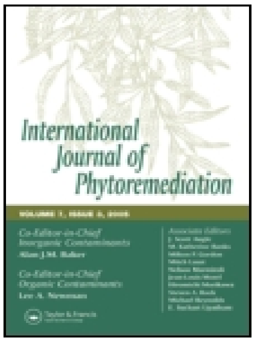


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# Effects of co-cropping *Bidens pilosa* (L.) and *Tagetes minuta* (L.) on bioaccumulation of Pb in *Lactuca sativa* (L.) growing in polluted agricultural soils

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**ABSTRACT**

Polluted agricultural soils are a serious problem for food safety, with phytoremediation being the most favorable alternative from the environmental perspective. However, this methodology is generally time-consuming and requires the cessation of agriculture. Therefore, the purpose of this study was to evaluate two potential phytoextractor plants (the native species *Bidens pilosa* and *Tagetes minuta*) co-cropped with lettuce growing on agricultural lead-polluted soils. The concentrations of Pb, as well as of other metals, were investigated in the phytoextractors, crop species, and in soils, with the potential risk to the health of consumers being estimated. The soil parameters pH, EC, organic matter percentage and bioavailable lead showed a direct relationship with the accumulation of Pb in roots. In addition, the concentration of Pb in roots of native species was closely related to Fe (*B. pilosa*, r = 0.81; *T. minuta* r = 0.75), Cu (*T. minuta*, r = 0.93), Mn (*B. pilosa*, r = 0.89) and Zn (*B. pilosa*, r = 0.91; *T. minuta*, r = 0.91). Our results indicate that the interaction between rhizospheres increased the phytoextraction of lead, which was accompanied by an increase in the biomass of the phytoextractor species. However, the consumption of lettuce still revealed a toxicological risk from Pb in all treatments.

**KEYWORDS**
co-cropping phytoremediation; food safety; *B. pilosa*; *T. minuta*; *Lactuca sativa*



## Introduction

The accumulation of toxic metals in agricultural soils and thereby crops is nowadays considered to be one of the most serious environmental problems (Rajmohan *et al.* 2014; Salazar *et al.* 2012). Regarding lead (Pb), it has been shown that this metal is a highly toxic element, even at low concentrations, leading to serious consequences in human health and ecosystems. Thus, many studies have reported Pb concentrations in crops above the European threshold of 0.2 mg/kg FW for Pb (Rodriguez *et al.* 2014; Salazar *et al.* 2012; Zhao *et al.* 2014).

The remediation of heavy metal polluted soils represents a technological challenge for both industries and governments, with various physico-chemical and biological remedial technologies having been developed (Mulligan *et al.* 2001). However, those based on engineering have the disadvantage of impairing or even destroying the biological soil functionality, besides being costly (Ghosh and Singh 2005). As a result, phytoremediation studies have been proposed as an environmentally friendly alternative and also cost-effective method compared to conventional ones (Ghosh and Singh 2005; Kidd *et al.* 2009). Pb phytoremediation is not common, with some species being mentioned such as *Thlaspi caerulescens* (Robinson *et al.* 1998), *Trifolium repens* and *Achyroctine alata* (Bech *et al.* 2016), in field and laboratory studies. However, many studies showed that non-accumulating plants such as *Brassica juncea* and *Zea mays* have been shown to hyperaccumulate lead in their shoots once that lead solubility in the soil was greatly enhanced by synthetic chelates (Komárek *et al.* 2007b). The drawback is, however, that phytoremediation technologies are usually time-consuming and require the cessation of agriculture for a number of years, which represents a non-economical alternative for agricultural producers. Recently, co-cropping systems have been applied that involve the growth of a metal hyperaccumulator plant associated with a low metal accumulating crop, in order to improve the remediation of heavy metals (Wei *et al.* 2011; Wu *et al.* 2007; Xiaomei *et al.* 2005; Xu *et al.* 2013).

Co-cropping can enhance the growth and metal uptake of the hyper-accumulating plant, by producing a synergistic effect between the species through the sharing of their rhizospheres, in contrast to mono-cropping (Gove *et al.* 2002; Wu *et al.* 2007). Furthermore, it may be possible to use hyperaccumulators to alleviate the metal uptake of conventional plants by depletion of the potentially toxic metals within shared rhizospheres, which has been given the name 'phytoprotection' (Whiting *et al.* 2001). However, rhizosphere interactions of co-cropped species still require a better understanding in order to be able to optimize phytoremediation technologies (Wenzel 2009). Therefore, the objectives of this study were (i) to assess the phytoprotection capacity of two potential phytoextractor species (*Bidens pilosa* and *Tagetes minuta*) co-cropped with edible species (*Lactuca sativa*) in lead polluted soils, and (ii) to analyze the influence of the rhizosphere interaction on the phytoextraction efficiency of accumulator plants by comparing co-cropping and mono-cropping methods.

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## Materials and methods

### Soil

Agricultural topsoils were collected from the surrounding area of a former battery recycling plant in the town of Bouwer, Córdoba Province, Argentina. Soils corresponding to three different levels of lead (pseudo-total concentrations) were chosen following a systematic sampling according to previous studies (Rodriguez *et al.* 2014; Salazar and Pignata 2014): low (~20 mg/kg; Latitude 31°34′7.05″S; Longitude 64° 11′10.59″W), medium (~ 300 mg/kg; Latitude 31° 33′29.39″S; Longitude 64°11′9.36″W) and high (~1,200 mg/kg; Latitude 31°33′33.42″S; Longitude 64°11′10.43″W).

The study area was cultivated mainly with soybean, which was subjected to zero tillage and had simple superphosphates fertilizers applied in general in proportions ranging from 50 to 100 kg/ha.

### Plant materials and experimental design

The seed collection of the native species *B. pilosa* and *T. minuta* was carried out during their mature stage (March-April) in the study area, since both plants have been previously described as being potentially suitable species for phytoremediation of lead contaminated soils (Salazar and Pignata 2014). In addition, commercial lettuce (*Lactuca sativa* L.) seeds were employed, as this vegetable has been reported to accumulate unsafe levels of lead (Bassuk 1986; Cobb *et al.* 2000; Lee *et al.* 2011).

*Bidens pilosa*, *Tagetes minuta* and *Lactuca sativa* seeds were germinated for 4 or 5 days at 25°C in sterilized sand. Seedlings of each species, at distance of 2.5 cm between plants, were grown in pots (40 cm × 20 cm × 20 cm) corresponding to the different treatments under controlled conditions (temperature range 16–35°C, relative humidity from 60 to 70%, corresponding to the summer photoperiod) in a greenhouse for a full growth period (4 months).

A split pot design was used to create conditions for mono-cropping and co-cropping in rectangular pots with 3 kg of soil. With the aim of achieving the same experimental conditions (such as soil and space availability) in both treatments, pots for mono-cropping were divided into two compartments by utilizing a nylon mesh with 1 $\mu$m of porosity to prevent root interaction or exchanges of exudates. In co-cropping pots, no mesh was used, with the species being free to interact. The experiment included three replicates for each treatment, and all species were exposed to different Pb soil concentrations (low, medium and high) and to mono-cropping or co-cropping conditions.

### Physico-chemical analyses

#### Soil

Topsoil pH and electrical conductivity (EC) were measured in a 1:5 soil:water suspension in triplicate (Bäckström *et al.* 2004). The organic matter percentage (OM%) was determined according to Peltola and Åström (2003), by combustion of the samples at 500°C for 4 hours. The ratio C/N was calculated using the autoanalyzer PE2400 Series II C, N, H, S, Perkin Elmer.

Heavy metal determinations were carried out after a sequential extraction consisting of three steps [mobile or exchangeable, mobilizable, and pseudo-residual fraction], according to Tessier *et al.* (1979), Maiz *et al.* (1997), Ketterer *et al.* (2001) and Salazar (2015).

