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Field study on the accumulation of trace elements by vegetables produced in the vicinity of abandoned pyrite mines



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HIGHLIGHTS

- Soils in kitchen gardens near pyrite mines are heavily contaminated with As, Cu, Pb, and Zn.
- Soil is mainly neutral, due to soil amendment, which contributes to the low bioavailability of TE.
- Generally, vegetables contain levels of these elements characteristic of uncontaminated plants.
- Risk is considerable where the soil pH is acidic, triggering high Zn bioavailable concentrations.
- If the soil pH is not controlled, there is a risk of consuming metal contaminated vegetables.

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ABSTRACT

To evaluate the accumulation of trace elements (TE) by vegetables produced in the vicinity of abandoned pyrite mines, eighteen different small farms were selected near three mines from the Portuguese sector of the Iberian Pyrite Belt (São Domingos, Aljustrel and Lousal). Total and bioavailable As, Cu, Pb, and Zn concentrations were analyzed in the soils, and the same TE were analyzed in three different vegetables, lettuce (*Lactuca sativa*), coriander (*Coriandrum sativum*), and cabbage (*Brassica oleracea*), collected at the same locations. The soils were contaminated with As, Cu, Pb, and Zn, since their total concentrations exceeded the considered soil quality guideline values for plant production in the majority of the sampling sites. The maximum total concentrations for those TE were extremely high in some of the sampling sites (e.g. 1851 mg As kg⁻¹ in São Domingos, 1126 mg Cu kg⁻¹ in Aljustrel, 4946 mg Pb kg⁻¹ in São Domingos, and 1224 mg Zn kg⁻¹ in Aljustrel). However, the soils were mainly circumneutral, a factor that contributes to their low bioavailable fractions. As a result, generally, the plants contained levels of these elements characteristic of uncontaminated plants, and accumulation factors for all elements < 1, typical of excluder plants. Furthermore, the estimated daily intake (EDI) for Cu and Zn, through the consumption of these vegetables, falls below the recommended upper limit for daily intake of these elements. The sampling site that stood out from the others was located at São João de Negrilhos (Aljustrel), where bioavailable Zn levels were higher, a consequence of the slight acidity of the soil. Therefore, the Zn content in vegetables was also higher, characteristic of contaminated plants, emphasizing the risk of Zn entering the human food chain via the consumption of crops produced on those soils.

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1. Introduction

The Iberian Pyrite Belt (IPB) is one of the largest metallogenetic provinces of massive sulphide deposits in the world (Pérez-López et al., 2008). The IPB exploitation dates back to the time of the Roman

Empire, and was very active during part of the nineteenth and the twentieth centuries (Matos and Martins, 2006). Populations have developed around these mining sites and tended to explore small farms in the vicinity of the mining areas (e.g. mine tailings, locally with ore waste). The incomplete closure process and abandonment of some of these mines did not reverse this situation and they still live there and farm soils that are potentially affected by mining activities. Several studies, aiming the assessment of the potential risk of accumulation of trace elements in food crops grown in soils potentially affected by mining activities, have been developed around the world (e.g. Boularbah

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et al., 2006; Dudka and Adriano, 1997; Liu et al., 2005; Gonzalez-Fernandez et al., 2011; Hamiani et al., 2010; Harmanescu et al., 2011; Ji et al., 2013; Roberts and Johnson, 1978; Xu et al., 2013; Zhuang et al., 2009). Some of these studies have pointed out the hazardous effects on consumer's health of eating food crops and/or vegetables produced in soils in the vicinity of mine works in Southern Morocco, due to Cd, Cu, Mn, Pb or Zn (Boularbah et al., 2006; Hamiani et al., 2010), in São Domingos, Portugal, especially due to Pb and As (Gonzalez-Fernandez et al., 2011), in Dabaoshan mine, South China, due to Cd, Pb and Zn (Zhuang et al., 2009), in the vicinity of Tongliang mining area, China, due to Cd and Pb (Xu et al., 2013), in abandoned metal mines in Goseong, Korea, due to rice contaminated with Cd (Ji et al., 2013), and in soils affected by the Chenzhou Pb/Zn mine spill (Hunan, China), due to As, Cu, Zn, Cd, and Pb (Liu et al., 2005).

According to Xu et al. (2013), the quality of garden soils in mine areas is still poorly characterized, pointing to a need for better knowledge of those soils to predict the risk of transfer of pollutants into the food chain. To our best knowledge, the study from Gonzalez-Fernandez et al. (2011) was the only one made in the Portuguese sector of the IPB, in São Domingos.

Micronutrients, such as Cu, Cr, F, Mo, Ni, Se or Zn, are essential for plant growth and/or human nutrition, being the cause of undesirable pathological conditions when in deficiency, but they can also be toxic to both animals and humans at high concentrations (Abbasi et al., 2013; Fraga, 2005; McLaughlin et al., 1999). Regulatory agencies should define intake, supplementation, and toxicity levels of micronutrients for humans, which can be used as dietary reference intake (DRI) values. The upper limit (UL) is intended as a maximum level of daily nutrient intake that is likely to pose no risk of adverse effects. Other trace elements (TE), for example As, Cd, Hg and Pb, may also accidentally enter the food chain and pose health risks to humans and animals (McLaughlin et al., 1999). For those TE, we are only interested in the UL values, since no recommended adequate intake can be outlined. In fact, all TE, whether essential or non-essential, above threshold concentration levels, can cause morphological abnormalities, reduced growth, increased mortality and mutagenic effects in humans (Abbasi et al., 2013; Fraga, 2005; McLaughlin et al., 1999).

There are different human exposure routes to TE from soils, like ingestion and inhalation of soil and dust particles, dermal absorption from soil and dust particles, and consumption of vegetables grown on contaminated land (Provoost et al., 2006). From those, the most important pathway is the consumption of contaminated vegetables (Baraud, and Leleyter, 2012; Provoost et al., 2006; Xu et al., 2013). In fact, the uptake and accumulation of metals in the edible portions of some food plants may make crops unsafe to be eaten if they are likely to be consumed over a long period of time (ICRCL 59/83, 1987). Uptake depends on the concentrations of the metals in the soil, and particularly, on the chemical forms in which they are present. Factors such as the soil pH value, the plant species, and the proportion of home-grown food in the diet also influence the importance of this hazard (Gonzalez-Fernandez et al., 2011; ICRCL 59/83, 1987; Ji et al., 2013; Xu et al., 2013; Zhuang et al., 2009).

In most countries, risk assessment and regulation of trace elements in soils are based on total concentration threshold values (Harmsen, 2007; Provoost et al., 2006). Despite the importance of their quantification, many studies suggest that the bioavailable fraction of the element is more suitable than their total concentration for predicting TE element transfer to plants or other soil organisms (Alexander, 2000; Alvarenga et al., 2013; Baraud, and Leleyter, 2012; Harmsen, 2007; ISO/DIS, 17402, 2006; NRC Committee, 2003; Peijnenburg et al., 2007; Semple et al., 2004; Xu et al., 2013). The bioavailable fraction of a contaminant may be tentatively assessed using chemical extraction procedures, which determine a specific available fraction of a well-defined class of contaminant, that can be functionally defined (e.g. plant-available, mobile, mobilizable, exchangeable form) (Alvarenga et al., 2013; Gupta et al., 1996; Peijnenburg et al., 2007).

Taking all these in account, the aims of the study were: (i) to evaluate the contamination of soils from small farms, or kitchen gardens, in the vicinity of abandoned mines (Iberian Pyrite Belt, Portugal), with potentially toxic TE (As, Cu, Pb, and Zn), assessing their total and bioavailable concentrations; (ii) to evaluate the accumulation of TE in vegetables produced in these soils; and (iii) to assess the potential health risk to TE via consumption of those vegetables.

The results from this study can give information not only on the potential risk of eating vegetables grown on these soils, but also on the soil properties that control the bioavailability of TE to plants. With this information, best suitable agricultural practices can be suggested to the local populations in order to produce vegetables with lower risk of TE uptake and accumulation.

2. Materials and methods

2.1. Characterization of the sampling sites

Eighteen different sampling sites were selected in kitchen gardens, or small farms, from three mines located in the Portuguese sector of the Iberian Pyrite Belt (IPB): Aljustrel (sites 1 to 8), São Domingos (sites 9 to 14) and Lousal (sites 15 to 18) (Figs. 1–3 – Supplementary Geospatial Data from Google Earth™). This region is characterized by a Mediterranean mesothermic humid climate, with hot and dry summers and with an annual average rainfall of 500–650 mm (Reis and Gonçalves, 1987). A control sampling site (site 19) was selected in a location without the influence of mining activities, near Alcácer do Sal (38°22'2.71"N; 8°29'32.68"W).

