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ORIGINAL ARTICLE

Accumulation of lead and associated metals (Cu and Zn) at different growth stages of soybean crops in lead-contaminated soils: food security and crop quality implications

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Abstract The Pb, Cu and Zn content, the physicochemical parameters in soils (EC, OM%, soil texture and pH) and the metal accumulation of *Glycine max* plants at different growth stages were evaluated. Topsoil and soybean samples were collected in the vicinity of a former battery-recycling plant, with the results showing that only the concentrations of Pb in soils corresponding to sites located near to the lead emission source were above the maximum permissible levels. However, soybean crops accumulated Pb above the permitted levels at all studied sites, revealing a potential toxicological risk for direct consumption. Thus, the accumulation of Pb in soybean was directly related to the translocation factor of the metal from roots to aerial parts of the plant. This was evidenced as a lower accumulation at early growth stages and a higher accumulation at maturity, with the distribution between organs coinciding with nutrient incorporation and remobilization in the plant. Moreover, the bioconcentration factor revealed that the bioaccumulation of lead in soybean was a consequence of the lead-recycling plant activity in the past. Taken together, results of the present study demonstrated that soybean crops can incorporate and accumulate potentially toxic metals, such as lead.

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Introduction

Heavy metal pollution in soils can have serious consequences for surrounding ecosystems, groundwater, agricultural productivity and human health, due to its persistence and high toxicity, which has a great impact on humanity as agriculture is not sustainable without soils under optimal conditions (Gunawardana et al. 2011). Regarding heavy metal-polluted agricultural soils, the principal risk lies in toxic accumulation of metals in crops, with numerous investigations having been performed concerning deleterious effects on human health and crops yield (Wahsha et al. 2014). Some of the effects of this metal on human health are referred to neurological damage, degeneration of the central nervous system, neurotoxic diseases and anemia (Cabral et al. 2015). Other authors show that there is a great number of effects in plants of different species which grow in Pb-polluted soils, including inhibition of germination, seedlings growth, root and stem elongation, and also abnormal morphology and physiology with alteration in enzymes activity (Nagajyoti et al. 2010). Although little is known about the mechanisms of incorporation in plants, some authors mention that Pb is preferably absorbed passively in the roots (Kabata-Pendias 2010). Once on the root, it is believed that Pb could passively move through the flow of transpiration, thus achieving other organs (Liao et al. 2006).

The main industrial practice that contributes to heavy metal pollution in soils is Pb smelting (Fernandez-Turiel et al. 2001). Related to this, previous studies carried out around Pb-smelter plants have reported the contamination of soil, vegetation, animals and humans (Dahmani-Muller et al. 2000). More recently a former battery-recycling plant in Córdoba, Argentina, was reported to have dangerously high levels of Pb in soils and native plants (Salazar and Pignata 2014). As this region is primarily an agricultural area cultivated with soybeans and associated rotation crops such as wheat, sorghum and maize, it is important to evaluate the toxicological risk in the area due to contamination via the food chain when consuming food from the polluted sites, especially as soybean has been mentioned several times recently as a being potential lead accumulator species (Lavado et al. 2001; Lavado 2006; Rodriguez et al. 2011; Salazar et al. 2012; Zhao et al. 2014). Translocation of metals in soil to crops has been reported in a wide variety of crop species (Li et al. 2012). Polluted soils have a reduced quality of their physical and chemical properties that causes changes in their metal retention capacity, being a complex process the metal transfer from soil to plant determined by several factors such as biological, geochemical or climatic (Kabata-Pendias and Sadurski 2004).

In this context, the purpose of this study was to evaluate the accumulation and distribution of Pb, Cu and Zn at different growth stages of soybean cultivated in lead-polluted soils taking into account the toxicological risk and crop quality.

Materials and methods

Site and sampling points

Four sampling sites, which were chosen taking into a preliminary study (Salazar and Pignata 2014), were located in Bouwer a peri-urban town in the Province of Córdoba in Argentina, which is characterized by a former battery-recycling plant (31°33′34.02″S; 64°11′9.05″W) (Table 1; Fig. 1). Climatic conditions and agricultural practices are described in Rodriguez et al. (2014). Briefly, the climate is mild (annual mean temperature and rainfall of 15 °C and 500–900 mm, respectively); the soil on site can be classified as Entic Haplustoll according to taxonomic soil keys (USDA 2006). This area is surrounded by agricultural crops (mostly soybean and associated crops such as sorghum, corn and wheat).

Topsoil and soybean sampling procedures

Topsoil (rhizosphere and bulk) and crop samples were collected throughout a full growing season of soybean (three different times) and thus represented the different growth stages of plant development as follows: prior to sowing, vegetative–early reproductive stage (R1–R5) and maturity (R8) according to Fehr and Caviness (1977).

