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ORAL BIOACCESSIBILITY AND HUMAN HEALTH RISK ASSESSMENT OF TRACE ELEMENTS IN AGRICULTURAL SOILS IMPACTED BY ACID MINE DRAINAGE

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ABSTRACT

Cultivated soils around the historic mine site of Tharsis (Spain) contain elevated concentrations of As (up to 621 mg kg^{-1}), Cu (752 mg kg^{-1}) and Pb (2395 mg kg^{-1}), exceeding the regional background levels and the statutory limits set for agricultural use. A site-specific health risk assessment of occupational and environmental exposures was conducted using an approach based on guidelines from regulatory agencies, refined by combining bioaccessibility and bioavailability data.

Oral bioaccessibility, as determined by simulating the human digestion process in vitro (Unified BARGE Method), was largely related to total trace element concentrations in soil. Arsenic seemed to be evenly distributed among the gastric and gastro-intestinal phases (about 31%), whereas the bioaccessible fraction of pH-dependent metal cations, like Pb and Zn, was noticeably higher in the stomach (nearly 50%) than in the gastro-intestinal tract (less than 10%). Bioaccessibility assessed by single extraction with 0.43 M HNO3 was overestimated by a factor of 1.2-1.4 relative to that obtained from the BARGE method.

Site-specific relative bioavailability (RBA) values of As (27.7%) and Pb (42.6%), predicted from bioaccessibility measurements through linear regression models, had little effect on the overall risk estimates. For the ingestion pathway, the RBA-adjusted cancer risk values (9.7E-05 to 2.0E-04) exceeded the regulatory threshold in all plots, and the hazard index re-calculated after adjustment of oral dose was also above the allowable limit, with values ranging from 2.5 to 4.8. However, no detrimental health effects are expected to occur through inhalation of soil particles in nearby residents.

1. Introduction

Soil heavy metal contamination is a worldwide environmental concern, particularly in regions with a long history of mining and metal processing (Fernández-Caliani et al., 2009a). Evidence of increased total concentrations of harmful trace elements in mine-impacted soils is not sufficient for an assessment of their potential health risks (Gupta et al., 1996), as it provides only a conservative estimate for screening purposes. The actual health risks depend not only on the exposure concentration but also on the intake rate and the biologically available fraction of contaminants. In the absence of site-specific data, soil exposure assessment is usually set by assuming that all ingested potentially toxic elements (PTE) are fully bioavailable (Ng et al., 2015), as adopted by many environmental guidelines and regulatory agencies. Considering the potential large difference between oral bioavailability and total trace element content routinely measured, human exposure to soil contamination may be overestimated, thereby influencing risk calculations, remediation decisions and costs (Bruce et al., 2007; Juhasz et al., 2007; Bradham et al., 2011; Ng et al., 2015). Hence, reliable site-specific bioavailability factors derived from experimental tests are increasingly used in human health risk assessments.

In vitro bioaccessibility (IVBA) tests provide a rapid and cost-effective alternative to *in vivo* assays for predicting relative bioavailability (RBA) of PTE in soils and sediments (USEPA, 2007; 2017), after being correlated with *in vivo* bioavailability data (Ruby et al., 1996; Rodriguez and Basta, 1999; Juhasz et al., 2009, 2014; Denys et al., 2012; Li et al., 2015; Diamond et al., 2016). Several *in vitro* assays have been developed over recent years (Yan et al., 2016, Kumpiene et al., 2017), in attempts to

simulate the effects of the human digestion process using synthetic digestive juices. These approaches provide a valuable tool for estimating the effect of the soil matrix on bioaccessibility and oral bioavailability (Oomen et al., 2003).

In an effort to harmonize the use of bioaccessibility in human health risk assessment of contaminant soils in Europe, the BioAccessibility Research Group of Europe (BARGE) has developed an *in vitro* test, commonly known as the Unified BARGE Method (UBM), that supports site-specific estimates of oral bioavailability (Oomen et al., 2002; Wragg et al., 2011). The UBM method (ISO certification 17924) has been extensively validated against an *in vivo* model for a variety of PTE (Caboche, 2009; Wragg et al., 2011; Denys et al., 2012). Measurements of IVBA using this assay appear to be a reliable predictor of RBA in risk assessment. Results from a recently developed single extraction method using 0.43 M HNO_3 (Rodrigues et al., 2018) showed that the extraction efficiency of PTE in soil is related to that of *in vitro* methods, including the UBM.

The purpose of this study was two-fold. First, it was intended to quantify the bioaccessibility of PTE for the ingestion pathway in cultivated soil around Tharsis, one of the oldest and most outstanding mine sites in Spain. The second aim was to assess the site-specific health risks associated with occupational and environmental exposures to PTE of concern, after adjustment for RBA, by using a Risk Based Corrective Action (RBCA) approach based on the USEPA's guidelines for risk analysis.

2. Study area

The mining district of Tharsis, in southwestern Spain (Fig. 1), is a historic mine site of world-class importance in terms of both its ore geology and its environmental geochemistry. Past and recent large-scale mining, ore processing and smelting activities have left a legacy of dereliction and environmental degradation. Open-pit mining operations led to destruction of the natural vegetation, transforming soils into degraded mine spoil lands (Fernández-Caliani et al., 2009b; Fernández-Caliani and Barba-Brioso, 2010). Nowadays, huge quantities of waste rock piles, spoil heaps and tailings impoundments constitute major sources of acid mine drainage (AMD). The metallic pollutants are being transferred from tailings and waste rock dumps to nearby agricultural soils by AMD and atmospheric deposition of wind-blown dust (Chopin et al., 2007a,b). Notwithstanding the above, a number of small orchards have persisted on limited areas of arable soils adjacent to the mining area, by adopting traditional farming methods that enable cultivation of food crops (Madejón et al., 2011), all at risk of contamination with PTE. The arable land close to the mining area is devoted to agriculture with families growing vegetables and fruits for their own consumption, despite the fact that soil is occasionally flooded by extremely acid mine waters.

