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LONG-TERM FIELD METAL EXTRACTION BY *PELARGONIUM:* PHYTOEXTRACTION EFFICIENCY IN RELATION TO PLANT MATURITY

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The long length of periods required for effective soil remediation via phytoextraction constitutes a weak point that reduces its industrial use. However, these calculated periods are mainly based on short-term and/or hydroponic controlled experiments. Moreover, only a few studies concern more than one metal, although soils are scarcely polluted by only one element.

In this scientific context, the phytoextraction of metals and metalloids (Pb, Cd, Zn, Cu, and As) by Pelargonium was measured after a long-term field experiment. Both bulk and rhizosphere soils were analyzed in order to determine the mechanisms involved in soil-root transfer.

First, a strong increase in lead phytoextraction was observed with plant maturity, significantly reducing the length of the period required for remediation. Rhizosphere Pb, Zn, Cu, Cd, and As accumulation was observed (compared to bulk soil), indicating metal mobilization by the plant, perhaps in relation to root activity. Moreover, metal phytoextraction and translocation were found to be a function of the metals' nature. These results, taken altogether, suggest that Pelargonium could be used as a multi-metal hyperaccumulator under multi-metal soil contamination conditions, and they also provide an interesting insight for improving field phytoextraction remediation in terms of the length of time required, promoting this biological technique.

KEY WORDS: phytoremediation, kinetics, plant maturity, long-term field experiment, metals, rhizosphere

INTRODUCTION

Soil pollution by metals and metalloids is a widespread, global dilemma with negative environmental and sanitary consequences (Dumat et al. 2006; Shahid et al. 2011a, 2011b; Uzu et al. 2011). According to Cutright et al. (2010) and Lai et al. (2010), Pb, As, Cd, Cu, Zn, Cr, Ni, and Hg are the main inorganic pollutants of the food chain. Therefore, over the last

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few last decades, numerous remediation techniques were developed in order to reduce total and/or available soil metal concentrations (Rascioa and Navari-Izzob 2011; Xu et al. 2010).

Among these remediation techniques, phytoextraction makes use of hyperaccumulator plants that can accumulate high concentrations of metals amounts in their shoots without incurring damage to their metabolic functions (Feng et al. 2011; Rascioa and Navari-Izzob 2011). In comparison to non-biological soil remediation techniques, solar driven phytoextraction is cost effective, has a high level of public acceptance and is environmentally friendly (Wu et al. 2010). However, its generally long-term calculated performance period, constitutes a weak point that strongly reduces its industrial use on polluted soils (Arshad et al. 2008). But, according to Liang et al. (2009), the calculated lengths of these periods are mainly based on short-term and/or hydroponic controlled phytoextraction experiments (KrishnaRaj et al. 2000). Moreover, due to the complexity of the soil-plant system, the prediction of plant metal uptake through mathematical modelling has received very little attention (Liang et al. 2009; Mathur and Yadav 2009; Shahid et al. 2011a). Consequently, supplementary long-term field data are needed to improve the knowledge on phytoextraction kinetics and to pertinently discuss its efficiency.

Although more than 400 plant species have been identified as hyperaccumulators, most of these studies were performed on only one metal (Cutright et al. 2010). These hyperaccumulators either do not have the ability to act as multi-metal hyperaccumulators or they were not tested under multi-metal contaminated soil conditions (the majority of the studies). Phaenark et al. (2009) reported that from among 36 plants tested for their metal extraction abilities in five different Cd and Zn contaminated sites, only one was able to hyperaccumulate both Cd and Zn.

Although total soil metal concentrations are high in contaminated sites due to the association of metals with soil components (Dumat et al. 2006, 2001; Quenea et al. 2009), they are often low in soil solutions; for example, soil solutions typically have less than 1% Pb (Sarkar et al. 2008). However, rhizosphere activity (such as the production of organic exudates or changes in pH) or the presence of organic ligands can modify metal speciation and increase its availability (Shahid et al. 2011a). In the phytoextraction context, this phenomenon could be of great interest for lead, which is generally considered as having low mobility in soil and has been widely found in polluted soils (Uzu et al. 2009). Therefore, a study of rhizosphere soil (in addition to bulk) could increase the level of knowledge on the mechanisms involved in soil-plant metal transfers (Lin et al. 2004; Shahid et al. 2011b).

Thus, the main objective of the current investigation was a long-term field experiment to study the lead phytoextraction performance of a plant in relation to rhizosphere characteristics and plant maturity. A three-year field phytoextraction experiment was performed on soil contaminated by the atmospheric fallout from a battery recycling factory. The attar of rose *Pelargonium* cultivar which was previously identified as being a high biomass accumulator of lead by Arshad et al. (2008) (in the context of a short-term experiment), was tested. In addition to lead, the uptake of As, Cu, Zn, and Cd was measured in relation to rhizosphere activity.

