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Effect of biochar amendments on As and Pb mobility and phytoavailability in contaminated mine technosols phytoremediated by *Salix*

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ABSTRACT

Mining activities lead to widespread environmental pollution of terrestrial ecosystems due to the presence of metal(loid)s in tailings. These contaminated areas present a health risk and hence need to be rehabilitated. *Ex situ* methods for soil remediation have been used for a long time but are expensive and disruptive to soil. Phytoremediation techniques for the stabilization or extraction of metal(loid)s could be an efficient alternative as they provide a low-cost and environmentally friendly option. However, due to the often poor nutrient content of these contaminated soils, amendments must be added to enhance plant growth and to improve phytoremediation efficiency. Biochar, a pyrogenic product, is a promising amendment for assisted phytoremediation. The aims of our study were (i) to evaluate the effect of a pinewood biochar on the physico-chemical properties of a former mine contaminated technosol, (ii) to assess the mobility and phytoavailability of As and Pb and (iii) to investigate the remediation potential of three willow species (*Salix alba*, *Salix viminalis* and *Salix purpurea*). A greenhouse experiment was conducted with contaminated technosols amended with biochar and garden soil, single or combined, revegetated with the 3 willow species. The physicochemical properties of soil pore water (SPW) as well as metal(loid) concentrations were determined. Plant growth, *Salix* organ dry weight and metal(loid) uptake were determined in order to evaluate the phytoremediation potential of the three *Salix* species studied. Biochar increased the pH and electrical conductivity of SPW. Biochar addition had no effect on As mobility but decreased SPW Pb concentration by 70%. For the three *Salix* species investigated, biochar addition to the polluted soil induced a better growth and a higher dry weight production. In most modalities tested, the metal(loid) content in the *Salix* organs increased due to the biochar application. Globally, a positive effect of biochar was noticed on the soil qualities (pH and electrical conductivity increase) and plant growth. Metal(loid)s were mostly confined to the roots. Among the species tested, *Salix alba* presented the lowest metal(loid) concentrations in the aerial parts, making it a particularly suitable tool for Pb soil phytostabilization.

1. Introduction

Potential toxic elements (PTE) such as metal(loid)s are naturally present in the environment at rather low concentrations. However, since the beginning of the industrial era, and due to anthropogenic activities such as mining and smelting, contamination by PTEs has increased drastically. The number of sites potentially contaminated in Europe is estimated at 3.5 million (Petruzelli, 2012). In addition, PTEs do not remain fixed and stabilized on site but can be disseminated to the surrounding environment by wind erosion and to the ground and surface water through leaching and run-off/on (Puga et al., 2016) and consequently they can enter the food chain (Kloss et al., 2014). Metal(loid) contaminants are therefore a major issue, not only for the environment but also for human health (Ali et al., 2013). As a result, remediation of these polluted sites has become an important societal objective.

Physical and chemical techniques to remediate contaminated soils have been used for a long time, but these conventional methods have many flaws: they are expensive, difficult to implement and disruptive to soil (Ali et al., 2013). An alternative is phytoremediation, defined as the use of plants to remediate polluted soils. It is performed *in situ* to stabilize or to extract soil pollutants (Moosavi and Seghatoleslami, 2013). Phytoremediation uses mainly solar energy (Borišev et al., 2009), and maintains or can even improve soil structure (Mleczek et al., 2010). Briefly, due to its capacity to install a plant cover which (i) limits

Abbreviations: PTE, potential toxic element; SPW, soil pore water; EC, electrical conductivity; DOC, dissolved organic carbon.

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erosion, (ii) creates an aerobic environment in the rhizosphere, (iii) provides organic matter in the soil, and (iv) aggregates and binds metal(loid)s to soil components, it is perceived as an environmentally friendly method (Vamerali et al., 2009; Ali et al., 2013). However, when PTE soil concentrations are very high, phytoextraction will take decades and poses the problem of PTEs returning to the ground when leaves and branches are shed. For this reason, phytostabilization, which strongly constrains these pollutants in soil and in the plant root system without translocation to the harvestable parts, is an efficient alternative.

To improve phytoremediation and because contaminated soils are often poor in available nutrients and often acidic for plants and the associated microbiota, organic and/or inorganic amendments must be used (Park et al., 2011). Moreover, when added to soil these amendments can contribute by their own properties to reducing contaminant levels in water soil solution, by reducing PTE leaching (Melo et al., 2016). Furthermore, Agegnehu et al. (2015) demonstrated that two organic amendments (biochar and compost) improved peanut seed and pot yields, as well as the chlorophyll contents of plants. These positive effects are associated to a better C, N, P, K plant uptake and to an increase in soil soluble organic carbon availability. Biochar is one of these organic amendments, resulting from the pyrolysis of organic materials under limiting oxygen conditions (Anawar et al., 2015). It is a porous, carboneous product characterized by a large surface area, a low density, a high cation exchange capacity (CEC), an alkaline pH (Paz-Ferreiro et al., 2014) and usually lasts longer than other amendments. Its beneficial use in agronomy has been known for a long time (*cf.* Terra Petra) (Lehmann and Joseph, 2009). Moreover, Fellet et al. (2011), Beesley and Marmiroli (2011) and Zhang et al. (2013) demonstrated the effectiveness of biochar to remediate PTE contaminated mine soils by reducing their concentrations in soil pore water and in the plants grown in the amended soils.

>400 plant species, either associated with amendments or not, have proved to be efficient phytoremediators (Moosavi and Seghatoleslami, 2013). Among them, a few Brassicaceae have been described as PTE hyperaccumulators (Sarma, 2011). However, their low biomass and slow growth rate diminish their potential use in phytoremediation (Ghosh and Singh, 2005). To overcome these drawbacks, tree species, which present a rapid growth, a large biomass production, a deep root system and sometimes a high accumulation capacity for PTEs, are interesting phytoremediation options. Among woody species, Salicaceae have already been proposed as phytoremediator plants (Marmiroli et al., 2011). Indeed, Bart et al. (2016) demonstrated the capacity of *Salix viminalis* and *Salix purpurea* to grow on a mine soil contaminated by As, Pb and Sb.