3. Materials and methods

The studied soil is silty loam in texture, neutral or slightly alkaline (pH= 7.1-7.6), and has a relatively high content of total organic carbon (about 7.1%) and carbonates (up to 14%) owing to the use of organic and lime amendments to improve soil fertility and to prevent soil acidity. Further details about the soil constituents and properties can be found in a complementary paper (Madejón et al., 2011).

3.1 Soil sampling and pretreatment

Five agricultural plots close to waste dumps of the nearby open pit were sampled (Fig. 1). Thirteen soil grab samples were taken at random from the root zone to a depth of 20 cm, using an Edelman hand auger. In addition, five samples of the plough layer were collected in each plot, and the extracted soil was bulked and thoroughly mixed to provide a composite sample (3-4 kg) per plot for bioaccessibility analysis.

The samples were air-dried at room temperature and disaggregated by gently crushing to pass through a 2-mm sieve. For bioaccessibility assays, bulk soil samples were sieved to <250 µm, as recommended by USEPA (2007, 2008). After homogenization, aliquots of the sieved material were ground in an agate mortar to fine powder (<63 µm) for chemical analysis.

3.2 Total trace element chemical analysis

Total concentrations of As, Cd, Co, Cr, Cu, Ni, Pb and Zn in soil were measured by ICP-MS after a multi-acid (HF-$HClO_4$-HNO_3-HCl) digestion. Quality control included the use of a method reagent blank, international certified reference materials (SARM-1 and SARM-4) and replicates by the laboratory to check accuracy and precision of the analytical data. The RSD was less than 10%.

3.3 *In vitro* bioaccessibility test

The UBM has been the assay adopted in this work because it provides a reliable

estimate of the bioaccessibility, has undergone inter-laboratory trials (Wragg et al., 2009) and has been correlated with bioavailability data from soils contaminated by either mining and smelting activities (Denys et al., 2012). Four synthetic digestive fluids (saliva, gastric fluid, duodenal fluid and bile) were used to mimic the human digestion model (Table S1). A full description of the validated UBM methodology can be found in Wragg et al. (2009), Roussel et al. (2010) and Pelfrêne et al. (2011).

In vitro bioaccessibility (IVBA) was calculated by dividing the extractable amount of each PTE of concern in the gastric and gastro-intestinal phases by its total concentration in the ingested soil, and expressed on a percentage basis:

Eq. 1 $$IVBA\,(\%) = \frac{C_{ext} \times V_{ext}}{C_{soil} \times M_{soil}} \times 100$$

where: C_{ext} is the amount of *in vitro* extractable PTE (mg L^{-1}); V_{ext} is the extraction solution volume (L); C_{soil} is the concentration of PTE in soil (mg kg^{-1}); and M_{soil} is the mass of soil sample being assayed (kg).

The extractable concentrations were measured by ICP-MS on an Agilent 7700 ICP-MS instrument. All measurements were done in triplicate to assess the precision of the digestion procedure. The accuracy of the extraction protocol was evaluated by analyzing the standard reference material NIST 2710a, finding good agreement with values reported in literature (Koch et al., 2013). Quality control included the use of reagent blanks of gastric and intestinal juices (Table S2).

Furthermore, for evaluating the potential suitability of the recently developed

method (ISO-17586:2016) using dilute nitric acid (0.43 M HNO_3), a single extraction test was performed to estimate the oral bioaccessibility of PTE (Rodrigues et al., 2018).

3.4 Health risk assessment

Quantitative health risk analysis of site-specific PTE was performed using the RBCA Tool Kit for Chemical Releases© (Connor et al., 2007). Reference dose (RfD) and cancer slope factor (CSF) were used to evaluate the health effects associated with soil surface exposure, while inhalation reference concentration (RfC) and inhalation unit risk factor (URF) were the toxicological parameters used for outdoor air exposure assessment (Table S3).

The mean dose received by an individual through incidental soil ingestion, vegetable ingestion, dermal contact and inhalation of outdoor dust was calculated on a daily basis by using the following exposure equations (USEPA, 1989):

Eq. 2 $$ADD_{ing} = \frac{C_{soil} \times IR_{soil} \times EF \times ED}{BW \times AT} \times CF$$

Eq. 3 $$ADD_{veg} = \frac{[(C_{soil} \times TC_{abg} \times IR_{abg}) + (C_{soil} \times TC_{bg} \times IR_{bg})] \times EF \times ED}{BW \times AT}$$

Eq. 4 $$ADD_{der} = \frac{C_{soil} \times SA \times AF \times ABS \times EF \times ED}{BW \times AT} \times CF$$

Eq. 5 $$ADD_{inh} = \frac{C_{air} \times IR_{inh} \times EF \times ED}{BW \times AT}$$

where ADD_{ing}, ADD_{veg}, ADD_{der} and ADD_{inh} are the average daily dose of PTE from incidental soil ingestion, vegetable ingestion, dermal contact and inhalation, respectively, expressed as mass of contaminant per unit body weight over time (mg kg^{-1}-day); C_{soil} is the concentration of PTE in soil (mg kg^{-1}); C_{air} is the concentration of PTE in ambient air at the point of exposure (mg m^{-3}); TC is the fraction of contaminant transferred from soil to above-ground vegetables (TC_{abg}) and below-ground vegetables (TC_{bg}); IR_{soil} is the ingestion rate of soil particles (mg/day); IR_{abg} and IR_{bg} are the ingestion rate (kg/day) of above-ground and below-ground vegetables, respectively; IR_{inh} is the inhalation rate (m^3/day); EF is the exposure frequency (days/year); ED is the exposure duration (years); BW is the body weight of the exposed individual (kg); AT is the time period over which the exposure is averaged (days); SA is the exposed skin surface area (cm^2); AF is a skin-soil adherence factor (mg cm^{-2}); ABS is the soil dermal absorption factor or dermal bioavailability (unitless); and CF is a units conversion factor (kg mg^{-1}).