The different fractions were analyzed for the determination of the Cu, Fe, Mn, Pb and Zn contents using total reflection X-ray fluorescence (TXRF) at the National Synchrotron Light Laboratory (LNLS) in Brazil. In this study, we calculated the "pseudo-total" fraction, which is the sum of the mobile, mobilizable and pseudo-residual fractions. For potential bioavailable metal quantification, we used the sum of the concentrations of the exchangeable and mobilizable fractions.

#### Plant tissues

After 3 months of growth, plants were harvested and separated into roots, stems and leaves. It is important to note that in the current study lettuce roots were not analyzed due to the removal of soil causing their loss as these were fragile and undeveloped. However, analysis of lettuce roots is irrelevant to the study considering that only leaves are consumed. Plants were washed, first with distilled water and then with ultrapure water, before being oven-dried at 60°C to dry weight (DW), weighed for biomass determination, ground, homogeneously mixed, and subsequently stored in the dark until analytical procedures were carried out.

The Cu, Fe, Mn, Zn and Pb contents in roots and aerial parts were analyzed using 100 mg DW of plant material, which was digested with 2 mL of concentrated nitric acid for 48 hours in a fume hood at room temperature (Ferri *et al.* 2012). After digestion, samples were centrifuged and filtered.

In this study, the Pb content ($\mu$g) extracted from the above-ground biomass produced by plants for each treatment (half pot) was calculated and is referred to from now on as "Pb extraction."

### Heavy metal determination by TXRF

The concentrations of Cu, Fe, Mn, Pb and Zn in plant and soil samples were obtained by total reflection X-ray fluorescence (TXRF) at the LNLS according to Rodriguez *et al.* (2010). The analysis utilized the known content of Ga, which was used as an internal standard. Standard solutions with known concentrations of different elements were prepared to calibrate the system. The samples were measured for 200 s, using the total reflection setup at the X-ray fluorescence beamline. For excitation, a white beam (approximately 0.3 mm wide and 2 mm high) was used. For the X-ray detection, a Ge detector was used with an energy resolution of 148 eV, at 5.9 keV, with a 0.8 mm collimator in the detector.

### Quality control

As a quality control, blanks and samples of the standard reference material "CTA-OTL-1" (oriental tobacco leaves, Institute of Nuclear Chemistry and Technology, Poland) for plants, and "BAM-U113" (soil, Federal Institute for Material Research and Technology, Germany) for soils, were prepared in the same way as described above for plants and soils, which were run after ten determinations to calibrate the instrument and to check the potential sample contamination during analysis.

These results were found to be between 87% and 93% of the certified value for CTA-OTL-1 and between 85% and 91% for BAM-U113, with the data errors being low and typically less than 15%. The coefficients of variation of the replicate analyses were calculated for different determinations, which were found to be less than 10%.

## Data analyses

### Statistical analyses

The data were subjected to ANOVA ($p < 0.05$) using the general linear model, with the ANOVA assumptions having been previously verified. Heteroscedasticity was found in some cases and was included in the model for correction. Spearman correlations and linear regressions were also carried out for the purpose of identifying relationships between soil variables and heavy metal content in plants. All analyses were performed using the software Infostat® coupled with R (Di Rienzo *et al.* 2011), version 2012.

### Translocation and bioconcentration factors

In this study, the translocation factor (TF) and the bioconcentration factor (BCF) were calculated using the ratio of the concentration of Pb in the aerial parts and roots ($TF = C_{aerial}/C_{roots}$) and the ratio of the concentration of Pb in the aerial parts and soils ($BCF = C_{aerial}/C_{soil}$), respectively (Salazar and Pignata 2014). TF values higher than one indicate that the metal was easily translocated, while values below unity show a higher accumulation in roots.

In this study a modified bioconcentration factor (BCF) was calculated, by using the ratio of the metal concentration in the aerial parts and the "potentially available" metals in the rhizospheric soils, which corresponds to the sum of the exchangeable and mobilizable fractions of the sequential extraction of metals (Komárek *et al.* 2007a; Salazar *et al.* 2012). Since the BCF indicates the ability of plants to accumulate metals (Yoon *et al.* 2006), this factor and the TF can be used to compare the ability of the different species employed in the phytoremediation programs. A BCF < 1 whereas a BCF>10 indicates hyperaccumulator species (Yoon *et al.* 2006).

### Risk assessment

The risk to human health resulting from the consumption of lettuce grown for the different treatments was calculated by employing the estimated dietary intake (EDI $\mu$g/kg/day Bw) and target hazard quotients (THQ), as described by Zheng *et al.* (2007) and US EPA (1989), respectively.

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:

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

where for the present study, C is the median concentration of a heavy metal in lettuce ($\mu$g/kg); Con is the ingestion rate of lettuce (g/person/day); EF is the exposure frequency (365 days/year); ED is the exposure duration (70 years for adults); Bw is the average body weight (70 kg for Argentine adults), and AT expresses the average exposure time for non-carcinogenic effects (ED × 365 days/year). The average daily intake of lettuce for an Argentinean adult is 55 g/person/day (Mariani *et al.* 2013).

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

$$THQ \times = \frac{EDI}{RfD}$$

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 to without any appreciable risk of deleterious effects during a lifetime. The RfD values used were 40, 700, 140 and 300 $\mu$g/kg/day for Cu, Fe, Mn and Zn, respectively (US EPA 2010). However, as the US EPA has not yet established RfD values for Pb, the one used in this paper was 4 $\mu$g/kg/day (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 US EPA (2010) bib24as follows:

$$Hi = \sum \frac{EDI}{RfD1} + \frac{EDI}{RfD2} + \ldots + \frac{EDI}{RfDi}$$

If either THQ or HI exceeds unity, a high risk of non-carcinogenic effects is implied.

## Results and discussion

### Physico-chemical characteristics of soils

Properties of the three studied soils, including pseudo-residual and bioavailable concentrations of metals, are listed in Table 1. Soils are Entic Haplustoll (USDA 2006), and presented similar pH, EC, OM%, particle size distribution, C/N ratios and concentrations of metals, except for Pb. Considering soil quality guidelines, the results revealed that only the soil with high lead concentration was above the limit for agricultural use, according to the Argentinean and Canadian legislation for contaminated soils (Argentina 1992; Canada CCME 1991). However, taking into account the Canadian criteria for the protection of environmental and human health (Canada CCME 2007), soils corresponding to medium lead content were also above the maximum permitted level for agricultural use (Table 2). In contrast, the Cu, Fe and Mn contents in soils were within the expected values for natural soils (Kabata-Pendias and Mukherjee 2007).

### Plant growth

Biomass above ground revealed significant differences among mono and co-cropped species (Figure 1). Regarding *T. minuta*, this species produced a significantly higher aerial biomass in co-cropping for most of the tested soils (low and high Pb content), being up to 2.13 fold higher compared to mono-cropping.

