Journal of Geochemical Exploration 166 (2016) 18–26

Contents lists available at ScienceDirect

Journal of Geochemical Exploration

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

Multidisciplinary study of chemical and biological factors related to Pb accumulation in sorghum crops grown in contaminated soils and their toxicological implications

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ARTICLE INFO

Article history:
Received 5 November 2015
Revised 20 January 2016
Accepted 30 January 2016
Available online 21 March 2016

Keywords:
Sorghum bicolor
Lead
Battery recycling plant
Phytostabilization
Food security

ABSTRACT

In this study, the content of Pb, the physico-chemical and biological parameters in soils, and the metal transfer to vegetative and reproductive *Sorghum bicolor* plants were evaluated along with their relationship with the toxicological risk of crop consumption. To carry this out, soil and sorghum samples at different growth stages were collected near to a former battery recycling plant. The results showed that the concentrations of Pb in soils at several sites were above the maximum permissible levels. Metal bioavailability was not directly related to the pH, OM% or EC, while no association between metals and the different genera of fungi was observed. Sorghum crops accumulated Pb mainly in the roots in all of the growth stages, and therefore presented low levels of Pb in aerial parts without toxicological risk due to direct consumption. Taken together, our results revealed that sorghum could be employed as a potential phytostabilizator of lead in soils associated with crop production. However, further studies are necessary to extend these findings.

1. Introduction

Heavy metal pollution in soils is of considerable concern worldwide due to the potential risk to the environment and to human health. Large areas of farmland have been contaminated by metals owing to anthropogenic input from mining, smelting, fossil fuel burning, phosphate fertilizers and sewage sludge (Navarro et al., 2008). Due to the high toxicity of heavy metals, the resulting polluted agricultural soil affects crop growth and the quality of agricultural products, as well as being a serious threat to human health through contamination of the food chain (Salazar et al., 2012; Zhao et al., 2014; Zhuang et al., 2009a). Industrial practices that contribute to heavy metal pollution include lead smelting and secondary lead smelting (recycling of Pb from Pb-containing products) (Cala and Kunimine, 2003; Dahmani-Muller et al., 2000; Fernandez-Turiel et al., 2001; Ramírez, 2008). Many studies have been carried out in industrial areas near to agricultural soils, and have reported Pb concentrations in crops above the European threshold of 0.2 mg kg^{-1} FW (fresh weight) for Pb (Caggiano et al., 2005; González-Miqueo et al., 2010; Honour et al., 2009; Rodriguez et al., 2014; Salazar et al., 2012). In another investigation, Salazar and Pignata (2014) reported high lead concentrations exceeding national and international norms in soils and native plants near to a former battery recycling plant in Córdoba, Argentina. Taking into account that this occurred in an agricultural area cultivated with sorghum, soybeans, wheat and maize, it is important to evaluate the toxicological risk for consumers. Concerning these crops, sorghum has been previously cited as being a potential accumulator of metals in roots in both field and laboratory studies (Al Chami et al., 2015; An, 2006; Salazar and Pignata, 2014; Soudek et al., 2014).

Pollution in soils results in a reduction in the quality of their physical and chemical properties and causes changes in their metal retention capacities (Simon et al., 2000). Metal transfer from soil to plant is a complex process determined by several factors of generally a biological, geochemical or climate nature, with both natural and anthropogenic processes controlling mobility and availability (Kabata-Pendias and Sadurski, 2004).

Numerous studies have indicated that in addition to climatic factors and soil properties, the plant-microorganism interaction is an important factor related to the metal bioavailability of soils (de Souza et al., 1999; Kuffner et al., 2008; Lin et al., 2004), with the rhizosphere being the soil compartment where the interactions among microorganisms, roots and soil modify the nutrient and also the heavy metal availability (Lin et al., 2004). Thus, numerous studies proposed arbuscular mycorrhizal fungi

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http://dx.doi.org/10.1016/j.gexplo.2016.01.020
0375-6742/

as a good tool for phytoextraction of heavy metals in soils (Redon et al., 2008; Vodnik et al., 2008). Moreover, there are some studies with bacteria and bioremediation of heavy metals, which reviewed the latest studies detailing the most important phenotypes and properties such as peptides and proteins that are used in bioremediation of heavy metals (Valls and de Lorenzo, 2002; Mejaré and Bülow, 2001).

Taking into account the findings mentioned above, it is pertinent to carry out a multidisciplinary study to consider all the chemical, physical and biological factors affecting the bioavailability of toxic metals in soils which can potentially be incorporated into crops. Therefore, the purpose of this study was to evaluate whether physical, chemical and biological factors determine metal mobilization in soils, metal accumulation in sorghum crops and its toxicological risk during the growing season.

2. Material and methods

2.1. Study area and sampling points

The study area was located in Bouwer, a peri-urban commune with a population of approximately 2000 inhabitants, 18 km south of Córdoba city in central Argentina (Fig. 1). The climate is mild, with an annual mean temperature of about 15 °C and an annual rainfall range of 500–900 mm. The soil is an Entic Haplustoll, and this area is characterized by a former battery recycling plant (31°33′34.02″S; 64°11′9.05″W) surrounded by agricultural crops (mostly soybean and associated crops such as sorghum). Bouwer is one of the environmentally most affected areas in the province of Córdoba, being characterized by a waste disposal area, car scrapyards, and a former battery recycling plant that was closed in the year 2005 due to functional problems with lead emissions being c. 35 times higher than the permitted values (Salazar and Pignata, 2014).

The study area is cultivated with *Sorghum bicolor*, which is subjected to zero tillage and has superphosphate fertilizers applied in general in proportions ranging from 50 to 100 kg ha^{-1}.

Fig. 1 shows the location of the sampling sites, which were chosen taking into account the main directions of the winds and the distance to the emission source according to a preliminary study (Salazar and Pignata, 2014).

2.2. Sampling procedure and analysis preparations

Topsoil (rhizosphere and bulk) and crop samples were collected for a full growing season of sorghum for the following different growth stages of plant development: prior to sowing (with only bulk soil fraction being collected), half bloom and maturity (November, 2012; February, 2013; April, 2013, respectively).