The fraction of PTE that is presumed to be transferred in the soil-crop system, allowing to predict the health risk associated to vegetable intake, was estimated by using the transfer coefficients calculated experimentally by Madejón et al. (2011) on samples taken from the same agricultural plots.

The ambient air concentration of PTE (C_{air}) was estimated by dividing the soil concentration by a pathway-specific natural attenuation factor (NAF). The NAF was calculated from the ADF/PEF ratio values, where ADF is the lateral air dispersion factor derived from a steady-state 3-D Gaussian dispersion model available in the RBCA Tool Kit, and PEF is the particulate emission factor.

175

176 The inhalation exposure concentration was calculated by multiplying the PTE
177 concentration in air by the exposure multiplier of each receptor, as given by:

178

179 Eq. 6 $$EC_{inh} = \frac{C_{air} \text{ x } ET \text{ x } EF \text{ x } ED}{AT \text{ x } 365 \text{ days}}$$

180

181 where EC_{inh} is the inhalation exposure concentration (mg m^{-3}); ET is the exposure time
182 (hours/day); and C_{air}, EF, ED, and AT are as previously defined.

183

184 The carcinogenic risk values for oral and dermal exposure to As were estimated by
185 using the following relationship (USEPA, 1989):

186

187 Eq. 7 $$CR = ADD \text{ x } CSF$$

188

189 where CR is the cancer risk; ADD is the chronic daily dose averaged over a 70-year
190 lifetime (mg kg^{-1}-day), and CSF is the cancer slope factor (mg kg^{-1}-day).

191

192 Similarly, the probability of increased cancer incidence over a lifetime was
193 estimated in terms of inhalation slope factor when the exposure is via inhalation, as
194 follows:

195

196 Eq. 8 $$CR = ADD_{inh} \text{ x } SFI$$

197

198 where CR is the cancer risk; SFI is the inhalation slope factor (μg kg^{-1}-day). The SFI
199 can be interpreted from the unit risk utilizing the following relationship (Greene and

Morris, 2006):

Eq. 9 $$SFI = URF \times \frac{1}{IR_{inh}} \times BW \times 1000$$

where URF is the inhalation unit risk factor (μg m^{-3}); IR_{inh} and BW are as previously defined.

Thus, in combining the equations Eq. 5 and Eq. 8, the cancer risk associated to As exposure via inhalation of ambient air was estimated by multiplying the inhalation exposure concentration (EC_{inh}) by its inhalation unit risk factor (URF), as follows:

Eq. 10 $$CR = EC_{inh} \times URF$$

For non-carcinogens, the risk was based on the hazard quotient, in accordance with the standard guidelines (USEPA, 1989):

Eq. 11 $$HQ = \frac{ADD}{RfD}$$

where HQ is the hazard quotient (unitless); ADD is the average daily dose (mg kg^{-1}-day); and RfD is the chronic reference dose or acceptable intake level (mg kg^{-1}-day).

When the exposure is via inhalation, the above expression becomes:

Eq. 12 $$HQ = \frac{EC_{inh}}{RfC}$$

where EC_{inh} is the inhalation exposure concentration (mg m^{-3}) and RfC is the inhalation reference concentration (mg m^{-3}).

For multiple PTE (*n*), the overall pathway hazard index (HI) was calculated by summing individual hazard quotients (HQ_i):

Eq. 13 $$HI = \sum_{i=1}^{n} HQ_i$$

If the HI exceeds a value of 1.0 there may be concern for potential non-carcinogenic risk. Otherwise, there are likely to be no adverse health effects.

4. Results and discussion

4.1 Trace element concentrations

The soil contains elevated concentrations of mine-sourced PTE (Table 1), notably As, Cu, Pb and Zn, which are well beyond the normal ranges reported for Spanish agricultural soils (López-Arias and Grau-Corbí, 2004) and for regional soils (Galán et al., 2008; Locutura et al., 2012). The areal distribution of the PTE concentrations varied meaningfully depending on the location of the plot. The maximum concentrations of As (621 mg kg^{-1}), Cu (752 mg kg^{-1}), Pb (2395 mg kg^{-1}) and Zn (593 mg kg^{-1}) were found in the grab samples from plots located in the immediate vicinity of the mine waste dumps. These levels are more than one order of magnitude above the soil geochemical baseline, and are comparable to those reported in other severely polluted mine soils of the region

(López et al., 2008; Fernández-Caliani et al., 2009a; Gabari and Fernández-Caliani, 2017).