**Table 1.** Mean values ± SD and results of the ANOVA of physico-chemical properties of soils.

| Parameter | Soil treatment: Low | Medium | High | ANOVA |
|---|---|---|---|---|
| Distance to the smelter | 1011 m | 141 m | 31 m | |
| pH | 6.53 ± 0.07 | 6.54 ± 0.07 | 6.83 ± 0.08 | ns |
| EC ($\mu$s/cm) | 61.4 ± 3.91 | 70.77 ± 3.64 | 76.54 ± 6.43 | ns |
| OM% | 8.91 ± 0.76 | 9.5 ± 1.3 | 8.08 ± 0.16 | ns |
| C:N (%) | 9.70 ± 0.08 | 10.11 ± 0.09 | 9.30 ± 0.10 | ns |
| Particle size distribution (%) | | | | |
| Sand (2–0.05 mm) | 16.04 ± 0.35 | 16.88 ± 1.23 | 15.91 ± 0.93 | |
| Silt (0.05–0.002 mm) | 82.01 ± 1.13 | 82.46 ± 1.48 | 78.02 ± 0.14 | |
| Clay (<0.002 mm) | 1.96 ± 0.78 | 0.66 ± 0.25 | 6.08 ± 0.78 | |
| $Pb_{bioav}$ (mg/kg) | 2.81 ± 0.22c | 124.41 ± 30.4b | 464.06 ± 34.65a | *** |
| $Pb_{Pst}$ (mg/kg) | 24.72 ± 3.73c | 295.5 ± 35.5b | 1222.63 ± 255.59a | ** |
| $Mn_{bioav}$ (mg/kg) | 64.03 ± 6.50 | 120.69 ± 14.26 | 92.87 ± 16.84 | ns |
| $Mn_{Pst}$ (mg/kg) | 392 ± 47.04 | 516.21 ± 14.01 | 389.88 ± 44.27 | ns |
| $Fe_{bioav}$ (mg/kg) | 112.53 ± 12.76 | 137.69 ± 18.63 | 148.78 ± 22.59 | ns |
| $Fe_{Pst}$ (mg/kg) | 12495 ± 4721 | 9276 ± 433.0 | 13708 ± 5960 | ns |
| $Cu_{bioav}$ (mg/kg) | 6.17 ± 0.48a | 4.22 ± 0.18b | 6.67 ± 0.435a | * |
| $Cu_{Pst}$ (mg/kg) | 8.6 ± 1.35 | 9.95 ± 0.19 | 7.61 ± 0.41 | ns |
| $Zn_{bioav}$ (mg/kg) | 12.43 ± 0.46a | 10.81 ± 0.33b | 14.39 ± 0.72a | * |
| $Zn_{Pst}$ (mg/kg) | 24.51 ± 5.31 | 28.56 ± 1.05 | 29.66 ± 6.81 | ns |

Abbreviations: Bioav = bioavailable metal concentration; Pst = pseudo-total metal concentration.
Values in each row (ANOVA) followed by the same letter do not differ significantly at $p < 0.05$. (ns, not significant. * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$.

In contrast, *B. pilosa* showed a higher biomass in co-cropping only for the soil with a medium Pb level. Moreover, the native species studied revealed a capacity for adaptation to polluted soils, since growth was not markedly affected by the increase of the Pb level in soils. Similarly, Jiang *et al.* (2010) and Whiting *et al.* (2001) found better growth of *Thlaspi caerulescens* when co-cropped with ryegrass and *Thlaspi arvense,* respectively. However, in the present study, lettuce growth was affected not only by increasing Pb concentrations in soils, but also by the co-cropping conditions (especially with *B. pilosa*) for every Pb treatment. This effect could have been due to strong competition for nutrients or growth inhibition action by *B. pilosa*. Thus, for lettuce, there were no benefits obtained by co-cropping.

### *Lead content in plants*

Table 3 shows Pb accumulation by organ under different planting and soil treatments. For the native species, the TF and BCF values decreased significantly in contaminated soils (Figure 2). Since *B. pilosa* showed similar Pb concentrations (Table 3) and TF and BCF values (Figure 2) in both cropping systems, the rhizosphere interaction did not appear to affect Pb accumulation in this species. In contrast, Pb accumulation and TF and BCF in *T. minuta* were significantly increased in co-cropping (Figure 2). Similar results were reported by Gove *et al.* (2002) with greater accumulation of Cd, Zn and Pb for *T. caerulescens* co-cropped with other species, and higher BCF and Cd accumulation in Japanese clover co-cropped with tobacco by Liu *et al.* (2011).

It is important to note that despite the Pb concentration for *B. pilosa* in co-cropping not being higher than in mono-cropping, the Pb extraction due to biomass production was higher in co-cropping for soils with low and medium Pb content (Figure 3). Thus, as the Pb extraction by biomass production was enhanced for *T. minuta* in co-cropping for medium and high lead content in soils (Figure 3A), our results indicate that plant coexistence tends to improve Pb extraction from soils, especially in the case of *T. minuta*. Regarding this, Whiting *et al.* (2001) also reported higher Zn uptake in *T. caerulescens* on considering its biomass production in co-cropping conditions.

For lettuce, the highest concentrations of Pb were found in soils with high and medium lead content (Table 3), and contrary to our predictions, co-cropping did not alleviate the Pb concentration in lettuce. Furthermore, lettuce significantly increased even Pb extraction for medium Pb content in soils when co-cropped with *T. minuta,* indicating that lettuce has an important capacity to extract Pb that can be enhanced by co-cropping. However, for high Pb content in soils, the Pb extraction was lower due to the reduced biomass production (Figure 3B).

It is important to note that even at low Pb content in soil, lettuce accumulated Pb at concentrations that exceeded the tolerance limit for human consumption, 2 mg/kg (Codex Alimentarius Commission 2001). Related to this, other studies have consistently reported that leafy vegetables tend to accumulate elevated amounts of heavy metals in their edible parts, lettuce being reported as an indicator for heavy metals as it

**Table 2.** Soil quality guidelines for total metal concentrations (mg/kg DW).

| | Land use and soil guidelines | | | | | |
|---|---|---|---|---|---|---|
| | Agricultural | | Residential | | Industrial | |
| Metal | 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 |
| Zn | 600 | 200 | 500 | 200 | 1500 | 360 |

[a]Argentinean legislation (Law 24051).
[b]CCME (Canadian Council of Ministers of the Environment) (1991) Soil Quality Guidelines.
[c]CCME (2007) Soil Quality Guidelines for Environmental Health.

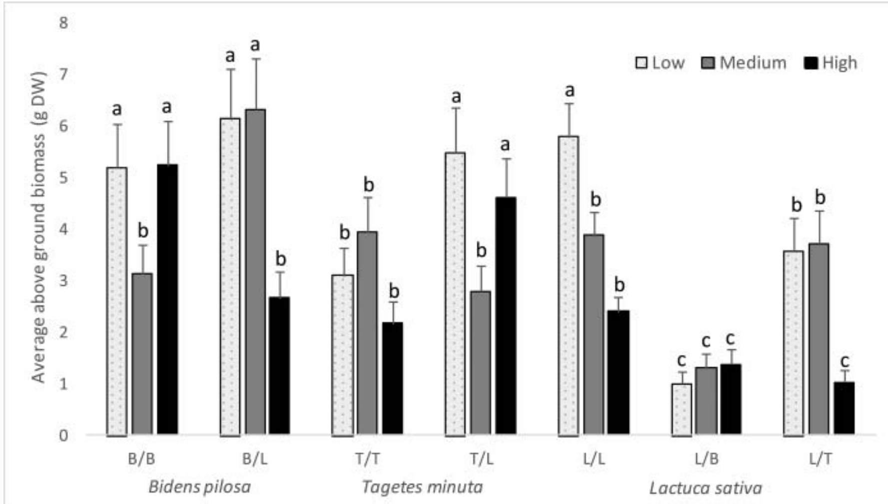
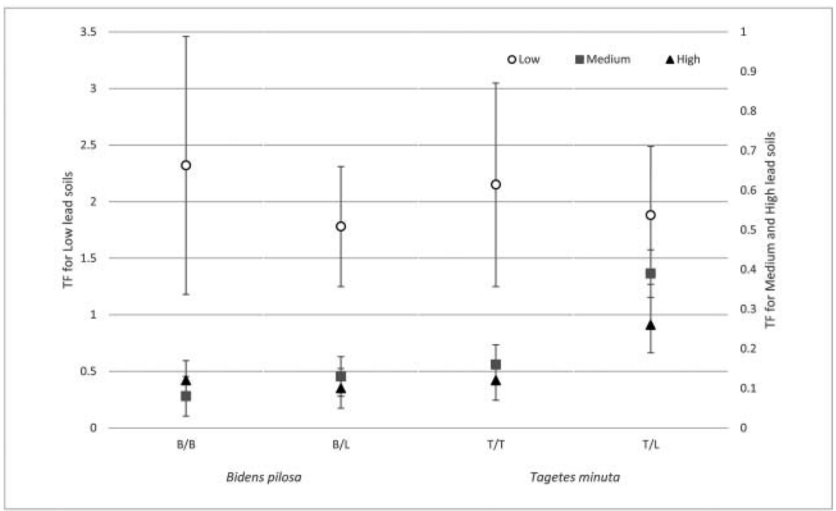



