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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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Abstract

Vegetation cover can be used in the phytomanagement of polluted areas by adding value to abandoned sites and reducing the dispersion of pollutants by erosion. Appropriate amendments, that allow both efficient plant growth and the immobilization of contaminants in the soil must be chosen in order to optimize the efficiency of this process. We used a mining technosol mainly contaminated by arsenic (1068 mg.kg^{-1}) and lead (23387 mg.kg^{-1}) to study the effect of three amendments (biochar, compost and iron grit) on (i) physico-chemical properties of the soil and soil pore water, (ii) metal(loid) mobility, bioavailability and bioaccessibility ($CaCl_2$ and Simple Bioaccessibility Extraction Test (SBET)), and (iii) the capability of *Trifolium repens* to germinate and grow. All the amendments used increased the pH and electrical conductivity of the SPW, resulting in a 90% decrease in the concentration of lead in the soil pore water (SPW). We also demonstrated a decrease in Pb phytoavailability. The amendments allowed the establishment of a plant cover, although the addition of iron grit alone did not allow any clover germination. For the Pontgibaud technosol, the combination of the three amendments resulted in a significant decrease in As and Pb concentrations in clover tissues, mainly in the aerial organs. The amendments also made it possible for some of them to halve the phytoavailable fraction of arsenic. However, for compost, both the As concentrations in the SPW, and the bioavailable fraction of As increased. All the amendments used had contrasting effects on the bioaccessible fractions of metal(loid)s. The most efficient amendment combination was the addition of 5 % biochar and 5 % compost.

Keywords

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

Highlights

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

-Amendments decreased Pb concentration in soil pore water

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

-Lead was more bioaccessible on amended soils

Introduction

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

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

To reduce these risks, remediation methods such as controlled backfilling, soil fixation, or leaching can be very useful. However, these methods are generally expensive, and some may even have negative effects on the biodiversity, biological activities, soil structure, or fertility (Ali et al., 2013). In contrast, environment-friendly remediation options, such as “aided phytostabilisation”, could improve soil functionality and allow a vegetation cover to be established, at the same time as reducing the mobility and availability of pollutants (Cundy et al., 2013, 2016). Plant cover may also reduce contaminant leaching (Houben et al. 2012), as well as stabilizing soils, and it controls water and wind erosion (Reubens et al. 2007). The implementation of plants will stabilize contaminants by adsorption or accumulation in roots (Vangronsveld et al. 2009). However, the translocation to the shoot system should remain limited to avoid the transfer of contaminants into the food chain through grazing (Henry et al., 2013; Pérez-de-Mora et al., 2011; Kidd et al. 2009).

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

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

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

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

However, biochar does not generally contain enough nutrients to allow plant growth on polluted soils, which are often characterized by low nutrient content (Fischer and Glaser, 2012). Extra nutrients, such as those found in compost must therefore be added alongside the biochar. The addition of compost will allow plant growth as it contains N, P, K, Ca, Mg, and S, as well as humus and different kinds of microorganisms (Fischer and Glaser, 2012; Gil-Loaiza et al., 2016). Moreover, it can reduce the phytoavailability of metal(loid)s due to their association with organic matter (OM), carbonates, or metal oxides (Mench et al., 2000), resulting in a reduction in the plant uptake of metals. But the effect of compost on metal bioavailability depends on the nature of the organic matter, the mineral species content, the potential degradability by microbiota, the pH and redox potential of the soil, and the soil type and metal(loid)s concerned (Walker et al., 2003).

