A field study of lead phytoextraction by various scented *Pelargonium* cultivars

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Abstract

Phytoremediation appears to be a promising technique for metal soil clean up, although its successful application on a large scale still remains a challenge. Field experiments for six scented *Pelargonium* cultivars, conducted on two Pb-contaminated calcareous and acidic soils, revealed vigorous plant growth, with no symptoms of morpho-phytotoxicity in spite of high Pb accumulation levels. Lead contents in the harvestable parts of all plants grown on the acidic and more contaminated soil were significantly higher than those grown on the calcareous soil. Three cultivars (Attar of Roses, Clorinda and Atomic Snowflake) are Pb-hyperaccumulator plants: they accumulated more than 1000 mg Pb kg^{-1} DW, with high biomass produced.

Keywords: Phytoremediation; Pelargonium; Cultivar; Pb; Soil–plant transfer

1. Introduction

Owing to its high persistence and several past and present uses (Chen et al., 2006), Pb is one of the most frequently encountered inorganic pollutants in soils (Alkorta et al., 2004). It is potentially toxic even at low concentrations, and above 400–500 mg Pb kg^{-1} soil is considered as a risk for human health by the US-EPA (2001). Clean up of contaminated soils, is a major challenge in environmental engineering.

Ex situ decontamination using physico-chemical techniques is labour intensive, expensive, and affects the soil's biological properties. The use of plants to decontaminate soils, known as phytoremediation could therefore offer an environment-friendly solution to soil remediation (Tanhan et al., 2007). The possibility of generating additional value from by-products of the biomass would improve the economic balance of this *in situ* decontamination technique. The most limiting factor is the time required to successfully clean contaminated soil to reach the goals set by legislation (Keller et al., 2005). Its successful application is inherently dependent upon the choice of an appropriate plant which should produce high biomass through rapid growth and accumulate abundant quantities of metals in easily harvestable parts of the plant (Prasad and Freitas, 2003). However, the combination of these two characteristics is rarely observed, as most of the high biomass producing plants, such as *Brassica juncea*, concentrate only moderate amounts of metals (Lasat, 2002) and hyper accumulating plants often produce low biomass (Reeves and Baker, 2000). Current research on the development of phytoremediation is thus targeted towards the identification of new plant species (Prasad and Freitas, 2003).

In a greenhouse study, cuttings of *Pelargonium sp.* "Frensham" grown on artificial soil and fed with different

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metal solutions, were capable of taking up large amounts of Pb over a 14 days treatment period while producing high biomass (Saxena et al., 1999). Most of the data available to-date regarding phytoremediation concerns hydroponics and pot experiments. In this context, field experiments on two contaminated contrasting soils were conducted to explore Pb phytoextraction capacity of six *Pelargonium* cultivars.

2. Materials and methods

2.1. Plant material

Six scented *Pelargonium* cultivars, propagated from cuttings of plantlets marketed by Heurtebise nursery, Clansayes, France, (http://www.pepinieres-heurtebise.com/), were grown in 0.5 l pots containing well-washed coarse Perlite, in the greenhouse, and regularly irrigated with nutrient solution containing 5000 μM KNO_3, 2000 μM $Ca(NO_3)_2$, 5000 μM KH_2PO_4, 1500 μM $MgSO_4$, 46 μM H_3BO_3, 9 μM $MnSO_4 \cdot H_2O$, 0.1 μM $MoNaO_4 \cdot 2H_2O$, 0.9 μM $CuSO_4 \cdot 5H_2O$, 15 μM $ZnSO_4 \cdot 7H_2O$ and 90 μM Fe–EDTA. The notations used in the present publication for the six scented *Pelargonium* cultivars are Attar, Atomic, Concolor, Clorinda, Rose, and Sweet, respectively, for *P. capitatum* cultivars Attar of Roses, Atomic Snowflake and Concolor Lace, *P. domesticum* cultivar Clorinda, *P. graveolens* cultivar Rose Geranium, and *Pelargonium sp.* cultivar Sweet Leaved.

2.2. Soil characteristics

Two top soils in the vicinity of active Pb recycling plants run by the company "Société de Traitement Chimique des Métaux" (STCM), contaminated by atmospheric fallout, were used for the field experiments. The first, (soil-B) is a calcic cambic soil, located near the factory of Bazoches in North-Western France, and surrounded by agricultural land. Lead contamination is present to a major extent, but other minor metal elements (Zn, Cu, Ni, As, Cr) have also been detected (Cecchi et al., 2008). The second, (soil-T) is an acidic soil, from urban Toulouse, located in South-Western France. Some physico-chemical characteristics of these two soils were determined by Lara Europe Analyses laboratory and are presented in Table 1. Soil-B is approximately 20 times less contaminated than soil-T, has a higher pH and is calcareous (2.1%). Organic matter (OM) concentration in soil-B is less than that in soil-T. Soil texture is also significantly different for the two soils.

