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ABSTRACT

Mine soils often contain metal(loid)s that may lead to serious environmental problems. Phytoremediation, consisting in covering the soil with specific plants with the possible addition of amendments, represents an interesting way of enhancing the quality of mine soils by retaining contaminants and reducing soil erosion.

In order to study the effect of an assisted phytoremediation (with willow and ryegrass) on the properties of soil pore water (SPW), we investigated the impact of amendment with biochar (BC) combined with the planting of willow and ryegrass on the behavior of several metal(loid)s (Pb, Zn, Ba, As, and Cd) in a mine soil. Data on the physicochemical parameters and concentrations of the different metal(loid)s in both SPW and in plant tissues of willow and ryegrass highlight the importance of BC for SPW properties in terms of reductions in soluble con-centrations of Pb and Zn, although there was no effect on the behavior of As and Cd. BC also increased soluble concentrations of Ba, probably related to ion release by the BC. By improving major ions available in mine soil, BC improved the lifetime of plants and enhanced their growth. Plant development did not appear to significantly affect the physicochemical parameters of SPW. Willow and ryegrass growing on soil with BC incorporated Cd and Ba into their tissues. The influence of plants on the behavior of metal(loid)s was noticeable only for ryegrass growing in soil with 2% BC, where it modified the behavior of Pb and Ba.

1. Introduction

The exploitation of a large number of mining sites over hundreds of years has generated large amounts of wastes such as tailings resulting from crushing and ore concentration, and consisting of fine particles and/or fine sand or silt (Li et al., 2014). Mine soils developing on these mine residues have particular physical and chemical properties that may lead to serious environmental problems due to the structure of the soils (e.g. diffusion of particles resulting from erosion) or to release of high metal(loid) concentrations (Barrutia et al., 2011). The main toxi-city problems of such contaminated soils are often related to metal(loid) s such as Cd, Pb, Hg, As, Cu, Zn, and Cr (Ghosh, 2010). While the poisonous and/or carcinogenic effects of Pb, As and Cd no longer need to be proven, those related to Ba (different disorders and metabolic problems) remain to be defined (Martin and Griswold, 2009 and Kravchenko et al., 2014). Ba concentrations range from 13 to 2050 $mg.kg^{-1}$ in the subsoil and from 30 to 1870 $mg.kg^{-1}$ in topsoil (De Vos and Tarvainen, 2006). To date, no maximum limit of Ba for food safety has been established (Whelan, 1993) and the effect of Ba on plants, especially in the presence of biochar (BC), has not been thor-oughly investigated.

From amongst all of the remediation options, assisted phytor-emediation – i.e. techniques for the management of polluted environ-ments (waters, soils) which utilizes the natural abilities of plants to immobilize, degrade, reduce or remove contaminants with the possible addition of amendments, microorganisms or chemical compound – of-fers an environmentally sound alternative that re-establishes a self-sustaining vegetation cover while reducing the mobility and toxicity of metal(loid)s in the environment. The efficiency of phytoremediation depends primarily on the selected plant species and their rhizospheres (including associated microorganisms), as well as on the (phyto)avail-ability of metal(loid)s (Khalid et al., 2016). Herbaceous and woody species, which show adequate rates of growth and biomass production and a tolerance for or even an ability to accumulate or retain (i.e.

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phytostabilization) metal(loid)s, are interesting candidates for phytoremediation (Sharma et al., 2014; Vamerali et al., 2010). Lolium perenne L. (perennial ryegrass) can accumulate and tolerate metal(loid)s without its growth being affected by high metal(loid) concentrations. Ryegrass is therefore a species commonly used for surface stabilization (Arienzo et al., 2004). Salix viminalis L. (basket willow) has a different root system from that of ryegrass and therefore explores a different and deeper soil volume compared with the herbaceous plant. It is known that willow does not accumulate high concentrations of metal(loid)s (Sytar et al., 2016), although some authors suggest an accumulating capability for Cd, Cr, Pb, and Hg (Schmidt, 2003) and a capability to survive on an As, Pb and Sb contaminated soil (Bart et al., 2016). Willow species are suitable candidates for remediation of metal con-taminated soils given their high resistance to many pollutants and their high biomass production and fast growth (Greger and Landberg, 1999).

Phytoremediation can be assisted and reinforced by adding organic or inorganic amendments to potentially enhance the organic content of degraded soils by increasing plant establishment and survival (Fellet et al., 2014) and also by reducing the mobility, transfer and bioaccu-mulation of metal(loid)s (Ahmad et al., 2014). BC appears to be one of the most promising amendments given its ability to improve both the physicochemical and biological properties of soils (Beesley et al., 2011). BCs are stable carbon-rich pyrolysis products from various organic matters (wood, manure, rice husk, etc.). With its singular porous structure and alkaline properties, BC helps to increase soil pH (neu-tralization of acidity) and redox potential (E_h) and also improves soil water retention capacity (reduction of leaching) (Atkinson et al., 2010) while enhancing cation exchange capacity (CEC) (Cheng et al., 2006). Furthermore, BC can even reinforce soil enzymatic and microbial ac-tivities and diversity (Ahmad et al., 2014, 2016) thereby providing possible diverse niches with suitable conditions for microorganisms while improving plant growth (Sohi et al., 2010; Quilliam et al., 2012). With its large specific surface, BC may adsorb metal(loid)s through multiple interaction mechanisms such as electrostatic adsorption, ion exchange interactions or co-precipitation, and by the complexing function of oxygen functional groups and π electrons (Sohi et al., 2010; Beesley et al., 2011; Ahmad et al., 2014). More specifically, metal ca-tions may interact with active sites provided by BCs' functional groups (carboxylic, amine, hydroxyl, carbonyl, alcoholic and phenolic groups and some inorganic mineral species; Gul et al., 2015) leading to highly stable BC-metal cation complexes (Baldock and Smernik, 2002). BC may also uptake metal cations by chemical precipitation with inorganic components (Wang et al., 2015). Organic functional groups on BCs influence the pH increase in soil and can therefore improve the stability of metal cations. Moreover, negatively charged organic functional groups increase over time with their oxidation in the soil (Cheng et al., 2008). Several studies have evaluated the effect of BC amendments on assisted phytoremediation of contaminated mine soils (Paz-Ferreiro et al., 2014) but few studies have aimed at determining the availability and behavior in mine soil pore water (SPW) and the absorption of metal (loid)s by plants (Lebrun et al., 2017; Bart et al., 2016; Beesley and Marmiroli, 2011).

The present study aims to determine the behavior of Pb, Zn, Ba, As and Cd in a contaminated mine soil amended with BC and planted with willow and ryegrass. We investigated the potential impacts of BC from hardwood feedstock (2% and 5% BC (w/w)) on: (1) physicochemical properties of SPW; (2) total dissolved concentrations of metal(loid)s in SPW; and (3) distribution of metal(loid)s in plant organs and their ca-pacities to promote plant growth in a mine soil. To our knowledge, there have been only a few reports on the behavior of Ba, and even fewer in the presence of BC. This metal has, accordingly, been the focus of special investigation.

2. Materials and methods

2.1. Study site and mine soil characterization

The study covered a mine soil formed by Ag-Pb ore mining. The mine soil was located in the mining district of Pontgibaud (Roure-Les-Rosiers, Massif Central region, France; 45°47′29″ N, 2°49′17″ E). The site is located at an average altitude of 750 m with annual rainfall of 579 mm and average temperature ranging from 1.3 °C (winter) to 16.6 °C (summer). Geologically, the site is located on metamorphic rocks of the migmatite type (metatexites) with nearby inclusions of basalts and amphibolites posterior to the emplacement of the meta-morphic soil (Bouladon et al., 1964).

The site has diversified mineralogy rich in secondary minerals (ar-senopyrite, anglesite, barytine, pyrite, quartz, etc.). Quartz and/or barite gangues include sulphides. The site was formed through the deposition of laundry spills and slag from the ore processing and en-richment phases (Dommanget, 2005). Operation dates back to Gallo-Roman times (100–300 A.D.) and ceased in 1897, with a resumption of activities during the Second World War. Mining activity was most in-tense during the 19th century.

The samples were collected from a settling pond of the Ag-Pb ore mine. Sampling was systematic at a depth of 0–20 cm in the mine soil and was conducted using a 5 m mesh over an area of 300 m^2. A total of 20 samples was collected and these were carefully mixed to form a single composite sample. Mining settling ponds were used mainly during the XIXth century to remove suspended solids from water pro-duced by a mining plant using physical treatment to concentrate metals. Due to the processed used at that time, the grain size of the tailings produced by the plant was rather coarse compared to more recent processes. Thus, in the pond the material deposited was manly sandy. After settling in the pond, the water was released in a nearby stream. The place from which the mine soil samples were taken for experiments was almost bare, with only very small patches of spontaneous vegeta-tion (trees and herbaceous) at the edge.

2.2. Mine soil and biochar

Composite soil samples were taken over a depth of 0–20 cm from the top layer of a mine soil from a settling pond and were air-dried, sieved through a 2-mm mesh, and stored at room temperature until analysis. The sandy mine soil had a pH of 4.7. The tailing material was dried at 40 °C, and crushed for sieving at 250 μm, according to standard NF ISO 11464. The tailing material (250 mg ± 0.5 mg) was then en-tirely solubilized with fluorhydric acid (5 mL) and perchloric acid (1.5 mL) by heating to 160 °C, and then taken up in hydrochloric acid (1 mL) according to NF X 31–147. The total concentrations of metal (loid)s in the mine soil measured by ICP/AES (detection limit (DL) in the $mg.kg^{-1}$ range) were as follows: Pb = 12,519 $mg.kg^{-1}$, Zn = 143 $mg.kg^{-1}$, Ba = 1701 $mg.kg^{-1}$, As = 466 $mg.kg^{-1}$. Cd was below the DL of 2 $mg.kg^{-1}$. Analysis of major elements of the mine soil gave the following results: 2.54% K_2O (ICP/AES), 0.01% MnO (ICP/ AES), 438 mg kg^{-1} P_2O_5 (ICP/AES), 1.12% SO_4 (NF ISO 11048), and 84.8% SiO_2, with CaO, Fe_2O_3, and MgO below DL (ICP/AES). The samples were extracted by a sequential extraction procedure for the fractionation of metal(loid)s (Tessier et al. 1979) after air drying and sieving through a 2-mm mesh. The water-soluble fraction and cation exchangeable fraction enabled determination of the phytoavailable part of the metal(loid)s, with the following results: 43.9 $mg.kg^{-1}$ Pb, 7.5 $mg.kg^{-1}$ Zn, 27.2 $mg.kg^{-1}$ Ba, 0.2 $mg.kg^{-1}$ Cd and 0.3 mg.kg $^{-1}$As. The cation exchange capacity (CEC), determined using the 0.05 N co-baltihexamine at soil pH method (standard NF ISO 31–130), was 4.56 $cmol.kg^{-1}$ pH of the mine soil was 4.7 ± 0.01.

