Effects of biochar, ochre and manure amendments associated with a metallicolous ecotype of Agrostis capillaris on As and Pb stabilization of a former mine technosol

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Corresponding Author:	Sylvain Bourgerie, Ph.D University of Orleans Orleans, FRANCE
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Orleans
Corresponding Author's Secondary Institution:	
First Author:	Manhattan Lebrun
First Author Secondary Information:	
Order of Authors:	Manhattan Lebrun
	Romain Nandillon
	Florie Miard
	Lydie Le Forestier
	Domenico Morabito
	Sylvain Bourgerie, Ph.D
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Abstract:	Metal(loid)soil pollution is a major environmental and health issue, requiring these areas to be remediated, for example through phytoremediation processes. In order to allow proper plant establishment and growth, amendments must be applied to highly contaminated and poorly fertile soils. Amendments are diverse, but many studies have shown the beneficial effects of biochar, manure and ochre, although studies on their combined use are scarce. Moreover, no studies have evaluated the effect of these combined amendments on endemic plant growth. Endemic plants growing on contaminated soils showed higher tolerance towards pollutants compared to plants coming from unpolluted areas. Therefore, the aim of the present study was to evaluate both the effect of amendments (single or combined) on the physico-chemical properties of a former mining technosol, and the growth and metal(loid) accumulation ability of endemic Agrostis capillaris plants. This study revealed an improvement in the soil physico-chemical properties following the application of amendments, with combined amendments showing better results than the application of just one. On top of this, Agrostis plants performed better on the amended technosols, especially the ones receiving manure, due to its high nutrient content. Finally, based on soil properties, plant growth and the metal(loid) accumulation profile, the use of biochar combined with manure seems to be the most appropriate treatment. Indeed, this treatment showed an improvement in both soil fertility and plant growth. Moreover, Agrostis plants grown in these conditions were among those showing higher root metal(loid) concentration associated to a lower translocation toward aerial parts.
Response to Reviewers:	Dear Dr. Xunwen Chen (guest editor EGAH),

We were pleased to learn your interest in the resubmission of our manuscript entitled "Effects of biochar, ochre and manure amendments associated to a metallicolous ecotype of Agrostis capillaris on As and Pb stabilization of a former mine technosol".

The reviewers suggested some revisions to be done to the manuscript. We thank them for their work and for helping us to improve the quality of our paper. In this amended version, we have addressed their comments and have rewritten the text accordingly. Below is our item-by-item reply to each of their observations.

Having addressed all the issues raised by both reviewers, we hope that our work will be now considered suitable for publication in the Environmental Geochemistry and Health.

Looking forward to hearing from you.

Yours sincerely,

Dr. Sylvain BOURGERIE

Reviewer #1: All suggestions have been considered

Minor errors can be corrected at proof stage (for instance L205)
Following Reviewer #1 comment, the sentence was modified (line 217: "In addition, the supply of soluble organic matter and organic anions by the organic amendment…").

Reviewer #3: In the revised ms, author explain the explanation of plant choose and the inner mechanism of metal absorption. However, the role of P on As leaching has already been reported. the adsorption of metall ions on biochar also have been demonstrated eles where. The core problem is the scientific reason on the mixture of these materilas for phytoremediation. however, authors did not explain clearly. Is there any data related to phosphrous or any other own information could be supported? if yes, please add these data, if not, please explain the advantage of the mixture on phytoremediation.
As suggested by Reviewer #3, some explanations were provided (lines 87-103) in introduction part. A reference (Liang et al. 2017) was added.

Effects of biochar, ochre and manure amendments associated to a metallicolous ecotype of *Agrostis capillaris* on As and Pb stabilization of a former mine technosol

Manhattan Lebrun[ab], Romain Nandillon[acd], Florie Miard[a], Lydie Le Forestier[e], Domenico Morabito[a] and Sylvain Bourgerie[a]

[a] Université d'Orléans, LBLGC INRA USC1328, rue de Chartres, BP 6759, 45067 Orléans Cedex 2, France
[b] Università degli Studi del Molise, Dipartimento di Bioscienze e Territorio, 86090, Pesche, Italy
[c] IDDEA, Environmental consulting engineering, 45160 Olivet, France
[d] BRGM, ISTO, UMR 7327, BP 36009, 45060 Orléans, France
[e] Université d'Orléans, CNRS, BRGM, ISTO, UMR 7327, 45071 Orléans, France

Abstract

Metal(loid)soil pollution is a major environmental and health issue, requiring these areas to be remediated, for example through phytoremediation processes. In order to allow proper plant establishment and growth, amendments must be applied to highly contaminated and poorly fertile soils. Amendments are diverse, but many studies have shown the beneficial effects of biochar, manure and ochre, although studies on their combined use are scarce. Moreover, no studies have evaluated the effect of these combined amendments on endemic plant growth. Endemic plants growing on contaminated soils showed higher tolerance towards pollutants compared to plants coming from unpolluted areas. Therefore, the aim of the present study was to evaluate both the effect of amendments (single or combined) on the physico-chemical properties of a former mining technosol, and the growth and metal(loid) accumulation ability of endemic *Agrostis capillaris* plants. This study revealed an improvement in the soil physico-chemical properties following the application of amendments, with combined amendments showing better results than the application of just one. On top of this, *Agrostis* plants performed better on the amended technosols, especially the ones receiving manure, due to its high nutrient content. Finally, based on soil properties, plant growth and the metal(loid) accumulation profile, the use of biochar combined with manure seems to be the most appropriate treatment. Indeed, this treatment showed an improvement in both soil fertility and plant growth. Moreover, *Agrostis* plants grown in these conditions were among those showing higher root metal(loid) concentration associated to a lower translocation toward aerial parts.

Keywords: *Agrostis capillaris*; amendment; metal(loid)s; mine; stabilization

Introduction

Metals and metalloids are significant pollutants of soils. Indeed, there are more than three million potentially polluted sites in Europe, of which about 250 000 are contaminated by metal(loid)s (Khalid et al. 2016). This pollution is a big issue, as metal(loid)s, contrary to organic pollutants, cannot be degraded, and thus accumulate in the soil. Moreover, the negative effects of the metal(loid) soil pollution on both the environment and human health mean that metal(loid) polluted soils must be remediated (Ali et al. 2013). Indeed, polluted soils often present a low biodiversity and a loss of ecosystemic functions, and the lack of vegetation induces the transportation of polluted soil particles towards the unpolluted surrounding area. Furthermore, metal(loid)s have detrimental effects on human health. For instance, lead (Pb) can affect and damage many organs and systems in the body as well as altering enzyme activities (Sharma and Agrawal 2005, Su et al. 2014). Arsenic (As) can affect the reproduction, neurological and respiratory systems, among others (Mandal and Suzuki 2002). Moreover, both are considered carcinogenic elements (Ahmad et al. 2015, Jarup 2003, Sharma and Agrawal 2005).

Different remediation techniques, *i.e* physical, chemical and biological approaches, aiming to diminish pollutant toxic effects can be employed. However, phytoremediation, defined as the use of plants, and their associated microorganisms, to remediate and re-vegetate contaminated sites, is an environment-friendly, energy efficient and cost-effective technique (Khalid et al. 2016). Several processes of phytoremediation exist. Among them, phytostabilisation is more suitable for large and highly contaminated areas. In this case, the pollutants are immobilized in the root area through (i) absorption into the roots, (ii) adsorption onto the roots and/or (iii) precipitation in the rhizosphere area (Cristaldi et al. 2017). This technique relies on the re-vegetation of the polluted area rather than the real depollution of the soil, which can be difficult and time consuming when large amounts of soils are contaminated. The re-vegetation of the polluted areas in the phytostabilisation process is important because, in addition to the immobilization of the pollution to the rhizosphere, it will also lessen the potential risk of contaminated soil spreading off-site by wind or water erosion (Gray et al. 2006). Such process has been studied for many years as the best option to reduce metal(loid) spreading as well as minimize the entry of pollutants into the food chain (Wong 2003).

Moreover, in some cases, the extreme conditions (acidic pH, low nutrient content associated to high pollution concentrations) of the polluted soils imply the need of amendments to allow plant growth. Different amendments can be used, both organic and inorganic, although some have attracted more attention than others over the last few years.

For instance, manure is commonly used as a fertilizer in agriculture. It is rich in organic materials and essential nutrients (Kiran et al. 2017). Its application to soil, whether contaminated or not, allows an improvement to the fertility and thus promotes plant growth (Walker et al. 2004). Moreover, previous studies have demonstrated its ability to immobilize Pb (Tang et al. 2015), Cd, Cu, Zn (Huang et al. 2018, Kiran et al. 2017) and Al (Whalen et al. 2000). However, its positive effects are usually short-lasting and repeated application is necessary (Mosaddeghi et al. 2009).