MATERIALS AND METHODS

Plant Culture

Attar from the rose cultivar *Pelargonium* (herein called Attar) was obtained from cuttings of commercial plantlets (http://www.pepinieres-heurtebise.com/). The cuttings were grown in a greenhouse in well-washed coarse 0.5 l Perlite pots. They were regularly

Table 1 Main physico-chemical characteristics of the contaminated soil studied

pH-$CaCl_2$	6.5
Organic matter (%)	8.5
Clay (g kg^{-1})	112
Silt (g kg^{-1})	337
Sand (g kg^{-1})	550
Total nitrogen (g kg^{-1})	3.8
P_2O_5 Joret-Hebert (mg kg^{-1})	95

irrigated with a complete nutrient solution containing 5000 μM KNO_3, 5000 μM $Ca(NO_3)_2$, 2000 μM KH_2PO_4, 1500 μM $MgSO_4$, 46 μM H_3BO_3, 9 μM $MnSO_4.H_2O$, 0.1 μM $MoNaO_4.2H_2O$, 0.9 μM $CuSO_4.5H_2O$, 15 μM $ZnSO_4.7H_2O$, and 90 μM Fe-EDTA. After a growth period of three weeks, the seedlings were transported and cultured in an experimental area.

Polluted Soil

The experiment was carried out on an acidic soil located in the urban zone of Toulouse (south-western France; 43°38′ 12″ N, 01° 25′34″ E) in the vicinity of active Pb recycling factory from the Chemical Metal Treatment Company (STCM), contaminated by atmospheric fallouts. Table 1 presents some of the physico-chemical characteristics of this soil measured according to normalized AFNOR procedures: NF ISO 10390, NF ISO 14237, NF X 31-107, NF ISO 13878 and NF X 31-161 for pH-$CaCl_2$, organic matter amount, texture, total nitrogen, and exchangeable Joret-Hebert phosphorus, respectively.

Field Experiment and Sampling

All phytoextraction experiments were performed in the field, from May 2004 to July 2006. Attar from the *Pelargonium* cultivar was tested in five replicates (one separate plant was considered as a replicate). A plant density of 15 m^{-2} was used on an experimental plot of 10 m^2. The plants were grown without NPK fertilization or optimum irrigation conditions in order to test the feasibility of remediation in the context of an industrial site. The first Pb extraction experiment was performed from May to September 2004. The shoots were harvested after 150 days of field culture while the roots were left in place to perform a second experiment with the same plants. From October 2004 to March 2005, the plants remained in a ‘still’ state, and then the second extraction experiment was performed from April to July 2005 (120 days). Finally, a third experiment was performed during 2005–2006, with the shoots, roots, and rhizosphere being sampled in July 2006. The roots were collected by digging around and under the plants. On the first day after harvest, the roots were hand agitated in order to remove the bulk soil. Then, root-adhering soil, called the rhizosphere soil, was sampled by gently brushing it away with soft toothbrushes.

Metal Concentrations, Biomass, and Dissolved Organic Carbon (DOC) Measurements

Metals (from the atmospheric fallouts) potentially adsorbed on the leaves were determined according to the desorption protocol used for roots by Ferrand et al. (2006). Due to the objectives of the present long-term experiment and because phytoextraction efficiency is a function of metal accumulation in harvested shoot biomass, fresh, and dried (two days at 80°C) biomass weights were only recorded for shoot samples (leaves and stems). The roots were sampled on the third year of the experiment in order to measure their total metal concentrations.

The metal content analysis was realized as described by Uzu et al. (2009). Plant and soil samples were milled in a micronizing mill and mineralized (1 g) in a 1:1 mixture of 65% HNO_3 and 30% H_2O_2 at 80°C for 6 h. The digestate was analyzed for metal and metalloid (Pb, As, Cu, Zn, and Cd) tissue levels using an IRIS Intrepid II XDL ICP-OES. Accuracy of analytical procedure was verified using a reference material: Virginia tobacco leaves (CTA-VTL-2, Polish certified reference material; ICHTJ). Calculations were performed on the basis of the dry weight (DW) of each sample.

The 0.01 M $CaCl_2$ extracted metals and pH_{CaCl2} were determined in both bulk and rhizosphere soils (after three years of culture) according to Uzu et al. (2009). Moreover, the DOC was measured using a Shimadzu 5000A TOC Analyzer on $CaCl_2$ supernatants.

