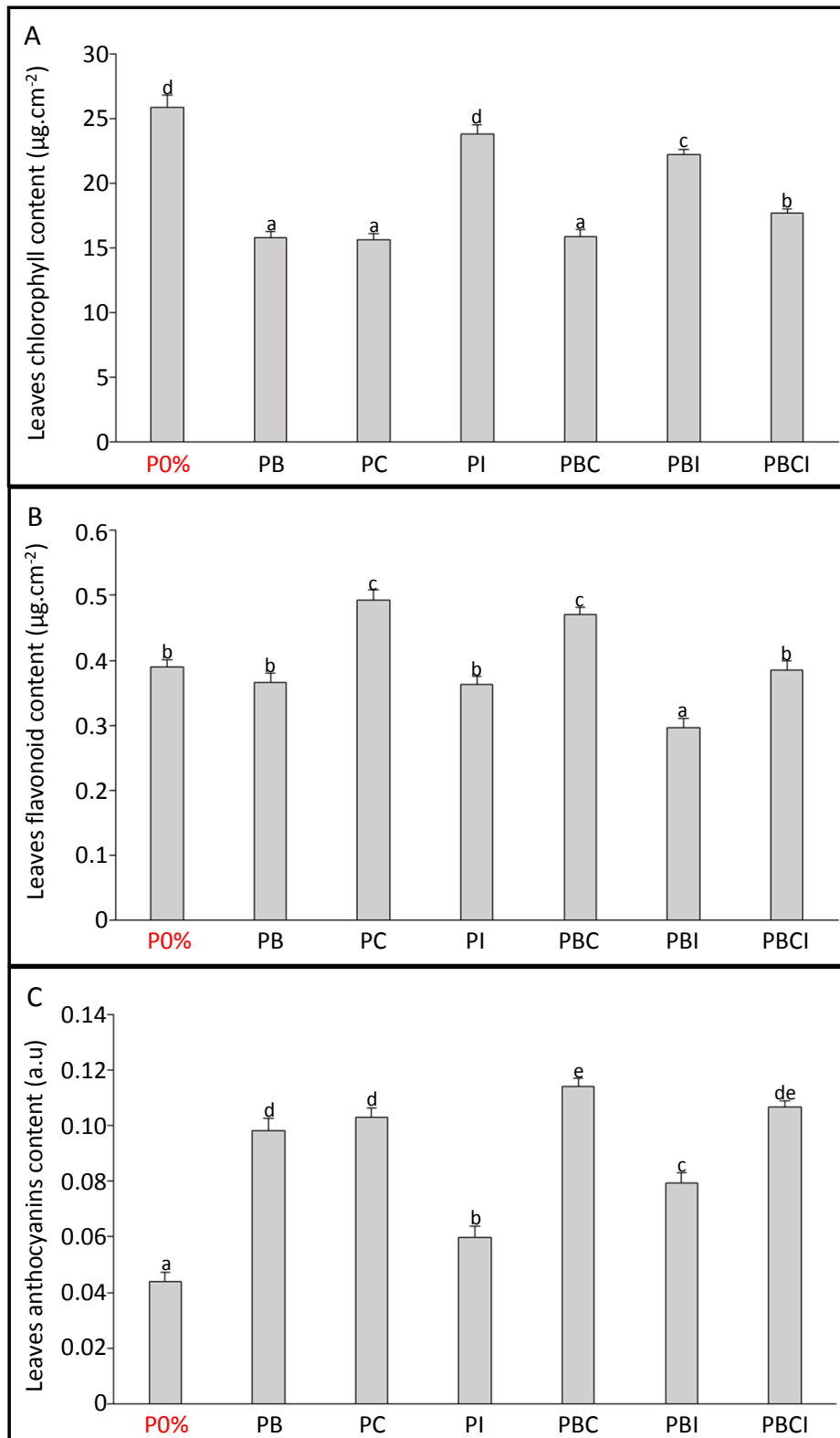


Fig 3. *Salix viminalis* leaf pigment contents (chlorophyll (A), flavonoids (B) and anthocyanins (C)) determined after 69 days of growth on Pontgibaud technosol (P) alone (P0%) and amended with 5% biochar (B), 5% compost (C) or 1.5% iron grit (I), alone or combined. Letters indicate significant difference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

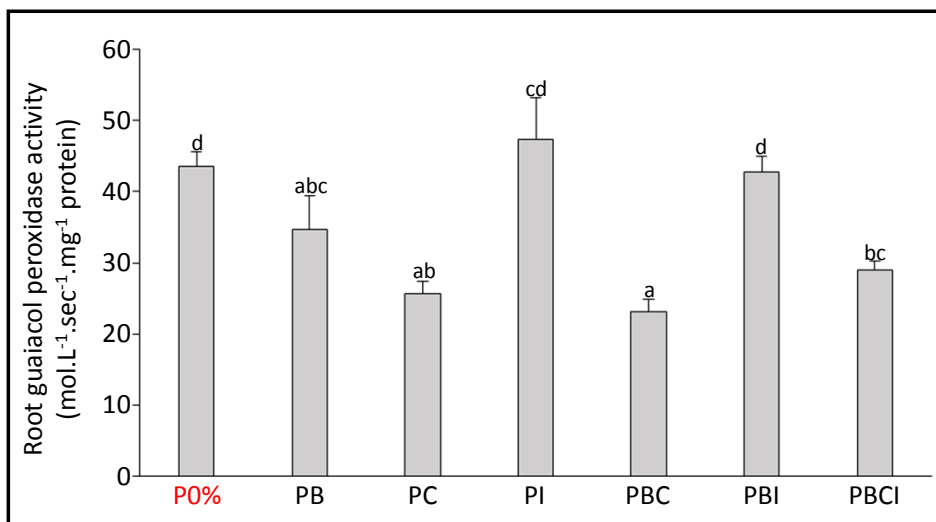


Fig 4. *Salix viminalis* root guaiacol peroxidase activity (mol.L⁻¹.sec⁻¹.mg⁻¹ protein) determined after 69 days of growth on Pontgibaud technosol (P) alone (P0%) and amended with 5% biochar (B), 5% compost (C) or 1.5% iron grit (I), alone or combined. Letters indicate significant difference ($p < 0.05$)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

1

2 **Highlights**

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

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

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

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

7

ACCEPTED MANUSCRIPT