Ochre, on the other hand, is not a typical agricultural amendment. Ochre is a by-product found in the outflows of some mining systems (Olimah et al. 2015). It is a brown or yellowish-brown product, and rich in Fe(III) oxyhydroxides (Abed et al. 2017, Olimah et al. 2015). For this reason, ochre is a very valuable product for the remediation of arsenic, known to have an affinity for iron (Doi et al. 2005, Olimah et al. 2015).

Finally, biochar is produced through the pyrolysis of biomass under conditions of limited oxygen (Anawar et al. 2015). It is characterized by an alkaline pH, a large surface area and the presence of functional groups on its surface, such as phenolic, carboxyl and hydroxyl (Cha et al. 2016, Lebrun et al. 2018c). Such properties induce an immobilization of metallic pollutants, such as Pb, when added to polluted soils (Lebrun et al. 2018a, b, Lomaglio et al. 2017). Moreover, biochar soil application improves soil fertility and plant growth and tends to reduce metallic cation concentrations in plant tissues (Chen et al. 2018).

Taken separately, all these amendments have benefits for contaminated soils but they also have limits, especially for soils that have a poor fertility and contaminated by both metals and metalloids. To overcome the first issue, the poor fertility, nutrient-rich organic amendments can be applied, such as manure or compost. Biochar has a high capacity to immobilize cation metals and can be used to overcome the second issue. Moreover, biochar and organic amendments can interact once applied together in the soil (Liang et al. 2017): biochar increases the humification and properties of organic amendments, whereas biochar properties are modified by the microorganisms present in the manure. However, both amendments are not efficient regarding metalloids. Anions can even be mobilized due to the organic matter and phosphates contained in manure, arsenic and P being chemically similar and competing for the same sorption sites (Cao et al. 2003). Thus, it can be necessary to add a third amendment, rich in iron, such as iron sulfate. The iron present will interact with arsenic and immobilize it (Fresno et al. 2020). ~~Such amendments demonstrated different effects on soil properties, manure being mainly used for fertility improvement, biochar for metal immobilization and ochre for metal(loid) immobilization. However, when soils are contaminated with more than one element, especially a combination of metals and metalloids, it can be difficult to find one amendment efficient for all the pollutants.~~ Therefore, the complexity of multi-contaminated soils can require ~~it is necessary to apply~~the application of a combination of amendments. Several previous studies showed that amendment combinations had better effects than the single application of the same amendments (Beesley et al. 2010, Fresno et al. 2016, Oustriere et al. 2016).

Plant selection is an important criterion for phytoremediation success, and the use of endemic species has shown better results in the past. For instance*, Agrostis capillaris* L., endemic species found on a studied mine technosol, has been shown to be a good Pb phytostabilizer (Rodríguez-Seijo et al. 2016). Many studies aimed at comparing metal-tolerant and non-tolerant ecotypes. All research showed that the metal tolerant ecotype, collected on a polluted site, presented a higher metal(loid) tolerance with a lower metal(loid) uptake and root-to-shoot translocation (Doubkovà and Sudovà 2016, Sudovà et al. 2008). This higher metal(loid) tolerance of populations grown on polluted sites was associated to a long-term pollutant selection pressure (Sudovà et al. 2008).

Nevertheless, even though manure, ochre and biochar have been much studied in polluted soil remediation, combinations of the three have not yet been investigated. Moreover, to the best of our knowledge, this is the first study associating a combination of amendments and *Agrostis capillaris* from a metallicolous population. In this context, the aims of this study were to evaluate the effect of the application of manure, ochre and biochar, alone or combined, on: (i) the physico-chemical properties of a former mining site and (ii) *Agrostis capillaris* growth and metal(loid) stabilization performance.

Material and methods

1. Site description

The mesocosm experiment was realized using soil collected from a former silver-lead extraction mine site, located in Pontgibaud (Auvergne-Rhône-Alpes, France) (GPS coordinates 45° 47′ 27″ North and 2°49′38″ East). This site has been described in previous studies (Lebrun et al. 2018a, b) and is mainly contaminated by lead (11 453 mg.kg^{-1}) and arsenic (539 mg.kg^{-1}) (Lebrun et al. 2017).

2. Amendments

The commercial biochar was provided by La Carbonerie (Crissey, France). It was produced from the slow pyrolysis at 500 °C of hardwood biomass (mixture of oak, beech and charm), followed by a sieving to obtain a particle size between 0.5 and 1 mm.

The ochre amendment was collected at a former charcoal mine (Alès) in France, as described in Thouin et al. (2019). Before its incorporation into the soil, it was dried and crushed to obtain a fine powder.

Finally, cow manure was provided by a local farmer located in Pontgibaud (GPS coordinates 45° 47′ 26′′ North and 2° 49′ 44′′ East).

All three amendments were analyzed for pH, electrical conductivity (EC), redox potential and NH_4NO_3-extractable As, Fe, K, P and Pb concentrations. pH and EC as well as redox potential of the three amendments were determined using the same procedure as described in Lebrun et al. (2018a) and measured using a multimeter (Metler-Toledo, Serveur excellence, Columbus, Ohio, USA). Concentrations in NH_4NO_3-extractable metal(loid)s were determined after shaking a mixture of soil and 1 mol.L^{-1} NH_4NO_3 solution (2:5 solid:liquid ratio) at room temperature for two hours (Pueyo et al. 2004), followed by ICP-AES (Inductively Coupled Plasma- Atomic Emission Spectroscopy) (ULTIMA, HORIBA, Labcompare, San Francisco, USA) analyses. Amendment characteristics are given in Table 1.

Field Code Changed

3. Plant

Agrostis capillaris was chosen as the phytoremediator plant. It is a *Poaceae* characterized as a facultative metallophyte. It has been found to grow spontaneously on contaminated soils (Austry et al. 2013, Rodriguez-Seijo et al. 2016). Studies also showed its potential for the phytostabilization of soils polluted by As (Austruy et al. 2013) and Pb (Rodriguez-Seijo et al. 2016) and as presenting an adaptive tolerance to contaminated sites (Austruy et al. 2013). Moreover, *Agrostis capillaris* was chosen because such plants have been found on the Pontgibaud polluted site and the use of seeds collected from the site showed a potential for the phytoremediation of PG soil (Nandillon et al. 2019).

Similarly here, seeds of the metallicolous ecotype found on the site were collected to be used in this study.

4. Experimental design

All three amendments were applied at 1 % (w/w), either alone or in combination, which gave eight treatments in total: i) non-amended Pontgibaud technosol (P0%), ii) Pontgibaud + 1 % biochar (PB), ii) Pontgibaud + 1 % ochre (PI), iii) Pontgibaud + 1 % biochar + 1 % ochre (PBI), iv) Pontgibaud + 1 % manure (PM), vi) Pontgibaud + 1 % biochar + 1 % manure (PBM), vii) Pontgibaud + 1 % ochre + 1 % manure (PIM) and viii) Pontgibaud + 1 % biochar + 1 % ochre + 1 % manure (PBIM). Ten pots were prepared per treatment. The different mixtures were placed in 2 L pots and half the pots were sown with *Agrostis capillaris* seeds (quantity equivalent to 2000 seeds.m^{-2}) collected at Pontgibaud site. The experiment lasted for 26 days in a phytotron (16h-8h photoperiod, 800 µmol·m^{-2}·s^{-1} light intensity, temperature 24 °C day and 21 °C night).

5. Soil analysis

The eight treatments were analyzed at the beginning of the experiment to determine soil NH_4NO_3-extractable As, Fe, K, P and Pb concentrations. The procedure was the same as the one used for the amendment characterization.

6. Soil pore water (SPW) sampling and analysis

SPWs were sampled in all pots twice over the length of the experiment: at the beginning, before seed sowing (T0) and at the end, before plant harvest (TF). Sampling was performed using a Rhizon® (model MOM, Rhizosphere Research Products, Wageningen, The Netherlands), as described in Lebrun et al. (2017). SPWs were used directly to measure pH, EC and redox potential, using a multimeter (Metler-Toledo, Serveur excellence, Columbus, Ohio, USA). SPWs were then acidified and As, Fe, K, P and Pb concentrations were determined by ICP-AES.

Field Code Changed

7. Plant analysis

At the end of the experiment, *Agrostis* plants were harvested in each pot. Aerial and root parts were separated, washed twice with tap water then distilled water, and dried (72 h at 60 °C) to determine dry weight production per pot. Aerial and root biomasses were then submitted to acid digestion (0.2 g of plant material + 6 mL HNO_3 70 % + 3 mL HCl 35 %) in a microwave (15 min increase up to 180 °C, 15 min hold at 180 °C, cooling) (Multiwave 5000, Anton Paar, Courtaboeuf, France), and plant As, Fe and Pb concentrations were determined by ICP-AES, using standard solutions prepared from mother solutions of As and Pb provided by Alfa Aesar-Fisher (Karlsruhe, Germany).

