Traditional agricultural practices enable sustainable remediation of highly polluted soils in Southern Spain for cultivation of food crops.

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Abstract

This study relates elemental content of a range of edible crops grown in soils severely polluted by metals and metalloids as affected by traditional smallholder management practices. Five agricultural plots close to a sulfidic waste dump were monitored. Soil analysis demonstrated elevated concentrations of As, Cu, Pb and Zn that were greatly in excess of maximum statutory limits for agricultural soils in this region. The main vegetables (lettuce, chard, onion, potatoes) and lemon, together with their associated soils, were measured for elemental content. Extractable soil element concentrations were very low. There were differences in elemental accumulation between crops, but none exceeded statutory concentrations in edible parts. Soil-plant transfer factors were uniformly low for all elements and crops. It is concluded that traditional soil management practices (annual liming and application of animal manures) have created conditions for sustainable long-term safety use, with potential for multiple end-use, of these highly polluted soils

Keywords: Trace elements, edible plants, soil pollution, Tharsis mines, Spain

1. Introduction

Plants are the first compartment of the terrestrial food chain. Due to their capacity to accumulate potentially toxic trace elements, they can represent a threat to animals and humans that consume them (Intawongse and Dean, 2006; Liu et al., 2006).

The risk that trace elements pose to the environment and human health is a function of their speciation in soils and subsequent accumulation and partitioning within plants (Kabata-Pendias, 2004). The solubility of trace elements varies widely because many factors influence their concentration in the soil solution: edaphic characteristics (pH, texture and organic matter content), climatic conditions, and agronomic management (Alloway, 1995, Chojnacka et al., 2005; Tokalioğlu and Kartal 2006). The rate of metal translocation from the soil to edible and harvested parts of cultivated plants depends, in addition, on vegetation type and metal involved, besides the soil and climatic factors (Alloway, 1995; McLaughlin et al., 1999).

Chaney (1980) introduced the concept of the 'soil-plant barrier' and classified trace elements in four groups with respect to their potential for food chain transfer: Group 1 (Ag, Cr, Sn, Ti, Y and Zr) with low risk to human health because they are not usually taken up by plants due to their low solubility in soil. Group 2 (As, Hg and Pb) which are strongly sorbed by soil colloids, or may be immobilized in plant roots with very limited transfer to edible shoot tissues, and therefore pose marginal risks. Group 3 (B, Cu, Mn, Mo, Ni and Zn) are phytotoxic at concentrations that pose little risk, although 'the soil-plant barrier' protects the food chain from these elements. Group 4 consists of Cd, Co, Mo and Se, which pose human or animal health risks at plant tissue concentrations that are not phytotoxic.

Soil pollution with trace elements, mainly those from Groups 2 and 3 described above, represents one of the most prominent environmental hazards from abandoned mine sites in the Iberian Pyrite Belt (Fernández-Caliani et al. 2009a, b; Fernández-Caliani and Barba-Brioso, 2010). Past mining and ore processing activities resulted in deforestation and destruction of the natural vegetation, transforming soils into marginal lands unable to sustain commercial agriculture. However, traditional small-scale agriculture has persisted on limited areas of arable land adjacent to the mining villages in this region.

The goals of the study reported here were to: 1) determine total concentrations of trace elements in different parts of fruit and vegetables, and in the soil in which they grew, focusing on their edible parts; 2) assess the mobile and potentially-mobile fractions of the soil trace element pools and the soil-plant transfer factors for the crops grown here; and 3) evaluate the long-term sustainability of the traditional soil management practices used in this region for marginal or soils of low fertility in relation to soil-plant transfer of inorganic pollutants.

2. Site description

The Tharsis Mines (UTM coordinates: X= 666,746 and Y= 4,162,779) are located in one of the oldest and best-known mining districts in the Iberian Pyrite Belt (South-West Spain), with a lengthy history of exploitation that dates back to pre-Roman times (Checkland, 1967). The most intensive period of mining operations occurred between the end of the 19th century and the middle of the 20th century, when pyrite was the main raw material used for sulphuric acid manufacture by the European chemical industry. Past mining and smelting activities were carried out without concern or even awareness of their negative environmental impact, resulting a present-day landscape of large opencast mines, waste rock piles and tailings dams that encompass an area of about 350 ha (Figure 1a).

During mining and after closure, pollutants have been transferred from the mine wastes to nearby soils by acid mine drainage and/or atmospheric deposition of wind-blown dust. Soils and vegetation show elevated trace element concentrations up to 2-3 km away from the mining area (Chopin and Alloway, 2007a,b). There are also a number of small holdings, none of which exceeds one hectare in extent, located in the immediate vicinity of the mine waste dumps, all at risk of contamination with metals.

Five agricultural plots, close to the sulfidic waste dumps of the “Filón Norte” open pit (Figure 1 b, c) were selected for this study. The alluvial soils of these plots are devoted to traditional horticulture, despite the fact that they are occasionally flooded by acid waters emanating from nearby waste dumps and surface mine workings. To correct soil acidity, farmers routinely amend soils with lime, together with regular additions of animal manure. These practices have taken place for at least 25 years in the investigated plots, a fact established by interviews with each plot owner. The main crops cultivated by the smallholders are vegetables, typically lettuce (*Lactuca sativa* L.), chard (*Beta vulgaris* (L.) var. *cicla* K. Koch), onion (*Allium fistulosum* L.) and potato (*Solanum tuberosum* L.). Some plots also contain mature Lemon trees (*Citrus limon* L. Brum).

3. Methodology

3.1 Materials and methods

A suite of vegetable samples, comprising leaf, bulb, tuber and fruit crops, along with their associated soils were randomly collected from each plot in April 2008 at the indicated stage of maturity (Table 1).

Composite soil samples were taken to a depth of 20 cm (Ap horizon) from each plot where sampled vegetables were growing. These were transported to the laboratory in polyethylene bags, air dried, disaggregated with a wooden roller, passed through a 2 mm stainless steel sieve, and homogenized prior to analysis. The particle-size distribution was determined by a combination of sieving and laser diffraction (Malvern MasterSizer instrument) methods. The pH, Eh and electrical conductivity values were measured with calibrated glass electrodes in a 1:2.5 (w/v) soil to water suspension. The content of total organic matter was determined by oxidation with potassium dichromate in a strong acid medium (Walkley-Black method), as described by Pansu and Gautheyrou (2006), and the carbonate content was measured by the Bernard calcimetry method.