**Figure 1.** Comparison among species above ground biomass (g DW) in mono- and co-crop. Note: $B/_B$: *B. pilosa* mono-crop; $B/_L$: *B. pilosa* co-crop with *L. sativa*; $L/_L$: *L. sativa* mono-crop; $L/_B$: *L. sativa* co-crop with *B. pilosa*; $L/_T$: *L. sativa* co-crop with *T. minuta*; $T/_T$: *T. minuta* mono-crop; $T/_L$: *T. minuta* co-crop with *L. sativa.*



**A**




**B**

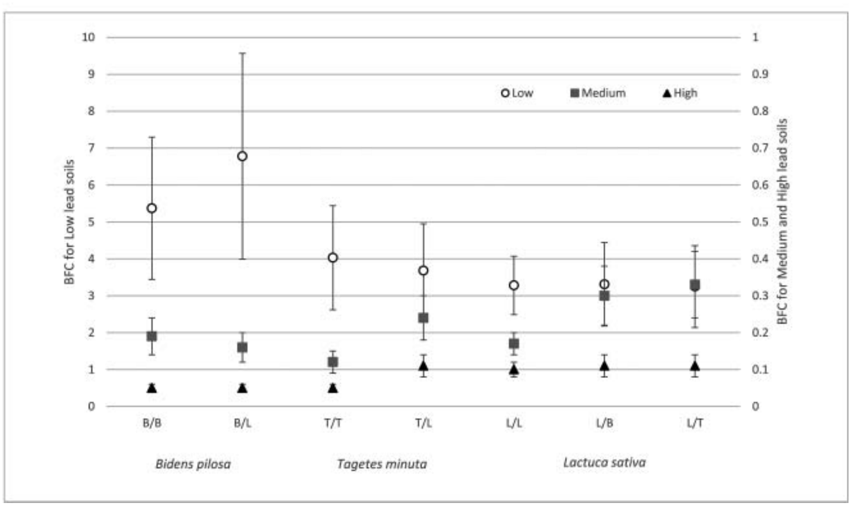



**Figure 2.** Translocation factor (TF) of native species (A), and bioconcentration factor (BCF) of native species and lettuce (B) in mono- and co-crop. Note: $B/_B$: *B. pilosa* mono-crop; $B/_L$: *B. pilosa* co-crop with *L. sativa*; $L/_L$: *L. sativa* mono-crop; $L/_B$: *L. sativa* co-crop with *B. pilosa*; $L/_T$: *L. sativa* co-crop with *T. minuta*; $T/_T$: *T. minuta* mono-crop; $T/_L$: *T. minuta* co-crop with *L. sativa*.

**Table 3.** Mean values ± S.E. and ANOVA results for Pb concentrations ($\mu$g/g DW) of roots and aerial parts of the studied species.

| Pb in soils | Crop | Root*** | Aerial*** |
|---|---|---|---|
| High | $B/_B$ | 204.44 ± 4.09 a | 24.45 ± 6.54 b |
| | $B/_L$ | 313.97 ± 173.92 a | 21.15 ± 5.91 b |
| | $T/_T$ | 226.98 ± 83.98 a | 23.39 ± 6.34 b |
| | $T/_L$ | 205.37 ± 13.62 a | 52.29 ± 11.15 a |
| | $L/_L$ | — | 47.08 ± 6.55 a |
| | $L/_B$ | — | 55.23 ± 11.29 a |
| | $L/_T$ | — | 49.06 ± 10.67 a |
| Medium | $B/_B$ | 298.13 ± 34.11 a | 25.58 ± 6.38 b |
| | $B/_L$ | 151.64 ± 4.96 b | 20.35 ± 5.75 b |
| | $T/_T$ | 92.34 ± 4.13 b | 14.46 ± 4.52 b |
| | $T/_L$ | 90.53 ± 31.53 b | 30.31 ± 7.61 a |
| | $L/_L$ | — | 14.69 ± 3.37 b |
| | $L/_B$ | — | 37.49 ± 8.83 a |
| | $L/_T$ | — | 41.14 ± 4.52 a |
| Low | $B/_B$ | 6.59 ± 1.56 c | 15.08 ± 4.66 b |
| | $B/_L$ | 10.81 ± 1.01 c | 19.06 ± 5.49 b |
| | $T/_T$ | 4.76 ± 1.00 c | 11.33 ± 3.81 b |
| | $T/_L$ | 5.86 ± 1.04 c | 10.34 ± 3.57 b |
| | $L/_L$ | — | 9.20 ± 2.33 b |
| | $L/_B$ | — | 9.30 ± 3.32 b |
| | $L/_T$ | — | 9.14 ± 3.28 b |

Abbreviations: $B/_B$ = *B. pilosa* mono-crop; $B/_L$ = *B. pilosa* co-crop with *L. sativa*; $L/_L$ = *L. sativa* mono-crop; $L/_B$ = *L. sativa* co-crop with *B. pilosa*; $L/_T$ = *L. sativa* co-crop with *T. minuta*; $T/_T$ = *T. minuta* mono-crop; $T/_L$ = *T. minuta* co-crop with *L. sativa*.

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

can easily accumulate high metal concentrations (Wang *et al.* 2009). Malandrino *et al.* (2011) reported a Pb concentration in lettuce close to 130 mg/kg when grown in soils with 686 mg/kg of Pb.

In the current study, neither *B. pilosa* nor *T. minuta* was able to provide phytoprotection to lettuce, as Pb concentrations were not reduced and the biomass was not improved in contaminated soils used for growing lettuce. In this regard, Lee *et al.* (2009) evaluated different amendments to threat contaminated soils, but conditions for the safe consumption of lettuce were not achieved. Thus, diminishing metal uptake in lettuce is a challenging task.

Our results show that co-cropping may modify the availability of metals in shared rhizospheres. In this regard, Whiting *et al.* (2001) reported that the co-crop between *T. caerulescens* and *T. arvense* ameliorated Zn uptake by the latter species, whereas a different behavior was found for Cd during co-crop of barley and *T. caerulescens*, with an increase being observed in both species (Gove *et al.* 2002). In another investigation, Fuksová *et al.* (2009) reported a decrease of As, Cd, and Pb concentrations in *Thlaspi caerulescens* shoots in co-crop with *Salix dasyclados*. Therefore, the accumulation of metals depends on which co-cropped species are used that can either confer phytoprotection or phytoextraction.

In the present study, as neither of the native species showed concentrations > 1000 mg/kg in shoots, or TF and BCF values > 1 in contaminated soils, they do not fit the classic criteria for hyperaccumulators (Baker *et al.* 2000). It is worth noting that Pb is poorly accumulated in aboveground tissues, so few species are able to meet the criteria for hyperaccumulators (Dahmani-Muller *et al.* 2000; Vamerali *et al.* 2010; Yoon *et al.* 2006). In fact, although *Brassica juncea* is widely recognized as Pb hyperaccumulator species, it only exceeds *B. pilosa* and *T. minuta* efficiency when soils are treated with the metal chelator EDTA, which is considered to be environmentally harmful (Blaylock *et al.* 1997; Clemente *et al.* 2005; Marchiol *et al.* 2004).