In order to remediate a multi-PTE contaminated soil using assisted phytoremediation, the goals of our study were to investigate the effect of two organic amendments, a garden soil and a pinewood biochar, single or in combination, on i) the physicochemical properties of a multi-contaminated soil and ii) the growth and metal(loid) uptake by three willow species (*Salix alba*, *Salix viminalis* and *Salix purpurea*). To our knowledge, this paper is the first study describing the effect of biochar on the remediation capacities of willow species towards an acidic and highly multi-contaminated PTE soil.

2. Materials and methods

2.1. Study site

The study focused on a technosol derived from a former silver-lead mine extraction site located in Pontgibaud, Puy-de-Dôme, Auvergne, France. It was one of the largest mining and metallurgical districts in Europe during the nineteenth century but has been disused since 1897. Due to the mining activities, the site is an acidic sandy soil contaminated mostly by high concentrations of arsenic ($539.06 \pm 0.01\ mg \cdot kg^{-1}$) and lead ($11{,}453.63 \pm 0.18\ mg \cdot kg^{-1}$) (Cottard, 2010). Soil samples were collected in a settling pond (between 0 and 20 cm of depth) in the area called Roure-les-Rosiers (GPS coordinates: 45°49′59″ North and 2°51′04″ East).

2.2. Amendments

Two different organic amendments, single or combined, were used: i) a garden soil collected in the park of Orleans University, France; ii) biochar, produced from pinewood woody biomass (VT Green Company, Saint Bonnet de Rochefort, France). The main physico-chemical properties and total PTE concentrations in the biochar are presented in Table 1.

2.3. Soil mixtures preparation

Three different 2 mm diameter sieve soils were prepared: i) Garden soil (named G, control soil), ii) Pontgibaud technosol (named P) and iii) a mixture of 50% Technosol and 50% Garden soil (v/v) (named PG). These three soils were amended with 0%, 2% or 5% (w/w) biochar and placed in plastic pots (87 × 113 mm). Six pots were prepared per modality and per *Salix* species tested. Potted soils were allowed to equilibrate 5 days at field capacity using tap water before the introduction of non-rooted *Salix* cuttings (T0).

2.4. Technosol analysis

pH and EC of the different technosols were measured using a pH meter (FE20/EL20, Mettler-Toledo AG 2007) and a multimeter (WTW Multi 1970i, GEOTECH, Denver, Colorado) according to the following protocol: 10 g of technosol were put in solution with 25 mL distilled water, the solutions were stirred during 1 h (150 rpm), then left to settle for half an hour before measurements were made.

Table 1
Main biochar physico-chemical properties provided by VT Green.

Parameter	Value	Unit
pH	82	
Conductivity	9	mS/cm
Resistivity	115,207	Ohm cm
Density without compaction	0.125	kg/L
Density with compaction	0.167	kg/L
Water-insoluble	85.2	%
Insoluble in acid	84.8	%
Total exchange capacity	46	Me/kg
total porosity	96	%
Water retention capacity	85	%v/v
Retention capacity for air	11	%v/v
Major elements secondary		
Total nitrogen	<0.20	%
Total organic carbon	73.7	%
Mineral materials	1.27	%
Lost on ignition at 450 °C	89.0	%
P_2O_5 total (soluble in mineral acids)	<0.07	%
P_2O_5 soil. water%	<0.20	%
K_2O	0.14	%
K_2O water soluble	0.06	%
Total CaO	0.36	%
Total MgO	0.10	%
Total Na_2O	<0.03	%
Total sulfur	<0.10	%
Trace elements		
Total arsenic	<0.50	mg/kg
Total cadmium	0.050	mg/kg
Total chrome	16.5	mg/kg
Total cobalt	0.54	mg/kg
Total mercury	0.004	mg/kg
Total molybdenum	0.62	mg/kg
Total nickel	11.1	mg/kg
Total lead	2.36	mg/kg
Total selenium	<1	mg/kg

2.5. Soil pore water (SPW) analysis

SPWs were collected twice during the growth experiment: at the end of the equilibration period (T0) and at the end of the experiment, day 63, before harvesting the plants (TF). SPW sampling was performed using soil moisture samplers (Rhizon) (model MOM, Rhizosphere Research Products, Wageningen, The Netherlands), placed in pots at an angle of 45° and allowed to equilibrate for 4 h under vacuum (Cattani et al., 2006).

Collected SPWs were used directly to measure: pH (pHmeter, FE20/EL20, Mettler-Toledo AG 2007), electrical conductivity (EC) (multimeter, WTW Multi 1970i, GEOTECH, Denver, Colorado) and dissolved organic carbon (DOC) concentration (Pastel UV spectrophotometer, SECOMAM, Ales, France). Total dissolved PTE concentrations (As, Pb) were determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (ULTIMA 2, HORIBA, Labcompare, San Francisco, USA) after acidification, according to Bart et al. (2016).

2.6. Plant growth conditions and analysis

Non-rooted cuttings (*S. alba*, *S. viminalis*, *S. purpurea*) were planted individually in plastic pots and placed in mesocosm: temperature was maintained at 23 °C ± 2 °C during the day with a light intensity of 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and at 20 °C ± 2 °C during the night. After bud break, a single stem per cutting was grown in order to produce stable continuous growth and to minimize the variability induced by different numbers of stems per plant (Monclus et al., 2006).

During the experiment time course (day 21 to day 63), plant growth rate was determined weekly by measuring stem height. At harvesting time, leaf, root and stem dry weights were measured after a 3-day drying period at 60 °C. PTE concentrations in the different organs (leaves, roots and stem) formed during the growth period were measured by ICP-AES according to Bart et al. (2016).