Soil mineralogy was investigated in both bulk samples (<2 mm) and clay-size fractions (<2 µm) by powder X-ray diffraction (XRD) on a Bruker-AXS D8-Advance diffractometer, using monochromatic CuKα radiation at 40 kV and 30 mA. The fine fraction was separated by sedimentation and analyzed in oriented aggregates of air-dried, ethylene glycol-treated and thermal-treated samples for clay mineral identification. Selected soil samples were examined by scanning electron microscopy using a JEOL JSM-5410 instrument coupled with an energy dispersive X-ray spectrometer (SEM-EDS).

Total concentrations of potentially toxic trace elements (As, Cu, Pb and Zn) in soil samples were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES), (Jobin Yvon ULTIMA 2), after 4-acid (HF-$HClO_4$-HNO_3-HCl) digestion of 0.1 g soil sample oven dried for 24 hours at 110 °C. Quality control included the use of a method reagent blank and several certified reference materials (SARM-1 and SARM-4 for soil analysis) to check accuracy and precision of the analytical data (relative standard deviation below 10%).

In order to determine the most labile metal pools, an aliquot of each soil sample (2 g) was subjected to single extractions by shaking for one hour with deionised water, a mild neutral salt solution (0.01 M $CaCl_2$) or a complexing agent (0.05 M EDTA at pH 7) at a soil:solution ratio of 1:10 (w/v) (e.g. Houba et al. 1996; Ure et al., 1996). The first two soil extract solutions were analyzed after centrifugation (for 10 min at 4500 rpm) by inductively coupled plasma mass spectrometry (ICP-MS), using a Hewlett Packard 4500 instrument with detection limits of 0.01 μgL^{-1}, whilst EDTA-extractable metals were determined by ICP-OES. All trace element concentrations were reported on an oven-dry basis.

All plant samples (leaves, roots, bulbs, peel and seeds) were washed (for 10 s approximately) with a solution of phosphate-free detergent, then with a 0.1 N HCl solution and finally with distilled water. Plant material was oven-dried at 70ºC, ground and passed through a 500 µm stainless steel sieve. A 0.5 g aliquot was digested by wet oxidation with concentrated HNO_3 under pressure in a microwave digester (Jones and Case, 1990). Three consecutive steps (5 min. each) of power (250 W, 450 W and 600 W) were applied, and then these extracts were diluted to 50 ml volume with deoinised water of 18 mΩ quality. The analysis of trace metals in the digests was performed by ICP-MS. The accuracy and precision of the analytical method were assessed by routine analyses of the reference sample CRM-279 (Sea lettuce) and CS DC73350 (poplar leaves). Recovery rates for reference plant samples were between 90 and 110%.

3.2 Treatment of analytical data

Quantitative assessment of overall soil pollution was based on the pollution load index (PLI), as defined by Tomlinson et al., (1980), taking into consideration the concentration factor (CF), which is the ratio between each trace element in the soil and its background value. The PLI of each soil sample was calculated by deriving the *n*-th root of the *n* factors ($CF_1 x CF_2 x CF_3 x ... CF n$). Thus, values of PLI close to one indicate heavy metal loads near the background level, while values >1 indicate soil pollution (Cabrera et al., 1999).

In order to find out what proportion of the total soil metal concentration was available and transferred to different organs of the vegetables grown by the smallholders, the transfer coefficient (TC) was calculated. This is defined as the ratio of metal concentration in the plant, [M]plant, to the total metal concentration in the soil, [M]soil (Adriano, 2001).

TC = [M]plant / [M] soil

The data was statistically analysed using StatSoft Statistica 7.0 to recognize variables trends and groupings. Kolmogorov-Smirnov and Shapiro-Wilk's normality tests were carried out for all variables, refusing the normal distribution for almost all of them. Because the variables required a non-parametric analyses, a Spearman correlation matrix (significance level $p<0.01$) was obtained, correlating total trace element contents in soils with those measured in soil extracts and plants.

4. Results and discussion

4.1 Soil constituents and properties

The upper part of the soil profile shows a well-developed organic horizon with granular structure and had similar edaphic properties in all the sampled soils (Table 2). The soil is brown (10YR 5/3, 10YR 6/3 dry) in colour and has a silty loam texture with <10% of clay-sized particles. Some soil samples contain significant amounts of coarse-grained components (greater than 2 mm), consisting of sub-angular lithic fragments of heterogeneous waste rocks and slag residues.

The soil had an average pH value of 7.4, and electrical conductivity ranged between 0.15 and 0.95 mS cm^{-1}, indicating a low salinity level in the soil solution. The soil appears to be well drained and aerated spanning a narrow range of positive Eh values (457-505 mV) that reflect moderately oxidizing conditions.

The soil has a high content of total organic matter (8.1-14.9%) and carbonates (up to 14%) resulting from the application of organic amendments and lime to improve soil fertility and to prevent soil acidity.

Soil minerals, identified by XRD, were composed of phyllosilicates (50-60%), quartz (30-40%), feldspars (5-10%), calcite (<5%) and dolomite (<5%). The clay mineral assemblage was dominated by illite and kaolinite, with minor vermiculite and/or poorly defined mixed-layer phases. In addition, SEM-EDS analysis revealed the occurrence of amorphous or poorly crystalline Fe oxyhydroxides and a number of accessory minerals, such as baryte, apatite and monazite.

4.2 Total concentrations of trace elements in soil

The sampled soils contained high levels of As, Cu, Pb and Zn (Table 3), although total concentrations varied, depending on the location of the sampling plot (Figure 1). The highest concentrations of trace elements (up to 621 mg kg^{-1} As, 752 mg kg^{-1} Cu, 2395 mg kg^{-1} Pb, and 593 mg kg^{-1} Zn) were found in plots 4 and 5, located in the immediate vicinity of the mine wastes. Similar total soil element concentrations have been reported for cultivated soils adjacent to other abandoned mine sites in the Iberian Pyrite Belt (López et al., 2008; Fernández-Caliani et al. 2009a).

