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## Potential use of biochar, compost and iron grit associated with *Trifolium repens* to stabilize Pb and As on a multi-contaminated technosol

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### ► To cite this version:

Romain Nandillon, O. Lahwegue, F. Miard, M. Lebrun, M. Gaillard, et al.. Potential use of biochar, compost and iron grit associated with *Trifolium repens* to stabilize Pb and As on a multi-contaminated technosol. *Ecotoxicology and Environmental Safety*, 2019, 182, pp.109432. 10.1016/j.ecoenv.2019.109432 . insu-02269931

**HAL Id: insu-02269931**

**<https://insu.hal.science/insu-02269931>**

Submitted on 28 Apr 2021

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1 **Potential use of biochar, compost and iron grit associated with**  
2 ***Trifolium repens* to stabilize Pb and As on a multi-contaminated**  
3 **technosol**

4

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11

12 **Abstract**

13 Vegetation cover can be used in the phytomanagement of polluted areas by adding  
14 value to abandoned sites and reducing the dispersion of pollutants by erosion. Appropriate  
15 amendments, that allow both efficient plant growth and the immobilization of contaminants in  
16 the soil must be chosen in order to optimize the efficiency of this process. We used a mining  
17 technosol mainly contaminated by arsenic (1068 mg.kg<sup>-1</sup>) and lead (23387 mg.kg<sup>-1</sup>) to study  
18 the effect of three amendments (biochar, compost and iron grit) on (i) physico-chemical  
19 properties of the soil and soil pore water, (ii) metal(loid) mobility, bioavailability and  
20 bioaccessibility (CaCl<sub>2</sub> and Simple Bioaccessibility Extraction Test (SBET)), and (iii) the  
21 capability of *Trifolium repens* to germinate and grow. All the amendments used increased the  
22 pH and electrical conductivity of the SPW, resulting in a 90% decrease in the concentration of  
23 lead in the soil pore water (SPW). We also demonstrated a decrease in Pb phytoavailability.  
24 The amendments allowed the establishment of a plant cover, although the addition of iron grit  
25 alone did not allow any clover germination. For the Pontgibaud technosol, the combination of  
26 the three amendments resulted in a significant decrease in As and Pb concentrations in clover  
27 tissues, mainly in the aerial organs. The amendments also made it possible for some of them  
28 to halve the phytoavailable fraction of arsenic. However, for compost, both the As  
29 concentrations in the SPW, and the bioavailable fraction of As increased. All the amendments  
30 used had contrasting effects on the bioaccessible fractions of metal(loid)s. The most efficient  
31 amendment combination was the addition of 5 % biochar and 5 % compost.

32

33

34 **Keywords**

35 Phytomanagement, Biochar, Compost, Iron grit, metal(loid)s, *Trifolium*

36

37 **Highlights**

38 -Biochar and compost amendments, alone or combined, improved soil fertility and plant  
39 growth

40 -Amendments decreased Pb concentration in soil pore water

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

42 -Lead was more bioaccessible on amended soils

43

44 **Introduction**

45

46 After more than 200 years of industrialization, the European Union now faces a major  
47 problem with metal and organic contaminated soils (EEA, 2007). It was estimated that  
48 approximately 342,000 sites are currently contaminated, and that industrial activities,  
49 including mining, are among the main sources, representing 33% of the contamination  
50 (Panagos et al., 2013).

51 To the extent that this industrial waste contain organic and inorganic pollutants, they  
52 participate as source of pollution for soil, water and air. Thus, these wastes can have negative  
53 health consequences through direct ingestion or inhalation (Uzu et al., 2011) or by entering  
54 the food chain (Schreck et al., 2012).

55 To reduce these risks, remediation methods such as controlled backfilling, soil  
56 fixation, or leaching can be very useful. However, these methods are generally expensive, and  
57 some may even have negative effects on the biodiversity, biological activities, soil structure,  
58 or fertility (Ali et al., 2013). In contrast, environment-friendly remediation options, such as  
59 “aided phytostabilisation”, could improve soil functionality and allow a vegetation cover to be  
60 established, at the same time as reducing the mobility and availability of pollutants (Cundy et  
61 al., 2013, 2016). Plant cover may also reduce contaminant leaching (Houben et al. 2012), as  
62 well as stabilizing soils, and it controls water and wind erosion (Reubens et al. 2007). The  
63 implementation of plants will stabilize contaminants by adsorption or accumulation in roots  
64 (Vangronsveld et al. 2009). However, the translocation to the shoot system should remain

65 limited to avoid the transfer of contaminants into the food chain through grazing (Henry et al.,  
66 2013; Pérez-de-Mora et al., 2011; Kidd et al. 2009).

67 The selection of an appropriate metal tolerant vegetation cover is critical, as it affects  
68 the efficiency of the phytoremediation process.

69 *Trifolium repens* has characteristics which have led to it being selected for restoring  
70 plant cover. Clover mainly stores metals and metal(loid)s in its roots (Bidar et al., 2009), and  
71 it enriches poor soils by fixing atmospheric nitrogen, which allows the development of other  
72 plant species. In addition, it has a high germination rate and good resistance to environmental  
73 stress conditions. It appears that its stoloniferous growth makes *Trifolium repens* able to  
74 colonize bare spaces in lawns and it can therefore be used in various revegetation strategies  
75 (Bidar et al., 2007). Previous studies using *Trifolium repens* in contaminated zones have  
76 focused on metal uptake and remediation (Bidar et al., 2007, 2009; Lopareva-Pohu et al.,  
77 2011), and have shown that *Trifolium repens* could therefore play a positive role in the metal  
78 phytoremediation strategy.

79 However, the extreme conditions encountered on polluted sites, i.e the high  
80 concentrations of toxic elements, the low pH, and the low organic matter content, do not  
81 usually allow plant growth. It is therefore essential to add mineral and organic amendments  
82 that could reduce the mobility of soil pollutants, and therefore their (phyto)availability  
83 (Houben et al., 2012; Kumpiene et al., 2008; Lambrechts et al., 2011; Mench et al., 2003), at  
84 the same time as increasing soil fertility in order to improve plant development (Lopareva-  
85 Pohu et al., 2011).

86 Biochar, used as an organic amendment, has received increasing attention in recent  
87 years. It is a calcined carbon material produced by heating biomass in a closed system with a  
88 limited oxygen supply, using thermochemical technologies such as pyrolysis, gasification, or  
89 hydrothermal technologies (Xu et al., 2013). In degraded soils it is used for its potentially  
90 beneficial effects on the environment. This includes increased soil fertility through  
91 improvement of the soil microbial activity, and reduction of pollutant mobility. It can also  
92 help reduce the greenhouse effect by long-term sequestration of carbon in the soil (Inyang et  
93 al., 2016). Biochar can adsorb pollutants from the soil due to its microporous structure, high  
94 pH, cation exchange capacity, and its surface functional groups (Jiang et al., 2012; Ding et al.,  
95 2017).

96 However, biochar does not generally contain enough nutrients to allow plant growth  
97 on polluted soils, which are often characterized by low nutrient content (Fischer and Glaser,  
98 2012). Extra nutrients, such as those found in compost must therefore be added alongside the

99 biochar. The addition of compost will allow plant growth as it contains N, P, K, Ca, Mg, and  
100 S, as well as humus and different kinds of microorganisms (Fischer and Glaser, 2012; Gil-  
101 Loaiza et al., 2016). Moreover, it can reduce the phytoavailability of metal(loid)s due to their  
102 association with organic matter (OM), carbonates, or metal oxides (Mench et al., 2000),  
103 resulting in a reduction in the plant uptake of metals. But the effect of compost on metal  
104 bioavailability depends on the nature of the organic matter, the mineral species content, the  
105 potential degradability by microbiota, the pH and redox potential of the soil, and the soil type  
106 and metal(loid)s concerned (Walker et al., 2003).