8. Statistical analyses

All data was analyzed using R software version 3.1.2 (R Development Core Team, 2009). Firstly, the homogeneity and homoscedasticity of the data was evaluated using Shapiro and Bartlett tests respectively. Next, Anova or Kruskal tests were performed, followed by a post hoc test to assess two-by-two differences. In addition, for the SPW data, a “time effect” was assessed by comparing the means at T0, TF-*Agrostis* and TF+*Agrostis* for each treatment: normality and homoscedasticity were evaluated as previously described and mean comparison was realized using an Anova test for parametric data or a Kruskal test for non-parametric data, followed by a post-hoc test, Tukey HSD or Pairwise-Wilcox, respectively. Differences were considered significant at $p < 0.05$.

Finally, Pearson correlation coefficients were calculated between the different parameters measured. Correlation was considered significant at $p < 0.05$.

Results and Discussion

1. Soil NH_4NO_3-extractable elements

Soil NH_4NO_3-extractable elements were analyzed at the beginning of the experiment in order to investigate the phytoavailable fraction of metal(loid) in the soil. NH_4NO_3-extractable As concentration was 2.29 µg.g^{-1} on P0% and it only increased in two conditions (Table 2): the addition of biochar and biochar + manure led to a 1.42 and 1.56-fold increases, respectively. In 2013, Alvarenga et al. observed an increase in available As concentrations following compost amendment, while Namgay et al. (2010) showed that biochar increased available As (from 12.7 to 14.9 mg.kg^{-1}) when applied at 1.5 % on a metal(loid) spiked soil. Both studies attributed this increase to a rise in soil pH and organic matter, induced by amendment application. Moreover, Moreno-Jiménez et al. (2016) found that the rise in As solubility following biochar amendments was likely to be related to changes in available P. The same explanation was found in this study: NH_4NO_3-extractable As concentration was positively correlated with NH_4NO_3-extractable P concentration ($R^2 = 0.51$, $p < 0.001$) (Table S1). With increasing P concentrations, As is displaced from sorption sites and thus becomes more available (Moreno-Jiménez et al. 2016). In addition, the ~~addition~~ supply of soluble organic matter and organic anions by the organic amendment can lead to the formation of soluble As complexes (Namgay et al. 2010).

The NH_4NO_3-extractable Fe concentration was low for P0% (0.02 µg.g^{-1}), and only increased following the ochre amendment (27-fold) (Table 2), and can be explained by the NH_4NO_3-extractable Fe concentration of the ochre (1.04 µg.g^{-1}) (Table 1). Such elevated Fe content in the ochre has been previously observed by Abed et al. (2017) , Doi et al. (2005) and Thouin et al. (2019). However, one surprising fact was that although NH_4NO_3-extractable Fe concentration in manure was high (2.41 µg.g^{-1}), no increase was observed following its addition to the Pontgibaud technosol, maybe due to its low application rate.

NH_4NO_3-extractable K concentration was 22.14 µg.g^{-1} for P0%, and it increased with all treatments containing manure (Table 2). This improvement can be directly related to the amendment composition, as shown by the NH_4NO_3 extractable K concentrations of manure. Manure is a known fertilizer characterized by elevated K concentrations and other nutrients that can be released into the soil matrix, as shown in Celik et al. (2004), Mokolobate and Haynes (2002) and Materechera and Mkhabela (2002).

NH_4NO_3-extractable P concentration was low on P0% (1.11 µg.g^{-1}) and increased with all treatments, except manure alone (Table 2). Increases went from 1.8-fold for biochar to 3.0-fold for biochar + manure. The amendments presented NH_4NO_3-extractable P concentrations of 0.63, 250.28 and 8.40 µg.g^{-1} for ochre, manure and biochar, respectively (Table 1), which could explain the observed increase in NH_4NO_3-extractable P concentration following their addition to Pontgibaud. However, as for Fe, contrary to the high concentrations of P found in manure, NH_4NO_3-extractable P concentration did not increase when manure was added alone to Pontgibaud.

NH_4NO_3-extractable Pb concentration was 4198.27 µg.g^{-1} for P0% (Table 2) and was observed to increase following PB, PI and PBM treatments, and at the same rate (1.34-fold on average). Only the combination of the three amendments (PBIM) decreased NH_4NO_3-extractable Pb concentration (by 30 %). These results contradict previous studies, which showed that biochar, ochre and manure were able to decrease the extractable fractions of cationic elements (Namgay et al. 2010, Walker et al. 2014). However, such an increase could be due to the increase in dissolved organic carbon following these amendments, which mobilizes the Pb (Lee et al. 2009, Lebrun et al. 2019). Organic carbons can decrease the sorption of Pb onto the soil surfaces, through competition with the free metal ions and the formation of soluble complexes or by its preferential sorption on the soil surface over metallic ions (Lee et al. 2009).

2. Soil pore water physico-chemical properties

SPW pH was determined at the beginning (T0) and at the end of the experiment, in both non-vegetated (TF-*Agrostis*) and vegetated (TF+*Agrostis*) pots. At T0, SPW pH of P0% was acidic (pH 4.3) (Table 3) and increased with all amendment treatments, from 1.0 (PB and PM) to 3.4 units (PBIM). The same trends were observed for TF-*Agrostis* and TF+*Agrostis*, with acidic pH on P0% (pH 3.8 and pH 3.9, respectively), which increased following treatments, between 0.8 (PB) and 3.2 units (PBIM) for TF-*Agrostis* and between 0.9 (PB) and 3.0 units (PBIM) for TF+*Agrostis*. It can be noted that all amendments, alone or combined, increased SPW pH compared to P0% at all sampling times, with

average rises between 1.1 (PB) and 3.2 units (PBIM). Finally, the results showed that SPW pH increases were higher when amendments were combined than when they were applied alone, showing neutral values compared to the slightly acidic values still observed with single amendment application. Such increases in SPW pH following biochar, ochre and/or manure amendments have previously been observed and attributed to the alkalinity of the three amendments (Marques et al. 2008, Olimah et al. 2015, Van Poucke et al. 2018), which induces a liming effect. Indeed, biochar, ochre and manure presented alkaline pH values of 8.46, 8.29 and 9.52, respectively. However, although manure presented the highest pH among the amendments, it did not induce the highest increase in SPW pH when applied alone. This low increase compared to the high amendment pH was also observed by Walker et al. (2004) when a cow manure with a pH of 9.09 was added to a contaminated soil at a rate of 29 $g.kg^{-1}$ (1.12 units after 31 days). Such an effect could be explained by the presence of more carboxyl groups and on biochar and redmud surfaces than on the manure surface. These carboxyl groups can consume H^+ (Lebrun et al. 2019).
The evolution of SPW pH with time was assessed, revealing that in almost all conditions (except PM and PBM), SPW pH decreased with time, between 0.6 and 0.8 units, and with no effect of plant growth. Such decreases with time have been observed previously (Hattab-Hambli et al. 2016, Marseille et al. 2000), and explained by the oxidation/acidification of the substrates due to watering.