Although the application of biochar and compost has been shown to improve soil agronomic characteristics, as well as its ability to sorb and reduce metal cation availability such as Pb in soil, neither amendment is effective in decreasing the mobility of metal anions, such as arsenic. They even tend to increase anion soil mobility due to the rise in soil pH (Beesley et al., 2011). This can cause problems in soils polluted with both cationic and anionic metal(loid)s. In such polluted sites, one possible strategy would be to combine compost and biochar with iron-rich amendments, or Mn oxides for metal(loid) immobilization (Ruttens et al., 2006). Among these amendments, zero-valent iron, in the form of iron grit, is the most relevant type of iron (Kumpiene et al., 2006), because its high adsorption potential has proved valuable in immobilizing inorganic contaminants (Liu and Zhao, 2007; Satapanajaru et al., 2008; O'day and Vlassopoulos, 2010). In soil, iron grit is known to be transformed to reactive iron (hydr)oxydes (e.g. ferrihydrite) (Komárek et al., 2013). Moreover, iron (hydr)oxydes capacity to sorb ions is modulated according to soil pH. A strong sorption of anions as arsenic is implemented at low pH due to a higher net positive surface charge on iron, whereas at high pH a stronger sorption of cations will be possible. In addition to sorption reactions, co-precipitation of metals and metalloids with iron oxides is another important mechanism for stabilizing metal and metalloid elements in amended soils (Komárek et al., 2013). Thus, it has been shown that the formation of relatively insoluble mineral precipitates Fe(II/III)-As (e.g. $FeAsO_4^{-}H_2O$, $FeAsO_4^{-}2H_2O$ and $Fe_3(AsO_4)_2$) influences the immobilization of arsenic and its bioavailability in soils (Kumpiene et al., 2008; Drahota and Filippi 2009).

Thus, to demonstrate the effectiveness of amendments and revegetation, it is essential to study the metal(loid) concentrations in the soil, soil pore water and plants *continuum*. However, as Lebrun et al. (2018a) observed, the metal(loid) concentration in soil pore water did not always reflect the amount available for plants. It is therefore interesting to measure this phytoavailable quantity by simple extraction tests. These plant availability tests can be performed using extractant salts to determine the more or less mobile fraction of metal(loid)s, to understand their availability for plants (Qasim et al., 2015; Black et al., 2011).

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

The objectives of this study were to evaluate: (1) the capacity of biochar, compost and iron grit to reduce As and Pb phytoavailability when added to a multi-contaminated technosol by measuring their concentrations in soil pore water and by using a $CaCl_2$ neutral salt extractant; (2) whether clover can be used to set up a vegetative cover and (3) the bioaccessibility of Pb and As in soil and in the aerial part of clover plants grown on amended soil, using the SBET approach.

Materials and methods

Origin of contaminated soil (Pontgibaud site)

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

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

Preparation of amended soils - Seed sowing and plant biomass

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

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

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

Soil pore water (SPW) sampling and analysis

SPW was collected using Rhizon® (Rhizosphere Research Product, Wageningen, The Netherlands), from each pot. 20mL of SPW was sampled to determine the pH (pH meter, FE20/EL20, Mettler-Toledo AG 2007), electrical conductivity (EC) (conductimeter, SOLEA, Tacussel électronique, Lyon, France), dissolved organic carbon (DOC) concentration (Pastel UV spectrophotometer, SECOMAM, Ales, France) and metal(loid) concentrations (ICP-AES, ULTIMA 2, HORIBA, Labcompare, San Francisco, USA), according to Bart et al. (2016). The sampling was done before sowing the clover seeds (T0) and at the end of the greenhouse experiment (TF) (30 days).

Metal(loid) quantification in tested soils and in plant organs

Metal(loid) contents (As, Fe and Pb) of soil samples and plant organs were determined by aqua regia digestion treatment (HNO_3 (65%) : HCl (37%), 1:3 v/v) (Zhang and Selim 2008), with a solid/liquid ratio of 1/45. The digestion program consisted of a gradual increase in temperature to 180°C in 15 minutes, a 15 minutes digestion step at 180°C followed by a 15 minutes cooling step. The mineralized samples were then adjusted to a volume of 50 mL with ultrapure water (18 $M\Omega.cm^{-1}$) and filtered through a nitrocellulose membrane at 0.45 µm. Arsenic, iron and lead concentration were measured by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES, Ultima 2, HORIBA, Labcompare, San-Francisco, USA). The accuracy of the assay technique was verified by enriching the samples analyzed with a solution whose concentration of the element to be assayed was known.