107         Although the application of biochar and compost has been shown to improve soil  
108 agronomic characteristics, as well as its ability to sorb and reduce metal cation availability  
109 such as Pb in soil, neither amendment is effective in decreasing the mobility of metal anions,  
110 such as arsenic. They even tend to increase anion soil mobility due to the rise in soil pH  
111 (Beesley et al., 2011). This can cause problems in soils polluted with both cationic and  
112 anionic metal(loid)s. In such polluted sites, one possible strategy would be to combine  
113 compost and biochar with iron-rich amendments, or Mn oxides for metal(loid) immobilization  
114 (Ruttens et al., 2006). Among these amendments, zero-valent iron, in the form of iron grit, is  
115 the most relevant type of iron (Kumpiene et al., 2006), because its high adsorption potential  
116 has proved valuable in immobilizing inorganic contaminants (Liu and Zhao, 2007;  
117 Satapanajaru et al., 2008; O'day and Vlassopoulos, 2010). In soil, iron grit is known to be  
118 transformed to reactive iron (hydr)oxydes (e.g. ferrihydrite) (Komárek et al., 2013).  
119 Moreover, iron (hydr)oxydes capacity to sorb ions is modulated according to soil pH. A  
120 strong sorption of anions as arsenic is implemented at low pH due to a higher net positive  
121 surface charge on iron, whereas at high pH a stronger sorption of cations will be possible. In  
122 addition to sorption reactions, co-precipitation of metals and metalloids with iron oxides is  
123 another important mechanism for stabilizing metal and metalloid elements in amended soils  
124 (Komárek et al., 2013). Thus, it has been shown that the formation of relatively insoluble  
125 mineral precipitates Fe(II/III)-As (e.g.  $\text{FeAsO}_4 \cdot \text{H}_2\text{O}$ ,  $\text{FeAsO}_4 \cdot 2\text{H}_2\text{O}$  and  $\text{Fe}_3(\text{AsO}_4)_2$ )  
126 influences the immobilization of arsenic and its bioavailability in soils (Kumpiene et al.,  
127 2008; Drahotka and Filippi 2009).

128         Thus, to demonstrate the effectiveness of amendments and revegetation, it is essential  
129 to study the metal(loid) concentrations in the soil, soil pore water and plants *continuum*.  
130 However, as Lebrun et al. (2018a) observed, the metal(loid) concentration in soil pore water  
131 did not always reflect the amount available for plants. It is therefore interesting to measure  
132 this phytoavailable quantity by simple extraction tests. These plant availability tests can be

133 performed using extractant salts to determine the more or less mobile fraction of metal(loid)s,  
134 to understand their availability for plants (Qasim et al., 2015; Black et al., 2011).

135 However, the metal(loid) phytoavailability is not the only parameter to be measured,  
136 as human exposure to pollutants must also be taken into account. This exposure can include  
137 inhalation, oral ingestion or dermal absorption. One of the most common exposure pathways  
138 is the accidental ingestion of polluted soils (Luo et al., 2012). Such risk of metal(loid)  
139 exposure is often evaluated by a conservative approach in which the total metal(loid) content  
140 of the soil is measured. However, it is often pointed out that the risk for the exposed  
141 individual is only related to a small fraction of the total metal(loid)s, which is referred to as  
142 the bioaccessible fraction. To measure the bioaccessible fraction, various *in vitro* methods  
143 have been developed. They are usually simple and quick to manage, relatively inexpensive  
144 and do not raise ethical concerns (Deshommes et al., 2012). Some of these methods evaluate  
145 the fraction of ingested metal(loid)s that are soluble in the gastrointestinal tract (Mendoza et  
146 al., 2017; Wijayawardena et al., 2017) and therefore available in the blood flow. The Simple  
147 Bioaccessibility Extraction Test (SBET) procedure consists of an acid extraction that imitates  
148 the gastric compartment where the bioaccessibility is the strongest (Ruby et al., 1996; Casteel  
149 et al., 1997).

150 The objectives of this study were to evaluate: (1) the capacity of biochar, compost and  
151 iron grit to reduce As and Pb phytoavailability when added to a multi-contaminated technosol  
152 by measuring their concentrations in soil pore water and by using a CaCl<sub>2</sub> neutral salt  
153 extractant; (2) whether clover can be used to set up a vegetative cover and (3) the  
154 bioaccessibility of Pb and As in soil and in the aerial part of clover plants grown on amended  
155 soil, using the SBET approach.

156

## 157 **Materials and methods**

158

### 159 **Origin of contaminated soil (Pontgibaud site)**

160 The studied site in Roure-les-Rosiers (France) is a former silver-lead extraction mine,  
161 located in the Massif Central near Clermont-Ferrand (Saint-Pierre-le-Chastel (63)), in the  
162 Pontgibaud mining district (P<sub>s</sub>), Lambert II coordinates are: X: 638147.54 Y: 2087962.79.  
163 The mine has been in operation since Gallo-Roman times, and was industrially exploited only  
164 during the 19th century and ceased in 1897, with a resumption of activities during the Second  
165 World War. Mining activities stopped definitively in 1947.

166 The old mining site is subject to a semi-continental climate, with snowy winters, and  
167 hot summers, which can be marked by severe and localized thunderstorms. On average, the  
168 Roure-les-Rosiers sector receives about 770 mm of rainfall per year, and is located at around  
169 700 meters above sea level.

170

### 171 **Preparation of amended soils - Seed sowing and plant biomass**

172 The soil chosen for this study came from the slag heaps of the former Pontgibaud mine  
173 site. Six different soil samples were tested: polluted soil ( $P_s$ ), ( $P_s$ ) amended with 5 % (w/w) of  
174 hardwood-derived biochar ( $P_sB$ ), ( $P_s$ ) amended with 5 % (w/w) of compost ( $P_sC$ ), ( $P_s$ )  
175 amended with 1.5 % (w/w) of iron grit ( $P_sI$ ); ( $P_s$ ) amended with 5 % (w/w) of hardwood-  
176 derived biochar and 5 % (w/w) of compost ( $P_sBC$ ) and ( $P_s$ ) amended with 5 % (w/w) of  
177 hardwood-derived biochar and 1.5 % (w/w) of iron grit ( $P_sBI$ ). Biochar, was provided by La  
178 Carbonerie (Crissey, France). It was obtained by the slow pyrolysis of hardwood biomass (*Quercus*  
179 *sp.*, *Carpinus sp.* and *Fagus sp.*) at 500 °C (residence time: 3 h, heating rate: 2.5 °C.min<sup>-1</sup>), followed  
180 by a sieving to obtain a particle size between 0.2 and 0.4 mm. Physico-chemical properties of  
181 amendments including biochar is provided in table 1 SM.

182 Three replicates of the different mixtures were placed in rectangular pots with a  
183 capacity of 5 liters, and a surface area of 360cm<sup>2</sup> (24cm x 15cm). The bases of the pots were  
184 covered with clay beads to keep the soil mixture in the pots when watering. Each pot was  
185 divided into 2 compartments: one non-vegetated (NV), and the other vegetated (V), with 500  
186 clover seeds, which had a measured germination rate of 91%. The experiment was carried out  
187 in a controlled growth chamber under the following conditions: day/night temperatures  
188 (25°C/16°C), 16 hours of light/8 hours of darkness and with a light intensity of 800 μmol.m<sup>-2</sup>.s<sup>-1</sup>.

190 The experiment lasted 30 days. The plants were then separated from the soil. The roots  
191 were washed thoroughly with tap water and rinsed with distilled water to remove soil particles  
192 attached to the roots. Next, the plants were divided into two: the root system and the aerial  
193 parts (shoots and leaves). Finally, the plant organs were dried in an oven at 55 °C (until they  
194 were a constant weight), and the dry weight of the organs (DW) was determined.