Sorghum plants and topsoil samples were collected at the sampling sites following a systematic sampling. Each site was a 3 m^2 square, with 9 sub-sampling points systematically arranged with a 1 m gap between them. At each sampling site, three pools of samples (soils for November, soils and plants for February and April) were collected.

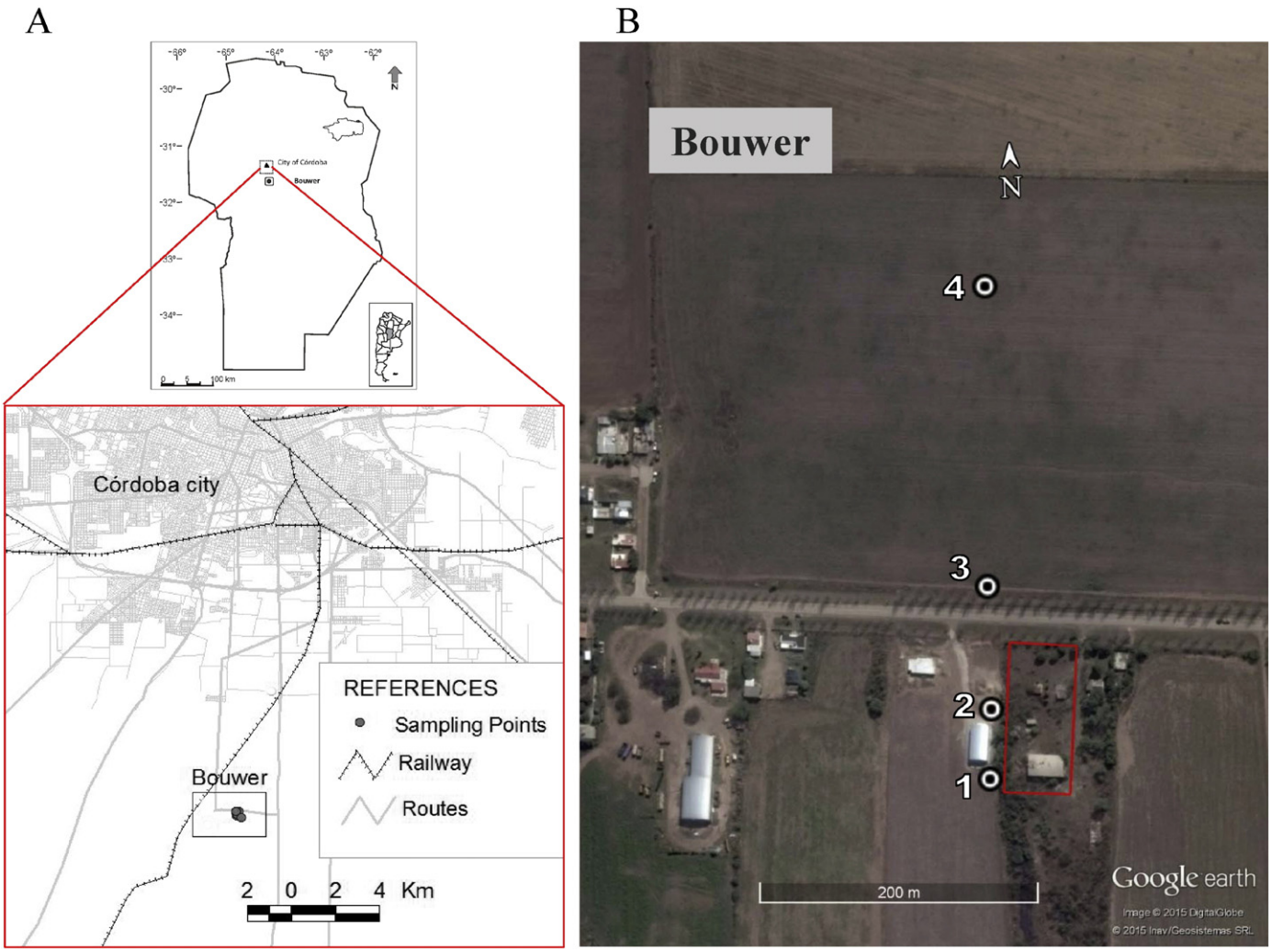

Fig. 1. Satellite image of the commune of Bouwer. (a) Location of Bouwer with respect to Córdoba city. (b) Location details of the smelter and sampling points.

Plants were gathered using a stainless steel shovel to extract the whole root and immediate soil, considered in this study to be rhizospheric soil. In addition, bulk topsoil subsamples were obtained at the sampling points using a blasthole at a depth of 0–10 cm ($n = 9$ for rhizosphere and bulk soil). All soil and plant samples were kept in plastic bags until being processed in the laboratory.

It should be noted that typical agrochemicals were applied to the sorghum crops. Before sowing, the chemical fallow was applied, which consisted of a mixture of flumioxazin 2,4D and Dicamba. Then, after sowing, pre-and post-emergence agrochemicals were applied (atracine, 2-4D, MCPA, Dicamba).

Sorghum samples were separated into roots, aerial parts and flowers/fruits, and then washed and sonicated with ultrapure water for the purpose of removing the soil remains attached to the organs. Subsequently, the samples were oven-dried at 60 °C to constant dry weight (DW), homogeneously mixed, and stored in the dark until analytical procedures were carried out. The morphological parameters biomass of roots, aerial parts, flowers and roots were analyzed and expressed as dry weight. In addition, soil samples were dried at room temperature and then sieved to <2 mm (polyethylene sieve), homogeneously mixed, and stored in the dark until being used for heavy metal determination. Each composite sample was made by mixing and homogenizing 150 g of each of the topsoil sieved subsamples.

2.3. Physical, chemical and biological analyses

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

In topsoil samples (rhizosphere and bulk), the pH and electrical conductivity (EC) were measured in 1:5 soil:water suspension in triplicate (Bäckström et al., 2004). In order to calculate the dry weight (DW), samples were oven-dried at 105 °C to constant weight (Al-Khashman and Shawabkeh, 2006). The organic matter percentage (OM%) was determined according to Peltola and Åström (2003), by combustion of the samples at 500 °C for 4 h. In addition, the grain size was measured by laser-diffraction size analysis using a Horiba LA-950 particle size analyzer according to Gaiero et al. (2013), for which the presence of organic matter was eliminated using 30% v/v of H_2O_2 analytical grade.