Soybean plants and topsoil samples were collected at the sampling sites at the different growth stages using a systematic sampling following the procedure of composite soil sampling, for which each site consisted of a square of 3 m^2 with nine subsampling points systematically arranged with a 1-m gap between them. At each sampling site, three pools of samples (soils and plants) were collected taking into account previous results in the study area (Salazar and Pignata 2014). It is important to note that for the first sampling corresponding to the period before planting only bulk soil samples were collected, as there were no plants.

Plants were collected carefully extracting the whole root and soil in close contact, considered in this study to be rhizospheric soils. Rhizosphere soil samples were isolated from roots by shaking them in a plastic box. In addition, at the sampling points bulk topsoil samples (0–10 cm) were collected using a blast hole ($n = 9$ for rhizosphere and bulk soil).

In the study area typical agrochemicals were applied to soybean crops. Before sowing a chemical fallow was applied, consisting of a mixture of glyphosate (2–3 L ha^{-1}) and atrazine (2–3 L ha^{-1}). Then, subsequent to sowing, pre- and postemergence agrochemicals were applied (glyphosate 48%, 2 L ha^{-1} and endosulfan).

Soybean samples were washed and sonicated with ultrapure water removing any soil remains attached to the organs. Next, the samples were oven dried at 60 °C to constant dry weight (DW) and stored until analytical procedures were performed.

All soil samples were sieved to <2 mm, homogeneously mixed and stored in the laboratory under controlled conditions before chemical determinations.

Physicochemical and biological analyses

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. In order to calculate the dry weight (DW), samples were oven dried at 105 °C to constant weight, with the organic matter percentage (OM%) being determined according to Peltola and Åström (2003) by combustion of the samples at 500 °C for four hours. In addition, the grain size was measured by laser diffraction size analysis using a Horiba LA-950 particle size analyzer, following Gaiero et al. (2013), for which the presence of organic matter was eliminated using 30% v/v of analytical grade H_2O_2.

Heavy metal sequential extraction in topsoils

Sequential extraction was performed for the purpose of determining the mobile and pseudototal fractions, with

Table 1 Mean values and standard deviation (±SD) of mobile (Mob) Pb and pseudototal (Pst) heavy metal concentrations (Pb, Cu and Zn) in rhizosphere and bulk soils, and their relation to the source distance

Site	Distance to the source (m)	Type of soil	Sampling	Pb_{Mob} (mg kg^{-1})	Pb_{Pst} (mg kg^{-1})	Cu_{Pst} (mg kg^{-1})	Zn_{Pst} (mg kg^{-1})
1	176	Bk	1	19.1 ± 2.8 A	186 ± 22 A	6.83 ± 0.23 A	15.2 ± 0.1 B
			2	19.7 ± 2.9 A	205 ± 13 A	5.88 ± 0.65 B	10.1 ± 1.3 E
			3	18.5 ± 1.8 A	197 ± 48 A	7.41 ± 0.13 A	15.2 ± 1.1 B
		Rz	2	19.6 ± 4.0 A	180 ± 12 A	6.18 ± 0.14 B	12.6 ± 0.1 C
			3	19.6 ± 1.8 A	177 ± 5 A	6.04 ± 0.13 B	13.3 ± 0.0 C
2	224	Bk	1	20.3 ± 2.9 A	91 ± 1 C	7.02 ± 0.24 A	15 ± 0.4 B
			2	20.9 ± 3.3 A	106 ± 1 C	6.02 ± 0.16 B	9.9 ± 0.3 E
			3	21.7 ± 4.2 A	140 ± 20 B	7.16 ± 0.54 A	12.2 ± 0.9 C
		Rz	2	22.6 ± 4.2 A	126 ± 19 B	7.23 ± 0.47 A	13.4 ± 1 C
			3	19.6 ± 2.0 A	142 ± 19 B	7.45 ± 0.02 A	12.7 ± 1.4 C
3	720	Bk	1	15.3 ± 1.4 B	31 ± 1 D	6.95 ± 0.10 A	14.6 ± 0.2 B
			2	15.2 ± 0.9 B	34 ± 2 D	6.28 ± 0.04 B	9.4 ± 0.2 E
			3	13.3 ± 1.9 B	32 ± 1 D	7.03 ± 0.25 A	10.8 ± 0.2 D
		Rz	2	14.0 ± 0.9 B	37 ± 5 D	7.31 ± 0.14 A	12.6 ± 0.3 C
			3	14.9 ± 2.1 B	40 ± 4 D	6.18 ± 0.05 B	13.9 ± 1.2 E
4	1011	Bk	1	19.2 ± 2.5 A	27 ± 4 D	8.01 ± 0.79 A	18.3 ± 2.4 A
			2	19.7 ± 5.8 A	33 ± 8 D	6.58 ± 0.43 A	10.1 ± 0 E
			3	20.6 ± 4.0 A	28 ± 2 D	6.61 ± 0.05 A	12 ± 0.7 C
		Rz	2	21.2 ± 0.7 A	32 ± 13 D	5.91 ± 1.22 B	9.8 ± 0.7 C
			3	21.2 ± 5.4 A	29 ± 5 D	5.88 ± 0.81 B	9.3 ± 0.5 E
Anova				*	***	***	***