2.7. Statistical analysis

Results were analyzed with the 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. For each soil studied (garden soil, contaminated soil, mixtures), the biochar effect (0%, 2% and 5%) was tested. Differences were considered significant when $p < 0.05$.

3. Results

3.1. Technosol analysis

Table 2 shows the technosol physico-chemical characteristics of the different treatments.

Table 2
Technosol physico-chemical characteristics (pH, EC ($\mu S \cdot cm^{-1}$)) determined at the beginning of the experiment in the 3 conditions, garden soil (G), contaminated soil (P) and the mixture of 50% garden soil and 50% contaminated soil (PG), amended with 0%, 2% or 5% of biochar. Letters indicate a significant difference ($p < 0.05$) ($n = 3$).

	pH	EC ($\mu S \cdot cm^{-1}$)
G0%	7.45 ± 0.03 a	433 ± 12 a
G2%	7.38 ± 0.03 a	444 ± 23 b
G5%	7.39 ± 0.02 a	508 ± 6 b
P0%	4.60 ± 0.02 a	68 ± 1 a
P2%	5.13 ± 0.05 b	112 ± 4 b
P5%	6.44 ± 0.02 c	197 ± 17 c
PG0%	7.25 ± 0.04 a	348 ± 34 a
PG2%	7.29 ± 0.00 a	388 ± 18 a
PG5%	7.38 ± 0.02 a	395 ± 13 a

The initial pH of the garden soil and the mixture were 7.45 and 7.25, respectively, and were not affected by biochar addition. However, the contaminated soil was acidic (4.60) and biochar application significantly increased the pH. A stronger effect was observed at 5% biochar (6.44) compared to 2% biochar (5.13).

Garden soil EC was 433 $\mu s \cdot cm^{-1}$, while contaminated soil and mixture EC were 68 $\mu s \cdot cm^{-1}$ and 348 $\mu s \cdot cm^{-1}$, respectively. With a 2% application of biochar, contaminated soil EC increased by 1.6 while a 5% biochar application led to an EC increase of 1.2 and 2.9 in garden soil and contaminated soil respectively, compared to the non amended treatment.

3.2. SPW physico-chemical characteristics

Table 3 shows the physico-chemical characteristics of SPW of the different treatments, collected at the beginning of the experiment.

The initial pH of the contaminated soil (P0%) was acidic (4.62), while the garden soil (G0%) and the PG0% mixture had a slightly alkaline pH of 7.99 and 8.12 respectively. Whatever the biochar concentration added to the garden soil or to the PG mixture, no significant pH change was observed. However, when P soil was amended by 2% or 5% biochar, pH increased significantly by 2.2 units and 2.9 units respectively, when compared to P0%.

For the contaminated soil P0%, EC was lower (285 $\mu s \cdot cm^{-1}$) compared to the garden soil G0% and to the PG0% mixture (912 $\mu s \cdot cm^{-1}$ and 1136 $\mu s \cdot cm^{-1}$ respectively). For the two biochar concentrations, garden soil EC increased approximately by a factor of 1.7 whereas for P soil, a 2% biochar amendment induced a twofold increase in EC and a 5% biochar amendment induced a threefold EC increase. When mixing Pontgibaud soil with the garden soil, no biochar amendment effect was observed on EC.

Without biochar, dissolved organic carbon (DOC) content in the garden soil (26.47 $mg \cdot L^{-1}$) was higher than in the contaminated soil and the mixture (10.58 $mg \cdot L^{-1}$ and 15.09 $mg \cdot L^{-1}$ respectively). In garden soil (G), when adding 2% or 5% biochar, DOC concentration was 1.8 times higher than in G0%. In contaminated soil P, for 2% and 5% biochar concentrations, DOC concentration decreased by 30% and 45% respectively, compared to P0%. For the P and G mixtures, no significant DOC concentration difference between the 3 levels of biochar was observed.

3.3. PTE concentrations in SPW

The SPW metal(loid) total dissolved concentrations (As and Pb) in the different tested soils are presented in Table 4.

At T0 and for the 3 different soils, the initial As concentrations in the SPW were rather low, <0.1 $mg \cdot L^{-1}$. No Pb was detected in garden SPW. The initial Pb concentration in P0% was relatively high (22.509 $mg \cdot L^{-1}$) and a 132-fold decrease in Pb concentration (0.171 $mg \cdot L^{-1}$) was observed when P was amended with G.

Table 3
Soil pore water (SPW) physico-chemical characteristics (pH, EC (μS.cm-1), DOC ($mg \cdot L^{-1}$)) determined at the beginning of the experiment in the 3 conditions, garden soil (G), contaminated soil (P) and the mixture of 50% garden soil and 50% contaminated soil (PG), amended with 0%, 2% or 5% of biochar. Letters indicate a significant difference ($p < 0.05$) ($n = 6$).

	pH	EC ($\mu S \cdot cm^{-1}$)	DOC ($mg \cdot L^{-1}$)
G0%	7.99 ± 0.09 a	912 ± 54 a	26.47 ± 3.20 a
G2%	8 ± 0.03 a	1474 ± 95 b	47.23 ± 7.19 b
G5%	8.08 ± 0.02 a	1567 ± 96 b	48.28 ± 6.43 b
P0%	4.62 ± 0.06 a	285 ± 44 a	10.58 ± 0.71 a
P2%	6.85 ± 0.14 b	600 ± 77 b	7.45 ± 0.49 b
P5%	7.51 ± 0.07 c	827 ± 64 b	5.73 ± 0.52 b
PG0%	8.12 ± 0.02 a	1136 ± 88 a	15.09 ± 1.80 a
PG2%	7.95 ± 0.21 a	984 ± 15 a	9.96 ± 0.81 a
PG5%	8.03 ± 0.06 a	1161 ± 85 a	14.97 ± 3.46 a

Table 4
Soil pore water (SPW) metal(loid)s concentrations (As and Pb) ($mg \cdot L^{-1}$) determined at the beginning (T0) and at the end (TF) of the experiment in the 3 conditions, garden soil (G), contaminated soil (P) and the mixture of 50% garden soil and 50% contaminated soil (PG), amended with 0%, 2% or 5% of biochar. Letters indicate a significant difference ($p < 0.05$) ($n = 6$).