Calculated Shoot Bio-Concentrations, Translocation and Solubility Factors

In this study, the shoot bio-concentration factor (BCF_{shoot}) was calculated as the ratio of metal concentrations in plant shoots to those in bulk soil. The translocation factor (TF_t) was the ratio of metal concentrations in plant shoots to those in plant roots (Zhang et al. 2010). The mobilization factor (TF_m) was defined as the ratio between concentrations of metals in the rhizosphere soil to those in the bulk soil.

Statistical Analysis

The data obtained were subjected to analysis of variance (ANOVA) with one factor, using Statistica, version 8 software (StatSoft Inc., Tulsa, OK, USA). For each experiment, the mean values with different letters or a symbol (*) represent a significant difference ($p < 0.05$), as measured by LSD Fisher test.

RESULTS

Metal and Metalloid Uptake by *Pelargonium*

Under our experimental conditions, the concentrations of the metals of interest adsorbed on *Pelargonium* leaves, as determined by the desorption procedure, were under the detection limits or represented less than 1% of the total shoot metals for all three culture periods. Therefore, these are neglected in further discussions. In the studied range of total metal concentrations in soils and the hyperaccumulator *Pelargonium* plants, the soil-plant transfer of metals was considered as the main route of metal absorption by plants. The uptake of metals from atmospheric fallouts by the leaves, as observed by Uzu et al. (2010) in the case of lettuces cultivated on uncontaminated soils, was not investigated.

Figure 1 shows the mean Pb, As, Cu, Zn, and Cd concentrations in bulk soil and various plant parts (roots and shoots) for the third culture period (2006). The uptake and accumulation of metals in roots and shoots varied with the type of metal. The Attar cultivar ranked as a strong Pb hyperaccumulator due to the uptake and accumulation of high concentrations of lead (5550 and 8644 mg Pb kg^{-1} DW in roots and shoots, respectively)

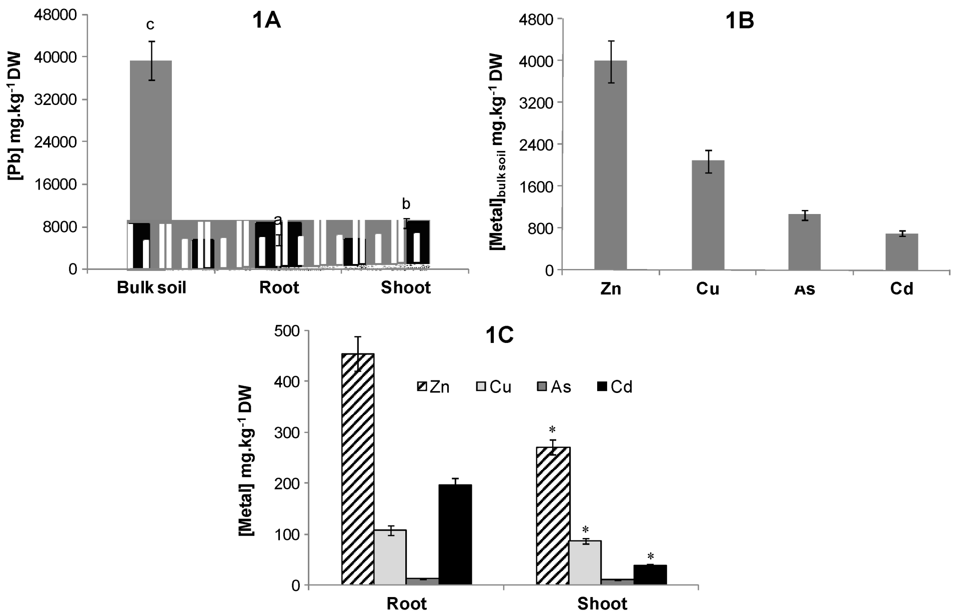

Figure 1 Mean bulk soil, root and shoot concentrations of metals for the 2006 culture period. Values are means ±SD (n = 5). Different letters or a symbol (*) represent significant differences among the bulk soil, root, or shoot metal concentrations. a. Mean bulk soil, root, and shoot concentrations of Pb. b. Mean bulk soil concentrations of As, Cu, Zn, and Cd. c. Mean root and shoot concentrations of As, Cu, Zn, and Cd.

from highly contaminated acidic soil (39,250 mg kg^{-1} of total Pb) (Figure 1a) with no morpho-phytotoxicity symptoms.