The total mean concentrations of As and Pb exceeded the health-based screening levels for agricultural land use (Junta de Andalucía, 2015). The reference level for Cu was also exceeded in samples from the plots 1, 2 and 3. Therefore, As, Pb and Cu are the key contaminants as they are potentially harmful at the concentrations found in the soil. Based on the precautionary principle, the plots of this study are potentially contaminated and require site-specific quantitative risk assessment. In contrast, the mean concentrations of Cr, Ni, Co, Cd, and Zn did not exceed the maximum allowed limits, and unlikely to pose a human health threat.

4.2 Bioaccessibility of trace elements

Average concentrations of PTE extracted from the soil plough layer and associated standard deviations in each extraction phase, as determined by using the UBM method, are given in Table 2 along with the results obtained from the single extraction test with 0.43 M HNO_3.

The As concentrations of the gastric phase (UBM-G) and gastro-intestinal phase (UBM-GI) were rather similar, varying from about 80 mg kg^{-1} to about 160 mg kg^{-1} (plot 5), with mean values of 117 and 122.6 mg kg^{-1}, respectively. The average UBM-G levels of Cd, Co, Cr and Ni, and especially their respective UBM-GI mean values were relatively low compared with those of As. Conversely, Pb ranked the highest contents of metals solubilized in the UBM-G extracts (577-996 mg kg^{-1}), followed by Zn (181-

334 mg kg^{-1}) and Cu (175-304 mg kg^{-1}), although the concentrations extracted from the UBM-GI compartment showed a strong decrease, particularly for Pb (less than 112 mg kg^{-1}) and Zn (less than 64 mg kg^{-1}) which represent the maximum amounts of contaminants that are available for intestinal absorption.

A good linear relationship between bioaccessible and total concentrations of PTE was observed particularly for Zn, Cu and As, with coefficients of determination (R^2) ranging between 0.79 and 0.99 in both extraction phases, and for Pb in the UBM-G phase (R^2= 0.97). The bioaccessible concentrations of these elements increased significantly ($p< 0.05$) as the total metal content in the soil increased. Bioaccessibility is not dependent upon the readily available metal fraction since the amount of PTE extractable with water and $CaCl_2$ 0.01 M in soil samples from the same orchards is negligible, according to Madejón et al. (2011).

For all PTE, the bioaccessible fractions (Fig. S1) varied within a short range of values in both digestive extracts. According to their mean values (shown in brackets), the relative bioaccessibility of PTE in the UBM-G phase increased in this order: Cr (4.56%) < Ni (16.92%) < Cd (18.58%) < As (30.30%) < Co (34.58%) < Cu (41.18%) < Zn (48.70%) ≈ Pb (48.72%). Interestingly, the metal bioaccessibility in the UBM-GI phase followed the order: Cr (1.80%) < Pb (7.50%) < Cd (8.20%) < Zn (9.66%) < Ni (14.94%) < Co (21.48%) As (31.66%) < Cu (37.52%), indicating that a considerable proportion of the total concentrations may be tightly bound to soil matrix, and so may not be available for intestinal absorption. This is consistent with the fact that most PTE in regional mine sites are specifically bound to reducible phases, notably Fe oxides and oxy-hydroxides (Barba-Brioso et al., 2008; Pérez-López et al., 2008; Fernández-Caliani

et al., 2009a), which are not easily dissolved by the digestive solutions used during the UBM extraction (Denys et al., 2012).

The IVBA results showed that As appears to be evenly distributed among the gastric and gastro-intestinal phases, with a relative bioaccessibility of about 31%, suggesting that As solubility was similar in both compartments. In agreement with previous studies (e.g. Roussel et al., 2010), the extractable As concentration did not change noticeably when the pH shifted from acidic values in the gastric phase to near-neutral values in the intestinal phase of the sequential *in vitro* extraction. However, the remaining PTE were preferentially extracted in the UBM-G phase, with Pb and Zn being the most bioaccessible metals in the gastric fluid. A similar partitioning pattern between gastric and gastro-intestinal digestion models has been reported in the literature for a range of soil types contaminated with PTE (Ruby et al., 1996; Navarro et al., 2006; Juhasz et al., 2007; Bosso et al., 2008; Roussel et al., 2010; Pelfrêne et al., 2011; Ng et al., 2015).

A plausible explanation for the variability of the bioaccessibility results is based on the strongly pH-dependent behavior of certain metals. The highly acidic environment of the stomach phase increased the solubility of Pb, Zn, and other divalent metallic cations in the gastric extracts making them more bioaccessible and available for uptake by the body. The decrease of bioaccessibility of the metal cations observed in the UBM-GI, particularly Pb and Zn with percent bioaccessibility lowered by around 40%, may be due to re-adsorption onto the soil particles, complexation by biomoleculas or chemical precipitation from solution under the alkaline conditions prevailing in the intestines (Ellickson et al., 2001).

The amounts of As, Cu, Pb and Zn released from the soil by the 0.43 M HNO_3 single extraction test were greater than the IVBA measurements (Fig. S2). Gastric bioaccessibility was overestimated by the single extraction test by a factor of 1.2-1.4. Nevertheless, there is a strong linear relationship (R^2= 0.95-0.99) between the concentrations of such elements in the UBM-G and HNO_3 soil extracts, indicating that the extraction efficiency of both methods is statistically similar, as found by Rodrigues et al. (2018).

4.3 Site-specific health risk assessment

Two scenarios were considered in the risk evaluation process. An occupational on-site farm worker scenario (Scenario I) in which an adult receptor would be exposed to surface soil through multiple pathways: a) dermal absorption from direct skin contact with surface soil while performing agricultural activities; b) inhalation of re-suspended particles generated during soil tillage and harvesting; c) incidental ingestion of soil particles by hand-to-mouth transfer; and d) consumption of vegetables that take up PTE from the AMD-affected soil. An off-site residential scenario of ambient air exposure (Scenario II) in which the surrounding population may be environmentally exposed to PTE by outdoor inhalation of dust blown from the plots, with receptor distances of about 150 m.