[As]($mg \cdot L^{-1}$)					[Pb]($mg \cdot L^{-1}$)			
	T0	TF			T0	TF		
		S. alba	*S. viminalis*	*S. purpurea*		*S. alba*	*S. viminalis*	*S. purpurea*
G0%	0.091 ± 0.014 a	0.103 ± 0.032 a	0.162 ± 0.026 a	0.155 ± 0.038 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.101 ± 0.028 a	0.003 ± 0.003 a
G2%	0.077 ± 0.011 a	0.142 ± 0.037 a	0.071 ± 0.023 a	0.097 ± 0.031 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.052 ± 0.023 ab	0.00 ± 0.00 a
G5%	0.082 ± 0.011 a	0.158 ± 0.037 a	0.138 ± 0.032 a	0.081 ± 0.027 a	0.00 ± 0.00 a	0.195 ± 0.053 b	0.00 ± 0.00 b	0.00 ± 0.00 a
P0%	0 ± 0 a	0.176 ± 0.066 a	0.024 ± 0.012 a	0.144 ± 0.079 a	22.509 ± 0.730 a	1.815 ± 0.280 a	2.145 ± 0.302 a	2.850 ± 0.248 a
P2%	0.042 ± 0.017 a	0.182 ± 0.083 a	0.008 ± 0.005 a	0.053 ± 0.037 a	7.081 ± 0.036 b	2.541 ± 0.403 a	3.756 ± 0.537 a	2.879 ± 0.541 a
P5%	0.004 ± 0.002 a	0.155 ± 0.046 a	0.132 ± 0.060 a	0.144 ± 0.054 a	0.720 ± 0.036 c	1.972 ± 0.348 a	0.482 ± 0.081 b	1.658 ± 0.281 a
PG0%	0.015 ± 0.010 a	0.138 ± 0.057 a	0.569 ± 0.100 a	0.501 ± 0.023 a	0.171 ± 0.043 ab	0.098 ± 0.048 a	0.043 ± 0.016 a	0.287 ± 0.049 a
PG2%	0.056 ± 0.021 a	0.103 ± 0.050 a	0.222 ± 0.059 b	0.277 ± 0.082 ab	0.052 ± 0.014 a	0.108 ± 0.041 a	0.227 ± 0.051 a	0.229 ± 0.044 a
PG5%	0.090 ± 0.043 a	0.120 ± 0.042 a	0.199 ± 0.066 b	0.165 ± 0.052 b	0.147 ± 0.019 b	0.186 ± 0.042 a	0.162 ± 0.036 a	0.268 ± 0.082 a

Biochar addition had no effect on As concentrations in the SPW collected at the beginning of the experiment (T0) for the 3 conditions (garden soil, contaminated soil and mixture), whereas biochar addition at 2% or 5% induced a significant decrease in Pb concentration (68.5% and 96.8% respectively) in the contaminated soil. A 5% biochar amendment to Pontgibaud soil (P) induced a tenfold decrease in Pb SPW concentration when compared to a 2% biochar amendment. For G and PG, no significant biochar amendment effect was observed on lead SPW concentration.

PTE concentrations in SPW collected at the end of the experiment differed depending on the willow species. As concentrations in SPW collected from soils (G, P, PG) vegetated by *S. alba* were not affected by biochar addition. However, when growing *S. viminalis* on PG with 2% or 5% biochar, As concentration decreased by 63% when compared to PG0%. For *S. purpurea*, it was only when 5% biochar was added to PG that a significant decrease in As concentration of 67% compared to PG0% was observed. Finally, when growing *S. viminalis* on 5% biochar-amended G and P soil, Pb SPW concentrations decreased respectively to a non-detectable level and by 78%. When *S. alba* was cultivated on the garden soil amended with 5% biochar, a 0.195 $mg \cdot L^{-1}$ Pb concentration in SPW was observed whereas no Pb was detected in SPW when *S. alba* was cultivated on G0% and G2%. For all the remaining soils and mixtures tested, no biochar effect was observed.

3.4. Plant growth

The growth rates (cm/day) measured during the last 42 days of the experiment time course are shown in Table 5.

When growing the three *Salix* species (*S. alba*, *S. viminalis* and *S. purpurea*) on P0% soil, growth rate was 5.6, 4.4, and 4.3 times lower than on G0% soil, respectively. In the three soils, G0%, P0% and PG0%, *S. viminalis* exhibited systematically a faster growth compared to the other two species, between 33% and 50%.

Biochar addition (2% or 5%) in G and PG tested soil did not affect the growth rate of the three species. The application of 2% or 5% biochar to P soil, however, led systematically to a significant improvement in growth rate. With 5% biochar, the growth rates of *S. alba*, *S. viminalis* and *S. purpurea* were 6.3, 3.25 and 2.23 times higher than P0%, respectively.

3.5. Biomass production

The dry weight (DW), expressed in milligrams, of the different organs collected at the end of the experiment for the three *Salix* species is shown in Fig. 1.

As observed for the growth rate, the three willow species when growing on the non-amended contaminated soil (P0%) demonstrated a lesser DW than that measured on G0% and PG0%.