2.3.2. Heavy metal sequential extraction in topsoils

Lead determinations were conducted to determine two phases of interest, the mobile (plant available) and the pseudototal fractions (for comparison with land use guides).

The mobile or exchangeable fraction (Mob) was obtained according to Tessier et al. (1979). Topsoils were dried at room temperature and then sieved at 63 μm with a stainless steel mesh and then the first step of the extraction was performed. A solution of $MgCl_2$ 1 M (1:8 w/v) was added to soil samples, which were agitated for 1 h and then centrifuged at 350 rpm for half an hour. Subsequently, the concentration of Pb_{Mob} in the mobile fraction was determined using a Perkin-Elmer AA3110 flame atomic absorption spectrometer (Norwalk, CT, USA).

In addition, soil samples were used to extract the "pseudototal" metal (Pst fraction), which was carried out using an acid digestion (HNO_3 60%), with the Pb_{Pst} concentration being analyzed by flame AAS.

As a quality control, blanks and Pb standard solution were prepared in the same way and were run after ten determinations to calibrate the instrument and monitor potential sample contamination during analysis. Moreover, samples of the certified reference material "BAM-U113 Soil" (heavy metal polluted soil, Germany) were measured to check the quality of the analytical procedures of the samples analyzed. The results were found to be between the 86% and 92% of the certified value, with the data indicating a low error of typically less than 15%. The coefficient of variation of replicate analyses ($n = 3$) was calculated for different determinations, and variations were found to be less than 10%.

The detection limit of the flame AAS was 2 mg kg^{-1} for Pb, which was calculated considering the calibration curve of lead, the weight of the sample and the volume of extraction.

2.3.3. Metal concentrations in plants

The content of Pb was analyzed in fruits, flowers, aerial parts and roots (1 g DW), which were ashed at 450 °C for 4 h and then digested using 20% HNO_3 for 24 h. The solid residue was separated by centrifugation, and the volume adjusted to 5 mL with Milli-Q water. Then, 10 ppm of a Ga solution was added as an internal standard. Aliquots of 5 μL were taken from this solution and dried on an acrylic support. Standard solutions with known concentrations of different elements, using Ga as an internal standard, were prepared for the calibration of the system.

The samples were measured for 200 s, using the total reflection set up at the X-ray fluorescence beamline of the National Synchrotron Light Laboratory (LNLS), Campinas, Brazil. For the excitation, a white beam (approximately 0.3 mm wide and 2 mm high) was used. For the X-ray detection, a HPGe detector was used with an energy resolution of 148 eV at 5.9 keV.

As a quality control, blanks and samples of the standard reference materials "Oriental Tobacco Leaves (CTA-OTL-1, ICTJ) and CRM 281 (ryegrass, European Commission/BCR)" were prepared in the same way and were run after five determinations to test the accuracy of the measurement. Moreover, standard calibration curves were prepared and run to calibrate the instrument. The results were found to be between 96% and 98% of the certified value, with the data indicating a low error of typically less than 12%.

The coefficient of variation of replicate analysis was calculated for different determinations. Variations were found to be less than 10%.

2.3.4. Mycological determination

2.3.4.1. Characterization of soil fungi. Analysis of the mycobiota was obtained using the plate dilution spread method onto dichloran rose bengal chloranphenicol agar (DRBC), a general medium used for estimating total mycobiota and dichloran glycerol 18% agar (DG18) (Pitt and Hocking, 2009). Briefly, 10 g of each soil sample (both bulk and rhizospheric) was homogenized with 90 mL of 0.1% peptone water solution for 30 min in an orbital shaker. Serial dilutions (10^{-2} to 10^{-3}) were made, and 0.1 mL aliquots were inoculated in duplicate onto the culture media above. Plates were incubated at 25 °C for 7–10 days in darkness, being results expressed as colony-forming units per gram of sample (CFU g^{-1}). The results were expressed as isolation frequency (sample percentages of each genera) and relative density (percentages of isolation of each species among strains of the same genera).

2.3.4.2. Arbuscular mycorrhizal fungal (AMF) morphospecies community. Rhizospheric soil samples of April were sieved through a 1 cm mesh size to remove litter, stones and sticks. AMF spores were extracted by wet sieving and decanting of 100 g of dry soil, followed by centrifugation in sucrose (Walker et al., 1982). A fine sieve (38 mm) was used to collect the small spores, and the top sieve (125 mm) was also checked for sporocarps and larger spores. Only apparently healthy spores were isolated, using direct observation with a stereomicroscope. For quantification and taxonomic identification, fungal spores were mounted onto slides using PVA with or without Melzer's reagent (Omar et al., 1979), and examined with a compound microscope (Nikon, E200). AMF morphospecies identification was based on current species classification (Redecker et al., 2013) and the identification manuals of Schenck and Perez (1990) and INVAM (2014).

Total spore density was determined as the AMF spore number per 100 g of soil dry weight. Also, as an approximation of the soil volume occupied by spores, the biovolume of each morphospecies was calculated (Egerton-Warburton et al., 2007; van Veen and Paul, 1979).

Table 1
Distance to the smelter (m) and mean values and standard deviation (±SD) of mobile (Mob) and pseudo-total (Pst) Pb concentrations (mg kg^{-1}), pH, electrical conductivity (EC) and organic matter percentage (OM%)) in rhizosphere (Rz) and bulk (Bk) soils for the different sampling months.