Applicable exposure parameters characterizing the potential duration, frequency, and ingestion rate of vegetables were site-specific values based on a population survey, whereas averaging time, soil dermal adherence and absorption factors, and ingestion

rate of soil particles were the conservative default values recommended by USEPA (2011) for on-site worker and age-adjusted residential receptors (Table S4). Standard values for applicable outdoor air parameters and site-specific soil properties and partitioning parameters listed in Table S4 were also provided as input data in the algorithm. In agreement with the Spanish regulatory guidelines (Tarazona et al., 2005), which are fully integrated within the European legal framework, the target health risk value considered for the assessment was 1.0E-05 for genotoxic carcinogens, i.e. a cancer risk of one excess case of cancer in 100,000 individuals exposed over a 70-year lifetime, and the unity value for systemic chemicals.

Results from the quantitative health risk assessment for each exposure scenario under consideration are given in Table S5 (soil exposure pathways) and Table S6 (ambient air exposure pathway).

4.3.1 Surface soil exposure

The carcinogenic dose received by agricultural workers varied by sample location and by exposure pathway. The carcinogenic daily intake rate of As ranged from 7.5E-05 to 1.6E-04 mg kg^{-1}-day for incidental soil ingestion, from 2.8E-05 to 5.8E-05 for vegetable consumption, and from 2.1E-05 to 4.5E-05 for dermal contact, resulting occupational cancer risk values greater than the regulatory threshold value (1.0E-05) in all the plots studied. The cancer risk was calculated to be between 1.9E-04 (plot 1) and 3.9E-04 (plot 3), with a mean value of 2.9E-04. Consequently, As may pose health risks to farm workers at any soil exposure pathway, under the conditions and assumptions of the assessment.

The non-carcinogenic hazard index (HI) varied between 3.33 (plot 1) and 6.36 (plot 5), with a median value four-fold higher than the acceptable toxic risk for the receptor under consideration. When categorizing the PTE by individual hazard quotient (HQ), the threshold value of 1.0 was exceeded for As in all plots. However, the largest contributor to the HI was Pb, with HQ values ranging from 2.10 to 3.89. Therefore, Pb and As can be regarded as the major PTE exceeding risk criteria for human health protection. The highest health risks are being experienced by farmers performing agricultural activities in the plots closest to the mine waste dumps, with cancer risks of 3.9E-04 (plot 3) and 3.8E-04 (plot 5) and HI values around 6 in both plots.

4.3.2 Bioavailability adjustment for As and Pb

In our risk assessment, the bioaccessibility value of the gastric phase of each composite sample was taken as the fraction of the PTE that an individual is exposed to upon soil ingestion, and then used to estimate the RBA as the stomach compartment alone is a good analogue of *in vivo* bioaccessibility (Caboche, 2009; Roussel et al., 2010; Pelfrêne et al., 2011). Accordingly, the approach was based on the UBM-G phase, which represents a worst-case scenario. The UBM method provides a robust *in vitro* tool that has been validated against an *in vivo* model for Pb and As (Caboche, 2009; Wragg et al., 2011). Thus, an *in vivo-in vitro* correlation model that predicts RBA from measurements of IVBA was applied to estimate the site-specific bioavalability of As and Pb in soil, by using simple linear regression equations:

Eq. 14 $\mathrm{RBA}_{Arsenic}(\%) = (0.59 \times \mathrm{IVBA}_{Arsenic}) + 9.8 \quad (R^2 = 0.90)$ (Juhasz et al., 2014)

399

400 Eq. 15 $IVBA_{Lead}(\%) = (1.10 \times RBA_{Lead}) + 1.86 \quad (R^2 = 0.93)$ (Caboche, 2009)

401

402 Predicted RBA values of As and Pb ranged from 23.5 to 31.3% and from 37.9 to
403 48.9%, with mean values of 27.7±2.90% and 42.6±4.41%, respectively. It is apparent,
404 therefore, that the default exposure assumption commonly used for the risk assessment
405 of soil contaminants may in fact overestimates the potential risk of trace element
406 exposure. In the absence of site-specific data for oral bioaccessibility of PTE in edible
407 plants, it was assumed that 80% of the bioaccessible As content (Schoof et al., 1999)
408 and 25% of the bioaccessible Pb concentration (Intawongse and Dean, 2008) in
409 vegetables grown on cultivated soil are absorbed into the body.

410

411 In order to reduce uncertainty when conducting the site-specific risk analysis of
412 potential carcinogenic effects of As exposure, the cancer risk was adjusted ($CR_{adjusted}$)
413 incorporating RBA into the assessment (USEPA, 2007), as follows:

414

415 Eq. 16 $CR_{adjusted} = ADD \times CSF \times RBA$

416

417 where ADD is the average daily dose from incidental soil ingestion and vegetable
418 ingestion (mg kg^{-1}-day), CSF is the As cancer slope factor (mg kg^{-1}-day), and RBA is
419 the predicted relative bioavailability adjustment factor (unitless). Similarly, for non-
420 cancer effects the exposure estimate was adjusted when estimating hazard quotient
421 (HQ) of As, Cu and Pb, as follows:

422

Eq. 17 $HQ_{adjusted} = \frac{ADD}{RfD}$ x RBA

Actually, when considering the soil exposure adjusted for RBA (Fig. 2), the potential health risk estimate to farmers was lower than that previously estimated based on a default value (100% bioavailability). The RBA-adjusted cancer risk values (Fig. 2a) exceeded the regulatory threshold value in all the plots, ranging from 9.7E-05 (plot 1) to 2.0E-04 (plots 3 and 5). The HI re-calculated after adjustment of oral dose (Fig. 2b) was also above the allowable limit, with values ranging from 2.5 (plot 1) to 4.8 (plot 5). The concerned soil should be classified as polluted and, therefore, unsuitable for agricultural use.