For the three *Salix* species and whatever the organ measured, when applying biochar at 2% or 5% on G soil or PG soil, no significant effect was observed. However, when added in the contaminated soil, biochar amendment at 2% and 5% had a positive effect on total plant DW. It should be noted that although *S. viminalis* produced about 190.15 mg of root DW on P0% after 63 days of treatment, no significant DW production was observed in *S. alba* and *S. purpurea* under the same conditions. For *S. viminalis* grown on P soil, root, stem and leaf DW also increased when 2% or 5% biochar were applied, *i.e.* by 3.3, 4.2 and 2.9 times, respectively. For *S. alba*, biochar did not affect stem DW whereas for leaves and roots, DW was positively affected as a function of the biochar concentration: with 5% biochar, leaf and root DW increased by 6.9 and 23.6 times, respectively. As observed for *S. alba* stems, *S. purpurea* stem DW was not affected by biochar amendment. When growing on 2% biochar, *S. purpurea* demonstrated an increase in root and leaf DW production of 9.8 and 4.2 times, when compared to P0%.

3.6. PTE concentration in plants

For the three species and for the 9 treatments, As (Fig.2) and Pb (Fig.3) concentrations were higher in the roots compared to the leaves and stems. Moreover, in roots, As concentration was not affected by biochar amendment.

3.6.1. Arsenic

For *S. alba, S. viminalis and S. purpurea,* biochar addition in G and PG did not affect As concentrations in leaves, stems or roots (Fig. 2a, b and c). Moreover, for *S. purpurea* grown on the P soil, whatever the *Salix* organ and the biochar concentration applied, no As variation was observed. For *S. alba* grown on the P soil, when 5% biochar was applied,

Table 5
Growth rates (cm/day) of the 3 willow species (*Salix alba*, *Salix viminalis*, *Salix purpurea*) exposed to the different soils, garden soil (G), contaminated soil (P) and the mixture of 50% garden soil and 50% contaminated soil (PG), amended with 0%, 2% or 5% of biochar. Different letters indicate a significant difference ($p < 0.05$) ($n = 6$).

	G0%	G2%	G5%	P0%	P2%	P5%	PG0%	PG2%	PG5%
Salix alba	0.56 ± 0.10 a	0.75 ± 0.04 a	0.58 ± 0.12 a	0.10 ± 0.04 a	0.41 ± 0.05 b	0.63 ± 0.08 b	0.42 ± 0.06 a	0.67 ± 0.09 a	0.54 ± 0.06 a
Salix viminalis	0.89 ± 0.11 a	0.69 ± 0.03 a	0.60 ± 0.04 a	0.20 ± 0.05 a	0.60 ± 0.07 b	0.65 ± 0.04 b	0.63 ± 0.05 a	0.72 ± 0.04 a	0.64 ± 0.05 a
Salix purpurea	0.56 ± 0.06 a	0.66 ± 0.05 a	0.60 ± 0.07 a	0.13 ± 0.04 a	0.38 ± 0.00 b	0.29 ± 0.06 ab	0.30 ± 0.11 a	0.33 ± 0.05 a	0.62 ± 0.07 a

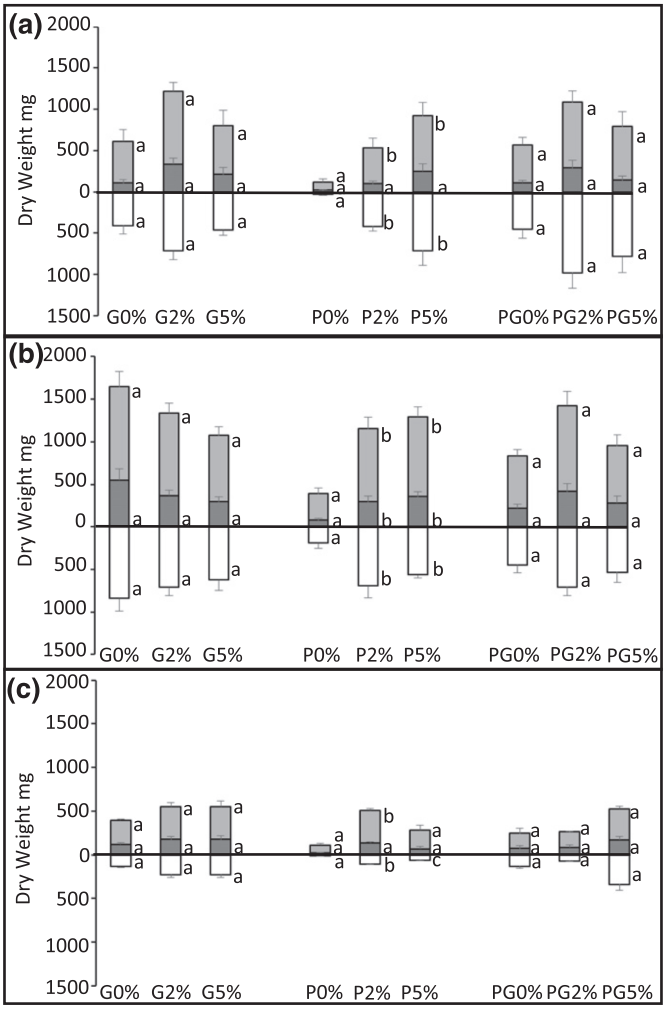

Fig. 1. Dry weight (mg) of the different organs (leaves, stems, roots) after 63 days of treatment: (a) *Salix alba*, (b) *Salix viminalis*, (c) *Salix purpurea*, exposed to the different soils, garden soil (G), contaminated soil (P) and the mixture of 50% garden soil and 50% contaminated soil (PG), amended with 0%, 2% or 5% of biochar. Letters on bar graphs indicate a significant difference ($p < 0.05$) ($n = 6$).