Soil metal(loid) simple extraction procedures ($CaCl_2$ treatment)

The phytoavailable fractions of As and Pb were obtained as described by Qasim et al (2015), using $CaCl_2$ extractant at 0.01M with a solid: liquid ratio 1: 10. Metal(loid) concentrations were determined by ICP-AES (ULTIMA 2, HORIBA, Labcompare, San Francisco, USA).

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

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

Data processing and statistical analysis

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

Results and discussions

Effect of amendments on soil characteristics

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

None of the treatments affected the As pseudo-total concentrations compared to P_s. The iron concentration in Pontgibaud soil was 6325 mg kg^{-1}, and the addition of amendments, apart the iron grit, did not induce a significant change in iron soil concentration. The iron grit addition in P_sI and P_sBI caused the pseudo-total Fe soil concentration to increase by approximately 3 times. Lastly, the pseudo-total Pb soil concentrations in the different tested soils were the same for P_s, P_sI, P_sC, P_sB and P_sBI, whereas for P_sBC, Pb concentration decreased by 30% compared to Ps due to a dilution effect produced by the addition of compost and biochar (Table 1).

Effect of amendments and clover plants on SPW characteristics

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

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

Finally, for P_sBI the pH decreased by two units over the time period of the treatment. This can be explained by the fact that the iron was oxidized during the experiment thus inducing the SPW pH decrease.

At T0, the SPW electrical conductivity (EC) for P_s and P_sI was 0.220 $mS.cm^{-1}$ and 0.315 $mS.cm^{-1}$ respectively. For the other treatments, at T0, EC value was approximately 1.1 $mS.cm^{-1}$. At the end of the experiment, an increase in EC was observed compared to T0 on all tested non-vegetated soils, and corresponded to 4.2 fold, 2.5 fold, 2 fold, 2.4 fold, 3 fold and 2.1 fold for P_s, P_sI, P_sC, P_sB, P_sBI, P_sBC, respectively. The presence of clover had no effect on P_s, P_sI and P_sBC, while for P_sC, P_sB and P_sBI it caused a 1.3 fold EC decrease when compared to the corresponding non-vegetated pots. Overall, biochar and compost significantly increased SPW EC ($p < 0.05$), which is consistent with previous studies (Lebrun et al., 2017, 2018a). Indeed, organic matter and mineral compounds such as Ca^{2+}, Mg^{2+}, K^+ or inorganic carbonates present on biochar and compost can be released into the soil and thus increase SPW electrical conductivity (Chintala et al., 2013). Kloss et al. (2014) demonstrated an increase in EC from 0.04 $dS.cm^{-1}$ to 0.19 $dS.cm^{-1}$ when 3% straw biochar was applied to a planosol. Finally, iron grit must be solubilized and mostly precipitated in the form of iron oxide on soil particulates, which is why it did not induce a large increase in the specific EC value.

The DOC content of SPW at T0 (Table 2) for P_s was 5.7 mg.L^{-1}, which is identical to that of P_sBI. Moreover, the DOCs for P_sB and P_sBI were 2.8 fold and 1.2 fold smaller than P_s. On the contrary, DOC for P_sC and P_sBC were 3.7 and 1.9 fold higher than for P_s. It should be noted that neither the time nor the presence of clover modified the DOC.