4.3.3 Ambient air exposure

The analysis focuses on potential health risks that may result from the inhalation of soil particles in the investigated plots. The estimated lifetime cancer risk values associated with inhalation exposure of As ranged between 7.8E-11 and 1.6E-10 for farmer receptors (Scenario I), with a mean value of 1.2E-10. This implies that they did not exceed the regulatory threshold limit for carcinogens exposure (1E-05). The non-cancer hazards due to inhalation of soil particles were also at a safe level. All HI values (6.4E-07 to 1.0E-06) were much less than the guideline value hence dust inhalation is unlikely to pose health risks to the farmers. Accordingly, no detrimental health effects would be expected to occur through the inhalation of soil particles in worker receptors. In the case of Scenario II, the average cancer risk value (8.2E-10) and the HI values (8.3E-07 to 1.3E-06) were well below the regulatory limits for any PTE of concern,

indicating that ambient air pathway does not pose a significant health risk to nearby residents living downwind.

5. Conclusions

This paper has highlighted the importance of assessing site-specific oral bioavailability to provide improved estimates of human health risks from exposure to PTE in soils impacted by mining activities. The bioaccessible concentrations of the mine-sourced PTE were largely related to their high total concentrations in cultivated soil. The UBM-G phase can be used as a conservative surrogate or proxy for RBA in the light of the IVBA results achieved in this study. Although somewhat overestimated, gastric bioaccessibility assessed by the 0.43 M HNO_3 test seems to be suitable as a first-tier for screening purposes.

The RBA estimates showed that PTE are not fully bioavailable, which implies that the use of a default value of 100% bioavailability is not adequate in determining the actual risk associated with PTE in soil. Insertion of site-specific RBA values had little effect on the overall risks, but it reduces the uncertainty associated with the human exposure estimates and the extent of required remediation efforts.

The results of the RBCA analysis based on the USEPA's criteria, after RBA-adjusted exposure assessment, provide risk evidence for decision-makers. Incidental ingestion of contaminated soil particles is expected to be the major exposure pathway for contaminants, with As and Pb being identified as priority control pollutants to prevent adverse effects on the health of the farmers. The results can be generalized to

other similar metal-contaminated soils elsewhere in the region, and the approach may be of value in preventing health risks and hazards associated with exposures to PTE in soils near abandoned mine sites with a long history of mining activities.

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686 LIST OF ACRONYSMS AND ABBREVIATIONS

687

688 ABS: Dermal Absorption Factor

689 ADD: Average Daily Dose

690 ADF: Air Dispersion Factor

691 AF: Adherence Factor

692 AMD: Acid Mine Drainage

693 ASTM: American Society for Testing and Materials

694 AT: Averaged Time Period

695 BARGE: BioAccessibility Research Group of Europe

696 BW: Body Weight

C: Trace Element Concentration

CR: Carcinogenic Risk

CSF: Cancer Slope Factor

EC_{inh}: Inhalation Exposure Concentration

ED: Exposure Duration

EF: Exposure Frequency

HI: Hazard Index

HQ: Hazard Quotient

ICP-MS: Inductively Coupled Plasma-Mass Spectrometry

IR: Ingestion Rate

IR_{inh}: Inhalation Rate

IRIS: Integrated Risk Information System

ISO: International Organization for Standardization

IVBA: In Vitro BioAccessibility

NAF: Natural Attenuation Factor

PEF: Particulate Emission Factor

PTE: Potentially toxic element

RBA: Relative BioAvailability

RBCA: Risk Based Corrective Action

RfC: Inhalation Reference Concentration

RfD: Reference Dose

RSD: Relative Standard Deviation

SA: Exposed Skin Area

SFI: Inhalation Slope Factor

TC: Soil-Plant Transfer Coefficient

722 UBM: Unified BARGE Bioaccessibility Method

723 UBM-G: Gastric phase of the UBM test

724 UBM-GI: Gastro-intestinal phase of the UBM test

725 URF: Inhalation Unit Risk Factor

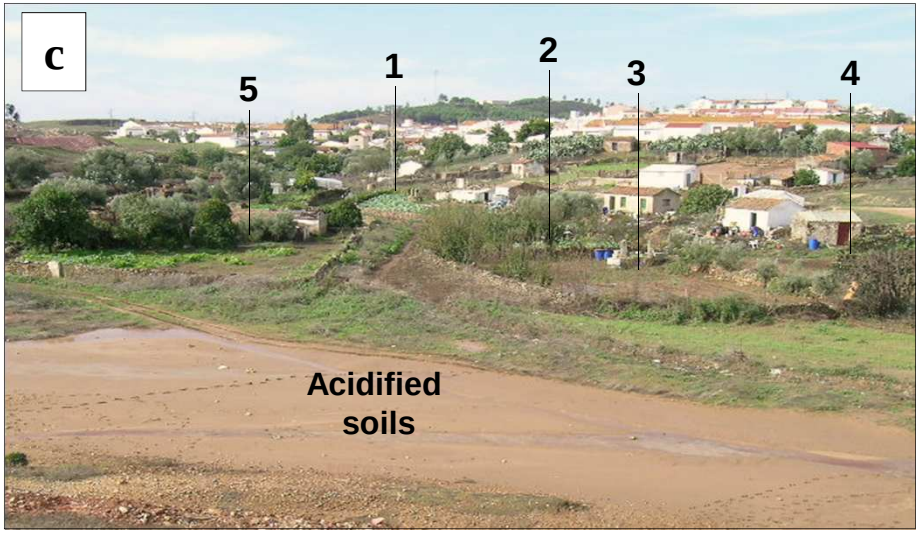
726 USEPA: United States Environmental Protection Agency