Biochar (BC) from hardwood (source La Carbonerie, Crissey, France) was obtained after slow pyrolysis (500 °C) of platelets and chips of oak, beech and charm. It was ground (< 100 μm) for use as a soil amendment in pot experiments. Brunauer-Emmett-Teller (BET) surface area analysis, Barrett-Joyner-Halenda (BJH) pore size and volume and pH analysis were performed for this material. BC characterization showed a specific area of 242.4 $m^2.g^{-1}$. Total pore volume was 0.17 $cm^3.g^{-1}$ with a mean pore diameter of 2.80 nm. The CEC de-termined using the 0.05 N cobaltihexamine at soil pH method (standard NF ISO 31–130) was 0.65 $cmol.kg^{-1}$ pH of BC was 9.5 ± 0.01.

Measurements of total carbon, nitrogen and hydrogen were per-formed on the prepared mixtures of mine soil and BC prior to the pot experiment, using an elemental flash pyrolyser analyzer (Flash 2000; Thermo Fischer Scientific) on samples previously dried and crushed to obtain a fine powder < 70 μm. The total carbon concentrations were respectively 0.2%, 1.6%, and 4.7% for the mine soil without BC and for the mine soils with 2% and 5% BC. Total nitrogen concentrations of the mine soil were respectively 0.03% and 0.01% for the mine soil without BC and with 5% BC. No nitrogen was detected in the mine soil with 2% BC. Total hydrogen concentrations of the mine soil were 0.1% for the mine soil without BC and, respectively, 0.2%, and 0.2% for the mine soils with 2% and 5% BC. Elementary analysis of BC showed 81.2% carbon, 1.7% hydrogen, and 0.6% nitrogen.

2.3. Pot experiment

The experiment design is presented in Table 1. This was a pot ex-periment with full factorial design set up with 45 pots (3 amendments x 3 planting conditions x 5 replicates for each condition). Mine soil (equivalent to 400 g air-dried) was placed in 87 × 113 mm pots. BC was blended manually with mine soil at mass fractions (w/w) of 2% and 5% dry weight. Fifteen pots were planted with one non-rooted cutting (7.5 cm length x 1 cm diameter) of Salix viminalis L. (basket willow) (W) (source ONF, Guémené-Penfao France) in mine soil without BC (5 pots), with 2% BC (5 pots) and with 5% BC (5 pots). Fifteen pots were sown with 30 seeds of Lolium perenne L. (perennial ryegrass) (R) (source Phytosem SAS, Gap, France) with the same soil treatments as for the willow. Fifteen pots were non-vegetated (NV), with the same treat-ments as for the willow and ryegrass.

Pots were kept for 5 weeks (ryegrass) or 7 weeks (willow) in a growth chamber with a 16 h day/8 h night photoperiod (20 ± 5 °C), 80% relative humidity and 800 $\mu mol\ m^{-2}.s^{-1}$ of photosynthetically active radiation (PAR). Control pots were kept in the same conditions as the planted pots. Data from these controls correspond to analyses conducted at 5 and 7 weeks to match the duration of cultivation of the willow and of the ryegrass. The number of samples is not the same for each treatment because of mortality of certain plants, (see legend of Figures and Tables). The differences between the duration of cultivation of willows and of ryegrass are due to the fact that ryegrass showed signs of weakness. Pots with limited drainage were watered daily with a quantity of distilled water calculated to match 70% of the water holding capacity (WHC) previously measured for each blend of mine tailing-BC. The field capacity was measured using a pressure plate extractor to determine the water content of the soil considered to be ideal for plant growth. The pF unit is derived from the soil moisture tension in cm H_2O by taking the logarithm (pF = log (cm H_2O)) (Kirkham, 2005). Eva-luation of WHC allowed determination of the quantity of water to be provided (by weighing) for good plant growth in each of the conditions.

Table 1
The experiment design is a pot experiment with full factorial design set up with 45 pots (3 amendments x 3 planting conditions x 5 replicates for each condition).The factors were (1) mine soil without biochar, (2) mine soil with 2% biochar, and (3) mine soil with 5% biochar. For each of the soil treatments:
(a) 5 pots were non-vegetated, (b) 5 pots were planted with one non-rooted willow cutting, and (c) 5 pots were sown with ryegrass seeds.

		(a)	(b)(c)	
		Non-vegetated	Willow	Ryegrass
(1)	Without biochar	NV0	W0	R0
(2)	2% biochar	NV2	W2	R2
(3)	5% biochar	NV5	W5	R5

A soil-moisture sampler (SMS) with a mean pore size of 0.15 μm (Rhizon, MOM model, Rhizosphere Research Products, Wageningen, The Netherlands) was placed at the center of each pot (at 45°) to collect the soil pore water (SPW) (Cattani et al., 2006). The water yield with a pressure differential of 100 kPa was greater than 1 $mL.min^{-1}$ (Di Bonito et al., 2008). SPW (~10 mL) was collected weekly, using syringes, two hours after watering. Given the SMS′ cut off threshold (0.15 μm), samples were not filtered. Each SPW sample was separated into several aliquots to allow a series of measurements and analyses. Measurements of pH, Eh, and electrical conductivity were performed directly on SPW samples, whereas dissolved organic carbon (DOC) and dissolved or-ganic nitrogen (DON) were analyzed the day of sampling after dilution of the aliquot. A portion of the aliquots was diluted and acidified with concentrated HNO_3 to determine cation and metal(loid) concentrations, then stored (at 4 °C) until analysis.

2.4. Physicochemical analysis

2.4.1. Soil pore water analysis

pH, Eh (platinum silver/silver chloride reference electrode) and electrical conductivity (EC) of SPW were measured at the initial and final steps with a Multi 197i portable multiparameter meter (WTW, Weilheim, Germany). DOC and DON concentrations were determined using a TOC 5050/SSM 5000-A elemental analyzer (Shimadzu, Kyoto, Japan) with $C_8H_5KO_4$ (1000 $mg_{Carbon}.L^{-1}$) and KNO_3 (100 $mg_{Nitrogen}.L^{-1}$) used as standards. Ion chromatography (IC), using a 940 Professional IC Vario instrument (Metrohm, Switzerland) equipped with conductivity detectors, was used for major ion analyses. For anions (F^-, Br^-, Cl^-, NO_2^-, NO_3^-, SO_4^{2-}, and PO_4^{3-}), a Metrosep A Supp 16 (150 mm × 4 mm) ionic resin column (Metrohm, Switzerland) and a Metrosep A Supp 4/5 guard column (Metrohm, Switzerland) were used, along with a self-regeneration suppressor (Model 833 suppressor unit,
Metrohm, Switzerland). The eluent was Na_2CO_3 7.5 mM and NaOH 0.75 mM. For cations (Li^+, Na^+, NH_4^+, K^+, Ca^{2+}, and Mg^{2+}), a
Metrosep C6 (150 mm × 4 mm) separation column (Metrohm, Switzerland) was used with HNO_3 1.7 mM and $C_7H_5NO_4$ 1.7 mM solu-
tions as eluents. Some major ions were analyzed but were not detected in SPW (F^-, Br^-, NO_2^-, PO_4^{3-}, and Li^+).

2.4.2. Plant analysis

At harvest, the surviving plants were counted and each of the plant organs (leaves, stems, cuttings and roots) was isolated and washed for 30 min with 0.5 mM $CaCl_2$ with slow agitation to remove adsorbed metal(loid)s (Zacchini et al., 2009); they were then rinsed thoroughly with doubly de-ionized water. $CaCl_2$ was used to remove the adhering metal(loid)s from the cell wall and to avoid plasma membrane altera-tion. This desorption allows quantification of the intracellular fraction of metal(loid)s only, not of the metal(loid)s adsorbed on the plant surface. Plants organs were oven dried at 40 °C until constant weight.

Microwave-assisted digestion of plant organs was conducted with a pressurized closed-vessel microwave system (Multiwave 3000, Anton Paar GmbH, Germany). Around 200 mg (± 0.5 mg) of dry and pre-viously crushed sample were put into PFA-Teflon vessels with a mixture of 6 mL of 70% nitric acid and 3 mL of 37% hydrochloric acid. Samples were placed in the microwave and digested as follows: heating of samples over 15 min to a temperature of 180 °C; temperature of 180 °C maintained for 15 min; temperature allowed to drop over 15 min to reach 55 °C. Power was 850 W for all steps. The solution was then fil-tered at 0.45 μm (nitrocellulose filter, Sigma-Aldrich), transferred into a flask, and the volume adjusted to 50 mL with doubly de-ionized water. Blank controls using reagents (6 mL 70% nitric acid and 3 mL 37% hydrochloric acid) were treated in the same way.

2.4.3. Metal(loid) concentrations

Concentrations of metal(loid)s (Pb, Zn, Ba, As and Cd) in SPW and in plants were determined by inductively coupled plasma mass spec-trometry (ICP-MS; Finnigan Element XR, Thermo Electron, Germany) (DL in the $\mu g.kg^{-1}$ range). The amounts of metal(loid)s found in plants were expressed either as a concentration ($\mu g.g^{-1}$) in the dry material or as mineralomass (μg) (mineralomass = biomass of plant organs x $[metal(loid)s]_{plant\ organs}$).

2.4.4. Metal(loid) transfer into plant organs

Three factors were determined to evaluate the capabilities of the metal(loid)s to transfer into the different plant organs. The transfer of metal(loid)s from the root to shoot parts (leaves or leaves and stems) was determined by the Translocation Factor (TF) (TF = $[metal(loid)\ s]_{shoot}$/[$metal(loid)]_{root}$). The ability of the plant to accumulate metal (loid)s from mine soil in its tissues was determined by the Bioconcentration Factor (BCF) (BCF = = $[metal(loid)]_{root}$/[metal $(loid)]_{SPW}$) and by the Biological Absorption (or Accumulation) Factor (BAF) (BAF = $[metal(loid)]_{shoot}$/[$metal(loid)]_{SPW}$). The metal(loid) concentration in each plant part was obtained by calculating net bio-mass uptake.