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

SPW metal(loid) concentrations and phytoavailable metal(loid)s

At T0 (Table 2), no As content was detected for P_s, P_sI, P_sB and P_sBI SPW whereas for P_sC and P_sBC, As concentrations were 0.09 mg.L^{-1} and 0.06 mg.L^{-1}, respectively. Both the passage of time, and the presence of clover plants increased As SPW for these last two treatments, by 2 fold and 4 fold respectively. For P_s, at TF, As SPW concentration could be quantified, and corresponded to 0.03 mg.L^{-1} and 0.02 mg.L^{-1}, for TF-NV and TF-V, respectively. The As soil phytoavailable fractions ($CaCl_2$-extractable fraction) for P_s and P_sC at T0 were not significantly different, and corresponded on average to 0.11 % of the total soil As concentration (Table 1). Whereas for the other treatments P_sI, P_sB, P_sBI and P_sBC, As phytoavailability was 1.9 times smaller than P_s. Arsenic is usually found as an oxyanion in solutions, and presents specific challenges to sanitation, because its mobility increases when pH increases (Beesley et al., 2011, Nandillon et al., 2019). It was shown that the addition of compost may increase the mobility and leacheability of metal(loid)s, particularly arsenic (Mench et al., 2003). In the present study, a significant increase in arsenic SPW concentration was observed when compost was added to soils, probably because DOC competed with arsenic for sorption sites, such as those present in iron oxides, resulting in its increased mobility (Bolan et al., 2014). Compost with a high degradable organic matter content, associated to a neutral pH, present an active microbial activity which uses the organic matter as energy source (Balasoiu et al., 2001). This means that compost and its microbiota could reduce the soil redox potential, and consequently support the transformation of As(V) to As(III), which is more mobile (Kim et al., 2003). This high mobility could also be explained by the presence of phosphorus. Indeed, Fresno et al. (2018) showed that phosphorus-Olsen had a significant effect on mobilizable As, as phosphate and arsenate have similar chemical properties, and they compete for organic and inorganic binding sites (Bolan et al., 2014; Moreno-Jiménez et al., 2013; Adriano, 2001). Other authors (Clemente et al., 2012; Moreno-Jiménez et al., 2013; Beesley et al., 2014) have already reported that competition for mineral sorption sites between these anions can be expected, which will lead to arsenic mobilization from less labile soil fractions and increase its availability. It is important to note that the phytoavailable fraction of arsenic was identical for the P_s and P_sC treatments, suggesting that compost influenced the easily mobilizable fraction found in pore water, but not the $CaCl_2$ extractable fraction. Moreover, the decrease in As concentration observed between P_sB and P_sBC both at T0 and at TF (Table 2) could be explained by: (i) the mobile part of arsenic potentially being trapped in the biochar structure; (ii) DOC decrease after biochar addition resulting in less competition between dissolved organic matter and arsenate; and (iii) the calcite present in the biochar could form stable spherical compounds with arsenate anions on the biochar's surface (Alexandratos et al., 2007; Yin et al., 2016).

At T0, the SPW iron concentration (Table 2) for P_s was 0.05 $mg.L^{-1}$. No soluble Fe was quantified in SPW for P_sC, P_sC and P_sBC. As expected, Fe concentration in P_sI and P_sBI treatments were 450 times and 50 times higher than P_s, respectively. Moreover, for P_sI and P_sBI, no significant difference in SPW Fe concentration was observed at TF between non-vegetated and vegetated pots. Although at TF, SPW Fe concentration increased by 2.4 fold for P_sI and 38 fold for P_sBI. The values for the Fe-$CaCl_2$-extractable fraction (Table 1) for P_s, P_sI and P_sBI, were not significantly different, and were all between 0.06 % and 0.08 %. Finally, compost or biochar added to P_s soil, alone or in combination (P_sB, P_sC and P_sBC), led to a significant decrease in the Fe-$CaCl_2$-extractable fraction (3.5 fold).

As expected, lead was the most prevalent ion in Pontgibaud SPW (11.2 $mg.L^{-1}$), at T0, and on top of that, no significant difference was measured between T0 and TF for P_s, or any of the other studied treatments. The SPW Pb concentrations for P_sI, P_sC, P_sB, P_sBI and P_sBC were 4.8 $mg.L^{-1}$, 0.3 $mg.L^{-1}$, 0.6 $mg.L^{-1}$, 1.4 $mg.L^{-1}$ and 0.12 $mg.L^{-1}$, respectively. For the Pb-$CaCl_2$-extractable fraction (Table 1) four different significant groups were identified P_sI 2.75 %; P_s 2.15 %; P_sB and P_sBI 1.6 %; and P_sC and P_sBC 0.15 %.

