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Phytoextraction of Ni from a toxic industrial sludge amended with biochar

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ABSTRACT

Agromining is a technology based on the phytoextraction of metals by hyperaccumulator plants, combining agronomic and hydrometallurgical processes, to produce metal-based compounds. So far, it has been primarily developed to recover Ni from ultramafic soils, but secondary materials, namely industrial wastes containing Ni, may offer new opportunities for agromining. However, because of the toxicity of such materials, plants cannot be grown without formulating suitable substrates. The aim here was to assess the feasibility of growing Ni-hyperaccumulating plants on a Technosol containing a toxic industrial sludge and to test the influence of a biochar amendment on plant growth and Ni uptake. A constructed soil was prepared by mixing a decontaminated soil with an industrial sludge containing high concentrations of Fe, Ni, P and Zn, and amending it with biochar at different rates (0 to 5 wt%, dry matter). An ultramafic, Ni-rich soil was used as a reference material. Pot experiments were conducted with the hyperaccumulator *Alyssum murale* and the non-accumulating plant *Lolium multiflorum* used as a reference plant. After twelve weeks of growth, plant shoots and roots and soil samples were collected and analysed. Soil pore water was also collected over the experiment and analysed.

Results showed that the growth of both plants was higher on the constructed soil than on the ultramafic soil, and increased with biochar amendments. The highest amounts of phytoextracted Ni were reached by *A. murale* on the ultramafic soil in the presence of biochar, whereas they remained low on the constructed soil. Contrary to the ultramafic soil, the constructed soil contained high amounts of Zn which was shown to impair Ni uptake as a result of the strong competition between Ni and Zn. Further investigations should therefore focus on practical solutions for decreasing this competition in order to maximize Ni uptake. In conclusion, agromining was proven feasible on soils constructed from industrial waste containing metals, providing that such soils are carefully designed to meet hyperaccumulator requirements.

1. Introduction

In the context of rarefaction of primary resources, agromining is a new way to recover strategic metals from contaminated soils, sediments or wastes too weakly concentrated for conventional mining or direct metal recovery. Agromining is a production chain of metal compounds (*e.g.* oxides, salts, complexes) based on the combination of agronomic and hydrometallurgical processes (van der Ent et al., 2018; Morel, 2013). Agromining consists of growing hyperaccumulator plants (HA) on metal-bearing substrates, harvesting the shoots, and finally recovering the metal with pyro/hydrometallurgical processes to produce compounds of economic interest (Barbaroux et al., 2012; van der Ent et al., 2018). Agromining has been tested at field scale for the recovery of nickel (Ni) from ultramafic soils with the hyperaccumulator plant *Alyssum murale* Waldst. & Kit (Bani et al., 2015a, 2015b; Pardo et al., 2018). For example, up to 120 kg of Ni ha^{-1} were harvested on ultramafic soils in Albania, allowing the production of Ni salts of high purity (Barbaroux et al., 2011). Ultramafic soils are characterized by Ni concentrations that can be higher than 1 g kg^{-1} (Proctor and Woodell, 1975), high concentrations of iron (Fe), magnesium (Mg) and chromium (Cr), calcium (Ca) deficiency, low water retention capacity and low nitrogen (N), phosphorus (P) and potassium (K) nutrient levels (Proctor et al., 1981).

As well as natural soils, secondary materials such as urban and industrial wastes offer another opportunity to recover strategic metals. However, contrary to soils, industrial wastes containing metals of interest seldom exhibit favourable conditions for plant establishment and growth, and subsequent phytoextraction of metals (Rosenkranz et al., 2017). Wastes are generally very poor in organic matter, present an extreme pH, have unbalanced nutrient contents, may have a high

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salinity and are poorly structured, with limited oxygen and water supply. Moreover, they often contain mixtures of metallic elements, which can inhibit root growth and impair plant development and metal uptake. Furthermore, competition between elements for plant uptake may reduce the accumulation of the targeted elements (*e.g.* Deng et al., 2014 for Ni:Zn interactions in *A. murale*; Broadhurst et al., 2009; Ghaderian et al., 2015 for Ni:Mn and Mnasri et al., 2015 for Ni:Cd interaction), leading to the accumulation of undesired elements (*e.g.* Co, Zn). Agromining from industrial wastes may therefore fail without preliminary operations to build a suitable growth medium. Growing plants on industrial material has been achieved with soil construction processes using a variety of secondary materials (*e.g.* sewage sludge, composts, treated contaminated soil) (Séré et al., 2010, 2008; Yilmaz et al., 2018). It has allowed the establishment of agro-forestry practices on brownfields subsequently used to produce biomass for energy, fibres, and agromining of metals contained in contaminated soils. In the case of toxic industrial wastes, specific conditions must be created to allow plant growth, and avoid any release of harmful compounds into water bodies or into the air; the latter being achieved with the implementation of closed growth systems with the collection of drainage water.

A wide range of amendments can be used to improve a given substrate, including composts, steel slags, lime and biochars (Álvarez-López et al., 2016; Lagomarsino et al., 2011; Meers et al., 2008; Puschenreiter et al., 2005). An increase in plant biomass production with biochar amendments has often been reported and has been attributed to the increase of soil water- and nutrient-holding capacities, cation exchange capacity (CEC), and the improvement of the biological properties of the amended soils (Liang et al., 2006; Paz-Ferreiro et al., 2014; Streubel et al., 2011; Chintala et al., 2014). Biochar also allows germination and plant establishment on phytotoxic matrices (Beesley et al., 2011; Rees et al., 2015). The addition of biochar frequently decreases metal mobility, through direct sorption of heavy metals in soils or indirect phenomena. *e.g.* an increase in soil pH (Beesley et al., 2011; Rees et al., 2014; Uchimiya et al., 2010). Accordingly, a decrease in plant metal uptake is generally reported (Fellet et al., 2014; Houben et al., 2013a; Lu et al., 2014; Rees et al., 2015). However, this effect may depend on several parameters such as soil properties, biochar type or metal, and is not always observed (Beesley et al., 2011; Fellet et al., 2014; Rodríguez-Vila et al., 2014). In particular, the application of biochar led to a decreased competition between cations and metal and then increased Cd uptake by the hyperaccumulator *Noccaea caerulescens* (J. Presl & C. Presl) F. K. Mey. (Rees et al., 2015). Moreover, the total root surface developed by the plant can increase in biochar-amended soils, leading to a higher plant metal uptake (Rees et al., 2016). To the best of our knowledge, no investigation has been published regarding the application of biochar for improving hyperaccumulator plant growth on ultramafic soils. Only one study has reported an experimentation on an ultramafic soil (pH 5.5), where the application of 5% (%w) wood biochar increased biomass production, and strongly reduced the uptake of Cr, Ni and Mn by > 90% in tomato plants (Herath et al., 2015).