The total concentrations of As, Cu, Pb and Zn in these soils are between one and two orders of magnitude above both the regional geochemical baseline (Galán et al. 2008) and normal levels found in Spanish agricultural soils (López-Arias and Grau-Corbí, 2005). Furthermore, these values greatly exceed the maximum allowable concentrations for agricultural soils established by the Regional Government of Andalusia (Aguilar et al., 1999). This indicates potential health risks associated with consuming edible crops grown in these soils.

The Concentration Factor, CF, defined as the ratio between each trace element in the soil sample and its background value (50th percentile), was particularly elevated for As (up to 24.8), Cu (up to 23.5), and Pb (up to 63.0), indicating soil pollution. The lowest CF values were found for Zn, although the ratio was higher than 1 for all samples. Pollution load indices (PLI) at each plot are shown in Table 3. The PLI values varied between 9.4 (plot 1) and 22.8 (plot 5), reflecting the high pollution loads of As, Cu, Pb and Zn, especially in plots near the mine wastes.

4.3 Trace element concentrations extracted with water, $CaCl_2$ and EDTA

Water, $CaCl_2$ and EDTA remove trace elements from different compartments within the overall soil matrix. The water-soluble fraction and the fraction assessed by $CaCl_2$-extraction are considered to simulate the proportion of the total soil metal pool that may be available for uptake by plants and play a key role in many transfer pathways (Gupta et al. 1996; Houba et al. 1996). The EDTA-extractable fraction is composed of those ions present in the soil solution, as well as those that are loosely bound to sites in the solid phase of the soil and potentially able to move into the plant root system (Madejón et al., 2009). In some cases, EDTA-extractable metal concentrations have been reported to be closely correlated with metal concentrations in plants (e.g. Sahuquillo et al., 2003), although methodology for assessing the bioavailability of metals in soil is a controversial issue (Menzies et al., 2007).

The mean water-soluble and $CaCl_2$-extractable concentrations of As, Cu, Pb and Zn were found to be less than 1 mg kg^{-1} in all the agricultural plots (Table 3), whereas the EDTA-extracted mean concentrations were higher, and varied widely depending on the trace element involved (2.9-7.2 mg kg^{-1} for As, 22.3-32.6 mg kg^{-1} for Cu, 46.4-95.8 mg kg^{-1} for Pb, and 17.8-20.3 mg kg^{-1} for Zn). Accordingly, the mobile and very active metal fraction was practically negligible (lower than 0.22%), and the EDTA-extractable fraction was below 10% (Figure 2), indicating a limited potential mobility of all elements despite their high total concentrations in soil. No significant correlation was observed between the extractable concentrations and the total concentrations in soil for all the investigated elements, suggesting that the pollution load did not have a noticeable effect on the proportion of water soluble, exchangeable and complexed fractions. These results are in good agreement with previous studies on soils from this area (Barba-Brioso et al., 2007; Chopin and Alloway, 2007a,b).

In general, trace elements showed the following order of relative abundance in the mobile fraction: As > Cu > Zn > Pb. Therefore, As seems to be the most easily extractable trace element in soil. This can be explained by the fact that, under circumneutral pH conditions, As mobility can be increased in soils (Hartley et al., 2004). Arsenic usually forms water-soluble oxyanion species which are repelled by the negatively-charged surfaces of soil particles, thus preventing the adsorption of As oxyanions. A further finding supports our assumption: As concentration up to 138 μg L^{-1} compared to much lower to Cu and Zn concentrations (< 5 μg L^{-1}) were measured in groundwater from a nearby well (unpublished data); the guideline value for As irrigation water is 0.1 mg L^{-1} (FAO, 1985). On the other hand, the low extractability of Pb with water and $CaCl_2$ indicated that this trace element was more strongly bound to constituents of the soil than As, Zn, and Cu.

Differences between elemental concentrations removed by the 3 extractants show that each provides different information on the metal status of the tested soils. As EDTA is a chelating agent that extracts elements by forming complexes with cations, it can access trace elements associated with stable binding sites within the soil matrix. The higher extractability of trace elements with EDTA (Table 3) compared with water and $CaCl_2$ extraction could be due to EDTA removing trace elements bound to organic matter (soil OM contents were between 8 and 15%, Table 2).

Speciation of trace elements in soils is mainly related to pH (McLaughlin et al., 2000). Although there were no correlation patterns between pH and extractable trace elements, the low extractability of Cu and Zn compared to their total concentrations could also be related to the neutral pH of the soil (Madejón et al., 2009). Correlations between pH and both water and $CaCl_2$ extraction were significant ($P<0.01$) for As ($r_{H_2O}= 0.59$ and $r_{CaCl_2}= 0.62$), Cu ($r_{H_2O}= 0.49$ and $r_{CaCl_2}= 0.52$) and Pb ($r_{H_2O}= 0.60$ and $r_{CaCl_2}= 0.82$). Significant correlations between pH and EDTA extraction were only found for Zn ($r= 0.34$).

4.4 Trace element content of plants

The elements that most commonly produce concerns about food safety are Cd, Hg, Pb, As and Se (Reilly, 1991). In addition, some micronutrients (e.g. Cu, Cr, Ni, Zn) may be toxic to both plants and animals when present at high concentrations (McLaughlin et al., 1999). Of the trace elements investigated here, As is considered to pose the major risk for human food-chain contamination (Kabata-Pendias and Pendias, 1999, 2001).

Trace element content of onion bulb differed; this could be related depending on the stage of development when bulbs were sampled. In young onions bulbs, As concentration reached values up to 23.5 mg kg^{-1} (above the statutory limits, Table 4). When the onion ripened and increased its biomass, this concentration decreased, analysis showing values below the statutory limit. This could be related to a ‘dilution effect’ of elements due to biomass increase with no concomitant increase in trace element uptake; this has been previously considered in the literature (e.g. Jarrel and Beverly 1981). Leafy vegetables such lettuce and chard are classified as crops with a high potential for trace element transfer from soil to the edible foliage (Pillay and Jonnalagadda, 2007), however Juhasz et al. (2008) found that such vegetables were poor As accumulators. In the case of the lettuces and chard sampled here, As contents in the edible leaves were within normal and statutory levels for vegetables. It is interesting to note that the highest As concentrations were found in roots and outer leaves of lettuce (the most external dark green leaves), both non-edible parts of this vegetable (Figure 4).