Compost and biochar + compost appeared to be the treatments that decreased the phytoavailable Pb fraction the most significantly, suggesting that the high pH and organic content of these amendments reduce the lead mobility in the soil. As is well known, SPW metal mobility is strongly influenced by organic carbon, which is brought to the soil by the biochar and compost (Bolan et al., 2014). Furthermore, higher pH values are known to increase the surface charges of soil particles including amendments, which induces the retention of metal (Perez-Esteban et al., 2014; Boisson et al., 1999; Mench et al., 1998, 2000). These amendments can immobilize metal(loid)s via three mechanisms (Mench et al., 2000): (i) adsorption of metals to highly accessible sites on the surface of modified aluminosilicates, soil components and of biochar or compost through interactions with oxygenated functional groups (Lebrun et al. 2018b); (ii) precipitation with Al, Fe and Mn oxides (Oustrière et al., 2017); (iii) formation of minerals (such as metal silicates) and diffusion through mineral surfaces (Lee et al., 2013) and (iv) precipitation with phosphates and carbonates contained by the biochar (Lomaglio et al. 2016).

Metal(loid) bioaccessibility in soil

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

The bioaccessible arsenic concentration for P_s was 3.2% and decreased for P_sI and P_sBI treatments by 2.1 and 1.8 times respectively, compared to P_s. This is because the iron oxide formed strongly adsorbed As. Many studies (Martin and Ruby 2003; Subacz et al., 2007) have shown that use of Fe-rich amendments ($FeCl_3$, $FeCl_2.4H_2O$, Ferrihydrite, Fe^0 and $FeBr_3$) could be an effective strategy for reducing bioavailability and bioaccessibility, and for remediating soils contaminated with As. Cui et al. (2010) demonstrated that the addition of $FeSO_4$ to a mining technosol decreased gastric and intestinal bioaccessibility of arsenic in all treatments. Finally, the bioaccessible arsenic fraction for P_sC, P_sB and P_sBC treatments was almost 1.4 times higher than for P_s. This could be explained by the fact that anions such as bicarbonate, phosphate, silicate, and organic acids present in the soil solution due to the addition of compost and biochar to P_s could compete with As for chemisorption sites, and thus mobilize As to the aqueous phase. The links between biochar and arsenic, and between compost and arsenic are weak and therefore easily broken by the glycine present in SBET solution. This would explain the greater As accessibility for P_sC, P_sB and P_sBC.

The iron bioaccessible fraction for P_s was 1.3 % , which demonstrates its high stability in soil. When compost and biochar were added to P_s (P_sC and P_sB), Fe accessibility was unchanged. As expected, the addition of iron to P_s or P_sB led to an increase in accessible-Fe, by 1.9 and 3.1 fold, respectively. Interestingly, for P_sBC, the iron bioaccessible fraction was almost twice the value measured for P_s. This was probably due to the destabilization of iron oxides by the increase in organic ligands following the addition of biochar and compost.

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

Plant growth and metal(loid) uptake

After 30 days of growth, the clover dry weight (DW) and the metal(loid) concentrations in the roots and aerial parts were measured for the different treatments. Firstly, it was noted that when iron was added alone to P_s the seeds could not germinate. This was probably due to the high iron concentration in the soil pore water at T0. Indeed, when just iron grit was added to P_s the concentration of Fe in the SPW was 22.5 mg L^{-1}, whereas when iron grit was added to P_s at the same time as biochar (P_sBI), the Fe SPW concentration was 2.5 mg L^{-1} due to the biochar's physico-chemical characteristics. Iron alone seemed to have an acidifying effect, certainly due to the oxidation of Fe(II) followed by the precipitation of $Fe(OH)_3$. Thus, the combination of high Fe supply and acidity induced a high solubility and Fe availability. In the presence of biochar, the pH effect was attenuated. However, it was noted that the smallest increase in the biomass of aerial parts was observed for P_sBI treatment (0.623 g). For the Ps treatment, the dry weights of the roots and aerial parts were smaller, and corresponded to 0.124 g and 0.170 g, respectively. For the roots, P_sC, P_sB and P_sBC DW were not significantly different and corresponded to four times the value for P_s.