Sampling 1, correspond to soil prior to planting; Sampling 2, correspond to soil in the vegetative–reproductive growth stage of soybean; Sampling 3, correspond to soil in the maturity growth stage of soybean. Values in each column (ANOVA) followed by the same letter do not differ significantly at $p < 0.05$. (*ns* not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

heavy metal values being obtained using a PerkinElmer AA3110 atomic absorption spectrometer (Norwalk, CT, USA) to measure the amounts of Cu, Pb and Zn.

Topsoils were sieved at 63 μm with a stainless steel mesh before the sequential extraction was carried out. The mobile or exchangeable fraction (Mob) was obtained according to Tessier et al. (1979), with a solution of $MgCl_2$ 1 M (1:8 W/V) being added to soil samples, which was then shaken for 1 h and subsequently centrifuged at 1370 g for half an hour. The concentrations of Pb_{Mob}, Cu_{Mob} and Zn_{Mob} were determined by AAS, and the pseudototal fraction (Pst) was determined using an acid digestion (HNO_3, 60%), with the Cu_{Pst}, Pb_{Pst} and Zn_{Pst} concentrations being analyzed by AAS.

As a quality control, blanks and samples of the standard reference material "BAM-U113 Soil" (Germany) were prepared in the same way and were run to calibrate the instrument. These results were found to be between 86 and 92% of the certified value, with the data indicating a low error of typically less than 15%. The coefficient of variation of the replicate analyses ($n = 3$) was calculated for different determinations, being less than 10% of variations.

Metal content in soybean

The Cu, Pb and Zn contents were obtained from the seeds, pods, stems, leaves and roots (1 g DW), which were ashed at 450 °C for 4 h before being 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. X-ray fluorescence preparation of samples and characteristics of measurement are described in Graziani et al. (2015).

As a quality control, blanks and samples of the standard reference materials "Soybean flour (INCT-sbf-4), Oriental Tobacco Leaves (CTA-OTL-1, ICTJ) and CRM 281 (rye-grass, European Commission/BCR)" were prepared in the same way and were run after five determinations to calibrate the instrument, and these results were found to be within ± 2% of the certified value. The coefficient of variation of the replicate analysis showed variation lowers than 10%.

Soybean quality parameters

In order to evaluate the effect of soybean crop quality, the following parameters were determined according to ISTA

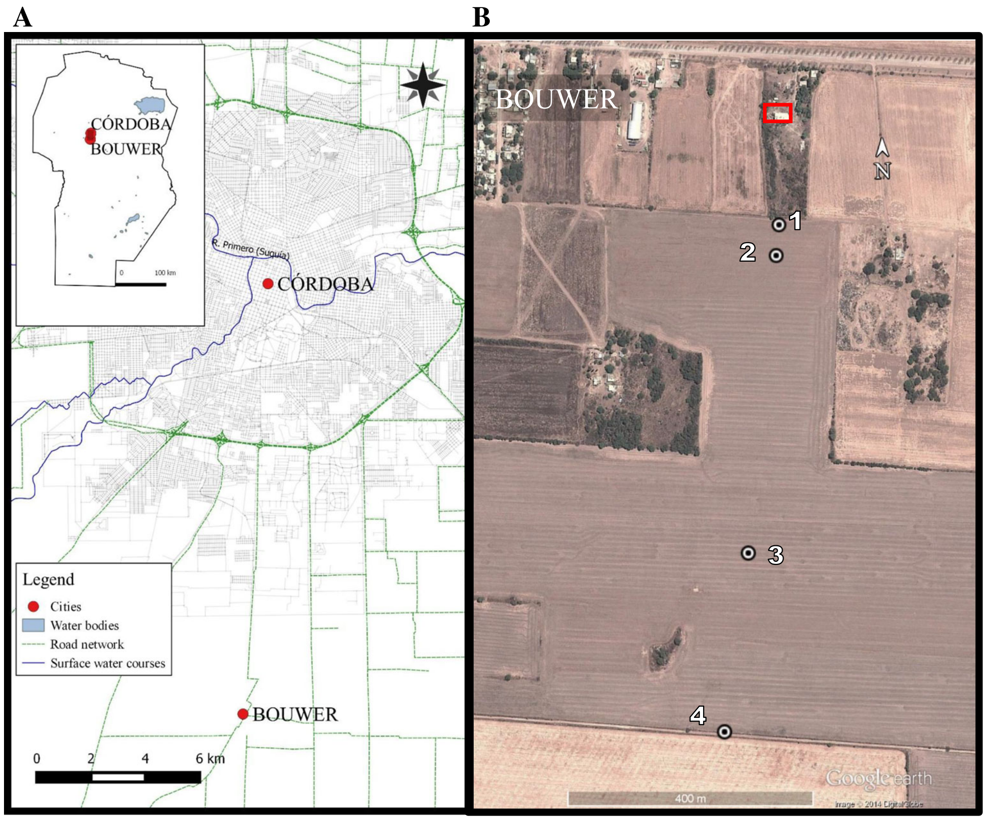

Fig. 1 Sample site location. **a** Position of Bouwer and Córdoba city. **b** Satellite image showing location of the smelter and sampling points