The sampling sites in the mining areas were selected taking in account the location of the kitchen gardens explored by the local population. The potential locations were first selected in a map and afterwards in situ, with the collaboration of the residents. The sampling locations were identified by their geographical coordinates (Figs. 1–3) and by the owner's identification (data not shown). Sites 1 to 8 were located in Aljustrel mining district (1 to 5, near Aljustrel village, and 6 to 8, near São João de Negrilhos village), sites 9 to 14 were located in São Domingos area, and sites 15 to 18 in Lousal area. São João de Negrilhos village, in Aljustrel mining district, is located in an ancient mine waste deposit, which points out to a high contaminated background material.

The samples were mainly collected at kitchen gardens with a small area, located in the backyard of the houses (maximum acreage size of 500 m²), but some of them were located outside the village, and the size of the cultivated area was large enough to be considered as a small farm (sites 10, 13 and 14, mean acreage size of 2000 m²). In the majority of the sampling sites the application of organic amendments (e.g. manure) on the soils in order to improve their quality from the background soils was obvious.

The mine works at these mines were very intensive during part of the nineteenth and the twentieth centuries: Aljustrel mine was extensively exploited from 1850 to 1991, São Domingos was exploited from 1857 to 1966, and Lousal from 1900 to 1988 (Matos and Martins, 2006). Afterwards, the production was discontinued in those mines, and only the production in Aljustrel was again reestablished in 2008. Presently, the Aljustrel mining project is developed by Almina Company, which activity is focused on the Feitais and Moinho ore lense exploitation, and Cu concentrate production at the Santo Antão ore plant. Each selected IPB deposit presents different ore grades: São Domingos is characterized by a single ore lense with 1.25% Cu, 2–3% Zn and 45–48% S (Matos et al., 2012); several ore lenses were exploited at Lousal until 500 m depth, with an annual production of 230,000–250,000 Mg of concentrate with 45% S and 0.7% Cu (Relvas et al., 2012), and at Aljustrel the mining works were developed in the Moinho, Algarves, S. João and Feitais ore deposits, with high Zn and Cu reserves (Silva et al., 1997): Feitais 54 Mt (0.43% Cu, 3.43% Zn, and 1.12% Pb); Moinho 44.1 Mt (0.85% Cu, 2.983% Zn, and 1.10% Pb); and São João 45 Mt (0.87% Cu, 3.37% Zn,

and 1.20% Pb). All three IPB sites present acid mine drainage related with old tailings and acid water dams and lagoons (Abreu et al., 2010).

2.2. Sampling and sample pre-treatments

The vegetables were selected considering the regional consumption habits of the population, and the season, in order to increase the likelihood of finding those vegetables. Three plants were selected, lettuce (*Lactuca sativa*), coriander (*Coriandrum sativum*), and cabbage (*Brassica oleracea*), which were found in the majority of the gardens.

The sampling took place during the spring of 2012. Topsoil (0–20 cm) and vegetables, whenever available, were collected at each sampling site, using a random sampling method. Each individual sample of soil was collected as a composite sample, representative of the soil at that kitchen garden, and the plant sample was also a composite sample of the edible part of different plants of the same species collected at that site. Samples were stored in polyethylene bags and transported to the lab.

Soils were air-dried, passed through a 2 mm sieve, and stored in the dark in hermetic flasks until analysis.

Plant fresh samples were weighed, thoroughly washed with tap water to remove any attached particles, and then rinsed three times with deionized water. The samples were dried at 70 °C for 48 h, weighed again, and ground in an electric mill. Dried samples were stored in polyethylene bags, in the dark, until analysis.

2.3. Soil general physicochemical characterization

Soils were subjected to general physicochemical characterization. Particle-size distribution was determined by the pipet method (Gee and Bauder, 1986). Soil pH was determined in a soil to deionized water suspension of 1:2.5 (w/v), and electrical conductivity (EC) in a soil to deionized water suspension of 1:5 (w/v). Total oxidizable organic carbon (Corg) was determined according to Walkley and Black (1934). Soil cation exchange capacity (CEC) was determined using the ammonium acetate (pH 7) method (Sumner and Miller, 1996). Total nitrogen was analyzed by the Kjeldahl method. Extractable P and K were determined using the Egner–Riehm method (Riehm, 1958). All measurements, except particle-size distribution, were carried out in triplicate and reported in a dry weight basis.

2.4. Quantification of trace elements in soils

Total trace element (As, Cu, Pb and Zn) concentrations were determined by either flame or electrothermal atomic absorption spectrometry after digestion of the samples with aqua regia according to ISO 11466 (1995); using a Varian apparatus (SpectrAA 220FS, 220Z, and 110Z). Three independent replicates were performed for each sample and blanks were measured in parallel. All concentrations were reported in a dry weight basis. A certified reference material, Buffalo Lake Sediment (SRM 2704), from NIST (National Institute of Standard Technology), was analyzed to validate the aqua regia extraction procedure. Results showed a good agreement between the obtained and the certified values for the metals analyzed (mean relative errors < 10%).

Trace element extractable fractions were determined using two different single step extractions: a mobile fraction (extracted by 0.01 M CaCl₂, pH 5.7, without buffer) (Houba et al., 1996; Pueyo et al., 2004), sometimes referred as the effective bioavailable metal fraction, and a mobilizable fraction (extracted by a solution of 0.5 M NH₄CH₃COO, 0.5 M CH₃COOH and 0.02 M EDTA, pH 4.7) (Hammer and Keller, 2002), considered as a potentially bioavailable metal fraction (Gupta et al., 1996; Harmsen, 2007). Extractions were performed with 2 h horizontal reciprocate shaking, on a 1:10 (w/v) soil to solution ratio, at room temperature. The extract was separated from the solid residue by centrifugation at 3000 g for 10 min. Three independent replicates were performed for each sample and blanks were also prepared. Arsenic, Cu, Pb and Zn concentrations in both extracts were also determined by flame

or electrothermal atomic absorption spectrometry, using the referred equipment. All concentrations were reported in a dry weight basis.

2.5. Quantification of trace elements in the plant tissues

Approximately 2 g of dried plant sample was ashed in a muffle furnace at 500 °C for 6 h, dissolved with 10 mL of 3 M HCl and evaporated to near dryness twice, dissolved again with the same acid solution, filtered (Whatman® 40), and adjusted to a volume of 100 mL with ultra-pure water. Plant digested samples were analyzed for total As, Cu, Pb and Zn by flame or electrothermal atomic absorption spectrometry, using a Varian apparatus (SpectrAA 220FS, 220Z, and 110Z). All concentrations were reported in a dry weight basis.

2.6. Data treatment

Accumulation factors (AF), i.e. the ratios of the trace element concentration in the shoot to the total trace element concentration in the soil, were calculated. The AF for each pair trace element/plant are related to the ability of the vegetable, *L. sativa*, *B. oleracea*, and *C. sativum*, to accumulate a particular trace element with respect to its concentration in the soil.

The estimated daily intake (EDI) of trace elements, via the consumption of these vegetables, depends on both trace element concentrations in the vegetable and the amount of the vegetable which is consumed per day. The EDI was calculated using the formulae adapted from Zhuang et al. (2009):

$$EDI = C_{\text{trace element}} \times \text{MDC}$$

where:

EDI estimated daily intake ($\mu\text{g dia}^{-1}$);
 $C_{\text{trace element}}$ trace element concentration in the plant ($\mu\text{g g}^{-1}$ fresh weight);
 MDC mean daily consumption of each vegetable ($\text{g fresh weight dia}^{-1}$).

The concentration of trace elements in the plant ($C_{\text{trace element}}$), reported in a dry weight basis, was converted to a fresh weight basis ($\mu\text{g g}^{-1}$ fresh weight) by multiplying the trace element concentration of each element ($\mu\text{g g}^{-1}$ dry weight) by the ratio of the dry weight to the fresh weight of the sample (both fresh and dry weights were registered for each sample).

The mean daily consumption of each vegetable (MDC), by an adult person, was estimated considering a study made in Portugal (Lopes et al., 2006), and was: 100 g for cabbage, 50 g for lettuce and 5 g for coriander. We found homegrown information more suitable to be used than the international standard portion sizes provided by the World Health Organization (WHO), that lacks the standard portion size for vegetables WHO (2006).

In the absence of Portuguese or European Community regulations, the calculated EDIs were compared with the “Dietary Reference Intakes” from the National Academies (USA) (National Academies, 2001).

2.7. Statistical analysis

All data were checked for homogeneity of variance and normality (Kolmogorov–Smirnov test) and, when possible, subjected to one-way ANOVA. Data not satisfying assumptions for ANOVA were analyzed non-parametrically using the Kruskal–Wallis ANOVA by Ranks test. Whenever significant differences were found ($P < 0.05$) a post-hoc Tukey HSD test was used to further elucidate differences among means ($P < 0.05$). Pearson’s correlation coefficients (r) were calculated between the soil main physicochemical properties and their level of trace elements in the different fractions ($P < 0.05$), and between the

levels of trace elements in plants, the main physicochemical properties of soils, and the levels of trace elements in the different fractions. All statistical analysis was carried out with the software Statistica 6.0 (StatSoft Inc., 2001).