Non-amended Pontgibaud technosol presented a low EC at T0 (210 $\mu S.cm^{-1}$), which increased following amendment application, except for with biochar alone (Table 3). SPW EC increases were between 9.6 (PI) and 26.5-fold (PBIM). SPW EC increased only with PIM and PBIM, at TF-*Agrostis*, compared to P0%. At TF+*Agrostis*, there was no effect with biochar alone and an increase with all other treatments, between 2.5 (PI) and 6.6-fold (PBIM) for TF+*Agrostis*. Except for with biochar alone, all treatments increased SPW EC compared to P0%, at all sampling times, with average increases ranging from 2.9 (PI) to 7.9-fold (PBIM). As for SPW pH, amendment combinations tended to lead to a greater increase in SPW EC than when applied alone. Such improvements are related to the EC of the amendments (Walker et al. 2004): biochar 302 $\mu S.cm^{-1}$, ochre 7765 $\mu S.cm^{-1}$ and manure 9476 $\mu S.cm^{-1}$ (Table 1). Biochar alone did not have an effect on SPW EC. This could be related to biochar's low EC compared to the two other amendments, and having similar EC values to that of the SPW P0% at T0. It could also be related to the low application rate in this study. Indeed, in a previous study, Lebrun et al. (2018a) used the same biochar and did not observe a SPW EC rise after biochar application to a mine soil at T0 compared to P0%, although SPW EC increased with time, and was higher than P0% at the end of the experiment. However, biochar application rates were 2% and 5%, compared to the 1% used here. Furthermore, as observed by Lebrun et al. (2018a), SPW EC increased after 26 days for the PB treatment, by 2.5-fold on average, with no plant effect, which could be due to the biochar ageing. An increase was also observed in the P0% treatment, with a 3.2-fold rise on average. However, all the other treatments observed a decrease in SPW EC with time. For PBI and PBM, the decrease was only significant in non-vegetated pots while for PM it was significant in both conditions, with no difference between TF-*Agrostis* and TF+*Agrostis*. Finally, for PIM and PBIM, the decrease was higher in non-vegetated pots than in *Agrostis* vegetated pots. Such decreases can be explained by a leaching of salts and/or root exudation/collection.
P0% SPW redox potential was 427 mV at T0 (Table 3) and all amendments caused it to decrease between 6 % (PB) and 43 % (PIM). At TF, SPW redox potential of P0% was 439 and 421 mV, for non-vegetated and vegetated conditions, respectively. Again, all amendments decreased SPW redox potential between 10 % (PB) and 27 % (PIM and PBIM) for TF-*Agrostis,* and between 10 % (PB) and 23 % (PBI and PBIM) for TF+*Agrostis*. Contrary to pH and EC, all amendments decreased SPW redox potential compared to P0% at all sampling times, and between 9 % (PB) and 31 % (PIM) on average. Few changes in SPW redox potential were observed over time: SPW redox potential decreased by 4 % for TF-*Agrostis* compared to T0 on PBM while on PI, PIM and PBIM, it increased with time, between 1.1 and 1.4-fold, with no plant effect, following a reverse trend compared to pH. Indeed, redox potential is known to have the opposite behavior to pH (Rinklebe et al. 2015).

3. Soil pore water element concentrations

At T0, SPW of P0% substrate presented a low As concentration (0.05 $mg.L^{-1}$) (Table 4), and there was no immediate effect from amendment application, either alone or combined, which is consistent with a previous study on biochar (Lebrun et al. 2018a). Similarly, amendment application had no effect on SPW As concentrations, compared to P0%. However, for TF+*Agrostis*, SPW As concentration increased by 2.3 times following the application of the three amendments together. The behavior of SPW As concentrations for TF+*Agrostis* was linked to the amendment induced pH increase (R^2 = 0.38, $p < 0.05$) (Table S1). Additionally, the increase in SPW As concentration with the PBIM treatment could be related to the P concentration measured in the same treatment. Indeed, As and P are chemically similar and thus compete for the same sorption site. Thus, with an increase in P concentration, As mobility also increases (Fitz and Wenzel 2002, Cao et al. 2003). Furthermore, it should be noted that contrary to what previous studies showed, ochre did not decrease As solubility. Indeed, several studies showed that ochre was able to reduce soluble metal concentrations, and particularly As, through sorption by Fe oxides (Abed et al. 2017, Doi et al. 2005, Olimah et al. 2015). However, due to the input of other elements such as P, K but also Pb, by the amendments, the Fe oxides could have been occupied by these elements and thus not be available for As sorption. Finally, increases in SPW As concentration were observed with time for PBI, PBM, PIM and PBIM, with no plant effect, except for PBM where the increase was only significant in the vegetated conditions.

SPW Fe concentrations were below detection limit for all treatments and at all times (Table 4).

At T0, SPW K concentrations was 14.83 $mg.L^{-1}$ for P0% (Table 4) and increased with all amendments (except for biochar and ochre alone), by between 1.1 (PBI) and 26.7-fold (PBIM). At TF, in the non-vegetated condition, SPW K concentrations increased only with PM, PBM and PBIM. Moreover, in the vegetated conditions, only the manure treatments increased SPW K concentrations. Such increases in K may be explained by a supply of potassium provided by the different amendments, especially manure, which showed the highest NH_4NO_3-extractable K concentrations among the three tested amendments (Table 1). Finally, SPW K concentrations were found to decrease with time for PI, PM and PBIM with no plant effect, while the decreases for PBM and PIM were higher in the non-vegetated pots. Such decreases can be attributed to both the leaching of K, and the effect of root exudates in the case of PBM and PIM (Badri and Vivanco 2009, Erickson et al. 2005). Furthermore, K's behavior can be related to the pH. Indeed, positive correlations were observed between the SPW pH and SPW K concentrations at T0 (R^2 = 0.35, $p < 0.01$), TF-*Agrostis* (R^2 = 0.41, $p < 0.01$) and TF+*Agrostis* (R^2 = 0.39, $p < 0.05$) (Table S1). This shows that SPW K concentrations tended to increase with increasing SPW pH. This backs up observations made by Arienzo et al. (2009) who showed that potassium availability is strongly related to pH, and is sustained for plants in neutral or slightly acidic soils, which was the pH range observed after the incorporation of amendments into PG.

At T0, SPW P concentration was 0.14 $mg.L^{-1}$ for P0% (Table 4) and decreased following biochar + manure and biochar + ochre amendments by 71 % and 36 %, respectively, which could be due to P sorption onto the amendments, especially biochar, and/or onto the soil's surface. On the contrary, when ochre and manure were applied alone, SPW P concentrations increased by 1.2 and 1.4-fold, which can be explained by a supply of P from manure and ochre. Indeed, ochre and manure especially showed high extractable concentrations in K. Moreover, they have no structure, compared to biochar; therefore their nutrients are easily leachable. At TF, in non-vegetated and vegetated conditions, SPW P increased after adding the different amendments, except for with biochar. This could be attributed to a P supply from the amendments, especially manure, which showed the highest NH_4NO_3-extractable P concentrations among the three amendments tested (Table 1). Finally, SPW P concentrations decreased with time for P0%, PB and PI, without plant effect, which was probably due to P leaching. On the contrary, SPW P concentration increased in PBI and in vegetated PBM. This could have been due to leaching of the P from the amendments, as well as root exudates, such as amino acids, carboxylic acids, sugars and flavonoids, which affect the behavior of nutrients by modifying soil acidity, chelation, precipitation as well as altering the microbial activity and other physical and chemical soil properties (Kidd et al. 2009).

P0% presented high SPW Pb concentrations at T0 (22.26 mg.L^{-1}) (Table 4), and decreased with all amendments except manure alone. Decreases in SPW Pb concentration ranged from 30 % (PB) to 95 % depending on the amendments used (PBI, PIM and PBIM). For TF-*Agrostis*, SPW Pb concentrations decreased for all amendments, except biochar alone, with decreases observed between 20 % (PB) and 80 % (PIM). All amendments decreased SPW Pb concentrations for TF+*Agrostis*, between 24 % (PM) and 80 % (PIM) compared to P0%. Such results are consistent with the sorption capacity of the amendments. Indeed, Huang et al. (2018) showed that pig manure was able to reduce Cd, Cu, Pb and Zn concentrations through precipitation onto phosphates and chelation with organic matter, while Olimah et al. (2015) showed that ochre could adsorb As due to its high content of Fe(III) oxyhydroxides. Moreover, Lebrun et al. (2018b) explained the reduction in SPW Pb concentrations following biochar amendment to be partly due to its functional groups. Drops in SPW Pb concentration can also be attributed to the increase in SPW pH induced by the application of amendments (Kiran et al. 2017), as shown by the negative correlation observed between SPW pH and SPW Pb concentrations at T0 (R^2 = - 0.91, $p < 0.001$), for TF-*Agrostis* (R^2 = - 0.93, $p < 0.001$) and TF+*Agrostis* (R^2 = - 0.90, $p < 0.001$) (Table S1). Regarding the evolution of SPW Pb with time, and depending on the treatments tested, both increases and decreases were observed. For P0% and PB, it decreased, with no plant effect, while for PM and PBM, the decrease was higher for plant growth, which can be explained by both leaching, and ageing of the amendment, allowing higher sorption and plant uptake. Finally, SPW Pb concentrations were observed to increase on PIM and PI, with and without plants, respectively. On PBI and PBIM, SPW Pb concentrations increased in both conditions, although it was higher without plants for PBI.