2.3. Field experiments

The six *Pelargonium* cultivars were all field-tested in five replicates on both sites from May 2004 to July 2005. A density of 15 plants per m^2 was used on an experimental plot of 50 m^2 for soil-B and 10 m^2 for soil-T. The plants were grown under natural agro-climatic conditions, with neither fertilisation nor optimum irrigation so as to assess the feasibility of the remediation process in the natural context of an industrial site. In both cases, all above ground parts were trimmed off the plants after 150 days of growth i.e. in September 2004. Since *Pelargonium* does not tolerate temperatures below –6 °C, the plants grown in soil-B in North Western France, did not survive the frosts. Data for soil-B was from a single harvest. On the other hand, plants grown on soil-T under the warmer climate of South Western France, had two growing seasons. Shoots in soil-T were harvested 120 days after the onset of sprouting during the second season.

2.4. Shoot sample analyses

After harvest, the aerial parts of five replicated plants of each cultivar were weighed fresh. Lead adsorbed on to the leaf surfaces was determined according to the method as described by Ferrand et al. (2006). Shoots were then oven-dried at 80 °C for 48 h and dry weight determinations carried out. The dried plant material was ground to powder and 1 g of each sample was treated with a 1:1 acid mixture of trace metal grade HNO_3 and H_2O_2 at 80 °C for 4 h in a closed reactor for complete mineralization. Total Pb contents for the various *Pelargonium* samples were measured in solution by inductively coupled plasma optical emission spectrometry (ICP-OES), IRIS Intrepid II XDL/Thermo Electron Corporation. Standard reference materials were used to assess the accuracy of the acidic digestion procedure and measurements. Measurements on the reference samples (Virginia tobacco leaves, CTA-VTL-2, ICHTJ) demonstrated the validity of the assays: the concentration we found was 21.6 ± 1 mg kg^{-1}, for a certified value of 22.1 ± 1.2 mg kg^{-1}. Calculations were performed relative to shoot dry weight (DW).

2.5. Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) with two factors, using the software Statistica, Edition'98 (StatSoft Inc., Tulsa, OK, USA). For each bioassay, mean values with different letters represent a signif-

Table 1
Characteristic properties of the two soils

Soil	Clay (g kg^{-1})	Silt (g kg^{-1})	Sand (g kg^{-1})	pH_{H_2O}	N_{Total} (g kg^{-1})	P_2O_5[a] (mg kg^{-1})	Pb (mg kg^{-1})	OM
Soil-B	221	527	251	8.0	3.3	88	1830	5.0
Soil-T	112	337	550	6.0	3.8	95	39250	8.5

[a]Joret–Hebert method, OM = Organic matter.

icant difference ($p < 0.05$) as measured by the LSD Fisher test.

3. Results and discussion

3.1. Screening of *Pelargonium* cultivars for Pb accumulation potential

The *Pelargonium* cultivars grown on the two soils were screened for their Pb accumulation capacity. Under our experimental conditions, Pb adsorbed on *Pelargonium* leaves, as determined by a desorption procedure, was below the ICP-OES detection limit (0.05 mg Pb kg^{-1} DW) or represented less than 1% of the total shoot Pb. It was therefore ignored in all further calculations. The results presented in Table 2 indicate that there are significant differences in the total biomass in terms of dried matter of cultivars grown on contaminated calcareous soil-B as well as total Pb contents of different cultivars and lead quantity extracted per plant. According to Baker et al. (2000), shoot concentrations of Pb in hyper-accumulators should be in the range of 1000 mg kg^{-1} DW. The field experiments on soil-B showed the ability of Attar, Clorinda and Atomic *Pelargonium* cultivars to accumulate Pb in the same range. Schnoor (1997) reported that any plant useful for phytoremediation, should be vigorously growing, easily harvestable and exhibit a biomass of more than three tons DW ha^{-1} y^{-1}. Our results demonstrate that such is the case with the scented *Pelargonium* cultivars used in this study. On the basis of calculations, taking into account the planting density and number of cultures performed per year, the shoot dry weight produced was more than three tons DW ha^{-1} y^{-1} for all the *Pelargonium* cultivars and reached 45.3 tons ha^{-1} y^{-1} with Atomic cultivated on soil-T in 2005.