The mean concentrations of Zn, Cu, As, and Cd in bulk soil were 3995, 2085, 1060, and 706 mg kg^{-1} DW, respectively (Figure 1b). The mean concentrations of these metals in the roots (454, 108, 14, and 197 mg kg^{-1} DW for Zn, Cu, As, and Cd, respectively) were significantly higher compared to the shoots (272, 88, 12 and 39 mg kg^{-1} DW for Zn, Cu, As and Cd, respectively), except for As (Figure 1c).

Comparison of Rhizosphere and Bulk Soil Characteristics

Total metal concentrations of the rhizosphere (mg kg^{-1}) were 73,327 $\pm$ 5662, 5990 $\pm$ 268, 3296 $\pm$ 139, 1403 $\pm$ 71, and 816 $\pm$ 47 for Pb, Zn, Cu, As, and Cd, respectively. Comparison of the total metal concentrations (Figure 1) between the bulk soil and rhizosphere soil demonstrated an accumulation of all of the studied metals and metalloids in the rhizosphere.

Table 2 shows the $CaCl_2$ extraction results for bulk and rhizosphere soils (2006 culture period) expressed as ratios between the extracted and total metal quantities (%). Pollutant availability increased in the rhizosphere soil compared to the bulk soil. The following sequence was observed: Pb (3.6) > Zn (3.4) > Cu (2.9) > As (2.7) > Cd (2.1); the figures within parentheses are the availability ratios calculated between the rhizosphere and bulk soils.

The pH-$CaCl_2$ for the bulk and rhizosphere soils were 6.5 $\pm$ 0.1 and 5.8 $\pm$ 0.08, respectively. The DOC levels for the bulk and rhizosphere soils were 55 $\pm$ 8 mg l^{-1}

Table 2 Relative contents (extracted quantity/total quantity) of metals and metalloids (Pb, As, Cu, Zn, and Cd) extracted with 0.01 M $CaCl_2$ for both bulk and rhizosphere soils (2006 culture period). The values are expressed as percentages (%) and are the means of two extractions

Element	Bulk	Rhizosphere
Pb	1.2	4.3
Zn	0.8	2.7
Cu	0.7	2
As	0.45	1.2
Cd	0.66	1.4

and 173 ± 21 mg l^{-1}, respectively. Therefore, it was concluded that rhizosphere activity modified both pH and DOC soil characteristics.

Influence of Plant Maturity on Lead Phytoextraction

Figure 2 presents the results of the dry weights of shoots (g), the total lead concentration in the shoots (mg Pb kg^{-1} DW plant) and the amount of lead extracted by the plant (mg Pb $plant^{-1}$) for three culture periods of the *Pelargonium* Attar cultivar. The shoot biomass of the rose Attar cultivar increased from 2004 to 2006 (two-fold for 2006 compared to 2004) (Figure 2a). However, the total lead contents decreased (Figure 2b) for 2005 compared to 2004, while for 2006 the Pb contents increased by 1.5 and 1.2 times compared to 2004 and 2005, respectively. Moreover, the amount of Pb extracted per plant remained

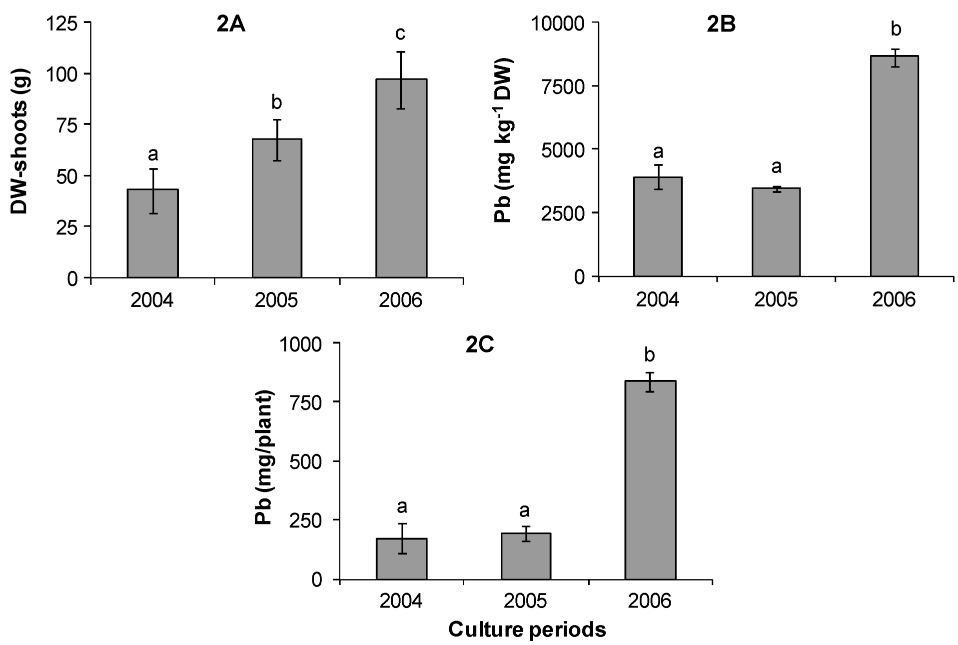

Figure 2 Comparison of shoot biomass (a), total lead contents (b) and lead extraction per plant (2C) for the three culture periods. Values are means ± SD (n = 5).

