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María Julieta Salazar, María Luisa Pignata

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Lead accumulation in plants grown in polluted soils. Screening of native species for phytoremediation.

María Julieta Salazar[1*], María Luisa Pignata[1].

[1] Multidisciplinary Institute of Plant Biology, Pollution and Bioindicator section, Faculty of Physical and Natural Sciences, National University of Córdoba,
Av. Vélez Sársfield 1611, X5016CGA Córdoba, Argentina.

* Corresponding author. Tel/fax: +54 351 4344983 int. 6 / +54 351 4334139

E-mail address: mjulietasalazar@gmail.com (M. J. Salazar)

Abbreviations

EC: Electrical Conductivity. %OM: Organic Matter percentage. DW: Dry Weight. TF: Translocation factor. BCF: Bioconcentration Factor.

ABSTRACT

In the present work, we focused on soils contaminated with elevated lead concentrations in an agricultural and residential area surrounding a lead smelter plant in Bouwer, province of Córdoba, Argentina. The aim of this research work was to assess the phytoextraction suitability of native plant species growing in the vicinity of a former lead smelter.

The lead concentration in leaves, stems and roots was determined in ten species collected at ten sampling sites along a lead concentration gradient in soil. It was found that at circa 1,600 μg g^{-1} Pb HCl 0,5 M extractable concentration in soil two native species, *Tagetes minuta* L. and *Bidens pilosa* L. accumulated high values of Pb concentration in leaves (380.5 μg g^{-1} DW and 100.6 μg g^{-1} DW, respectively). Therefore, *Tagetes minuta* L. and *Bidens pilosa* L. have a considerable phytoremediation potential for lead polluted soils. At the same sampling site, *Sorghum halepense* (L.) Pers., a non-native species, only bioconcentrate lead in roots (1,406.8 μg g^{-1} DW) showing a phytostabilization potential. The results of this study should be further developed in order to confirm the potential use of these species in soil remediation programs.

Keywords: Lead polluted soils; phytoextraction; phytostabilization; *Tagetes minuta*; *Bidens pilosa*; *Sorghum halepense*.

1. Introduction

Heavy metal soil pollution is currently considered one of the most serious environmental problems due to its persistence and toxicity, having a great impact as the development of areas without soil in good condition is difficult (Becerril Soto et al., 2007).

Anomalous concentrations of heavy metals in soils can result from natural or anthropogenic factors, with latter being the most common. There are many studies indicating that the soil is a sink of heavy metals acquired through the aerial deposition of particles emitted by urban and industrial activities (Fabietti et al., 2009), vehicle exhausts (Hernandez et al., 2003) and agricultural practices (Fabietti et al., 2009), among other sources. Thus, the accumulation of metals in soils may produce unwanted changes in its properties (Navarro-Aviñó et al., 2007).

Heavy metal soil pollution implies complex and costly measures, being necessary in order to utilize the soil, due to the high residence time of metals in soils with the additional possibility of groundwater migration (Becerril Soto et al., 2007). The remediation of heavy metal polluted soils represents a technological challenge for both industries and government institutions, with phytoremediation being an alternative that contemplates soil conservation by harnessing the potential of plants to transform or eliminate the contaminants accumulating in their tissues (Alvarez and Illman, 2006). This emergent technology has many advantages over traditional decontamination techniques, especially when the plants used are native or non-invasive. It is important to note the favorable results obtained in cost-benefit terms, ecological features of social and aesthetic value which not only eliminates heavy metals but also recovers soil quality, functionality and sustainability (Alvarez and Illman, 2006). Although phytoremediation applicability requires long term actions, the transport and storage risks are lower compared to chemical ex-situ treatment (Betancur et al., 2005; Montes Botella, 2001).

On the other hand, some studies have reported good results in the use of contaminated sites as a gene-tolerant bank for use in the phytoremediation of soil, with this practice involving a minimum intervention impact (Becerril Soto et al., 2007).

However, the implementation of this technology has certain conditions that must first be studied in order to select suitable species for each situation. Among these conditions, the most mentioned are the plant`s ability to raise the metals, biomass production, the plant organ in which metals are accumulated, the effect of weather and seasonality on the species, bioavailability of metals in soil and its toxicity levels and the competition between different metal ions (Montes Botella, 2001). Many of these problems, however, can be avoided if extensive knowledge is obtained regarding species selection and the establishment of optimal working conditions by considering the above factors. Despite phytoremediation being a method that is currently being used in many parts of the world, studies related to this are scarce in Argentina, especially those using native species for heavy metals removal (Arreghini et al., 2006; Bonfranceschi et al., 2009; Flocco et al., 2002; Torri et al., 2009).

In the present study, we focused on soil and plant metal concentrations in an agricultural and residential area around a former battery recycling plant. Numerous studies have reported that this kind of smelter is a source of Pb contamination in the surrounding soils (Cala and Kunimine, 2003; Ramírez, 2008). Therefore, as the vegetal community growing around the smelter represented a great opportunity to study native plant suitability for phytoremediation of lead contaminated soils, the purpose of this study was to investigate lead transfer to native species and their potential application to soil phytoremediation.

2. Materials and Methods

2.1. Study area

The study area was located in Bouwer, which is 18 km South from Córdoba City, Argentina (Figure 1), and has a population of 1,500 inhabitants. The soil at the site is an Entic Haplustoll and the climate is mild, with an annual mean temperature of about 15 °C and an average annual rainfall of 500-900 mm (Gorgas and Tassile, 2003). This is an area characterized by a former battery recycling plant that once operated here from 1984 to 2005 (31°33'34.02"S; 64°11'9.05"W). This smelter was closed down in 2005 due to functional problems associated with a lack of emission control and improper disposal of waste. In addition, diseases usually related to lead were reported to affect the workers and neighbors of the industrial site (La Voz del Interior, 2005). Consequently, measurements of the emissions were carried out by the provincial authorities of Córdoba, which indicated that these exceeded the permitted lead value by 35 times (Comuna de Bouwer, 2008). Although the factory was then closed, the site has not been remediated and there is still a potential risk to human health, and it is still common to find slag scattered around the town and its vicinity. In the proximity of the abandoned lead smelter, the land use is principally agricultural (mostly soybean), with the rest being residential or subject to the growth of uncultivated plants.