195

### 196 **Soil pore water (SPW) sampling and analysis**

197 SPW was collected using Rhizon® (Rhizosphere Research Product, Wageningen, The  
198 Netherlands), from each pot. 20mL of SPW was sampled to determine the pH (pH meter,

199 FE20/EL20, Mettler-Toledo AG 2007), electrical conductivity (EC) (conductimeter, SOLEA,  
200 Tacussel électronique, Lyon, France), dissolved organic carbon (DOC) concentration (Pastel  
201 UV spectrophotometer, SECOMAM, Ales, France) and metal(loid) concentrations (ICP-AES,  
202 ULTIMA 2, HORIBA, Labcompare, San Francisco, USA), according to Bart et al. (2016).  
203 The sampling was done before sowing the clover seeds (T0) and at the end of the greenhouse  
204 experiment (TF) (30 days).

205

### 206 **Metal(loid) quantification in tested soils and in plant organs**

207 Metal(loid) contents (As, Fe and Pb) of soil samples and plant organs were determined  
208 by aqua regia digestion treatment ( $\text{HNO}_3$  (65%) :  $\text{HCl}$  (37%), 1:3 v/v) (Zhang and Selim  
209 2008), with a solid/liquid ratio of 1/45. The digestion program consisted of a gradual increase  
210 in temperature to 180°C in 15 minutes, a 15 minutes digestion step at 180°C followed by a 15  
211 minutes cooling step. The mineralized samples were then adjusted to a volume of 50 mL with  
212 ultrapure water ( $18 \text{ M}\Omega\cdot\text{cm}^{-1}$ ) and filtered through a nitrocellulose membrane at 0.45  $\mu\text{m}$ .  
213 Arsenic, iron and lead concentration were measured by Inductively Coupled Plasma Atomic  
214 Emission Spectroscopy (ICP-AES, Ultima 2, HORIBA, Labcompare, San-Francisco, USA).  
215 The accuracy of the assay technique was verified by enriching the samples analyzed with a  
216 solution whose concentration of the element to be assayed was known.

217

### 218 **Soil metal(loid) simple extraction procedures ( $\text{CaCl}_2$ treatment)**

219 The phytoavailable fractions of As and Pb were obtained as described by Qasim et al  
220 (2015), using  $\text{CaCl}_2$  extractant at 0.01M with a solid: liquid ratio 1: 10. Metal(loid)  
221 concentrations were determined by ICP-AES (ULTIMA 2, HORIBA, Labcompare, San  
222 Francisco, USA).

223

### 224 **Metal(loid) bioaccessibility test for soil samples (SBET) and plant organs**

225 According to the method developed by Mendoza et al. 2017, crushed dry material from  
226 tested soils or plant upper parts were put in a glycine solution  $0.4 \text{ mol}\cdot\text{L}^{-1}$  adjusted to pH 1.5  
227 at a concentration of 1%. The suspension was shaken for 1 h at  $37 \pm 1 \text{ }^\circ\text{C}$ , then filtered  
228 through a 0.45  $\mu\text{m}$  pore size membrane with syringe filter and stored at 4  $^\circ\text{C}$  until analysis.  
229 Metal(loid) concentrations were determined by ICP-AES (ULTIMA 2, HORIBA,  
230 Labcompare, San Francisco, USA).

231

## 232 **Data processing and statistical analysis**

233 Data was analyzed using R statistical software Version 3.1.2 (R Development Core  
234 Team, 2009). The normality and homogeneity were tested using Shapiro and Bartlett tests,  
235 and the means were compared using a parametric Anova test for normal data and the non-  
236 parametric Kruskal-Wallis test for non-normal data. Differences were considered significant  
237 when  $p < 0.05$ .

238

## 239 **Results and discussions**

240

### 241 **Effect of amendments on soil characteristics**

242 All tested amendments apart from iron induced an increase in WHC, averaging 10% for  
243 compost, and 30% for biochar when added alone at 5% (w/w) to Pontgibaud soil. Biochar  
244 showed real efficiency in retaining water due to its porous structure, its hydrophobicity and  
245 high surface area (Pietikäinen et al., 2000; Warnock et al., 2007; Molnar et al., 2016).  
246 However, these results are inconsistent with those obtained when measuring water holding  
247 capacity (WHC) on isolated compost and biochar, which were 299% and 183% respectively  
248 (Table 1SM). This difference is certainly related to the fact that the compost is compressed  
249 when it is added to Pontgibaud soil, meaning it is no longer able to expand in order to retain  
250 such a large quantity of water. Biochar is less subject to compressive stress when added to the  
251 Pontgibaud soil due to its porous and rigid structure, and therefore maintains its water  
252 retention characteristics.

253 None of the treatments affected the As pseudo-total concentrations compared to Ps. The  
254 iron concentration in Pontgibaud soil was  $6325 \text{ mg kg}^{-1}$ , and the addition of amendments,  
255 apart the iron grit, did not induce a significant change in iron soil concentration. The iron grit  
256 addition in PsI and PsBI caused the pseudo-total Fe soil concentration to increase by  
257 approximately 3 times. Lastly, the pseudo-total Pb soil concentrations in the different tested  
258 soils were the same for Ps, PsI, PsC, PsB and PsBI, whereas for PsBC, Pb concentration  
259 decreased by 30% compared to Ps due to a dilution effect produced by the addition of  
260 compost and biochar (Table 1).

261

### 262 **Effect of amendments and clover plants on SPW characteristics**

263 At the beginning of the experiment, neither time nor the presence of clover significantly  
264 modified the SPW pH of Pontgibaud soil (Ps) which was approximately 4.08 (Table 2).

265 Iron grit application to P<sub>s</sub> allowed a 0.5 unit increase in SPW pH, whereas time and  
266 clover growth induced a significant decrease in SPW pH (0.5 units). Compost (C), biochar  
267 (B), biochar + iron grit in combination (BI) and biochar + compost in combination (BC)  
268 increased P<sub>s</sub> SPW pH by 2.3 units, 2.7 units, 2.6 units and 3.3 units, respectively. It is noted  
269 that clover growth impacted SPW pH for P<sub>s</sub>B and P<sub>s</sub>BC by 0.45 units, whereas for these  
270 treatments on non-vegetated pots, time did not allow any significant pH modification. The  
271 alkalization of SPW due to the addition of biochar has already been demonstrated by  
272 Lebrun et al. (2018a) when 5% pinewood biochar was added to Pontgibaud soil, inducing a  
273 2.7 pH unit increase. Moreover, Beesley et al. (2014) showed that the SPW pH of a  
274 contaminated soil increased by more than three units when compost combined with biochar  
275 was added. The improvement in pH following the application of biochar or compost can be  
276 attributed to: (i) the alkalinity of the biochar (Table 1SM) which induced a liming effect  
277 (Lebrun et al., 2017); (ii) the dissolution or addition of alkali metals, and (iii) the presence of  
278 functional groups on the surface of the biochar, such as carbonyls, phenols, carboxyls and  
279 pyrones (Marks et al., 2014; Lebrun et al., 2018a).

280 Finally, for P<sub>s</sub>BI the pH decreased by two units over the time period of the treatment.  
281 This can be explained by the fact that the iron was oxidized during the experiment thus  
282 inducing the SPW pH decrease.