3. Results and discussion

3.1. Soil general characteristics

The soils were mainly characterized by a moderately coarse texture (sandy loam), and some by a coarse texture (loamy sand) (Table 1). Taking the texture in account, the organic matter (OM) level of the soils was medium, except at sites 2, 7, 11, 13, 15 and 18, in which the levels of organic matter were considered high (Santos, 1996). These OM levels in soils contrast with the background levels of soils in the south of Portugal, where OM levels in soils are expected to be lower than 1% (JRC, 2008). In fact, the soil from the control soil (site 19) was the one with the lower level of soil OM. From the results, it is obvious the effort of the local farmers to improve the quality of the soils by adding organic amendments, like manure. The organic matter addition also contributed to the increase in the soil CEC, which were higher than can be expected for coarse to medium coarse soils. The main macronutrient (N, P and K) concentrations in soils were also high (Santos, 1996), which indicated the good nutritional status of these soils.

The majority of the soils were neutral, with pH values between 6.6 and 7.5. Soil from site 12 was slightly alkaline, and soils from sites 2 and, particularly, site 7, with a pH value of 5.87, were slightly acidic (Santos, 1996). These last two sites were more similar to the pH values from background soils, which are sometimes affected by acid mine drainage (Alvarenga et al., 2004, 2012; Pérez-López et al., 2008).

3.2. Trace element total and extractable concentrations

In order to evaluate if soils were contaminated with TE, and in the absence of Portuguese or European Community regulations, soil quality guideline values (SQGV) from different countries were used. The use of SQGV from a different country could be criticized, due to the existing differences between soil types, climates, and so on. In this study, the total TE concentrations in soils were compared with the Canadian SQGV (CCME, 2006) and with the tentative UK trigger concentrations for selected inorganic contaminants (ICRCL 59/83, 1987). The Canadian SQGV are more conservative than some of the soil clean-up standards

proposed in many European countries for some elements, but the opposite is true for others (Provoost et al., 2006). They have the merit of considering more TE than other regulations, and of taking into account the soil use (CCME, 2006; Provoost et al., 2006). Moreover, the Canadian SQGV used to be recommended by the Portuguese Environmental Agency to assess contaminated soils in Portugal. The Canadian SQGV considered that, if a soil is to be used for agricultural purposes, the total As, Cu, Pb, and Zn concentrations should be lower than 12, 63, 70 and 200 mg kg⁻¹, respectively (CCME, 2006). As for the tentative UK trigger concentrations (ICRCL 59/83, 1987), As and Pb are considered in a group of elements which “may pose hazards to health”, while Cu and Zn are considered in a different group, with “phytotoxic elements, but not normally hazardous to human health”. In this case, As, Cu, Pb, and Zn concentrations should be lower than 10, 130, 500 and 300 mg kg⁻¹, respectively, if the soils are to be used as domestic gardens, when considering As and Pb, or for any use where plants are supposed to grow, when considering Cu and Zn. Considering the results (Tables 2, 3, 4 and 5), the soils were considered contaminated with TE, since the total As, Cu, Pb, and Zn concentrations exceeded the lower SQGV of the above-mentioned guidelines in 100, 72, 94 and 75% of the sampling sites, respectively. The maximum total concentrations for those trace elements were extremely high in some of the sampling sites: e.g. 1851 mg As kg⁻¹ in São Domingos (site 9), 1126 mg Cu kg⁻¹ in Aljustrel (site 4), 4946 mg Pb kg⁻¹ in São Domingos (site 9), and 1224 mg Zn kg⁻¹ in Aljustrel (site 3).

However, as discussed before, the soil pH values were mainly neutral, a factor that contributed to the low TE bioavailability, especially their effective bioavailable fraction (extracted with CaCl₂ 0.01 M), was very low. The effective bioavailable fraction of As and Cu was below 1% of the total concentration at all sites. The effective bioavailable fraction of Pb was below the detection limit at all sites, and Zn effective bioavailable fraction was below 1% of the total concentration, or below the detection limit, except at site 7 where 3.8% of the total concentration could be considered as effective bioavailable. Considering these results, it is possible to say that, despite the high total concentration of TE in the soils, the risk of their uptake by the vegetables is very low since they are mostly in a non-extractable or non-bioavailable form. This fact can be mainly attributed to the organic amendment of those soils, which was evident from their OM content, much higher than can be found in background soils (Alvarenga et al., 2004, 2012), and even in the non-contaminated soils in Portugal (JRC, 2008). Several studies have proved that organic materials, such as biosolids, manures and composts, rich in

Table 1
Soil general physicochemical characteristics (mean ± standard deviation, n = 3). Values in a column marked with the same letter are not significantly different (Tukey HSD test, P > 0.05).

| Sampling sites | Texture ^a | CEC (meq 100 g ⁻¹) | pH(H ₂ O) (1:2.5) | EC (dS m ⁻¹) | OM (%) | N _{Kjeldahl} (%) | P _{extractable} (mg P ₂ O ₅ kg ⁻¹) | K _{extractable} (mg K ₂ O kg ⁻¹) |
|----------------|----------------------|-----------------------------------|---------------------------------|-----------------------------|----------------|------------------------------|--|---|
| Aljustrel | 1 Sandy loam | 16.9 ± 0.1cdef | 7.28 ± 0.04ghi | 0.44 ± 0.02i | 3.8 ± 0.0i | 0.29 ± 0.00bcd | 2351 ± 169d | 532 ± 12cd |
| | 2 Sandy loam | 19.5 ± 0.8def | 6.51 ± 0.04b | 0.22 ± 0.02def | 6.8 ± 0.1def | 0.4 ± 0.1defg | 2438 ± 134d | 776 ± 10ef |
| | 3 Sandy loam | 14.4 ± 2.5bcde | 7.29 ± 0.00hi | 0.80 ± 0.03k | 3.2 ± 0.1k | 0.25 ± 0.02bc | 7611 ± 23a | 152 ± 5a |
| | 4 Loamy sand | 14.8 ± 0.4bcdef | 7.11 ± 0.04e | 0.26 ± 0.02fg | 4.3 ± 0.2fg | 0.34 ± 0.00bcdef | 2483 ± 135d | 389 ± 12bc |
| | 5 Loamy sand | 9.7 ± 0.7abc | 7.54 ± 0.03k | 0.25 ± 0.02fg | 3.2 ± 0.1fg | 0.21 ± 0.01ab | 1080 ± 72ab | 320 ± 18b |
| | 6 Sandy loam | 12.6 ± 5.2bcd | 7.19 ± 0.07efgh | 0.21 ± 0.01def | 3.8 ± 0.2def | 0.23 ± 0.00bc | 2284 ± 87d | 370 ± 4b |
| | 7 Sandy loam | 19.0 ± 1.0def | 5.87 ± 0.03a | 0.72 ± 0.01j | 8.3 ± 0.2j | 0.5 ± 0.2fg | 2269 ± 129d | 826 ± 17f |
| | 8 Sandy loam | 19.6 ± 2.1def | 7.22 ± 0.08efgh | 0.10 ± 0.01a | 4.9 ± 0.1a | 0.33 ± 0.00bcde | 4040 ± 557f | 1048 ± 43g |
| São Domingos | 9 Loamy sand | 3.2 ± 1.0a | 7.29 ± 0.02hi | 0.11 ± 0.00ab | 4.2 ± 0.1ab | 0.25 ± 0.00bc | 3811 ± 74ef | 140 ± 6a |
| | 10 Sandy loam | 3.1 ± 0.2a | 7.37 ± 0.04ij | 0.19 ± 0.00de | 4.8 ± 0.1de | 0.30 ± 0.01bcd | 993 ± 59ab | 634 ± 15de |
| | 11 Loamy sand | 14.8 ± 0.4bcdef | 7.26 ± 0.02fghi | 0.47 ± 0.02i | 5.7 ± 0.1i | 0.30 ± 0.00bcd | 1469 ± 198bc | 1189 ± 193g |
| | 12 Loamy sand | 22.9 ± 0.1ef | 7.69 ± 0.00l | 0.26 ± 0.01fg | 4.8 ± 0.3fg | 0.47 ± 0.01efg | 3828 ± 195ef | 644 ± 5de |
| | 13 Loamy sand | 33.7 ± 1.2g | 7.14 ± 0.01ef | 0.38 ± 0.05h | 9.5 ± 0.4h | 0.71 ± 0.03h | 3371 ± 75e | 1374 ± 24h |
| | 14 Sandy loam | 20.2 ± 0.3def | 7.46 ± 0.00jk | 0.13 ± 0.00abc | 4.9 ± 0.1abc | 0.34 ± 0.01cdef | 1646 ± 130c | 595 ± 20d |
| Lousal | 15 Loamy sand | 23.4 ± 9.3ef | 7.22 ± 0.04efgh | 0.13 ± 0.01abc | 8.1 ± 0.2abc | 0.56 ± 0.01gh | 3411 ± 90e | 259 ± 7ab |
| | 16 Sandy loam | 20.0 ± 0.4def | 7.3 ± 0.1hi | 0.30 ± 0.02g | 4.2 ± 0.1g | 0.26 ± 0.03bc | 765 ± 75a | 281 ± 16ab |
| | 17 Sandy loam | 18.9 ± 0.1def | 7.15 ± 0.01efg | 0.18 ± 0.00cde | 4.2 ± 0.3cde | 0.36 ± 0.01cdef | 678 ± 78a | 655 ± 69de |
| 18 Sandy loam | 23.8 ± 0.2f | 6.87 ± 0.01d | 0.22 ± 0.02ef | 5.2 ± 0.0ef | 0.47 ± 0.07efg | 2246 ± 231d | 310 ± 23b | |
| Control | 19 Loamy sand | 8 ± 1ab | 6.69 ± 0.04c | 0.16 ± 0.00bcd | 1.6 ± 0.0bcd | 0.07 ± 0.03a | 1062 ± 98ab | 283 ± 7ab |