(2013): biomasses of stems, leaves, pods, seeds and roots, expressed as dry weight, weight of 1000 seeds and number of seeds per plant, were analyzed.

Data analyses

Statistical analyses

The Analysis of Variance (ANOVA) assumptions were previously verified. ANOVA was performed to compare the metal concentrations in plants with physicochemical parameters in soils at the sampling sites. Whenever the ANOVA indicated significant effects ($p < 0.05$), a pair-wise comparison of means was undertaken using Fisher's least significant difference (LSD) and the formation of exclusive groups (DFG) test (Di Rienzo et al. 2002). The Pearson correlation coefficient was performed with the purpose to identify the relationships between soil variables in the different soil compartments (rhizosphere and bulk topsoil). Moreover, Pearson correlations were also used to evaluate possible relationships among the metal exchangeable concentrations in rhizospheric soil, and soybean parameters. In addition, simple regressions were made between the soil metal concentrations and the metal content in soybean.

All analyses were performed using the software Infostat®.

Bioaccumulation and translocation factors

In this study, a modified bioconcentration factor (BCF) was calculated according to Salazar et al. (2012). This factor is computed as the ratio of the metal concentration in seeds and the "potentially available" metals in the rhizospheric soils (mobile fraction of metals):

$$\mathrm{BCF} = C_{\mathrm{seed}}/C_{\mathrm{soil-mob}}$$

where C_{seed} is the median concentration of a heavy metal in seeds (mg kg^{-1}); $C_{\mathrm{soil\text{-}mob}}$ is the median concentration of a heavy metal in the mobile fraction of the rhizospheric soil compartment.

On the other hand, a translocation factor (TF) was calculated in order to identify the accumulation organs of heavy metals as described in Rodriguez et al. (2011). TFs were computed between roots and stem (TFr/st) and between stem and seed (TFst/s) using the following formula:

$$\mathrm{TFr/st} = C_{\mathrm{stem}}/C_{\mathrm{root}}$$
$$\mathrm{TFst/s} = C_{\mathrm{seed}}/C_{\mathrm{stem}}$$

where C_{stem}, C_{roots} and C_{seed} are the median concentration of a heavy metal in stem, root and seed (mg kg^{-1}), respectively. Values higher than one suggest that the elements were easily translocated.

Health risk assessment of exposure to heavy metals

The health risk from consumption of soybean grown in heavy metal-polluted sites was calculated by employing the estimated dietary intake (EDI mg kg^{-1} day^{-1} Bw) and target hazard quotients (THQs), as described by Zheng et al. (2007) and EPA (1989). In the present study, Chinese, European and Argentine inhabitants were considered potential consumers, considering the exports of soybean and local consumption.

The 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:

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

where C is the median concentration of a heavy metal in soybean (μg g^{-1}); Con is the ingestion rate of soybean (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 European or 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 soy products for a Chinese individual was 100 g person^{-1} day^{-1}, while the daily intake in Western inhabitants was less than 1 g person^{-1} day^{-1} (Keinan-Boker et al. 2002; Franco 2010).

THQ gives the potential non-cancer risk of individual heavy metals and can be calculated as follows:

$$\mathrm{THQ} = \mathrm{EDI}/\mathrm{RfD}$$

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 to without any appreciable risk of deleterious effects during a lifetime. The reference oral dose (RfD) values employed in this study were 40 and 300 mg kg^{-1} day^{-1} for Cu and Zn (EPA 2010) and 4 mg kg^{-1} day^{-1} for Pb according to Huang et al. (2008).

In order to assess the overall potential for non-carcinogenic effects from more than one heavy metal, a hazard index (HI) has been formulated based on the Guidelines for Health Risk Assessment of Chemical Mixtures of EPA (EPA 1989) as follows:

$$\begin{aligned}\mathrm{HI} &= \sum \mathrm{THQ} \\ &= \mathrm{EDI}/\mathrm{RfD}_1 + \mathrm{EDI}/\mathrm{RfD}_2 + \cdots + \mathrm{EDI}/\mathrm{RfD}_i\end{aligned}$$

THQ and HI values exceeding the unity implied a high risk of non-carcinogenic effects.