4. Plant growth and metal(loid) accumulation

Agrostis capillaris grown on P0% produced a low biomass: 0.48 g.pot^{-1} and 0.92 g.pot^{-1} for aerial and root parts, respectively (Fig. 1). Regarding the aerial dry weight (DW), it only increased following the manure amendments, with no difference between the four treatments containing manure. Root DW increased for all amendments containing manure. This improvement in DW production was observed previously, and can be explained by (1) a reduction in SPW As and Pb concentrations, (2) an improvement in the soil physico-chemical properties (increases in pH and EC), and (3) the provision of nutrients, especially K, by the amendments added, in particular manure (Jiang et al. 2014, Lebrun et al. 2017, Walker et al. 2004). Indeed, manure was the only amendment leading to an increase in aerial and root DW production compared to P0%, and the application of additional amendments did not increase it any further. Manure treatments were also the ones presenting the highest NH_4NO_3-extractable and SPW K and P concentrations. Moreover, K concentrations meet the concentrations that are recommended for most of the plants to grow, *i.e.* 150-250 mg.kg^{-1} (Rodriguez-Vila et al. 2017), only in the treatments containing manure. Therefore, the improvement of *Agrostis* growth in this experiment was due to the fertilizing ability of manure, compared to biochar and redmud, which are not fertilizers *per se*. These explanations were confirmed by the significant correlations found. Aerial DW was positively correlated with NH_4NO_3-extractable K concentrations (R^2 = 0.52, $p < 0.001$) and P concentrations (R^2 = 0.73, $p < 0.001$), SPW K concentrations at TF-*Agrostis* (R^2 = 0.74, $p < 0.001$) and TF+*Agrostis* (R^2 = 0.90, $p < 0.001$) and SPW P concentrations at TF-*Agrostis* (R^2 = 0.71, $p < 0.001$) and TF+*Agrostis* (R^2 = 0.63, $p < 0.001$) (Table 5). Root DW correlated positively with NH_4NO_3-extractable K concentrations (R^2 = 0.56, $p < 0.001$) and P concentrations (R^2 = 0.73, $p < 0.001$), SPW K concentrations at TF-*Agrostis* (R^2 = 0.69, $p < 0.001$) and TF+*Agrostis* (R^2 = 0.76, $p < 0.001$) and SPW P concentrations at TF-*Agrostis* (R^2 = 0.65, $p < 0.001$) and TF+*Agrostis* (R^2 = 0.56, $p < 0.001$) (Table S1).

Aerial As concentration was 19.70 mg.kg^{-1} in plants grown on P0% substrates (Fig. 2A) and decreased only following PBM amendment, by 64 %. Such decreases contradict the observed increases in NH_4NO_3-extractable As concentrations, but could be due to a dilution effect. This dilution effect was consistent with the negative correlation between aerial DW and aerial As concentrations (R^2 = - 0.63, $p < 0.01$) (Table 5). When plants were grown on P0%, they accumulated 58.84 mg As kg^{-1} in their roots. Root As concentrations were found to increase with only with PBM. Such increment could be explained by the affinity of As for organic matter, which can increase its availability to plants. Moreover, a positive correlation was observed between root As concentrations and SPW P concentrations for TF+*Agrostis* (R^2 = 0.55, $p < 0.001$) and NH_4NO_3-extractable P concentrations (R^2 = 0.51, $p < 0.001$) (Table S1). It could be caused by the fact that the supply of P from the amendments increased the availability of As. This relation was previously observed by Gunes et al. (2009), where they observed an increasing concentration of As in chickpea shoots when increasing concentrations of As and P were applied to a clay loam soil. On the contrary, in the present case, the correlation between aerial As concentrations and SPW P concentrations for TF+*Agrostis* was negative and lower (R^2 = - 0.38, $p < 0.05$) (Table S1). However, a negative correlation between rice grain As concentration and available P was observed by Jiang et al. (2014). Similarly, this study showed a negative correlation between aerial As concentration and NH_4NO_3-extractable P concentrations (R^2 = - 0.62, $p < 0.001$) (Table S1). It can be hypothesized that P SPW concentrations, and its availability, increased As uptake by the roots, although the transport system from roots to shoots was reduced. This can be explained by the fact that, in the plants, As and P compete for the same transporters, as shown by Cao et al. (2003) in *Pteris vittata* L.

Aerial Fe concentration was 193.73 $mg.kg^{-1}$ on P0% (Fig. 2B), and decreased following the biochar+manure treatment, by 68 %. This could be due to a dilution effect, as plants grew better on manure-amended technosols, and was also corroborated by the negative correlation between aerial DW and aerial Fe concentrations (R^2 = - 0.63, $p < 0.001$) (Table S1). On the contrary, root Fe concentration, which was 515.11 $mg.kg^{-1}$ on P0%, increased with PBM, PIM and PBIM treatments.

Aerial Pb concentration was 257.16 $mg.kg^{-1}$ on P0% and decreased with all the amendments. This decrease was contradictory to the increase in NH_4NO_3-extractable Pb concentrations as well as the study of Norini et al. 2019, which observed a decrease in Pb plant availability following the biochar amendment. This could be due once again to a dilution effect. This was confirmed by the negative correlation between aerial DW and aerial Pb concentrations (R^2 = - 0.71, $p < 0.001$) (Table S1). Root Pb concentrations was decreased with PI and PBI, whereas it increased following PBM treatment.

For all three metals and metalloids, As, Fe and Pb, the concentrations observed in *Agrostis* plants were at levels considered normally toxic for plants, although *Agrostis* has been shown to tolerate such high amounts (Rodríguez-Seijo et al. 2016). The concentrations were higher than the ones found in previous studies (Dahmani-Muller et al. 2000), but the soil studied here presented higher pollution concentrations.

In general, metal(loid) concentrations tended to decrease following amendment application, especially in the aerial parts of plants grown on manure treated soils. This could have been the results of the presence of higher contents in nutrients in these amendments. Abbas et al. (2018) showed that a proper mineral nutrition of the plants could decrease plant Cd concentrations, due to the competition between the essential elements and Cd. Since As and Pb are not essential nutrients, and are even toxic to plants, plants tend to preferentially take up essential nutrients, especially when they are in high concentrations. With more nutrients available in the soils amended with manure, and other amendments to a lesser extent, they were preferentially taken up by *Agrostis capillaris* compared to metal(loid)s.

Furthermore As, Fe and Pb concentrations in *Agrostis* plants were higher in the roots compared to the aerial parts, which could be a tolerance mechanism (Rodríguez-Seijo et al. 2016). Indeed, roots act as a trap organ for both metals and metalloids (Austruy et al. 2013), and the root cell wall, which is negatively charged, can bind to pollutants with positive charges (Gautam and Agrawal 2017).

Finally, the metal(loid) concentrations showed a higher increase in the roots associated to a lower translocation toward aerial parts in the PBM treatment, followed by PM and PIM.

Conclusion

In the present study, a phytoremediation mesocosm experiment was set up using a former mining technosol amended with three different amendments, biochar, ochre and manure, applied alone or combined. *Agrostis capillaris* plants were grown from seeds collected at the polluted site to test its metal(loid) accumulation ability. The results showed that the different amendments, alone or combined, improved soil conditions, which in turn allowed a better *Agrostis* plant growth, especially with manure, due to its high nutrient supply. Based on this experiment, manure seems the best amendment to ameliorate soil conditions and thus plant growth. However, it is a well-known fact that the manure amendment has a short-term effect and would need to be re-applied regularly (Mosaddeghi et al. 2009).. Therefore, when combined and added with ochre, and especially with biochar, this will allow a longer term effect. Indeed, the SPW measurements showed that Pb concentrations continued to decrease with time in PBM, contrary to PIM and PBIM treatments, which encountered an increase in SPW Pb concentration with time. Moreover, the biochar + manure treatment led to the lowest metal(loid) translocation to the aerial biomass. This study also showed that *Agrostis*, metallicolous ecotype, seeds collected from the site could be used in a rehabilitation process associated to the use of biochar and manure amendments. Indeed, the amendment treatments will improve soil conditions (reduction of acidity and metal(loid) concentrations, supply of nutrients) which will allow efficient *Agrostis* plant growth. Such treatments will reduce contaminant spreading by soil erosion and water leaching.

However, the soil microbiota of the different substrates in particular, in both the bulk and the rhizosphere, needs to be evaluated in order to assess the effect of amendments and *Agrostis* growth on the soil microbial diversity and activity, as microorganisms are known to play an important role in soil rehabilitation. Moreover, a test at the macrocosm level will be performed to confirm the present results.