2.5. Statistical analysis

All statistical tests were conducted in R 3.2.3 (R Development Core Team, 2014). After mortality of some plants, the corresponding samples were removed from the dataset, thereby reducing the number of re-plicates per treatment. The accuracy of the measurement of metal(loid) s, DOC and DON, assessed by the intra-species variance, was taken into account in the calculations of inter-species variance (total standard deviations). To analyze these data, inter-species averages were used. The other standard deviations were calculated directly from inter-spe-cies average. ANOVA and a post hoc test for homogeneity of variance were performed. Multiple comparisons were performed by the Dunnett test for comparing several treatments with a control with a 95% con-fidence level (mine soil without BC compared to mine soil with 2% and 5% BC). Multiple comparisons of means were performed by Tukey test for comparing treatments at levels *p < 0.05.

An unsupervised pattern recognition method by a hierarchical ag-glomerative cluster analysis (HCA) was performed on standardized data using the Ward method of linkage and using Euclidean distance be-tween samples as the similarity measurement. Correlations of metal (loid) concentration analyses were calculated using the non-parametric Spearman's correlation coefficients because most chemical parameters analyzed were not normally distributed and included outliers.

3. Results

3.1. Physicochemical parameters of soil pore water

Measurements of physicochemical parameters of soil pore water (SPW) (except dissolved organic carbon (DOC) and dissolved organic nitrogen (DON)) revealed changes in the composition of SPW due to biochar (BC) (Table 2). Overall, Eh dropped greatly while pH and electrical conductivity (EC) increased with the presence of BC. Con-centrations of major ions – especially K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, and SO_4^{2-} – were also influenced by BC amendment (mostly 5% BC) (SM 1). Some anions, such as NO_2^- and PO_4^{3-}, were not detected in SPW. The concentrations of major ions had decreased at the end of the ex-periment in both planted and non-planted pots, suggesting that this decrease is mainly attributable to time rather than to plant cultivation. For DOC, and especially for DON, variations among samples did not seem to be controlled by BC (Table 2). At the end of the experiment, pH and DOC values had fallen overall, whereas redox values had increased overall, except for R5. EC also decreased overall, except for NV5. At the final step there was almost no more DON. Closer examination of the individual parameters showed that the duration of the experiment had more impact on the physicochemical parameters than the presence of plants (Table 2). For all treatments, physicochemical parameters were modified during the experiment. Redox potential increased between the beginning and the end of experiment for all treatments (by 57 mV for W2), except for NV0 and R0 which showed decreases (of 4 and 13 mV, respectively) and for R5, which remained constant. EC decreased during the experiment for all treatments (by 543.7 μS for NV2), with the exception of NV5 and W0 which showed increases (of 64.6 and 22.9 μS, respectively). pH values either remained constant compared to the average of initial values (for NV5 and R5) or decreased during experi-ment (drop of 0.4 for R2 and W5, and of 1 for W2). DOC decreased during the experiment whatever the treatment (respective losses of 3.2 $mg_C.L^{-1}$ and 5.6 $mg_C.L^{-1}$ for W5 and R2 compared to average of initial values). DON decreased for NV0 and W5 compared to initial values, with respective losses of 1.1 and 0.5 $mg_N.L^{-1}$.

Table 2

Physicochemical properties of soil pore water (Redox potential (Eh), electrical conductivity (EC), pH, dissolved organic carbon (DOC), and dissolved organic nitrogen (DON)) at the beginning and at the end of experiment. I: average of initial values, NV: non-vegetated, W: willow, and R: ryegrass. 0, 2, and 5 for 0%, 2% and 5% biochar (BC). Data in parentheses indicate the standard error with n = 5 for NV0, NV2, NV5, R0 and R2; n = 4 for W2, W5, and R5; n = 3 for W0. ANOVA was followed by a post-hoc Dunnett test at level P < 0.05. Differences were evaluated comparing several treatments with a control (initial samples, I). Letters a, b, c refer to statistics on samples without BC, letters A, B, C to statistics on samples with 2% BC and letters α, β tor statistics on samples with 5% BC. For pairwise comparisons, analysis of variance was performed by a post-hoc Tukey test at different levels *P < 0.05 and n.s. for not significant statistics.

	Eh	Electrical conductivity	pH	DOC	DON
	mV	$\mu S.cm^{-1}$		$mg_C.L^{-1}$	$mg_N.L^{-1}$
I0	323 a	99.1 b	5.0 a	6.6 a	1.1 ab
I2	226 C *	828.7 A *	7.2 A *	6.9 A n.s.	0.7 A n.s.
I5	235 α *	1473.4 α *	7.5 α *	5.7 α n.s.	0.6 α n.s.
NV0	319 a	91.4 b	4.4 b	1.4 b	0.0 b
NV2	266 AB *	285.0 B *	6.6 BC *	1.4 B n.s.	0.0 B n.s.
NV5	252 αβ *	1538.0 α *	7.5 α *	2.0 β *	0.0 α n.s.
W0	334 a	122.0 a	4.2 ab	2.2 ab	0.4 a
W2	283 A *	304.3 B n.s.	6.2 C *	2.3 B n.s.	0.1 B n.s.
W5	255 β *	1407.0 α *	7.1 α *	2.5 αβ n.s.	0.1 α n.s.
R0	310 a	85.0 b	4.5 ab	1.3 b	0.1 b
R2	250 B *	372.0 B *	6.8 BC *	1.3 B n.s.	0.0 B *
R5	235 αβ *	1219.3 α *	7.5 α *	1.9 αβ n.s.	0.0 α *

3.2. Metal(loid) concentrations in soil pore water

Total dissolved metal(loid) concentrations in SPW are given in Fig. 1. With the exception of Ba, concentrations of metal(loid)s de-creased with time. The presence of BC also induced a decrease in metal (loid) concentrations in SPW for Pb and Zn but had no significant effect on As and Cd. While $[Pb]_{spw}$ decreased during the experiment whatever the treatment, in the presence of plants significant differences were observed between the mine soils without BC and those with 2% BC. BC seemed to be a more influential parameter than plant presence (Fig. 1). Only the 2% BC and ryegrass combination showed a significant dif-ference, with higher $[Pb]_{spw}$ than the other treatments with 2% BC. It seemed that the behavior of $[Zn]_{spw}$ was similar to that of $[Pb]_{spw}$. For the final samples, the addition of 5% BC tended to better reduce $[Zn]_{spw}$ whatever the treatment. As for Pb, BC did not have any influence on $[Zn]_{spw}$ in the presence of ryegrass. Multivariate statistical analysis of the sum of metal(loid)s accumulated by the plant (for each metal(loid) s), carried out using centered reduced principal component analysis

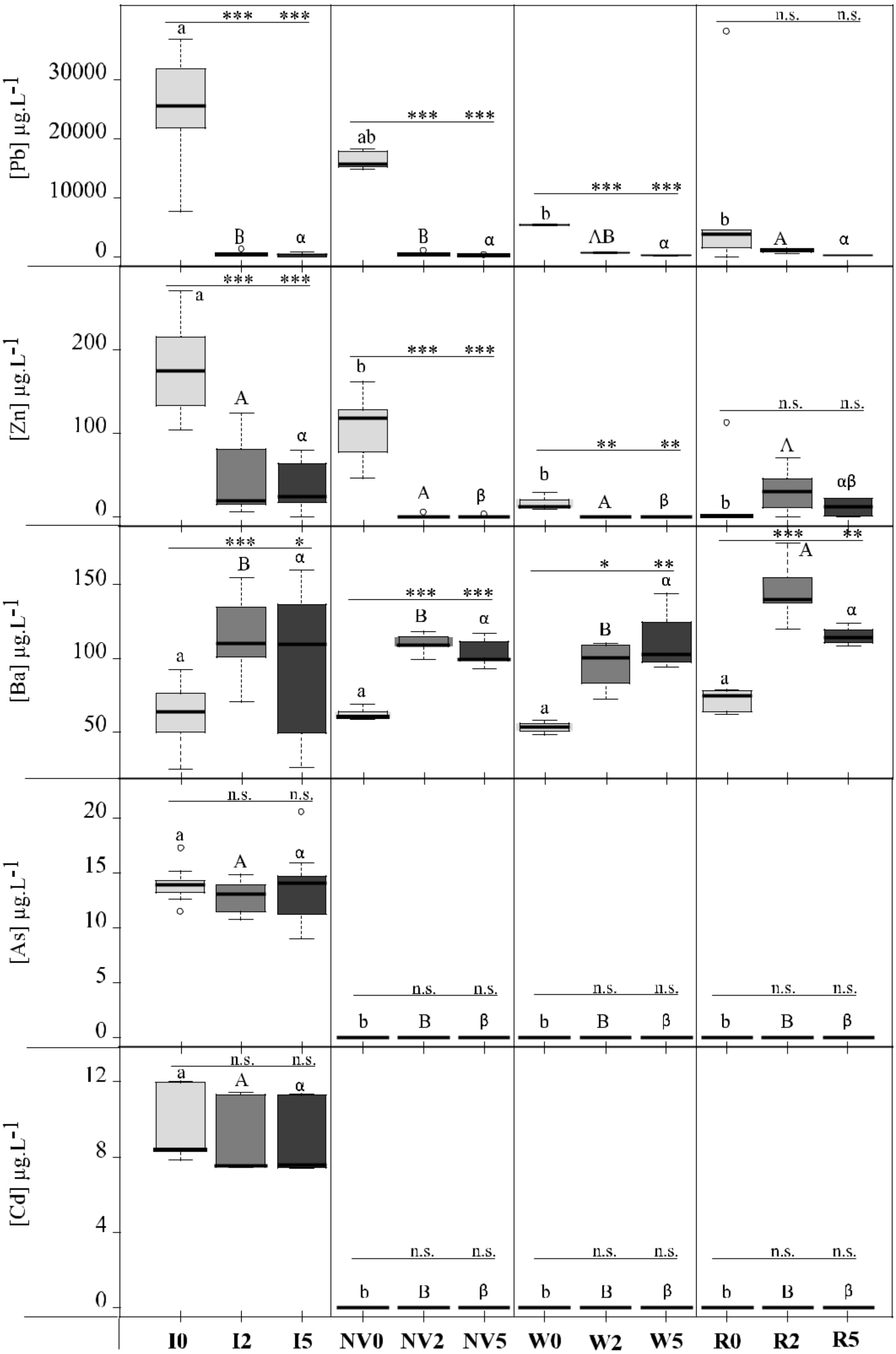

Fig. 1. Total dissolved metal(loid) (Pb, Zn, Ba, As, and Cd) concentrations (µg.L^{-1}) in the mine soil pore water at the beginning and at the end of experiment. BC: biochar, I: average of initial values, NV: non-vegetated, W: willow, and R: ryegrass. 0, 2, and 5 for 0%, 2% and 5% biochar. ANOVA was followed by a post-hoc Dunnett test at level $P < 0.05$ with n = 5 for NV0, NV2, NV5, R0 and R2; n = 4 for W2, W5, and R5; n = 3 for W0. Differences were evaluated comparing several treatments with a control (initial samples, I). Letters a, b, c refer to statistics on samples without BC, letters A, B, C to statistics on samples with 2% BC and letters α, β to statistics on samples with 5% BC. For pairwise comparisons, analysis of variance was performed by a post-hoc Tukey test at different levels $^*P < 0.05$ and n.s. for not significant statistics.