^a Particle-size and soil texture classification were performed according to the United States Department of Agriculture (USDA) textural classes of soils.

Table 2

Concentration of As in different soil extracts (mean \pm standard deviation, n = 3). Values in a column marked with the same letter are not significantly different (Tukey HSD test, $P > 0.05$).

| Sampling sites | | As (LC = 12 mg kg ⁻¹) ^a | | | | |
|----------------|----|--|----------------------|------------------------|---------------------|--------------------------|
| | | Total | | Effective bioavailable | | Potentially bioavailable |
| | | mg kg ⁻¹ | mg kg ⁻¹ | % total | mg kg ⁻¹ | % total |
| Aljustrel | 1 | 68.9 \pm 4.5a | 0.135 \pm 0.003abc | 0.20 | 4.1 \pm 0.1cd | 5.9 |
| | 2 | 23.4 \pm 1.2a | 0.049 \pm 0.001ab | 0.21 | 0.52 \pm 0.03a | 2.2 |
| | 3 | 173.5 \pm 27.9abc | 0.065 \pm 0.001abc | 0.04 | 1.9 \pm 0.3abc | 1.1 |
| | 4 | 97.9 \pm 4.7ab | 0.151 \pm 0.008bc | 0.15 | 3.6 \pm 0.3bcd | 3.7 |
| | 5 | 91.3 \pm 9.0ab | 0.059 \pm 0.009abc | 0.06 | 1.9 \pm 0.2abc | 2.1 |
| | 6 | 167.0 \pm 66.2abc | 0.18 \pm 0.02cd | 0.11 | 7.1 \pm 0.5e | 4.2 |
| | 7 | 381.9 \pm 27.7cd | 0.8 \pm 0.2g | 0.22 | 6.2 \pm 0.2de | 1.6 |
| São Domingos | 8 | 329.1 \pm 27.8bcd | 0.30 \pm 0.2de | 0.09 | 18.5 \pm 2.1g | 5.6 |
| | 9 | 1850.8 \pm 282.5e | 0.42 \pm 0.01f | 0.02 | 16.9 \pm 2.8g | 0.9 |
| | 10 | 106.1 \pm 6.9ab | 0.06 \pm 0.01abc | 0.06 | 1.2 \pm 0.1ab | 1.1 |
| | 11 | 140.6 \pm 17.3abc | 0.05 \pm 0.01ab | 0.04 | 0.40 \pm 0.04a | 0.3 |
| | 12 | 570.9 \pm 203.6d | 0.38 \pm 0.01ef | 0.07 | 13.7 \pm 1.3f | 2.4 |
| | 13 | 71.7 \pm 32.9a | 0.100 \pm 0.003abc | 0.14 | 3.5 \pm 0.2bc | 4.8 |
| | 14 | 86.7 \pm 8.0ab | 0.078 \pm 0.003abc | 0.09 | 2.7 \pm 0.4abc | 3.1 |
| Lousal | 15 | 74.3 \pm 22.2a | 0.045 \pm 0.006ab | 0.06 | 1.8 \pm 0.2abc | 2.4 |
| | 16 | 76.3 \pm 1.5ab | 0.06 \pm 0.02abc | 0.08 | 3.3 \pm 0.3bc | 4.3 |
| | 17 | 27.4 \pm 2.7a | 0.037 \pm 0.005ab | 0.13 | 0.433 \pm 0.004a | 1.6 |
| | 18 | 40.4 \pm 4.8a | 0.063 \pm 0.007abc | 0.16 | 1.58 \pm 0.05abc | 3.9 |
| Control | 19 | 1.7 \pm 0.3a | 0.014 \pm 0.001a | 0.81 | 0.09 \pm 0.01a | 5.4 |

^a LC: limit concentration of the Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health considering agricultural use (CCME, 2006); total trace element content: aqua regia digestion; potentially bioavailable trace element content: extracted by a solution of 0.5 M NH₄CH₃COO, 0.5 M CH₃COOH and 0.02 M EDTA, pH 4.7; effective bioavailable trace element content: extracted by CaCl₂ 0.01 M.

organic matter, have proved to be successful in reducing the mobility of contaminants in multi-element polluted soils (Adriano et al., 2004; Alvarenga et al., 2008; Karami et al., 2011).

A potentially bioavailable TE fraction was also determined, in order to ascertain if the potential risk of metal mobilization is higher in soils with lower pH or with higher content of soluble OM. Those soil conditions are expected to induce higher metal mobilization for the same total TE content (Alvarenga et al., 2008; Xu et al., 2013), but contradictory effects on the mobility and bioavailability of specific elements were also reported as a consequence of the soil amendment with

organic materials (Beesley et al., 2010; Beesley and Dickinson, 2010; Xu et al., 2013), emphasizing the importance of the measurement of that TE fraction. The potentially bioavailable As content was low, with values ranging from 0.3 to 5.9% of the total, but the same was not true for the other TE, with potentially bioavailable fractions ranging from 16.4 to 37.8% of the total, for Cu, from 1.8 to 52.3% of the total for Pb, and from 1.66 to 79.88% of the total for Zn. Again, the highest value for Zn potentially bioavailability content was at site 7. This aspect is of great concern and it should be considered as an alert to the gardens' owners. In fact, although the present risk of TE availability is controlled,

Table 3

Concentration of Cu in different soil extracts (mean \pm standard deviation, n = 3). Values in a column marked with the same letter are not significantly different (Tukey HSD test, $P > 0.05$).

| Sampling sites | | Cu (LC = 63 mg kg ⁻¹) ^a | | | | |
|----------------|----|--|------------------------|---------|--------------------------|---------|
| | | Total | Effective bioavailable | | Potentially bioavailable | |
| | | mg kg ⁻¹ | mg kg ⁻¹ | % total | mg kg ⁻¹ | % total |
| Aljustrel | 1 | 206.2 \pm 2.8bcd | 0.30 \pm 0.01abcd | 0.15 | 78.0 \pm 2.7def | 37.8 |
| | 2 | 74.1 \pm 1.3ab | 0.18 \pm 0.01a | 0.24 | 24.4 \pm 2.2ab | 33.0 |
| | 3 | 488.7 \pm 34.3g | 0.71 \pm 0.01e | 0.14 | 114.5 \pm 13.1fg | 23.4 |
| | 4 | 1126.1 \pm 182.0h | 0.91 \pm 0.02f | 0.08 | 440.4 \pm 55.3h | 39.1 |
| | 5 | 334.9 \pm 47.1def | 0.26 \pm 0.04abc | 0.08 | 70.2 \pm 7.3cde | 21.0 |
| | 6 | 370.3 \pm 46.8efg | 0.39 \pm 0.02cd | 0.10 | 123.2 \pm 5.9g | 33.3 |
| | 7 | 390.9 \pm 38fg | 0.9 \pm 0.2f | 0.24 | 91.6 \pm 6.4efg | 23.4 |
| | 8 | 349.5 \pm 5.1efg | 0.4 \pm 0.1d | 0.12 | 88.1 \pm 11.1efg | 25.2 |
| São Domingos | 9 | 137.6 \pm 7.2abc | 0.21 \pm 0.02ab | 0.15 | 32.7 \pm 4.3abc | 23.8 |
| | 10 | 60.5 \pm 0.9a | 0.16 \pm 0.02a | 0.26 | 22.2 \pm 1.7ab | 36.7 |
| | 11 | 52.6 \pm 1.5a | 0.19 \pm 0.02ab | 0.35 | 8.6 \pm 0.2a | 16.4 |
| | 12 | 239.0 \pm 18.3cde | 0.33 \pm 0.01bcd | 0.14 | 52.5 \pm 3.9bcde | 22.0 |
| | 13 | 60.1 \pm 1.4a | 0.1352 \pm 0.0003a | 0.23 | 15.4 \pm 0.7ab | 25.6 |
| | 14 | 147.5 \pm 1.4abc | 0.147 \pm 0.006a | 0.10 | 40.3 \pm 5.1abcd | 27.3 |
| Lousal | 15 | 104.8 \pm 3.0abc | 0.134 \pm 0.010a | 0.13 | 29.1 \pm 1.5abc | 27.8 |
| | 16 | 46.2 \pm 1.7a | 0.099 \pm 0.012a | 0.21 | 14.5 \pm 0.8ab | 31.3 |
| | 17 | 40.7 \pm 1.6a | 0.106 \pm 0.006a | 0.26 | 14.8 \pm 0.3ab | 36.4 |
| | 18 | 113.7 \pm 1.7abc | 0.18 \pm 0.02a | 0.16 | 25.0 \pm 3.2ab | 22.0 |
| Control | 19 | 17.3 \pm 0.8a | 0.1111 \pm 0.0001a | 0.64 | 8.9 \pm 1.7a | 51.6 |