unchanged between the first and second culture periods (2004 and 2005, respectively), while an increase of more than double was observed for the third culture period (2006) compared to first and second periods (Figure 2c). Therefore, the lead phytoextraction efficiency strongly increased with the increasing maturity of the *Pelargonium* cultivar over the 3-year culture period.

DISCUSSION

Metal and Metalloid Uptake by *Pelargonium* in Relation with Rhizosphere Activity

According to Maestri et al. (2010), specific hyperaccumulators can concentrate more than 1000 ppm Pb or Cu, 100 ppm Cd, or As and 10,000 ppm Zn within their tissues. The Attar accumulated 8644 mg Pb kg^{-1} DW, which was nine times greater than the hyperaccumulator threshold level (Figure 1a), therefore proving to be a good hyperaccumulator of Pb. For Cd, the concentrations in the roots were twice as high as the threshold level. In contrast, As, Cu, and Zn concentrations in the different plant parts were lower than their hyperaccumulator threshold levels (Figure 1c). These results suggest that *Pelargonium* is not just a hyperaccumulator of Pb, but that it can also accumulate Cd under multi-metal contaminated soil conditions. Wu et al. (2010) found that *Viola baoshanensis* (a Cd hyperaccumulator) is also a strong accumulator of Pb and Zn. Based on these results, it is suggested that specific metal-hyperaccumulators could be tested for the remediation of other toxic metals under multi-metal contaminated soil conditions.

The shoot bio-concentration factors (BCF_{shoot}) calculated for all metals (Figure 3a) indicated the soil-plant transfer efficiency of the Attar cultivar. The results showed that lead was the element most efficiently transferred from the soil to the plant, followed by Cd, Zn, Cu, and As with BCF_{shoot} values of 0.22, 0.07, 0.05, 0.04, 0.01, and 0.05, respectively. These low BCF_{shoot} values were due to very high concentrations in the bulk soil. Indeed, according to Liu et al. (2010) and Franco-Hernández et al. (2010), BCF_{shoot} values of heavy metals decrease with increasing metal concentrations in the soil. The calculated metal translocation factors (Figure 3b) indicate that most of the lead absorbed by roots is translocated to aerial parts (TFt = 1.6). *Pelargonium* is therefore a useful plant for lead phytoextraction. Enhanced metal uptake from the roots and translocation to the shoots in hyperaccumulators is generated by specific carrier protein members. Recently, Maestri et al. (2010) reviewed several studies that reported the presence of metal transporters such as ZIP (ZRT/IRT-like protein), CDF (cation diffusion facilitator), and HMA (heavy metal ATPase) among plant cells. They are also known to have internal detoxification mechanisms such as the induction of non-protein thiol (NP-SH), cysteine, glutathione, ascorbic acid, proline, and antioxidant enzymes to deal with the toxic effects of metals (Shahid et al. 2011b). However, the physico-chemical properties of soil affect the expression of metal transporter and chelator proteins inside the plant, which in turn play a determinant role in the phytoremediation potential (Couselo et al. 2010; Shahid et al. 2011b).

In the case of Cd, Zn, Cu, and As, the major portions were sequestered in roots with TFt values <1 (i.e. 0.2, 0.6, 0.8, and 0.8, respectively). The co-presence of Cd with other metals in the soil could affect its uptake and translocation to aerial parts (Oliver et al. 1994; Wu et al. 2010) and may explain the relatively low phytoextraction efficiency of this element. Recently, Xin et al. (2010) reported that Cd shoot/soil ratio concentrations were increased by the presence of medium amounts of Pb but reduced by the presence of high