Results and discussion

Heavy metals, pH, EC and OM% in soil

The results of the concentrations of heavy metals and physicochemical characteristics found in the monitored sites are shown in Tables 1 and 2 (Zn_{Mob} and Cu_{Mob} were below the detection limit of the AAS). These levels did not exceed the limits set by Argentine legislation for cropping lands for any of the metals (Pb 375 ppm, Cu 150 ppm, Zn 600 ppm; Argentinian National Law 24501). However, when compared with other legislation, which also considers human and environmental health, sites 1 and 2 exceeded the permitted limit values (Pb 70 ppm, Cu 63 ppm, Zn 200 ppm; CCME 2007).

No significant differences between rhizosphere and bulk soil compartments were observed, which indicates a lack of association between the plant and the availability of metals. As expected, for Pb_{Pst} significant differences were found related to distance from the emission source, which was confirmed with a potential least squares model of decreasing Pb for greater distance from the source ($y = 40{,}346.701\ x^{-1058}$***; R^2: 0.95; $p < 0.001$). However, it is important to note that residues of slag from Pb were found near the site 4, and although these did not affect the pseudototal distribution, they had an impact on the concentrations of mobile Pb, with concentrations matching those of sites 1 and 2 (Table 1).

Concerning Cu and Zn, although significant differences occurred for the pseudototal concentrations no distinct pattern was observed. For the physicochemical soil parameters, the soils are Entic Haplustoll and presented similar particle size distributions, without any significant differences between sites for OM% or any defined patterns for EC relative to site, soil type or month sampling (Table 2). In contrast, more acidic pH values were found in sites close to the former Pb smelter, which was supported by linear regressions between pH and the bioavailable concentration of lead in soil (Supplementary Fig. 1). Similar results have been previously reported by other authors, which revealed variations in pH, particularly acidification, with an increase in the heavy metal bioavailability in soils (Sauvé et al. 1997).

Table 2 Physicochemical properties of soils (pH, EC, OM% and texture) in rhizosphere and bulk soils in relation to the source distance

Site	Type of soil	Sampling	pH	EC (µs cm^{-2})	OM%	Particle size distribution (%)			Texture
						Sand (2–0.05 mm)	Silt (0.05–2.10^{-3} mm)	Clay (<2.10^{-3} mm)	
1	Bk	1	6.47 ± 0.06 B	84.67 ± 21.82 C	9.28 ± 0.6	22.56 ± 0.42	66.72 ± 0.97	10.72 ± 1.39	Silt loam
		2	6.51 ± 0.13 B	69.7 ± 30.45 C	10.55 ± 1.67				
		3	6.2 ± 0.05 C	35.01 ± 2.51 D	9.99 ± 2.17				
	Rz	2	6.34 ± 0.11 B	80.97 ± 8.01 C	7.27 ± 0.52	15.47 ± 0.05	67.85 ± 0.71	16.67 ± 0.67	Silt loam
		3	6.36 ± 0.16 B	65.83 ± 13.51 C	10.37 ± 3.14				
2	Bk	1	6.54 ± 0.11 B	68.07 ± 3.88 C	10.06 ± 1.2	22.38 ± 1.07	64.62 ± 0.33	13 ± 0.75	Silt loam
		2	6.61 ± 0.03 A	80.13 ± 50.39 C	10.09 ± 1.01				
		3	6.37 ± 0.06 B	36.37 ± 2.67 D	9.16 ± 4.46				
	Rz	2	6.4 ± 0.17 B	88.93 ± 8.84 C	8.84 ± 1.77	16.04 ± 0.35	71.37 ± 1.82	12.59 ± 1.48	Silt loam
		3	6.5 ± 0.08 B	72.47 ± 20.84 C	8.34 ± 6.37				
3	Bk	1	6.67 ± 0.03 A	77.1 ± 10.33 C	10.4 ± 1.9	19.97 ± 0.04	71.09 ± 0.29	8.94 ± 0.24	Silt loam
		2	6.68 ± 0.13 A	57.13 ± 1.69 C	9.52 ± 1.3				
		3	6.73 ± 0.02 A	69.97 ± 3.86 C	9.69 ± 1.55				
	Rz	2	6.77 ± 0.1 A	117.8 ± 3.27 B	7.84 ± 1.28	18.44 ± 0.06	68.05 ± 0.2	13.52 ± 0.14	Silt loam
		3	6.61 ± 0.06 A	66.3 ± 7.89 C	9.57 ± 0.57				
4	Bk	1	6.67 ± 0.06 A	64.5 ± 5.81 C	9.93 ± 1.07	19.96 ± 0.37	69.09 ± 1.99	10.96 ± 1.63	Silt loam
		2	6.47 ± 0.09 B	50.77 ± 21.21 C	9.3 ± 1.64				
		3	6.63 ± 0.17 A	60.87 ± 9.67 C	9.15 ± 1.81				
	Rz	2	6.46 ± 0.15 B	149.65 ± 12.95 A	7.14 ± 0.43	16.17 ± 0.53	73.12 ± 1.46	10.72 ± 0.93	Silt loam
		3	6.47 ± 0.17 B	58.3 ± 7.74 C	9.67 ± 2.43				
Anova			***	***	ns	–	–	–	