^a LC: limit concentration of the Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health considering agricultural use (CCME, 2006); total trace element content: aqua regia digestion; mobilizable trace element content: extracted by a solution of 0.5 M NH₄CH₃COO, 0.5 M CH₃COOH and 0.02 M EDTA, pH 4.7; mobile trace element content: extracted by CaCl₂ 0.01 M.

Table 4
Concentration of Pb in different soil extracts (mean \pm standard deviation, n = 3). Values in a column marked with the same letter are not significantly different (Tukey HSD test, $P > 0.05$).

| Sampling sites | | Pb (LC = 70 mg kg ⁻¹) ^a | | | | | |
|----------------|----|--|-----|------------------------|-------------------|--------------------------|---------|
| | | Total | | Effective bioavailable | | Potentially bioavailable | |
| | | mg kg ⁻¹ | | mg kg ⁻¹ | % total | mg kg ⁻¹ | % total |
| Aljustrel | 1 | 242.0 \pm 8.8a | <DL | <0.7 | 74.0 \pm 2.2e | 30.6 | |
| | 2 | 103.4 \pm 2.6a | <DL | <1.6 | 32.9 \pm 3.8abc | 31.8 | |
| | 3 | 336.7 \pm 20.6a | <DL | <0.5 | 22.8 \pm 2.2ab | 6.8 | |
| | 4 | 230.4 \pm 18.1a | <DL | <0.7 | 42.2 \pm 2.5bcd | 18.3 | |
| | 5 | 231.4 \pm 21.8a | <DL | <0.7 | 30.3 \pm 2.1ab | 13.1 | |
| | 6 | 373.3 \pm 33.1a | <DL | <0.4 | 76.0 \pm 2.1e | 20.4 | |
| | 7 | 1597.5 \pm 648.7c | <DL | <0.1 | 28.2 \pm 4.7ab | 1.8 | |
| São Domingos | 8 | 972.9 \pm 29.1b | <DL | <0.2 | 71.7 \pm 5.4ab | 7.4 | |
| | 9 | 4945.7 \pm 192.8d | <DL | <0.0 | 257.6 \pm 30.9g | 5.2 | |
| | 10 | 269.8 \pm 13.7a | <DL | <0.6 | 62.1 \pm 3.0de | 23.0 | |
| | 11 | 334.8 \pm 24.3a | <DL | <0.5 | 58.8 \pm 1.4cde | 17.6 | |
| | 12 | 1919.3 \pm 354.6c | <DL | <0.1 | 387.0 \pm 16.5h | 20.2 | |
| | 13 | 382.8 \pm 100.9a | <DL | <0.4 | 152.1 \pm 3.3f | 39.7 | |
| | 14 | 341.8 \pm 13.2a | <DL | <0.5 | 73.1 \pm 5.2e | 21.4 | |
| Lousal | 15 | 253.0 \pm 18.8a | <DL | <0.7 | 57.9 \pm 1.9cde | 22.9 | |
| | 16 | 316.3 \pm 7.3a | <DL | <0.5 | 81.1 \pm 2.9e | 25.6 | |
| | 17 | 62.2 \pm 2.2a | <DL | <.7 | 20.6 \pm 0.8ab | 33.1 | |
| | 18 | 260.5 \pm 48.4a | <DL | <.6 | 136.3 \pm 4.1f | 52.3 | |
| Control | 19 | 23.6 \pm 6.3a | <DL | <7.1 | 14.3 \pm 1.2a | 60.6 | |

^a LC: limit concentration of the Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health considering agricultural use (CCME, 2006); total trace element content: aqua regia digestion; potentially bioavailable trace element content: extracted by a solution of 0.5 M NH₄CH₃COO, 0.5 M CH₃COOH and 0.02 M EDTA, pH 4.7; effective bioavailable trace element content: extracted by CaCl₂ 0.01 M; DL: detection limit; DL (Pb) = 1.667 mg kg⁻¹.

namely by the neutral to alkaline soil pH values, if the chemical characteristics of the soils change, like in site 7, there is a risk of metal mobilization which can be uptaken by the vegetables raised on those gardens.

The calculation of the correlation coefficients (r) between the main physicochemical properties of soils and their level of TE in the different fractions: total, effective bioavailable and potentially bioavailable (Table S1 – Supplementary material), allows us to validate the importance of the pH control in the low effective bioavailable levels of Cu and Zn, which is evident from the negative correlation observed between pH and Cu, and Zn effective bioavailable fractions. The soil OM content correlates positively with the levels of As and Zn in their effective bioavailable fraction, suggesting that the risk of

mobilization of these elements is higher in soils where the OM content of the soil is also higher, perhaps due to the rise in soluble organic compounds, which can contribute to the increased mobilization of those elements. Beesley et al. (2010) found a 30 fold increase in Cu and As concentrations in soil pore water after adding biochar and greenwaste compost, associated with significant increases in dissolved organic C and pH, whereas, in their experiment, Zn and Cd significantly decreased.

On the other hand, there were significant positive correlations between the total contents of As, Cu, Pb and Zn and their potentially bioavailable fractions, which allows us to observe that, if the soil properties with a direct influence in TE mobilization change, TE are

Table 5
Concentration of Zn in different soil extracts (mean \pm standard deviation, n = 3). Values in a column marked with the same letter are not significantly different (Tukey HSD test, $P > 0.05$).

| Sampling sites | | Zn (LC = 200 mg kg ⁻¹) ^a | | | | | |
|----------------|----|---|------------------|------------------------|---------------------|--------------------------|---------|
| | | Total | | Effective bioavailable | | Potentially bioavailable | |
| | | mg kg ⁻¹ | | mg kg ⁻¹ | % total | mg kg ⁻¹ | % total |
| Aljustrel | 1 | 341.4 \pm 52.9defg | <DL | <0.05 | 67.1 \pm 39.2abcd | 19.66 | |
| | 2 | 212.1 \pm 12.9abcde | 0.26 \pm 0.02a | 0.12 | 75.6 \pm 9.8bcde | 35.62 | |
| | 3 | 1223.7 \pm 115.0k | <DL | <0.01 | 20.3 \pm 1.7a | 1.66 | |
| | 4 | 1022.9 \pm 37.8j | 0.25 \pm 0.04a | 0.02 | 191.5 \pm 8.7f | 18.72 | |
| | 5 | 413.5 \pm 62.4fgh | <DL | <0.04 | 76.3 \pm 11.7cde | 18.46 | |
| | 6 | 763.7 \pm 89.6i | 0.26 \pm 0.06a | 0.03 | 343.7 \pm 33.4g | 45.00 | |
| | 7 | 280.9 \pm 32.5cdef | 3.8 \pm 0.1c | 1.36 | 224.4 \pm 13.7f | 79.88 | |
| | 8 | 1171.6 \pm 61.2jk | 0.24 \pm 0.11c | 0.02 | 379.5 \pm 19.4g | 32.39 | |
| São Domingos | 9 | 250.0 \pm 21.5bcdef | <DL | <0.07 | 125.0 \pm 21.6e | 49.98 | |
| | 10 | 176.2 \pm 7.9abcd | <DL | <0.09 | 65.2 \pm 3.9abcd | 37.02 | |
| | 11 | 119.3 \pm 9.7abc | <DL | <0.14 | 25.61.5a | 21.45 | |
| | 12 | 547.8 \pm 148.9h | <DL | <0.03 | 204.5 \pm 10.9f | 37.34 | |
| | 13 | 157.7 \pm 5.6abc | <DL | <0.11 | 85.0 \pm 17.2de | 53.86 | |
| | 14 | 141.4 \pm 2.2abc | <DL | <0.12 | 55.4 \pm 5.2abcd | 39.20 | |
| Lousal | 15 | 360.4 \pm 2.5efg | <DL | <0.05 | 213.6 \pm 3.3f | 59.28 | |
| | 16 | 180.4 \pm 62abcd | <DL | <0.09 | 25.9 \pm 1.1ab | 14.37 | |
| | 17 | 80.3 \pm 2.9ab | <DL | <0.21 | 32.0 \pm 9.7abc | 39.84 | |
| | 18 | 503.5 \pm 21.4gh | 0.6 \pm 0.2b | 0.13 | 359.5 \pm 9.4g | 71.40 | |
| Control | 19 | 44.0 \pm 2.3a | <DL | <0.38 | 34.8 \pm 39.2abcd | 79.17 | |

^a LC: limit concentration of the Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health considering agricultural use (CCME, 2006); total trace element content: aqua regia digestion; potentially bioavailable trace element content: extracted by a solution of 0.5 M NH₄CH₃COO, 0.5 M CH₃COOH and 0.02 M EDTA, pH 4.7; effective bioavailable trace element content: extracted by CaCl₂ 0.01 M; DL: detection limit; DL (Zn) = 0.167 mg kg⁻¹.

