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# Capability of amendments (biochar, compost and garden soil) added to a mining technosol contaminated by Pb and As to allow poplar seed (*Populus nigra* L.) germination

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**Abstract** The germination capacity of poplar seeds has never been studied in the context of metal(loid)-contaminated soils, even though poplars are present over a vast geographical area. In this study, black poplar seeds from the Loire Valley (France) were grown for 28 days in mesocosm on a heavily polluted soil that was subjected to different amendments. This phytomanagement process aimed to allow the revegetation of an As and Pb-contaminated mining soil by adding appropriate amendments, resulting in metal(loid) soil stabilisation and efficient plant growth. The objectives were to evaluate the effect of three amendments (garden soil, compost

and biochar) when added alone or combined to a technosol on (i) the soil physicochemical properties, (ii) the mobility of As and Pb in the soil pore water (SPW), (iii) the capacity of poplar seeds to germinate and to grow and (iv) the metal(loid) distribution within the plant organs. The addition of amendments alone or combined allowed a 90% decrease in SPW Pb concentrations, while the arsenic concentrations were between 18 and 416 times higher. However, we were only able to obtain seed germination and plant growth on amended soils. These promising results will allow us to explore the use of such amendments in rehabilitating areas that are sources of significant metal(loid) dissemination, as well as allowing a natural plant recolonisation of these sites by seeds from the surrounding environment.

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Biochar · Metal(loid)s · Seeds · *Populus nigra* · As · Pb

## Introduction

Out of all the inorganic soil pollutants, metal(loid)s are the main cause of soil contamination on a global level. In the European Union, metal(loid) contamination accounts for more than 37% of the polluted areas, followed by mineral oils (33.7%) and polycyclic aromatic hydrocarbons (PAHs, 13.3%) (EEA 2007). Localised contaminated soils, known as brownfields (French et al. 2006), are frequently associated to abandoned industrial areas or to urban soils (EEA-UNEP 2000). Mining activities are the main sources of environmental

contamination as they release large amounts of metallic and metalloid elements through erosion caused by runoff or wind (Razo et al. 2004).

Many industrialised countries are now focusing on using regulations to reduce the impact of pollution. For example, the European Pollutant Release and Transfer Register (Regulation 166 EC 2006) lists potentially hazardous activities and pollutants and establishes the thresholds for releases to air, water and soil for all major contaminants (Vamerali et al. 2009). Most of the remediation methods developed for metallised soils are physical or chemical approaches, such as soil washing, excavation and rewetting (Wang et al. 2017). Phytoremediation uses plants to absorb or to stabilise metals in the roots and in the associated rhizosphere and is a low-cost technique carried out directly on the contaminated spot (Salt et al. 1998; Vamerali et al. 2009). Phytoremediation could help maintain or even improve soil structure (Mleczek et al. 2010). Vamerali et al. (2009) and Ali et al. (2013) defined this strategy as an alternative method which is positively perceived by the population due to its significantly reducing metal(loid) availability without using chemical processes. It also creates a favourable environment around the root system by providing organic and mineral nutrients, resulting in a decrease in the amount of pollutants released into the environment through erosion. Phytoremediation is a general term comprising different techniques, with the two major methods being phytoextraction and phytostabilisation.

Phytostabilisation is based on root characteristics such as biomass, exchange surface, exudates production and the associated microorganism activities. Root metal(loid) accumulation is also indirectly linked to plant transpiration, which could allow greater ion absorption. Finally, when fixed in the root and the rhizosphere, metal(loid) leaching is then controlled (Sarwar et al. 2017). Many abandoned or post-operative mining dams are sterile or have minimal natural vegetative colonisation due to limited soil physicochemical characteristics such as acidic or alkaline pH, high ion content, lack of required nutrients, metal toxicity, high bulk density, lack of structure, low water retention capacity and, sometimes, low air permeability (Lebrun et al. 2019). To overcome these limitations, amendments can be added, which can be considered as an aided phytostabilisation (Alvarenga et al. 2009a, b; Galende et al. 2014). An efficient way of establishing an efficient phytostabilisation may therefore involve the addition of organic matter to soil, which will improve its

structure and fertility, as well as its ability to retain water or create niches favourable to the development of microorganisms (Pérez-Esteban et al. 2014; Cha et al. 2016; Lebrun et al. 2019).

The selection of suitable plant species is crucial for a successful phytostabilisation. Such plants must be able to develop both an extensive root system and a large amount of biomass in the presence of metal(loid)s while mainly compartmentalising metal(loid)s in the root zone and keeping their translocation from the roots to the shoots as low as possible. This will limit their spread to the food chain or their return to the ground when the leaves fall (Henry et al. 2013).