Element (mg kg^{-1})	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Composite samples (< 250 µm)								
Plot 1	247	3.27	24	60	477	32	1199	532
Plot 2	341	3.00	21	54	455	26	1291	409
Plot 3	518	3.67	28	57	695	30	1748	695
Plot 4	336	3.26	20	44	466	23	1300	469
Plot 5	505	3.31	27	45	639	27	2215	628
Arithmetic mean	389.4	3.30	24	52	546.4	27.6	1550.6	546.6
Standard deviation	117.7	0.20	3.5	7.2	112.1	3.5	428.4	116.0
Median	341	3.27	24	54	477	27	1300	532
SRM 2710a (measured)	1620	12.8	6.5	25	3439	8.4	4406	4266
SRM 2710a (certified)	1540	12.3	6.0	23	3420	8.0	5520	4180
Grab samples (< 2 mm)								
Plot 1-1	215	-	-	-	305	-	902	342
Plot 1-2	221	-	-	-	289	-	911	308
Plot 1-3	172	-	-	-	385	-	778	293
Plot 2-1	372	-	-	-	471	-	1308	512
Plot 2-2	374	-	-	-	526	-	1432	557
Plot 2-3	360	-	-	-	579	-	1410	485
Plot 2-4	349	-	-	-	499	-	1396	493
Plot 3-1	393	-	-	-	580	-	1422	584
Plot 3-2	207	-	-	-	355	-	857	448
Plot 3-3	453	-	-	-	615	-	1760	553
Plot 3-4	561	-	-	-	615	-	1963	573
Plot 4-1	575	-	-	56	692	23	2395	593
Plot 5-1	621	-	-	48	752	24	2260	589
Arithmetic mean	375	-		-	513	-	1446	487
Standard deviation	148	-	-	-	146	-	527	108
Median	372	-	-	-	526	-	1410	512
Reference values (< 2 mm)								
Regional baseline[a]	25	-	19	95	32	35	38	76
Regulatory level[b]	36	25	24	10000	595	1530	275	10000

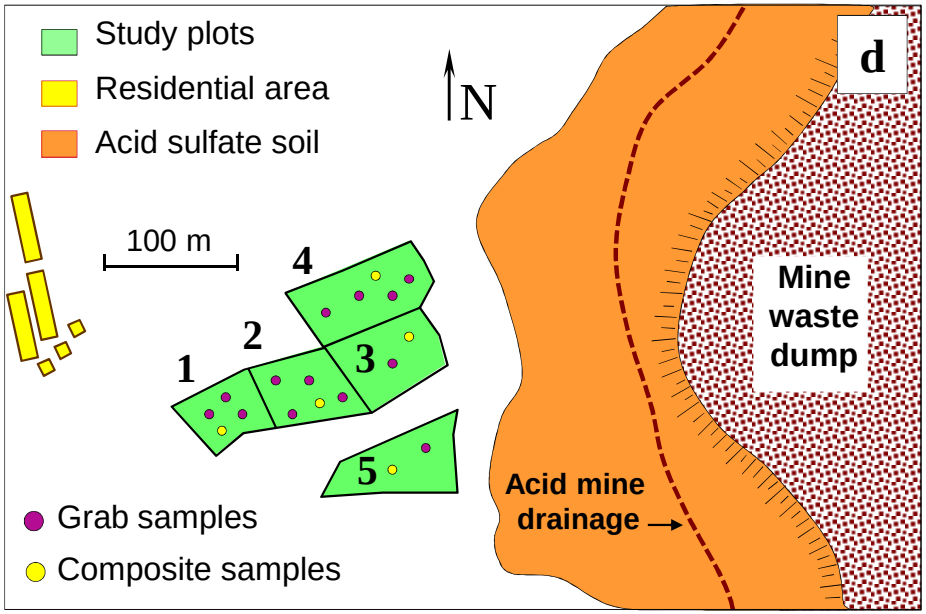
[a] Galán et al. (2008). [b] Junta de Andalucía (2015).