In the present study, ten vegetation and soil sampling sites (Figure 1) were chosen in order to contemplate a Pb concentration gradient and they were categorized in decreasing order of lead concentration in pseudototal fraction (site 1 having the highest Pb concentration and sites 9 and 10 being the reference sites). In order to determine suitable native plant species to be used in phytoremediation, the most abundant herbaceous plants growing naturally were sampled at ten points with different lead soil concentrations. A total of ten species was assessed, but not all of these were present at every point.

2.2. Sampling procedure

Topsoil composite samples were collected in the study area at a depth of 0-10 cm, with foreign objects removed. At each point, three composite samples were obtained. Topsoils were kept in plastic bags, and once in the laboratory they were oven-dried at 40 °C for 24 h. All samples were sieved to < 2 mm (using a polyethylene sieve) and stored in darkness until analytical procedures were carried out (Bäckström et al., 2004).

Plant sampling was performed at the end of summer season when plants were flowering. Approximately 10 exemplars of each of the most abundant herbaceous species at each site (including the roots, stems, leaves) were collected and identified by their scientific names at the Botanical Museum of IMBiV, CONICET (registration codes from 365854 to 365864).

Samples were divided in two pieces in the field, roots were kept in plastic bags while shoots (stems and leaves) were kept in paper bags. Once in the laboratory, they were washed with ultrapure water and then oven-dried at 40 °C to dry weight (DW). For each test site, species, and plant organ, three composite samples were made with three exemplars each, and then these were triturated and stored in darkness until carrying out analytical. The tenth exemplar was registered in the Botanical Museum herbarium.

2.3. Physico-chemical analysis

2.3.1. Electrical conductivity, pH and percentage of organic matter in topsoils

The topsoil pH and electrical conductivity (EC) were measured in 1:5 soil:water suspension triplicates at room temperature (Bäckström et al., 2004). In order to calculate the dry weight (DW), samples were oven-dried for four hours at 105 °C to constant weight (Al-Khashman and Shawabkeh, 2006), and the percentage of organic matter (%OM) was determined according to Peltola and Åström (2003) by the combustion of the samples at 500 °C for four hours.

2.3.2. Heavy metals in topsoils

With the aim of analyzing non-residual and anthropogenic metals in the topsoils, a 0.5 M-hydrochloric acid extraction was performed. The extraction was accomplished by mixing 7 g DW of topsoil (63 µm sieved) with 25 mL 0.5 M HCl and shaking it at room temperature for 30 minutes (Sutherland et al., 2004).

Pseudototal metal concentrations in the topsoils were measured using a hydrochloric and nitric acid extraction (Ketterer et al, 2001). First, 5 g DW of topsoil were burnt at 450 ºC for four hours. The extraction solution was prepared by mixing the ashes of 5 g DW of topsoil previously burnt at 450 ºC for four hours, with 10 mL of HCl/HNO_3 3:1 (V/V) and shaking them at room temperature for 30 minutes.

After 24 h, the solution was filtered and analyzed using a Perkin-Elmer AA3110 spectrophotometer to measure extractable Co, Cu, Ni, Mn, Zn, Pb and Fe (Sutherland, 2002).

2.3.3. Lead concentration in plants

The concentrations of Pb in plant tissues were determined using a 20% hydrochloric acid and nitric acid extraction, with each sample being analyzed in triplicate (Bermudez et al., 2009). First, 3 g DW of each organ (leaves, stems and roots) were burnt at 450 ºC for four hours. Then, the extraction solution was prepared by mixing the ashes with 2.5 mL of 20% HCl and 0.5 mL of analytical commercial pure HNO_3. After 4 h, the solution was filtered and analyzed using a Perkin-Elmer AA3110 spectrophotometer to measure the Pb concentration.

2.4. Quality control

As a quality control, blanks and samples of the standard reference certificated material "CTA-OTL-1" (oriental tobacco leaves, Institute of Nuclear Chemistry and Technology) for plants, and reference certificated material (CRM GBW07405 Soil-NRCCRM, China) for soils, were prepared in the same way, and were run after ten determinations to monitor the potential sample contamination during analysis. The results were found to be within 89% and 92% of the certified value for CTA-OTL-1 and within 86% and 90% for CRM GBW07405 Soil-NRCCRM, with the data indicating a low error of typically less than 15%. The coefficients of variation of replicate analyses were found to be less than 10%.

2.5 Data Analyses

2.5.1 Statistical analyses

The Shapiro–Wilks test for normality was applied, and non-normal distributed elements were not found so log-transformation was not necessary. Heteroscedasticity was found in almost all cases, so it was included in the model using Infostat/E coupled to R to perform an Analysis of Variance (ANOVA) to determine Pb soil effects on the Pb concentration in plant. Whenever the ANOVA indicated significant effects ($p < 0.05$), a pairwise comparison of means was undertaken using the Tukey test.

2.5.2 Translocation and Bioaccumulation factors

In this study, translocation factor (TF) and bioaccumulation factor (BCF) were calculated using the ratio of Pb concentration in roots and shoots [$TF = C_{shoot}/C_{root}$] and the ratio of Pb_{HCl} concentration in soils and shoots [$BCF = C_{shoot}/C_{soil}$], respectively (Bu-Olayan and Thomas, 2009). To obtain Pb concentration in shoots the values found for leaves and for stems were multiplied by the total biomass of each organ and they were added to each, finding the total mass of Pb in aerial organs. Then, this result was divided by the total mass of the aerial organs (stems plus leaves).

3. Results

3.1. Heavy metals in soils

The lead concentrations for pseudototal and extractable soil fraction, %MO, pH, EC, and distance to the lead source for the ten sampling sites are shown in Table 1.

According to the Argentinean and Canadian soil quality guidelines (Table 2), the mean Pb pseudototal concentrations at sampling sites 1, 2, 3, 4, 5 and 6 were above the limits for agricultural, residential and industrial land use, while at sampling sites 7, 8, 9 and 10, values were within these limits. The pseudototal concentrations for Pb were significant higher at sites 7 and 8 than those at sites 9 and 10.

It is important to note that sampling site 3 was chosen for the presence of a slag pile. Although it was at a distance of 1,260 m from the lead smelter, it revealed lead concentrations as high as at the sites next to the smelter.