Sampling 1, correspond to soil prior to planting; Sampling 2, correspond to soil in the vegetative–reproductive growth stage of soybean; Sampling 3, correspond to soil in the maturity growth stage of soybean. Values in each column (ANOVA) followed by the same letter do not differ significantly at $p < 0.05$ (*ns* not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Particle size distribution was measured in the maturity growth stage

Metals in plants

Metal distribution in organs

Results corresponding to the concentrations of Pb, Zn and Cu in different organs of soybean plants (root, stem, leaf, pod and seed) corresponding to the different growth stages of soybean in the study area are summarized in Table 3. No significant differences between sites were observed for any of the metals studied (data no shown), which is consistent with the little variation observed for the Pb bioavailable concentrations. However, the comparison between organs and Pb accumulation for both sampling months revealed the highest values for the leaves. Moreover, regarding Cu and Zn, variations between organs were observed, with a greater accumulation in roots, leaves and seeds observed for Cu, whereas Zn was mainly accumulated in leaves in the vegetative–early reproductive period, while in the maturity growth stage it was concentrated in the roots and seeds.

Correlation analysis among mobile Pb concentration (rhizosphere and bulk), the physicochemical parameters in soil and the metals in plants

In order to assess the relationship between the accumulation of metals in soybean and the physicochemical parameters of soils, a Pearson correlation analysis was performed (data not shown). For the vegetative–early reproductive stage, the accumulated Pb in the root correlated significantly with the EC (r: 0.69; $p = 0.02$), as has been reported in other studies which showed an increase in the electrical conductivity of soils in agreement with a rise in the availability of metals in plants (Lim et al. 2004; Reed et al. 1995). Furthermore, the accumulated Pb in roots correlated negatively with the mean particle size of the soil (r: -0.72; $p = 0.01$), since smaller soil particles permitted a higher metal adsorption, as previously observed in other studies (Abouelnasr 2010; Li et al. 2015).

Positive correlations between Pb in soil and Cu and Zn in leaves (r: 0.70; $p = 0.02$ and r: 0.66; $p = 0.03$, respectively) were found. Related to this, as mentioned above, the leaves were the plant organ with the highest concentrations of metals. This behavior has been reported in other studies, which observed higher levels of Cu and Zn due to an increase in the enzymes involved in the antioxidant system of the plant, such as one of the enzymes of the superoxide dismutase family, which uses these metals as electron carriers (Chongpraditnun et al. 1992; Goldstein et al. 2006; Szőllősi 2014).

On the other hand, soybean quality parameters revealed a negative correlation between the content of mobile Pb of the rhizosphere and the number of seeds produced by the plant (r: -0.63, $p = 0.03$), which indicated a reduction in the seed quality in lead-polluted soils, as mentioned in a previous study (Rodriguez et al. 2014).

Heavy metal accumulation in seeds

The concentrations of Pb, Zn and Cu in soybean and food consumption permitted levels are shown in Fig. 2. With respect to Pb, it is important to note that at all study sites the maximum permitted levels for human consumption (0.2 mg kg^{-1} FW) according to the European legislation were exceeded (EC 2006), while only site 2 was below the permitted value according to Argentina legislation (maximum value of 2 mg kg^{-1} DW, (CAA 2010). These results are consistent with previous investigations, which reported concentrations of lead above maximum permitted levels in soybeans in the study area as well as at other sites (Rodriguez et al. 2014; Salazar et al. 2012). Furthermore, it is important to note that this crop has recently been mentioned as being a potential lead accumulator by several authors (Lavado 2006; Rodriguez et al. 2011; Zhuang et al. 2013).

Regarding Zn, the concentrations found in soybean were within permitted levels in food (CAA 2010). In contrast, values above the maximum permitted level for foods of 10 mg kg^{-1} DW (CAA 2010) were found for Cu at site 2, while most of the remaining sites showed values close to the permitted level. Similar results for Cu in soybeans have been previously reported in Pampean soils (Lavado 2006; Lavado et al. 2001).