Plants showed similar patterns of Pb uptake and partitioning as found for As; maximum contents in roots, up to 51.8 mg kg^{-1} in onions (Table 4) and 13 mg kg^{-1} in lettuce (Figure 4). This is in agreement with other observations on Pb uptake and distribution in plants (Adriano, 2001). In these soils, the soil–plant barrier may act to protect the human food chain against Pb toxicity (Chaney, 1989). Similar results for As and Pb uptake in onion and lettuce were reported by Lim et al. (2008). The effect of biomass increase in onions was also evident, bulbs of young onions showed Pb contents up to 8 mg kg^{-1} in the edible part, but in ripe onions Pb content decreased to values below statutory levels (Table 4). This reflects the nature of bulb development, with biomass added as a result of carbohydrate transport from leaves. The Pb content of lettuce and chard foliage was also below statutory limits. There were also no age-dependant differences in Pb distribution in mature lettuce heads: the older, outer leaves had very similar Pb content to their less mature counterparts in the centre of the head (Figure 4).

The concentrations of As and Pb detected in whole potatoes, peel and tuber, were negligible (0.1 mg kg^{-1}). There was no evidence of As contamination in potato tubers, which is in agreement with other findings (Dahal et al. 2008).

Copper and Zn have important physiological roles as micronutrients in plants, however excess concentrations in edible plant parts may pose a risk to both humans and animals. Maximum values for Cu and Zn were found in onion roots (up to 80 mg kg^{-1} Cu and 135 mg kg^{-1} Zn, Table 4), although that was not the case for lettuce roots. In case of Cu, concentrations in onion leaves (maximum of 30 mg kg^{-1}, above normal levels in plants) were higher than in bulbs in contrast to data for Zn. The dilution effect of trace elements (bulbs of mature *vs* young onions) was also observed for Cu and Zn. In general, concentrations in onions were above normal concentrations found in this plant according to Mohamed et al. 2003 (2.81 mg kg^{-1} for Cu and 17.6 mg kg^{-1} for Zn) and Kabata-Pendias and Pendias 1999, 2001 (Table 4). Likewise the contents of Cu and Zn in lettuce and chard were within the normal range reported in plants, although for Cu these contents were higher than contents in the same species from non-polluted sites (lettuce 3.81 mg kg^{-1} Cu and 81.5 mg kg^{-1} Zn and chard 5.49 mg kg^{-1} Cu and 150 mg kg^{-1} Zn, Pillay and Jonnalagadda, 2007). The maximum concentrations of Cu and Zn in lettuce were detected in the inner leaves (Figure 4), so the distribution patterns of both micronutrients were different from those found for As and Pb.

The relatively high concentrations of Cu in onion leaves, lettuce and chard could have been influenced by foliar absorption of Cu-based agrochemicals, which are applied by the local farmers. Plants growing on Cu-polluted sites tend to accumulate increased amounts of this metal, especially near industrial areas and in soils treated with Cu-bearing pesticides (Kabata-Pendias and Mukherjee, 2007).

Elemental concentrations in different organs of lemon trees are shown in Figure 5. Arsenic and Pb reached the highest contents in leaves (although within the normal range in plants) whereas concentrations of these potentially toxic elements in different parts of the fruit were very low, especially in seed (0.1 mg kg^{-1}). Both trace elements in lemon leaves and fruits followed the same pattern found in other trees growing on polluted soils: high content in leaves and much lower in fruits and seeds (Madejón et al., 2006). Ernst et al. (1992) reported that plants do not totally exclude trace elements (with no essential function in the plant) from their reproductive organs although the content in fruits and seed is usually very low. On the other hand, Cu and Zn showed a similar distribution pattern, with high concentrations in leaves and in seeds when compared to As and Pb.

Finally, the concentrations of As and Pb detected in whole potatoes, peel and tuber, were negligible (0.1 mg kg^{-1}), whereas the concentrations of Cu in tubers (up to 12.0 mg kg^{-1}) and Zn (up to 28.8 mg kg^{-1}) were greater than those compiled by Kabata-Pendias and Pendias (1999, 2001) as possible background values.

4.5 Trace element correlations between soil and plants and soil-plant transfer coefficients

Significant correlations between soils and plants (taking together all species and plant parts) were found for As with water and $CaCl_2$ extraction (r_{H_2O}= 0.45 and r_{CaCl_2}= 0.46). Correlation coefficients for each species could be only calculated for young onions, lettuce and chard. There were significant correlations between As in lettuce leaves and EDTA extractable As (r= 0.86) and chard leaves and pH (r= -0.87). For Pb, significant negative correlations were found between lettuce and chard and total soil Pb content; this may be indicative of a non-soil pollution source (wind-blown dust). Significant soil-plant correlations for Cu were found for lettuce and chard and for total, EDTA and $CaCl_2$ extraction concentrations, but these are less robust due to the low number of samples.

The soil-plant transfer coefficients were calculated for As, Cu, Pb and Zn (Table 5). The transfer factors may depend not only on plant species, but also on the element concentration and bioavailability in soil (Huang et al., 2006). The values calculated were, in general, very low, especially in the case of As and Pb, and in the different tissues of lemon and potato that were analyzed. It has been observed that transfer factors tended to decrease with increasing soil concentrations (Alan et al., 2003). In the case of onions, the high As TC in roots, (TC= 3.03) coupled with the greatly reduced transfer to aerial parts (TC= 0.03) and bulb (TC= 0.001) would indicate that the As accumulated in root tissues. In case of Cu and Zn, TC were always low, with maximum values found in onion roots (TC= 0.15 for Cu and 0.18 for Zn). Similar results were found for other plants growing in multi-element polluted soils by Madejón et al. (2007). The present results imply that there is a low transfer of pollutants from these soils to primary producers.

5. Conclusions

There are several conclusions that can be drawn from this study:

First: There is limited soil-plant transfer of potentially hazardous trace elements (As, Cu, Pb, Zn) to selected vegetables and fruit cultivated on a mine-polluted soil. One reason may be the current management practices employed on the agricultural small holdings under investigation such as regular inputs of organic matter and lime to maintain soil pH close to neutrality.