Table 6
Trace element concentrations (mg kg⁻¹) in the edible plant material (mean ± standard deviation, n = 3). Values in a column marked with the same letter are not significantly different (Tukey HSD test, P > 0.05).

| Sampling sites | Lettuce (<i>L. sativa</i>) | | | | | | Coriander (<i>C. sativum</i>) | | | | | | Cabbage (<i>B. oleracea</i>) | | | | | | | | | | | |
|----------------|------------------------------|--------------|---------------|------------|----------------|----------------|---------------------------------|-----|-----------------|---------------|--------------|-----|--------------------------------|----|----|----|----|----|----|----|----|----|----|----|
| | As | | Cu | | Pb | | Zn | | As | | Cu | | Pb | | Zn | | As | | Cu | | Pb | | Zn | |
| | As | Cu | Pb | Zn | As | Cu | Pb | Zn | As | Cu | Pb | Zn | As | Cu | Pb | Zn | As | Cu | Pb | Zn | As | Cu | Pb | Zn |
| Aljustrel | 1 | 0.7 ± 0.03ab | 17.4 ± 1.9bc | <DL | 63.1 ± 2.4cde | 0.2 ± 0.1a | 21.9 ± 12.7b | <DL | 39.0 ± 4.1ab | <DL | 6.9 ± 0.2bc | <DL | 53.1 ± 1.8cde | | | | | | | | | | | |
| | 2 | 0.3 ± 0.02a | 13.6 ± 1.3abc | <DL | 60.3 ± 5.8bcde | 0.4 ± 0.2ab | 18.1 ± 10.0ab | <DL | 83.1 ± 16.0def | <DL | 7.1 ± 1.2bc | <DL | 32.7 ± 2.3abc | | | | | | | | | | | |
| | 3 | 0.9 ± 0.3abc | 20.5 ± 0.7cd | <DL | 80.3 ± 3.2ef | <DL | 17.7 ± 0.9ab | <DL | 78.6 ± 2.5def | <DL | 7.1 ± 0.3bc | <DL | 75.0 ± 10.7ef | | | | | | | | | | | |
| | 4 | 1.0 ± 0.1abc | 36.5 ± 1.2e | <DL | 85.4 ± 5.8f | 0.2 ± 0.1a | 12.8 ± 0.7ab | <DL | 85.1 ± 3.5ef | <DL | 12.4 ± 0.9d | <DL | 64.4 ± 3.8def | | | | | | | | | | | |
| | 5 | 1.8 ± 1.3c | 37.2 ± 11.5e | <DL | 68.3 ± 5.5def | <DL | 13.1 ± 3.4ab | <DL | 47.6 ± 20.5abc | 0.24 ± 0.02ab | 9.5 ± 3.9cd | <DL | 31.4 ± 14.8abc | | | | | | | | | | | |
| São Domingos | 6 | 1.8 ± 0.04c | 28.6 ± 0.9de | <DL | 123.1 ± 12.2g | <DL | 12.2 ± 2.0ab | <DL | 90.5 ± 4.8f | <DL | 12.4 ± 2.8d | <DL | 89.2 ± 22.9f | | | | | | | | | | | |
| | 7 | 1.6 ± 0.1bc | 28.8 ± 3.9de | <DL | 427.3 ± 15.6i | 0.19 ± 0.01a | 16.8 ± 4.6ab | <DL | 306.3 ± 9.4g | 0.3 ± 0.1c | 10.6 ± 0.4cd | <DL | 157.3 ± 17.2g | | | | | | | | | | | |
| | 8 | 1.7 ± 0.1bc | 22.5 ± 3.3cd | <DL | 147.3 ± 10.0h | NA | NA | NA | NA | 0.21 ± 0.02ab | 10.9 ± 0.3d | <DL | 74.6 ± 7.8ef | | | | | | | | | | | |
| | 9 | NA | NA | NA | NA | 0.37 ± 0.04a | 13.3 ± 1.4ab | <DL | 68.2 ± 12.3cdef | 0.27 ± 0.05bc | 2.9 ± 0.2a | <DL | 26.3 ± 1.1ab | | | | | | | | | | | |
| | 10 | 0.3 ± 0.1a | 6.9 ± 0.2a | <DL | 41.0 ± 4.0ab | <DL | 8.0 ± 0.2a | <DL | 61.7 ± 13.3bcde | <DL | 2.1 ± 0.5a | <DL | 14.4 ± 3.5ab | | | | | | | | | | | |
| Lousal | 11 | 0.2 ± 0.03a | 6.8 ± 0.1a | <DL | 46.9 ± 2.5abc | 0.7 ± 0.3b | 12.7 ± 2.7ab | <DL | 32.1 ± 1.1a | <DL | 5.3 ± 0.1ab | <DL | 36.7 ± 0.5abc | | | | | | | | | | | |
| | 12 | NA | NA | NA | NA | <DL | 14.3 ± 1.3ab | <DL | 68.6 ± 6.4cdef | 0.20 ± 0.01a | 3.5 ± 0.3ab | <DL | 21.6 ± 2.0ab | | | | | | | | | | | |
| | 13 | 0.2 ± 0.01a | 6.8 ± 0.2a | <DL | 40.5 ± 1.1ab | 0.257 ± 0.003a | 6.0 ± 0.3a | <DL | 28.1 ± 2.1a | NA | NA | NA | NA | | | | | | | | | | | |
| | 14 | 0.3 ± 0.2a | 10.2 ± 3.0ab | 16.8 ± 0.7 | 51.1 ± 0.2bcd | <DL | 12.8 ± 1.1ab | <DL | 38.2 ± 1.3ab | <DL | 2.8 ± 0.04a | <DL | 12.6 ± 1.2a | | | | | | | | | | | |
| | 15 | <DL | 8.8 ± 0.3ab | <DL | 86.3 ± 2.2f | 0.17 ± 0.02a | 12.2 ± 1.3ab | <DL | 38.1 ± 1.1ab | <DL | 3.1 ± 0.1a | <DL | 39.1 ± 4.4bcd | | | | | | | | | | | |
| Control | 16 | < | 9.1 ± 0.3ab | <DL | 54.8 ± 10.7bcd | <DL | 15.2 ± 1.0ab | <DL | 50.1 ± 6.4abc | <DL | 2.5 ± 0.3a | <DL | 21.1 ± 3.2ab | | | | | | | | | | | |
| | 17 | <DL | 7.0 ± 0.1a | <DL | 47.2 ± 1.7bcd | <DL | 11.4 ± 0.3ab | <DL | 25.7 ± 0.8a | <DL | 2.8 ± 0.2a | <DL | 24.0 ± 1.6ab | | | | | | | | | | | |
| | 18 | 0.17 ± 0.01a | 8.8 ± 0.1ab | <DL | 69.0 ± 1.7def | <DL | 13.1 ± 1.0ab | <DL | 59.1 ± 1.0bcd | <DL | 2.0 ± 0.4a | <DL | 33.2 ± 0.9abc | | | | | | | | | | | |
| | 19 | <DL | 4.2 ± 0.7a | <DL | 29.1 ± 5.8a | <DL | 9.5 ± 0.2ab | <DL | 47.8 ± 0.7abc | <DL | 3.6 ± 0.3ab | <DL | 39.3 ± 3.2bcd | | | | | | | | | | | |

NA: not analyzed; DL: detection limit; DL (As) = 0.170 mg kg⁻¹; DL (Pb) = 16.7 mg kg⁻¹.

solubilized in a direct proportion to their total content. This is a real risk in places where total levels of TE are higher.

Another possible observation was that the high OM content of the soils was, as could be expected, very important to the soil nutritional status and to their CEC, as a significant positive correlation was observed between soil OM content and soil macronutrient contents (N, P and K) and CEC.