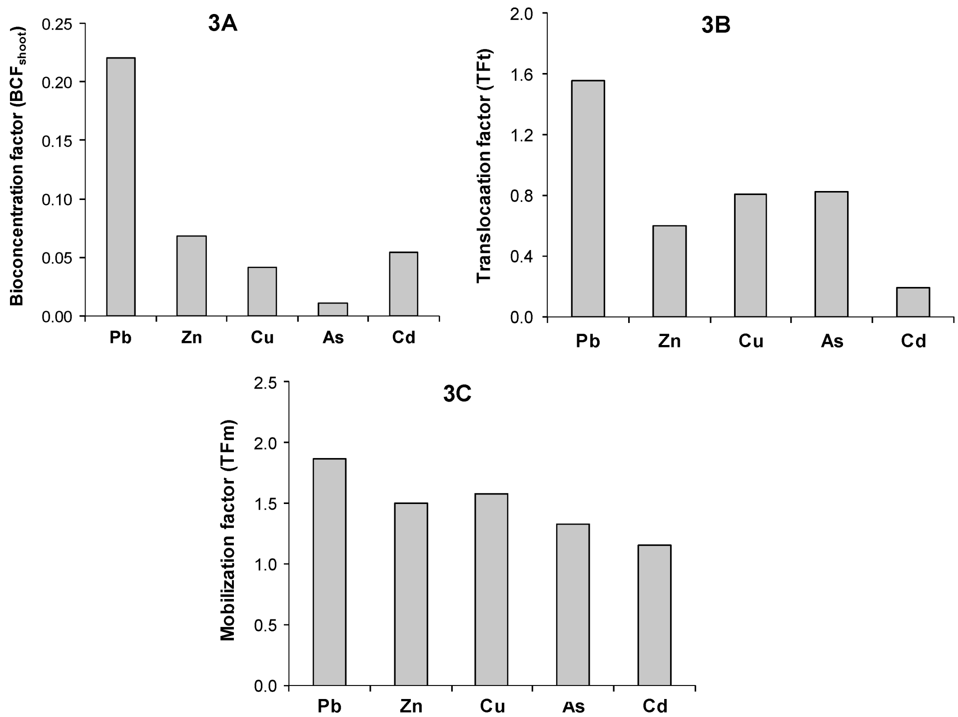

Figure 3 Comparison of shoot bio-concentration factors, BCF_{shoot} (a), translocation factors, TF_t (b), and mobilization factors, TF_m (c), of Pb, As, Cu, Zn, and Cd.

amounts of Pb. Moreover, Zhang et al. (2010) reported that Cd translocation could strongly vary with the Cd level in the soil or hydroponic solution, and with plant and soil types.

Figure 3c shows the metal mobilization ratios of all of the studied metals. Lead was more efficiently mobilized and accumulated in the rhizosphere, followed by Cu, Zn, As, and Cd, with TFm values of 1.9, 1.6, 1.5, 1.3, and 1.2, respectively. According to our results, the mobilization of metals by the *Pelargonium* Attar cultivar could occur via two possible mechanisms: acidification of the medium (a decrease of 0.7 pH units was observed) or secretion of organic root exudates (an increase of 118 mg DOC l^{-1} was observed), which can form soluble metal complexes. Arshad et al. (2008) concluded that rose Attar cultivars could favor Pb transfer from the solid phase of contaminated soils to their soil solutions. The increased bioavailability of Cu, Pb, and Zn in the rhizosphere by *Lupinus albus* exudates was reported by Martinez-Alcala et al. (2009). Lin et al. (2004) also observed that concentrations of exchangeable lead were much higher in the rhizosphere of *Oryza sativa* than in bulk soil. Shilev et al. (2007) found that introducing 1 and 5 mmol l^{-1} of organic chelates into the rhizosphere of maize and sunflower plants significantly enhanced soluble and accumulated concentrations of Pb, Cd, and Zn. Recently, Kim et al. (2010) also reported increased soil solution concentrations of Cu, Pb, and Zn in contaminated soils using *Brassica juncea* and *Helianthus annuus* due to a marked increase in DOC linked to plant root exudates.

Indeed, these compounds induce modifications in biogeochemical behavior (sorption and desorption) and directly influence the fate of metals in soil by affecting acidification, chelation, precipitation and redox reactions, or indirectly through their effects on microbial activity, and physical and chemical properties of the rhizosphere and root growth patterns (Kim et al. 2010). They can form soluble complexes with metal cations, allowing further dissolution of metals from soil organic matter, oxides, and clays, and enhance their release (Marchand et al. 2010). In the rhizosphere, ground organic elements (plant or microbial) and microbiological activity seem to play a larger role than acidification (Kim et al. 2010). In the rhizosphere, organic acids such as citric, malic, and oxalic acids have the ability to desorb metals and promote their solubilization in soil.