Month	Site	Distance	Soil	pH	EC (μs.cm^{-2})	OM%	Pb_{Mob}	Pb_{Pst}
November	1	176	Bk	6.71 ± 0.07 B	74.23 ± 5.34	8.63 ± 0.20 A	30.8 ± 5.3 A	1217 ± 74 A
	2	224	Bk	6.66 ± 0.02 B	60.90 ± 5.00	8.10 ± 0.14 A	15.8 ± 3.2 B	365 ± 131 A
	3	720	Bk	6.40 ± 0.08 C	69.77 ± 4.03	12.38 ± 3.28 A	16.0 ± 1.2 B	180 ± 29 A
	4	1011	Bk	6.46 ± 0.02 C	69.23 ± 7.18	8.25 ± 0.29 A	17.3 ± 1.6 B	60 ± 5 B
February	1	176	Bk	7.03 ± 0.14 A	69.77 ± 17.32	8.91 ± 1.17 A	37.8 ± 11.0 A	996 ± 681 A
			Rz	6.96 ± 0.05 B	80.53 ± 7.53	7.54 ± 0.04 B	56.8 ± 32.0 A	1317 ± 328 A
	2	224	Bk	6.72 ± 0.08 B	47.93 ± 4.8	8.95 ± 0.52 A	48.8 ± 22.1 A	1223 ± 288 A
			Rz	6.60 ± 0.10 B	55.13 ± 7.13	8.20 ± 0.21 A	57.6 ± 8.1 A	1495 ± 652 A
	3	720	Bk	6.74 ± 0.14 B	64.23 ± 17.95	9.69 ± 1.35 A	14.1 ± 1.4 B	158 ± 46 A
			Rz	6.55 ± 0.07 B	64.17 ± 3.90	7.94 ± 0.25 A	15.6 ± 4.0 B	107 ± 4 A
	4	1011	Bk	6.74 ± 0.04 B	46.37 ± 5.8	8.09 ± 0.52 A	14.2 ± 3.0 B	70 ± 13 B
			Rz	6.82 ± 0.07 B	73.83 ± 4.53	7.82 ± 0.13 A	12.8 ± 0.5 B	72 ± 7 B
April	1	176	Bk	6.86 ± 0.24 B	74.8 ± 29.1	11.43 ± 5.57 A	29.7 ± 4.8 A	1459 ± 438 A
			Rz	6.83 ± 0.15 B	74.87 ± 18.43	8.07 ± 0.25 A	27.8 ± 2.0 A	1104 ± 871 A
	2	224	Bk	7.12 ± 0.18 A	81.47 ± 15.39	8.04 ± 0.51 A	40.5 ± 6.0 A	1314 ± 861 A
			Rz	6.67 ± 0.12 B	64.37 ± 12.78	8.37 ± 0.23 A	31.1 ± 13.1 A	868 ± 536 A
	3	720	Bk	6.7 ± 0.12 B	61.07 ± 7.41	8.47 ± 0.55 A	16.9 ± 0.7 B	147 ± 61 A
			Rz	6.66 ± 0.06 B	78.37 ± 3.00	8.18 ± 0.36 A	16.3 ± 1.1 B	145 ± 42 A
	4	1011	Bk	7.07 ± 0.03 A	58.07 ± 2.73	7.41 ± 0.15 B	15.4 ± 2.3 B	69 ± 9 B
			Rz	6.89 ± 0.06 B	89.23 ± 4.22	7.76 ± 0.31 A	12.3 ± 1.0 B	71 ± 13 B
ANOVA				***	ns	**	***	*

Values in each column (ANOVA in capital letters) followed by the same letter do not differ significantly at $p < 0.05$. (ns. not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

2.4. Data analyses

2.4.1. Statistical analyses

The analysis of variance (ANOVA) assumptions were previously verified graphically (residual vs. fitted values, box plots, and steam leaf plots). ANOVA was performed to compare metal concentrations with the pH and OM percentages in the rhizosphere and bulk topsoils at the sampling sites. Moreover, ANOVA was also employed to compare the metal concentrations at the sorghum in organs and sites. Whenever the ANOVA indicated significant effects ($p < 0.05$), a pairwise comparison of means was undertaken using Fisher's Least Significant Difference (LSD) test. The Pearson correlation matrix was used to identify the relationships among soil variables in the rhizosphere and between the same variables in the bulk topsoil, in order to try to explain differences between these compartments. This method was also used to evaluate possible relationships among metal exchangeable concentrations in rhizospheric soil, morphological variables and lead content in sorghum organs.

Also ANOVA was performed with the purpose of compare fungal richness at the different sampling sites. A PCA was performed with the purpose of reducing the dimensionality of the data matrix, to avoid redundancy and to highlight relationships in order to identify associations between fungal richness and the concentrations of lead in soils. It should be noted that the assumptions of PCA were met (that the continuity of the variables and the number of elements observed should be greater than the number of original variables). Moreover, simple regressions were performed between the soil lead concentrations and the presence of this metal in sorghum organs, with power regressions carried out between lead concentrations in soils and smelter distance. Analyses were performed using the software Infostat® (Di Rienzo et al., 2011), and the power regressions were carried out using PASW Statistics® (2009).

2.4.2. Risk assessment

The risk to human health resulting from the consumption of sorghum grown in the different sample soil sites was calculated by employing estimated dietary intake (EDI) and the target hazard quotients (THQ) as described by Zheng et al. (2007) and EPA (1989). For the present study, Chinese and Argentine inhabitants were considered to be potential consumers. Despite Africa being the world's largest consumer, the Argentine production is not exported to this destination.

EDI exposure is expressed as the mass of a substance per unit body weight per unit time, averaged over a long period of time (a lifetime), and is calculated as follows:

$$EDI = C \times Con \times EF \times ED/(Bw \times AT)$$

where for this study C is the median concentration of Pb in sorghum (mg kg^{-1}); Con is the ingestion rate of sorghum (g $person^{-1}$ day^{-1}); EF is the exposure frequency (365 days $year^{-1}$); ED is the exposure duration (70 years for adults); Bw is the average body weight (65 kg for Chinese adults and 70 kg for Argentine adults), and AT expresses the average exposure time for non-carcinogenic effects (ED × 365 days $year^{-1}$). Keinan-Boker et al. (2002) reported that the average daily intake of traditional sorghum products for a Chinese individual was 11.23 g $person^{-1}$ day^{-1}, while the daily intake in Argentine inhabitants was about 0.27 g $person^{-1}$ day^{-1} (FAO, 1997).

THQ indicates the potential non-cancer risk for Pb and is calculated as follows:

$$THQ = EDI/RfD$$

where RfD is the reference oral dose, and represents an estimation of the daily exposure to which the human population is likely to be subjected

Table 2
Power regressions for metal concentrations in soils (mg kg^{-1} DW) for different smelter distances (m).