## Lead uptake related to other metals and physico-chemical parameters of soil

Table 4 shows regressions among Pb and other metal concentrations in different organs, and with soil physicochemical parameters. In roots, *B. pilosa* presented multiple positive relations among Pb and other metals in co-cropped experiments: Zn ($r = 0.91$ $p < 0.05$); Fe ($r = 0.81$ $p < 0.05$); Mn ($r = 0.89$ $p < 0.05$). In contrast, Pb concentration in roots for mono-cropped *B. pilosa* depended only on Fe ($r = 0.86$ $p < 0.05$). Regarding *T. minuta*, regression coefficients among Pb and other metals in co-cropped roots were: Zn ($r = 0.91$ $p < 0.05$); Fe ($r = 0.75$ $p = 0.08$); Cu ($r = 0.93$ $p < 0.05$). The mono-cropped roots of *T. minuta* behaved similar to *B. pilosa* roots for the same growing condition, revealing a common regression model for Pb and Fe, and lower regression coefficients among Pb and other metals: Fe ($r = 0.95$ $p < 0.01$); Mn ($r = 0.93$ $p < 0.05$). Moreover, both species had prevailing metal correlations for the two planting conditions: Fe-Mn ($r = 0.7$–$0.98$) and Cu-Zn ($r = 0.6$–$0.95$). Thus, our results suggest that root interactions promote metal mobilization, which led to the uptake of various metals together with Pb in these species, with competition for nutrients and combined root exudates, being related to these observations. In an experiment performed with Indian mustard, Kim *et al.* (2010) found that increased Pb concentrations resulted in the release of more Zn. Moreover, Yoon *et al.* (2006) reported correlations of BCFs (metal in roots/metal in soil) on multiple plants between Pb-Cu and Pb-Zn, indicating a common transport into the plant for these metals.

In stems, the correlation between Pb and other metals was weaker in comparison to roots. For *T. minuta,* positive correlations and regressions were found between Pb and Cu ($r = 0.71$ $p < 0.05$) in the co-crop, being also correlated in the mono-crop with Cu ($r = 0.80$ $p < 0.05$) and Zn ($r = 0.82$ $p < 0.05$) whereas a negative regression coefficient was found between Pb and Fe.

In relation to *L. sativa* in co-crop, only a significant regression was shown among Pb and other metals when grown with *T. minuta*.

Our results showed, in most of the treatments, the following metal correlations: Zn-Cu, Fe-Zn and Fe-Mn. In this respect, it is known that Fe and Mn are interrelated in their metabolic functions in plants (Kabata-Pendias and Mukherjee 2007; Somers and Shive 1942). However, in the present study, with respect to leaves, metal contents were poorly related to Pb in comparison to the other organs, with a positive relation found between Pb with Zn, but a negative relation with Cu for *T. minuta* in co-cropping. In the case of *L. sativa*, a relationship between Pb and Fe in mono-cropped individuals was observed.

Our results collectively indicate that Pb uptake in relation to other metals was lower in leaves and stems, compared to roots. In agreement, Yoon *et al.* (2006) reported correlations among Pb BCFs with Cu and Zn, but no relationships bebib12tween

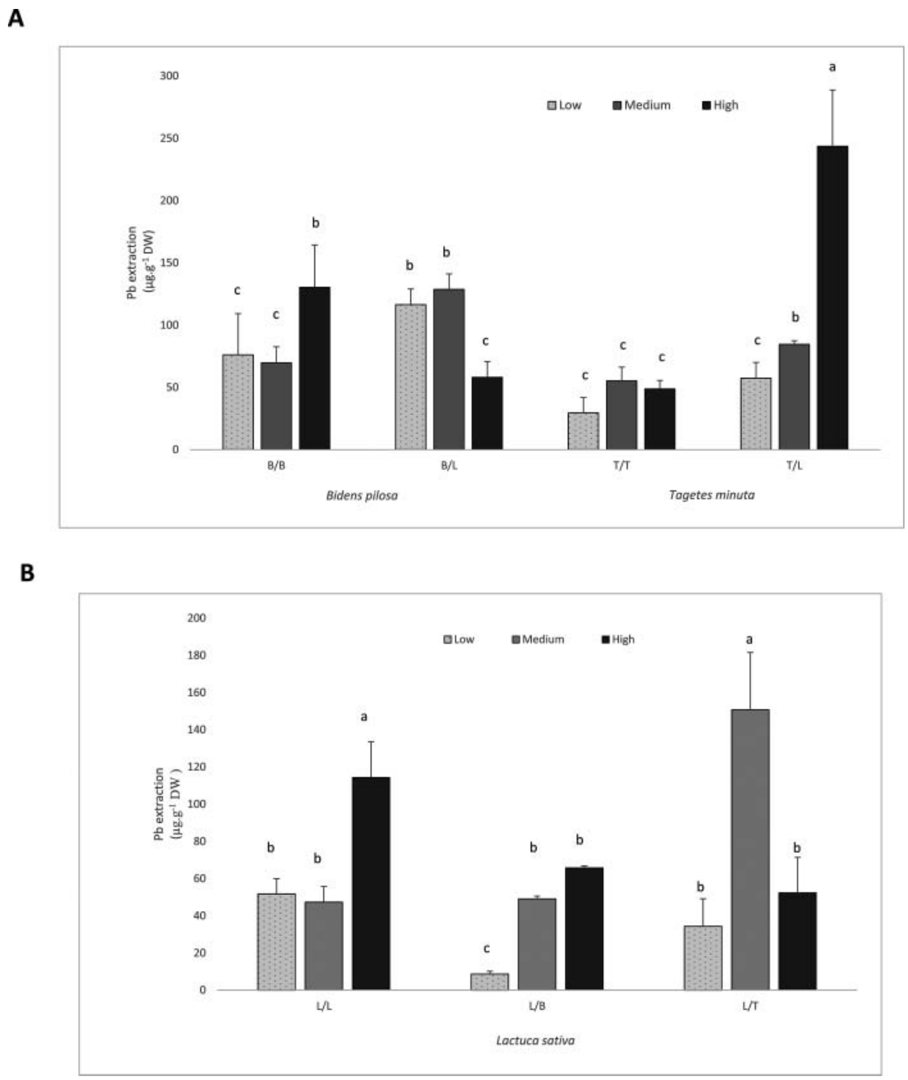



**Figure 3.** Pb extraction by aerial biomass production ($\mu$g/g DW) of native species (A) and lettuce (B) in mono- and co-crop. Note: $B/_B$: *B. pilosa* mono-crop; $B/_L$: *B. pilosa* co-crop with *L. sativa*; $L/_L$: *L. sativa* mono-crop; $L/_B$: *L. sativa* co-crop with *B. pilosa*; $L/_T$: *L. sativa* co-crop with *T. minuta*; $T/_T$: *T. minuta* mono-crop; $T/_L$: *T. minuta* co-crop with *L. sativa*.



**Table 4.** Significant regression models for Pb concentration (mg/kg DW) as a function of other metals and soil physicochemical characteristics.