For P_sC, P_sB and P_sBC, the average DW of the aerial parts was 1.24 g, i.e. 7 times the value measured in P_s pots. The same beneficial effect for the DW of *Salix viminalis* and *Populus euramericana* cuttings was obtained by Lebrun et al. (2018a) when adding biochar produced from different feedstocks (hardwood, lightwood and pinewood, at 2% and 5% w/w) to Pontgibaud technosol. Fresno et al., (2018) showed that iron combined with bio-char on an As and Cu contaminated technosol slightly increased lupine shoot biomass. In our case, even though biochar allowed a better biomass production of clover aerial parts, the compost addition to biochar improved DW production by 30%. Indeed, organic matter, provided by the compost improved the N, P, K nutrient cycle (Marques et al., 2008, Abbas et al., 2018), allowing better plant growth, as observed by Marques et al. 2008 on *Solanum nigrum* and Gregory et al. (2014) on *Lolium perenne* grown on polluted soils

Concerning the concentrations of ions in clover organs (Fig 2), we noticed that the amendments systematically decreased the As and Pb concentrations in aerial parts by about 1.8 times for arsenic and between 1.8 and 3.8 times for lead, whereas As and Pb concentrations in P_s plants were 396 mg kg^{-1} and 4577 mg kg^{-1}, respectively. In roots, As concentrations were only decreased by P_sB and P_sBC treatments, and corresponded to 500 mg kg^{-1}, whereas for P_s, P_sC and P_sBI, As concentrations were approximately 812 mg kg^{-1}. For Pb concentrations in roots, P_s and P_sBI demonstrated the same accumulation i.e 13300 mg kg^{-1}, whereas P_sC, P_sB and P_sBC decreased root Pb concentration by 2.4 fold. As expected, iron concentration was highest in clover plants grown on the P_sBI soil, with concentrations of 5000 mg kg^{-1} and 17000 mg kg^{-1} in aerial parts and roots, respectively. Whereas for P_s, P_sC, P_sB and P_sBC treatments Fe concentrations in aerial parts were 2500, 1200, 1300 and 800 mg kg^{-1}, respectively. Finally, Fe concentrations in roots for P_s, P_sC, P_sB and P_sBC were not significantly different, and corresponded to 3100 mg kg^{-1}, which was 5.5 times lower than the Fe concentration measured in roots of plants grown on the P_sBI soil. In the present study, for all treatments, As, Fe and Pb concentrations were higher in the roots than in the aerial parts of clover. This is consistent with the results of Bidar et al., (2007, 2009) and Lopareva-Pohu et al., 2011 for *Trifolium repens* growing on metal(loid) contaminated soil. However, it is difficult to distinguish between the elements fixed to the root surface and those that are absorbed. We observed, a significant As, Fe and Pb concentration decrease in the aerial parts for all treatments, apart from for plant Fe accumulation levels on the P_sBI treatment. For Pb and Fe, this decrease could be associated to a significant diminution of the two ions in the soil pore water, correlated to the amendments. Interestingly we demonstrated that compost being added to P_sC and P_sBC soils was associated with an increase in As concentrations in the soil pore water, whereas As concentrations in the aerial parts decreased compared to P_s. Such results could be associated to a probable change in the chemical form of As, which is certainly less assimilable for clover plants. In fact, the plant metal(loid) absorption depends on different parameters, relative both to the plant (such as root exudations), and to the soil characteristics (such as oxidation-reduction processes in the soil, pH, cation exchange capacity, dissolved oxygen, and temperature), which modify the metal(loid) speciation (Cheng, 2003).

The high concentration of As in the roots for the P_sBI treatment compared to the P_sB and P_sBC treatments, which have similar biomasses and phytoavailable arsenic, could be due the mobilization of As by root exudates in soils treated with iron (Fresno et al. 2017). Another explanation could be that the precipitation of iron oxides on the roots causes an indirect increase in arsenic on the roots. The SBET bioaccessible fraction (Table 4) was only measured for the aerial parts of clover plants grown on amended soil. We were not able to do these measurements for aerial parts of clover grown on Pontgibaud soil (P_s) due to poor plant biomass.