The Zn, Co, Cu, Ni concentrations for the pseudototal fraction presented values within the legal thresholds [Zn: (34 ± 8) mg kg^{-1}; Co: (12 ± 5) mg kg^{-1}; Cu: (9 ± 4) mg kg^{-1}; Ni: (5 ± 2) mg kg^{-1}]; and did not show a relation to the smelter. There are no legal thresholds for Mn and Fe, however their concentrations in pseudototal fraction were within expected values for the region if they are compared to previous research (Bermudez et al. 2010), [Mn: (435 ± 105) mg kg^{-1}; Fe: (10000 ± 2000) mg kg^{-1};].

The extraction performed with 0.5 M HCl has been successfully used to assess total non-residual or non-lattice-held heavy metal concentrations in streams, road sediments and soils (Sutherland, 2002; Sutherland et al., 2004; Sutherland and Tolosa, 2001). In this method, 0.5 M HCl was used because dilute HCl liberates adsorbed detrital and non-detrital carbonate-bound metals together with much of the Fe/Mn oxide and organic-associated metals, while minimizing the loss of residual silicate-bound metals (Sutherland and Tolosa, 2001). The Pb concentration in this fraction presented high values, almost reaching pseudototal concentrations at several sites (table 1). The Zn, Co, Cu, Ni, Mn, Fe concentrations for the 0.5 M HCl extractable fraction presented the following values: Zn: (9 ± 3) mg kg^{-1}; Co: (3.0 ± 0.5) mg kg^{-1}; Cu: (4 ± 1) mg kg^{-1}; Ni: (2.7 ± 1.1) mg kg^{-1}; Mn: (270 ± 50) mg kg^{-1}; Fe: (600 ± 200) mg kg^{-1}.

Organic matter percentage presented values between 5.4% and 10.5%, there was not a clear trend but it is remarkable that taking into account the most polluted soils as a group (1 to 6), they had lower %MO compared to the others (7 to 10). The soil pH was found to be near neutrality, with values being slightly acidic except for sites 1 and 4 that presented strong acidity. Electrical conductivity presented variable results, and that variability was not associated to pseudototal or 0.5 M HCl extractable heavy metal concentration.

3.2. Screening of native plant species for phytoremediation of lead polluted soils

Table 3 shows the 10 species studied, the family they belong to, their status, habit, sampling sites and the mean, maximum and minimum Pb concentrations (μg g^{-1} DW) measured in leaves. To evaluate the potential of the plant species in phytoremediation, lead concentration in leaves was considered in this work as the most significant variable.

Since not all species were found at all sampling sites, and as Pb soil concentrations were variable, we consider that the most important value to analyze, according to the aim of this work, was the maximum one. The highest maximum Pb leaf concentration was found for *Tagetes minuta* L. (396 µg g^{-1} DW), followed by *Bidens pilosa* L. (111 µg g^{-1} DW).

3.3. Lead concentration in plants.

Table 4 shows the Pb concentrations in leaves, stems and roots for each species in the study area and it gives ANOVA results for the seven species found at more than one site. *B. pilosa* showed high Pb root concentrations, principally at site 1 (741 ± 64 µg g^{-1} DW) and site 5 (448 ± 75 µg g^{-1} DW). Furthermore, the Pb accumulation was found to be relatively high for leaves at site 5 (101 ± 9 µg g^{-1} DW) and for stems at site 9 (99 ± 2 µg g^{-1} DW).
Regarding to *T.minuta*, this species was only found at sampling sites 2 and 5. The leaves, stems and roots showed high Pb concentrations at site 5 (380 ± 22 µg g^{-1} DW, 72 ± 2 µg g^{-1} DW and 859 ± 36 µg g^{-1} DW, respectively). Despite Pb plant accumulation at site 2 was low, it is important to note that Pb and Pb_{HCl} soil content at site 2 were significantly higher than at site 5.

The rest of species studied did not reveal high Pb concentrations in leaves, limiting their use for phytoremediation. In addition, *Sorghum halepense* had a high Pb concentration in the roots at site 5 (1407 ± 64 µg g^{-1} DW), so this species can not be used in Pb phytoextraction since it did not translocate Pb to leaves. However, it may be useful for Pb immobilization in soil.

4. Discussion

4.1. Heavy metal concentration in topsoils

Comparing the results of this study with others about soil metal concentration around lead battery smelters, the total lead concentration found in this study were lower than the ones reported in the USA by Elliott and Brown (1989) and Austin et al. (1993), (21% w/w and 30,000 mg kg^{-1} respectively), but similar to those found by Kim et al. (2003) (13,260 mg kg^{-1}), also in the USA, and higher than those found by Eckel et al. (2002) in the USA (between 306 and 2,550 mg kg^{-1}), or by Cala and Kunimine (2003) who reported 5,900 mg kg^{-1} in Spain.

The relation between pseudototal and 0.5 M HCl extractable concentrations for Pb was surprisingly elevated, these values could indicate that Pb is not strongly bounded to soil. Relation for the rest of the heavy metal studied was the expected according to Sutherland and Tolosa (2001),

4.2. Lead content in plants

Although lead content in plants depends on different environmental factors (Blaylock and Huang, 2000; Blaylock et al., 1997), the Pb in plants growing in uncontaminated soils is usually between 0.1 and 10 µg g^{-1} DW with an average of 2 µg g^{-1} DW (Kabata Pendias and Pendias, 1984; Palacios et al., 2002). Despite most authors using 1,000 µg g^{-1} DW as the criterion for defining Pb hyperaccumulation, nominal threshold criteria currently provide the only practical operational framework for recognizing hyperaccumulators (van der Ent et al., 2013). Van der Ent et al. (2013) proposed to additionally set hyperaccumulation threshold criteria at a level that is (i) 2–3 orders of magnitude higher than in plant leaves on normal soils, and (ii) at least one order of magnitude greater than the usual range in plant leaves on metalliferous soils. Taking this into account, despite not reaching 1,000 µg g^{-1} DW both species *T. minuta* and *B. pilosa* showed values that make them candidates for further studies about their capabilities as phytoremediators [*T. minuta* meeting requirement (i) and *B. pilosa* meeting requirement (ii)]. Nevertheless, as it was pointed by van der Ent et al. (2013) these species must be confirmed as Pb accumulators in further glasshouse experiments.

In addition, both species are annual and fast-growing herbs, features considered to be optimal for phytoremediation (Blaylock et al., 1997). Other species from genera *Tagetes* and *Bidens* were reported for phytoextraction of heavy metals from soils, but *T. minuta* and *B. pilosa* have never been reported for these purpose. Most of the other studied species also showed higher concentrations than plants growing on uncontaminated soils, but with relatively low differences, indicating that lead was transferred to plant tissues but not accumulated at concentrations to be of interesting for phytoremediation.