| Parameters | | | | | |
|---|---|---|---|---|---|
| Y | X | Species and treatment | *f(x)* | $R^2$ | p |
| $Pb_{ROOT}$ | $Zn_{ROOT}$ | *B. pilosa, $B/_L$* | $Y = -0.14 + 5.48\ Zn_{ROOT}$ | 0.78 | 0.010 |
| $Pb_{ROOT}$ | $Mn_{ROOT}$ | *B. pilosa, $B/_L$* | $Y = 15.10 + 1.29\ Mn_{ROOT}$ | 0.74 | 0.010 |
| $Pb_{ROOT}$ | $Fe_{ROOT}$ | *B. pilosa, $B/_B$* | $Y = 41.89 + 0.11\ Fe_{ROOT}$ | 0.7 | 0.020 |
| $Pb_{ROOT}$ | $Mn_{ROOT}$; $Cu_{ROOT}$ | *T. minuta, $T/_L$* | $Y = -63.81 + 5.53\ Zn_{ROOT} + 1.16\ Mn_{ROOT}$ | 0.94 | 0.006 |
| $Pb_{ROOT}$ | $Fe_{ROOT;}$ $Zn_{ROOT}$ | *T. minuta, $T/_L$* | $Y = -81.24 + 0.17\ Fe_{ROOT} + 5.20\ Zn_{ROOT}$ | 0.94 | 0.007 |
| $Pb_{ROOT}$ | $Zn_{ROOT}$; $Mn_{ROOT}$ | *T. minuta, $T/_L$* | $Y = -63.81 + 5.53\ Zn_{ROOT} + 1.16\ Mn_{ROOT}$ | 0.94 | 0.006 |
| $Pb_{ROOT}$ | $Fe_{ROOT}$ | *T. minuta, $T/_T$* | $Y = 29.66 + 0.14\ Fe_{ROOT}$ | 0.88 | 0.003 |
| $Pb_{ROOT}$ | $Mn_{ROOT}$ | *T. minuta, $T/_T$* | $Y = -8.52 + 3.08\ Mn_{ROOT}$ | 0.83 | 0.007 |
| $Pb_{STEM}$ | $Fe_{STEM}$; $Cu_{STEM}$ | *T. minuta, $T/_L$* | $Y = 53.65–0.90\ Fe_{STEM} + 4.39\ Cu_{STEM}$ | 0.96 | 0.003 |
| $Pb_{STEM}$ | $Zn_{STEM}$; $Fe_{STEM}$ | *T. minuta, $T/_L$* | $Y = 63.44 + 1.55\ Zn_{STEM}$ - $0.96\ Fe_{STEM}$ | 0.94 | 0.007 |
| $Pb_{STEM}$ | $Zn_{STEM}$ | *T. minuta, $T/_T$* | $Y = -2.57 + 0.92\ Zn_{STEM}$ | 0.72 | 0.04 |
| $Pb_{STEM}$ | $Mn_{STEM}$; $Zn_{STEM}$ | *L. sativa, $L/_T$* | $Y = -30.59 + 1.94\ Mn_{STEM} + 1.39\ Zn_{STEM}$ | 0.086 | 0.02 |
| $Pb_{LEAF}$ | $Cu_{LEAF}$; $Zn_{LEAF}$ | *T. minuta, $T/_L$* | $Y = 34.90–3.11\ Cu_{LEAF} + 0.72\ Zn_{LEAF}$ | 0.88 | 0.01 |
| $Pb_{LEAF}$ | $Fe_{LEAF}$ | *L. sativa, $L/_L$* | $Y = -2.62 + 0.13\ Fe_{LEAF}$ | 0.54 | 0.002 |
| $Pb_{ROOT}$ | $Pb_{BA}$; %OM; pH | *B. pilosa, $B/_L$* | $Y = 184 + 0.55\ Pb_{BA} + 337.84\%MO$ - 246.96 pH | 0.95 | 0.02 |
| $Pb_{ROOT}$ | $Pb_{BA}$; pH | *B. pilosa, $B/_B$* | $Y = 6248.68–935.80\ pH + 0.66\ Pb_{BA}$ | 0.8 | 0.04 |
| $Pb_{ROOT}$ | $Pb_{BA}$; %OM; EC | *T. minuta, $T/_L$* | $Y = 1515.52 + 0.75\ EC$ - $237.85\%MO + 0.67\ Pb_{BA}$ | 0.99 | 0.007 |
| $Pb_{ROOT}$ | $Pb_{BA}$ | *T. minuta, $T/_T$* | $Y = 16.70 + 0.46\ Pb_{BA}$ | 0.71 | 0.02 |

Abbreviations: $B/_B$ = *B. pilosa* mono-crop; $B/_L$ = *B. pilosa* co-crop with *L. sativa*; $L/_L$ = *L. sativa* mono-crop; $L/_B$ = *L. sativa* co-crop with *B. pilosa*; $L/_T$ = *L. sativa* co-crop with *T. minuta*; $T/_T$ = *T. minuta* mono-crop; $T/_L$ = *T. minuta* co-crop with *L. sativa*; $Pb_{BA}$ = Pb bioavailable.

Pb and Cu or Pb and Zn for TFs. This could indicate that metals tend to entry simultaneously in roots, but the proportions and relations among metal concentrations are modified during translocation. Furthermore, other metal uptakes (Cu, Fe, Mn and Zn) were also evaluated, which revealed differing results on the species and planting conditions (supplementary material Table S1 and S2).

Regarding the Pb uptake by roots with respect to Pb bioavailability and physico-chemical parameters in soils, both species showed a direct association between bioavailable Pb in soils and its content in roots (Table 4). For *B. pilosa*, we found that lower pH values were related to higher Pb concentrations in roots for all cropping conditions. *T. minuta* in co-cropping, on the other hand, presented a positive association with EC and a negative one with OM% for Pb uptake. These results were consistent with other reports, which mentioned that lead solubility could be affected by biotic and abiotic factors, such as organic matter, pH, sorption on clays and oxides and precipitation such as carbonates, hydroxides, and phosphates (Kabata-Pendias and Mukherjee 2007).

### *Toxicological risk associated with lettuce consumption*

Significant THQ values (>1) for Pb and Mn are shown in Figure 4A, with THQ values for Pb being higher than unity in all cases, even for plants cultivated in soils with low Pb concentration. Thus, co-cropping lettuce with the other species did not alleviate Pb uptake by this crop, as mentioned above. In addition, despite soils not presenting high amounts of Mn, the THQ values for this element were also above one for soils with medium and high Pb concentration. Concentrations of Mn fluctuate greatly within species and organ parts (range 20–500 $\mu$g/g) (Kabata-Pendias and Mukherjee 2007), and it is important to note that Mn is an essential micronutrient related