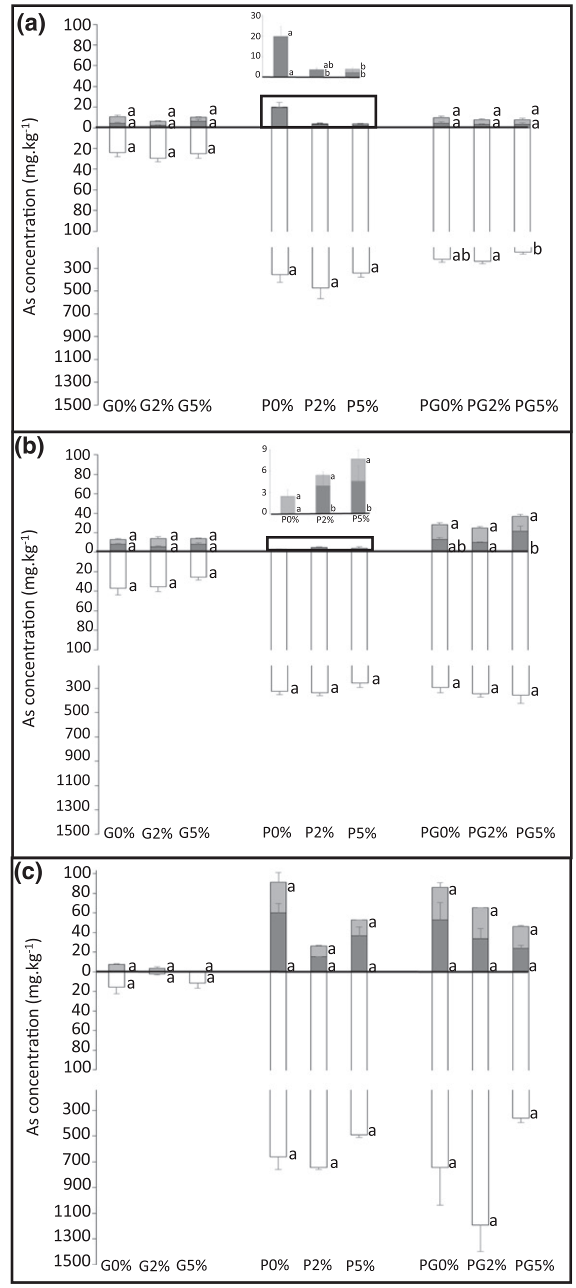

Fig. 2. Arsenic concentration ($mg \cdot kg^{-1}$) determined in the 3 organs (leaves, stem and roots) of (a) *Salix alba*, (b) *Salix viminalis* and (c) *Salix purpurea* after 63 days of experiment in the 3 conditions, garden soil (G), contaminated soil (P) and the mixture of 50% garden soil and 50% contaminated soil (PG), all amended with 0%, 2% or 5% of biochar. Letters indicate a significant difference ($p < 0.05$) ($n = 6$).

As concentration decreased significantly by 88% in stems but increased 41-fold in leaves, respectively (Fig. 2a). For *S. viminalis* in the P condition, the addition of 2% or 5% biochar induced an increase in As concentration in stems, while no effect was observed in leaves (Fig. 2b).

3.6.2. Lead

When grown on garden soil, no Pb was measured in the upper parts of the three *Salix* species studied, whatever the biochar concentration used (Fig. 3a, b, c). A significant 1.8-fold Pb decrease was observed in the roots of *S. viminalis* only when applying 5% biochar to G soil (Fig. 3b). On P soil, when biochar was applied, no significant difference in root Pb concentration was observed in *S. purpurea*, while for *S. alba* and for *S. viminalis*, a significant root Pb decrease of 57% and 70% respectively was observed, mainly for P5%. In stems, even though *S. alba* presented a Pb concentration of 64.7 $mg \cdot kg^{-1}$ when grown on P0%, no biochar effect was observed. For *S. viminalis* in the same conditions, stem Pb concentration for P0% was approximately 3 times higher than in *S. alba*, at 218 $mg \cdot kg^{-1}$. No biochar effect was observed. Finally, for *S. purpurea*, the biochar amendment induced a significant increase in Pb stem concentration. For P5%, Pb stem concentration was 5 times higher than P0%, rising to 428 $mg \cdot kg^{-1}$. In *S. purpurea* leaves under P conditions, Pb concentration was approximately 65 $mg \cdot kg^{-1}$ and was not affected by biochar addition, whereas when grown on Pontgibaud soil amended with 5% biochar, Pb leaf concentration increased in *S. alba* and decreased in *S. viminalis* to 44.5 and 35.7 $mg \cdot kg^{-1}$, respectively. When adding garden soil and biochar to P, we observed a significant 30% Pb root concentration decrease for PG5% only for *S. alba*, which reached 2322 $mg \cdot kg^{-1}$. For *S. viminalis* and *S. purpurea*, whatever the PG conditions tested, no significant Pb root concentration variations were noticed. Similarly, for *S. alba*, *S. viminalis* and *S. purpurea*, no variations in stem and leaf Pb concentrations were observed when biochar was added to the PG soil.