The present work was undertaken in order to assess the feasibility of agromining of Ni from toxic industrial wastes, and to test the influence of biochar on plant growth and Ni uptake. To achieve this goal, an industrial waste was chosen. It was a sludge derived from a metal surface treatment in the automotive industry containing high amounts of metals and phosphate, exhibiting a very low pH, a high salinity, and a low organic matter content. In order to decrease the high phytotoxicity induced by the sludge, we used a similar strategy to that developed for soil construction on brownfields to formulate suitable mixtures. The industrial sludge was mixed with a soil material unsuitable for food production, and amended with various doses of biochar. An ultramafic soil naturally rich in Ni was selected as a reference soil material. Based on previous data (Bani et al., 2015a, 2007) we chose the Ni-hyperaccumulator plant *A. murale* for its ability to accumulate Ni from ultramafic soils and to grow in various pedoclimatic conditions. We also selected the grass *Lolium multiflorum* Lam. as a reference non-accumulating plant in order to highlight possible specific interactions between biochar amendments and metal-hyperaccumulating plants, as suggested in previous works (Rees et al., 2015, 2016). A pot experiment was the set-up to i) test the feasibility of phytoextracting Ni from an industrial sludge in comparison with a natural soil and ii) determine how biochar amendments may affect Ni uptake by *A. murale* in comparison with *L. multiflorum* in such soils, and iii) determine the optimal biochar rate to be used in field scale for maximizing Ni extraction for the purpose of process optimization.

2. Materials and methods

2.1. Materials

Soil materials, sludge and biochar were analysed by the Laboratoire d'Analyse des Sols at INRA-Arras (France) using standard techniques (AFNOR 2013): granulometry (NF X 31-107), pH in water (1/5 ratio (v/v), NF ISO 10390), conductivity (water extract at 25 °C, 1/5 (w/v)), total N (NF ISO 13878), total organic C (NF ISO10694), available P (Olsen method, NF ISO 11263), CEC (NF ISO 23470 for soils and Metson method NF 31-130 for biochar), total trace elements (extraction with HF-$HClO_4$, NF X 31-147), pseudo-total elements (extraction with

Table 1
Main characteristics of the materials.

Parameter	Unit	Ultramafic soil (U)	Sludge	Soil treated by biopile	Constructed soil (C)	Biochar
Clay	$g\,kg^{-1}$	290	n.a.	117	158	n.a.
Silt	$g\,kg^{-1}$	367	n.a.	145	233	n.a.
Sand	$g\,kg^{-1}$	343	n.a.	738	609	n.a.
pH	–	6.58	3.83	8.45	6.46	9.62
Conductivity	$mS\,cm^{-1}$	0.0677	2.08*	0.214*	0.873	1.99*
Total N	$g\,kg^{-1}$	2.14	13.9	0.405	1.79	5.25
Organic C	$g\,kg^{-1}$	28.7	2.11	24.2	25	685
Available P	$g\,kg^{-1}$	0.007	6.19	0.292	0.515	0.0913
CEC	$cmol^{+}\,kg^{-1}$	39.7	n.a.	8.74	8.00	3.20
Water holding capacity	%	59.2*	159*	17.2*	25.9*	n.a.
Ni_{HF}	$mg\,kg^{-1}$	1780	5070	41.1	553	13.2
$Mn_{HF(or\ AR)}$	$mg\,kg^{-1}$	1830	$9940^{(AR)*}$	631	1490	521
Zn_{HF}	$mg\,kg^{-1}$	177	65,700	181	6230	94.4
Ni_{DTPA}	$mg\,kg^{-1}$	64.3	815*	0.392	73.8	0.154
Mn_{DTPA}	$mg\,kg^{-1}$	8.11	179*	13.3	53.9	27.5
Zn_{DTPA}	$mg\,kg^{-1}$	1.17	776*	16	90.8	6.05

The analyses were carried out by the Laboratory of Laboratoire d'Analyse des Sols of INRA-Arras excepted the values followed by *, which were obtained in the Laboratory Soil and Environment. n.a.: not available. $^{(AR)}$: values obtained after aqua regia extraction.

aqua regia, ISO NF 11466) and exchangeable trace elements with DTPA (diethylene triamine pentaacetic acid) with soil/solution ratio 1/2 (w/v) at pH 7.3 (NF ISO 31-121) (Table 1). The water-holding capacity (WHC) was also determined by measuring the water content of the soil samples adjusted to a water pressure of 104 Pa (pF 2) using a ceramic plate under pressure (Labotest 11,500, 11,600) (Table S1).

Ultramafic soil material was collected from the top layer (10–40 cm) on a single determined area of an hypermagnesic hypereutric Cambisol covered by a natural forest (Vosges Mountains, north-eastern France, 48°11′03.7″N, 07°06′42.2″E). This silty clay soil exhibited a slightly acidic pH (6.58), contained 28.7 g kg^{-1} of C organic (C_{org}), and showed a CEC of 39.7 $cmol^{+}$ kg^{-1} (Table 1 and Table S2). As expected, it was rich in Ni (0.178%), Fe (9.88%) and Cr (0.254%) and presented a Ca/Mg quotient < 1 (Table S3).

The industrial sludge was generated by the automotive industry, using metal and phosphate solutions for anti-corrosion treatment by galvanizing. Sludge exhibited an acidic pH (3.9), and contained high amounts of metals, primarily Fe (25%) and Zn (6.7%), and secondarily Mn (0.99%) and Ni (0.57%). It also contained a significant amount of N (1.4%) and a large amount of P (49%). DTPA-extractable Ni, Zn and Mn were also very high.

The filling material was a sandy-loam soil excavated from an industrial soil polluted by organic compounds and metals and treated by biological treatment (biopile) by SITA company (Jeandelaincourt, France). The treated soil was alkaline (pH 8.45) and exhibited a high conductivity of 0.21 mS cm^{-1}.

Both materials were air-dried, crushed when necessary and sieved to 2 mm. They were then thoroughly mixed by hand three times for 5 min using a dry weight ratio of industrial sludge over treated industrial soil material of 1:9. Once homogenized, the mixture, hereafter referred to as 'constructed soil', was sampled for analysis. This constructed soil exhibited a balanced texture for the three particle classes. The pH was close to neutrality and the CEC rather low; other parameters are presented in Table 1.

Biochar was produced by the company Maschinenring (Wängi, Switzerland) from 50% coniferous and 50% hardwood chips pyrolyzed at *ca.* 650 °C using the Pyreg process. Before use, it was dried at 20 °C, manually crushed and sieved at 2 mm.