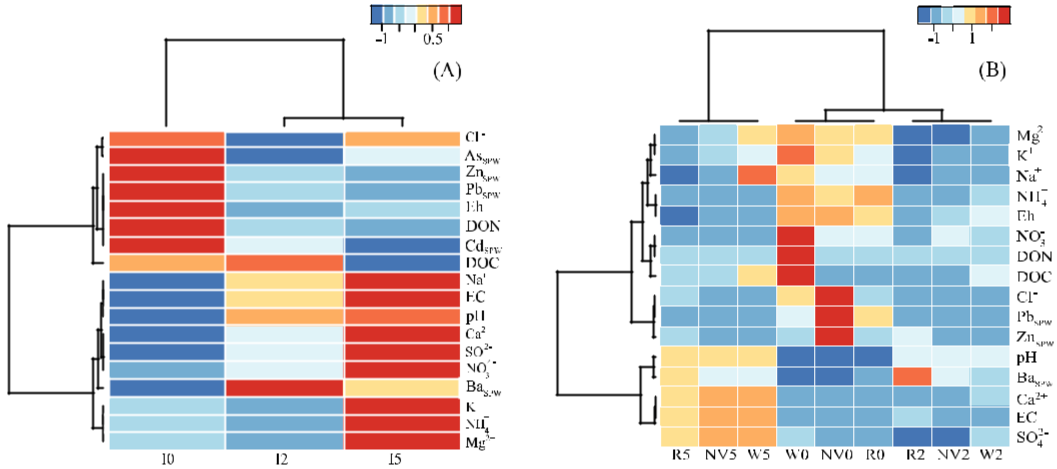

Fig. 2. Heatmap analysis based on the correlation matrix of (A) 18 (initial step) or (B) 16 (final step) variables measured in the mine soil pore water at the beginning and at the end of the experiment. Heatmap analysis was obtained by hierarchical agglomerative clustering using Ward method of linkage and Euclidean distance as a measure of similarity. Warmer colors represent lower values and cooler colors represent higher values. The double hierarchical dendrogram reveals the correlation among treatments (column) and among chemical parameters (row). BC: biochar, I: average of initial values, NV: non-vegetated, W: willow, and R: ryegrass. 0, 2, and 5 for 0%, 2% and 5% biochar. Eh: Redox potential, EC: electrical conductivity, DOC: dissolved organic carbon, DON: dissolved organic nitrogen, and SPW: soil pore water. Heatmap analysis was obtained with n = 5 for NV0, NV2, NV5, R0 and R2; n = 4 for W2, W5, and R5; n = 3 for W0. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(PCA) (SM 2), showed close trends. The second principal component (F2) of PCA, which explained 29.1% of the total variability, did not separate the treatments, suggesting that $[Pb]_{spw}$ and $[Zn]_{spw}$ did not discriminate the samples. BC amendment seemed to significantly in-crease the $[Ba]_{spw}$ (Fig. 1). The presence of ryegrass also tended to increase $[Ba]_{spw}$, especially for R2. PCA (SM 2) showed a similar trend. The first principal component (F1) of PCA explained 37.0% of the total variability, driven mainly by Ba concentration in SPW. Bi-dimensional plots which represent variable loadings and sample scores clearly se-parated the W0 samples from the R2 samples along the F1 axis (SM 2). No As and no Cd were detected in SPW at the end of experiment. Dif-ferences in initial metal(loid) concentrations were not due to BC amendment but to varying levels of contamination between samples.

3.3. The relationships between physicochemical parameters and metal(loid) concentration in soil pore water

The relations between all of the parameters measured in the SPW were shown by a heatmap built using Ward method of linkage and the Euclidean distance measurement (Fig. 2). This two-dimensional visua-lization of correlation was obtained from the 18 or 16 variables mea-sured, respectively, at the beginning and at the end of the experiment. The difference in the number of variables was due to the lack of de-tection of Cd and As in SPW at the end of the experiment. The heatmap yielded a combined dendrogram which clearly differentiates three groups of samples according to treatment (0% BC, 2% BC, 5% BC), at the beginning of the experiment (Fig. 2A) and at the end (Fig. 2B). The double hierarchical dendrogram reveals the correlation among treat-ments (column) and among chemical parameters (row). The column hierarchical dendrogram distinguishes treatments without BC, treat-ments with 2% BC and treatments with 5% BC, indicating that BC amendment was the most discriminating parameter acting on this group of variables. However, BC effect was not constant over time. At the beginning of the experiment, the treatments with 2% and 5% BC could be clearly distinguished from the treatment without BC, whereas at the end of the experiment, treatments with 5% BC exhibited a more marked difference (Fig. 2A). Over time, a 2% BC amendment no longer changed the composition of SPW. At the initial step, mine soil without BC was essentially characterized by high concentrations of metal(loid)s in SPW and by a low Eh. Variables influenced by the 5% BC amendment were a higher EC and ion concentrations (especially SO_4^{2-} and Ca^{2+}) com-pared to mine soils without BC. Similarly, the dendrogram of variables indicated that at the beginning of the experiment Pb, Zn, and As con-centrations in SPW were related to Eh, DOC and Cl^{-}, while Ba con-centration was linked to pH, EC and other major ions (Fig. 2A). Cor-relation matrices (Fig. 3) of aggregated data at initial step (Fig. 3A) showed that Pb and Zn concentrations in SPW were strongly positively correlated (0.83), and also negatively correlated with pH (−0.87 and −0.89 for Pb and Zn, respectively) and with EC (−0.77 and −0.71 for Pb and Zn, respectively). $[Ba]_{SPW}$, $[As]_{SPW}$, and $[Cd]_{SPW}$ presented no correlations above 0.60. Correlation of aggregated data failed to high-light relations between the analyzed data. This showed that either the phenomena that govern the dynamics of these metal(loid)s were not highlighted by these correlations or that the aggregated data did not make it possible to highlight the correlations. It will be necessary to study correlations by treatment (SM 3). At the final step, samples amended with 5% of BC were ranked according to their pH and EC and also according to Ca^{2+}, SO_4^{2-} and Ba concentration (Fig. 2B). Thus, $[Ba]_{SPW}$ seemed to be linked to pH and conductivity whatever the time step considered, while Pb and Zn were linked to Eh at the beginning of the experiment and to Cl^{-} at the end of the experiment. At the final step of the experiment, correlation of aggregated data (Fig. 3 B) showed a positive correlation between $[Pb]_{SPW}$ and $[Zn]_{SPW}$ (0.77). The con-centrations of these metal(loid)s were less related to the other para-meters than at the beginning of the experiment: correlation coefficient of $[Pb]_{SPW}$ and $[Zn]_{SPW}$ with pH (−65 and −0.50 for Pb and Zn, re-spectively) and EC (−0.41 and −0.37 for Pb and Zn, respectively). $[Ba]_{SPW}$ was correlated positively with pH (0.48 and 0.77 at the be-ginning and at the end of experiment, respectively) and correlated negatively with Eh (−0.57 and −0.78 at the beginning and at the end of experiment, respectively) (Fig. 3B). Regarding the study of correla-tions by treatment, the observation at the final step was different (SM 3). The positive correlation between $[Pb]_{SPW}$ and $[Zn]_{SPW}$ was sig-nificant only for certain treatments (1 for NV2, NV5 and W2, and 0.99 for R0).

3.4. Plant biomasses, metal(loid) concentrations in plant organs and mineralomasses

Willows and ryegrasses grown on mine soil without BC had no or

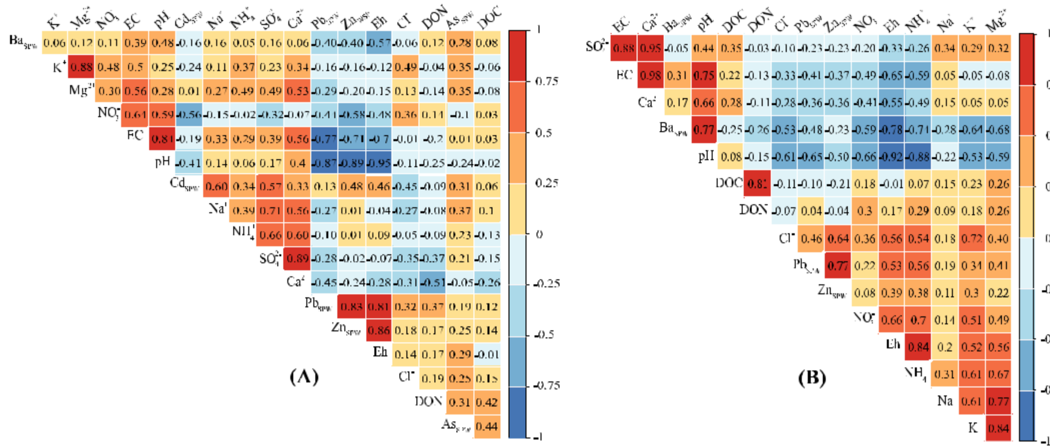

Fig. 3. Matrices of Spearman's correlation coefficients for (A) 18 (initial step) or (B) 16 (final step) variables measured in the mine soil pore water at the beginning and at the end of the experiment. Warmer colors represent lower values and cooler colors represent higher values. Eh: Redox potential, EC: electrical conductivity, DOC: dissolved organic carbon, DON: dissolved organic nitrogen, and SPW: soil pore water. Correlation matrices were obtained with n = 5 for NV0, NV2, NV5, R0 and R2; n = 4 for W2, W5, and R5; n = 3 for W0. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Survival rate and average biomasses dry weight (DW) (mg) W: willow, and R: ryegrass. 0, 2, and 5 for 0%, 2% and 5% biochar (BC). ANOVA was followed by a post-hoc Dunnett test at level P < 0.05. Differences were evaluated com-paring several treatments with a control (without BC namely W0 and R0) with n = 5 for NV0, NV2, NV5, R0 and R2; n = 4 for W2, W5, and R5; n = 3 for W0. Letters a, b, c refer to statistics on samples without BC, letters A, B, C to sta-tistics on samples with 2% BC and letters α, β to statistics on samples with 5% BC. For pairwise comparisons, analysis of variance was performed by a post-hoc Tukey test at different levels *P < 0.05; n.s. for not significant statistics, and n.d. not determined for unstated statistic (no data).