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

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

Conclusion

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

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

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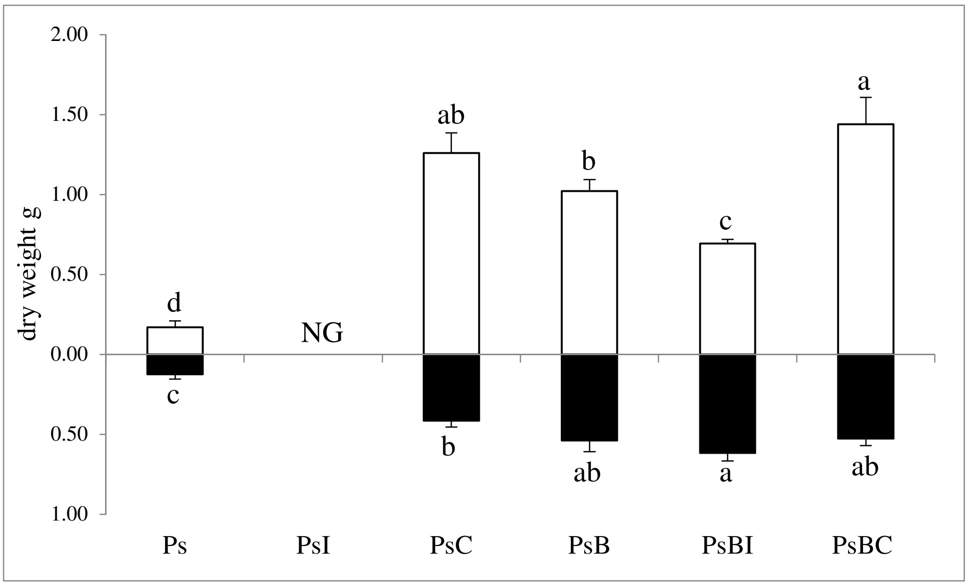

Figure 1: *Trifolium repens* dry weight (DW) (g) of organs collected at the end of the experiment (30 days); white columns: aerial parts, black columns: roots. Results are obtained from 500 seeds sown on tested soils. Ps: polluted soil, PsI: Ps + iron grit (I) 1.5%, PsC: Ps + compost (C) 5%, PsB: Ps + 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.