283 At T<sub>0</sub>, the SPW electrical conductivity (EC) for P<sub>s</sub> and P<sub>s</sub>I was 0.220 mS.cm<sup>-1</sup> and 0.315  
284 mS.cm<sup>-1</sup> respectively. For the other treatments, at T<sub>0</sub>, EC value was approximately 1.1  
285 mS.cm<sup>-1</sup>. At the end of the experiment, an increase in EC was observed compared to T<sub>0</sub> on all  
286 tested non-vegetated soils, and corresponded to 4.2 fold, 2.5 fold, 2 fold, 2.4 fold, 3 fold and  
287 2.1 fold for P<sub>s</sub>, P<sub>s</sub>I, P<sub>s</sub>C, P<sub>s</sub>B, P<sub>s</sub>BI, P<sub>s</sub>BC, respectively. The presence of clover had no effect  
288 on P<sub>s</sub>, P<sub>s</sub>I and P<sub>s</sub>BC, while for P<sub>s</sub>C, P<sub>s</sub>B and P<sub>s</sub>BI it caused a 1.3 fold EC decrease when  
289 compared to the corresponding non-vegetated pots. Overall, biochar and compost  
290 significantly increased SPW EC (p <0.05), which is consistent with previous studies (Lebrun  
291 et al., 2017, 2018a). Indeed, organic matter and mineral compounds such as Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup> or  
292 inorganic carbonates present on biochar and compost can be released into the soil and thus  
293 increase SPW electrical conductivity (Chintala et al., 2013). Kloss et al. (2014) demonstrated  
294 an increase in EC from 0.04 dS.cm<sup>-1</sup> to 0.19 dS.cm<sup>-1</sup> when 3% straw biochar was applied to a  
295 planosol. Finally, iron grit must be solubilized and mostly precipitated in the form of iron  
296 oxide on soil particulates, which is why it did not induce a large increase in the specific EC  
297 value.

298 The DOC content of SPW at T0 (Table 2) for P<sub>s</sub> was 5.7 mg.L<sup>-1</sup>, which is identical to that of  
299 P<sub>s</sub>BI. Moreover, the DOCs for P<sub>s</sub>B and P<sub>s</sub>BI were 2.8 fold and 1.2 fold smaller than P<sub>s</sub>. On the  
300 contrary, DOC for P<sub>s</sub>C and P<sub>s</sub>BC were 3.7 and 1.9 fold higher than for P<sub>s</sub>. It should be noted  
301 that neither the time nor the presence of clover modified the DOC.

302 A decrease in DOC was observed in previous studies when biochar was added to  
303 various soils (Lu et al. 2014; Kloss et al., 2014; Jain et al., 2014). This has been explained by  
304 the structure of the biochar, which contains sorption sites able to bind soluble organic  
305 compounds (Hass et al., 2012). Treatments containing compost (i.e P<sub>s</sub>C and P<sub>s</sub>BC) showed  
306 higher levels of DOC, which could be explained by the fact that even though the compost is  
307 composed of refractory humified material, labile fresh organic matter is also present (Lefevre,  
308 2015).

309

### 310 **SPW metal(loid) concentrations and phytoavailable metal(loid)s**

311 At T0 (Table 2), no As content was detected for P<sub>s</sub>, P<sub>s</sub>I, P<sub>s</sub>B and P<sub>s</sub>BI SPW whereas  
312 for P<sub>s</sub>C and P<sub>s</sub>BC, As concentrations were 0.09 mg.L<sup>-1</sup> and 0.06 mg.L<sup>-1</sup>, respectively. Both the  
313 passage of time, and the presence of clover plants increased As SPW for these last two  
314 treatments, by 2 fold and 4 fold respectively. For P<sub>s</sub>, at TF, As SPW concentration could be  
315 quantified, and corresponded to 0.03 mg.L<sup>-1</sup> and 0.02 mg.L<sup>-1</sup>, for TF-NV and TF-V,  
316 respectively. The As soil phytoavailable fractions (CaCl<sub>2</sub>-extractable fraction) for P<sub>s</sub> and P<sub>s</sub>C  
317 at T0 were not significantly different, and corresponded on average to 0.11 % of the total soil  
318 As concentration (Table 1). Whereas for the other treatments P<sub>s</sub>I, P<sub>s</sub>B, P<sub>s</sub>BI and P<sub>s</sub>BC, As  
319 phytoavailability was 1.9 times smaller than P<sub>s</sub>. Arsenic is usually found as an oxyanion in  
320 solutions, and presents specific challenges to sanitation, because its mobility increases when  
321 pH increases (Beesley et al., 2011, Nandillon et al., 2019). It was shown that the addition of  
322 compost may increase the mobility and leacheability of metal(loid)s, particularly arsenic  
323 (Mench et al., 2003). In the present study, a significant increase in arsenic SPW concentration  
324 was observed when compost was added to soils, probably because DOC competed with  
325 arsenic for sorption sites, such as those present in iron oxides, resulting in its increased  
326 mobility (Bolan et al., 2014). Compost with a high degradable organic matter content,  
327 associated to a neutral pH, present an active microbial activity which uses the organic matter  
328 as energy source (Balasoiu et al., 2001). This means that compost and its microbiota could  
329 reduce the soil redox potential, and consequently support the transformation of As(V) to  
330 As(III), which is more mobile (Kim et al., 2003). This high mobility could also be explained  
331 by the presence of phosphorus. Indeed, Fresno et al. (2018) showed that phosphorus-Olsen

332 had a significant effect on mobilizable As, as phosphate and arsenate have similar chemical  
333 properties, and they compete for organic and inorganic binding sites (Bolan et al., 2014;  
334 Moreno-Jiménez et al., 2013; Adriano, 2001). Other authors (Clemente et al., 2012; Moreno-  
335 Jiménez et al., 2013; Beesley et al., 2014) have already reported that competition for mineral  
336 sorption sites between these anions can be expected, which will lead to arsenic mobilization  
337 from less labile soil fractions and increase its availability. It is important to note that the  
338 phytoavailable fraction of arsenic was identical for the P<sub>s</sub> and P<sub>s</sub>C treatments, suggesting that  
339 compost influenced the easily mobilizable fraction found in pore water, but not the CaCl<sub>2</sub>  
340 extractable fraction. Moreover, the decrease in As concentration observed between P<sub>s</sub>B and  
341 P<sub>s</sub>BC both at T<sub>0</sub> and at T<sub>F</sub> (Table 2) could be explained by: (i) the mobile part of arsenic  
342 potentially being trapped in the biochar structure; (ii) DOC decrease after biochar addition  
343 resulting in less competition between dissolved organic matter and arsenate; and (iii) the  
344 calcite present in the biochar could form stable spherical compounds with arsenate anions on  
345 the biochar's surface (Alexandratos et al., 2007; Yin et al., 2016).

346

347 At T<sub>0</sub>, the SPW iron concentration (Table 2) for P<sub>s</sub> was 0.05 mg.L<sup>-1</sup>. No soluble Fe was  
348 quantified in SPW for P<sub>s</sub>C, P<sub>s</sub>C and P<sub>s</sub>BC. As expected, Fe concentration in P<sub>s</sub>I and P<sub>s</sub>BI  
349 treatments were 450 times and 50 times higher than P<sub>s</sub>, respectively. Moreover, for P<sub>s</sub>I and  
350 P<sub>s</sub>BI, no significant difference in SPW Fe concentration was observed at T<sub>F</sub> between non-  
351 vegetated and vegetated pots. Although at T<sub>F</sub>, SPW Fe concentration increased by 2.4 fold for  
352 P<sub>s</sub>I and 38 fold for P<sub>s</sub>BI. The values for the Fe-CaCl<sub>2</sub>-extractable fraction (Table 1) for P<sub>s</sub>, P<sub>s</sub>I  
353 and P<sub>s</sub>BI, were not significantly different, and were all between 0.06 % and 0.08 %. Finally,  
354 compost or biochar added to P<sub>s</sub> soil, alone or in combination (P<sub>s</sub>B, P<sub>s</sub>C and P<sub>s</sub>BC), led to a  
355 significant decrease in the Fe-CaCl<sub>2</sub>-extractable fraction (3.5 fold).

356 As expected, lead was the most prevalent ion in Pontgibaud SPW (11.2 mg.L<sup>-1</sup>), at T<sub>0</sub>,  
357 and on top of that, no significant difference was measured between T<sub>0</sub> and T<sub>F</sub> for P<sub>s</sub>, or any of  
358 the other studied treatments. The SPW Pb concentrations for P<sub>s</sub>I, P<sub>s</sub>C, P<sub>s</sub>B, P<sub>s</sub>BI and P<sub>s</sub>BC  
359 were 4.8 mg.L<sup>-1</sup>, 0.3 mg.L<sup>-1</sup>, 0.6 mg.L<sup>-1</sup>, 1.4 mg.L<sup>-1</sup> and 0.12 mg.L<sup>-1</sup>, respectively. For the  
360 Pb-CaCl<sub>2</sub>-extractable fraction (Table 1) four different significant groups were identified P<sub>s</sub>I  
361 2.75 %; P<sub>s</sub> 2.15 %; P<sub>s</sub>B and P<sub>s</sub>BI 1.6 %; and P<sub>s</sub>C and P<sub>s</sub>BC 0.15 %.