Ultramafic and constructed soils had a close composition regarding pH (6.6 and 6.5), organic C (29 and 25 g kg^{-1}), and N (2.1 and 1.8 g kg^{-1}). However, high concentrations of Zn and P were measured in the constructed soil. Total Ni concentration was higher in the ultramafic soil than in the constructed soil, but the fraction of DTPA-extractable Ni was identical in both soils (Table S4). After mixing both soil with different rates of biochar (0, 1, 3 and 5), each of the eight treatments was homogenized in the same way as that used for the constructed soil. A subsample of the < 2 mm fraction dried at 40 °C for 72 h was used for pH measurement and another one used to measure the DTPA-extractable metals. pH was measured in a soil-water suspension (vol. ratio 1:5) according to the NF ISO 10390 standard. The soil suspension was then centrifuged and filtered through a 0.45 µm filter before measuring electrical conductivity. Extractable Ni, Zn and Mn were determined using a DTPA-TEA solution. These analyses were performed in triplicate. Throughout the whole text, all the percentages are weight percentages, referring to dry matter.

2.2. Plant growth experiment

Biochar was added to the substrates at rates of 0, 1, 3 or 5% relative to substrate (dry matter), and the mixture was manually homogenized. One rhizon sampler (model 192,122, Rhizosphere Research Products, 5 cm long and 2.5 mm in diameter) was introduced in each pot at an angle of 45° towards the centre of the pot and connected to a syringe. Pots were filled with either 220 g of ultramafic soil or 260 g of constructed soil. As a result, each pot contained either 392 mg total Ni (Ni_{HF}) and 13 mg Ni_{DTPA} for the ultramafic soil, or 144 mg total Ni (Ni_{HF}) and 17 mg Ni_{DTPA} for the constructed soil.

Pots were placed in a growth chamber with the following conditions: photoperiod of 16 h of day/8 h of night, temperatures of 25 °C day/18 °C night, 50% air humidity. Soil moisture was adjusted to 80% of the WHC by adding ultrapure water. Pots were left unplanted for four weeks before sowing. Soil moisture was controlled by weighing pots every two days and adding water if necessary.

Two plants were selected: *Alyssum murale*, collected from several plants in 2011 at the surface of an ultramafic soil from the area of Metsovo (Greece), and commercially available *L. multiflorum* cv. 'Lema' (Italian ryegrass). Ten seeds of *A. murale* were sown by pot and later only the three strongest individuals were retained. For *L. multiflorum*, 0.5 g of seeds was sown in 100 cm^2 (or 100–110 seeds per pot). The experiment included two soils, four rates of biochar, two plants per pot and an unplanted control, each replicated five times (Fig. S1). The 120 pots were arranged in five randomized blocks.

Pore-water was sampled in three of the five pots of each treatment. It was sampled just after sowing, and then every five weeks. On each sampling day, pots were watered to reach the WHC, and the pore-water was extracted 4 h later.

Both species were harvested twelve weeks after germination. The plants were cut at the root collar. After sieving the full content of each pot to 2 mm, roots were collected at the surface of the sieve using forceps.

2.3. Analysis of pore-water

pH was measured in pore-water samples according to NF ISO 10390 standard, with a HQ440d Multi (Hach) equipment and an IntelliCALTM pH PHC101 probe. Conductivity was measured with a CyberScan CON 100 conductometer and a V21 probe JJ0-043 (EUTECH INSTRUMENTS). Samples were acidified with HNO_3 (2%, v/v) prior to elemental analysis. The Ni, Zn and Mn concentrations of the solutions were obtained by Inductively-coupled Plasma – Optical Emission Spectrometry (ICP-OES, Thermo Fischer iCAP 6300 Duo), calibrated with certified solutions (PlasmaCAL, SCP Science). A standard solution was introduced into the sample series for analytical control over time (EU-H-4 CRM EnviroMAT, SCP Science). Dissolved organic and inorganic carbon were measured with TOC VSCN (Shimadzu). Total N was measured according to the principle of 'oxidative combustion-chemiluminescence' with a NOx adsorber (Shimadzu). Ionic concentrations were measured by ion chromatography (Dionex, IC25 chromatograph and EG40 eluent generator), with AS11-HC columns for anion analysis (NO_3^-, Cl^- and SO_4^{2-}) and CS12 A-5 µm for cation analysis (Na^+, K^+, Mg^{2+}, Ca^{2+} and NH_4^+).

2.4. Analysis of plant material

Shoots and roots were washed with ultrapure water. In the case of *A. murale* only, roots were scanned and root total length, surface area and mean diameter were measured using Winrhizo software (V. 2005c, Regent Instruments). Then all roots and shoots were weighed and dried at 40 °C for 72 h and ground using a tungsten pestle and mortar. A sample of 0.500 g was digested using 5 mL HNO_3 (65%) and 2.5 mL H_2O_2 (30%) (for smaller samples, 0.050 g with 2 mL HNO_3 and 1 mL H_2O_2). After 16 h, samples were placed in a DigiPREP® system (SCP Science, Baie-d'Urfé, QC, Canada) for 120 min at 95 °C and the resulting solutions were diluted to 25 mL (or 10 mL for smaller samples) with ultrapure water (Millipore 18.2 MΩ·cm at 25 °C) then filtered at 0.45 µm before analysis with ICP-OES (Thermo Fisher iCAP 6300 Duo) for Ni, Zn and Mn. Total C and N concentrations were measured with a CHNS (VARIO MICRO cube, ELEMENTAR) according to NF ISO 10694 and NF ISO 13878 standards. The amount of phytoextracted Ni (mg Ni per pot) was calculated for both plant species from the product of the shoot biomass yield x Ni concentration in the shoot biomass.