Table 3 Mean values (±SD) and results of the analysis of variance (ANOVA) of Pb, Cu and Zn (mg kg^{-1} DW) content in different organs of *Glycine max* at different growth stages

Metal	Vegetative–reproductive			Maturity				Anova
	Root	Stem	Leaf	Root	Stem	Pod	Seed	
Pb	4.42 ± 0.55 B	4.11 ± 0.09 B	**5.85 ± 0.17 A**	2.81 ± 0.55 B	3.1 ± 0.13 B	2.57 ± 0.17 B	2.81 ± 0.16 B	***
Cu	**9.68 ± 0.77 A**	5.16 ± 0.22 B	**14.15 ± 2.51 A**	**18.73 ± 1.21 A**	3.13 ± 0.69 B	3.49 ± 0.59 B	**8.94 ± 0.61 A**	***
Zn	12.55 ± 1.14 D	15.03 ± 0.28 C	**39.25 ± 2.14 A**	19.11 ± 0.97 B	11.47 ± 0.61 D	10.18 ± 0.35 D	26.05 ± 1.28 B	***

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

Bold indicates the highest metal value

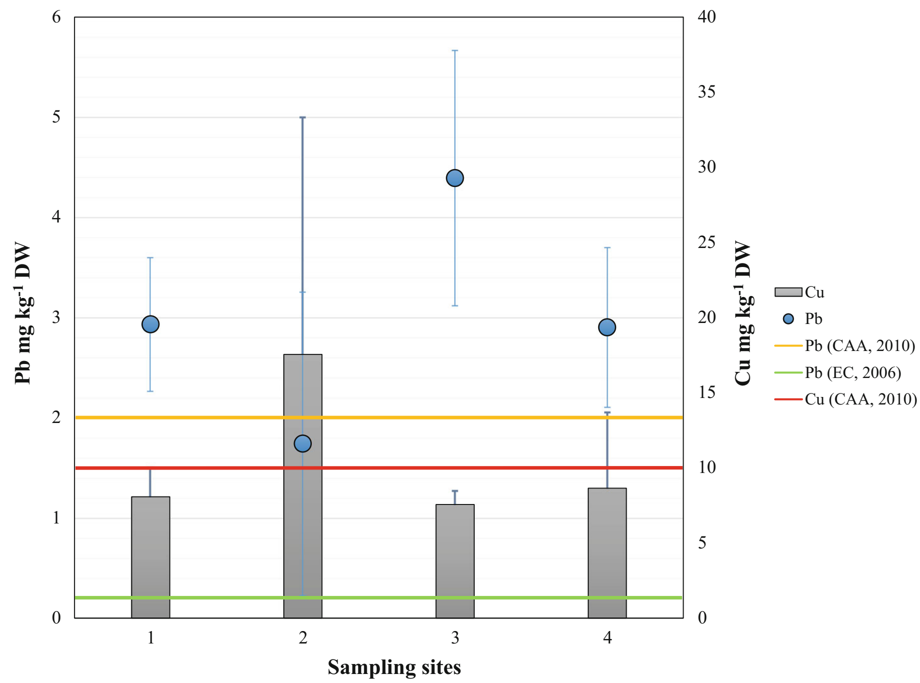

Fig. 2 Cu and Pb concentrations in seeds of *Glycine max* at the sampling sites

Considering the above results and the maximum permitted levels in agricultural soils (see Table 1), our findings clearly demonstrated that the total concentration of metals in soil is not sufficient to establish whether a site is appropriate for agricultural use. Thus, as has been discussed in previous studies, it is important to also consider the bioavailable concentration of the metal and the cultivated species (Salazar et al. 2012).

Pb translocation (TF) and bioconcentration (BCF) factors in soybean

Taking into account that only Pb showed higher values than those permitted in foods and soils, the translocation and bioconcentration factors were only calculated for this metal (Supplementary Table 1). The Pb translocation from roots to shoots in soybean revealed different responses depending on the vegetative growth stage, which was higher at the early growth stages and lower at maturity in most contaminated sites. This behavior is in agreement with nutrient incorporation in the plant through the roots, with it having been reported that toxic metals such as lead can be incorporated in plants using ionic channels, as in the case of calcium (Huang and Cunningham 1996; Pourrut et al. 2008; Wang et al. 2007).

High translocation values from stem to leaves and from pods to seed were found. Related to this, an effective translocation of Pb to soybean seeds was reported in a study conducted in climatic chambers (Rodriguez et al. 2011). However, other authors have observed lower levels of translocation to seeds at contaminated sites (Zhuang et al. 2013). Thus, it is still necessary to perform more studies to understand the mechanisms involved in the uptake and mobility of lead in soybeans.

Regarding BCF, this study revealed similar values to those reported in other studies performed in proximity to metal sources (Salazar et al. 2012; Zhuang et al. 2013), which indicates that the bioaccumulation of lead in soybeans was a result of human activity near to the crops.

Total accumulation of Pb in soybean

Considering that Pb was the only metal exceeding the maximum permitted values for agricultural use of soils and for consumption of seeds, the total accumulation was analyzed only for Pb (Fig. 3). For this purpose, the absolute amount (g) accumulated by each plant was measured, with a comparison between the total accumulation of lead in soybean during the vegetative–early reproductive (R1–R5) and maturity (R8) growth stages of soybean, and was carried out in order to evaluate the absorption and accumulation of Pb. Furthermore, to observe the changes in Pb distribution over time, a partition between the quantities of metal accumulated in each organ was used.