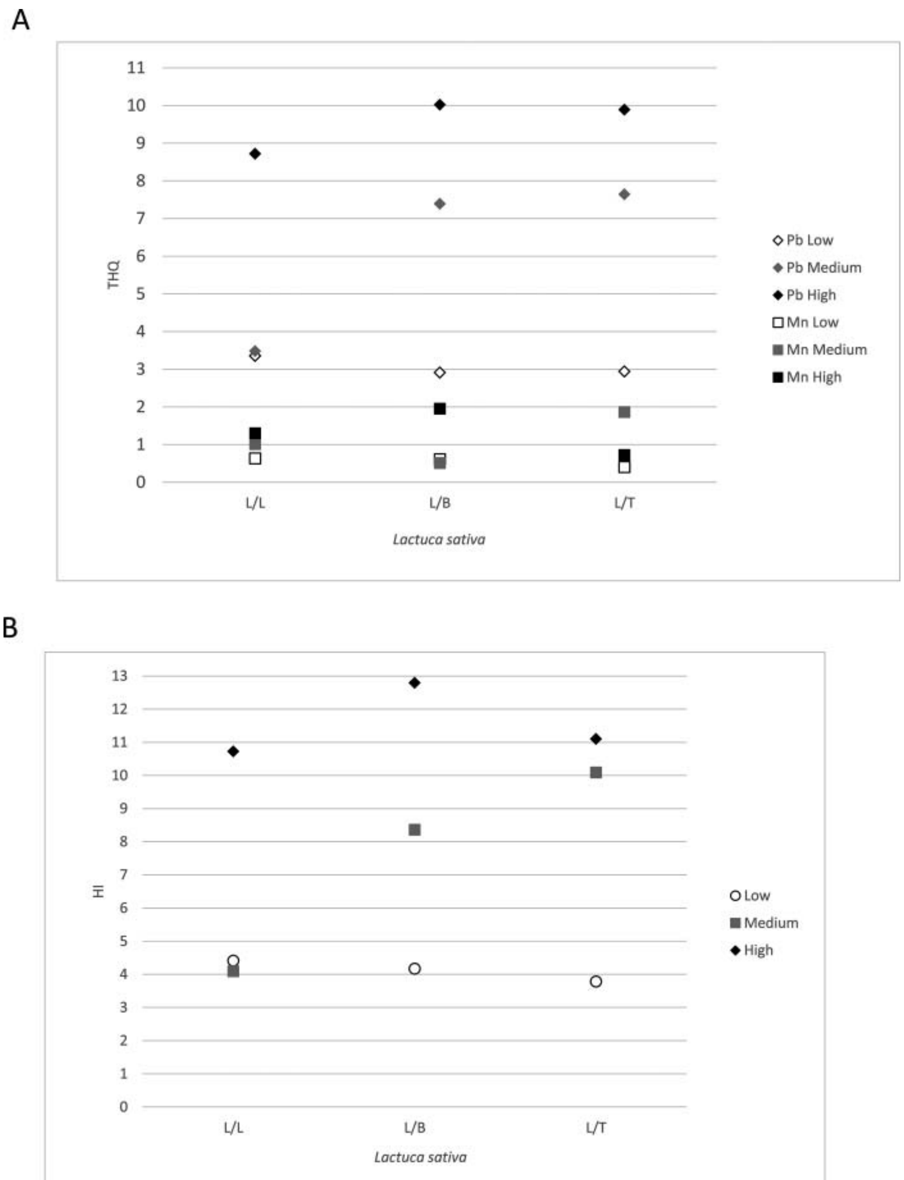



Figure 4. Target hazard quotients (THQ) (A), and hazard index (HI) (B) for metals in lettuce consumption in mono- and co-crop in soils with different lead content. Note: (A) Diamonds: Pb; square: Mn; (B) HI for Pb, Mn, Fe, Cu and Zn. Cropping system: $B/_B$: *B. pilosa* mono-crop; $B/_L$: *B. pilosa* co-crop with *L. sativa*; $L/_L$: *L. sativa* mono-crop; $L/_B$: *L. sativa* co-crop with *B. pilosa*; $L/_T$: *L. sativa* co-crop with *T. minuta*; $T/_T$: *T. minuta* mono-crop; $T/_L$: *T. minuta* co-crop with *L. sativa*.

to the oxidation-reduction processes, including the response to biotic and abiotic stress in plants (Kabata-Pendias and Mukherjee 2007; Mascher *et al.* 2002). Therefore, in this study the higher uptake of Mn in lettuce leaves with THQ > 1 may have been related to a detoxification process in Pb contaminated soils. In contrast, the THQ values for Zn, Cu and Fe were low, indicating no risk for health (data not shown).

Finally, the HI calculations for aggregate non-cancer risks also revealed values greater than unity due to the contributions of Pb and Mn (Figure 4B), indicating a health risk related to general heavy metal incorporation into crops in the studied soils.

## Conclusions

Our findings revealed that lead incorporation in plants was related to other metals, with uptake and transport between metals depending on their relative concentrations and interactions of rhizospheres. Moreover, in order to explain these relationships between metals further research should be performed considering the metabolic processes.

In this study, the physico-chemical parameters in soils were associated with the bioavailability of lead in soils, and consequently, with the accumulation in plants.

Our findings showed that co-cropping native species with lettuce did not alleviate the heavy metal content, being necessary to perform further studies in order to achieve safe cultivation of this vegetable. Although the native species studied did not behave as hyperaccumulators, they could be employed for phytostabilization with resulting slow metal removal in their aboveground tissues since they are tolerant to Pb contaminated soils. Related to this, as it has been indicated in other co-cropping studies, *T. minuta* developed a high biomass in co-cropping, which resulted in higher translocation, bioconcentration and lead extraction per plant. Hence, future work might reveal a co-cropping combination that gives phytoprotection for a particulate crop.

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## References

Argentina. 1992. Argentinean legislation, National Law 24051, Regimen de desechos peligrosos. Decreto Nacional reglamentario 831/93. Anexo II, Tabla 9 Secretaria de Ambiente y Desarrollo Sustentable, Poder Ejecutivo Nacional.

Bäckström M, Karlsson S, Allard B. 2004. Metal leachability and anthropogenic signal in roadside soils estimated from sequential extraction and stable lead isotopes. Environ Monit Assess 90(1–3):135–160.

Baker AJM, McGrath SP, Reeves RD, Smith JAC. 2000. Metal hyperaccumulator plants: A review of ecology and physiology of a biological resource for phytoremediation of metal polluted soils. In: Terry N, Banuelos G, editors. Phytoremediation of Contaminated Soil and Water. Boca Raton(FL): Lewis Publishers. p 129–158.

Bassuk NL. 1986. Reducing lead uptake in lettuce. Hortic Sci 21(4):993–995.

Bech J, Roca N, Tume P, Ramos-Miras J, Gil C, Boluda R. 2016. Screening for new accumulator plants in potential hazards elements polluted soil surrounding Peruvian mine tailings. Catena 136: 66–73.

Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD, Raskin I. 1997. Enhanced accumulation of pb in indian mustard by soil-applied chelating agents. Envir Sci Tech 31 (3):860–865.

Canada CCME. 1991. Canadian Soil Quality Guidelines. Winnipeg: Canadian Environmental Quality Guidelines, CCME.

Canada CCME. 2007. Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health Canadian. Winnipeg: Environmental Quality Guidelines, CCME.

Clemente R, Walker DJ, Bernal MP. 2005. Uptake of heavy metals and As by *Brassica juncea* grown in a contaminated soil in Aznalcóllar (Spain): The effect of soil amendments. Environ Pollut 138(1):46–58.

Cobb GP, Sands K, Waters M, Wixson BG, Dorward-King E. 2000. Accumulation of heavy metals by vegetables grown in mine wastes. Environ Toxicol Chem 19(3):600–607.

Codex Alimentarius Commission. 2001. Report of the 33rd session of the Codex Committee on food additives and contaminants. The Netherlands, Hague. pp. 285.

Dahmani-Muller H, van Oort F, Gélie B, Balabane M. 2000. Strategies of heavy metal uptake by three plant species growing near a metal smelter. Environ Pollut 109(2):231–238.

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. 2011. InfoStat. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Ferri R, Donna F, Smith DR, Guazzetti F, Zacco A, Rizzo L, Bontempi E, Zimmerman NJ, Lucchinni RG. 2012. Heavy metals in soil and salad in the proximity of historical ferroalloy emission. J Envir Protect (3):374–385.

Fuksová Z, Száková J, Tlustoš P. 2009. Effects of co-cropping on bioaccumulation of trace elements in *Thlaspi caerulescens* and *Salix dasyclados*. Plant Soil Environ 55(11):461–467.

Ghosh M, Singh SP. 2005. A review on phytoremediation of heavy metals and utilization of Its byproducts. Appl Ecol Env Res 3(1):18.

Gove B, Hutchinson JJ, Young SD, Craigon J, McGrath SP. 2002. Uptake of metals by plants sharing a rhizosphere with the hyperaccumulator *Thlaspi caerulescens*. Int J Phytoremediat 4(4):267–281.

Huang M, Zhou S, Sun B, Zhao Q. 2008. Heavy metals in wheat grain: Assessment of potential health risk for inhabitants in Kunshan, China. Sci Total Environ 405(1–3):54–61.

Jiang C-A, Wu Q-T, Sterckeman T, Schwartz C, Sirguey C, Ouvrard S, Perriguey J, Morel J-L. 2010. Co-planting can phytoextract similar amounts of cadmium and zinc to mono-cropping from contaminated soils. Ecol Eng 36(4):391–395.

Kabata-Pendias A, Mukherjee AB. 2007. Trace Elements from Soil to Human. Berlin Heidelberg: Springer-Verlag. 550 p.

Ketterer ME, Lowry JH, Simon Jr J, Humphries K, Novotnak MP. 2001. Lead isotopic and chalcophile element compositions in the environment near a zinc smelting–secondary zinc recovery facility, Palmerton, Pennsylvania, USA. Appl Geochem 16(2):207–229.