Y	X	Mobile fraction			Pseudo-total fraction		
		R^2	Model	Equation	R^2	Model	Equation
Pb	Smelter distance	0.535***	Power	$Y = 157{,}490^{***}X^{-0423^{***}}$	0.848***	Power	$Y = 104{,}495.448^{*}X^{-1266^{***}}$
Zn		ns	–	–	0.381***	Power	$Y = 30.192^{***}X^{-0160^{***}}$
Cu		ns	–	–	0.504***	Power	$Y = 14.746^{***}X^{-0151^{***}}$

Significant value: *$p < 0.05$; ***$p < 0.001$.

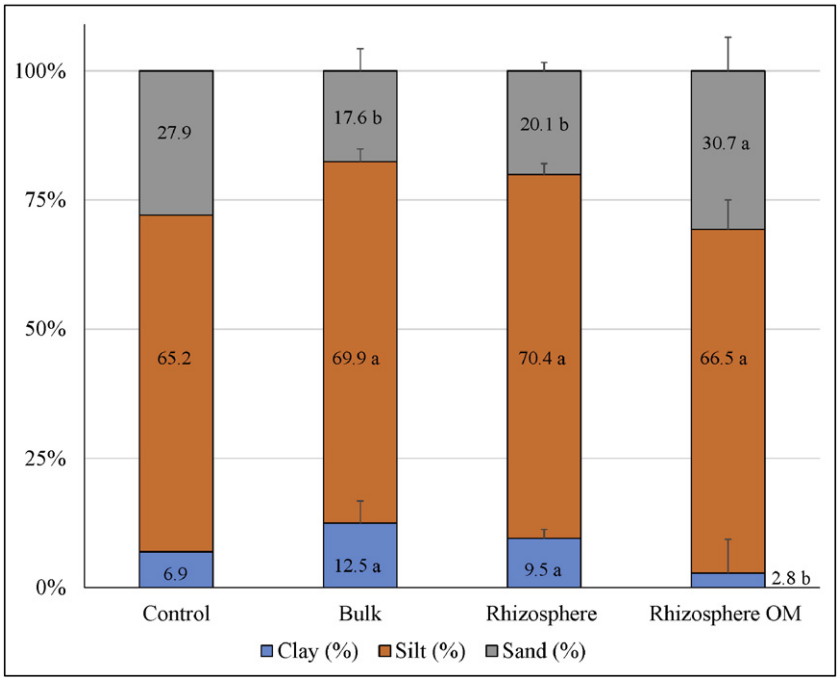

Fig. 2. Percentage composition of clay (<5 μm), silt (5 μm–50 μm) and sand (>50 μm) corresponding to the rhizosphere compartment, soil bulk sample and control in the study area. ANOVA was performed between different types of soil for each type of particle. Notes: Values followed by the same letter do not differ significantly at $p < 0.05$.

to without any appreciable risk of deleterious effects during a lifetime. The RfD value used was 4 mg kg^{-1} day^{-1} according to Huang et al. (2008).

3. Results and discussion

3.1. Heavy metal concentration in soils

The results of the Pb concentrations in soils are shown in Table 1. Differences of Pb concentrations between sites were founded, which were particularly accentuated for mobile Pb, where the sites 1 and 2 showed the highest concentration, with the influence of the distance to the foundry being noteworthy in both cases. Regarding the pseudo-total Pb concentrations, the lowest values were found for site 4.

A regression analysis between the Pb concentration and smelter distance was performed (Table 2) and statistically significant models were developed. These results confirmed a main gradient of lead contamination in soils as a consequence of the battery recycling plant emission in the past.

With regard to the bioavailable or mobile Pb fractions in soils, no significant differences between the rhizosphere and bulk compartments were observed (Table 1). However, a trend was observed for higher concentrations in the rhizospheric soil at the vegetative stage of growth, which corresponded to February. Related to this, during February, the rhizospheric soil was more acidic than the bulk soil when considering all the sites during this period (pH, $p < 0.05$: rhizospheric soils, 6.73 ± 0.04; bulk soil, 6.88 ± 0.04), thereby indicating an acidification process caused by the root system. Indeed, many studies have reported that the rhizosphere exudates such as organic acids, which may acidify the soils, induce a greater availability of nutrients, and also could consequently affect undesirable toxic metal mobility or even uptake (Crowley et al., 1991; Ghosh and Singh, 2005; Romheld and Marschner, 1986). However, although some studies have shown acidification in the rhizosphere related to a greater incorporation of metals in plants (Li et al., 2013), other studies did not show a marked trend. Regarding to this Shuman and Wang (1997) showed pH differences similar to this study, around 0.5 units of pH lower in soils with plants. On the contrary, Marschner and Römheld (1983) demostrate both acidification and alkalinization of the soil pH in the rhizoshere. Finally, Lin et al. (2004) made a review where they showed that some plants could modified the pH of the soil even in 1 or 2 units, but they disscus about the real influence of this parameter in the metal biodisponibility.

It is important to note that all the monitored sites showed values of pseudo-total Pb concentration in soils above the permitted levels of total concentrations for agricultural land use in Argentina (375 mg kg^{-1} DW Pb; Argentinean Legislation Law 24051, 1992),

Table 3
Total and genera fungal counts (CFU g^{-1}) in sorghum-soil samples contaminated with natural variable Pb levels.

Kind of soil	Site	Total counts (CFU g^{-1} $.10^4$)	Fungal genera (CFU g^{-1} $.10^4$)						
			Aspergillus spp.	*Penicillium* spp.	*Cladosporium* spp.	*Trichoderma* spp.	Yeasts	Mucorals	Mycelia
Bulk[a]	1	1.54 ± 0.78^{B}	0.45	0.77	0.01	0.02	ND	0.02	0.76
	2	5.27 ± 3.32^{AB}	1.63	1.66	ND	0.012	ND	ND	0.53
	3	10.63 ± 7.72^{A}	0.76	7.02	ND	0.27	0.03	0.017	1.33
	4	2.92 ± 2.73^{B}	0.5	1.3	ND	0.24	ND	0.012	0.27
Rhizosphere[b]	1	42.30 ± 41.35^{a}	1.1	14.4	ND	0.3	0.3	0.2	2.6
	2	12.33 ± 2.57^{ab}	2.39	8.12	ND	ND	0.9	ND	1.65
	3	42.70 ± 43.22^{a}	2.1	15.5	0.1	0.3	ND	0.01	2.6
	4	4.75 ± 2.67^{b}	0.02	2.28	0.02	0.24	1.57	0.02	0.45

Note: Total fungal counts values in each row corresponding to Bulk ([a]ANOVA) in capital letters, and values corresponding to Rhizosphere in lowercase letters ([b]ANOVA) followed by the same letter do not differ significantly at $p < 0.05$.