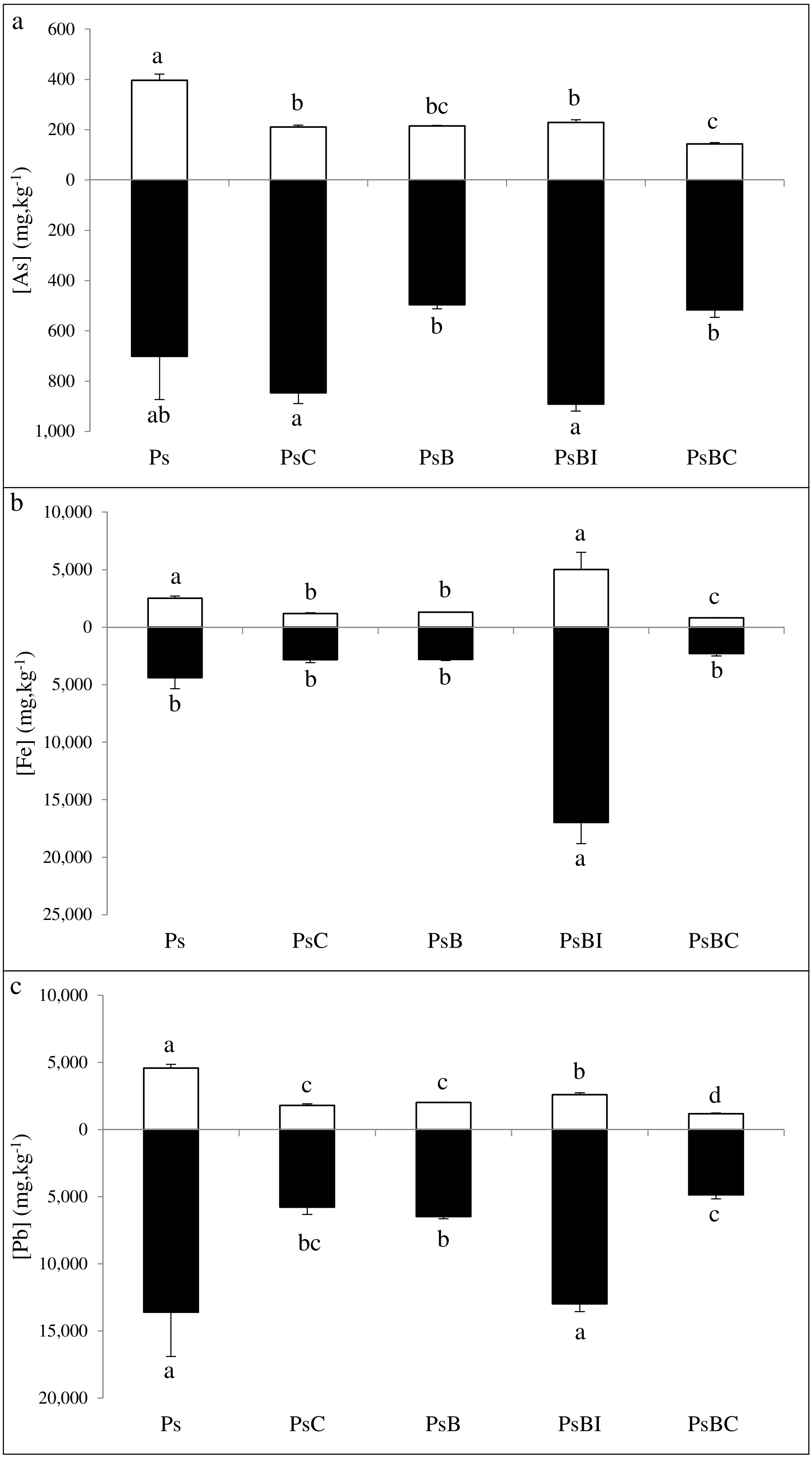

Figure 2 : Arsenic (a), iron (b) and lead (c) concentration (mg.kg^{-1}) in the different *Trifolium repens* organs collected at the end of the experiment (30 days); white columns: aerial parts, black columns: roots. Results are obtained from 500 seeds sown on tested soils. Ps: polluted soil, PsI: Ps + 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 ($mg.kg^{-1}$) and $CaCl_2$-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 ($p < 0.05$).

	WHC (%)	TOC (%)	Pseudo-total concentrations ($mg.kg^{-1}$)			$CaCl_2$-extractable fraction (%)		
treatments			[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^{-1}), DOC: dissolved organic carbon (mg.L^{-1}) and As, Fe and Pb concentrations (mg.L^{-1}). 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^{-1})	DOC (mg.L^{-1})	[As] (mg.L^{-1})	[Fe] (mg.L^{-1})	[Pb] (mg.L^{-1})
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 ± standard error (n = 5). Letters indicate treatment significant difference ($p < 0.05$).

	SBET bioaccessible fractions (%)		
treatments	[As]	[Fe]	[Pb]
Ps	3.24±0.07 c	1.32±0.21 b	51.12±0.61 d
PsI	1.58±0.09 d	2.43±0.24 c	53.00±2.36 cd
PsC	4.38±0.20 b	2.12±0.14 bc	60.54±1.04 b
PsB	4.12±0.15 b	1.68±0.07 b	59.52±2.04 bc
PsBI	1.97±0.12 d	4.14±0.32 a	54.08±2.79 cd
PsBC	5.11±0.14 a	2.46±0.10 c	69.49±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 ± standard error (n=5); letters indicate significant difference ($p<0.05$).

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