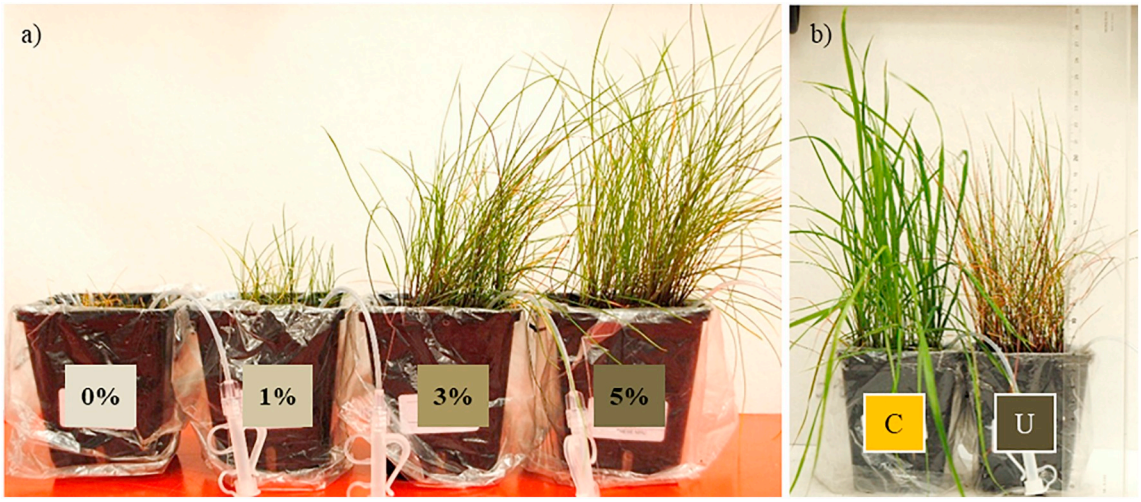

Fig. 1. *L. multiflorum* after a) four weeks on ultramafic soil amended with biochar (%w) and b) six weeks on constructed soil (C) and ultramafic soil (U) with 3% biochar.

2.5. Statistical analyses

Statistical analyses were conducted with R software, version 3.1.2 (2014-10-31). Under R, possible outliers were identified by the Dixon test, the outliers package. When the data followed a normal distribution (Shapiro test) and were homoscedastic (Levene test), a parametric test (Newman-Keuls or *t*-test) was applied. If the data did not meet both criteria, they were transformed to log values in order to meet the conditions of application of the parametric tests. If the data after transformation still did not follow a normal distribution and/or were not homoscedastic, a non-parametric test (Kruskal-Wallis) was run, then a *post hoc* test by the FDR method.

3. Results

3.1. Plant germination and growth

Both plant species responded in the same way to the different soil treatments. Shoot biomass production of both plant species was generally higher on the constructed soil than on the ultramafic soil (Figs. 1b, 2, 3 and Fig. S2). Seeds germinated earlier and developed faster with biochar amendments (Fig. 1a and Table S5). The two first pairs of leaves (those succeeding the cotyledonary leaves) appeared earlier with the highest biochar rate. At harvest, the shoot biomass of both plant species generally increased with increasing biochar rate on both soils.

Lolium multiflorum showed larger, greener and longer leaves on the constructed soil than on ultramafic soil (Fig. 1b). Its shoot biomass was 67–99% lower on ultramafic soil than on the constructed soil. Root biomass of *L. multiflorum* was increased in the presence of biochar in ultramafic soil, but no significant change was recorded on the constructed soil.

Similar to *L. multiflorum, A. murale* produced significantly more shoot biomass on the constructed soil than on the ultramafic soil (Fig. 3). Biomass production significantly increased with biochar on both soils, and the highest biomass was obtained at the highest biochar rate. Similar trends were recorded for the root biomass of *A. murale*, which increased with biochar rates on both soils. The root surface of *A. murale* grown on the ultramafic soil increased with the biochar rate from 247 ± 58 cm^2 with 1% to 452 ± 11 cm^2 with 5%. However, it was lower in the constructed soil (245 ± 11 cm^2) than in the ultramafic soil at 5% of biochar.

3.2. Ni uptake by plants

Ni concentrations in *L. multiflorum* in both shoots and roots were higher for the constructed soil than for the ultramafic soil (Fig. 4). On the constructed soil, Ni concentration in roots and shoots decreased with increasing rates of biochar, whereas no significant effect of biochar was observed with the ultramafic soil.

Nickel concentration of the hyperaccumulator grown on the ultramafic soil reached 2.4% in shoots (Fig. 5). However, shoot concentrations of *A. murale* reached only 0.1% Ni on the constructed soil. Contrary to the case of *L. multiflorum*, Ni concentrations in shoots of *A.*

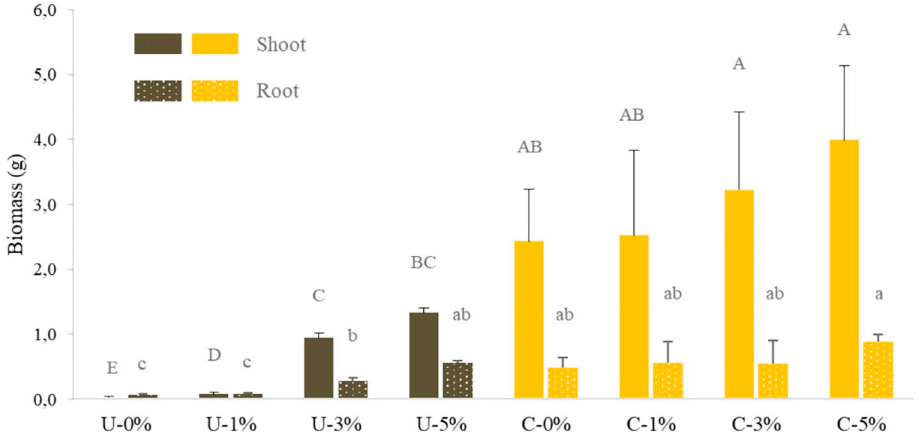

Fig. 2. Shoot and root biomass of *Lolium multiflorum* after 12 weeks of culture on ultramafic soil (U) and constructed soil (C) at different biochar doses (0, 1, 3 and 5%). Vertical bars represent standard deviations. Values for shoot affected by different capital letter were significantly different (Newman Keuls, $p \leq 0.05$), values for roots affected by different lowercase letter were significantly different (*t*-test, $p \leq 0.05$), log-transformed data.

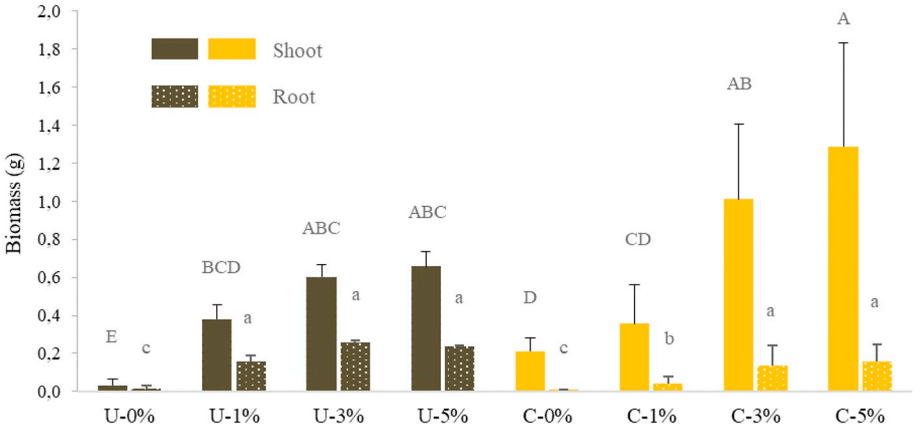

Fig. 3. Shoot and root biomass of *A. murale* after 12 weeks of culture on ultramafic soil (U) and constructed soil (C) at different biochar doses (0, 1, 3 and 5%). Vertical bars represent the standard deviations. Values for shoots affected by different capital letter were significantly different (Newman Keuls, $p \leq 0.05$); roots values affected by different lowercase letter were significantly different (Newman Keuls, $p \leq 0.05$), log-transformed data.