Table 4
Mean values and standard deviation (±SD) of total number of spores and total number of morphospecies found at the different sampling sites.

Site	Spores	Morphospecies
1	133.33 ± 99.23	6
2	274.33 ± 143.51	7
3	90.33 ± 15.37	5
4	174.67 ± 184.53	7

with the first two sites even surpassing the soil limit for industrial land use.

3.2. Analysis of pH, EC, OM percentages and granulometry in soils

The pH and EC values along with the organic matter percentages (OM%) of soils at the study sites are shown in Table 1. Although significant differences for pH and OM% were found, these parameters were not apparently related to the distribution of the bioavailable metal fractions in soil, despite many authors having reported this interaction (Kashem and Singh, 2001; Kim et al., 2010; Sauvé et al., 1997). In the present study, only the analysis of the bulk and rhizosphere soils revealed an influence of plant exudates on soil acidification, as mentioned above (see Section 3.1.).

Soil particle size (granulometry, G), was determined for all sites corresponding to the rhizosphere compartment (G Rz) and soil bulk sample (G Bk). In addition, samples without the soil organic matter of the rhizosphere (G RzOM) removed were analyzed in order to compare the effect of OM on the particle size and metals, with a control site located 6 km from the sampling area being chosen as a reference value. A comparison for the percentages of clay, silt and sand presented among a control soil and different soil compartments for all sites combined are shown in Fig. 2. On comparing the soil granulometry between bulk and rhizospheric compartments, no significant differences were found. However, regarding the rhizospheric compartment, an increase in the percentage of sand particles at the expense of clay was observed when organic matter was retained. These particles missing in the finest fraction were agglomerated by organic matter up to sand size, indicating that the organic matter controlled the soil structure, and consequently, may result in an indirect control of metal availability due to agglomerated particles being those with the strongest heavy metal adsorption (Abouelnasr, 2010; Li et al., 2015; Zhao et al., 2010).

3.3. Mycobiota

3.3.1. Toxicogenic fungi

Taking into account the difficulties involved in the identification of toxicological species of fungi, in many cases it was not possible to differentiate species with a morphological study. Therefore, quantitative analysis was performed considering genera (see Supplementary Table S1).

Is should be noted that toxigenic analysis was made combining all sampling months for each site. The total fungal count revealed significant differences ($p^{\wedge}0.01$) between bulk and rhizosphere soils as well as between sampling sites. However, these differences were no related with the Pb levels detected. In addition, considering the genera of fungi abundance it was observed that both *Aspergillus* and *Penicillium* were dominant in the study area (Table 3). Moreover, *Aspergillus* spp. showed a positive association with the most contaminated sites (see biplot of the Supplementary Fig.S1). In fact, *Aspergillus* species have been previously mentioned as potential bioremediators of soils contaminated with Pb, since this species may be involved in the processes of mineral transformation in soils, thereby reducing the bioavailability of the metal (Mulligan and Galvez-Cloutier, 2003; Ren et al., 2009; Sayer et al., 1999). In addition, other studies have mentioned an increase of *Aspergillus* species in agricultural soils with soybeans or maize, as a result of the application of the herbicide glyphosate usage and associated with the production of aflatoxins, and consequently affecting food security in the province of Córdoba, Argentina (Barberis et al., 2013; Carranza et al., 2014). Regarding this, it is important to note that in the present study typical agrochemicals were applied to soybeans in rotation with other crops, which included the use of glyphosate, and endosulfan, with atrazine being applied to sorghum.

3.3.2. Mycorrhizae

No significant differences between sampling sites and spore number were found in the study area (Table 4), with the arbuscular mycorrhizae

Table 5
Mean values ± standard deviation (SD) and results of the analysis of variance (ANOVA) of Pb, Cu and Zn (mg kg^{-1} DW) content in different organs of *Sorghum bicolor* at different sampling sites and months.