Several hundred different plant species could be used in a phytoremediation process (Moosavi and Seghatoleslami 2013). But, many of these plants are small (small leaf area and small root system) and would not be efficient, due to their evaporating limited amounts of water, and their roots being unable to explore a large volume of soil. To overcome these disadvantages, woody species with rapid growth, high biomass production and high metal(loid) storage capacity within a deep root system are attractive options. Among the woody species, willow and poplar have already been proposed as phytoremediating plants for metal-contaminated soils (Marmiroli et al. 2011). Poplar, in particular, has several interesting features rendering it a good candidate for phytoremediation (Radojčić Redovniković et al. 2017). For instance, *Populus nigra*, along with a few other poplar species, are recognised for both their root water purification abilities, which improve soil water quality. They are known, in particular, for their highly developed root system, which acts as a natural filter by trapping organic and inorganic pollutants (Chamaillard 2012; Houda et al. 2016; Radojčić Redovniković et al. 2017) and allows a good cohesion and fixation of soil materials (Foussadier 2003; Rodrigues et al. 2007). Poplar have a large geographical distribution zone from Western Europe to the western tip of China through a narrow North African fringe (Euforgen 2015), demonstrating a large adaptability to different soils and climates. In addition, its capacity to be used in short rotation coppices allows its use in biomass production. Baldantoni et al. (2014) showed that two poplar clones (*Populus alba* AL22 and *P. nigra* N12) cultivated on a soil collected from an urban and industrial area have a

remarkable ability to absorb and accumulate Cd and Zn and to phytostabilise Cu, Fe and Pb.

Among the available amendments used in phytomanagement, biochar obtained mainly by carbon-rich biomass pyrolysis has attracted attention in recent years due to its beneficial effects on soils by increasing electrical conductivity, soil pore water (SPW) pH, microorganism activities (Beesley et al. 2011) and heavy metal sorption (Beesley et al. 2014; Lebrun et al. 2017). However, the integration of biochar into the soil may have some disadvantages such as (i) SPW carbon release, which could increase arsenic mobility, and (ii) cationic nutrient immobilisation. When these two phenomena occur, they interact negatively with plant growth, especially if soil nutrient capacities are already low. It therefore seems necessary to combine biochar with other amendments such as compost, allowing the addition of micronutrients (Beesley et al. 2011). Various studies discuss using combinations of amendments (Bart et al. 2016; Lebrun et al. 2017), in which various types of organic amendments (garden soil and biochar) have been tested alone or associated in order to ameliorate plant growth and decrease SPW Pb concentration. Many other articles also reported the use of several types of organic amendments, such as compost and/or biochar (Beesley et al. 2014; Pérez-Esteban et al. 2014; Yin et al. 2016; Pardo et al. 2017).

Lastly, all previous experiments using poplar as phytoremediator plants have been undertaken using cuttings, which is an efficient way of producing monoclonal short rotation coppices (Ruttens et al. 2011; Van Slycken et al. 2015; Janssen et al. 2015). To our knowledge, no experiments have been conducted on poplar trees originating from seed germination with the aim of screening potential efficient phytoremediator genotypes and vegetating and remediating soils contaminated by metal(loid)s. We hypothesise that by planting poplar seeds instead of cuttings in an open field, we will (1) observe spontaneous poplar recolonisation on polluted sites by adding amendments when necessary and (2) perpetuate spontaneous plant propagation by improving the agronomic capacity of technosols while maintaining the biochar's ability to immobilise metal(loid)s.

The aims of our study were (i) to evaluate, in mesocosm, the germination capacity of black poplar seeds (*Populus nigra* L.) on a mining technosol, with and without the presence of amendments (biochar, compost or garden soil), and (ii) to determine the best combination of amendments (garden soil, compost and

biochar). For this purpose, six different conditions were studied: mining technosol (P), mining technosol and hardwood-derived biochar (P + B), mining technosol and garden soil (P + G), mining technosol and compost (P + C) and combinations of different amendments: (P + G + B) and (P + C + B). The soil pore water physicochemical properties (pH, electrical conductivity (EC), As and Pb) were measured in tested soils. Germination rate, plant growth and metal(loid)s accumulated into plant organs were determined.

## Materials and methods

### Soil sampling

The studied site in Roure-les-Rosiers corresponds to a former silver-lead extraction mine and is located in the Massif Central near Clermont-Ferrand (Saint-Pierre-le-Chastel (63, France)), in the Pontgibaud mining district (P). Two streams cross the site: the Veyssière, which constitutes the southern edge of the deposits, and the Faye, which borders the northwest of the deposits. The location of the study area in Lambert II coordinates is as follows:  $X = 638,147.54$  and  $Y = 2,087,962.79$ . The old mining site has a semi-continental climate, with hot summers, which can be marked by severe and localised thunderstorms and snowy winters. On average, the Roure-les-Rosiers sector receives about 770 mm of rainfall per year and is located at around 700 m above sea level. The area consists mainly of deposits of silver-bearing lead ore that still contain toxic elements (mainly lead and arsenic). Surface soils (0–20 cm) were sampled with a stainless steel shovel. The soil samples collected were dried in open air, screened using a 2-mm mesh sieve to remove crude plant material (Qasim and Motelica-Heino 2014) and homogenised manually. One fraction was used to determine the physicochemical soil properties, another one was used for the metal(loid) analysis and the third fraction was used to conduct germination tests.

### Soil physicochemical analysis

The As and Pb concentrations were determined using X-ray fluorescence (XRF) (DELTA Portable XRF Analyzer Model; Olympus, Hannover, Germany). The signal provided by the pXRF was previously calibrated using a curve obtained after total dissolution in fluorhydric acid

and inductively coupled plasma (ICP)-MS quantification of the original contaminated soil. The pH and EC were determined according to AFNOR NF T01-013, using a combined pH EC meter (ProfiLine 1970i; WTW, Germany), calibrated using pH 4.0 and pH 7.0 standards and a KCl standard solution with an electrical conductivity of  $1430 \mu\text{S cm}^{-1}$ . Water holding capacity (WHC) was determined according to Lebrun et al. (2017).