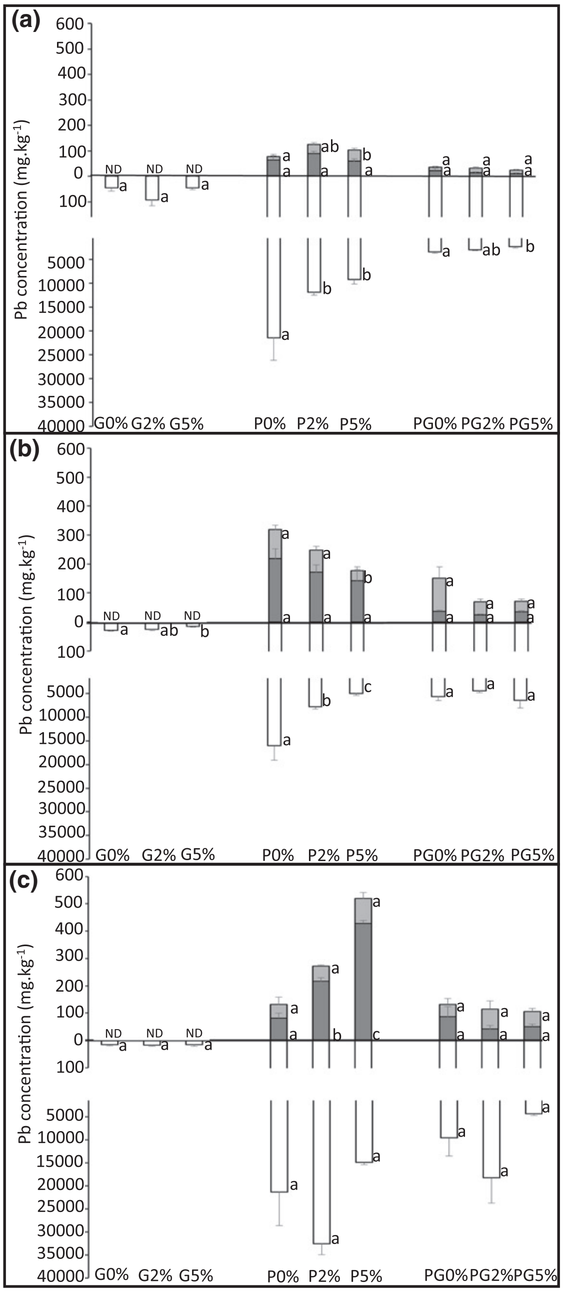

Fig. 3. Lead concentration (mg·kg^{-1}) determined in the 3 organs (leaves, stem and roots) of (a) *Salix alba*, (b) *Salix viminalis* and (c) *Salix purpurea* after 63 days of experiment in the 3 conditions, garden soil (G), contaminated soil (P) and the mixture of 50% garden soil and 50% contaminated soil (PG), all amended with 0%, 2% or 5% of biochar. Letters indicate a significant difference ($p < 0.05$) ($n = 6$). ND = non detectable level.

4. Discussion

4.1. SPW and technosol physico-chemical characteristics

When incorporating biochar to the contaminated soil (P, Pontgibaud technosol), a significant effect on pH, EC and DOC was observed. Biochar added at 2% or 5% to P increased SPW pH by 2.23 and 3.09 units, respectively, which is consistent with other studies (Forjàn et al., 2016; Molnàr et al., 2016; Beesley et al., 2014). Jones et al. (2016) found that an increase in pH of a French Cu contaminated site (loamy sand) correlated with biochar applications from 1% to 3%. In 2013, Chintala et al. observed an increase in pH and EC after application of biochar at 2, 4 and 6% on an acidic soil collected from a cultivated Entisol. This pH increase can be explained by 2 mechanisms: (i) the biochar alkaline pH induces a liming effect that increases the soil pH (Bian et al., 2014); (ii) biochar incorporation to soil releases cations and soil solution acidity is reduced by proton consumption reactions in the soil (Chintala et al., 2013). However, we did not observe any biochar effect on the pH of the SPW collected from the garden soil (G) or the mixture of P and G. These results can be attributed to the fact that both G and PG SPW pH were almost equal (around pH 8) and comparable to biochar pH (8.2).

The EC of P was very low, about 285 μs·cm^{-1} and was significantly increased by biochar amendment. To a lesser extent, the same results were observed for G. Molnàr et al. (2016) described a 24% EC increase when 0.1% grain husk and paper fibre sludge biochar was added to a sandy agricultural soil in Hungary.

SPW DOC concentration decreased for the P biochar amended soil as a function of biochar concentration. At least two mechanisms have been suggested to explain a DOC decrease after biochar application (Lu et al., 2014; Kloss et al., 2014; Jain et al., 2014): (i) due to its structure, biochar addition increases the number of soil organic matter sorption sites (Hass et al., 2012); (ii) biochar improves microbial activity. In fact, Jokinen et al. (2006) showed that an increase in pH led to an increase in microbial activity, and hence an increase in organic carbon degradation by microbiota (Hass et al., 2012).

4.2. PTE concentrations in the SPW

Before *Salix* plantation, for all treatments (G, P and PG), biochar application had no effect on SPW As concentration. At the end of the experiment time course, only PG soils amended with biochar and vegetated with *S. viminalis* demonstrated a SPW As concentration decrease. This was probably induced by an As soil or biochar immobilization associated to the properties of *S. viminalis* root exudates. This hypothesis will be tested by measuring the *S. viminalis* root exudates. Interestingly, when comparing SPW As concentration between T0 and TF, an increase was observed in all conditions tested, except for *S. viminalis* on P2%. This is not in favor of the use of biochar as a soil As stabilizer.

In the garden soil, at T0 and whatever the quantity of biochar amendment applied, we did not detect any Pb in SPW. In P soil, the lead SPW concentration was 22.5 mg·L^{-1}, which is 2000 times the European directive for human health (98/83/EC) (10 μg·L^{-1}). However, a beneficial biochar effect was observed for P soil, since lead SPW concentrations decreased by 68% and 96% when biochar was added at 2% or 5%, respectively. A few studies have described a similar positive effect on SPW Pb concentration when biochar was incorporated to a contaminated soil: Bian et al. (2014) applied a wheat straw biochar on a Pb hydroagric stagnic anthosol at three different rates (10, 20 and 40 t·ha^{-1}), while Houben et al. (2013) amended a natural reserve contaminated by Cd, Zn and Pb with 1%, 5% and 10% miscanthus straw biochar. The observed decrease was attributed to specific adsorbent or physico-chemical biochar properties, mainly due to the presence of oxygen functional groups on biochar surfaces. Uchimiya et al. (2011) showed that biochars containing high oxygen functional groups stabilize PTEs more efficiently, especially when applied to an acidic, low CEC and low organic carbon soil. At the end of our experiment (day 63), SPW Pb concentration declined compared to T0 in all tested soils and varied depending on the *Salix* species. For P0% soil, SPW Pb concentration was 12, 10 and 8 times lower, for *S. alba*, *S. viminalis* and *S. purpurea*, respectively, when compared to T0. This decrease can be attributed to a Pb uptake by plants and/or a specific root exudate effect, since root exudates affect acidification, chelation, precipitation and redox reactions, thus affecting the bioavailability of metal(loid)s (Kidd et al., 2009). However, we observed a specific SPW Pb concentration increase in 2 conditions: *S. alba* and *S. purpurea* when grown in the P5% condition. This could be explained by a specific root exudates production having a specific Pb mobilizer effect, which could favor Pb soil desorption favored by biochar addition (Kidd et al., 2009), since studies have shown that root exudates may differ among species (Kidd et al., 2009) and among amendments used (Mitton et al., 2012).