	Survival rate	Biomasses mg			
		Leaves	Stems	Roots	Total plant
W0	60% A	102 A	13 n.d.	n.d.	115 A
W2	100% a*	612 a*	584 n.d.*	459 a n.d.	1655 a*
W5	80% α*	681 α*	601 n.d.*	462 α n.d.	1743 α*
R0	70% A	90 A	-	n.d.	90 A
R2	74% b*	194 b*	-	26 b n.d.	220 b*
R5	61% β*	148 β*	-	52 β n.d.	199 β*

poorly developed roots. For this reason, analyses were not conducted on the roots for mine soils without BC (Table 3, Table 4, Fig. 4, and Fig. 5). Globally, the survival of the plants was improved by the presence of BC, especially for the 2% BC concentration (Table 3). Compared with the mine soil without BC, 2% and 5% BC favored higher plant biomasses, with significant differences depending on the species considered (Table 3). Thus, the global biomass of willow was, respectively, 14 times and 15 times higher for 2% and 5% BC; for ryegrass, overall biomass was, respectively, 2.4 times and 2.2 times higher for 2% and 5% BC. Growth difference was significant for all plant organs (Table 3).

Metal(loid) concentrations in plant organs were determined at the end of the experiment. Concentrations are given in Fig. 4 and metal (loid) mineralomasses are presented in Fig. 5. The metal(loid) miner-alomass, which represents the total amount of metal(loid)s accumu-lated in the plants, can be used to evaluate metal(loid) removal effi-ciency by the plant organs on dry weight. For willow growing on mine soil with BC, Pb concentration in cuttings, stems and leaves, as well as in the roots, decreased significantly ($P < 0.05$) mainly for plants growing on mine soil with 5% BC. For ryegrass growing in the presence of BC, $[Pb]_{plant}$ also decreased, even if a significant decrease was ob-tained only for plants growing on mine soil with 2% BC, due to high heterogeneity of plants in samples without BC (Fig. 4B). However, Pb mineralomasses (Fig. 5) seemed to depend on plant organs, with more Pb in roots of willow or in leaves of ryegrass. A similar but less marked trend was observed for Zn. Plants growing in the presence of BC (re-gardless of its percentage), had less Zn in their tissues (Fig. 4). There was no difference in Zn concentrations in willow leaves for the different treatments (Fig. 4A). For ryegrass, only concentration of Zn in plants growing on mine soil amended with 2% BC showed a noticeable dif-ference (Fig. 4B). A similar finding was established for Zn mineralomass (Fig. 5). Ba appeared to behave differently. The plants growing in the mine soil with BC and in the mine soil without BC had similar Ba concentrations in their organs (Fig. 4) or similar Ba mineralomass (Fig. 5). The willows growing in mine soil with BC tended to have lower concentrations of As and less As mineralomass in their organs, except for leaves (Figs. 4 and 5). When ryegrass growing on BC, a reduction of As in the leaves was noticeable although significant only for As miner-alomass (Fig. 5A). When the willow grew on mine soils with BC, Cd concentration and mineralomass were increased whatever the quantity of BC (Figs. 4 and 5).

Main contributing factors of F2 of the PCA (SM 2) were metal(loid)s in SPW (positively) and metal(loid)s accumulated in plants (nega-tively). This representation highlights opposing trends of concentra-tions of metal(loid)s in SPW and in plant organs, in particular for Pb and Zn (SM 2). This suggests that total metal(loid)s in plants depends on the level of concentration of metal(loid)s in SPW that the plant can absorb. These results were consistent with the influences of BC amendment on the amount of metal(loid)s in the SPW that can be up-taken by the plants.

3.5. Metal(loid) transfers into plant organs

The transfer of metal(loid)s was evaluated with the three factors: the translocation factor (TF), the bioconcentration factor (BCF), and the biological absorption (or accumulation) factor (BAF) (Table 4). These could not be determined in the absence of metal(loid)s in the SPW or when roots were nonexistent. The distribution of Pb in plant organs depended on the presence of amendments. For willow, Pb was mainly present in cuttings for plants growing on non-amended soil samples, whereas it was mostly present in the roots of plants growing on amended samples. Increasing BC content tended to reduce Pb con-centration in leaves of ryegrass growing on amended mine soils (Table 4). Regarding TF, none of the plants translocated Pb in aerial organs. The transport of Pb from SPW to aerial parts, expressed by the BAF, depended on the concentration of BC in the soil in which the

Table 4
Metal(loid) transfer into plant organs (translocation factors (TF), bioaccumulation factor (BAF), and bioconcentration factor (BCF)). W: willow, and R: ryegrass. 0, 2, and 5 for 0%, 2% and 5% biochar (BC). ANOVA was followed by a post-hoc Dunnett test at level P < 0.05 with n = 5 for NV0, NV2, NV5, R0 and R2; n = 4 for W2, W5, and R5; n = 3 for W0. Differences were evaluated comparing several treatments with a control (without BC namely W0 and R0). Letters a, b, c refer to statistics on samples without BC, letters A, B, C to statistics on samples with 2% BC and letters α, β to statistics on samples with 5% BC. For pairwise comparisons, analysis of variance was performed by a post-hoc Tukey test at different levels *P < 0.05; n.s. for not significant statistics, and n.d. not determined for unstated statistics (no data).

		Pb	Zn	Ba	As	Cd
Translocation factor (TF)	W0	n.d.	n.d.	n.d.	n.d.	n.d.
	W2	0.02 b n.d.	1.19 a n.d.	2.90 a n.d.	0.37 a n.d.	1.61 a n.d.
	W5	0.03 α n.d.	2.33 α n.d.	4.28 α n.d.	0.59 α n.d.	1.65 α n.d.
	R0	n.d.	n.d.	n.d.	n.d.	n.d.
	R2	0.16 a n.d.	0.29 b n.d.	1.40 a n.d.	0.14 b n.d.	0.09 b n.d.
	R5	0.28 α n.d.	0.52 β n.d.	1.56 α n.d.	0.29 α n.d.	0.30 β n.d.
Bioaccumulation factor (BAF)	W0	0.00 A	7.41 A	10.47 A	n.d.	n.d.
	W2	0.36 a n.s.	n.d.	0.40 a *	n.d.	n.d.
	W5	0.73 α *	n.d.	0.44 α *	n.d.	n.d.
	R0	0.00 A	2.04 B	3.95 A	n.d.	n.d.
	R2	0.00 b n.s.	0.33 n.d.	0.28 a *	n.d.	n.d.
	R5	0.00 β n.s.	1.05 β n.s.	0.31 α *	n.d.	n.d.
Bioconcentration factor (BCF)	W0	n.d.	n.d.	n.d.	n.d.	n.d.
	W2	15.73 a n.d.	n.d.	0.22 b n.d.	n.d.	n.d.
	W5	28.52 α n.d.	n.d.	0.19 β n.d.	n.d.	n.d.
	R0	n.d.	n.d.	n.d.	n.d.	n.d.
	R2	0.01 b n.s.	1.31 n.d.	2.28 a n.s.	n.d.	n.d.
	R5	0.01 β n.s.	2.36 n.d.	1.14 α n.s.	n.d.	n.d.

plants grew. Thus, BAF was higher for willows with 5% BC treatment (0.73) than for 2% BC (0.36). The level of transport of Pb from SPW to the root parts (expressed by BCF) was very high for all treatments planted with willow, whatever the BC concentration (15.73 and 28.52 respectively for plants growing in mine soils with 2% and 5% BC) (Table 4), whereas BCF was very low for ryegrass (0.01).

For Zn, only BAF and BCF calculated from $[Zn]_{SPW}$ had values greater than 1 (corresponding to W0 and R0, and R5 for BAF_{SPW} and corresponding to R2 and R5 for BCF_{SPW}) (Table 4). For Ba, the trans-location factors increased with the quantity of BC in which the plants grew. Ba translocation from the roots to the plants' aerial parts corre-lated positively with BC content. It was respectively 2 and 2.7 times higher for willow than for ryegrass in mine soils with 2% and 5% BC (Table 4). A high BAF_{SPW} was obtained for plants growing in soil without BC (~10.5 and ~4 for willow and ryegrass, respectively). BCF calculated from the $[Ba]_{SPW}$ was also high for R2 and R5. For Cd, willow presented a TF > 1 (Table 4).

4. Discussion

4.1. Effect of biochar on physicochemical parameters of soil pore water

The application of biochar (BC) had a significant effect on the physicochemical parameters of soil pore water (SPW), especially at the beginning of the experiment. It has already been evidenced (Ahmad et al., 2014) that BC can increase the values of pH, and electrical conductivity (EC) and Eh, and can provide several ions (NO_3^-, SO_4^{2-}, K^+, Ca^{2+}, Mg^{2+}, Cl^-) that can be quantified in SPW. The increase or decrease of dissolved organic carbon (DOC) depends on the BC used. Beesley et al. (2011) showed that BC contributes to increased soil or-ganic matter content, especially carbon and nitrogen, and also that it may react with metal(loid)s in soils more readily than native organic matter. However, our experiment did not show such an increase and the type of BC used in this experiment did not lead to increase in DOC (Table 2). This may be an obstacle for this experiment, but it has al-ready been demonstrated in other studies. As suggested by Ahmed et al. (2016), the carbon content of BC depends on the type of BC feedstock. According to Mukherjee and Zimmerman (2013), BCs contain a range of nutrient forms with different release rates (depending on BC feedstock and on the conditions of production of the BC). The physi-cochemical changes linked to BC clearly distinguished the amended soils from the soil without BC at the beginning of the experiment. However, this effect decreased over time since, by the end of the ex-periment, only the mine soil with the highest BC content (5%) differed from the other two conditions (without and with 2% BC) (Figs. 2 and 3).