Modelling *Pelargonium* Phytoremediation Efficiency

The lead extraction efficiency of the rose Attar cultivar increased with increasing plant maturity (Figure 2c). The literature to date shows data regarding the increasing ability of plants to absorb and translocate nutrients and metals with increasing maturity; variations largely depend on soil characteristics, plant species, and the metals/nutrients under consideration (Liang et al. 2009; Shelmerdine et al. 2009). In our soil-plant experiment, Pb extraction and uptake increased with age, suggesting that older Attar cultivars are more capable of solubilizing Pb in soil with increased uptake and translocation. Moreover, the quantity, proportion and type of organic acids in the root exudates of rose Attar cultivars that form soluble complexes with Pb may vary with age. Padmavathiamma and Li (2009) also reported an increased uptake of Cu, Pb, Mn, and Zn at 120 than at 90 days after sowing using *Lolium perenne, Festuca rubra, Helianthus annuus, Poa pratensis,* and *Brassica napus*. Recently, Lai, (2010) also observed an increased accumulation of Cr, Cu, Ni, and Zn in the shoots by extending the growth period. Joner et al. (2004) found that root density affects metal concentrations in plants, with increased metal concentrations in plants where the entire volume of the soil was efficiently exploited by roots. Using these data, the first annual removal percentages were calculated for the various inorganic pollutants and then the lengths of time required for full remediation (based on several hypotheses) were estimated.

From a general point of view, before calculating the period required for metals to be removed by phytoextraction, several preliminary questions must be asked: (1) what are the total metal quantities present in the soil? Do all soil profiles have to be taken into account or, for instance, only the strongly contaminated top soil? (2) What are the target metal concentrations? What natural geochemical background values or values fixed by regulations of a country can be chosen? (3) What hypotheses are needed to perform the calculations? Several parameters are generally estimated: soil mass and the variation of plant biomass with time, for example. In order to define the total metal quantities removed by the phytoextraction remediation technique, knowledge of the soil metal profile is needed. In the present study the metals were mainly present in the first 10 cm of the top soil. Using an average volume weight for the soil of 1.2×10^3 kg m^{-3}, the soil weight corresponding to a top soil (10 cm depth) sheet of 1 ha is 1200 tons. Therefore, the total initial quantities of Pb, Cd, Zn, Cu, and As that corresponded to 1 ha of contaminated top soil were calculated as 1200 tons $\times$ total initial metal concentration in the bulk soil. The values calculated were $S_0(Pb) = 4710$ kg of Pb, $S_0(Cd) = 85$ kg of Cd, $S_0(Zn) = 479$ kg of Zn, $S_0(Cu) = 250$ kg of Cu, and $S_0(As) = 127$ kg of As. The quantity of metal extracted per hectare per year (Q_{Metal}, kg ha^{-1} year^{-1}) can be expressed by the following equation: $Q_{Metal} = (10^{-3} \times \text{bDW} \times D) \times (10^{-6} \times [\text{Metal}]\text{DW}) \times c$, where, bDW corresponds to the dry weight of plant biomass per plant (g plant^{-1}, DW); D is the density of plants per hectare = 150,000

Table 3 Phytoextracted metal quantities for 2006 and the calculated annual removal percentages

Element	Extracted quantity (kg ha^{-1} yr^{-1})	Annual removal (%)
Pb	125.8 ± 3	2.7
Zn	3.7 ± 0.4	0.8
Cu	1.4 ± 0.2	0.6
Cd	0.54 ± 0.2	0.6
As	0.26 ± 0.07	0.2

plants ha^{-1}; [Metal]DW is the total metal concentration measured in the shoots (mg Pb kg^{-1} DW), and c is the number of cultures per year: one culture was performed per year.

Table 3 shows the quantities of extracted metals and annual percentages of metals removed from the polluted soil in 2006. The values of the extracted quantities are quite high but the annual removal percentages are low, due to the high level of pollution of the studied soil, as already discussed for the BCF_{shoot} values. According to Baize et al. (2000), total metal concentrations (mg kg^{-1}) for uncontaminated French soils are: 30–50 for lead, 0.05–0.45 for Cd, 10^{-}100 for Zn, 2–20 for Cu, and 1–25 for As. Using the maximum values (50 mg kg^{-1}, and 0.45, 100, 20, and 25 for Pb, Cd, Zn, Cu, and As, respectively),the following target values can be calculated: $S_f(Pb) = 60$ kg, $S_f(Cd) = 0{,}054$ kg, $S_f(Zn) = 12$ kg, $S_f(Cu) = 2.4$ kg and $S_f(As) = 3$ kg. It can be noticed that in comparison to uncontaminated soils, the total initial quantities of metals are 79 times, 1574 times, 40 times, 104 times, and 42 times greater for Pb, Cd, Zn, Cu, and As, respectively.