### Soil preparation

Three different organic amendments were added to Pontgibaud technosol, either individually or in combination: (i) a garden soil collected from the University of Orléans, Parc de la Source, France; (ii) a compost, which was a commercial product mix of potting soil and horse manure; and (iii) a biochar, made from tree wafer and chip feedstock (oak, beech and hornbeam) pyrolysed at  $500 \text{ }^\circ\text{C}$  (La Carbonerie, Crissey, France). The main physico-chemical properties of the various amendments are presented in Table 1. All amendments as well as Pontgibaud contaminated soil were homogenised and sieved to 2 mm. The mixtures tested corresponded to Pontgibaud contaminated soil (P), P amended with 10% garden soil (PG), P amended with 10% compost (PC), P amended with 2% hardwood-derived biochar (PB) and combinations of different amendments: PB + 10% garden soil (PBG) and PB + 10% compost (PBC). For each tested treatment, 300 g of soil were distributed individually in 400-mL pots, and 6 pots were prepared per treatment. The pots were then placed outside for 30 days for soil equilibration, at which point the poplar seeds were sown (T0) on the different soils tested. The growth of the young seedlings was measured after 28 days.

### Soil pore water metal(loid) concentrations

To evaluate the effects of amendments on soil As and Pb leachability, soil pore waters were collected at day 0 and at day 28, using Rhizon samplers (Rhizosphere Research Product, Wageningen, The Netherlands). The Rhizon samplers were inserted transversely over the height of the pots. Samples were collected in plastic tubes, after conductivity and pH measurements according to Bart et al. (2016). The samples were then acidified

with 1.6% (v/v) of 65%  $\text{HNO}_3$  and stored at  $4 \text{ }^\circ\text{C}$ . Total dissolved metal(loid) concentrations (As, Pb) were determined by ICP atomic emission spectroscopy (AES) (ULTIMA 2, HORIBA; Labcompare, San Francisco, USA).

### Seed germination

At day 0, twelve *Populus nigra* seeds were sown per pot and allowed to germinate and grow for 28 days. Pots were placed in a controlled chamber under a 16-h light regime at  $23 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ . The soil WHC was kept between 55% and 65% using deionised water. The germination rate was determined by the number of seedlings that had developed leaf and root systems at the end of the experiment (day 28).

### Plant analysis, dry weight and metal(loid) concentrations

The seedlings were harvested 28 days after sowing. They were rinsed thoroughly with double deionised water, then divided into roots, stem and leaves before being dried at  $40 \text{ }^\circ\text{C}$ , for 72 h, at which point their dry weight was measured. Three replicates per treatment and per organ were digested in a Regal water solution using a pressurised vacuum microwave system (Multiwave 3000; Anton Paar GmbH, Germany). The digestion program consisted of a gradual increase of temperature for 15 min up to  $180 \text{ }^\circ\text{C}$ , followed by a digestion step of 15 min at  $180 \text{ }^\circ\text{C}$ , then a cooling step to  $55 \text{ }^\circ\text{C}$  over 15 min. Once cooled to room temperature, the samples were diluted in 30 mL of ultra-pure water ( $18 \text{ M}\Omega \text{ cm}$ ) and then filtered on a  $0.45\text{-}\mu\text{m}$  filter nitrocellulose. Samples were measured three times using ICP-AES from plant digestions to determine the concentrations of As and Pb in different organs of *Populus nigra*.

### Statistical analysis

As described by Lebrun et al. (2017), R statistical software (version 3.1.2) was used to analyse data. Parametric data was analysed using ANOVA and Tukey HSD tests, and non-parametric data were compared with the Kruskal-Wallis test per pair.

**Table 1** Amendments of physicochemical properties

Granulometry (mm)	Compost (ND)	Biochar (0.2–0.4)	Garden soil (<2)
pH	7.4 ± 0.02	8.98 ± 0.02	6.5 ± 0.01
EC (µS cm <sup>-1</sup> )	801 ± 24	432 ± 2	253 ± 2
WHC (%)	51 ± 1	183 ± 3	31 ± 2
Specific area (m <sup>2</sup> g <sup>-1</sup> )	ND	43.91	ND
Total pore volume (cm <sup>3</sup> g <sup>-1</sup> )	ND	0.0364	ND
Mean pore diameter (nm)	ND	3.32	ND

EC electrical conductivity (µS cm<sup>-1</sup>), WHC water holding capacity (% mass), ND no data

## Results

### Effects of amendments on seed germination and soil and SPW properties

The germination rate on the control medium was obtained by sowing the seeds on a substrate composed of only sand. The germination rate for this case was 92%. We showed that Pontgibaud contaminated soil (P) was unsuitable for spontaneous seed germination (Fig. 1). All the amendments allowed the germination of black poplar seeds without any real difference between treatments. Germination rates were between 43 and 69%. The pH of the P soil used in this study was  $4.55 \pm 0.06$ , its electrical conductivity was low ( $54 \mu\text{S cm}^{-1} \pm 2 \mu\text{S cm}^{-1}$ ) and metal(loid) concentrations were  $728 \text{ mg kg}^{-1} \pm 26 \text{ mg kg}^{-1}$  for As and  $9944 \text{ mg kg}^{-1} \pm 227 \text{ mg kg}^{-1}$  for Pb. For the WHC (Table 2), the Pontgibaud soil plus garden soil (PG) treatment showed no improvement in WHC, whereas the other treatments improved soil water retention by 5%, 6%, 8% and 10% for PBG, PB, PC and PBC, respectively.