Comparison of Pb contents and extraction between Attar, Clorinda and Atomic *Pelargonium* cultivars grown on the two soils during 2004 is given in Fig. 1. Regardless of the cultivar, lead extraction was higher for *Pelargonium* plants, grown on soil-T. During the course of experiment, no morpho-phytotoxicity symptoms were observed on the *Pelargonium* cultivars cultivated on the two contaminated soils. The ability of the three *Pelargonium* cultivars to tolerate high concentrations of Pb on both calcareous and acidic soils satisfies the first prerequisite for use in phytoremediation. Taking high biomass into consideration, cultivars Attar, Clorinda and Atomic meet the two criteria to be qualified as Pb-hyperaccumulators.

Table 2
Biomass, total lead content in shoots and lead extracted per plant, for field cultures on soil-B

Cultivar	Shoot dry weight (DW) (g plant^{-1})	[Pb] Shoots DW (mg Pb kg^{-1})	Pb extracted per plant (mg Pb plant^{-1})
Attar	66 ± 13c	1467 ± 134c	96.7 ± 19b
Clorinda	56 ± 14c	1182 ± 336b	64 ± 15b
Atomic	28 ± 6ab	1107 ± 193b	30.5 ± 7b
Graveolens	39.5 ± 9.5b	435 ± 157a	17 ± 7a
Sweet	22.5 ± 6.5a	549 ± 76a	12 ± 1.5a
Concolor	16 ± 3a	468 ± 91a	8 ± 2.5a

For each bioassay, mean values with different letters are significantly different ($p < 0.05$) as measured by LSD Fisher test.

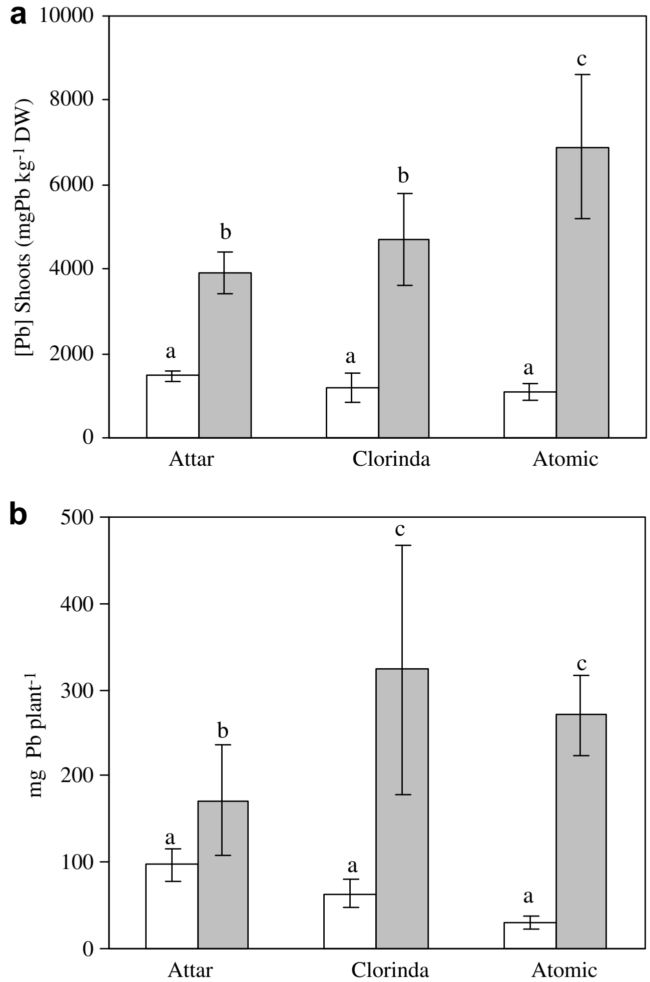

Fig. 1. Lead accumulation (2004) by three *Pelargonium* cultivars on two soils [B (□) and T (■)]. (a) Total Pb contents in shoots (mg Pb kg^{-1} DW). (b) Lead extracted per plant (mg Pb plant^{-1}).

3.2. Factors influencing the transfer of lead from soil to shoots

Data in Table 2 shows that the cultivar is determinant for the soil–plant transfer of Pb. The six cultivars grown on soil-B, could be divided into two groups for their Pb accumulation potential, the first one accumulating more than 1000 mg kg^{-1} DW (Attar, Clorinda and Atomic) and the second group (Rose, Sweet and Concolor) accumulating, on average, three times less. The differences observed in Pb accumulation between the various cultivars of *Pelargonium* can be accounted for by variations in the genetic background. Under similar pedo-climatic conditions, differences in the net Pb accumulation levels observed between the 6 cultivars could be the consequence of complex signalling and molecular dialogues between biotic and abiotic components of the rhizosphere together with those of the root cells, which will together influence metal speciation, modify metal availability and translocation (Ma and Nomoto, 1996).