Metal	Month	February			ANOVA[a]	April			ANOVA[a]
	Site	Root	Aerial	Flower		Root	Aerial	Fruit	
Pb	1	60.37 ± 21.63 Aa	11.98 ± 1.78 Aa	3.06 ± 0.05 Ba	**	20.48 ± 3.93 Aa	1.96 ± 1.05 Ba	1.08 ± 0.17 Ba	**
	2	24.9 ± 5.02 Aa	7.47 ± 1.08 Ba	1.95 ± 0.06 Bb	*	21.81 ± 7.67 Aa	8.97 ± 4.31 Aa	1.51 ± 0.49 Ba	*
	3	20.06 ± 3.63 Aa	6.62 ± 2.54 Ba	1.17 ± 0.59 Bb	**	13.24 ± 2.93 Aa	4.32 ± 0.92 Ba	1.15 ± 0.27 Ca	**
	4	8.21 ± 4.66 Ab	2.47 ± 1.24 Ab	0.64 ± 0.32 Ab	ns	9.75 ± 2.57 Aa	6.42 ± 3.71 Aa	0.69 ± 0.13 Ba	**
ANOVA[b]		*	**	***		ns	ns	ns	
		Root	Aerial	Flower		Root	Aerial	Fruit	
Cu	1	96.18 ± 73.36 Aa	2.39 ± 0.19 Bb	3.32 ± 0.11 Ab	**	10.19 ± 1.97 Aa	0.93 ± 0.56 Ba	1.27 ± 0.32 Bb	**
	2	2.97 ± 0.3 Aa	5.67 ± 1.9 Aa	3.67 ± 0.15 Ab	ns	6.05 ± 1.69 Aa	18.24 ± 11.46 Aa	2.38 ± 0.2 Aa	ns
	3	63.57 ± 54.68 Aa	3.76 ± 0.57 Aa	4.87 ± 0.34 Aa	ns	6.09 ± 3.39 Aa	8.14 ± 5.04 Aa	1.83 ± 0.16 Ab	ns
	4	6.28 ± 2.94 Aa	45.77 ± 29.71 Aa	8.65 ± 4.53 Aa	ns	10.28 ± 2.71 Aa	10.09 ± 7.68 Aa	1.57 ± 0.19 Bb	**
ANOVA[b]		ns	*	**		ns	ns	**	
		Root	Aerial	Flower		Root	Aerial	Fruit	
Zn	1	40.81 ± 7.85 Aa	28.8 ± 3.52 Aa	23.55 ± 1.83 Aa	ns	22.36 ± 5.63 Aa	17.88 ± 4.32 Aa	18.16 ± 1.64 Aa	ns
	2	20.76 ± 1.84 Aa	30.61 ± 5.34 Aa	21.58 ± 2.58 Aa	ns	27.64 ± 3.13 Aa	22.93 ± 7.71 Aa	14.01 ± 1.37 Ba	**
	3	63.99 ± 38.91 Aa	24.38 ± 1.67 Aa	26.97 ± 0.82 Aa	ns	20.74 ± 4.93 Aa	24.36 ± 4.99 Aa	17.77 ± 2.22 Ba	ns
	4	14.92 ± 1.34 Bb	48.77 ± 21 Aa	27.46 ± 2.19 Aa	**	23.89 ± 4.08 Aa	21.41 ± 3.95 Aa	14.31 ± 0.77 Ba	*
ANOVA[b]		**	ns	ns		ns	ns	ns	

Values in each row ([a]ANOVA in capital letters) and column ([b]ANOVA) followed by the same letter do not differ significantly at $p < 0.05$. (ns: not significant).
* $0.1 > p > 0.05$.
** $0.05 > p > 0.001$.
*** $p < 0.001$ Test DGC.
[a] ANOVA among soybean organs.
[b] ANOVA among exposure sites.

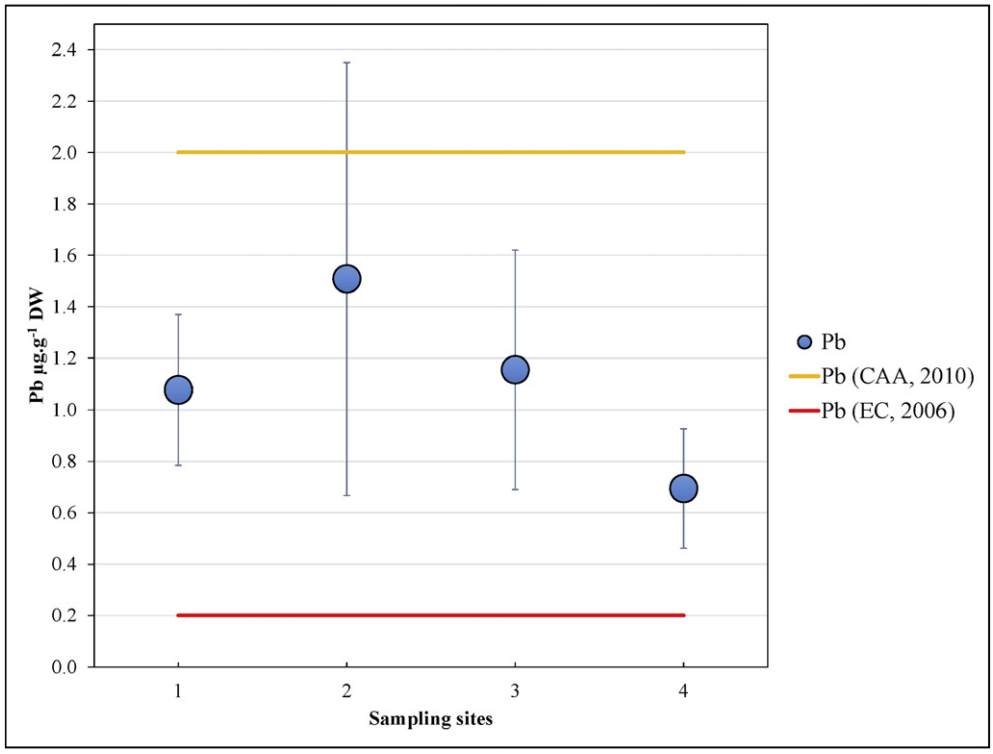

Fig. 3. Pb concentration (mg kg^{-1}) in sorghum fruits corresponding to the study area. Note: CAA (2010), Código Alimentario Argentino; EC (2006), European Comission.

fungi morphospecies being shown in Table S1. The study of the mycorrhizal community revealed a geometric distribution based on a rank-abundance diagram. This distribution is typical of disturbed environments (Begon et al., 2006), with this altered structure having been previously shown in heavy metal contaminated soils (Yang et al., 2015). *Glomus brohultii* was the dominant species in the community, and the genera *Glomus* spp. was associated with heavy metals (Kamal et al., 2010; Wu et al., 2007). However, no relationships were found between morphospecies and Pb in soils, suggesting that this soil biological factor did not control Pb availability.