SPW pH and EC increased significantly ( $p < 0.05$ ) after the addition of amendments (Table 2), but no significant differences were observed between T0 and T28. The pH of Pontgibaud SPW was 4.8, whereas for the amended soils, the pH was between 7.24 (PC) and 7.84 (PBC). EC was also positively affected by the addition of amendments; its value was multiplied by at least 2.3-fold for PBC, whereas when biochar was added alone, the EC value was three times higher than that in P ( $\sim 600 \mu\text{S cm}^{-1}$ ).

The various amendments induced a rise in SPW As concentrations for all cases (Table 2). The PC ( $4.161 \pm 0.556$  at T0) treatment showed the largest increase, with a value 400 times higher than that in P ( $0.010 \pm 0.007$  at T0). For all the other treatments, the increase was

smaller but significant ( $p < 0.05$ ), with values ranging between 1.8 and 50 times for PG and PBC, respectively. In contrast, SPW Pb concentration was decreased by adding amendments. In non-amended soil (P), SPW Pb concentration was  $12.3 \text{ mg L}^{-1}$ , whereas for all amended treatments, SPW Pb concentration was at least by 93% lower. No significant differences were found between T0 and T28 ( $p < 0.05$ ).

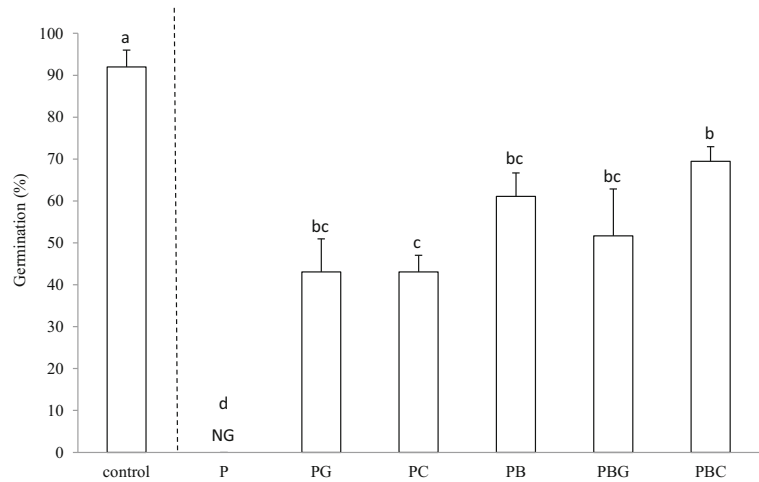
### Seedlings' dry weight and metal(loid) uptake

Measurements for the dry biomass of the different seedling organs (Fig. 2) showed that the highest biomass overall was found for the PC treatment. The highest root biomass was observed on PG and PC modalities, with 1.31 mg and 1.26 mg, respectively. Root biomasses for PB, PBG and PBC treatments were measured with values of 0.63 mg, 0.58 mg and 0.66 mg, respectively. Stem biomass was measured for all modalities; PB and PBG treatments showed values of 1.76 mg and 1.89 mg, respectively, while PG and PBC treatments showed intermediate values of 2.8 mg; the highest value was for the PC treatment with 3.6 mg. The highest foliar biomass was also obtained for plants grown on PC with 14.88 mg, with the lowest on PB with 6.46 mg. The remaining values for foliar biomass corresponded to 10.46 mg, 7.04 mg and 7.83 mg for PG, PBG and PBC, respectively.

For the five treatments allowing germination, As (Fig. 3) and Pb (Fig. 4) concentrations were higher in the root parts than in the aerial parts (leaves and stems) of the poplar, except for the Pb concentration in the stem of the PBC treatment, which was twice that of the roots. Moreover, in terms of aerial organs, the stem had higher As and Pb concentrations than leaves. Arsenic concentrations in the leaves were not significantly different between PG, PC, PB and PBC



**Fig. 1** Germination (%) of *Populus nigra* seeds at 28 days under 7 different treatments: control, polluted soil (P), P amended with 10% garden soil (PG), P amended with 10% compost (PC), P amended with 2% hardwood-derived biochar (PB) and a combination of different amendments: PB + 10% garden soil (PBG) and PB + 10% compost (PBC) (mean  $n = 6$ ;  $\pm$ SE). NG non-germinated. Letters on bar graphs indicate a significant difference ( $p < 0.05$ )



modalities, with values of  $0.035 \text{ mg g}^{-1}$ ,  $0.026 \text{ mg g}^{-1}$ ,  $0.043 \text{ mg g}^{-1}$  and  $0.020 \text{ mg g}^{-1}$ , respectively. The lowest value was obtained when P soil was amended with biochar and garden soil (PBG) and corresponded to  $0.002 \text{ mg g}^{-1}$ . In the stems, arsenic concentrations for PG, PC and PB treatments were not significantly different, and their values were  $0.12 \text{ mg g}^{-1}$ ,  $0.06 \text{ mg g}^{-1}$  and  $0.08 \text{ mg g}^{-1}$ , respectively. The lowest value was  $0.01 \text{ mg g}^{-1}$  and was observed for the PBG treatment, whereas the PBC treatment had the highest As concentration value ( $1.33 \text{ mg g}^{-1}$ ). For the root parts, the highest As concentration was calculated for the PC treatment with a value of  $4.10 \text{ mg g}^{-1}$ , and the other treatments had an arsenic concentration of  $2.60 \text{ mg g}^{-1}$ ,

$2.47 \text{ mg g}^{-1}$ ,  $1.80 \text{ mg g}^{-1}$  and  $0.99 \text{ mg g}^{-1}$  for PG, PBC, PB and PBG, respectively (Fig. 3).