The Pb accumulation found for *T. minuta* and *B. pilosa* leaves in our study were lower than those reported by other authors in herbaceous species, such as Kabata-Pendias and Pendias (1984) in Poland, and Palacios (2002) in Spain, where the Pb contents rose to 2,700 and 4,100 μg g^{-1} DW, respectively. However in these studies it was not clear if Pb had come from direct airborne deposition or from the soil, since the smelters were working during the research and it is known that translocation from soil to aerial tissues is normally low (Huang and Cunningham, 1996). In contrast, in our study the only source was the soil.

In this study we focused on the absolute concentrations of Pb in plant tissues, but also translocation and bioaccumulation factors were calculated as they are usual criteria to highlight phytorremediator plant species. Table 4 presents the TF and BCF for each species at each site. To categorize results, TF>1 indicates that plant translocate metals effectively from root to shoots; BCF>10 indicates hyper-accumulator species, BCF>1 means accumulator species, and BCF<1 points excluder species (Bu-Olayan and Thomas, 2009). Consequently, almost all the species studied behave as effective Pb translocators from roots to shoots when soils are not polluted (TF>1 at sites 8, 9 and 10). Taking into account BCF, most species presented higher BCF at not polluted sites ($BCF_{8,\ 9\ \mathrm{and}\ 10}$ > $BCF_{1\text{-}5}$), none of them behaved as hyper-accumulators, few did as accumulators, and most of them were excluder species. These translocation and accumulation capabilities are limited by increased Pb content in soils, so those species behave as excluder in polluted soils. Whereby, these species can be considered as good candidates for phytostabiliaztion, mainly *Sorghum halepense*, due to the high tolerance demonstrated in this study, preventing a metal transfer to the food web. This is an important result; as it was pointed by Närhi et al. (2012) recognition of plant species that tolerate high concentration of toxic elements is essential to phytostabilize them.

None of the species studied fits in to the classical criteria for phytoremediation. As a general trend, among most of the studied species and sampling sites, the Pb concentrations were higher in the roots than in aerial tissues, presenting relatively low TF and BCF. As pointed out by Blaylock and Huang (2000), roots are the principal impediment for lead translocation from soils, that is why there are few species known for hyper-accumulating Pb. It is important to highlight that Pb is an element hardly accumulated in shoots, and phytoremediation strategies include hyper-accumulators as well as accumulators that provide secondary products during the remediation process (Danh et al., 2010). In this regard, among the optimal features of *T. minuta* for phytoremediation are the adaptation to disturbed habitats and the use of this species oil. Absence of lead in oil must be demonstrated before use oil extracted from specimens grown at polluted soils. In that case, its oil could be use as flavor and perfumery raw material (Soule, 1993). In addition, *T. minuta* also presents flavonoids reported against microorganisms (Amat, 1983; Tereschuk et al., 1997) and has other important effects such as enzyme inhibition and antioxidant and cytotoxic effects (Middleton and Kandaswami, 1993). It also possesses a weed-suppressing ability and can be used as a natural herbicide (Batish et al., 2007).

4.3. Relation between soil properties and Pb accumulation in plants.

Results for most of the studied species presented a general declining trend of Pb concentrations in each tissue as the Pb in the soils decreased. Nevertheless, site 5 broke this trend, since the content of Pb_{HCl} in soil samples at site 3, 4 and 5 were not significantly different (Table 1) while Pb accumulation in plants was significantly higher at site 5 (Table 4).

This behavior suggests the effect of other factors influencing the uptake rate at site 5, for both *T. minuta* and *B. pilosa*. Taking into account soil physicochemical properties, organic matter content could be related to Pb concentration in soils but was not appropriated to explain the differences found for the Pb uptake by plants. Neither electrical conductivity nor pH in topsoils were related to its Pb concentration, and those variables did not explain either the Pb accumulation in plants. Acidic pH is usually related to heavy metal bioavailability (Sutherland and Tolosa, 2001), since most of the studied species did not accumulated high Pb quantities, it could be said that the slight acidic found in soils was not enough to enhance the uptake. Nevertheless, there were two sites with strong acidity, both presented evidence of battery breaking residues, so the acidity found could be related to the acid components from the batteries. Lead uptake at those site was not enhanced either. Summarizing, soil physicochemical properties were not associated to Pb uptake by plants.

A possible explanation for such behavior could be the availability of other metals, since plants exposed to stress activate the Cu-Zn superoxide dismutase enzyme to protect themselves. This mechanism can be activated only if there is Zn or Cu available (Goldstein et al., 2006). Figure 2 shows Cu and Zn concentration in topsoils at sampling sites from 1 to 6, where Pb concentration was elevated, and the ANOVA comparison among sites for each metal. Sampling site 5 presented significantly lower Zn concentration than sites 1, 2 and 6; and lower Cu concentration than sites 1, 6 and 4. These results could be indicating that Cu-Zn superoxide dismutase system was not activated at site 5 due to lack of those elements. Plants, devoid of such protection, could not stop the entry of Pb, and it resulted accumulated.

5. Conclusion

The findings obtained in this in situ research demonstrated that native tolerant species growing in lead polluted soils suffer a reduction of their natural translocation and bioaccumulation natural capability. This phenomenon implies a strategy change from hyper-accumulator or accumulator to excluder. However, some species conserved the ability to accumulate significant amounts of Pb in specific soil conditions. These species are *T. minuta* and *B. pilosa*, and they were metal-tolerant and could accumulate more Pb than other studied plants. On the other hand, these species present suitable characteristics for phytoremediation of Pb polluted soils. Therefore, further studies should be carried out and controlled experiments (with different conditions of soil, amendment uses, and other influencing factors) are required to determine the conditions in which these species are applicable and to evaluate the possibility of enhancing their Pb uptake rates to ameliorate TF and BCF.

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Table and Figure Captions

Table 1. Physico-chemical profile of study area topsoils.

Table 2. Soil quality guidelines for total metal concentrations (mg kg-1 DW) based on land use.

Table 3. Species assessed and Pb content in leaves.

Table 4. Plant Pb accumulation in leaves, stem and roots, traslocation factor (TF) and bioaccumulation factor (BCF); ANOVA results for Pb concentration in species found at more than one site.

Figure 1. Topsoil and vegetation sampling area in Córdoba (Argentina) in the surroundings of a lead smelter plant located in Bouwer. Site 9 is between Bouwer and Córdoba city, it is the isolated dot observable in the top square in this figure.