3.4. Metals in plants

3.4.1. Accumulation of Pb in different organs

The results of the Pb concentrations in different organs of sorghum plants (root, shoot and flower-fruit) for each sampling period (February

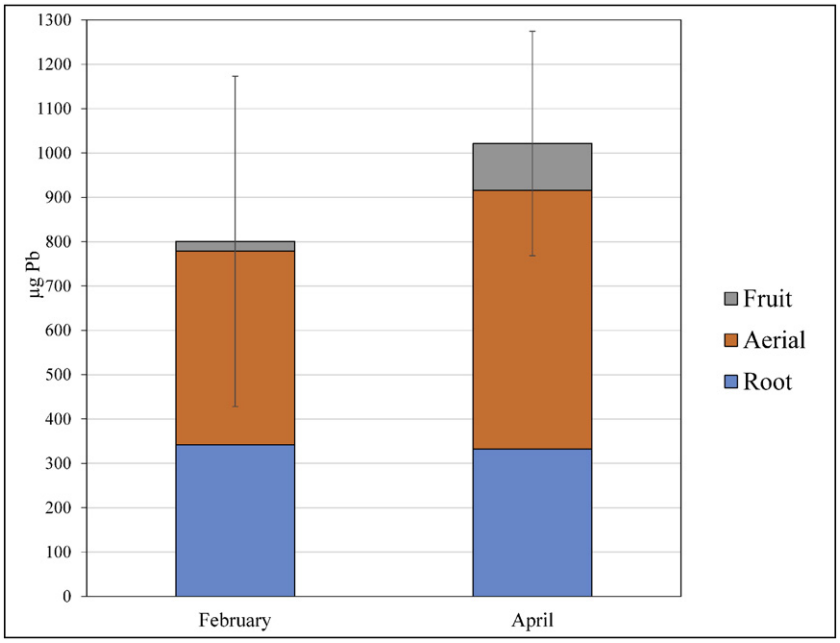

Fig. 4. Total content of Pb (µg) accumulated per sorghum plant for February and April samplings.

and April) in the study area are summarized in Table 5. Regarding the Pb accumulation in February (vegetative stage) significant differences among sites were observed, according the gradient of Pb in soils, with the least accumulation being at the site with the lowest Pb content in soil. However, in April (reproductive-maturity stage) no differences were revealed, which suggest different Pb accumulation strategies in plants, depending on the growth stage. In contrast, as a general trend, the highest concentrations of Pb were observed in the roots, a result also reported by others authors for sorghum (Al Chami et al., 2015; Kaplan et al., 2005; Murillo et al., 1999). This accumulation of toxic metals in roots could have been a defense mechanism of the plants, as it has been widely observed (Baker, 1981; Peralta-Videa et al., 2009; Raskin et al., 1994; Wallace and Romney, 1977).

3.4.2. Relationships among bioavailable Pb in soil, accumulation and morphological parameters in plants

A Pearson correlation analysis among the mobile Pb concentration in soil (Pb_{Mob}), morphological parameters (plant and fruit length, grain weight) and accumulation of Pb in plants was performed (data not shown), with the bioavailable Pb concentration in soil correlating negatively with the accumulation in roots and fruit length (fruit length-Pb_{Mob}: −0.730, $p < 0.001$; Pb root-Pb flower: 0.516; $p < 0.05$; Pb_{Mob}-Pb root: 0.651; $p < 0.001$). These results indicate a lower crop quality when Pb bioavailability was enhanced in soils, with similar results having been previously reported for soybean (Rodriguez et al., 2015; Rodriguez et al., 2014), and tea plants (Yongsheng et al., 2011).

3.4.3. Pb accumulation in fruits and toxicological risk

Although the Pb concentrations in sorghum fruits were less than the permitted level of 2 mg kg^{-1}, according to the Argentinean legislation (CAA, 2010), these values by far exceeded the European legislation of maximum permitted levels of 0.2 mg kg^{-1} (EC, 2006) and could therefore be a possible risk to consumers' health (Fig. 3).

Nevertheless, it is important to note that despite some of these sites showing high levels of pseudo-residual Pb in soils, there was no effect on sorghum toxicity, and the calculation of risk assessment for different metals (THQ) and the accumulative index (HI) did not indicate any toxicological hazard for human consumption even at industrial sites. Thus, these results reveal the importance of considering the crop species used in each place and not only the related soil parameters when evaluating the toxicological risk of a particular agricultural activity.

3.4.4. Total lead content in plants

In order to assess the different growth stages of sorghum and their relation with Pb accumulation, we compared the absolute amount of Pb (μg) accumulated by plants for the February and April samplings (Fig. 4). These results did not reveal significant differences between months, which may indicate that Pb was mainly accumulated during the early stages of growth, as mentioned above. This absolute accumulation, in addition to other characteristics of this crop such as the high levels of Pb found in roots and stems, the relatively low Pb concentrations in seeds with no toxicological risk and the high biomass of the crop, indicates the potential of using sorghum plants in phytoremediation programs associated with agricultural production. Regarding to this, other authors have proposed *S. bicolor* as a good candidate for phytoremediation (Al Chami et al., 2015; Zhuang et al., 2009b).

4. Conclusions

The analysis of different physical, chemical and biological parameters in soils showed that soil lead uptake and accumulation in sorghum crops did not have a direct relationship with pH, EC or OM. However, the results showed that organic matter alters soil grain size, thereby potentially increasing the adsorption of metal in the particles. Moreover, sorghum roots revealed an ability to alter the soil pH, and acidify the rhizosphere, although more studies are necessary to describe these processes. Regarding the soil mycobiota, diversity was a characteristic of a disturbed and Pb polluted soil, but it was not identified as being a factor controlling Pb availability or accumulation. Lead accumulation in sorghum is known to produce a lower crop quality. However, despite the fact that the Pb concentrations in the soils surpassed the permitted levels allowed in agricultural use, this crop did not present a toxicological risk for direct consumption by humans. In addition, the sorghum crop presented certain characteristics such as a high Pb accumulation in roots and a high biomass without any resulting toxicological risk, which are essential requirements for a potential phytostabilizator.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gexplo.2016.01.020.

Acknowledgments

This work was partially supported by the Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECYT-UNC "B" Res. 203/2014), Fondo para la Investigación Científica y Técnica (FONCyT, PICT 2011-2342; 2011-0084) and Proyectos Plurianuales del Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 2013-2015, 11220120100402CO). The authors Blanco, Salazar and Vergara Cid were funded by CONICET through scholarships. In addition, the authors Blanco and Vergara Cid are Ph.D. students in Biological Sciences at the National University of Córdoba. We would especially like to thank the Brazilian Synchrotron Light Source (LNLS) (partially supported under proposals XAFS1-15165 and XAFS1-15981) and Dr. D. Wannaz for laboratory support. Special thanks are due to Valeria Poloni from the Department of Microbiology and Immunology at the University of Río Cuarto, to the land owner and mayor of Bouwer (J. Lupi), and to Dr. P. Hobson (native speaker) for language revision.

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