362 Compost and biochar + compost appeared to be the treatments that decreased the  
363 phytoavailable Pb fraction the most significantly, suggesting that the high pH and organic  
364 content of these amendments reduce the lead mobility in the soil. As is well known, SPW

365 metal mobility is strongly influenced by organic carbon, which is brought to the soil by the  
366 biochar and compost (Bolan et al., 2014). Furthermore, higher pH values are known to  
367 increase the surface charges of soil particles including amendments, which induces the  
368 retention of metal (Perez-Esteban et al., 2014; Boisson et al., 1999; Mench et al., 1998, 2000).  
369 These amendments can immobilize metal(loid)s via three mechanisms (Mench et al., 2000):  
370 (i) adsorption of metals to highly accessible sites on the surface of modified aluminosilicates,  
371 soil components and of biochar or compost through interactions with oxygenated functional  
372 groups (Lebrun et al. 2018b); (ii) precipitation with Al, Fe and Mn oxides (Oustrière et al.,  
373 2017); (iii) formation of minerals (such as metal silicates) and diffusion through mineral  
374 surfaces (Lee et al., 2013) and (iv) precipitation with phosphates and carbonates contained by  
375 the biochar (Lomaglio et al. 2016).

376

### 377 **Metal(loid) bioaccessibility in soil**

378 The SBET was used to assess the oral bioaccessibility of As, Fe and Pb in soil samples  
379 (Mendoza et al., 2017). This method simulates the human gastric phase, and was validated by  
380 the EPA (USEPA, 2012). The results for the present study are presented in Table 3.

381 The bioaccessible arsenic concentration for P<sub>s</sub> was 3.2% and decreased for P<sub>s</sub>I and  
382 P<sub>s</sub>BI treatments by 2.1 and 1.8 times respectively, compared to P<sub>s</sub>. This is because the iron  
383 oxide formed strongly adsorbed As. Many studies (Martin and Ruby 2003; Subacz et al.,  
384 2007) have shown that use of Fe-rich amendments (FeCl<sub>3</sub>, FeCl<sub>2</sub>.4H<sub>2</sub>O, Ferrihydrite, Fe<sup>0</sup> and  
385 FeBr<sub>3</sub>) could be an effective strategy for reducing bioavailability and bioaccessibility, and for  
386 remediating soils contaminated with As. Cui et al. (2010) demonstrated that the addition of  
387 FeSO<sub>4</sub> to a mining technosol decreased gastric and intestinal bioaccessibility of arsenic in all  
388 treatments. Finally, the bioaccessible arsenic fraction for P<sub>s</sub>C, P<sub>s</sub>B and P<sub>s</sub>BC treatments was  
389 almost 1.4 times higher than for P<sub>s</sub>. This could be explained by the fact that anions such as  
390 bicarbonate, phosphate, silicate, and organic acids present in the soil solution due to the  
391 addition of compost and biochar to P<sub>s</sub> could compete with As for chemisorption sites, and  
392 thus mobilize As to the aqueous phase. The links between biochar and arsenic, and between  
393 compost and arsenic are weak and therefore easily broken by the glycine present in SBET  
394 solution. This would explain the greater As accessibility for P<sub>s</sub>C, P<sub>s</sub>B and P<sub>s</sub>BC.

395 The iron bioaccessible fraction for P<sub>s</sub> was 1.3 % , which demonstrates its high stability  
396 in soil. When compost and biochar were added to P<sub>s</sub> (P<sub>s</sub>C and P<sub>s</sub>B), Fe accessibility was  
397 unchanged. As expected, the addition of iron to P<sub>s</sub> or P<sub>s</sub>B led to an increase in accessible-Fe,  
398 by 1.9 and 3.1 fold, respectively. Interestingly, for P<sub>s</sub>BC, the iron bioaccessible fraction was

399 almost twice the value measured for P<sub>s</sub>. This was probably due to the destabilization of iron  
400 oxides by the increase in organic ligands following the addition of biochar and compost.

401

402 Finally, the bioaccessible Pb proportion was high regardless of the treatment. For the  
403 P<sub>s</sub> treatment, just over half of the total lead (51 %) was bioaccessible, which shows its low  
404 stability in soil. This could be explained by the low pH (pH = 1.5) and the presence of organic  
405 ligands in the gastric solution which may have favored the dissolution and desorption of the  
406 minerals containing lead (Cui et al., 2010). Iron did not affect the Pb accessible fraction when  
407 added to P<sub>s</sub> or P<sub>s</sub>B. Similarly, a recent study showed that a highly adsorbent Mn oxide added  
408 in large quantities (10% by weight) to a Pb polluted soil produced no change to the Pb  
409 bioaccessible fraction (McCann et al., 2015). When compost or biochar were added to P<sub>s</sub>,  
410 whether alone or in combination, the Pb accessible fraction increased between 1.2 and 1.4  
411 times . This result was probably due to the establishment of even weaker links between the  
412 lead and compost and lead and biochar than those that may exist between lead and soil  
413 particles in the technosol P<sub>s</sub>.

414

#### 415 **Plant growth and metal(loid) uptake**

416 After 30 days of growth, the clover dry weight (DW) and the metal(loid) concentrations  
417 in the roots and aerial parts were measured for the different treatments. Firstly, it was noted  
418 that when iron was added alone to P<sub>s</sub> the seeds could not germinate. This was probably due to  
419 the high iron concentration in the soil pore water at T0. Indeed, when just iron grit was added  
420 to P<sub>s</sub> the concentration of Fe in the SPW was 22.5 mg L<sup>-1</sup>, whereas when iron grit was added  
421 to P<sub>s</sub> at the same time as biochar (P<sub>s</sub>BI), the Fe SPW concentration was 2.5 mg L<sup>-1</sup> due to the  
422 biochar's physico-chemical characteristics. Iron alone seemed to have an acidifying effect,  
423 certainly due to the oxidation of Fe(II) followed by the precipitation of Fe(OH)<sub>3</sub>. Thus, the  
424 combination of high Fe supply and acidity induced a high solubility and Fe availability. In the  
425 presence of biochar, the pH effect was attenuated. However, it was noted that the smallest  
426 increase in the biomass of aerial parts was observed for P<sub>s</sub>BI treatment (0.623 g). For the P<sub>s</sub>  
427 treatment, the dry weights of the roots and aerial parts were smaller, and corresponded to  
428 0.124 g and 0.170 g, respectively. For the roots, P<sub>s</sub>C, P<sub>s</sub>B and P<sub>s</sub>BC DW were not  
429 significantly different and corresponded to four times the value for P<sub>s</sub>.