Figure 2. Topsoil Cu and Zn concentration and ANOVA comparison.

References

Al-Khashman, O.A., Shawabkeh, R.A., 2006. Metals distribution in soils around the cement factory in southern Jordan. Environmental Pollution 140, 387-394.

Alvarez, P.J.J., Illman, W.A., 2006. Bioremediation and natural attenuation: process fundamentals and mathematical models. Wiley-Interscience, New Jersey.

Amat, A., 1983. Pharmacological research for major taxons of Bonaerenses Compositae. Acta Farmacéutica Bonaerense 2, 23-36.

Argentinean legislation, 1991. Residuos Peligrosos. Generación, manipulación, transporte y tratamiento. Law 24051.

Arreghini, S., de Cabo, L., de Iorio, A.F., 2006. Phytoremediation of two types of sediment contaminated with Zn by Schoenoplectus americanus. International Journal of Phytoremediation 8, 223-232.

Austin, G., Brandvold, L., Hawley, J., Renault, J., 1993. Lead contamination at an old smelter site at Socorro, New Mexico: Part I-- Particle size and depth of contamination. Mining engineering Salt Lake City UT 45, 389-395.

Bäckström, M., Karlsson, S., Allard, B., 2004. Metal Leachability and Anthropogenic Signal in Roadside Soils Estimated from Sequential Extraction and Stable Lead Isotopes. Environmental Monitoring and Assessment 90,135-160.

Batish, D.R., Arora, K., Singh, H.P., Kohli, R.K., 2007. Potential utilization of dried powder of Tagetes minuta as a natural herbicide for managing rice weeds. Crop Protection 26, 566-571.

Becerril Soto, J.M., Barrutia Sarasua, O., García Plazaola, J.I., Hernández, A., Olano Mendoza, J.M., Garbisu Crespo, C., 2007. Especies nativas de suelos contaminados por metales: aspectos ecofisiológicos y su uso en fitorremediación. Ecosistemas 16 (2), 50-55.

Bermudez, G., Rodriguez, J.H., Pignata, M.L., 2009. Comparison of the air pollution biomonitoring ability of three Tillandsia species and the lichen Ramalina celastri in Argentina. Environmental Research 109, 6-14.

Bermudez, G., Moreno, M., Invernizzi, R., Plá, R., Pignata, M.L., 2010. Heavy metal pollution in topsoils near a cement plant: The role of organic matter and distance to the source to predict total and HCl-extracted heavy metal concentrations. Chemosphere 78, 375-381.

Betancur, L.M.A., Mazo, K.I.M., Mendoza, A.J.S., 2005. Fitorremediación: la alternativa para absorber metales pesados de los biosólidos. Revista Lasallista de Investigación 1, 57-60.

Blaylock, M.J., Huang, J.W., 2000. Phytoextraction of metals. Phytoremediation of toxic metals: using plants to clean-up the environment. John Wiley & Sons, New York.

Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D., Raskin, I., 1997. Enhanced accumulation of Pb in indian mustard by soil-applied chelating agents. Environmental Science &Technology 37, 860-866.

Bonfranceschi, B.A., Flocco, C., Donati, E., 2009. Study of the heavy metal phytoextraction capacity of two forage species growing in an hydroponic environment. Journal of Hazardous Materials 165, 366-371.

Bu-Olayan, A.H., Thomas, B.V., 2009. Translocation and bioaccumulation of trace metals in desert plants of Kuwait governorates. Research Journal of Environmental Sciences 3, 581-587.

Cala, V., Kunimine, Y., 2003. Distribución de plomo en suelos contaminados en el entorno de una planta de reciclaje de baterías ácidas. Revista Internacional de Contaminación Ambiental 19 (3), 109-116.

CCME (Canadian Council of Ministers of the Environment), 1991. Canadian Interim Environmental Quality Criteria for Contaminated Sites. CCME EPC-CS34. CCME, Manitoba.

CCME, 2007. Canadian soil quality guidelines for the protection of environmental and human health: summary tables (2007). In: Canadian Environmental Quality Guidelines, 1999. CCME, Winnipeg.

Comuna de Bouwer. Protocolo base. Presented by Comuna de Bouwer to the Environmental Department of Córdoba province (Secretaría de Ambiente de la Provincia de Córdoba) 2008.

Danh, L.T., Truong, P., Mammucari, R., Foster, N., 2010. Economic Incentive for Applying Vetiver Grass to Remediate Lead, Copper and Zinc Contaminated Soils. International Journal of Phytoremediation 13, 47-60.

Eckel, W.P., Rabinowitz, M.B., Foster, G.D., 2002. Investigation of unrecognized former secondary lead smelting sites: confirmation by historical sources and elemental ratios in soil. Environmental Pollution 117, 273-280.

Elliott, H., Brown, G., 1989. Comparative evaluation of NTA and EDTA for extractive decontamination of Pb-polluted soils. Water, Air, & Soil Pollution 45, 361-369.

Fabietti, G., Biasioli, M., Barberis, R., Ajmone-Marsan, F., 2009. Soil contamination by organic and inorganic pollutants at the regional scale: the case of Piedmont, Italy. Journal of Soils and Sediment 10, 290-300.

Flocco, C., Lobalbo, A., Carranza, M., Bassi, M., Giulietti, A., Mac Cormack, W., 2002. Some physiological, microbial, and toxicological aspects of the removal of phenanthrene by hydroponic cultures of Alfalfa (Medicago sativa L.). International Journal of Phytoremediation 4, 169-186.

Goldstein, S., Fridovich, I., Czapski, G., 2006. Kinetic properties of Cu, Zn-superoxide dismutase as a function of metal content—order restored. Free Radical Biology and Medicine 41, 937-941.

Gorgas, J., Tassile, J., 2003. Recursos Naturales de la Provincia de Córdoba. INTA, Córdoba.

Hernandez, L., Probst, A., Probst, J.L., Ulrich, E., 2003. Heavy metal distribution in some French forest soils: evidence for atmospheric contamination. Science of the Total Environment 312, 195-219.

Huang, J., Cunningham, S., 1996. Lead phytoextraction: species variation in lead uptake and translocation. New Phytologist 134: 75-84.

Kabata Pendias, A., Pendias, H., 1984. Trace elements in soils and plants. CRC Press, Boca Raton.