murale were either significantly higher than in roots in the case of the ultramafic soil or similar to the root concentrations in the case of the constructed soil. No effect of biochar on Ni concentrations was observed on the constructed soil for any biochar rate (*t*-test, $p \leq 0.05$), but significantly lower concentrations of Ni were measured at the higher biochar rates in plant shoots and roots harvested on the ultramafic soil (Fig. 5).

The translocation factor (quotient of shoot/root Ni concentrations) in *A. murale* was between 3 or 5 on ultramafic soil, and between 0.8 and 1.2 on constructed soil (Table S6 and S7). For both soils, biochar additions led to a significantly higher amount of phytoextracted Ni by *A. murale* as a result of the stimulation of biomass production (Fig. 6). With *A. murale*, amounts of Ni phytoextracted from the ultramafic soil were higher when biochar was added (9–11 mg of Ni per pot) than without biochar (< 1 mg). For a given biochar rate, lower amounts were recorded in the constructed soil (< 2 mg), than in the ultramafic soil. With *L. multiflorum*, the amounts were negligible on ultramafic soil, due to the low biomass and low Ni content. However, on the constructed soil, extraction by *L. multiflorum* reached amounts close to those observed with *A. murale* on this soil.

Zinc and Mn concentrations in shoots of *A. murale* were 2–7× higher on the constructed soil than the ultramafic soil (Table S6). Zn and Mn concentrations were close to those of Ni in *A. murale* shoots on the constructed soil. In the shoots of *A. murale* the Ni:Zn and Ni:Mn ratios were 40–100× higher on ultramafic soil than on the constructed soil; for *L. multiflorum* ratios were between 0.5 and 2 on both soils (Fig. 7). In the roots, the Ni:Zn ratio was inferior to the Ni:Mn on both soils and for both plants (Fig. S3).

3.3. Composition of the pore-water

Pore-water pH was generally higher in the ultramafic soil than in the constructed soil (Table 2). Increasing doses of biochar led to an increase in pH, particularly on the ultramafic soil. In the presence of a plant cover, higher pH values were measured compared to the unplanted control. The electrical conductivity was much higher on the constructed soil (13.1 ± 0.4 mS cm^{-1}) than on the ultramafic soil (0.71 ± 0.08 mS cm^{-1}). A decrease in conductivity was observed with addition of biochar to the constructed soil. However, with time, conductivity tended to increase with increasing rates of biochar. It was the opposite on the ultramafic soil.

Nickel concentration in the pore-water of the ultramafic soil was lower than 5 µM whereas it was very high for the constructed soil (1100 µM at the beginning of experimentation). In the presence of 3% and 5% of biochar, Ni pore-water concentration from ultramafic soil decreased over time, except under *L. multiflorum* cover. The opposite was recorded on the constructed soil for unplanted pots (Table 3). Compared to the unplanted controls, Ni pore-water concentration decreased on ultramafic soil with *A. murale* and on constructed soil with *L. multiflorum*. Zn concentration in pore-water was close to the Ni concentration in ultramafic soil and 6× lower in constructed soil (Table 3). Mn concentration in constructed soil was 3× higher than Zn and 2× lower than Ni (Table S8).

Phosphorus concentration was 30× higher with the constructed soil than with the ultramafic soil, as a result of the high supply of P from the industrial sludge (Table S9). At the last sampling, P concentration was near to zero for the ultramafic soil and reached half of the initial concentration for the constructed soil. Nitrate concentration was initially 150 mM in the constructed soil and 5.5 mM in the ultramafic soil (Table S10). On constructed soil, both species had a higher content of N and P in shoots (Tables S11, S12, S13 and S14).

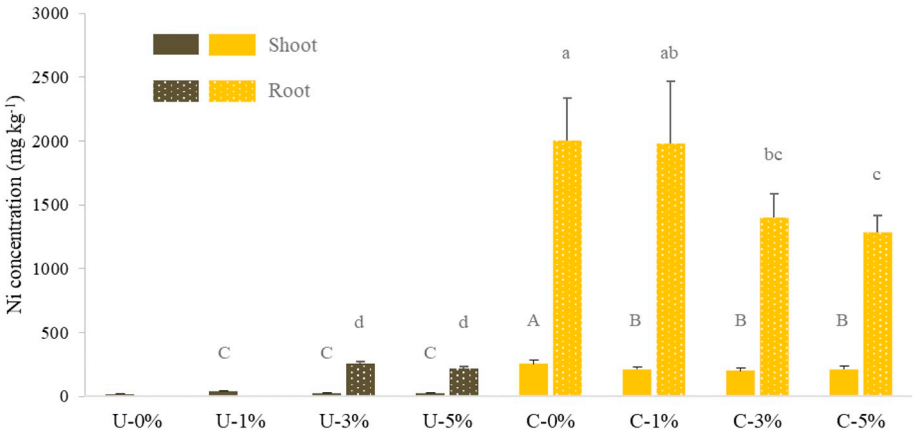

Fig. 4. Ni concentration in shoot and root of *L. multiflorum* after 12 weeks of culture on ultramafic soil (U) and constructed soil (C) at different biochar doses (0, 1, 3 and 5%). Vertical bars represent the standard deviations. Values for shoots affected by different capital letter were significantly different (Newman Keuls, $p \leq 0.05$); roots values affected by different lowercase letter were significantly different (Kruskal Wallis, $p \leq 0.05$).