Most current models for plant uptake of contaminants from soil were formulated on a differential mass-balance basis (Mathur and Yadav 2009; Verma et al. 2007). In order to assess the time required for a required removal percentage to be attained, an exponential decay function is therefore required. Each year a certain fraction of the total metal quantity in soil (noticed S_t) is removed; the next year a fraction of the remaining total is removed. Symbolically, this process can be described by the following differential equation: $dS/dt = -AS$, where S(t) is the metal quantity in the soil at the time and A is a positive number called the decay constant. The solution to this equation is: $S(t) = S_0 \times e^{-At}$ and $A = -\ln(S/S_0)/t$, where S_0 is the initial quantity of metal in the soil. In the case of lead, the constant A can be calculated from the metal concentration measured in the shoots: S(3 years) = S_0 – (extracted lead quantity in the first year + extracted lead quantity in the second year + extracted lead quantity in the third year) = 4710 – (25.7 + 27.5 + 125.7) = 4531 kg Pb ha^{-1}; A = 0.0129 $year^{-1}$ and the equation for the quantity of lead in the soil at time t is: $S_{Pb}(t) = S_0 \times e^{-0.0129t}$. If $S_{Pb}(t) = 60$ kg, then the calculated time is 338 years ($S_0 = 4710$). The same estimation performed with only one year of field culture would give 798 years: the interest in long-term experiments is therefore demonstrated.

In the case of highly contaminated soils, several actions could be taken in order to reduce the time needed for remediation: increasing the culture cycles, optimization of NPK and irrigation conditions and the use of organic chelates (assisted remediation). As explained in the experimental section, from October to March the plants remained in a 'still' state; however, it would only be possible to perform two culture cycles per year (two harvests) in order to reduce the period of remediation. According to KrishnaRaj et al. (2000), scented geraniums attained a total biomass of 5 kg fresh weight/plant within a 5-month growth period. In the present study, the maximum fresh biomass reached was 0.7 kg. Moreover, natural (e.g. low molecular weight organic acids) and synthetic chelates

(EDTA) can improve the availability and uptake of Pb (Saifullah et al. 2009; Shahid et al. 2011a). Chen et al. (2004) found that the addition of EDTA (2.5 and 5 mmol kg^{-1} EDTA) to a Pb-contaminated soil (2400 mg kg^{-1} total soil Pb) increased Pb concentrations in the shoots of ten plant species by 24–104-fold, with a greater increase in dicotyledonous species. Based on these previous studies, the time needed for remediation could be strongly reduced in the case of optimal phytoextraction conditions.

The estimated lengths of time discussed above correspond to the extraction of total Pb quantities in soils in order to reach natural background values, without information on bioavailability. However, plants absorb metals from soil solution and, according to Sarkar et al. (2008) and Huang et al. (1997), metal concentrations in soil solution are generally 100 to 1000 times lower than in the solid phase of the soil. *Pelargonium* cultivars could therefore be grown on various active industrial sites in order to decrease the available metal fractions (especially Pb) of moderately contaminated soils, i.e. less than 2000 mg Pb kg^{-1} (Huang et al. 1997), and thus reduce the risk to the biosphere.

CONCLUSIONS AND PERSPECTIVES

Our field experiments demonstrated the ability of the *Pelargonium* Attar cultivar to mobilize Pb, Zn, Cu, Cd, and As from a highly contaminated soil with no morpho-phytotoxicity symptoms. The plants accumulated high levels of Pb and Cd and therefore could be used multi-metal hyperaccumulators in the case of multi-metal contaminated soil. A strong increase in lead phytoextraction efficiency was observed with increasing plant maturity, significantly reducing the period required for remediation. Rhizosphere activity induced DOC, pH and metal availability changes, favoring phytoextraction. These results provide an interesting insight into improving calculated phytoextraction remediation times in the field experiment context, promoting this biological technique. In order to reach phytoextraction periods of decades, only moderately contaminated soils could be targeted. *Pelargonium* could be also cultivated at new plants in order to reduce available metal fractions. Optimization of NPK and irrigation conditions and/or the use of chelates and investigations concerning metal availability could also significantly reduce the time of treatment.

Moreover, the use of *Pelargonium* cultivars for remediation has several additional economic advantages that could offset the cost of deploying phytoremediation and render it a viable approach for remediating large areas of contaminated land: (1) the planting and harvesting of *Pelargonium* plants can be accomplished using conventional farming equipment; (2) the extraction of value-added essential oils from the harvested biomass and flash pyrolysis of the oil extracted from the biomass to yield commercial-grade organic acids and alcohols could be tested.

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