430 For P<sub>s</sub>C, P<sub>s</sub>B and P<sub>s</sub>BC, the average DW of the aerial parts was 1.24 g, i.e. 7 times the  
431 value measured in P<sub>s</sub> pots. The same beneficial effect for the DW of *Salix viminalis* and  
432 *Populus euramericana* cuttings was obtained by Lebrun et al. (2018a) when adding biochar

433 produced from different feedstocks (hardwood, lightwood and pinewood, at 2% and 5% w/w)  
434 to Pontgibaud technosol. Fresno et al., (2018) showed that iron combined with bio-char on an  
435 As and Cu contaminated technosol slightly increased lupine shoot biomass. In our case, even  
436 though biochar allowed a better biomass production of clover aerial parts, the compost  
437 addition to biochar improved DW production by 30%. Indeed, organic matter, provided by the  
438 compost improved the N, P, K nutrient cycle (Marques et al., 2008, Abbas et al., 2018),  
439 allowing better plant growth, as observed by Marques et al. 2008 on *Solanum nigrum* and  
440 Gregory et al. (2014) on *Lolium perenne* grown on polluted soils

441 Concerning the concentrations of ions in clover organs (Fig 2), we noticed that the  
442 amendments systematically decreased the As and Pb concentrations in aerial parts by about  
443 1.8 times for arsenic and between 1.8 and 3.8 times for lead, whereas As and Pb  
444 concentrations in P<sub>s</sub> plants were 396 mg kg<sup>-1</sup> and 4577 mg kg<sup>-1</sup>, respectively. In roots, As  
445 concentrations were only decreased by P<sub>s</sub>B and P<sub>s</sub>BC treatments, and corresponded to 500 mg  
446 kg<sup>-1</sup>, whereas for P<sub>s</sub>, P<sub>s</sub>C and P<sub>s</sub>BI, As concentrations were approximately 812 mg kg<sup>-1</sup>. For Pb  
447 concentrations in roots, P<sub>s</sub> and P<sub>s</sub>BI demonstrated the same accumulation i.e 13300 mg kg<sup>-1</sup>,  
448 whereas P<sub>s</sub>C, P<sub>s</sub>B and P<sub>s</sub>BC decreased root Pb concentration by 2.4 fold. As expected, iron  
449 concentration was highest in clover plants grown on the P<sub>s</sub>BI soil, with concentrations of 5000  
450 mg kg<sup>-1</sup> and 17000 mg kg<sup>-1</sup> in aerial parts and roots, respectively. Whereas for P<sub>s</sub>, P<sub>s</sub>C, P<sub>s</sub>B and  
451 P<sub>s</sub>BC treatments Fe concentrations in aerial parts were 2500, 1200, 1300 and 800 mg kg<sup>-1</sup>,  
452 respectively. Finally, Fe concentrations in roots for P<sub>s</sub>, P<sub>s</sub>C, P<sub>s</sub>B and P<sub>s</sub>BC were not  
453 significantly different, and corresponded to 3100 mg kg<sup>-1</sup>, which was 5.5 times lower than the  
454 Fe concentration measured in roots of plants grown on the P<sub>s</sub>BI soil. In the present study, for  
455 all treatments, As, Fe and Pb concentrations were higher in the roots than in the aerial parts of  
456 clover. This is consistent with the results of Bidar et al., (2007, 2009) and Lopareva-Pohu et  
457 al., 2011 for *Trifolium repens* growing on metal(loid) contaminated soil. However, it is  
458 difficult to distinguish between the elements fixed to the root surface and those that are  
459 absorbed. We observed, a significant As, Fe and Pb concentration decrease in the aerial parts  
460 for all treatments, apart from for plant Fe accumulation levels on the P<sub>s</sub>BI treatment. For Pb  
461 and Fe, this decrease could be associated to a significant diminution of the two ions in the soil  
462 pore water, correlated to the amendments. Interestingly we demonstrated that compost being  
463 added to P<sub>s</sub>C and P<sub>s</sub>BC soils was associated with an increase in As concentrations in the soil  
464 pore water, whereas As concentrations in the aerial parts decreased compared to P<sub>s</sub>. Such  
465 results could be associated to a probable change in the chemical form of As, which is certainly  
466 less assimilable for clover plants. In fact, the plant metal(loid) absorption depends on different

467 parameters, relative both to the plant (such as root exudations), and to the soil characteristics  
468 (such as oxidation-reduction processes in the soil, pH, cation exchange capacity, dissolved  
469 oxygen, and temperature), which modify the metal(loid) speciation (Cheng, 2003).

470

471 The high concentration of As in the roots for the P<sub>s</sub>BI treatment compared to the P<sub>s</sub>B  
472 and P<sub>s</sub>BC treatments, which have similar biomasses and phytoavailable arsenic, could be due  
473 the mobilization of As by root exudates in soils treated with iron (Fresno et al. 2017). Another  
474 explanation could be that the precipitation of iron oxides on the roots causes an indirect  
475 increase in arsenic on the roots. The SBET bioaccessible fraction (Table 4) was only  
476 measured for the aerial parts of clover plants grown on amended soil. We were not able to do  
477 these measurements for aerial parts of clover grown on Pontgibaud soil (P<sub>s</sub>) due to poor plant  
478 biomass.

479 The bioaccessible As fraction was significantly larger for clover plants grown on soils  
480 containing compost (Table 4). Thus, the SBET bioaccessible As fraction for clover plants  
481 collected on P<sub>s</sub>C and P<sub>s</sub>BC soils was 8.2 % and 9.5 %, respectively. These values were twice  
482 as high as those calculated for clover aerial parts collected on P<sub>s</sub>B and P<sub>s</sub>BI soils. The P<sub>s</sub>B and  
483 P<sub>s</sub>BI soils were the treatments with the lowest As SBET bioaccessible fraction. Finally, we  
484 demonstrated that the P<sub>s</sub>BI soil allowed the highest Fe and Pb bioaccessible fractions for  
485 clover aerial organs. On average this increase was 6.6 and 1.7 times the average value  
486 measured for the other amendments tested, respectively. It was also noted that lead was the  
487 most accessible element measured in the clover aerial organs. Between 34 % and 64% of all  
488 lead was part of the bioaccessible fraction and could therefore potentially be released into the  
489 gastric tract. In addition, our results for lead were significantly higher (2 to 6 times higher),  
490 than those obtained in the gastric phase for edible parts of spinach, lettuce, radish and carrot  
491 plant grown on a poly-metallic polluted soil (Intawongse and Dean, 2008).

492 It has been explained that the SBET bioaccessible fraction of ions in plant organs is correlated  
493 to the chemical form, location and concentration of the ion, as well as the compounds to  
494 which it is potentially associated within the plant tissues, such as fibers, polyphenols and  
495 phytates (Labronici-Bertin et al., 2016; Machado et al., 2017). These specific tissue locations  
496 for As and Pb are linked to plant metal(loid) tolerance. Indeed, Chou and Shen (2007) and Fu  
497 and Cui (2013) proposed that metal(loid)s are mainly found in the vacuole and cell wall,  
498 which contains polysaccharides and proteins which are composed of many oxhydryl,  
499 carboxyl, aldehyde and phosphate groups, which show affinity with metal(loid)s. Therefore,

500 these groups could form bonds to reduce metal(loid) activity, which could modify their  
501 bioaccessible fraction.

502

## 503 **Conclusion**

504

505 In the present study, we demonstrated that the addition of amendments (compost,  
506 biochar or iron grit, alone or combined) to an As and Pb polluted soil could be efficient in  
507 reducing As and Pb soil mobility and availability. In addition, the amendments allowed the  
508 establishment of a clover cover on a soil that had previously been resistant to any plant  
509 development. The most appropriate treatment for the studied technosol was 5 % biochar  
510 combined with 5 % compost. It resulted in a decrease in As and Pb concentrations in both  
511 clover roots and aerial parts. However, while the Pb concentration in SPW decreased  
512 significantly, the As concentration increased, although it did remain within environmentally  
513 acceptable values. It should also be noted that the bioaccessible As and Pb soil fractions  
514 increased significantly following the addition of amendments. In our case, the use of iron grit  
515 did not give the expected positive results.

516 Finally, field tests using the selected combination of amendments are currently being  
517 undertaken to demonstrate the role of clover leaf cover in stabilizing soil particles, and  
518 preventing the dispersion of arsenic and lead into the environment by wind or surface runoff.

519

## 520 **Acknowledgments:**

521 We would like to thank Ingrid Girardeau and Louis De Lary De Latour (DPSM / BRGM) for  
522 giving us the authorization to study the former Pontgibaud mine and Mr. JC. Léger (La  
523 Carbonerie) for the supply of biochar. We also wanted to thank the National Association of  
524 Research and Technology (ANRT) for funding R. Nandillon's thesis.