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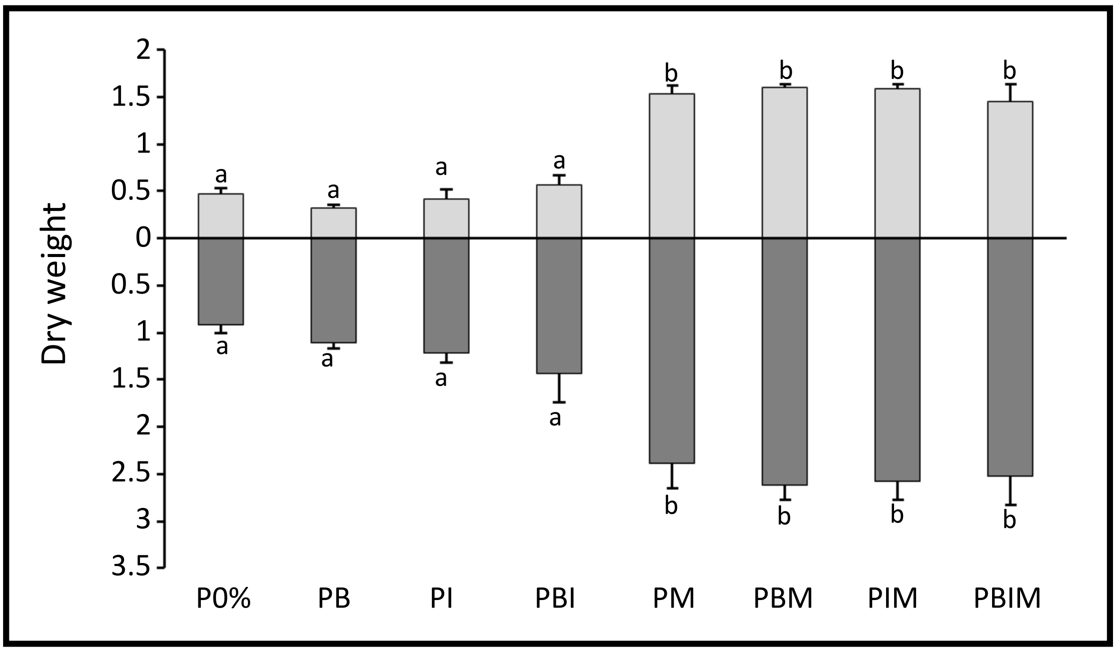

Fig 1: Dry weight ($g.pot^{-1}$) of the aerial (■) and root (■) parts of *Agrostis capillaris* plants grown on Pontgibaud technosol unamended (P0%) or amended with biochar (B), ochre (I) and manure (M), alone or combined. Letters indicate significant difference between treatments ($p < 0.05$) (n= 5 ± SE).

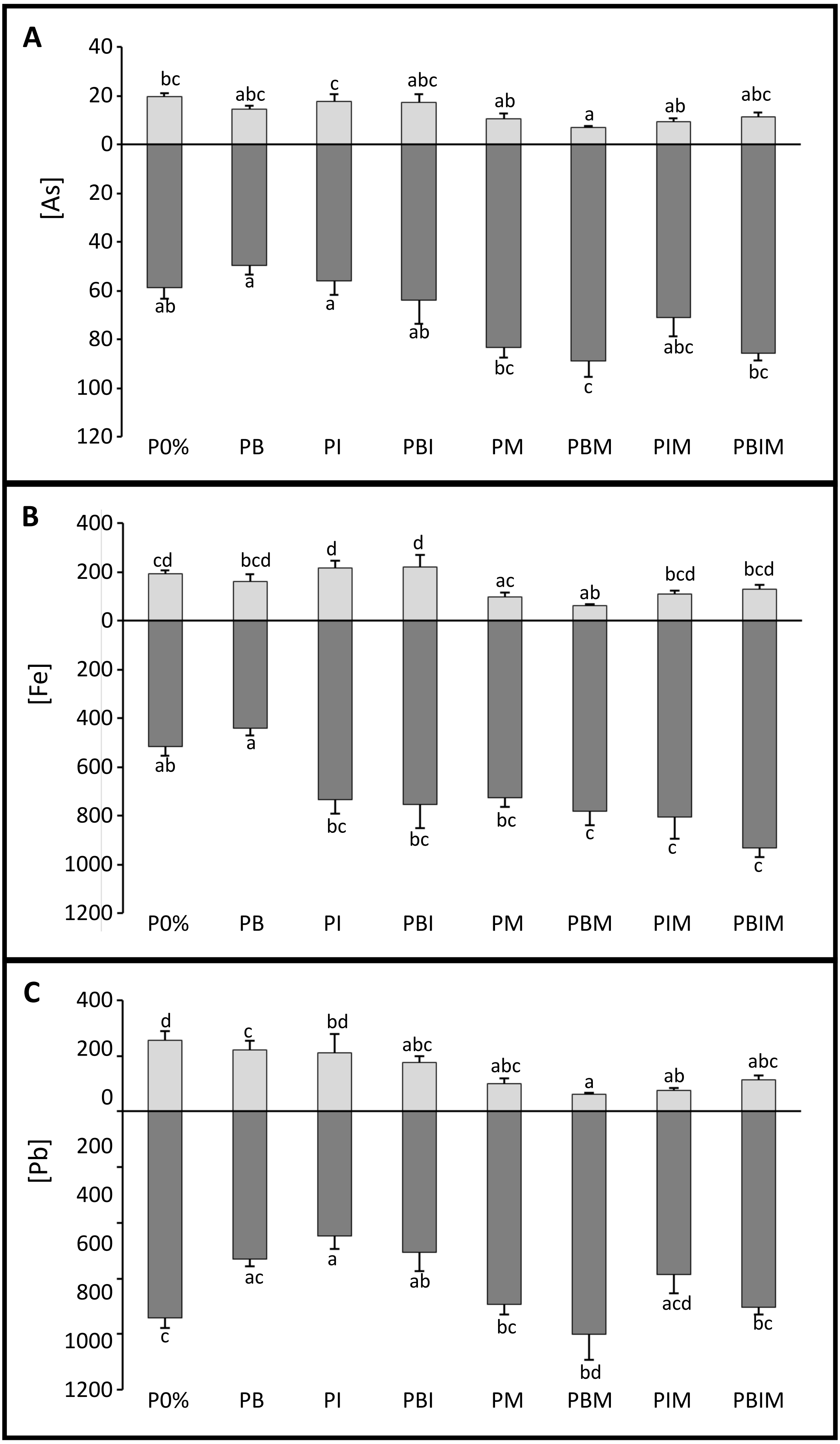

Fig 2: (A) As, (B) Fe and (C) Pb concentrations (mg.kg^{-1}) of the aerial (■) and root (■) parts of *Agrostis capillaris* plants grown on Pontgibaud technosol unamended (P0%) or amended with biochar (B), ochre (I) and manure (M), alone or combined. Letters indicate significant difference between treatments (p < 0.05) (n= 5 ± SE).

Table 1: Amendments (biochar, ochre and manure) physico-chemical properties (pH, EC = electrical conductivity (µS.cm^{-1}) and redox potential (mV)) and NH_4NO_3-extractable element (As, Fe, K, P, Pb) concentrations (µg.g^{-1}).

	Biochar	Ochre	Manure
Physico-chemical properties			
pH	8.46 ± 0.01	8.29 ± 0.03	9.52 ± 0.00
EC	302 ± 1	7765 ± 14	9476 ± 138
Redox potential	279.5 ± 6.5	217.4 ± 9.3	88.2 ± 0.9
NH_4NO_3 extractable concentrations			
As	0.90 ± 0.10	0.24 ± 0.09	0.75 ± 0.25
Fe	17.90 ± 5.10	1.04 ± 0.53	2.41 ± 0.20
K	752.30 ±27.90	355.50 ± 0.53	16096.56 ± 65.49
P	8.40 ± 0.60	0.63 ± 0.07	250.28 ± 3.21
Pb	1.60 ± 5.10	0.31 ± 0.05	0.57 ± 0.06

Table 2: Soil NH_4NO_3-extractable element (As, Fe, K, P, Pb) concentrations ($\mu g.g^{-1}$) determined at the beginning of the experiment in Pontgibaud technosol unamended (P0%) or amended with biochar (B), ochre (I) and manure (M), alone or combined. Letters indicate significant difference between treatments ($p < 0.05$) ($n = 5 \pm$ SE). < DL = under detection limit ($0.01\ mg.L^{-1}$)

	As	Fe	K	P	Pb
P0%	2.29 ± 0.25 **ab**	0.02 ± 0.02 **a**	22.14 ± 0.44 **a**	1.11 ± 0.14 **a**	4198.27 ± 138.94 **bc**
PB	3.25 ± 0.16 **c**	0.05 ± 0.04 **a**	25.76 ± 0.83 **a**	2.03 ± 0.11 **b**	5873.25 ± 288.53 **d**
PI	2.94 ± 0.21 **bc**	0.54 ± 0.09 **b**	24.56 ± 0.63 **a**	2.69 ± 0.12 **c**	5721.77 ± 123.12 **d**
PBI	3.23 ± 0.22 **bc**	0.13 ± 0.10 **a**	30.48 ± 0.63 **a**	3.24 ± 0.08 **e**	5215.40 ± 326.47 **cd**
PM	1.98 ± 0.19 **a**	< DL **a**	189.98 ± 8.85 **c**	1.42 ± 0.06 **a**	4005.37 ± 113.41 **b**
PBM	3.58 ± 0.35 **c**	< DL **a**	198.70 ± 9.85 **c**	3.35 ± 0.13 **e**	5706.37 ± 154.86 **d**
PIM	2.57 ± 0.26 **abc**	< DL **a**	165.12 ± 4.60 **b**	3.21 ± 0.09 **de**	3453.90 ± 397.50 **ab**
PBIM	2.30 ± 0.16 **ab**	< DL **a**	168.56 ± 6.58 **bc**	2.80 ± 0.10 **cd**	2962.18 ± 276.62 **a**