Second: The vegetables investigated are typical of those grown in this region, and no edible part showed element accumulation that could be considered as a significant risk to human health, based on statutory limits for food crops. The vegetables showed different patterns of trace element accumulation: highest concentrations in non-edible parts of onions and lowest concentrations in potatoes tubers.

Third: The data from this survey is a clear illustration of the use of simple, routine agronomic procedures as a means of a) cultivating crops on potentially phytotoxic soils and b) reducing the concentrations of potentially toxic elements (As, Pb) in their edible tissues. Routine application of a combination of livestock manure and agricultural lime has created a soil environment where the labile pools of As and Pb are very low, in contrast to the very elevated total content of these elements in the soil. In addition, this treatment has regulated plant uptake of both Cu and Zn, reducing potential phytotoxicity whilst still providing a source of trace concentrations of both micronutrients to maintain normal growth. The *ad hoc* treatments applied by the smallholders appear to have successfully contributed to the safe use of the polluted soils where it can sustain crop production over an extended period of time. There are clear lessons for future development of field scale remediation of soils with mixed metal/metalloid pollution from the successful and sustainable outcome of the ‘in situ’ treatments applied to these highly polluted soils by the smallholders who use traditional practices to farm these sites. However it is important to consider that the success obtained in this case cannot be generally extended to other situations where soil and climate conditions, as well as type of crop species, are different.

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FIGURE AND TABLE CAPTIONS

Figure 1. Panoramic view (a) of the Tharsis mining area (photo courtesy: E. Romero) showing the location of the agricultural plots selected for this study (b,c).

Figure 2. Percentages of elements extracted with (a) deionised water, (b) CaCl2, and (c) EDTA in soil growing young onion (C1, C3, C4, C5), ripe onion (C2), lettuce (L1, L2, L3), chard (A1, A2, A3), lemon (S5), and potato (S12).

Figure 3. Distribution of trace elements (mean values in mg kg^{-1}) in different parts of the onion.

Figure 4. Distribution of trace elements (mean values in mg kg^{-1}) in different parts of the lettuce.

Figure 5. Distribution of trace elements (mean values in mg kg^{-1}) in different parts of the lemon tree.

Table 1. Plant species, analyzed organs (number of samples in brackets) and their associated soil samples.

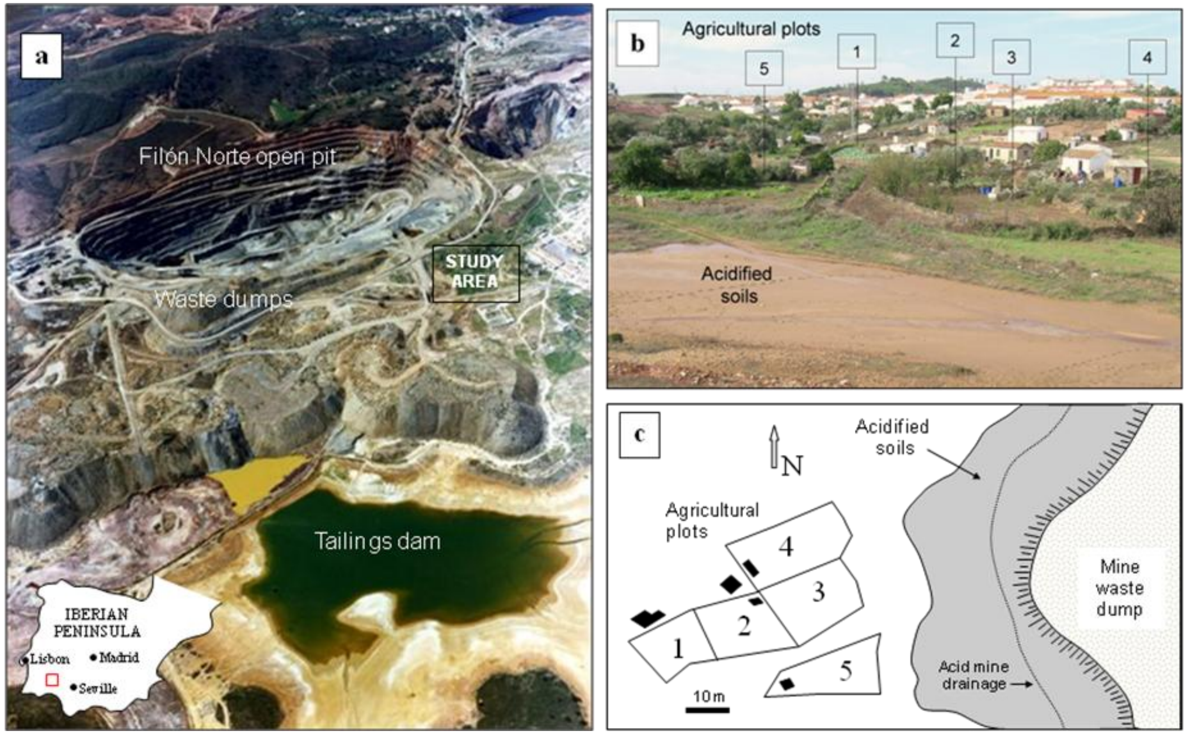
Table 2. Physico-chemical parameters of the soils where vegetables are cultivated.

Table 3. Mean and standard deviation of the total trace element concentrations and pollution load index (PLI) and trace elements extracted with deionized water, $CaCl_2$ and EDTA. All values are expressed in mg kg^{-1}. Number of samples (n) appears in brackets

Table 4. Trace element content (mean and range values in mg kg^{-1}) in different organs of vegetables growing in the agricultural plots.

Table 5. Transfer coefficients (TC) for As, Cu, Pb and Zn in the different organs of the studied vegetables.