Kidd P, Barcelo J, Bernal MP, Navari-Izzo P, Poschenrieder C, Shilev S, Clemente R, Monterroso C. 2009. Trace element behavior at the root-soil interface. Implications in phytoremediation. Environ Exp Bot 67: 243–259.

Kim K-R, Owens G, Kwon S-L. 2010. Influence of Indian mustard (*Brassica juncea*) on rhizosphere soil solution chemistry in

long-term contaminated soils: A rhizobox study. J Environ Sci 22(1):98–105.
Komárek M, Chrastný V, Štíchová J. 2007a. Metal/metalloid contamination and isotopic composition of lead in edible mushrooms and forest soils originating from a smelting area. Environ Int 33(5):677–684.
Komárek M, Tlustos P, Száková J, Chrastný V, Ettler V. 2007b. The use of maize and poplar in chelant-enhanced phytoextraction of lead from contaminated agricultural soils. Chemosphere 67:640–651.
Lee S-H, Lee J-S, Jeong Choi Y, Kim J-G. 2009. In situ stabilization of cadmium-, lead-, and zinc-contaminated soil using various amendments. Chemosphere 77(8):1069–1075.
Lee S-H, Park H, Koo N, Hyun S, Hwang A. 2011. Evaluation of the effectiveness of various amendments on trace metals stabilization by chemical and biological methods. J Hazard Mater 188(1–3):44–51.
Liu L, Li Y, Tang J, Hu L, Chen X. 2011. Plant coexistence can enhance phytoextraction of cadmium by tobacco (*Nicotiana tabacum* L.) in contaminated soil. J Environ Sci 23(3):453–460.
Maiz I, Esnaola MV, Millán E. 1997. Evaluation of heavy metal availability in contaminated soils by a short sequential extraction procedure. Sci Total Environ 206(2–3):107–115.
Malandrino M, Abollino O, Buoso S, Giacomino A, La Gioia C, Mentasti E. 2011. Accumulation of heavy metals from contaminated soil to plants and evaluation of soil remediation by vermiculite. Chemosphere 82(2):169–178.
Marchiol L, Sacco P, Assolari S, Zerbi G. 2004. Reclamation of polluted soil: Phytoremediation potential of crop-related BRASSICA species. Water Air Soil Poll 158(1):345–356.
Mariani S, Podversich R, Grosso JL, Leon C. 2013. Ensayo de lechuga Gallega INTA bajo condiciones agroecológicas. INTA.
Mascher R, Lippmann B, Holzinger S, Bergmann H. 2002. Arsenate toxicity: effects on oxidative stress response molecules and enzymes in red clover plants. Plant Sci 163(5):961–969.
Mulligan CN, Yong RN, Gibbs BF. 2001. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Eng Geol 60 (1–4):193–207.
Peltola P, Åström M. 2003. Urban geochemistry: A multimedia and multielement survey of a small town in Northern Europe. Environ Geochem Hlth 25(4):397–419.
Rajmohan N, Prathapar SA, Jayaprakash M, Nagarajan R. 2014. Vertical distribution of heavy metals in soil profile in a seasonally waterlogging agriculture field in Eastern Ganges Basin. Environ Monit Assess 186 (9):5411–5427.
Robinson BH, Leblanc M, Petit D, Brooks RR, Kirkman JH, Gregg PEH. 1998. The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. Plant Soil 203: 47–56.
Rodriguez JH, Pignata ML, Fangmeier A, Klumpp A. 2010. Accumulation of polycyclic aromatic hydrocarbons and trace elements in the bioindicator plants *Tillandsia capillaris* and *Lolium multiflorum* exposed at PM10 monitoring stations in Stuttgart (Germany). Chemosphere 80:208–215.
Rodriguez JH, Salazar MJ, Steffan L, Pignata ML, Franzaring J, Klumpp A, Fangmeier A. 2014. Assessment of Pb and Zn contents in agricultural soils and soybean crops near to a former battery recycling plant in Córdoba, Argentina. J Geochem Explor 145(0):129–134.
Salazar MJ. 2015. Fitorremediación de suelos contaminados con metales pesados. Evaluación de especies nativas en la Provincia de Córdoba. Facultad de Ciencias Exactas, Físicas y Naturales. PhD Thesis. Universidad Nacional de Córdoba, Argetnina. p. 285.
Salazar MJ, Pignata ML. 2014. Lead accumulation in plants grown in polluted soils. Screening of native species for phytoremediation. J Geochem Explor 137(0):29–36.
Salazar MJ, Rodriguez JH, Nieto GL, Pignata ML. 2012. Effects of heavy metal concentrations (Cd, Zn and Pb) in agricultural soils near different emission sources on quality, accumulation and food safety in soybean [*Glycine max* (L.) Merrill]. J Hazard Mater 233–234(0):244–253.
Somers I, Shive J. 1942. The iron-manganese relation in plant metabolism. Plant Physiol 17(4):582.
Tessier A, Campbell PGC, Bisson M. 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal Chem 51(7):844–851.
US EPA. 1989. Risk assessment guidance for superfund, human health evaluation manual (Part A), Interim final. In: Agency TUSEP, editor. Washington(DC): US EPA. p 1–89.
US EPA. 2010. Region 9, Preliminary Remediation Goals. Retrieved 12 June 2010.
USDA. 2006. Keys to Soil Taxonomy, United State Department of Agriculture, Natural Resources Conservation Service.
Vamerali T, Bandiera M, Mosca G. 2010. Field crops for phytoremediation of metal-contaminated land. A review. Environ Chem Lett 8(1):1–17.
Wang J, Yuan J, Yang Z, Huang B, Zhou Y, Xin J, Gong Y, Yu H. 2009. Variation in cadmium accumulation among 30 cultivars and cadmium subcellular distribution in 2 selected cultivars of water spinach (*Ipomoea aquatica* Forsk.). J Agr Food Chem 57(19):8942–8949.
Wei ZB, Guo XF, Wu QT, Long XX, Penn CJ. 2011. Phytoextraction of heavy metals from contaminated soil by co-cropping with chelator application and assessment of associated leaching risk. Int J Phytoremediat 13(7):717–729.
Wenzel W. 2009. Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321(1–2):385–408.
Whiting SN, Leake JR, McGrath SP, Baker AJM. 2001. Hyperaccumulation of Zn by *Thlaspi caerulescens* can ameliorate Zn toxicity in the rhizosphere of cocropped thlaspi arvense. Envir Sci Tech 35(15):3237–3241.
Wu QT, Wei Z-B, Ouyang Y. 2007. Phytoextraction of metal-contaminated soil by *Sedum alfredii H*: Effects of chelator and co-planting. Water Air Soil Poll 180(1–4):131–139.
Xiaomei L, Qitang W, Banks MK. 2005. Effect of simultaneous establishment of *Sedum alfredii* and *Zea mays* on heavy metal accumulation in plants. Int J Phytoremediat 7(1):43–53.
Xu T, Qiu J, Wu Q-T, Guo X, Wei Z, Xie F, Wong JWC. 2013. Fate of heavy metals and major nutrients in a sludge-soil-plant-leachate system during the sludge phyto-treatment process. Environ Tech 34(15):2221–2229.
Yoon J, Cao X, Zhou Q, Ma LQ. 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Environ 368(2–3):456–464.
Zhao Y, Fang X, Mu Y, Cheng Y, Ma Q, Nian H, Yang C. 2014. Metal pollution (Cd, Pb, Zn, and As) in agricultural soils and soybean, *Glycine max*, in southern China. B Environ Contam Tox 92:427–432.
Zheng N, Wang Q, Zheng D. 2007. Health risk of Hg, Pb, Cd, Zn, and Cu to the inhabitants around Huludao Zinc Plant in China via consumption of vegetables. Sci Total Environ 383(1–3):81–89.