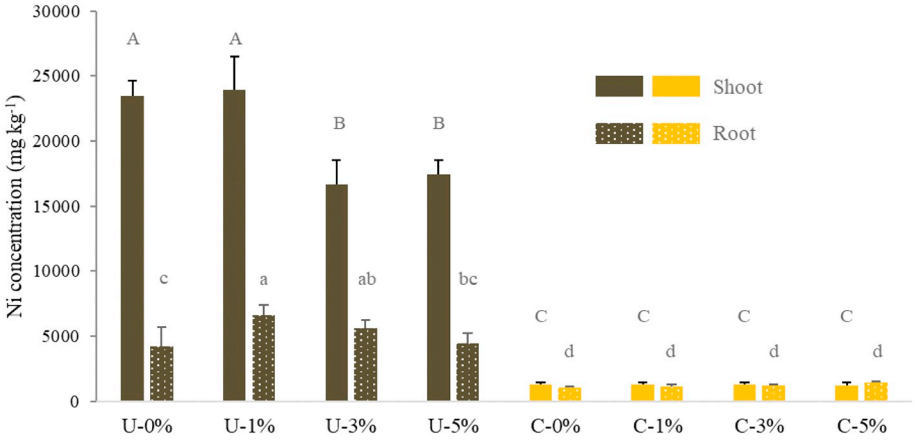

Fig. 5. Ni concentration in the shoot and root of *A. murale* on ultramafic soil (U) and constructed soil (C) at different biochar doses (0, 1, 3 and 5%). Values for shoot affected by different capital letter were significantly different (*t*-test, $p \leq 0.05$) and values for root affected by different lowercase letter were significantly different (*t*-test, $p \leq 0.05$), log-transformed data.

4. Discussion

Growing hyperaccumulator plants on industrial wastes to recover metals of interest requires the elaboration of methods, which can optimize not only plant establishment and growth, but also the uptake of the targeted metals by the plants. Here we have demonstrated that plant growth was possible on a soil constructed from a very phytotoxic industrial sludge (*e.g.* low pH, high salinity, high metal concentration) mixed with decontaminated soil. However, Ni hyperaccumulation was partly inhibited on this constructed soil, resulting in a lower amount of Ni phytoextracted from the sludge compared to the Ni recovered from an ultramafic soil. Biochar amendments improved plant growth and increased the amount of phytoextracted Ni, but did not completely succeed in alleviating the inhibition of Ni hyperaccumulation on the constructed soil.

4.1. Construction of a soil suitable for plant growth and Ni uptake

Preliminary experiments had shown that germination directly on the industrial sludge was impossible. For this reason, we chose to mix the sludge with a soil material derived from the treatment of a contaminated soil. Preliminary experiments had shown that a fraction of 90% of soil material was suitable for plant germination and growth. Our results show that this constructed soil was in fact more favourable to plant growth than a natural ultramafic soil, which exhibited similar characteristics as the one from which the hyperaccumulator originated (data not shown). The constructed soil made it possible to produce more plant biomass than the ultramafic soil for the two species tested, *L. multiflorum* and *A. murale*.

Plants grown on the constructed soils had a higher content of N (Tables S11 and S12) than plants grown on the ultramafic soil, suggesting that N availability had limited plant growth in the latter case. The amount of soluble mineral N and P was indeed much higher on the constructed soil than on the ultramafic soil, as shown by the comparison of pore-water concentrations between both soils. This shows that the industrial sludge can have a positive impact on plant growth because of its high nutrient content, providing that the toxicity caused by its high concentrations of metals is alleviated.

We have also shown that Ni uptake by the hyperaccumulator *A. murale* occurred under these conditions. However, shoot Ni concentrations in the hyperaccumulator grown on the constructed soil were much lower than those in the plant cultivated on the ultramafic soil. The hyperaccumulator grown on the constructed soil exhibited very low Ni concentration, 15–20× lower than the ones measured in plants grown on the ultramafic soil, despite an identical initial amount of DTPA-extractable Ni and pore-water Ni concentrations three orders of magnitude higher in the constructed soil compared to the ultramafic soil. A first explanation of this inhibition of Ni hyperaccumulation could be a dilution process caused by higher plant biomass production on the constructed soil. However, no differences in shoot Ni concentrations were observed between the plants grown on this soil at different biochar doses, despite a substantial increase in shoot biomass production. Furthermore, not only shoot Ni concentrations, but also translocation factors, were lower on the constructed soil than on the ultramafic soil. A more plausible explanation could be that Ni uptake and translocation were reduced on the constructed soil because of competition between Ni and other metals. Concentrations of Zn and Mn in the pore-water solutions were respectively about 20 and 500 x higher in the

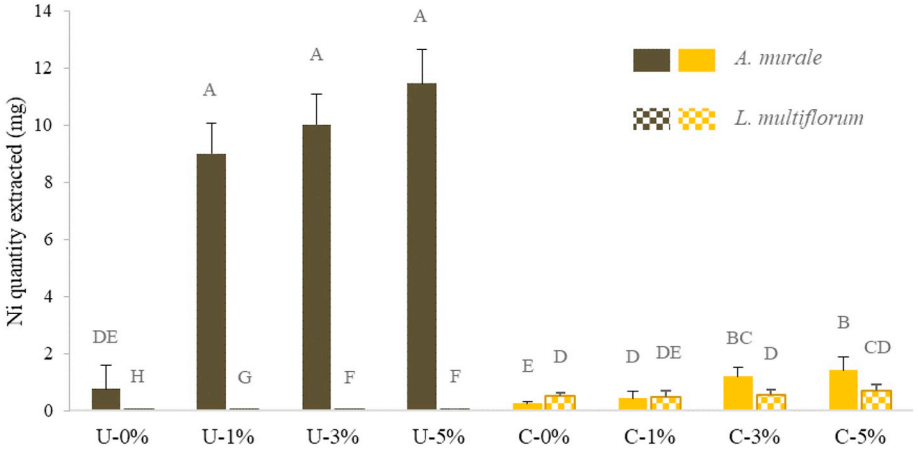

Fig. 6. Quantity of Ni extracted by *A. murale* and *L. multiflorum* per pot on ultramafic soil (U) and constructed soil (C) at different biochar doses (0, 1, 3 and 5%). Vertical bars indicate standard deviations. The affected values of different letter were significantly different (*t*-test, $p \leq 0.05$), log-transformed data.