Table 3: Soil pore water physico-chemical properties (pH, electrical conductivity (EC) ($\mu S.cm^{-1}$), redox potential (mV)) determined at the beginning (T0) and at the end of the experiment in both non-vegetated (TF-*Agrostis*) and vegetated (TF+*Agrostis*) pots in Pontgibaud technosol unamended (P0%) or amended with biochar (B), ochre (I) and manure (M), alone or combined. Capital letters indicate significant difference between treatments for each sampling time whereas minuscule letters indicate significant difference T0, TF-*Agrostis* and TF+*Agrostis* for each treatment (=Time effect) ($p < 0.05$) ($n = 5\text{-}10 \pm SE$).

		pH		EC ($\mu S.cm^{-1}$)		Redox potential (mV)	
			Time effect		Time effect		Time effect
P0%	T0	4.3 ± 0.1 **A**	**b**	210 ± 29 **A**	**a**	427 ± 7 **F**	**a**
	TF-*Agrostis*	3.8 ± 0.0 **A**	**a**	733 ± 53 **A**	**b**	439 ± 8 **D**	**a**
	TF+*Agrostis*	3.9 ± 0.0 **A**	**a**	596 ± 75 **A**	**b**	421 ± 9 **D**	**a**
PB	T0	5.3 ± 0.2 **B**	**b**	255 ± 66 **A**	**a**	403 ± 6 **E**	**a**
	TF-*Agrostis*	4.6 ± 0.0 **B**	**a**	617 ± 30 **A**	**b**	396 ± 11 **C**	**a**
	TF+*Agrostis*	4.8 ± 0.2 **B**	**ab**	661 ± 108 **AB**	**b**	378 ± 13 **C**	**a**
PI	T0	7.1 ± 0.1 **D**	**b**	2024 ± 59 **B**	**c**	318 ± 5 **BC**	**a**
	TF-*Agrostis*	6.3 ± 0.0 **D**	**a**	964 ± 77 **AB**	**a**	346 ± 7 **AB**	**b**
	TF+*Agrostis*	6.3 ± 0.0 **DE**	**a**	1508 ± 196 **BC**	**b**	347 ± 4 **AB**	**b**
PBI	T0	7.2 ± 0.1 **D**	**b**	2137 ± 53 **B**	**b**	332 ± 2 **C**	**a**
	TF-*Agrostis*	6.6 ± 0.1 **E**	**a**	1417 ± 184 **AB**	**a**	324 ± 6 **A**	**a**
	TF+*Agrostis*	6.6 ± 0.1 **E**	**a**	1895 ± 149 **CD**	**b**	323 ± 6 **A**	**a**
PM	T0	5.3 ± 0.2 **B**	**a**	2959 ± 140 **C**	**b**	383 ± 5 **D**	**a**
	TF-*Agrostis*	5.3 ± 0.1 **C**	**a**	1404 ± 211 **AB**	**a**	384 ± 5 **C**	**a**
	TF+*Agrostis*	5.3 ± 0.1 **C**	**a**	1964 ± 82 **CD**	**a**	368 ± 6 **BC**	**a**
PBM	T0	6.1 ± 0.1 **C**	**a**	2923 ± 188 **C**	**b**	367 ± 3 **D**	**b**
	TF-*Agrostis*	6.3 ± 0.1 **D**	**a**	1379 ± 161 **AB**	**a**	352 ± 5 **B**	**a**
	TF+*Agrostis*	6.1 ± 0.1 **D**	**a**	2387 ± 190 **D**	**b**	368 ± 4 **BC**	**b**
PIM	T0	7.5 ± 0.1 **E**	**b**	5451 ± 195 **D**	**a**	242 ± 5 **A**	**a**
	TF-*Agrostis*	6.8 ± 0.0 **E**	**a**	1778 ± 316 **B**	**b**	321 ± 7 **A**	**b**
	TF+*Agrostis*	6.8 ± 0.0 **E**	**a**	3338 ± 352 **E**	**c**	330 ± 8 **A**	**b**
PBIM	T0	7.7 ± 0.0 **E**	**b**	5557 ± 130 **D**	**c**	307 ± 3 **B**	**a**
	TF-*Agrostis*	7.0 ± 0.1 **E**	**a**	2741 ± 453 **C**	**a**	321 ± 3 **A**	**b**
	TF+*Agrostis*	6.9 ± 0.0 **E**	**a**	3927 ± 324 **E**	**b**	326 ± 2 **A**	**b**

Table 4: Soil pore water elements (As, Fe, K, P, Pb) concentrations ($mg.L^{-1}$) determined at the beginning (T0) and at the end of the experiment in both non-vegetated (TF-*Agrostis*) and vegetated (TF+*Agrostis*) pots in Pontgibaud technosol unamended (P0%) or amended with biochar (B), ochre (I) and manure (M), alone or combined. Capital letters indicate significant difference between treatments for each sampling time whereas minuscule letters indicate significant difference T0, TF-*Agrostis* and TF+*Agrostis* for each treatment (=Time effect) ($p < 0.05$) ($n = 5-10 \pm SE$).