Figure


a
Filón Norte open pit
STUDY AREA
Waste dumps
Tailings dam
IBERIAN PENINSULA
Lisbon
Madrid
Seville
b
Agricultural plots
5
1
2
3
4
Acidified soils
c
N
Acidified soils
Agricultural plots
4
3
2
1
5
10 m
Acid mine drainage
Mine waste dump

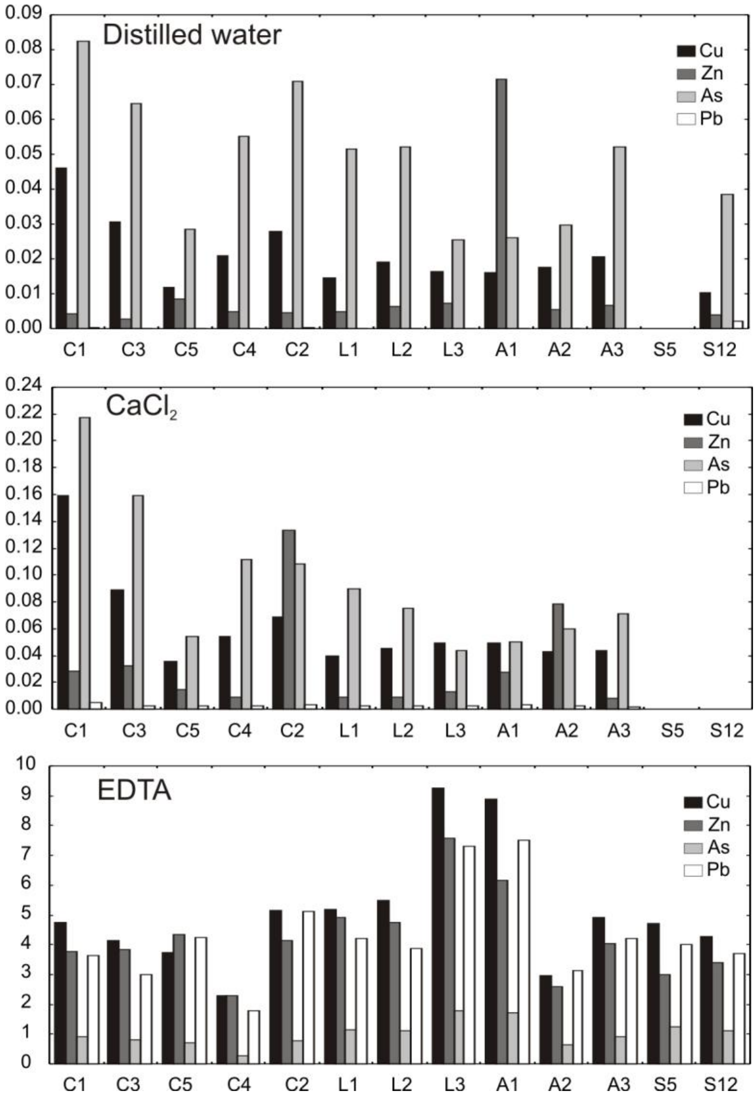

Distilled water
Cu
Zn
As
Pb
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
C1
C3
C5
C4
C2
L1
L2
L3
A1
A2
A3
S5
S12
CaCl$_2$
Cu
Zn
As
Pb
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0.20
0.22
0.24
C1
C3
C5
C4
C2
L1
L2
L3
A1
A2
A3
S5
S12
EDTA
Cu
Zn
As
Pb
0
1
2
3
4
5
6
7
8
9
10
C1
C3
C5
C4
C2
L1
L2
L3
A1
A2
A3
S5
S12

Figure 2

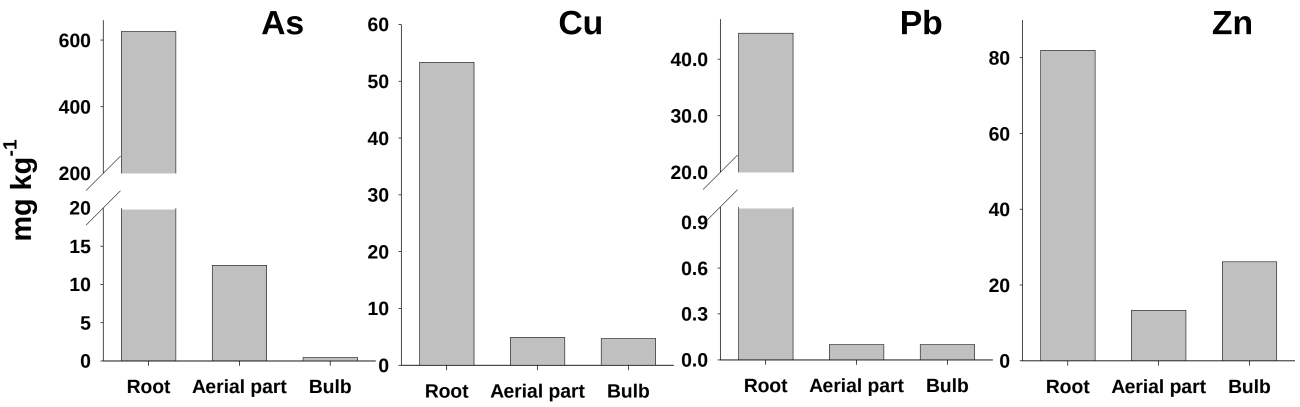

mg kg^{-1}
As
600
400
200
20
15
10
5
0
Cu
60
50
40
30
20
10
0
Pb
40.0
30.0
20.0
0.9
0.6
0.3
0.0
Zn
80
60
40
20
0
Root
Aerial part
Bulb