3.3. Plant trace element content

In order to evaluate the extent of the vegetable contamination with TE, and the health risks via their consumption, several regulations had to be considered in order to state maximum levels (MLs) for a contaminant in a food or feed commodity. In Europe, Commission Regulation No 1881/2006 of 19 December 2006 (EC, 2006) has set down MLs for certain contaminants in foodstuffs. Lead, Cd, Hg, and Sn-inorganic were the only metals considered in this Regulation. There, the ML for Pb in vegetables, excluding brassica vegetables, is 0.10 mg kg⁻¹ wet weight, and for brassica vegetables is 0.30 mg kg⁻¹ wet weight. For As, which was the other TE analyzed in this study, there is no ML defined by the European Commission. On the other hand, the “Codex Alimentarius for Contaminants and Toxins in Food and Feed”, from the Food and Agriculture Organization (FAO/WHO, 1995), which is a Regulation commonly used at an international level in food safety issues, has MLs for Pb, similar to those of the European Regulation, and some MLs for As, but only for oil or fat based products and canned foodstuffs, not for vegetables. Contaminants having only food and feed quality significance (e.g. Cu), but no public health implication, are not considered in this regulation (FAO/WHO, 1995). Since micronutrients, like Cu and Zn, are not considered in these Regulations, which are more concerned with TE that pose a risk to human health, their concentrations in the vegetables were compared with the total metal concentrations generally toxic to plant growth suggested by Kabata-Pendias and Pendias (2001). These authors have stated that plant leaf tissue toxicity limits for As are 5–20 mg kg⁻¹, for Cu are 2–20 mg kg⁻¹, for Pb are 30–100 mg kg⁻¹, and for Zn are 100–400 mg kg⁻¹, all in a dry matter basis (Kabata-Pendias and Pendias, 2001), based on mean values of toxic levels of TE accumulated in agricultural crops.

As a general tendency, As, Cu and Zn concentrations were higher in lettuce, followed by coriander and then by cabbage (Table 6). Again, taking a global look over the results, the TE concentration in plants collected at Aljustrel was higher than the concentrations of the same elements in São Domingos and, especially, in Lousal. Zinc was the metal absorbed in larger quantities by all three vegetables, followed by Cu. Arsenic and Pb concentrations in the plant tissues were below the detection limit (DL) of the technique at some sites. Considering that the DLs of both TE, DL (As) = 0.170 mg kg⁻¹ and DL (Pb) = 16.7 mg kg⁻¹, are below the lower limit for toxic concentrations in plant tissues, it is possible to say that the plants collected at all mine sites were not contaminated with As and Pb. This fact was expected, since the effectively bioavailable contents of these elements in soil were also low, which indicates a reduced availability of these elements to be assimilated by crops.

The same was not true for Cu and Zn. All lettuce, coriander and cabbage plants had Cu concentrations within or above the toxicity limits for that element. At some sites of Aljustrel, where the total Cu concentrations in soils were higher than at the other sites, those values were quite high for lettuce showing significantly higher values than in other mining areas, namely at sites 4 and 5, with 36.5 and 37.2 mg Cu kg⁻¹, respectively. Copper concentrations in cabbage had the same trend of variation than in lettuce, but with lower concentrations. For coriander, Cu concentrations were more homogeneous at all sampling sites, without significant differences among the majority of the values.

Zinc concentrations in the plants were considered not toxic at all sampling sites, with concentrations below the lower toxicity limits for that element, except for lettuce at sites 6, 7, and 8, and for coriander

and brassica at site 7. Especially at site 7, all plant samples presented abnormally high concentrations of Zn, which can be considered potentially toxic. In fact, the effective bioavailable Zn content at site 7 was significantly higher than others (3.8% of the total Zn content) (Table 5), which was a good indicator of the fact that this might happen.

To assess the relationship between the levels of TE in the plants, the main soils' physicochemical properties, and TE concentrations, Pearson's correlation coefficients (r) were calculated between the results (Table S2 – Supplementary material). Significant correlation was obtained ($P < 0.05$), with negative values for r , between soil pH and the concentrations of Zn in the shoots of all plants, indicating that the increase in soil pH is related to the immobilization of Zn, and to its reduced bioavailability. The opposite can be said for the behavior of the same metal in response to the soil OM: the observed correlations were significant for all plants, with positive r values, emphasizing the positive effect of the increased soil OM content on the Zn mobilization, which had an effect on the higher uptake of that element by all plants. This is due to the increased content of soluble organic compounds, which Zn may be associated with in the soil solution, thus increasing its bioavailability to crops. So, the high Zn content in the vegetables collected at site 7 can be explained by the combined effect of high OM content (8.3%), and to the lower pH(H₂O) of the soil (5.87) at that site.

Coriander was the vegetable with lower correlation coefficients between the levels of TE in plant tissues and their concentrations in the soil. In fact, only significant correlations were observed between the Zn content in the plant and the levels of Zn in effective and potentially bioavailable fractions in the soil. These results may be indicative of the fact that coriander is being able to exclude the other elements, As and Cu, from its aerial parts, keeping them in a constant concentration, independent from their concentration in the soil.

In the case of both cabbage and lettuce, significant correlations were observed between the levels of Zn, Cu and As in the aerial parts of the plants and the content in these TE in the soil. These significant correlations, all with positive r values, were obtained for all the elements in soil fractions: total, effective and potentially bioavailable, indicating that, for these plants, there is a more direct relationship between the concentrations of these elements in the soil and their uptake by cabbage and lettuce. Moreover, it is important to emphasize that, both extraction solutions were able to mimic the plant available As, Cu and Zn fractions.

3.4. Accumulation of trace elements in vegetables

In order to evaluate the behavior of the vegetables to the TE concentrations in soil, accumulation factors (AF) were calculated (Table 7). The AF for As, Cu and Zn for the vegetables used in the study were lower for As, followed by Cu, and then by Zn. Accumulation factors for As and Cu were low, $AF(As) \ll 1$ and $AF(Cu) < 1$, allowing the classification of these plants as excluders of As and Cu, in agreement with the studies made by other authors (Gonzalez-Fernandez et al., 2011). The AF for Zn were also low for the plants collected in the majority of the sites, but they were higher at some sites. Once more, we have to highlight the values found at site 7: $AF(Zn) > 1$ for lettuce and coriander, which indicates that Zn is accumulated by these vegetables at this site, again probably as a consequence of high Zn bioavailability, due to a lower soil pH value and high OM content.

3.5. Estimated daily intake of Cu and Zn via consumption of vegetables

The estimated daily intake (EDI) of Cu and Zn (Table 8) via consumption of lettuce (*L. sativa*), cabbage (*B. oleracea*) and coriander (*C. sativum*) was compared with the UL values from the "Dietary Reference Intakes" from the National Academies (National Academies, 2001). It was decided not to present the EDI values for As and Pb, since the levels of these trace elements in the vegetables were, for

most samples, below the detection limits of the technique. Therefore, the values are presented only for Cu and Zn.

The EDI values for Cu obtained in this study were below the UL values for the "Dietary Reference Intakes" from the National Academies (National Academies, 2001) for adults of both genders (700–900 $\mu\text{g Cu d}^{-1}$). Although the EDI was made for an adult person, the value is also lower than the UL for children (340–440 $\mu\text{g Cu d}^{-1}$), which eats lower portions than an adult. However, it is important to underline the higher EDI of Cu via consumption of those vegetables by Aljustrel's inhabitants (150–381 $\mu\text{g Cu d}^{-1}$), almost one third of the UL value, and only using those three vegetables as having the responsibility of the entry of Cu in the human food chain.

The EDI values for Zn were below the UL values for the "Dietary Reference Intakes" from the National Academies (National Academies, 2001) for adults of both genders (8–11 mg Zn d^{-1}). However, again for some Aljustrel's sampling sites, the EDI values were quite high, contributing significantly to the total ingestion of Zn by humans. Comparing the EDI value for Zn at site 7 with the UL for children (3–5 mg Zn d^{-1}), it is possible to see that it is within this range, stressing the risk of the entry of Zn in the human food chain by the consumption of crops produced in these soils. Although acute Zn toxicity from excessive ingestion is uncommon, gastrointestinal distress and diarrhea were reported following the ingestion of contaminated beverages (Goyer and Clarkson, 2001). Walsh et al. (1994) thoroughly discussed the effects related to Zn status, from deficiency to excess, and they reported changes in the structure, biochemistry and function of the pancreas in animals and in men following increased intake of zinc under experimental conditions or inadvertent exposures.

4. Conclusions

With the exception of the vegetables harvested at site 7, located at São João de Negrilhos (Aljustrel), with abnormally high levels of Zn, which can be considered toxic, all other concentrations were below the literature reference values for toxic concentrations in plants. Since almost all soils were heavily contaminated with TE, when we consider total TE content, the only explanation for this fact was that the soils were continuously amended with manure, which contributed to an overall raise in soil pH, soil OM content and CEC, which lead to the immobilization of TE, avoiding their uptake by plants.