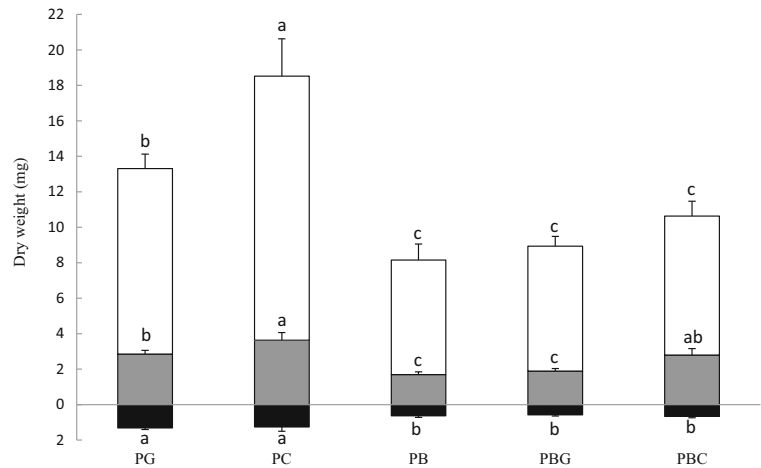
Pb concentration in PG leaves was  $0.55 \text{ mg g}^{-1}$  whereas for PBC, PB, PBG and PC, the concentrations were  $0.39 \text{ mg g}^{-1}$ ,  $0.22 \text{ mg g}^{-1}$ ,  $0.22 \text{ mg g}^{-1}$  and  $0.11 \text{ mg g}^{-1}$ , respectively. The PBC treatment demonstrated the highest Pb concentration in the stems and corresponded to  $6.50 \text{ mg g}^{-1}$ , whereas for PB, PBG, PG and PC, lead concentrations were  $1.09 \text{ mg g}^{-1}$ ,  $0.92 \text{ mg g}^{-1}$ ,  $0.60 \text{ mg g}^{-1}$  and  $0.41 \text{ mg g}^{-1}$ , respectively. For the root parts, the highest concentrations of Pb were obtained for PG and PB treatments and corresponded to  $9.11 \text{ mg g}^{-1}$  and  $8.46 \text{ mg g}^{-1}$ , respectively, while for PBG, PC and PBC, lead concentrations were  $5.98 \text{ mg g}^{-1}$ ,  $4.11 \text{ mg g}^{-1}$  and  $3.55 \text{ mg g}^{-1}$ , respectively (Fig. 4).

**Table 2** Technosol water holding capacity (WHC) and SPW physicochemical characteristics (pH, electrical conductivity (EC) and As and Pb concentration) at T0 (date of sowing poplar seeds) and at T28 (28 days) under 6 different treatments

	Soil WHC (%)	SPW							
		pH		EC ( $\mu\text{S cm}^{-1}$ )		[As] ( $\text{mg L}^{-1}$ )		[Pb] ( $\text{mg L}^{-1}$ )	
		T0	T28	T0	T28	T0	T28	T0	T28
P	$34 \pm 0.3$ a	$4.80 \pm 0.18$ a	$4.87 \pm 0.11$ a	$599 \pm 11$ a	$607 \pm 2$ a	$0.01 \pm 0.007$ a	$0.01 \pm 0.003$ a	$12.307 \pm 1.156$ a	$12.404 \pm 1.003$ a
PG	$33 \pm 1.8$ a	$7.41 \pm 0.03$ b	$7.36 \pm 0.05$ b	$1761 \pm 6$ b	$1748 \pm 5$ b	$0.018 \pm 0.016$ b	$0.023 \pm 0.005$ b	$0.287 \pm 0.018$ b	$0.321 \pm 0.093$ b
PC	$42 \pm 0.7$ b	$7.24 \pm 0.02$ c	$7.22 \pm 0.03$ c	$1572 \pm 15$ b	$1574 \pm 173$ b	$4.161 \pm 0.556$ c	$4.150 \pm 0.539$ c	$0.176 \pm 0.053$ c	$0.174 \pm 0.032$ c
PB	$40 \pm 0.4$ c	$7.37 \pm 0.01$ b	$7.39 \pm 0.01$ b	$1806 \pm 62$ b	$1776 \pm 24$ b	$0.309 \pm 0.041$ d	$0.257 \pm 0.050$ d	$0.750 \pm 0.065$ d	$0.958 \pm 0.096$ d
PBG	$39 \pm 0.2$ c	$7.73 \pm 0.04$ d	$7.84 \pm 0.03$ d	$1689 \pm 97$ b	$1645 \pm 161$ b	$0.176 \pm 0.041$ e	$0.192 \pm 0.063$ e	$0.496 \pm 0.052$ e	$0.406 \pm 0.21$ e
PBC	$44 \pm 0.4$ d	$7.84 \pm 0.03$ d	$7.87 \pm 0.03$ d	$1396 \pm 80$ c	$1367 \pm 32$ c	$0.562 \pm 0.133$ f	$0.591 \pm 0.110$ f	$0.105 \pm 0.013$ f	$0.092 \pm 0.012$ f