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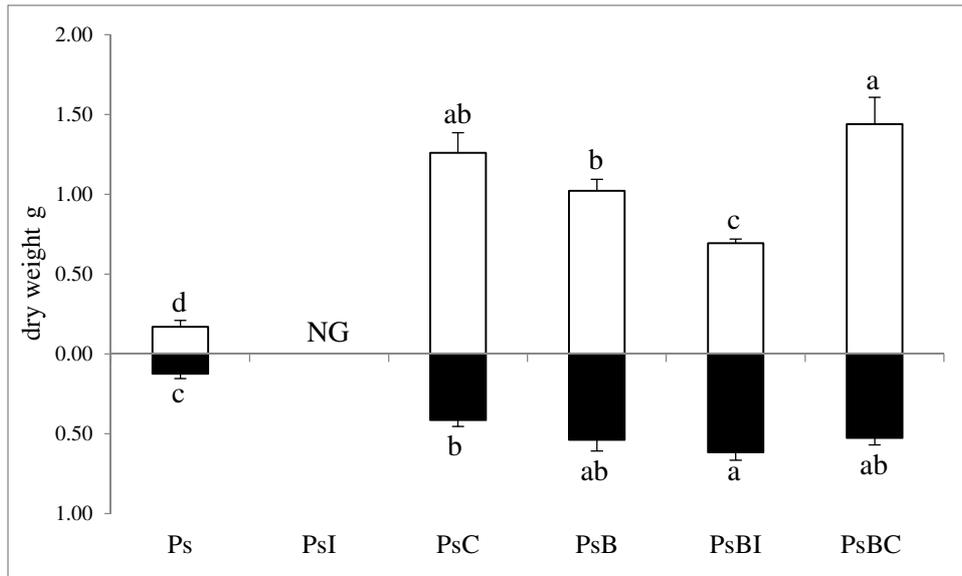
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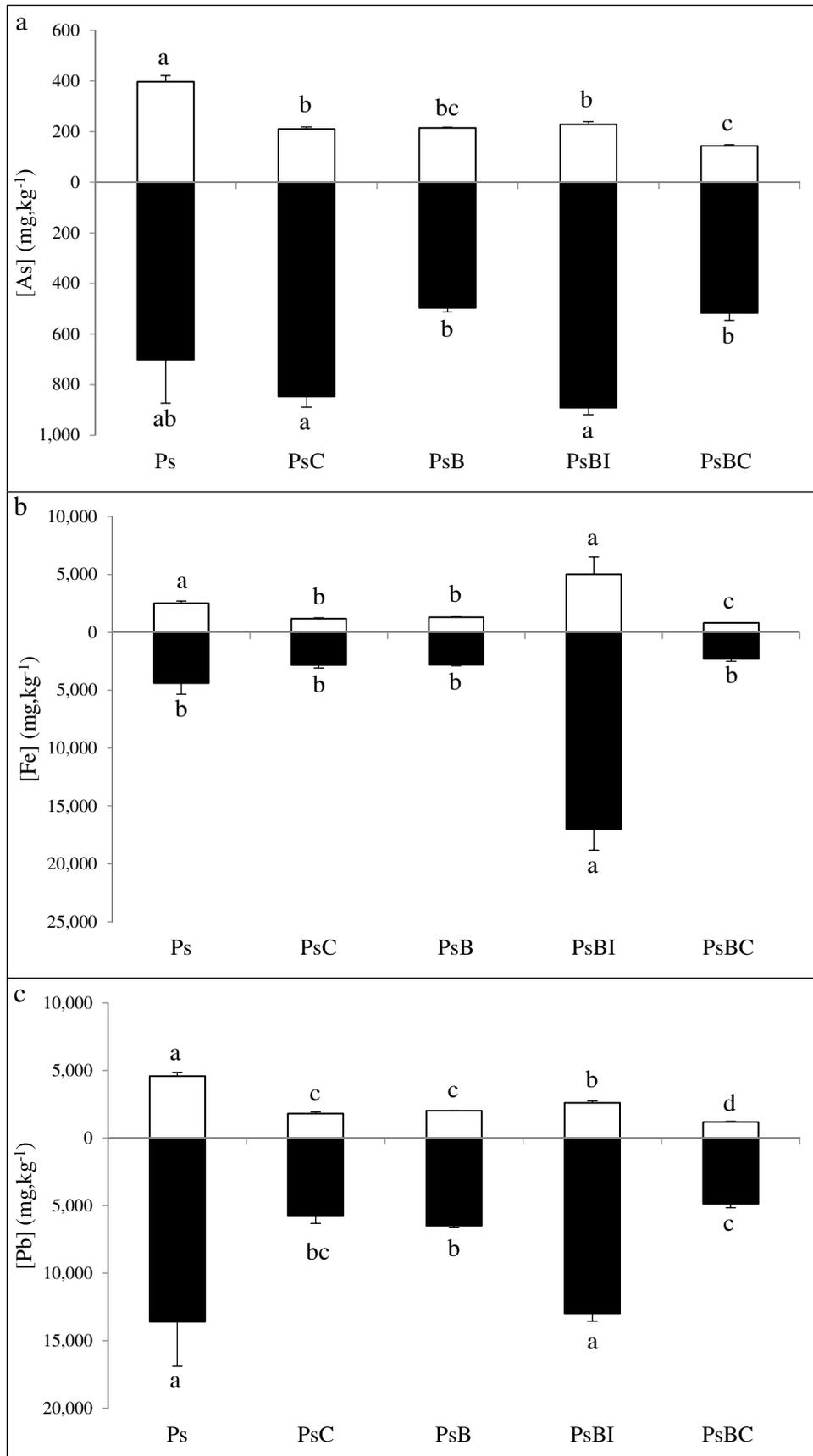
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**Figure 1:** *Trifolium repens* dry weight (DW) (g) of organs collected at the end of the experiment (30 days); white columns: aerial parts, black columns: roots. Results are obtained from 500 seeds sown on tested soils. Ps: polluted soil, PsI: Ps + iron grit (I) 1.5%, PsC: Ps + compost (C) 5%, PsB: Ps + biochar (B) 5%, PsBI: Ps + B 5% + I 1.5%, PsBC: Ps + B 5% + C 5%. Results are expressed as the mean value and bars indicate standard error (n=5); letters indicate significant difference (p<0.05). NG: no germination.



**Figure 2 :** Arsenic (a), iron (b) and lead (c) concentration (mg.kg<sup>-1</sup>) in the different *Trifolium repens* organs collected at the end of the experiment (30 days); white columns: aerial parts, black columns: roots. Results are obtained from 500 seeds sown on tested soils. Ps: polluted soil, PsI: Ps + iron grit (I) 1.5%, PsC: Ps + compost (C) 5%, PsB: Ps + biochar (B) 5%, PsBI: Ps + B 5% + I 1.5%, PsBC: Ps + B 5% + C 5%. Results are expressed as the mean value and bars indicate standard error (n=5); letters indicate significant difference (p<0.05).