Figure 3

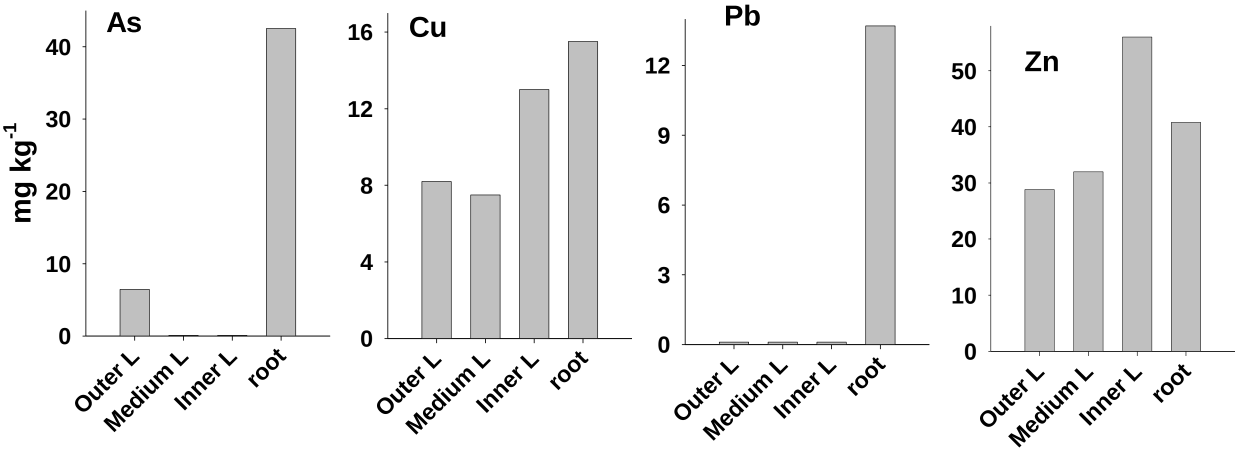

As
Cu
Pb
Zn
mg kg^{-1}
0
10
20
30
40
0
4
8
12
16
0
3
6
9
12
0
10
20
30
40
50
Outer L
Medium L
Inner L
root

Figure 4

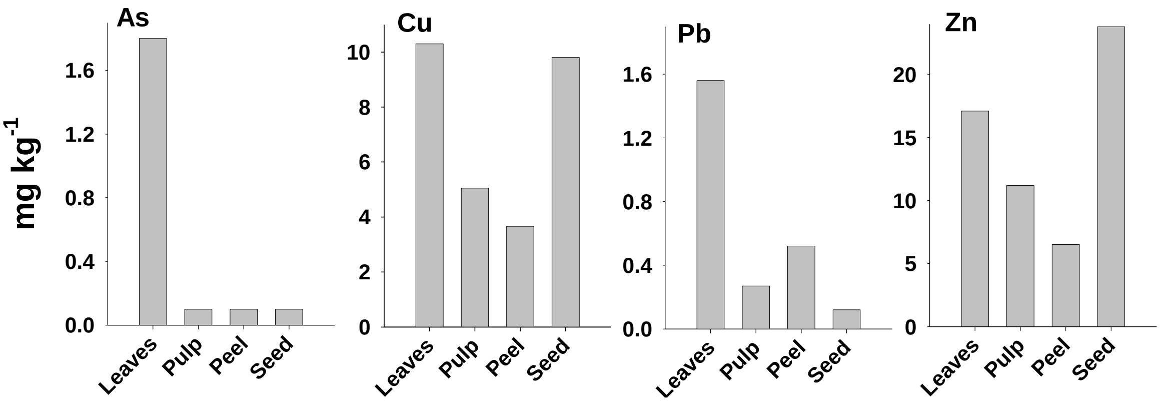

mg kg^{-1}
As
0.0
0.4
0.8
1.2
1.6
Cu
0
2
4
6
8
10
Pb
0.0
0.4
0.8
1.2
1.6
Zn
0
5
10
15
20
Leaves
Pulp
Peel
Seed

Figure 5

Table 1. Plant species, analyzed organs (number of samples in brackets) and their rhizospheric soil samples.

Plant species	Analyzed organs	Rhizosphere samples
Young onions	Bulb (7) and leaves (7)	C1, C3, C4,C5
Ripe onions	Bulb (6), leaves (6) and roots (3)	C2
Lettuce	Leaves (6) and roots (3) Inner, medium and outer leaves	L1, L2, L3
Chard	Leaves (6)	A1, A2, A3
Potato	Peel (5) and inner tissues (edible part) (5)	S12
Lemon	Leaves (3), peel (3), inner tissue (edible part) (3) and seeds (3)	S5

Table 2.. Physico-chemical parameters of the soils where vegetables are cultivated.

Plot	**Soil sample**	**Color (Munsell)**	**Coarse fragments (%)**	**Fine earths (<2 mm)**			**pH** (H_2O)	**Eh (mV)**	**Electrical conductivity (mS cm^{-1})**	**Organic matter (%)**	**Carbonates(%)**
				Sand (%)	Silt (%)	Clay (%)					
1	A1	10YR 5/3	15.3	14.2	76.8	9.0	7.5	491	0.20	13.7	9.0
1	L3	10YR 6/3	23.8	17.8	76.0	6.2	7.4	498	0.15	14.9	14.0
1	C5	10YR 6/3	19.3	30.0	57.7	4.3	7.1	461	0.28	14.8	8.1
2	A2	10YR 6/3	30.2	15.0	76.5	8.5	7.3	487	0.22	9.9	7.3
2	A3	10YR 6/3	20.0	18.2	75.3	6.5	7.1	486	0.14	11.8	11.8
2	L1	10YR 5/3	18.6	19.9	74.1	6.0	7.5	494	0.13	11.9	12.9
2	L2	10YR 6/3	24.8	20.5	73.6	5.9	7.1	505	0.20	13.9	10.1
2	C2	10YR 5/2	21.8	23.8	68.6	7.6	7.1	485	0.63	11.9	5.6
3	C1	10YR 5/3	16.1	18.3	74.4	7.3	8.2	457	0.43	12.8	9.3
3	C3	10YR 5/3	16.6	23.1	70.0	6.9	7.6	471	0.95	13.8	7.6
3	C4	10YR 6/2	18.5	28.8	71.0	7.2	7.5	468	0.56	11.8	8.7
4	S5	10YR 4/2	13.2	31.0	59.1	9.9	7.3	n.d.	0.69	10.4	n.d.
5	S12	10YR 5/3	9.9	33.1	59.0	7.9	7.5	n.d.	0.45	8.1	n.d.

Table 3. Mean and standard deviation of the total trace element concentrations and pollution load index (PLI) and trace elements extracted with deionised water, $CaCl_2$ and EDTA. All values are expressed in mg kg^{-1}. Number of samples (n) appears in brackets.