The six different treatments were as follows: polluted soil (P), P amended with 10% garden soil (PG), P amended with 10% compost (PC), P amended with 2% hardwood-derived biochar (PB) and a combination of different amendments: PB + 10% garden soil (PBG) and PB + 10% compost (PBC) (mean  $n = 5$ ;  $\pm$ SE). Letters indicate a significant difference ( $p < 0.05$ )

**Fig. 2** Dry weight (mg) of the different *Populus nigra* organs (leaves (white), stem (grey), root (black)) at 28 days under 5 different treatments: polluted soil (P), P amended with 10% garden soil (PG), P amended with 10% compost (PC), P amended with 2% hardwood-derived biochar (PB) and a combination of different amendments: PB + 10% garden soil (PBG) and PB + 10% compost (PBC) (mean  $n = 3$ ;  $\pm$ SE). Letters on bar graphs indicate a significant difference ( $p < 0.05$ )

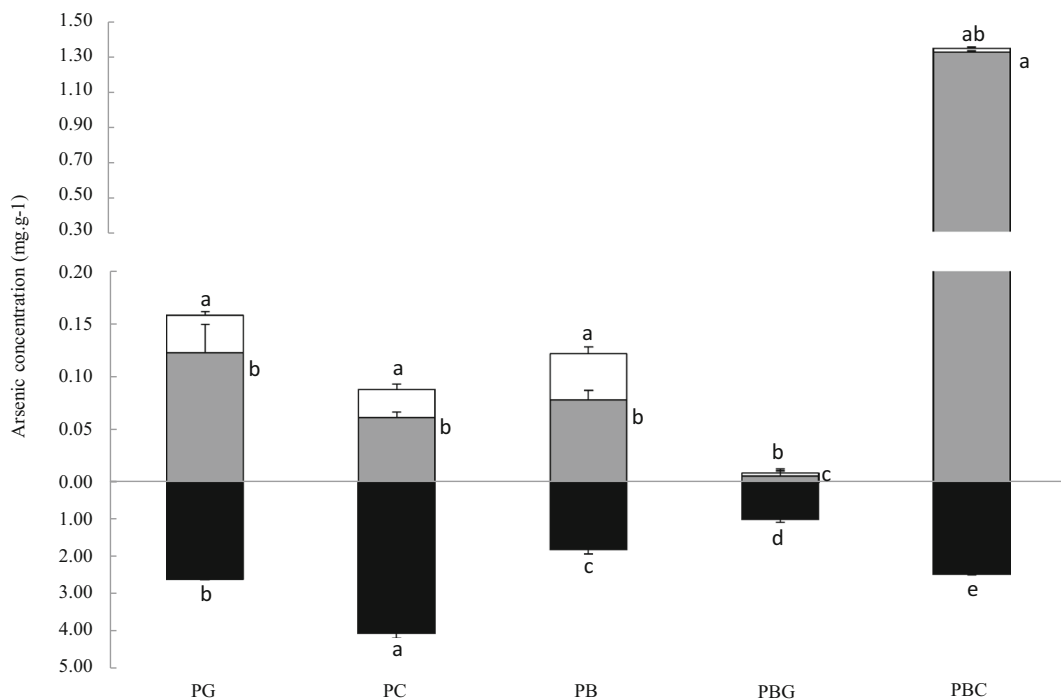


## Discussion

Physicochemical characteristics of soils and soil pore waters

In this study, all tested amendments increased SPW pH and EC values. Amendments reduced the mobility of Pb but increased arsenic mobility for the same