**Table 1:** Physico-chemical characteristics of tested soils at the beginning of the experiment (T0). WHC: water holding capacity (% mass), TOC: total organic carbon (% mass) and soil As, Fe and Pb concentrations (mg.kg<sup>-1</sup>) and CaCl<sub>2</sub>-extractable fraction (%). Ps: polluted soil, PsI: Ps + iron grit (I) 1.5%, PsC: Ps + compost (C) 5%, PsB: Ps + biochar (B) 5%, PsBI: Ps + B 5% + I 1.5%, PsBC: Ps + B 5% + C 5%. Results are expressed as the mean value ± standard error (n = 5). Letters indicate treatment significant difference (p < 0.05).

treatments	WHC (%)	TOC (%)	Pseudo-total concentrations (mg.kg <sup>-1</sup> )			CaCl <sub>2</sub> -extractable fraction (%)		
			[As]	[Fe]	[Pb]	[As]	[Fe]	[Pb]
Ps	29.80±0.47 e	0.04	1068±20 a	6325±114 b	23387±1020 a	0.11±0.01 a	0.06±0.02 a	2.15±0.19 b
PsI	28.11±0.38 f	0.04	1007±52 a	23248±4341 a	18420±1422 ab	0.07±0.01 b	0.08±0.01 a	2.75±0.06 a
PsC	32.75±0.49 d	0.87	906±44 a	5407±205 b	18882±670 ab	0.13±0.01 a	0.02±0.01 b	0.12±0.03 d
PsB	40.11±0.48 b	3.28	1003±18 a	5960±103 b	18927±555 ab	0.05±0.01 b	0.01±0.00 b	1.59±0.06 c
PsBI	36.83±0.26 c	4.03	990±17 a	20090±2410 a	18903±435 ab	0.05±0.01 b	0.06±0.01 a	1.60±0.09 c
PsBC	44.55±2.06 a	3.89	842±41 a	4973±296 b	16419±295 b	0.06±0.01 b	0.02±0.01 b	0.17±0.04 d

**Table 2:** Physico-chemical characteristics of the soil pore water of tested soils at the beginning of the experiment (T0) and after 30 days (TF). Non-vegetated soils corresponded to TF-NV and vegetated soils corresponded to TF-V. EC: Electrical Conductivity (mS.cm<sup>-1</sup>), DOC: dissolved organic carbon (mg.L<sup>-1</sup>) and As, Fe and Pb concentrations (mg.L<sup>-1</sup>). Ps: polluted soil, PsI: Ps + iron grit (I) 1.5%, PsC: Ps + compost (C) 5%, PsB: Ps + biochar (B) 5%, PsBI: Ps + B 5% + I 1.5%, PsBC: Ps + B 5% + C 5%. Results are expressed as the mean value ± standard error (n = 5). For each treatment, letters indicate time and vegetation effect (p < 0.05). Amendments effect is represented by asterisk, \*\*: p < 0.01, \*\*\*: p < 0.001.

treatments		pH	EC (mS.cm <sup>-1</sup> )	DOC (mg.L <sup>-1</sup> )	[As] (mg.L <sup>-1</sup> )	[Fe] (mg.L <sup>-1</sup> )	[Pb] (mg.L <sup>-1</sup> )
Ps	T0	3.98±0.20 a	0.220±0.09 a	5.7±0.3 a	0.00±0.00 a	0.05±0.02 a	11.25±0.41 a
	TF-NV	4.02±0.16 a	0.916±0.063 b	5.9±0.6 a	0.03±0.00 b	0.09±0.02 a	8.08±1.60 a
	TF-V	4.24±0.18 a	0.761±0.067 b	5.4±0.1 a	0.02±0.00 b	0.02±0.01 a	5.46±1.52a
PsI	T0	4.51±0.03 a	0.315±0.025 a	4.8±0.2 a	0.00±0.00 a	22.47±0.45 a	5.92±0.25 a
	TF-NV	4.02±0.03 b	0.797±0.044 b	4.9±0.4 a	0.00±0.00 a	59.03±6.04 b	5.06±0.51 a
	TF-V	4.09±0.06 b	0.822±0.039 b	4.8±0.5 a	0.00±0.00 a	50.16±14.61 b	3.46±0.52 a
PsC	T0	6.32±0.15 a	0.926±0.095 a	23.4±0.2 a	0.09±0.01 a	0.01±0.01 a	0.61±0.12 a
	TF-NV	6.97±0.19 a	1.824±0.085 b	21.9±6.8 a	0.43±0.09 b	0.00±0.00 a	0.18±0.06 a
	TF-V	6.95±0.11 a	1.295±0.134 a	24.9±6.7 a	0.34±0.11 b	0.01±0.01 a	0.15±0.07 a
PsB	T0	6.70±0.04 a	1.176±0.079 a	2.0±0.2 a	0.00±0.00 a	0.00±0.00 a	0.58±0.04 a
	TF-NV	7.04±0.13 ab	2.785±0.042 b	2.1±0.5 a	0.00±0.00 a	0.00±0.00 a	0.54±0.01 a
	TF-V	7.16±0.13 b	2.433±0.082 c	1.9±0.1 a	0.00±0.00 a	0.00±0.00 a	0.61±0.07 a
PsBI	T0	6.60±0.10 a	1.088±0.035 a	5.7±0.5 a	0.00±0.00 a	2.51±0.33 a	0.88±0.21 a
	TF-NV	4.53±0.09 b	3.196±0.047 b	5.8±0.4 a	0.00±0.00 a	107.64±16.24 b	1.78±0.18 a
	TF-V	4.47±0.04 b	2.634±0.064 c	5.6±0.4 a	0.00±0.00 a	83.09±23.34 b	1.44±0.13 a
PsBC	T0	7.31±0.09 a	1.232±0.094 a	10.7±0.2 a	0.06±0.01 a	0.00±0.00 a	0.15±0.02 a
	TF-NV	7.64±0.15 ab	2.573±0.019 b	10.6±0.9 a	0.15±0.06 b	0.00±0.00 a	0.06±0.01 a
	TF-V	7.75±0.13 b	2.255±0.199 b	10.8±0.8 a	0.12±0.05 b	0.00±0.00 a	0.15±0.04 a
amendments effect T0		***	***	***	***	***	***
amendments effect NV		***	***	***	***	***	***
amendments effect V		***	***	**	***	***	***

**Table 3:** As, Fe and Pb bioaccessible fractions (%) in tested soils using SBET (Simplified Bioaccessibility Extraction Test). Ps: polluted soil, PsI: Ps + iron grit (I) 1.5%, PsC: Ps + compost (C) 5%, PsB: Ps + biochar (B) 5%, PsBI: Ps + B 5% + I 1.5%, PsBC: Ps + B 5% + C 5%. Results are expressed as the mean value  $\pm$  standard error (n = 5). Letters indicate treatment significant difference ( $p < 0.05$ ).

treatments	SBET bioaccessible fractions (%)		
	[As]	[Fe]	[Pb]
Ps	3.24 $\pm$ 0.07 c	1.32 $\pm$ 0.21 b	51.12 $\pm$ 0.61 d
PsI	1.58 $\pm$ 0.09 d	2.43 $\pm$ 0.24 c	53.00 $\pm$ 2.36 cd
PsC	4.38 $\pm$ 0.20 b	2.12 $\pm$ 0.14 bc	60.54 $\pm$ 1.04 b
PsB	4.12 $\pm$ 0.15 b	1.68 $\pm$ 0.07 b	59.52 $\pm$ 2.04 bc
PsBI	1.97 $\pm$ 0.12 d	4.14 $\pm$ 0.32 a	54.08 $\pm$ 2.79 cd
PsBC	5.11 $\pm$ 0.14 a	2.46 $\pm$ 0.10 c	69.49 $\pm$ 1.18 a

**Table 4:** As, Fe and Pb bioaccessible fractions (%) in *Trifolium repens* aerial parts collected at the end of the experiment (30 days) on amended soils. Results are obtained from 500 seeds sown on tested soils. PsC: Ps + compost (C) 5%, PsB: Ps + biochar (B) 5%, PsBI: Ps + B 5% + I 1.5%, PsBC: Ps + B 5% + C 5%. Results are expressed as the mean value  $\pm$  standard error (n=5); letters indicate significant difference ( $p < 0.05$ ).

treatments	SBET bioaccessible fractions (%)		
	As	Fe	Pb
PsC	8.2 $\pm$ 0.5 a	2.3 $\pm$ 0.2 b	34.6 $\pm$ 2.2 b
PsB	5.5 $\pm$ 0.7 b	2.3 $\pm$ 0.4 b	34.7 $\pm$ 1.8 b
PsBI	3.6 $\pm$ 0.2 b	16.4 $\pm$ 3.0 a	64.2 $\pm$ 3.7 a
PsBC	9.5 $\pm$ 0.6 a	2.9 $\pm$ 0.2 b	42.5 $\pm$ 2.6 b