In conclusion, the variations in SPW As and Pb concentrations are linked to the *Salix* species used and to the combined contribution of soil composition and the type and rate of amendments applied. Finally, in the case of As and Pb soil co-contamination, the beneficial environmental effect produced by biochar, which induces a huge Pb SPW decrease, could be masked by a higher arsenic SPW availability.

4.3. Plant growth indicators

Biochar did not demonstrate a significant beneficial effect on plant growth rate when added to G or PG, whereas in P soil, the growth rate was enhanced by biochar application. This positive effect on plant growth was also observed by Carter et al. (2013), who found an improvement in lettuce and cabbage stem length after rice-husk biochar addition at 50 $g \cdot kg^{-1}$ on a sandy soil.

Similarly we demonstrated a positive biochar effect on the dry weight of the three *Salix* species studied when grown on P soil amended with biochar. Our findings are in accordance with the results of Gregory et al. (2014), who described a better *Lolium perenne* shoot dry weight production when a woody biochar was applied at 2% on an As contaminated site compared to a non-amended soil. Puga et al. (2015) also demonstrated a beneficial effect of biochar on plant dry weight when up to 5% sugar cane straw biochar was applied on a former zinc mining area.

At least two mechanisms can be proposed to explain this plant growth improvement induced by biochar addition in PTE contaminated soil.

Firstly, it is well known that biochar application improves soil in two ways: i) biochar application by itself adds nutrients to soil, enhances nutrient availability, increases soil pH and consequently induces a higher EC (Smider and Singh, 2014); ii) biochar addition improves water holding capacity (Agegnehu et al., 2015) and increases the SPW phosphorous (Puga et al., 2015), total nitrogen and major cation concentrations (Hossain et al., 2010).

Secondly, biochar diminishes metal(loid) availability, as shown in several studies (Al-Wabel et al., 2015; Bian et al., 2014). In fact, biochar can complex metal ions on its surface, thereby reducing their bioavailability (Beesley et al., 2011; Houben et al., 2013).

In the present study, in the case of the Pontgibaud polluted area (P), the soil characteristics were upgraded when amended with 2% biochar. Moreover, a 5% biochar amendment did not efficiently improve *Salix* growth and dry weight compared to a 2% biochar amendment. The three species can be ranked by growth rate and dry weight production as follows: *Salix viminalis* > *Salix alba* > *Salix purpurea*.

4.4. PTE concentration in the three willow species organs

As was mainly located in roots and biochar application did not affect its concentration whatever the biochar rate used. Among the three species tested, *S. purpurea* exhibited the highest root arsenic concentration, but also the highest As concentrations in the upper parts, whereas *S. alba* had the lowest plant As concentration whatever the soil or biochar concentrations tested, with an As leaf concentration of <2 $mg \cdot kg^{-1}$ in the P condition.

Compared to As, *Salix* Pb organ concentrations were systematically higher. As for arsenic, lead was mainly located in roots and *S. purpurea* demonstrated the highest Pb concentrations. *S. alba* had the lowest lead aerial parts concentrations (<150 $mg \cdot kg^{-1}$), while *S. viminalis* and *S. purpurea* translocated larger amounts of Pb to the upper parts.

The preferential location of As and Pb in roots has been pointed out in several studies. For instance, Zhivotovsky et al. (2010) observed in a hydroponic culture a higher lead concentration in roots (4164 to 14,146 $mg \cdot kg^{-1}$) than in woody tissues (71.9 to 403.5 $mg \cdot kg^{-1}$), when applying Pb concentrations from 48 to 241 μM. On a biochar amended arsenic polluted soil, Beesley et al. (2014) also found a higher accumulation in tomato roots. Tlustoš et al. (2007) concluded similarly for different *Salix* clones grown on three different multi-contaminated soils (As, Cd, Zn, Pb). Vamerali et al. (2009) tested several *Populus* and *Salix* species on a metal (Co, Cu, Pb, Zn) and As contaminated waste and found that the PTE concentrations were higher in the roots than in aboveground tissues. For instance, in *S. alba* leaves, concentrations of 5.0 $mg \cdot kg^{-1}$ As and 7.7 $mg \cdot kg^{-1}$ Pb, while in fine roots 85.9 $mg \cdot kg^{-1}$ As and 853.3 $mg \cdot kg^{-1}$ Pb were found. This confinement to the roots could make it possible to avoid PTE toxicity (Gupta et al., 2013). It has been proposed that the exclusion mechanisms of willows can protect the plant photosynthesis apparatus (Borišev et al.,2009).

In our study, unlike *S. viminalis* and *S. purpurea*, *S. alba* did not allow PTE aerial parts invasion, thus reducing the pollutant return to soil through biomass (stems and leaves) shedding. *S. alba* is therefore a good candidate for biomass production on contaminated areas by short rotation coppice.

Our results show that biochar added as soil amendment to Pontgibaud technosol improves soil fertility by increasing pH and EC, but also by reducing lead mobility. This improvement in soil properties induces a better willow plant growth. The metal(loid)s present in the soil (arsenic and lead) tend to be stabilized onto the root system and are not extracted and translocated towards upper parts during plant growth. *S. alba* seems to be an efficient species to stabilize soil Pb when assisted by soil biochar amendment. However, a long-term field study needs to be done to confirm these findings.

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