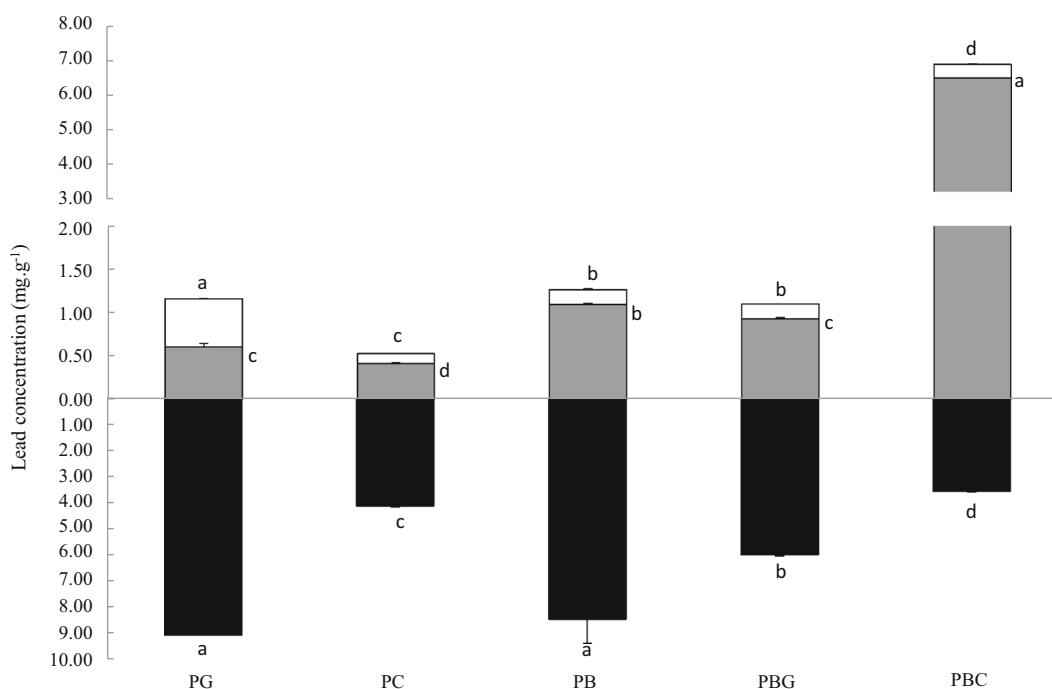
tested modalities. After the addition of amendments (biochar, compost or garden soil), and in particular for biochar, pH increase can be explained by two mechanisms: (i) the alkaline pH of biochar induced a liming effect (Bian et al. 2014; Lebrun et al. 2017) and (ii) the presence of functional groups such as  $\text{COO}^-$  and  $\text{O}^-$  on the amendments was able to bind protons (Houben and Sonnet 2015).



**Fig. 3** Arsenic concentration ( $\text{mg g}^{-1}$ ) in the different *Populus nigra* organs (leaves (white), stem (grey), root (black)) at 28 days under 5 different treatments: polluted soil (P), P amended with 10% garden soil (PG), P amended with 10% compost (PC), P amended with 2% hardwood-derived biochar (PB) and a combination of different amendments: PB + 10% garden soil (PBG) and PB + 10% compost (PBC) (mean  $n = 3$ ;  $\pm$ SE). Letters indicate a significant difference ( $p < 0.05$ )

amended with 2% hardwood-derived biochar (PB) and a combination of different amendments: PB + 10% garden soil (PBG) and PB + 10% compost (PBC) (mean  $n = 3$ ;  $\pm$ SE). Letters indicate a significant difference ( $p < 0.05$ )





**Fig. 4** Lead concentration ( $\text{mg g}^{-1}$ ) in the different *Populus nigra* organs (leaves (white), stem (grey), root (black)) at 28 days under 5 different treatments: polluted soil (P), P amended with 10% garden soil (PG), P amended with 10% compost (PC), P amended

with 2% hardwood-derived biochar (PB) and a combination of different amendments: PB + 10% garden soil (PBG) and PB + 10% compost (PBC) (mean  $n = 3$ ;  $\pm$ SE). Letters indicate a significant difference ( $p < 0.05$ )

The SPW electrical conductivity of Pontgibaud technosol (P) was very low and significantly increased by biochar addition. Similar results were also observed by Lomaglio et al. (2016) who described an increase in EC when 2% or 5% of hardwood-based biochar was added to a mining technosol contaminated mainly by arsenic.

SPW Pb concentration in amended soils was always lower than that in P, suggesting that the high pH of amendments reduced soil Pb mobility, as shown by the negative correlation ( $-0.97$ ) (Table 3) between the SPW Pb concentration and the pH values at T28. Indeed, a high pH value is known to increase the negative surface charges of soil particles and thus the retention of metals in soil (Pérez-Esteban et al. 2014). Moreover, as already described, metals such as Cd, Pb and Cu could be immobilised not only by the organic C content of biochar but also by humic acids contained in compost (Bolan et al. 2014). The mechanisms by which the amendments immobilise metal(loid)s are based on different properties, via different mechanisms (Boisson et al. 1999; Mench et al. 1998, 2000): (i) adsorption of metals to highly accessible sites located on the surface of biochar through interactions with oxygenated

functional groups (Lebrun et al. 2018), as well as on the humic substances contained in the compost (Lebrun et al. 2019), and (ii) precipitation with phosphates and carbonates contained by the biochar (Lomaglio et al. 2016).

Arsenic, unlike cationic metals (Cu, Zn, Pb, Cd), is known to be mainly present in the form of an oxyanion. Moreover, increasing soil pH allows an increase in As release from the solid phase to the soil pore water (Beesley et al. 2011). The effect of organic residues on the mobility and bioavailability of metal(loid)s in the soil depends on the type of soil and the characteristics of the amendment (EC, pH and degree of humification). This means that conditions such as a pH increase, induced by the addition of biochar to soils, could increase As mobility and therefore outweigh the capacity of biochar to sorb metal(loid)s. Finally, the organic and mineral residues brought about by the amendments are known to improve the agronomic potential of the new formed technosol, which should allow for better plant growth (Abbas et al. 2018).