		[As] ($mg.L^{-1}$)		[Fe] ($mg.L^{-1}$)		[K] ($mg.L^{-1}$)		[P] ($mg.L^{-1}$)		[Pb] ($mg.L^{-1}$)	
			Time effect		Time effect		Time effect		Time effect		Time effect
P0%	T0	0.05 ± 0.02 **A**	**a**	0.00 ± 0.00 **A**	**a**	14.83 ± 5.07 **A**	**a**	0.14 ± 0.00 **C**	**b**	22.26 ± 0.62 **E**	**b**
	TF-*Agrostis*	0.07 ± 0.02 **AB**	**a**	0.00 ± 0.00 **A**	**a**	9.93 ± 0.66 **A**	**a**	0.04 ± 0.01 **AB**	**a**	6.78 ± 0.71 **E**	**a**
	TF+*Agrostis*	0.04 ± 0.01 **A**	**a**	0.00 ± 0.00 **A**	**a**	12.31 ± 1.27 **A**	**a**	0.03 ± 0.01 **A**	**a**	9.19 ± 0.86 **E**	**a**
PB	T0	0.05 ± 0.01 **A**	**a**	0.00 ± 0.00 **A**	**a**	17.37 ± 4.11 **AB**	**a**	0.14 ± 0.00 **C**	**b**	15.51 ± 0.40 **D**	**b**
	TF-*Agrostis*	0.03 ± 0.00 **A**	**a**	0.00 ± 0.00 **A**	**a**	15.22 ± 1.03 **A**	**a**	0.02 ± 0.01 **A**	**a**	5.40 ± 0.18 **D**	**a**
	TF+*Agrostis*	0.05 ± 0.01 **AB**	**a**	0.00 ± 0.00 **A**	**a**	15.60 ± 0.38 **A**	**a**	0.02 ± 0.00 **A**	**a**	5.62 ± 0.50 **CD**	**a**
PI	T0	0.08 ± 0.03 **A**	**a**	0.00 ± 0.00 **A**	**a**	11.90 ± 0.51 **A**	**b**	0.17 ± 0.01 **D**	**b**	1.91 ± 0.19 **B**	**a**
	TF-*Agrostis*	0.05 ± 0.01 **AB**	**a**	0.00 ± 0.00 **A**	**a**	8.72 ± 0.42 **A**	**a**	0.06 ± 0.01 **BC**	**a**	3.42 ± 0.19 **BC**	**b**
	TF+*Agrostis*	0.03 ± 0.01 **A**	**a**	0.00 ± 0.00 **A**	**a**	6.80 ± 0.57 **A**	**a**	0.04 ± 0.01 **A**	**a**	2.52 ± 0.19 **AB**	**a**
PBI	T0	0.02 ± 0.01 **A**	**a**	0.04 ± 0.05 **A**	**a**	16.73 ± 0.65 **B**	**a**	0.04 ± 0.01 **A**	**a**	1.01 ± 0.12 **A**	**a**
	TF-*Agrostis*	0.07 ± 0.01 **AB**	**b**	0.00 ± 0.00 **A**	**a**	15.45 ± 1.39 **A**	**a**	0.08 ± 0.01 **CD**	**b**	2.85 ± 0.14 **B**	**c**
	TF+*Agrostis*	0.04 ± 0.01 **AB**	**b**	0.00 ± 0.00 **A**	**a**	15.01 ± 0.21 **A**	**a**	0.09 ± 0.01 **B**	**b**	2.10 ± 0.33 **A**	**b**
PM	T0	0.07 ± 0.01 **A**	**a**	0.01 ± 0.01 **A**	**a**	305.15 ± 16.75 **C**	**b**	0.19 ± 0.00 **D**	**b**	24.89 ± 2.92 **E**	**c**
	TF-*Agrostis*	0.03 ± 0.00 **AB**	**a**	0.00 ± 0.00 **A**	**a**	145.50 ± 31.97 **BC**	**a**	0.07 ± 0.00 **BC**	**a**	4.32 ± 0.18 **CD**	**a**
	TF+*Agrostis*	0.05 ± 0.01 **AB**	**a**	0.00 ± 0.00 **A**	**a**	223.09 ± 7.99 **B**	**a**	0.07 ± 0.01 **AB**	**a**	6.96 ± 0.54 **D**	**b**
PBM	T0	0.03 ± 0.00 **A**	**a**	0.00 ± 0.00 **A**	**a**	311.97 ± 19.04 **CD**	**c**	0.09 ± 0.00 **B**	**a**	10.24 ± 1.16 **C**	**c**
	TF-*Agrostis*	0.05 ± 0.00 **AB**	**ab**	0.00 ± 0.00 **A**	**a**	126.39 ± 24.48 **BC**	**a**	0.11 ± 0.01 **D**	**ab**	2.07 ± 0.19 **AB**	**a**
	TF+*Agrostis*	0.07 ± 0.01 **AB**	**b**	0.00 ± 0.00 **A**	**a**	232.00 ± 22.91 **B**	**b**	0.11 ± 0.01 **BC**	**b**	4.26 ± 0.43 **BC**	**b**
PIM	T0	0.04 ± 0.00 **A**	**a**	0.00 ± 0.00 **A**	**a**	382.59 ± 10.51 **DE**	**c**	0.13 ± 0.01 **C**	**a**	1.06 ± 0.08 **A**	**a**
	TF-*Agrostis*	0.08 ± 0.01 **B**	**b**	0.00 ± 0.00 **A**	**a**	94.71 ± 23.00 **AB**	**a**	0.11 ± 0.01 **D**	**a**	1.35 ± 0.15 **A**	**a**
	TF+*Agrostis*	0.06 ± 0.01 **AB**	**b**	0.00 ± 0.00 **A**	**a**	185.81 ± 20.41 **B**	**b**	0.11 ± 0.01 **BC**	**a**	1.85 ± 0.15 **A**	**b**
PBIM	T0	0.03 ± 0.00 **A**	**a**	0.00 ± 0.00 **A**	**a**	395.96 ± 8.01 **E**	**b**	0.13 ± 0.01 **C**	**a**	1.05 ± 0.04 **A**	**a**
	TF-*Agrostis*	0.08 ± 0.01 **B**	**b**	0.00 ± 0.00 **A**	**a**	191.11 ± 46.78 **C**	**a**	0.14 ± 0.01 **E**	**a**	1.46 ± 0.08 **A**	**b**
	TF+*Agrostis*	0.09 ± 0.02 **B**	**b**	0.00 ± 0.00 **A**	**a**	238.21 ± 22.59 **B**	**a**	0.15 ± 0.02 **C**	**a**	2.05 ± 0.28 **A**	**b**

Table S1: Pearson correlation coefficients between the soil physico-chemical characteristics (NH_4NO_3-extractable elements (As, Fe, K, P, Pb) contents), the soil pore water physico-chemical properties (pH, [As], [K] and [P], at T0, TF-*Agrostis* and TF+*Agrostis*) and *Agrostis capillaris* plant parameters (DW = dry wiehgt, As, Fe and Pb concentrations of the aerial and root parts). Signicant correlation are in bold, level of significance: * (p < 0.05), ** (p < 0.01) and *** (p< 0.001)

			Soil			Soil pore water											Plants							
			NH4NO3-extractable concentrations			T0			TF-*Agrostis*				TF+*Agrostis*				Aerial part				Roots			
			[As]	[K]	[P]	pH	[K]	[Pb]	pH	[K]	[P]	[Pb]	pH	[As]	[K]	[Pb]	DW	[As]	[Fe]	[Pb]	DW	[As]	[Fe]	[Pb]
Soil	NH4NO3-extractable concentrations	[As]	1	-0.19	**0.51*** **	-0.22	0.1	-0.29	-0.15	0.18	-0.11	0.10	-0.13	-0.01	0.24	0.18	0.15	**-0.4***	**-0.44****	-0.24	0.13	0.08	-0.16	0.1
		[K]		1	0.25	**0.69*****	**0.51*****	**-0.6*****	**0.74*****	**0.37***	**0.73****	**-0.78*****	**0.71*****	**0.42****	**0.47****	**-0.63*****	**0.52*****	**-0.41***	-0.27	**-0.51****	**0.56*****	**0.43****	**0.54*****	0.14
		[P]			1	**0.38***	**0.76*****	-0.09	**0.52*****	**0.58*****	**0.52*****	**-0.58*****	**0.49****	**0.31***	**0.77*****	**-0.35***	**0.73*****	**-0.62*****	**-0.58*****	**-0.65*****	**0.73*****	**0.51*****	**0.45****	0.16
Soil pore water	T0	pH				1	**0.35****	**-0.91*****	**0.94*****	0.27	**0.68*****	**-0.87*****	**0.94*****	**0.33***	0.27	**-0.91*****	0.31	-0.22	-0.02	**-0.37***	**0.43****	0.23	**0.66*****	-0.22
		[K]					1	-0.11	**0.52*****	**0.8*****	**0.75*****	**-0.64*****	**0.47****	**0.50****	**0.94*****	-0.27	**0.91*****	**-0.67*****	**-0.65*****	**-0.71*****	**0.84*****	**0.65*****	**0.54*****	**0.38***
		[Pb]						1	**-0.78*****	0.04	**-0.46***	**0.69*****	**-0.78*****	-0.28	0.04	**0.84*****	-0.02	0.04	-0.16	0.14	-0.17	-0.02	**-0.47****	0.28
	TF-*Agrostis*	pH							1	**0.39***	**0.76*****	**-0.90*****	**0.97*****	**0.38***	**0.45****	**-0.89*****	**0.49****	**-0.37***	-0.17	**-0.53*****	**0.57*****	**0.39***	**0.73*****	-0.13
		[K]								1	**0.64*****	**-0.47****	**0.36***	**0.44****	**0.81*****	-0.17	**0.74*****	**-0.53*****	**-0.50****	**-0.53*****	**0.69*****	**0.63*****	**0.51*****	**0.42****
		[P]									1	**-0.76*****	**0.74*****	**0.49****	**0.73*****	**-0.56*****	**0.71*****	**-0.42****	-0.3	**-0.54*****	**0.65*****	**0.55*****	**0.67*****	0.17
		[Pb]										1	**-0.93*****	**-0.42*****	**-0.57*****	**-0.80*****	**-0.61*****	**0.47****	0.30	**0.57*****	**-0.65*****	**-0.46****	**-0.70*****	-0.02
	TF+*Agrostis*	pH											1	**0.38***	**0.41****	**-0.90*****	**0.42****	-0.32	-0.07	**-0.47****	**0.49****	**0.32***	**0.70*****	-0.19
		[As]												1	**0.54*****	-0.32	**0.37***	**-0.42****	**-0.32***	**-0.43****	**0.36***	**0.47****	**0.43****	**0.36***
		[K]													1	-0.15	**0.90*****	**-0.64*****	**-0.64*****	**-0.70*****	**0.76*****	**0.72*****	**0.54*****	**0.48****
		[Pb]														1	-0.19	0.27	0.02	**0.39***	-0.37	-0.12	**-0.55*****	**0.36***
Plants	Aerial part	DW															1	**-0.63*****	**-0.63*****	**-0.71*****	**0.87*****	**0.75*****	**0.59*****	**0.48*****
		[As]																1	**-0.92*****	**0.83*****	**-0.67*****	**-0.37***	-0.21	-0.19
		[Fe]																	1	**0.78*****	**-0.60*****	**-0.37***	-0.09	-0.3
		[Pb]																		1	**-0.71*****	**-0.52*****	**-0.46****	-0.23
	Roots	DW																			1	**0.72*****	**0.62*****	**0.39***
		[As]																				1	**0.82*****	**0.78*****
		[Fe]																					1	**0.42****
		[Pb]																						1