Under our conditions of field experimentation in soil-T, Pb concentration in aerial parts was often greater than 3000 mg Pb kg^{-1} DW. These values are close to those of Krishna Raj et al. (2000) where plants were treated daily with 7.55 mM $Pb(NO_3)_2$ solutions (corresponding to 1564 mg Pb l^{-1}) over a 14 day-period. According to Huang et al. (1997), Pb concentrations in the soil solution were usually less than 0.1% of total Pb in the solid phase of the soil; i.e. 1.8 and 39 mg l^{-1} for soil-B and soil-T, respectively. In the field, plants were able to accumulate for much longer times (in comparison with hydroponic conditions) but were exposed to much lower concentrations. *Pelargonium* cultivars could favour the transfer of Pb from the solid phase of contaminated soils to their soil-solutions.

The soil type significantly influenced Pb content (Fig. 1a) of *Pelargonium* cultivars and the quantity of lead extracted per plant (Fig. 1b). The transfer factor (TF): ratio of lead concentration in plant to soil, was calculated for the three cultivars on the two soils. TF for the less contaminated soil-B were higher compared to soil-T. Attar had maximum values of TF (0.8), followed by Clorinda (0.65) and Atomic (0.6) on soil-B. For soil-T, the values of TF were ordered: Atomic (0.18) > Clorinda (0.12) > Attar (0.1) and the values were similar for both growing seasons. For a given plant biomass and total metal concentration in the soil, the higher the value of TF, the greater the phytoremediation efficiency. As soil-T presented a very high lead concentration in comparison with soil-B, the ratios between the values of total shoot Pb contents measured for the two soils (soil-T/soil-B) were also calculated. These ratios were: 6.2, 4 and 2.7, for Atomic, Clorinda and Attar *Pelargonium* cultivars, respectively, i.e. greater Pb concentrations in the more acidic and more contaminated soil-T compared to that in soil-B. According to Acidic pH is reported to favour the solubility of Pb (Sanchez-Camazano et al., 1994). The three cultivars also differed in Pb extraction per plant (Fig. 1b) and the cultivar accumulating maximum Pb in both soils was not the same. Attar accumulated maximum Pb per plant on soil-B and Atomic, the minimum. On soil-T, Clorinda extracted of the most Pb per plant followed by Atomic and Attar, respectively. Plant responses to metals can differ strongly as complex interactions exist, particularly in the rhizosphere, between soil components and metals (Dumat et al., 2006).

Fig. 2 compares the capacity of Pb extraction per plant on soil-T, over two years (2004 and 2005). The efficiency of Pb extraction increased threefold during two growing seasons for Atomic and remained unchanged for Clorinda and Attar. This change could be the result of biomass variation. For Atomic, dry shoot biomass was 12.4 and 45.3 tons ha^{-1} y^{-1} DW in 2004 and 2005, respectively, while the shoot biomass of other two cultivars did not significantly differ between the two growing seasons (24 and 15 tons ha^{-1} y^{-1} DW, respectively for Clorinda and Attar).

3.3. Phytoremediation efficiency under field experimental conditions

Fig. 3 represents the estimated time to clean contaminated top soils (0–10 cm) by the three hyper-accumulator cultivars grown on soil-B in 2004 and, soil-T in 2004 and 2005. Only the top 10 cm were considered because the lead pollution was mainly found in this layer (Cecchi et al., 2008): at 30 cm depth the soil Pb concentration was reduced 10-fold. Lead availability to plants is controlled by its interactions with the soil components and speciation (Dumat et al., 2001 and 2006), the more labile metal fraction being more readily absorbed by the plant roots (Bermond et al., 2005). The metal extracting potential increases with increasing biomass as observed for Atomic in 2004 and 2005. Moreover, in the rhizosphere we postulate that *Pelargonium* is able to displace the solid-solution equilibrium and favour further absorption of lead. But only few kinetic data on phytoextraction of metals (Tongtavee et al., 2005) are available in the literature and none concern the absorption of lead by *Pelargonium*. We therefore estimated the time needed for total lead extraction using the equation described below.