4.2. Effect of biochar and plants on behavior of metal(loid)s

4.2.1. Effect of biochar and plants on availability of metal(loid)s

The study of SPW enables accounting for the concentration of water-soluble contaminants. This concentration corresponds in fact to the most labile fractions of metal(loid)s, which are the most bioavailable for living organisms but also the most ecotoxicologically hazardous (Temminghoff et al., 1998). Biogeochemical processes (e.g. precipita-tion, desorption, dissolution, adsorption, complexation, redox reac-tions) (Adriano et al., 2004) determine metal partitioning between the aqueous and solid phases and influence the availability and mobility of metal(loid)s in soil (Shahid et al., 2012a, 2012b). As metal(loid)s such as Zn, Pb, and Cd are divalent cations, they could be adsorbed by the same sites and functional groups in soil and thus compete. The appli-cation of BC had variable effects depending on the metal(loid)s con-sidered. It is important to note that the application of BC used as an amendment in the experiment immediately reduced the availability of Pb and Zn in SPW. At the end of the experiment, the benefit of this amendment was less marked than at the beginning (Figs. 2 and 3). This can be attributed to a decrease in sorption efficiency of BC. The Pb removal in aqueous solution by BC was diffusion-controlled (BC pore size dependent), starting with a rapid initial step followed by a slow-down as sorption approached equilibrium (Mohan and Pittman, 2007; Inyang et al., 2012). The availability of metals in the soil thereby de-creased with time (Houben et al., 2013; Burges et al., 2015).

As plant uptake of metal(loid)s from soil is passive (mass flow of water into the roots) or active (transport across the plasma membrane of root epidermal cells), response to metal(loid)s depends on plant species (Yoon et al., 2006). L. perenne is a metal excluder, accumulation in the shoots being limited (Nowack et al., 2004). S. viminalis L. is able to survive in a soil contaminated with As, Pb and Sb (Bart et al., 2016). A difference in the growing times of the two species could be

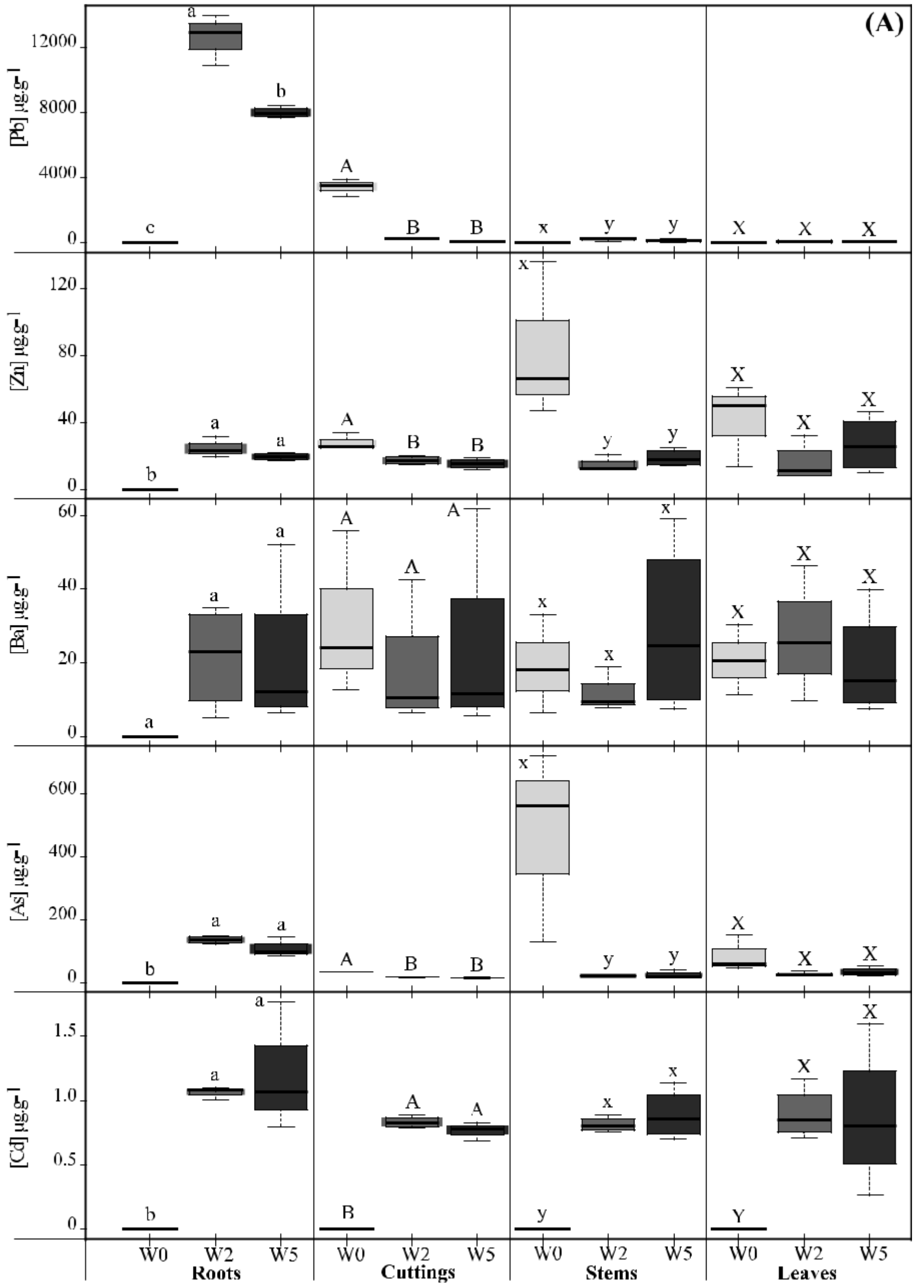

Fig. 4. Total metal(loid) (Pb, Zn, Ba, As, and Cd) concentrations ($\mu g.g^{-1}$) in plant organs for (A) willow and (B) ryegrass. W: willow, and R: ryegrass. 0, 2, and 5 for 0%, 2% and 5% biochar. ANOVA was followed by a post-hoc Tukey test at level $P < 0.05$ with n = 5 for NV0, NV2, NV5, R0 and R2; n = 4 for W2, W5, and R5; n = 3 for W0. For willow (A), letters a, b refer to statistics on roots, letters A, B to statistics on cutting, letters x, y to statistics on stem, and letters X, Y to statistics on leaves. For ryegrass (B), letters α, β refer to statistics on roots, and letters δ, ε to statistics on leaves.

problematic when comparing the removal of metals by these plants. Metal(loid) concentrations in the plants expressed as mineralomasses put the metal(loid)s accumulated in the plants at the same level. However, the primary effect of BC application to mine soil was to greatly improve plant survival and yields (Table 3). BC input to the mine soil improved plant lifetimes and biomasses by releasing ions used by plants for their nutrition, as has already been shown in other work (Fellet et al., 2014). This beneficial effect of BC is not always evidenced. Indeed, as suggested by Beesley et al. (2011), BC should be employed with a combination of composts, manures and other amendments be-cause some BC can retain nutrients useful for plants in degraded soils amended only with BCs. Without BC, neither ryegrass nor willow were able to develop a root system, as has already been observed for other mine soils (Park et al., 2011; Lebrun et al., 2017).

4.2.2. Effect on Pb behavior

Pb was the major contaminant among the metal(loid)s present in our mine soil. When BC was provided the amount of Pb in SPW reduced drastically without any influence from plants (Fig. 1). This may indicate that the BC had the potential to immobilize Pb. The immobilization of Pb in soils is mostly due to the strong affinity of Pb for soil organic matter, where it is strongly sorbed (Kushwaha et al.2018). The increase of the mine soil pH related to addition of BC could be the main factor explaining Pb immobilization (Fig. 2). $[Pb]_{SPW}$ and pH were correlated negatively at both the initial and final steps (Fig. 3). In addition, some authors have shown that BC application to water contributes to im-mobilization of metal(loid)s, and especially of Pb, through forces that are electrostatic (surface adsorption) and non-electrostatic (complexa-tion with functional groups) (Jiang et al., 2012; Lu et al., 2012). These

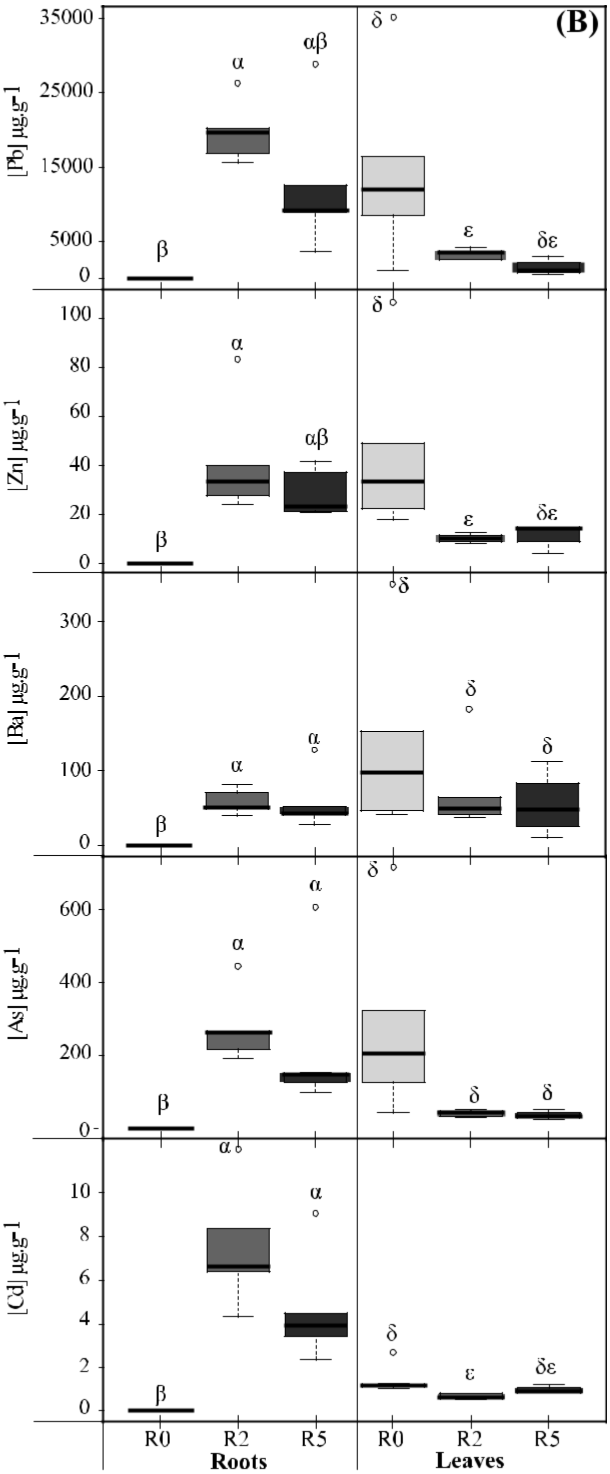

Fig. 4. (continued)

processes are strongly pH dependent. Indeed, as pH increases compe-tition between metal ions and protons decreases for binding sites on the BC or in the soil matrix, making adsorption more effective (Beesley et al., 2011). When BC is added to a soil (soil pH initially at about 4.7), it releases carbonates, phosphates and hydroxyl ions, promoting an overall liming effect (Fellet et al., 2014). Metal(loid)s present in the soil compete for binding sites on BC. Namgay et al. (2010) have demon-strated that BC sorption strength decreases in the order Pb > Cu > Cd > Zn > As. Pb could have greater affinity for the carboxylic and phenolic functional groups situated on the surface of BCs (Houben et al., 2013).