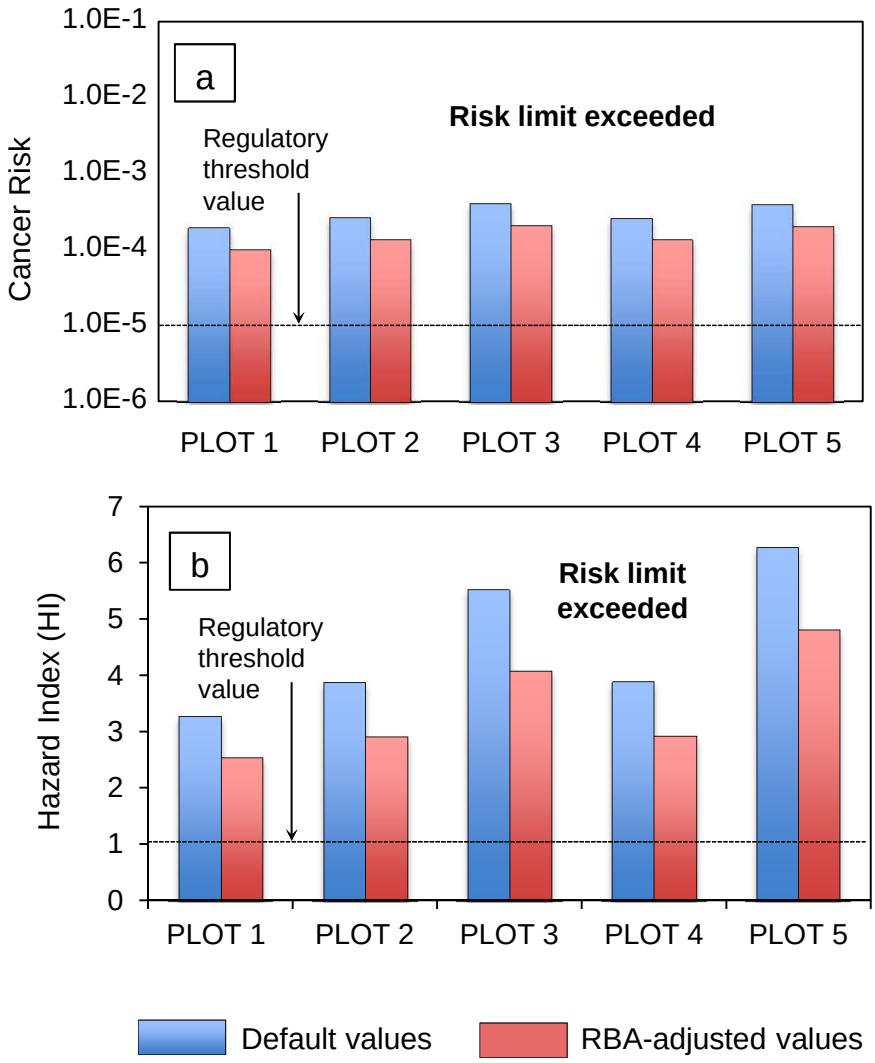
Element (mg kg^{-1})	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Gastric phase (UBM-G)								
Plot 1	80±12	0.64±0.15	7.2±1.2	2.5±0.4	198±35	4.5±0.7	609±85	266±31
Plot 2	79±12	0.55±0.12	7.2±1.1	1.7±0.3	175±25	4.1±0.6	577±74	181±16
Plot 3	147±23	0.64±0.18	9.6±1.9	2.8±0.6	271±47	5.3±1.0	761±114	314±26
Plot 4	123±16	0.57±0.13	7.6±1.3	2.0±0.4	183±29	4.4±0.7	631±92	238±21
Plot 5	156±23	0.67±0.17	9.9±1.8	2.7±0.5	304±56	4.8±0.9	996±157	334±32
Gastrointestinal phase (UBM-GI)								
Plot 1	80±13	0.27±0.01	4.1±0.7	1.0±0.3	174±28	4.0±0.7	112±18	57±4
Plot 2	89±13	0.25±0.01	4.4±0.7	1.0±0.3	163±23	3.6±0.6	86±12	38±2
Plot 3	157±25	0.30±0.02	5.6±1.0	0.9±0.3	239±38	4.4±0.8	108±17	54±3
Plot 4	126±18	0.26±0.01	4.7±0.8	0.8±0.3	175±25	3.8±0.6	108±18	48±2
Plot 5	161±25	0.27±0.01	6.9±1.1	1.0±0.3	277±43	4.6±0.8	108±16	64±2
Single extraction (0.43 M HNO_3)								
Plot 1	97±7	0.16±0.03	4.8±0.7	0.91±0.23	276±34	1.2±0.3	818±39	327±41
Plot 2	110±9	0.15±0.05	4.5±0.7	0.83±0.32	268±37	1.5±0.6	830±30	231±32
Plot 3	180±9	0.15±0.01	7.3±0.9	0.93±0.12	369±45	1.3±0.2	1001±59	401±49
Plot 4	150±84	0.16±0.05	5.4±0.6	0.90±0.31	272±35	1.4±0.5	911±19	293±37
Plot 5	203±26	0.16±0.02	10.0±1.0	1.07±0.18	467±55	1.4±0.2	1549±80	438±52


Cantabrian Sea
FRANCE
SPAIN
PORTUGAL
Madrid
Tharsis
Seville
Mediterranean Sea
AFRICA
a


c
5
1
2
3
4
Acidified soils


b
STUDY AREA
Filón Norte open pit
Waste dumps
Tailings dam


Study plots
Residential area
Acid sulfate soil
N
d
100 m
1
2
3
4
5
Mine waste dump
Acid mine drainage
Grab samples
Composite samples


a
Cancer Risk
1.0E-1
1.0E-2
1.0E-3
1.0E-4
1.0E-5
1.0E-6
Risk limit exceeded
Regulatory threshold value
PLOT 1
PLOT 2
PLOT 3
PLOT 4
PLOT 5
b
Hazard Index (HI)
7
6
5
4
3
2
1
0
Risk limit exceeded
Regulatory threshold value
PLOT 1
PLOT 2
PLOT 3
PLOT 4
PLOT 5
Default values
RBA-adjusted values

Bullet points:

- Bioaccessible fraction is largely related to total trace element content in soil
- Arsenic appears evenly distributed among the gastric and gastro-intestinal phases
- Bioaccessibility of pH-dependent metal cations is higher in the stomach phase
- Predicted bioavailability of As and Pb had little effect on the overall health risk