Significant differences between the different development growth stages were found, which corresponded to a higher accumulation in plants for the maturity growth stage, thereby suggesting a continued absorption during development. The findings also revealed the highest accumulation in leaves and seeds in the vegetative–reproductive and maturity growth stage, respectively. Thus, these results suggest that Pb was translocated from leaves

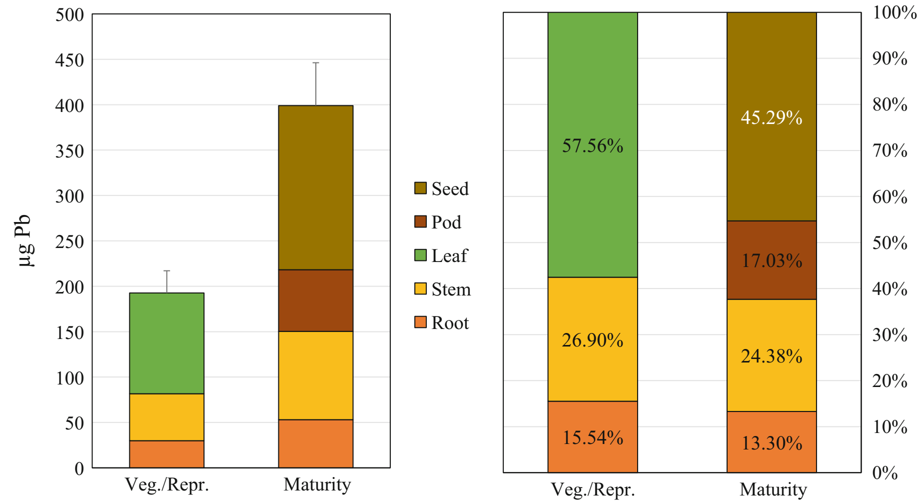

Fig. 3 Total amount of Pb (µg) accumulated by soybean plants during the study period. *Note* different colors represent soybean organs

to the seeds and pods, which coincided with nutrient translocation. In agreement, other studies have reported the mobilization of nutrients through the plant by the plant senescence process in order to transport them to the target organs (such as grains) (Himelblau and Amasino 2001). This behavior has also been observed in soybean leaves, which prior to falling remobilized some nutrients and translocated them to the seeds (Taiz and Zeiger 2002).

Risk assessment

The health risk resulting from the consumption of soybean grown in the study area was calculated for each metal and is shown in Supplementary Fig. 2A. Remarkable differences were observed between European–Argentinean consumers and Chinese consumers, as the latter have incorporated soy products in large quantities in their diet. Consequently, in this study only Chinese consumers revealed target hazard quotients above 1 for Pb in soybean in most places cultivated with this legume. Thus, the potential non-cancer risks from lead (THQ), due to its high toxicity, means that the daily intake of this metal through the consumption of soybean may have caused adverse effects on potential Chinese consumers. Moreover, regarding Cu and Zn, no significant risks for any consumers were detected (data not shown). In agreement, the hazard index, which summarizes the effect of more than one metal in the diet, also only showed values above 1 for Chinese consumers (Supplementary Fig. 2B). Therefore, the evaluation of the potential non-carcinogenic effect, through the sum of individual heavy metals (HI), was significant to the Chinese adult population in all the studied sites.

It is important to note that we have only considered the risk of direct consumption by humans, so more studies taking into account indirect incorporation through consumption of animals are necessary, since one of the main targets of soybean products is cattle (INTA 2010).

Conclusions

Although only the sites near the former battery-recycling plant exceeded the permitted lead values in agricultural soils, the lead content in seeds was above the maximum permitted levels for human consumption at all the studied sites, while the copper content in seeds was close to the permitted level. Thus, these findings revealed that the actual soil guidelines should be modified, as the total concentration of metals in soil alone is insufficient to establish whether a soil is appropriate for agricultural use, with it being necessary to consider the bioavailable concentration of the metals and cultivated species in new soil guidelines.

These findings also showed that the accumulation and translocation of heavy metals in soybean were dependent on the vegetative growth stage, which varied between organs throughout development, and coincided with nutrient incorporation (from roots to stems) at early growth stages and remobilization in the plant (mainly from leaves to seeds) at maturity. Further studies should now be performed in order to understand the mechanisms involved in the uptake and mobility of lead in soybean.

A reduction in seed quality in lead-polluted soils was observed as a consequence of their increased toxicity. Finally, our results revealed that the bioaccumulation of lead in soybean was a result of human activity near the crops, with a potential toxicological risk of lead for Chinese consumers. Taking into account these findings, future studies should also consider the toxicological risk of indirect incorporation through consumption of animals, as one of the main targets of soybean products is cattle.

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