Total contents	**As**	**Cu**	**Pb**	**Zn**
Plot 1 (PLI 9.4)	203 ± 26.7	326 ± 51.4	864 ± 74.3	314 ± 25.1
Plot 2 (PLI 14.5)	332 ± 70.9	486 ± 84.6	1281 ± 241	499 ± 39.9
Plot 3 (PLI 18.6)	469 ± 85.1	603 ± 20.2	1715 ± 274	570 ± 15.7
Plot 4 (PLI 22.2)	575	692	2395	593
Plot 5 (PLI 22.8)	621	752	2260	589
Water soluble	**As**	**Cu**	**Pb**	**Zn**
Plot 1 (3)	0.054 ± 0.004	0.047 ± 0.002	0.001 ± 0.0001	0.097 ± 0.128
Plot 2 (5)	0.164 ± 0.035	0.094 ± 0.011	0.001 ± 0.0005	0.029 ± 0.007
Plot 3 (3)	0.309 ± 0.015	0.195 ± 0.069	0.003 ± 0.0011	0.023 ± 0.007
Plot 4 (1)	n.d.	n.d.	n.d.	n.d.
Plot 5 (1)	0.240	0.078	0.045	0.024
$CaCl_2$-extractable	**As**	**Cu**	**Pb**	**Zn**
Plot 1 (3)	0.099 ± 0.007	0.144 ± 0.007	0.024 ± 0.005	0.058 ± 0.030
Plot 2 (5)	0.259 ± 0.040	0.227 ± 0.016	0.031 ± 0.003	0.225 ± 0.259
Plot 3 (3)	0.734 ± 0.116	0.602 ± 0.299	0.057 ± 0.016	0.131 ± 0.070
Plot 4 (1)	n.d.	n.d.	n.d.	n.d.
Plot 5 (1)	n.d.	n.d.	n.d.	n.d.
EDTA-extractable	**As**	**Cu**	**Pb**	**Zn**
Plot 1 (3)	2.95 ± 1.51	22.7 ± 7.22	55.8 ± 19.7	19.1 ± 5.56
Plot 2 (5)	3.06 ± 1.07	23.1 ± 6.70	51.7 ± 8.79	20.3 ± 4.43
Plot 3 (3)	2.88 ± 1.25	22.3 ± 7.31	46.4 ± 9.99	18.8 ± 4.89
Plot 4 (1)	7.18	32.6	95.7	17.7
Plot 5 (1)	6.86	32.1	83.9	20.1
Regional background*	25	32	38	76
Normal levels in agricultural soils**	n.d.	13	16	47
Threshold values for agricultural soils of Andalusia***	20	100	200	300

* Galán et al., (2008); ** López-Arias and Grau-Corbí, (2004); *** Aguilar et al., (1999). All these values are referred to total concentration

Table 4. Trace elements contents (mean and range values) in different organs of vegetables growing in the agricultural plots under study.

Plant species	Organ	As	Cu	Pb	Zn
Ripe Onion (n=6)	Leaves	7.14 (0.75-15.2)	12.1 (3.5-31.4)	0.65 (0.10-1.92)	14.1 (12.8-16.2)
	Bulbs	0.31 (0.1-1.2)	4.15 (2.9-5.0)	0.33 (0.1-0.83)	23.9 (14.9-29.1)
	Roots	627 (267-905)	53.3 (39.5-80.5)	44.5 (35.9-51.8)	82.0 (43.2-135)
*Onion bulbs**		-	*4.0-6.0*	*1.1-2.0*	*22-32*
Young Onion (n=6)	Leaves	9.56 (0.10-15.2)	20.4 (14.1-20.5)	3.44 (0.1-8.8)	24 (19.2-28.0)
	Bulbs	8.81 (1.95-23.5)	13.3 (9.0-18.0)	2.65 (3.01-8.3)	62.4 (34.9-101)
Lettuce (plot 1, n=3)	Leaves	1.00 (0.86-1.1)	13.5 (13.3-13.6)	0.76 (0.75-0.77)	64 (61.6-66.0)
Lettuce (plot 2, n=3)	Leaves	0.93 (0.2-1.6)	9.48 (7.6-11.7)	0.45 (0.35-0.65)	49 (37.6-60.3)
*Lettuce leaves**		-	*6.0-8.0*	*0.7-3.6*	*44-73*
Chard (plot 1, n=3)	Leaves	0.45 (0.20-0.70)	14.8 (13.6-15.9)	0.90 (0.75-1.1)	43.9 (41.4-46.4)
Chard (plot 2, n=3)	Leaves	1.07 (0.77-1.30)	18.4 (16.0-19.9)	1.50 (1.2-1.76)	79.8 (54.2-110)
Potato (n=5)	Peel	0.1	11.5 (8.9-12.6)	0.1	21.1 (19.2-24.0)
	Tuber	0.1	10.3 (8.2-12.0)	0.1	25.0 (20.8-28.8)
*Potato tubers**		-	*3.0-6.6*	*0.5*	*10-26*
* Normal levels in vegetables**		0.0001-0.46	0.04-2.4	0.0004-0.8	0.5-118
Statutory limits***		1.00	-	1.00	

* Possible background values compiled by Kabata-Pendias and Mukherjee (2007); **Szefer and Nriagu, (2007); *** Davis and White (1981)

Table 5. Transfer coefficients (TC) for As, Cu, Pb and Zn at the different organs of the studied vegetables.

Vegetable	organ	As	Cu	Pb	Zn
Ripe onion	Leaves (n=6)	0.034	0.034	0.001	0.03
	Bulbs (n=6)	0.001	0.011	0.0004	0.05
	Roots (n=3)	3.03	0.150	0.052	0.18
Young onion	Leaves (n=7)	0.020	0.03	0.002	0.04
	Bulbs (n=7)	0.017	0.02	0.001	0.11
Lettuce	Leaves (n=6)	0.003	0.03	0.0005	0.13
Chard	Leaves (n=6)	0.003	0.04	0.001	0.14
Lemon	Leaves (n=3)	0.003	0.015	0.0007	0.03
	Pulp (n=3)	0.0002	0.010	0.0001	0.02
	Peel (n=3)	0.0002	0.005	0.0002	0.01
	Seed (n=3)	0.0002	0.014	0.00005	0.04
Potato	Peel (n=5)	0.0002	0.015	0.00004	0.035
	Tuber (n=5)	0.0002	0.014	0.00004	0.04