As observed by Mench et al. (2003) at the Jales gold mine after adding municipal compost in combination

**Table 3** Correlations between pH, electrical conductivity (EC), organs' dry weight (DW) and metal(loid)s in soil pore water (SPW) and in *Populus nigra* organs

	pH	EC	SPW		DW			[As]			[Pb]				
			[As]	[Pb]	Leaves	Stem	Root	Leaves	Stems	Roots	Leaves	Stems	Roots		
pH	1	<i>0.87</i>	0.12	<i>-0.97</i>	<i>0.63</i>	<i>0.71</i>	<i>0.6</i>	0.45	0.38	0.56	0.57	0.46	<i>0.67</i>		
EC		1	0.16	<i>-0.93</i>	<i>0.69</i>	<i>0.66</i>	<i>0.74</i>	<i>0.69</i>	<i>-0.04</i>	0.59	0.52	0.04	<i>0.91</i>		
SPW			1	<i>-0.27</i>	<i>0.74</i>	<i>0.65</i>	<i>0.53</i>	0.18	<i>-0.09</i>	<i>0.76</i>	<i>-0.27</i>	<i>-0.13</i>	<i>-0.16</i>		
[As]				1	<i>-0.78</i>	<i>-0.82</i>	<i>-0.76</i>	<i>-0.58</i>	<i>-0.28</i>	<i>-0.71</i>	<i>-0.58</i>	<i>-0.34</i>	<i>-0.73</i>		
[Pb]					1										
Weight						1									
Leaves							0.96	<i>0.94</i>	0.52	0.05	<i>0.95</i>	0.37	0.03	0.44	
Stems								1	<i>0.91</i>	0.5	0.31	<i>0.96</i>	0.52	0.29	0.4
Roots									1	<i>0.64</i>	0	<i>0.89</i>	0.6	<i>-0.04</i>	<i>0.62</i>
[As]															
Leaves									1	0.04	<i>0.62</i>	0.48	0.04	<i>0.74</i>	
Stems										1	0.23	0.44	<i>0.99</i>	0.17	
Roots											1	0.39	0.19	0.35	
[Pb]															
Leaves												1	0.42	<i>0.61</i>	
Stems													1	<i>-0.11</i>	
Roots														1	

Significant correlations are represented in italics

with other amendments, the addition of compost to P increased the mobility and leaching of metal(loid)s, particularly arsenic. In the present study, the large increase in SPW arsenic concentration for the PC treatment was probably due to the fact that compost is able to discharge high quantities of dissolved organic carbon into the soil solution, which is known to compete with arsenic for sorption sites such as iron oxide, resulting in an increase in arsenic mobility (Bolan et al. 2014; Moreno-Jiménez et al. 2013). Compost could also contain a significant amount of soluble phosphate which may have displaced arsenic from organic and inorganic binding sites (Bolan et al. 2014).

However, the lower As concentrations observed in PBC SPW compared to PC suggest a possible and non-negligible As entrapment by biochar.

#### Plant growth and metal(loid) tolerance

The unamended Pontgibaud soil is unsuitable for poplar seed germination. This certainly explains why the plot selected to sample the soil used for this study is currently unvegetated. The

improvement to Pontgibaud soil quality through the addition of amendments clearly allows better seed germination and the production of young seedlings. This can be explained (i) by the improvement in soil physicochemical properties (WHC, pH, EC) as well as the supply of nutrient matter (Abbas et al. 2018) and by (ii) the decrease in SPW lead concentration. Interestingly, we observed concomitantly that although the highest dry weight production was when compost was added to P, the SPW As concentration for this treatment was higher than for the other amendments tested. This probably means that, even though As was found in high concentrations in the SPW in such conditions, the As was probably less phytoavailable. However, this increase in As in soil pore water could cause environmental problems for other organisms; therefore, this increase should be avoided or at least controlled.

It should be noted that the three lowest plant organ's dry weights were measured for PB, PBG and PBC treatments. This could be attributed to the presence of biochar, which probably partially adsorbed the nutrients present in soil pore water to its surface.

## Metal(loid) concentrations in plant organs

The highest concentrations of Pb and As were found in poplar seedling root tissues for all amendments added to the Pontgibaud technosol. This was also observed by Vamerali et al. (2009) who tested several species of *Populus* and *Salix* in metal- and arsenic-contaminated pyrite wastes (containing Co, Cu, Pb and Zn) and found that metal(loid) concentrations were higher in roots than in the upper organs. As demonstrated by Gupta et al. (2013), this root containment could prevent metal toxicity. Indeed, Borišev et al. (2009) proposed that willow metal(loid) exclusion mechanisms from the upper parts may protect the photosynthetic pathway, for example through complexation and sequestration of these metals in root cell vacuoles (Lasat et al. 2000). Moreover, it was also proposed from a cellular point of view that mechanisms governing metal(loid) tolerance in plant cells correspond to (i) cell wall binding, (ii) active transport of ions into the vacuoles and (iii) chelation through the induction of metal-binding peptides and the formation of metal complexes (Hazrat et al. 2013).

## Conclusion

The present work showed that the addition of compost, garden soil and biochar as soil amendments allows poplar seed germination and seedling development in a soil which was previously unfavourable to any vegetation. Such an approach could be proposed to remediate a soil, allowing it to be recolonised by the seeds present in the surrounding natural environment. Moreover, we demonstrated that such amendments could stabilise Pb in the soil effectively, allowing a decrease in concentrations found in the SPW, which, *in fine*, will protect the groundwater table. It is, however, important to precise that in the case of As, an increase in its mobility was observed for all amendments used and, in particular, when compost was added to the technosol. Further study into the behaviour of arsenic will be required, in particular concerning the choices for new amendments which will be efficient in stabilising it in the soil.

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