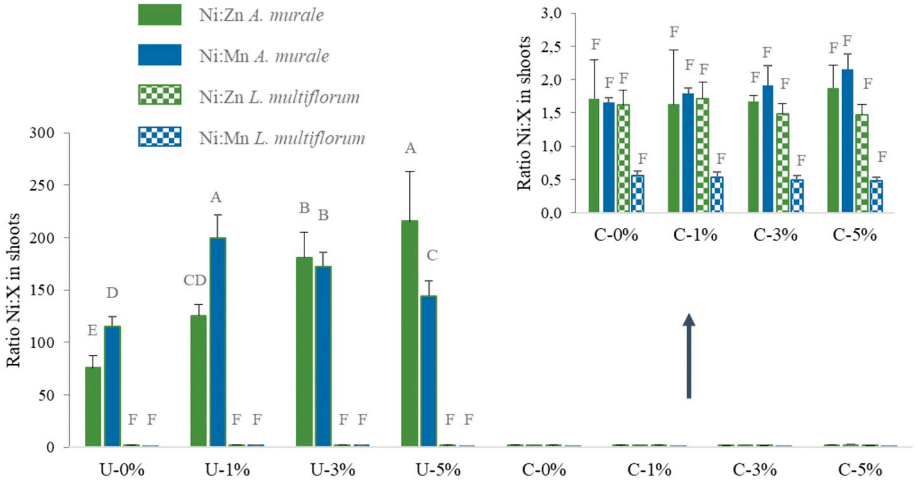

Fig. 7. Ratio of Ni:Zn and Ni:Mn mass concentrations in shoots of *A. murale* and *L. multiflorum* on ultramafic soil (U) and constructed soil (C) at different biochar doses (0, 1, 3 and 5%). Values affected by different letter were significantly different (Newman Keuls, $p \leq 0.05$).

Table 2
pH in pore-water at the sowing time and at the end of the experimentation on ultramafic soil and constructed soil at different biochar doses (0, 1, 3 and 5%), depending on cover.

Biochar (%)	Cover	Ultramafic soil		Constructed soil	
		Pore-water pH 1st sampling	Pore-water pH 3rd sampling	Pore-water pH 1st sampling	Pore-water pH 3rd sampling
0	Unplanted	6.28 ± 0.09	5.80 ± 0.04	5.45 ± 0.01	5.80 ± 0.11
1	Unplanted	6.51 ± 0.10	6.14 ± 0.08	5.58 ± 0.01	5.76 ± 0.24
3	Unplanted	6.86 ± 0.04	6.48 ± 0.05	5.76 ± 0.04	5.76 ± 0.05
5	Unplanted	7.14 ± 0.15	6.92 ± 0.07	5.88 ± 0.02	5.63 ± 0.06
0	*L. multiflorum*	6.32 ± 0.15	5.85 ± 0.02	5.47 ± 0.04	6.01 ± 0.16
1	*L. multiflorum*	6.58 ± 0.15	6.21 ± 0.03	5.58 ± 0.07	6.14 ± 0.04
3	*L. multiflorum*	6.89 ± 0.19	7.30 ± 0.08	5.70 ± 0.09	6.62 ± 0.26
5	*L. multiflorum*	7.21 ± 0.06	7.48 ± n.a.	5.92 ± 0.07	6.63 ± 0.27
0	*A. murale*	6.30 ± 0.05	5.79 ± 0.04	5.47 ± 0.03	5.76 ± 0.06
1	*A. murale*	6.55 ± 0.19	6.84 ± 0.00	5.64 ± 0.06	5.81 ± 0.16
3	*A. murale*	6.82 ± 0.06	7.20 ± 0.06	5.77 ± 0.03	6.02 ± 0.11
5	*A. murale*	7.12 ± 0.10	7.49 ± 0.02	5.96 ± 0.001	6.21 ± 0.59

Mean ± Standard errors (3 replicates). n.a.: not available.

constructed soil than in the natural soil. Furthermore, the Ni:Zn and Ni:Mn ratios of concentrations in the shoots of the hyperaccumulator were two orders of magnitude lower on constructed than on the ultramafic soils. This strongly suggests a role of Zn and Mn as inhibitors of Ni hyperaccumulation by *A. murale* grown on the constructed soil.

A strong competition between Ni and Zn and Mn can occur when Ni-transporters through the root cell membrane are involved (Broadhurst et al., 2009; Deng et al., 2016; Ghaderian et al., 2015). Contrary to the case of *A. murale*, Ni:Zn and Ni:Mn ratios of shoot concentrations in *L. multiflorum* did not differ between the two soils. This could imply that

Table 3
Ni and Zn concentrations in pore-water of ultramafic soil and constructed soil at different biochar doses (0, 1, 3 and 5%), depending on cover.

Biochar (%)	Cover	Ultramafic soil				Constructed soil			
		Ni concentration (μM)		Zn concentration (μM)		Ni concentration (μM)		Zn concentration (μM)	
		1st sampling	3rd sampling	1st sampling	3rd sampling	1st sampling	3rd sampling	1st sampling	3rd sampling
0	Unplanted	4.15 ± 0.40	6.41 ± 0.49	5.98 ± 2.10	8.00 ± 4.23	1240 ± 62	536 ± 75	213 ± 25	91.1 ± 1.0
1	Unplanted	3.37 ± 0.71	3.90 ± 0.35	5.33 ± 0.66	12.01 ± 0.04	1075 ± 210	835 ± 368	164 ± 33	148 ± 72
3	Unplanted	3.56 ± 1.19	1.95 ± 0.10	5.13 ± 1.09	6.48 ± 0.19	718 ± 44	1034 ± 114	92.6 ± 8.7	163 ± 26
5	Unplanted	3.16 ± 0.27	1.57 ± 0.04	4.15 ± 0.50	5.97 ± 1.82	576 ± 28	1106 ± 32	70.9 ± 6.9	191 ± 35
0	*L. multiflorum*	4.99 ± 0.26	5.84 ± 0.47	10.6 ± 4.8	12.9 ± 8.1	1109 ± 116	416 ± 68	181 ± 28	142 ± 96
1	*L. multiflorum*	4.02 ± 0.72	2.85 ± 0.17	8.08 ± 2.50	7.26 ± 2.44	991 ± 212	289 ± 201	150 ± 37	110 ± 30
3	*L. multiflorum*	3.31 ± 0.29	4.75 ± n.a.	5.79 ± 2.80	13.0 ± n.a.	750 ± 157	87.4 ± 59.4	105 ± 26	76.3 ± 54.7
5	*L. multiflorum*	3.52 ± 0.53	6.46 ± n.a.	6.84 ± 2.72	36.3 ± n.a.	488 ± 75	105 ± 114	59.4 ± 8.7	68.9 ± 47.5
0	*A. murale*	4.73 ± 0.20	5.64 ± 0.55	9.97 ± 4.31	7.61 ± 1.19	1100 ± 159	794 ± 153	185 ± 34	131 ± 38
1	*A. murale*	4.07 ± 0.24	2.57 ± 0.59	8.69 ± 6.53	14.9 ± 4.7	836 ± 20	911 ± 160	121 ± 1	160 ± 34
3	*A. murale*	3.43 ± 0.32	2.26 ± 0.24	3.98 ± 0.32	15.4 ± 1.7	598 ± 55	645 ± 223	78.2 ± 6.6	119 ± 11
5	*A. murale*	3.21 ± 0.19	1.52 ± 0.26	7.90 ± 6.78	9.50 ± 4.94	447 ± 28	505 ± 497	52.5 ± 5.1	92.1 ± 74.8

Mean ± Standard errors (3 replicates). n.a.: not available.

the process of Zn and Mn inhibition of Ni uptake is specific to the hyperaccumulator, further suggesting the existence of a specific metal transporter in this species. In fact, contrary to the hyperaccumulator, *L. multiflorum* accumulated more Ni when grown on the constructed soil than on the ultramafic one, in accordance with the high Ni concentrations in the corresponding pore-water solutions.