For willow and ryegrass growing on mine soil with 2% or 5% BC, Pb concentration in tissues was significantly lower than Pb concentration measured in plants growing in mine soil without BC. Ryegrass grown on mine soil with 2% BC influenced the behavior of Pb since only the 2% BC and ryegrass combination showed a significant difference with higher $[Pb]_{spw}$ than the other treatments. For both plants, even when growing on mine soil with BC, the majority of accumulated Pb was found in the roots (Figs. 4 and 5). Translocation of metal(loid)s from root to shoot is effective when the translocation factor (TF) > 1 (Baker and Brooks, 1989). Besides biomass production in the presence of metal (loid)s, metal(loid) bioconcentration efficiency is a parameter to eval-uate the feasibility of phytoremediation (McGrath and Zhao, 2003). Ryegrass leaves also accumulated Pb. Cheraghi et al. (2011) determined in their study that plants with a high biological absorption factor (BAF) (BAF > 1) are suitable for phytoextraction. Likewise, plants with a high bioconcentration factor (BCF) (BCF > 1) and low TF (TF < 1) have the potential for phytostabilisation. Even if the translocation factor was systematically below 1 for both plant species, this nonetheless leads to high phytoremediation efficiency parameters since BCF_{SPW} is > 1 for willow in mine soil with 2% and 5% BC (Table 4). These results agree with those of Wahsha et al. (2012) who showed that willows have the ability to accumulate Pb in roots more than in the aerial parts. With a high bioconcentration factor and a low translocation factor (BCF > 1 and TF < 1) willow seem to have the potential for phytostabilization of PB in this mine soil and under these conditions.

4.2.3. Effect on Zn and Cd behavior

If the effect of BC was obvious in reducing Pb availability, the mechanisms linked to BC are less clear for other metal(loid)s present in the mine soil (Fig. 1). Although the Cd concentration was below the detection limit (< 2 ppm) in the mine soil, $[Cd]_{SPW}$ was detectable at the initial step (Fig. 1). These differences were mainly due to the de-tection limit of the analysis method used (see materials and methods part). The presence of Cd at the same time as Zn could be of some importance, as the presence of Zn in the mine soil studied may have affected the behavior of Cd. Kabata-Pendias (2011) reported that Cd and Zn have similar ionic structures, electronegativities and chemical properties and Zn and Cd are water soluble, giving them high mobility in the soil. Beesley and Marmiroli (2011) have demonstrated the sorption of Zn onto BC particles, and that this retention was not im-mediately reversible.

Several authors have shown that Zn inhibits Cd uptake by plants. Zn competes with Cd because both metals are transported by a common carrier at the root plasma membrane (Pandey, 2012). Zn is more easily assimilated by plants than Cd, thus Cd phytoaccumulation decreases in the presence of Zn (Hart et al., 2005). Moreover, ryegrass induced a slight increase in Zn in SPW collected, in comparison with bared mine soil (Fig. 1). This may be due to the fact that the EC is higher in R samples than in NV samples (Table 2). Salimi et al. (2012) highlighted that high EC levels of irrigation water increased Cd concentration and decreased Zn concentration in the shoots of Helianthus annuus. Plants growing in BC amended mine soil tended to have lower Zn uptake but, for Cd, plant concentration and mineralomass were increased for plants growing in BC amended mine soil. However, unlike Pb, Zn was easily transferred to willow shoots, as were Ba and Cd. Zn and Cd were transferred from roots to aerial parts for the willow but not for the ryegrass (Table 4). BAF_{SPW} of Zn were > 1 for soil without BC but also with 5% BC, especially for ryegrass. This indicates a certain mobility of Zn but also an easy transfer into the aerial parts of the plants (Table 4). Most of the Zn was found in the leaves of the willows (Figs. 4 and 5). Cd uptake depends on the exposure time and the species (Grant et al.,

1997). Willows can tolerate and accumulate Cd in the root system: Landberg et al. (2011) have demonstrated that S. viminalis accumulates Cd in leaves.

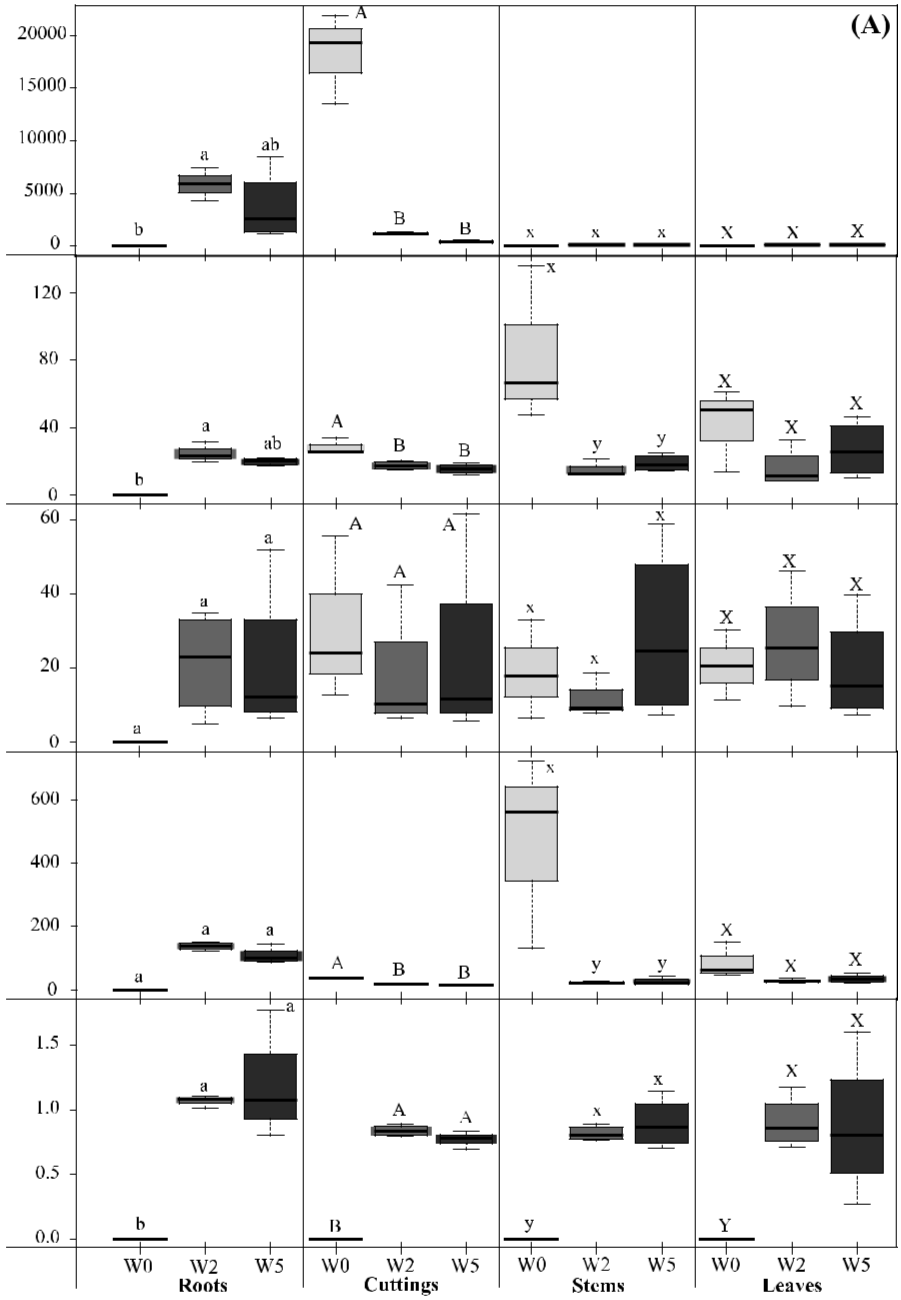

Fig. 5. Mineralomass (μg) of metal(loid)s (Pb, Zn, Ba, As, and Cd) from biomass for (A) willow and (B) ryegrass. W: willow, and R: ryegrass. 0, 2, and 5 for 0%, 2% and 5% biochar. ANOVA was followed by a post-hoc Tukey test at level $P < 0.05$ with $n = 5$ for NV0, NV2, NV5, R0 and R2; $n = 4$ for W2, W5, and R5; $n = 3$ for W0. For willow (A), letters a, b refer to statistics on roots, letters A, B to statistics on cutting, letters x, y to statistics on stem, and letters X, Y to statistics on leaves. For ryegrass (B), letters α, β refer to statistics on roots, and letters δ, ε to statistics on leaves.