Ketterer, M.E., Lowry, JH, Simon, J., Humphries K., Novotnak M.P., 2001. Lead isotopic and chalcophile element compositions in the environment near a zinc smelting-secondary zinc recovery facility, Palmerton, Pennsylvania, USA. Applied Geochemistry 16, 207-229.

Kim, C., Lee, Y., Ong, S.K., 2003. Factors affecting EDTA extraction of lead from lead-contaminated soils. Chemosphere. 51, 845-853.

La Voz del Interior. 19-December-2005. Estudios indicaron que 12 vecinos de la metalúrgica tenían niveles de plomo en sangre anormales. Last accessed 14/03/2013. http://archivo.lavoz.com.ar/06/09/23/secciones/sociedad/nota.asp?nota_id=2146.

Middleton, E., Kandaswami, C., 1993. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer in: Harborne, J.B. (Ed.), The flavonoids: Advances in Research Since 1986. Chapman and Hall, London, pp. 619-652.

Montes Botella, C., 2001. Condicionamientos ambientales de Erica Andevalensis Cabezudo & Rivera: respuesta frente a metales pesados. Agrónomos.

Närhi, P., Räisänen, M.L., Sutinen, M.L., Sutinen, R., 2012. Effect of tailings on wetland vegetation in Rautuvaara, a former iron-copper mining area in northern Finland. Journal of Geochemical Exploration 116-117, 60-65.

Navarro-Aviñó, J., Aguilar, A., López-Moya, J., 2007. Aspectos bioquímicos y genéticos de la tolerancia y acumulación de metales pesados en plantas. Ecosistemas. 16, 10-25.

Palacios, H., Iribarren, I., Olalla, M.J., Cala, V., 2002. Lead poisoning of horses in the vicinity of a battery recycling plant. Science of the Total Environment 290, 81-90.

Peltola, P., Åström, M., 2003. Urban geochemistry: a multimedia and multielement survey of a small town in northern Europe. Environmental Geochemistry and Health 25, 397-420.

Ramírez, A.V., 2008. Exposición a plomo en trabajadores de fábricas informales de baterías. Anales de la Facultad de Medicina 69, 104-108.

Soule, J., 1993. Tagetes minuta: A potential new herb from South America, in , J., Simon J.E. (Eds.), New crops. Wiley, New York, pp.649-654.

Sutherland, R.A., 2002. Comparison between non-residual Al, Co, Cu, Fe, Mn, Ni, Pb and Zn released by a three-step sequential extraction procedure and a dilute hydrochloric acid leach for soil and road deposited sediment. Appl. Geochem. 17:13.

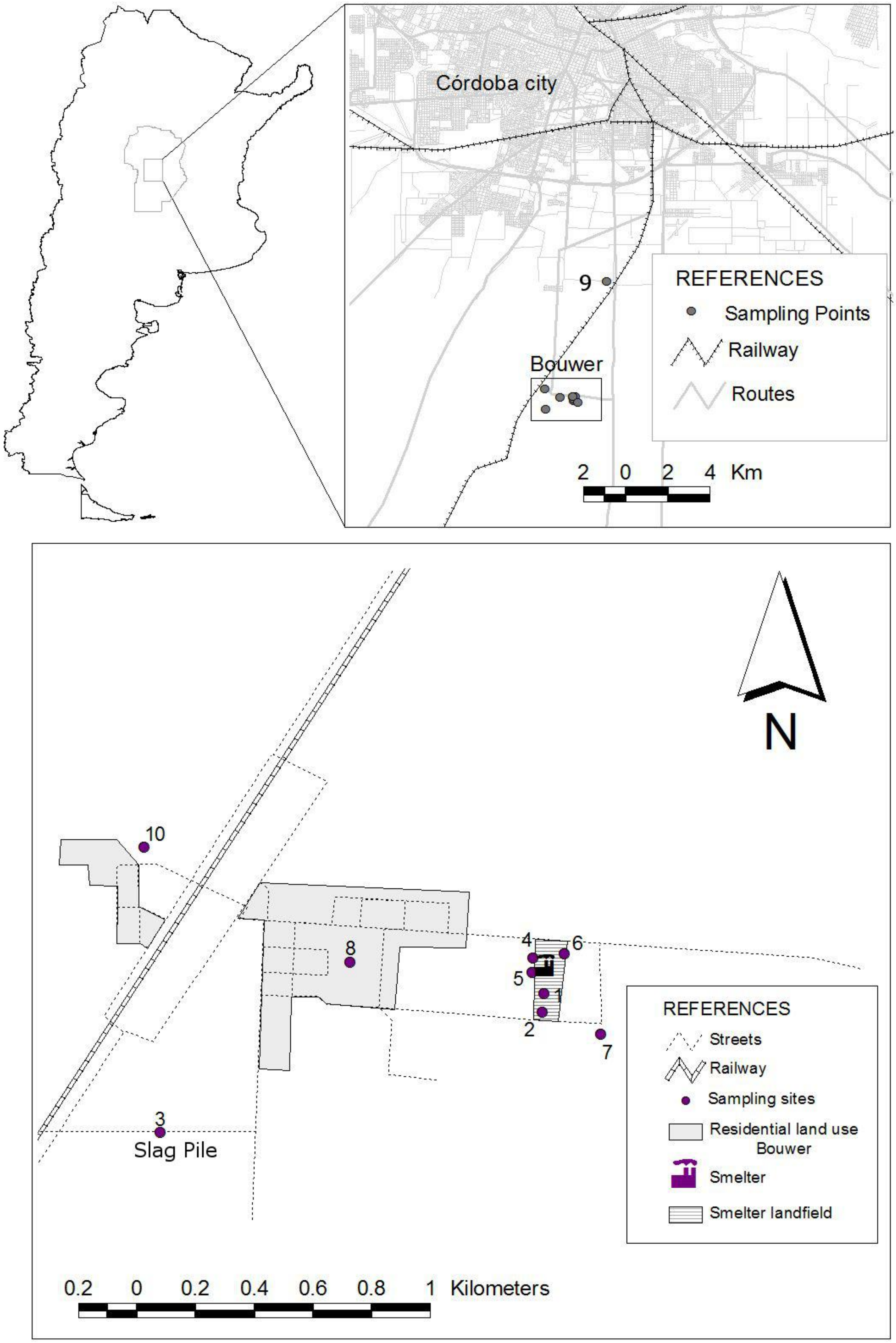
Sutherland, R.A., Tack, F.M.G., Ziegler, A.D., Bussen, J.O., 2004. Metal extraction from road-deposited sediments using nine partial decomposition procedures. Applied Geochemistry 19, 947-955.