Our results suggest that the efficiency of phytoextraction, hence agromining, depends on the proper management of the interactions between the targeted metal and all other cations that compete for transporters across the root cell walls. Although only one industrial sludge was tested in this study, we believe that this phenomenon of competition may occur with other industrial wastes where they contain significant amounts of competing elements. The pre-treatment of such wastes to remove easily recoverable metals through leaching, and/or the selective elimination of competing cations should therefore enhance phytoextraction. This would also decrease the volume of inert filling material needed to alleviate the toxicity or the inhibition of metal uptake by the constructed soil, making the process more efficient and less costly.

4.2. Optimization of Ni phytoextraction with biochar amendments

Biochar addition in both Ni-rich soils improved germination and growth, and both the hyperaccumulator and the non-accumulating plants responded positively in terms of shoot biomass production to increasing biochar rates, up to 5%. This confirms the results of many previous experiments in metal-contaminated soils as shown by various reviews (Beesley et al., 2011; Paz-Ferreiro et al., 2014). Different factors may explain this beneficial effect of biochar on plant growth. The decrease in pore-water metal concentrations in both soils with increasing amounts of biochar decreased soil metal toxicity. The decrease in Zn availability for *A. murale* and the decrease of Ni and Zn for *L. multiflorum* with biochar amendments probably favoured plant growth on the constructed soil, and, to a lesser extent on the ultramafic soil. Another explanation for plant growth improvements with biochar amendments could be a direct supply of nutrients from biochar, and a higher soil nutrient availability indirectly induced by biochar amendments. In this present work, no enhancement of N or P concentrations in soil pore-water was observed with increasing biochar dose. However, there was a good supply of Ca by biochar over time in the ultramafic soil, probably caused by the progressive dissolution of biochar calcium carbonate phases (Rees et al., 2017). This Ca supply reduced the imbalance between Ca and Mg typically observed in ultramafic soils (Proctor and Cottam, 1982), which may explain the net improvement of plant growth with biochar amendments in this soil. Biochar also seems to have provided available K, at least in the case of the ultramafic soil, and Mg to the constructed soil. Besides a decrease in soil toxicity and a better nutrition, other processes might also be involved in plant growth improvement with biochar additions. In particular, biochar amendments lowered bulk density and increased aeration of soil, and may have helped to prevent drought effects by holding more water in the pots in the intervals between the adjustments of soil moisture to 80% of the WHC in each treatment.

As expected, Ni uptake by the non-hyperaccumulating plant generally decreased with increasing biochar rate. This is related to the decrease in Ni pore-water concentrations observed in most cases and the general increase in pH observed in all cases with biochar amendments. This soil alkalinization has been shown to represent an important mechanism for indirect metal immobilization by biochar amendments (Houben et al., 2013b; Rees et al., 2015, 2014). Contrary to our expectations, Ni uptake by the hyperaccumulator did not increase with increasing biochar doses, and even decreased in the case of the ultramafic soil. An increase in Cd uptake by the Cd-hyperaccumulator *N. caerulescens* with 5% biochar was previously explained by i) a decreasing competition between Cd and major cations in solution (*e.g.* Ca) in their transport through root cell membranes (Rees et al., 2015), or ii) an increase in root surface caused by the development of finer roots in the presence of biochar (Rees et al., 2016). In the present study, no decrease in Ca concentrations was observed with biochar additions and the decrease in Zn and Mn concentrations initially observed in the constructed soil with increasing biochar amendments was not maintained over time. The fact that *A. murale* plants had lower shoot Ni concentrations at 5% biochar rate in the ultramafic soil despite having a larger root surface also contradicts the second possible explanation for biochar-improved hyperaccumulation through an increase in root surface. The factors positively or negatively affecting Ni hyperaccumulation by *A. murale* when grown in the presence of biochar should therefore deserve further investigations.

Nickel phytoextraction from the constructed soil by *A. murale* was improved by biochar additions, as the amendment improved plant growth while maintaining a similar plant Ni uptake, contrary to what was observed with the non-accumulating plant. However, the amount of Ni phytoextracted from the constructed soil remained low for any biochar rate in comparison with the ultramafic soil.

5. Conclusion

This work shows that agromining can be applied to other secondary sources of metals than ultramafic soils. We demonstrated that the Ni-hyperaccumulator *A. murale* could germinate and produce biomass on a Technosol containing an industrial sludge with very high metal concentrations. Biochar was shown to considerably improve the fertility of the constructed soil and of an ultramafic soil with a low intrinsic fertility, and in this way to result in a higher amount of phytoextracted Ni. However, we also demonstrated that phytoextraction of Ni from complex constructed matrices can be strongly impaired by an unbalanced initial composition in competing metals. In our work, Zn and Mn were confirmed to represent strong competitors for Ni uptake by *A. murale*. The high content and availability of Zn and Mn in the sludge considerably reduced Ni uptake despite higher biomass production in the constructed soil than in the natural ultramafic soil. Further agromining developments on industrial wastes will therefore need improved constructed soils, *e.g.* with a chemical composition adapted to meet the requirements of hyperaccumulation. Upscaling of the process in order to test the feasibility of agromining on industrial byproducts in conditions close to reality is underway.

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Appendix A. Supplementary data

Additional data are available online in a Supplementary data file: detailed properties of materials, pH, conductivity, WHC, Ni-, Zn-, Mn-extractable DTPA before and after experimentation, shoot biomass of *L. multiflorum* for each cut, Ni, Zn and Mn concentrations in shoots and roots for both species, ion concentrations (Mn, Ca, Mg, K, P, nitrate,

ammonium, sodium, sulphate), C_{org}, N concentration, pH, conductivity in pore-water during experimentation, C and N composition of *L. multiflorum* and *A. murale*, composition (Ca, K, Mg, Na, P, S concentrations) for both species, and figures (experimental design, ratios of concentrations (Ni:Zn and Ni:Mn in roots), principal components analysis of parameters measured on soils and plants, and the correlation matrix for each plant on each soil). Supplementary data to this article can be found online at https://doi.org/10.1016/j.gexplo.2018.10.007.

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