4.2.4. Effect on Ba behavior

Without BC, $[Ba]_{SPW}$ was relatively low and was not influenced by the presence of plants (Fig. 1). Even without plants, the mobility of Ba was less in mine soils without BC than in mine soils with BC, despite the more basic pH of SPW of the latter (Table 2). It would appear that in this study the BC contributed to Ba mobilization in SPW whereas pH of SPW increased (Table 2 and Fig. 1). The change in pH by adding BC was therefore not the factor that determined the mobility of Ba and its transfer to plants. These results agree with McBride et al. (2014), who reported no significant relationship between Ba in vegetables and soil pH. Lamb et al. (2013) and Myrvang et al. (2016) reported that the plant uptake of Ba was mainly controlled by the exchangeable Ba fraction in soil. Ball and Nordstrom (1991) noted that the concentration of free Ba in natural water is limited by equilibrium with barite ($BaSO_4$) and witherite ($BaCO_3$). For Suwa et al. (2008), Ba solubility can be increased by the presence of other anions, except for SO_4^{2-}. Thus, Ba solubilization was not controlled here by pH, but more probably by the ions released by BC (SM 1). This agrees with Bodek et al. (1998) who showed that the solubility of BaSO4 increases considerably in the pre-sence of chloride (Cl^-) and other anions (NO_3^- and CO_3^{2-}). Ba may be mobile in soil, since it associates primarily with soil colloids by ion exchange (Zhang et al., 2001). Zhang et al. (2001) also showed that sorption of Ba^{2+} on montmorillonite was not very sensitive to pH and that displacement of sorbed Ba increased with $NaNO_3$ concentration. Here the study of correlations by treatment (SM 3) highlighted the in-fluence of major ions rather than that of pH.

Ryegrass growing on soil treated with 2% BC showed a significant difference in relation to the other treatments by an increase in the

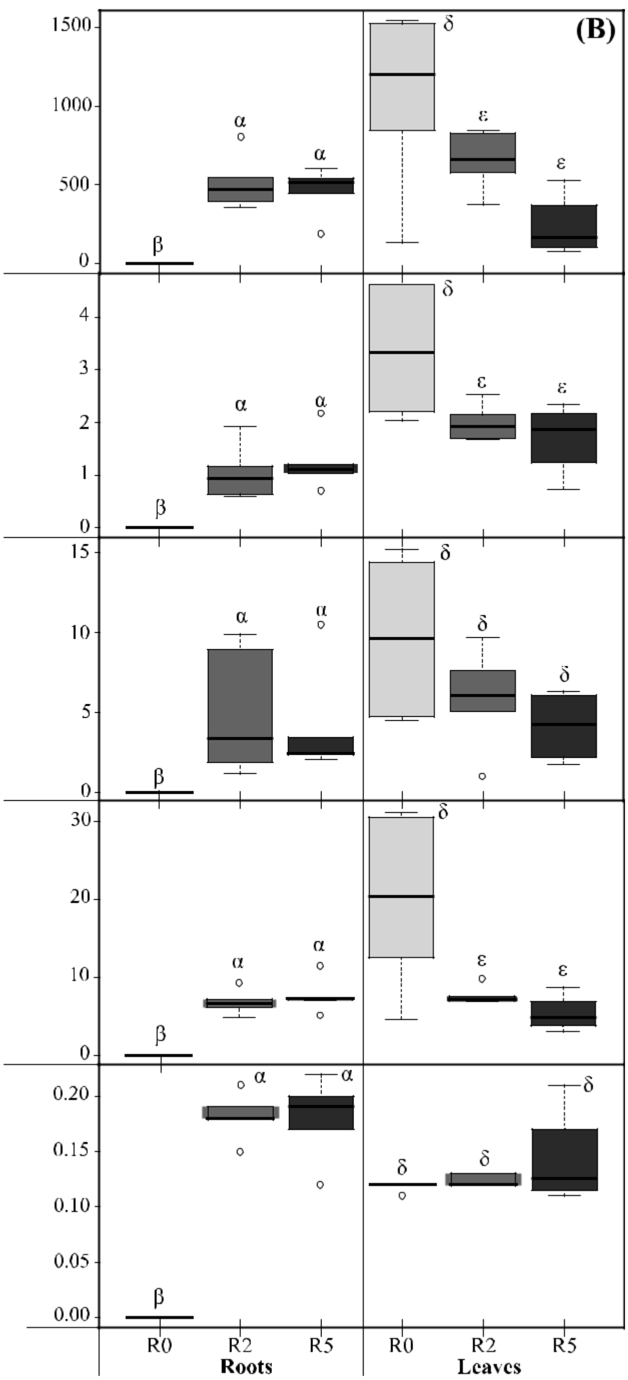

Fig. 5. (continued)

proportion of soluble Ba. Ba concentration in most plants ranged from 2 to 13 $mg.kg^{-1}$. Bowen and Dymond (1955) have reported a range of 0.5–400 $mg.kg^{-1}$ of Ba in different plant species growing in different soils. Ba concentrations of 200 $mg.kg^{-1}$ have been found to be mod-erately toxic for plants, and an excess of 500 $mg.kg^{-1}$ was considered to be toxic (Pais and Jones, 1997). Ba concentration or mineralomass for plants growing in BC amended mine soil was the same as for plants growing on mine soil without BC. Here the average concentrations (all organs combined) showed low Ba uptake by plant tissues (Fig. 4). Ba may accumulate in different parts of plants (IPCS, 1990), as shown here by translocation factors systematically above 1. Willows seemed to translocate more Ba than ryegrass (Table 4). Furthermore, even if $[Ba]_{SPW}$ was lower without BC (Fig. 1), BAF_{SPW} was clearly above 1 (Table 4). Here, the willow tended to translocate Ba in its aerial parts while the ryegrass accumulated it in its roots ($BCF_{SPW} > 1$ for the ryegrass only) (Table 4). Ba therefore showed the same plant-soil transfer mechanism as Ca (Bowen and Dymond, 1955) and they may substitute for each other (Kabata-Pendias, 2011). Here, the cumulative effect of the Ba absorption with the other metal(loid)s may reinforce the harmful effect of the contamination in this mine soil and should be considered as a potential concern as it may impact Ba movement in the ecosystem (Lamb et al., 2013).

4.2.5. Effect on As behavior

Arsenic can bind to anion exchange sites on soils (e.g. Fe, Al and Mn oxides and oxyhydroxides) (Dzombak and Morel, 1990; Masscheleyn et al., 1991; Moreno-Jimenez et al., 2012). Surprisingly, the addition of BC and an increase in the pH of the SPW did not increase the amount of As in SPW (Table 2 and Fig. 1). Nevertheless, high pH values can produce either a co-precipitation of As in the subsequently formed oxyhydroxides and sulfates (García et al., 2009), or a precipitate such as calcium arsenate in the presence of sulfates and carbonates. In our study, the $[As]_{SPW}$ was no longer detectable at the end of the experi-ment, whatever the presence of plants and amount of BC provided (Fig. 1). It can therefore be considered that neither the addition of BC nor the presence of plants seemed to be involved in this disappearance of As from the SPW, but that this may be due to physicochemical phenomena arising from the daily watering (probably leaching or im-mobilization by soil constituents). Several authors (Fitz and Wenzel, 2002; Beesley et al., 2014) have shown that competition may occur between DOC and As for soil retention sites. That work has linked the increase of DOC in SPW with the As concentration in solution (Table 2 and Fig. 1). Here, comparatively, DOC decreased during the experiment (Table 2), which may have reduced the competition between DOC and As for sorption sites. Finally, it can be assumed that the As present in the mine soil was not very mobile.

Concerning plant effects, BC addition generally significantly de-creased the mobility and availability of metal(loid)s in soil and reduced the phytotoxic concentrations of metal(loid)s (Abdelhafez et al., 2014; Lahori et al., 2017). As concentration and mineralomass tended to be reduced for plants growing in BC amended soil. Even so, the effect of plant cultivation on mine soil with BC was not very clear regarding As concentration in plants and disagreement exists in the literature. Hartley et al. (2009) reported no increase of As concentrations and transfer in the plant, while a decrease of these parameters was observed elsewhere (Namgay et al., 2010; Beesley et al., 2013; Lebrun et al., 2017). If As was no longer detectable in SPW at the end of the ex-periment, it was absorbed by plants (Figs. 4 and 5). This suggests that the immobilization (or leaching) of As present in the water takes place gradually over time. Whereas BC had no visible effect on $[As]_{SPW}$ (Fig. 1), it induced a decrease in $[As]_{plants}$, essentially in leaves (Figs. 4 and 5). The reduction of As uptake by plants in the presence of BC may be due the sorption of As by BC (Namgay et al., 2010). Another hy-pothesis would be linked to pH: as BC induces an increase of soil pH, precipitation of iron oxides in the rhizosphere may be favored, and As could be retained in these iron sinks. The higher As concentrations in the roots in the presence of BC may support this hypothesis.

5. Conclusion

This work suggests that the addition of biochar (BC) to a con-taminated mine soil improved the nutrient status of this mine soil (especially 5% BC) and contributed to a better establishment of plants. It appears that BC enhances root development and promotes willow and ryegrass growth. The effects of BC on plant growth and on the behavior of metal(loid)s depend on the metal(loid)s considered. The effect of BC on Pb behavior is clearer than for the other metal(loid)s investigated. By increasing pH, BC reduced soluble concentrations of Pb and Zn but, surprisingly, it did not seem to have any influence on the behavior of

As. The release of ions from the BC led to an increase in soluble con-centrations of Ba. The beneficial effect of BC was evidenced for accu-mulation of metal(loid)s in plants, especially for As. The effect of plants on behavior of metal(loid)s was shown only for ryegrass grown on mine soil with 2% BC (and only for Ba and Pb). Zn, Ba and Cd were con-centrated in willow and ryegrass leaves of plants growing in mine soil with or without BC.

Future work should be focused on gaining a better understanding of metal(loid) phyotoavailability and of speciation of metal(loid)s in mine soil solid fractions (electron microscopy and X-photon spectroscopy and/or sequential extractions) to understand the complex mechanisms driving their sorption/desorption in the presence of BC. As the behavior of Ba in contaminated environments is not fully understood, it would be interesting to extend knowledge underlying the mechanisms involved in its availability and potential transfer into environmental compart-ments. The phytoremediation studied here showed that the contribu-tion of an amendment (BC) combined with plant cultures (willow, ryegrass) modified the behavior of certain metal(loid)s (Pb, Zn, and Ba) in soil pore water (SPW) while it only slightly influenced others (As, Cd). Special attention could be paid to understanding which ions and/ or what ion concentration makes Ba more mobile. Thus, in the assisted phytoremediation strategy using BC, precautions must be taken if soils have high concentrations of Ba. In that case, it is also important to know the plants' affinity for Ba.

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Appendix A. Supplementary data

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