Assuming that Pb phytoextraction follows a linear pattern, the quantity of Pb extracted per hectare and per year (Q_{Pb}: kg Pb ha^{-1} y^{-1}) can be expressed as

$$Q_{Pb} = (10^{-3} \times b_{DW} \times D) \times (10^{-6} \times [Pb]_{DW}) \times c \qquad (1)$$

In this equation, b_{DW} corresponds to the dry weight of plant biomass per plant (g $plant^{-1}$ DW); D is the density of plants per hectare = 150,000 plants ha^{-1}; $[Pb]_{DW}$: total Pb concentration measured in shoots (mg Pb kg^{-1} DW); c

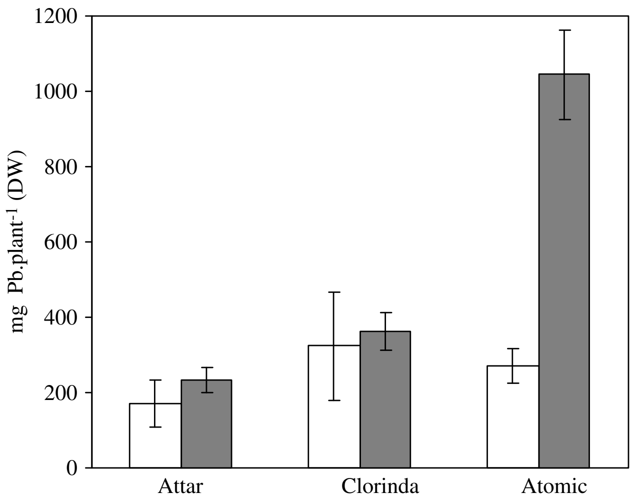

Fig. 2. Comparison of Pb extraction per plant (mg Pb $plant^{-1}$) on soil-T during two growing seasons. First season (□) from May to September 2004 and second (■) from April to July 2005.

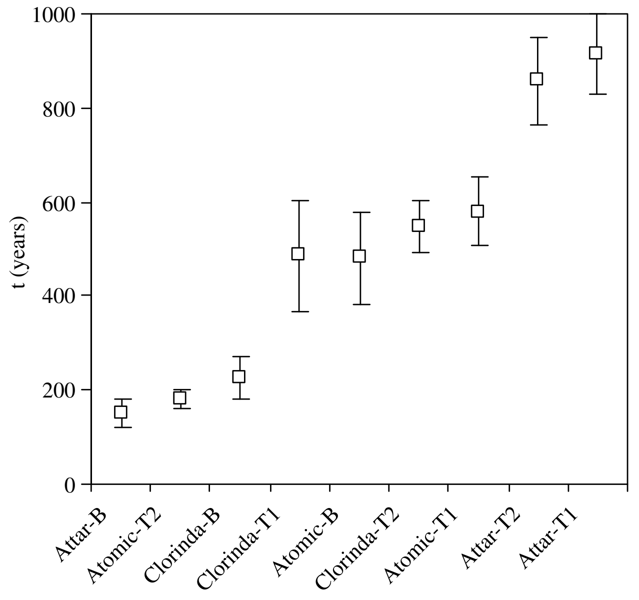

Fig. 3. Phytoremediation efficiency assessment of *Pelargonium* cultivars with respect to time required for cleaning contaminated soils (-B stands for culture on soil-B, -T1 and -T2 correspond to cultures on soil-T in 2004 and 2005, respectively).

is the number of crops per year: one for soil-B and two for soil-T. Using an average soil density of 1.2×10^3 kg m^{-3}, the weight corresponding to a top soil sheet of 1 m^2 surface would be 120 kg. Therefore, the quantities of Pb that correspond to one hectare of the contaminated top soils, termed S (total lead quantity in the top soil), are $S_B = 2.196$ tons and $S_T = 47.1$ tons, respectively, for soil-B and soil-T. The number of years necessary for the *Pelargonium* plants to perform total Pb extraction can be expressed by the following general equation:

$$t = S \times 10^3 / Q_{Pb} \quad (2)$$

The time estimated for total soil remediation ranges between 151 years for Attar grown on soil-B (minimum value observed) and 914 years for Attar on soil-T in 2004 (maximum value observed). The timing is site-specific and depends on future land use (Huang et al., 1997). However, *Pelargonium* cultivars could in any case reduce the available Pb fraction of contaminated soils and thus decrease the risk for the biosphere.

4. Conclusion

Our field experiments demonstrated the ability of several high biomass *Pelargonium* cultivars to hyper-accumulate Pb on both calcareous and acidic contaminated soils, without showing morpho-phytotoxicity symptoms. The estimated time scale for soil remediation with the *Pelargonium* plants was 151 years for soil-B with Attar and 182 years with Atomic for the soil-T. So *Pelargonium* cultivars could provide efficient remediation of moderately Pb contaminated soils.

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