Sutherland, R.A., Tolosa, C.A., 2001. Variation in total and extractable elements with distance from roads in an urban watershed, Honolulu, Hawaii. Water, Air, & Soil Pollution 127, 315-339.

Tereschuk, M.L., Riera, M.V.Q., Castro, G.R., Abdala, L.R., 1997. Antimicrobial activity of flavonoids from leaves of Tagetes minuta. Journal of Ethnopharmacology 56, 227-233.

Torri, S.I., Zubillaga, M., Cusato, M., 2009. Potential of *Discaria americana* for Metal Stabilization on Soils Amended with Biosolids and Ash-Spiked Biosolids. International Journal of Phytoremediation 11, 187-199.

van der Ent, A., Baker, A. J., Reeves, R.D., Pollard, A.J., Schat, H., 2013.Hyperaccumulators of metal and metalloid trace elements: Facts and fiction. Plant and Soil 362(1-2), 319-334.


Córdoba city
9
Bouwer
REFERENCES
Sampling Points
Railway
Routes
2 0 2 4 Km
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REFERENCES
Streets
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Sampling sites
Residential land use Bouwer
Smelter
Smelter landfield
0.2 0 0.2 0.4 0.6 0.8 1 Kilometers

Fig 1

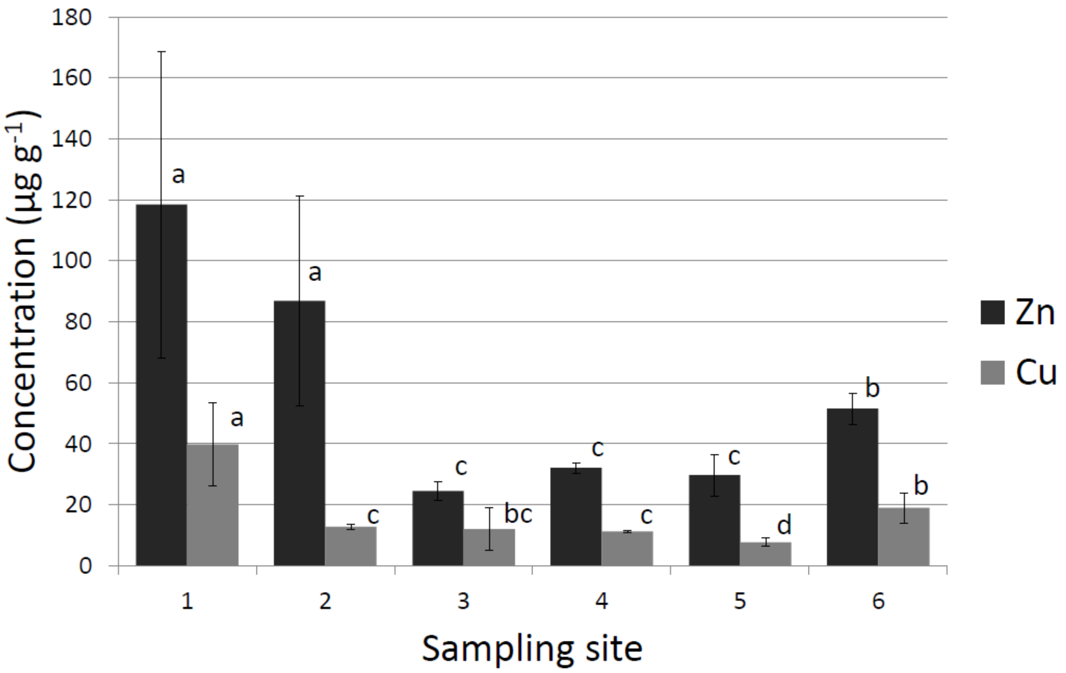
Concentration (µg g-1)
180
160
140
120
100
80
60
40
20
0
a
a
a
c
c
bc
c
c
c
d
b
b
1
2
3
4
5
6
Sampling site
Zn
Cu

Fig 2

Table 1. Physico-chemical profile of study area topsoils

Site	**Distance (m)**	**Pb $_{Pst}$ (µg g^{-1})**	**Pb $_{HCl}$ (µg g^{-1})**	**OM%**	**pH**	**EC (µs cm^{-1})**
1	80	11,936±1,144	3,000±592	5.5±0.7	4.7±0.2	332.5±34.2
2	140	2,645±622	2,376±301	5.8±0.6	6.6±0.2	107.1±27.7
3	1260	2,026±1,929	1,592±1,297	6.1±0.5	7.0±0.2	221.3±44.0
4	50	1,439±130	1,550±197	5.5±0.3	5.8±0.1	109.0±19.6
5	40	1,230±262	1,592±295	5.4±0.1	6.8±0.1	105.9±1.9
6	120	1,059±307	641±183	5.4±0.3	6.9±0.1	404.7±41.5
7	268	76.0±2.5	56.5±2.3	10.0±0.3	6.6±0.2	215.3±27.5
8	580	17.5±4.5	19.1±3.0	10.5±0.8	6.3±0.2	142.7±16.4
9	5630	9.9±1.7	9.1±0.9	5.8±0.4	6.9±0.1	96.8±21.3
10	1230	9.7±2.4	8.8±1.2	7.3±0.5	6.9±0.2	138.9±32.6

Abbreviations:
Pb $_{Pst}$ (pseudototal soil fraction); Pb $_{HCl}$ (extractable soil fraction); OM % (organic matter percentage); EC (Electrical conductivity).

Table 2. Soil quality guidelines for total metal concentrations (mg kg^{-1} DW) based on land use.

Element	**Land use**					
	Agricultural		**Residential**		**Industrial**	
	SQG [a,b]	**SQGEH** [c]	**SQG** [a,b]	**SQGEH** [c]	**SQG** [a,b]	**SQGEH** [c]
Pb	375	70	500	140	1000	600
Cu	150	63	100	63	500	91
Co	40		50		300	
Ni	150	50	100	50	500	50
Zn	600	200	500	200	1500	360

SQG = soil quality guidelines; SQGEH = soil quality guidelines for environmental health.

[a] Argentinean legislation (Law 24051).

[b] CCME (Canadian Council of Ministers of the Environment, 1991).

[c] CCME (2007).