Total Zn concentration in the soil at site 7 was not significantly higher than in the others, but its potentially bioavailable fraction was extremely high, 79.9% of the total, and effective bioavailable fraction was also high, 1.36% of the total. This high bioavailability can be a consequence of the slight acidity and high OM content of the soil at that site, which can contribute to an increased mobilization of metals in that soil. Therefore, the Zn content in the vegetables was also higher, characteristic of contaminated plants, emphasizing the risk of Zn entering the human food chain via the consumption of crops produced on soil from that site.

Bearing in mind that the EDI values for Cu and Zn at that site were close to the UL for adults, considered as the maximum level of daily element intake that is likely to pose no risk of adverse effects ("Dietary Reference Intakes", National Academies, 2001), it is important to advise those kitchen garden owners that they should amend the soil with some liming material, in order to increase the pH value and, concomitantly, decrease TE bioavailability.

One important message, at the local level, is that the pH value of the kitchen gardens, located in these ancient pyritic mines, should be monitored by the authorities, in order to evaluate the risk for the population to eat crops produced in these soils. Also, the land use management in the post-mining phase of each site must be carefully planned. Areas with tailings and old mine infrastructures should be avoided. The risk must be understood by the local communities.

Table 7

Accumulation factors (AF = [trace element concentration in the plant] / [trace element concentration in the soil]) calculated for the different plant species: *L. sativa*, *B. oleracea*, and *C. sativum*.

| Sampling sites | | Lettuce (<i>L. sativa</i>) | | | | Cabbage (<i>B. oleracea</i>) | | | | Coriander (<i>C. sativum</i>) | | | |
|----------------|----|------------------------------|-------|-------|-------|--------------------------------|-------|------|-------|---------------------------------|-------|------|-------|
| | | As | Cu | Pb | Zn | As | Cu | Pb | Zn | As | Cu | Pb | Zn |
| Aljustrel | 1 | 0.010 | 0.084 | n.c. | 0.185 | n.c. | 0.033 | n.c. | 0.155 | 0.003 | 0.106 | n.c. | 0.114 |
| | 2 | 0.014 | 0.183 | n.c. | 0.284 | 0.009 | 0.096 | n.c. | 0.154 | 0.017 | 0.244 | n.c. | 0.392 |
| | 3 | 0.005 | 0.042 | n.c. | 0.066 | n.c. | 0.014 | n.c. | 0.061 | n.c. | 0.036 | n.c. | 0.064 |
| | 4 | 0.010 | 0.032 | n.c. | 0.084 | n.c. | 0.011 | n.c. | 0.063 | 0.002 | 0.011 | n.c. | 0.083 |
| | 5 | 0.019 | 0.111 | n.c. | 0.165 | 0.003 | 0.028 | n.c. | 0.076 | n.c. | 0.039 | n.c. | 0.115 |
| | 6 | 0.011 | 0.077 | n.c. | 0.161 | n.c. | 0.034 | n.c. | 0.117 | n.c. | 0.033 | n.c. | 0.119 |
| | 7 | 0.004 | 0.074 | n.c. | 1.521 | 0.001 | 0.027 | n.c. | 0.560 | 0.001 | 0.043 | n.c. | 1.091 |
| | 8 | 0.005 | 0.064 | n.c. | 0.126 | 0.001 | 0.031 | n.c. | 0.064 | n.a. | n.a. | n.a. | n.a. |
| São Domingos | 9 | n.a. | n.a. | n.a. | n.a. | 0.000 | 0.021 | n.c. | 0.105 | 0.000 | 0.097 | n.c. | 0.273 |
| | 10 | 0.003 | 0.113 | n.c. | 0.233 | n.c. | 0.035 | n.c. | 0.082 | n.c. | 0.131 | n.c. | 0.350 |
| | 11 | 0.001 | 0.129 | n.c. | 0.393 | n.c. | 0.101 | n.c. | 0.308 | n.c. | 0.241 | n.c. | 0.269 |
| | 12 | n.a. | n.a. | n.a. | n.a. | 0.000 | 0.015 | n.c. | 0.040 | n.c. | 0.060 | n.c. | 0.125 |
| | 13 | 0.002 | 0.113 | n.c. | 0.257 | n.a. | n.a. | n.a. | n.a. | 0.004 | 0.100 | n.c. | 0.178 |
| | 14 | 0.004 | 0.069 | 0.049 | 0.362 | n.c. | 0.019 | n.c. | 0.089 | n.c. | 0.087 | n.c. | 0.270 |
| Lousal | 15 | n.c. | 0.084 | n.c. | 0.239 | n.c. | 0.030 | n.c. | 0.108 | 0.002 | 0.116 | n.c. | 0.106 |
| | 16 | n.c. | 0.196 | n.c. | 0.304 | n.c. | 0.054 | n.c. | 0.117 | n.c. | 0.329 | n.c. | 0.278 |
| | 17 | n.c. | 0.172 | n.c. | 0.588 | n.c. | 0.069 | n.c. | 0.299 | n.c. | 0.279 | n.c. | 0.321 |
| | 18 | 0.004 | 0.078 | n.c. | 0.137 | n.c. | 0.018 | n.c. | 0.066 | n.c. | 0.115 | n.c. | 0.117 |
| Control | 19 | n.c. | 0.245 | n.c. | 0.661 | n.c. | 0.208 | n.c. | 0.894 | n.c. | 0.552 | n.c. | 1.086 |
| Mean | | 0.007 | 0.110 | 0.049 | 0.339 | 0.002 | 0.047 | n.c. | 0.187 | 0.004 | 0.146 | n.c. | 0.297 |
| Minimum | | 0.001 | 0.032 | 0.049 | 0.066 | 0.000 | 0.011 | n.c. | 0.040 | 0.000 | 0.011 | n.c. | 0.064 |
| Maximum | | 0.019 | 0.245 | 0.049 | 1.521 | 0.009 | 0.208 | n.c. | 0.894 | 0.017 | 0.552 | n.c. | 1.091 |

n.a.: not analyzed; n.c.: not calculated, because trace element concentration in the plant was below detection limit.

Another important message, for the decision makers, is that legislation is needed regarding soil quality and guideline values for contaminants in soils in Portugal and in the European Community.

We dare to leave another message: it would also be important to define Dietary Reference Intakes and UL values for TE, not only for those elements with public health implication (e.g. As, Hg and Pb), but also for micronutrients (e.g. Cu and Zn), because in some specific environments, even essential elements can reach toxic concentrations in edible plants.

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Conflict of interest

I hereby declare that I do not have any conflict of interest, neither my co-authors.

Table 8

Estimated daily intake (EDI) of Cu and Zn ($\mu\text{g d}^{-1}$) via consumption of lettuce (*L. sativa*), cabbage (*B. oleracea*) and coriander (*C. sativum*) at the three different mine sites (mean \pm standard deviation, n = 3).

| Sampling sites | | EDI ($\mu\text{g d}^{-1}$) | |
|----------------|----|------------------------------|--------------------|
| | | Cu | Zn |
| Aljustrel | 1 | 150.3 \pm 11.3 | 796.7 \pm 9.9 |
| | 2 | 171.6 \pm 5.9 | 776.7 \pm 22.3 |
| | 3 | 167.5 \pm 2.2 | 1291.6 \pm 150.9 |
| | 4 | 270.1 \pm 7.0 | 1078.7 \pm 64.7 |
| | 5 | 380.6 \pm 71.3 | 931.9 \pm 220.5 |
| | 6 | 281.1 \pm 36.6 | 1633.9 \pm 231.0 |
| | 7 | 313.1 \pm 17.8 | 4699.6 \pm 293.8 |
| | 8 | 278.1 \pm 14.3 | 1856.4 \pm 98.3 |
| São Domingos | 9 | 75.8 \pm 4.9 | 546.0 \pm 21.9 |
| | 10 | 57.0 \pm 7.8 | 377.8 \pm 55.4 |
| | 11 | 87.5 \pm 2.6 | 565.4 \pm 4.0 |
| | 12 | 73.0 \pm 2.6 | 378.5 \pm 24.9 |
| | 13 | 46.6 \pm 0.7 | 312.7 \pm 2.6 |
| | 14 | 77.3 \pm 6.8 | 337.7 \pm 18.5 |
| Lousal | 15 | 61.2 \pm 1.0 | 610.5 \pm 45.2 |
| | 16 | 71.8 \pm 3.3 | 490.1 \pm 36.6 |
| | 17 | 53.2 \pm 1.8 | 375.1 \pm 19.9 |
| | 18 | 61.9 \pm 6.4 | 688.9 \pm 14.4 |
| Control | 19 | 63.5 \pm 5.7 | 588.1 \pm 58.6 |

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