

Contents lists available at ScienceDirect

Journal of Geochemical Exploration

journal homepage: www.elsevier.com/locate/jgeoexp

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



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ARTICLE INFO

Article history: Received 2 May 2013 Accepted 10 November 2013 Available online 16 November 2013

Keywords: Lead polluted soils Phytoextraction Phytostabilization Tagetes minuta Bidens pilosa Sorghum halepense

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 1600 μ g 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 μ g g⁻¹ DW and 100.6 μ g g⁻¹ DW, respectively). Therefore, *T. minuta* L and *B. 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 (1406.8 μ g 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.

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1. Introduction

Heavy metal soil pollution is currently considered as 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 the 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 their 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

* Corresponding author. Tel.: + 54 351 4344983 int.6; fax: + 54 351 4334139. *E-mail address:* mjulietasalazar@gmail.com (M.J. Salazar). 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 noninvasive. It is important to note the favorable results obtained in costbenefit terms, ecological features of social and esthetic 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, bio-availability 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

Abbreviations: EC, electrical conductivity; %OM, organic matter percentage; DW, dry weight; TF, translocation factor; BCF, bioconcentration factor.

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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 (Fig. 1), and has a population of 1500 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 (Fig. 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 $^{\circ}$ 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 procedure. 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 ovendried for 4 h 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 4 h.

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 min (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 °C for 4 h. The extraction solution was prepared by mixing the ashes of 5 g DW of topsoil previously burnt at 450 °C for 4 h, with 10 mL of HCl/HNO₃ 3:1 (V/V) and shaking them at room temperature for 30 min.

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 extraction 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 °C for 4 h. Then, the extraction solution was prepared by mixing the ashes with 2.5 mL of 20% HCl and 0.5 mL of analytically and commercially pure HNO₃. 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–Wilk's 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

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