Table 3. Species assessed and Pb content in leaves (μg g^{-1}).

Species	Family	Status	Habit	Present at sites	Pb		
					Me	**M**	**M**
Bidens pilosa L.	Asteracea	Native	Annual	1, 2, 3, 4, 5,	26.	5.	11
Tagetes minuta L.	Asteracea	Native	Annual	2, 5	157	8.	39
Solanum argentinum	Solanacea	Native	Perennial	2, 5, 7, 8, 10	10.	4.	24
Amaranthus sp.	Amaranth	Native	Annual	3, 4, 5, 6, 7,	11.	6.	21
Sorghum halepense (L.)	Poaceae	Introdu	Perennial	5, 8, 10	3.4	1.	5.
Ipomoea purpurea (L.)	Convolvul	Native	Annual	5, 6, 8, 9, 10	10.	3.	27
Mirabilis jalapa L.	Nyctagina	Adventi	Perennial	5, 9	18.	9.	27
Aristolochia argentina	Aristoloch	Native	Perennial	8	8.2	7.	9.
Lippia turbinata Griseb.	Verbenac	Native	Perennial	8	6.6	6.	6.
Eupatorium inulifolium	Asteracea	Native	Perennial	1	30.	30	31

Table 4. Plant Pb accumulation (μg g^{-1}) in leaves, stem and roots, traslocation factor (TF) and bioaccumulation factor (BCF); ANOVA results for Pb concentration in species found at more than one site.

Species		**Sampling sites**									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
***Bidens pilosa* L.**	Leaves***	30.5±0.9^{c}	16.3±0.7^{ab}	14±1^{ab}	13±1^{ab}	100±9^{d}			9.1±0.9^{a}	24±1^{bc}	5.5±0.6^{a}
	Stem***	29.3±0.9^{e}	22±3^{c}	23,2±0,3^{cd}	11.8±8^{b}	28±1de			5.1±0.7^{a}	99±2^{f}	5.8±0.7^{a}
	Root***	741±64^{c}	131±3^{a}	140±5^{a}	44.7±0.6^{a}	448±75^{b}			3.9±0.1^{a}	2.7±0.6^{a}	2.6±0.4^{a}
	TF	0.04	0.15	0.13	0.28	0.14			1.82	22.6	2.19
	BCF	0.01	0.01	0.01	0.01	0.04			0.37	6.76	0.64
***Tagetes minuta* L.**	Leaves***		9.0±0.4^{a}			380±22^{b}					
	Stem***		11±1^{a}			72±2^{b}					
	Root***		30.7±0.5^{a}			859±36^{b}					
	TF		0.31			0.26					
	BCF		0.01			0.14					
***Solanum argentinum* Bitter & Lillio**	Leaves***		8.2±0.2^{c}			24±1^{d}		6.8±0.4^{b}	6.2±0.2^{b}		5.1±0.4^{a}
	Stem***		8.1±0.2^{b}			20±3^{c}		3.6±0.5^{a}	3.5±0.2^{a}		4.5±0.6^{ab}
	Root***		10.8±0.1^{c}			68±3^{d}		undetectable	2.6±0.3^{a}		4.7±0.2^{b}
	TF		0.75			0.32			1.87		1.01
	BCF		0			0.01			0.25		0.54
***Amaranthus* sp.**	Leaves**			6.6±0.3^{a}	13.5±0.7^{abc}	16.7±0.7^{c}	14.2±0.5^{bc}	9.5±0.3^{ab}	14±6^{abc}	10.3±0.5^{abc}	8.6±0.6^{ab}
	Stem***			5.1±0.1^{a}	9±2^{bc}	9±1^{bc}	10.3±0.4^{c}	4.12±0.03^{a}	5.8±0.3^{ab}	7.4±0.1^{abc}	6.0±0.1^{ab}
	Root***			8±2^{a}	32.2±0.8^{c}	115±9^{d}	20.2±0.3^{b}	6.9±0.4^{a}	3.1±0.6^{a}	6±1^{a}	5.1±0.1^{a}
	TF			0.71	0.35	0.11	0.61	0.98	3.17	1.58	1.45
	BCF			0	0.01	0.01	0.02	0.12	0.51	0.97	0.83

***Sorghum halepense* (L.) Pers.**	Leaves**					5.2±0.6^{b}			2.8±0.5^{a}		2.1±0.6^{a}
	Stem*					3.1±0.6^{b}			1.2±0.1^{a}		1.6±0.3^{ab}
	Root***					1407±64^{b}			4±1^{a}		2.2±0.4^{a}
	TF					0			0.54		0.85
	BCF					0			0.11		0.21
***Ipomoea purpurea* (L.) Roth**	Leaves***					27.2±0.5^{c}	11.4±0.2^{b}		5±2^{a}	6.5±0.2^{a}	6±1^{a}
	Stem**					22±1^{a}	8.2±0.2^{a}		60±17^{b}	6±1^{a}	5.4±0.5^{a}
	Root***					299±24^{a}	23±1^{b}		not found	not found	not found
	TF					0.08	0.43				
	BCF					0.02	0.02				
***Mirabilis jalapa* L.**	Leaves***					26.9±0.4^{b}				9.8±0.4^{a}	
	Stem**					27±1^{b}				5.7±0.8^{a}	
	Root**					145±1^{b}				5.6±0.2^{a}	
	TF					0.19				1.38	
	BCF					0.02				0.85	
***Aristolochia argentina* Griseb.**	Leaves								14±1		
	Stem								3.2±0.2		
	Root								1.9±0.5		
	TF								3.62		
	BCF								0.6		
Lippia turbin	Leaves								6.6±0.3		

ata **Griseb.**											
	Stem								2.4±0.8		
	Root								2.0±0.1		
	TF								1.9		
	BCF								0.2		
Eupatorium inulifolium **Kunth**	Leaves	30.7±0.4									
	Stem	21.5±0.9									
	Root	441±23									
	TF	0.23									
	BCF	0.1									

Letters in order of increasing concentration. Same letter in each row represent concentration with no significant difference. ns= not significant. *= Significant at 0.05 probability level. ** = Significant at 0.01 probability level. ***= Significant at 0.001 probability level

Highlights

1. *Tagetes minuta* and *Bidens pilosa* are potential phytoextractors of Pb from soils.
2. Neither was previously reported as a Pb accumulator.
3. *Sorghum halepense* is a plant with a Pb phyto-stabilization potential.