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POTENTIALLY HARMFUL ELEMENTS IN SOIL-PLANT INTERACTIONS

Risk assessment of *Arbutus unedo* L. fruits from plants growing on contaminated soils in the Panasqueira mine area, Portugal

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Abstract
Purpose In the Panasqueira mine area, *Arbutus unedo* L. (arbutus tree) grows on soils developed on waste materials and on soils impacted by mining activity. The arbutus berry brandy is considered a product with economic value. The aims of this study were to evaluate the biogeochemical impact of the mining activity on soils and arbutus trees, to assess the possible risks associated with human consumption of the fruits and the derived brandy, and to evaluate the potential of the arbutus tree in phytostabilization.
Materials and methods Soil samples (10–15 cm deep) developed on waste materials, on schists affected by seepage water or treatment plant effluents and on colluvium-alluvium materials were characterized (fraction <2 mm) for pH, particle size distribution, organic carbon (C_{org}), cation exchange capacity (CEC) and NPK by classical methodologies. Plant (*A. unedo*) samples (roots, leaves and twigs, and fruits) were collected at the same sites as the sampled soils, washed with tap and distilled water and dried at 40 °C. The elements' concentrations in soils (total fraction—four-acid digestion and available fraction—diethylenetriaminepentaacetic acid extraction), plants (ashing followed by acid digestion) and brandy samples produced with fruits collected on contaminated and non-contaminated sites were determined by inductively coupled plasma atomic emission spectroscopy.
Results and discussion The soils are mainly acid, silty loam, with variable values for C_{org}, CEC and NPK. They are contaminated with As (158–7,790 mg/kg), Cd (0.6–79 mg/kg), Cu (51–4,080 mg/kg), W (19–1,450 mg/kg) and Zn (142–12,300 mg/kg). The available fraction of the soils is quite variable between <0.04 and 76 % of the total, depending on the element. Trace elements' concentrations, in leaves and twigs, are within the normal range for plants, except for Cd and Zn that, in some samples, are above the normal values, but without phytotoxic symptoms. Trace elements' concentrations in fruits are low. The calculated hazard quotient for all trace elements in arbutus berry was <0.1. In the brandy, elemental concentrations are within the legal standards, except for Pb, whose higher concentrations may result from distillery equipment.
Conclusions According to the EC 466/2001 legislation and with a hazard quotient of <1, the arbutus berry consumption does not constitute health risks for humans. The fruits can be used to produce local brandy. The concentration of copper in brandy is within the range established by the Portuguese legislation. *Arbutus unedo* can be used in the phytostabilization programs in the Panasqueira area, for it is a pioneer species and a non-accumulator of trace elements.

Keywords Arbutus berry brandy · Health risks · Mine soils · Panasqueira mine

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1 Introduction

The world famous Panasqueira tungsten and tin mine is one of the few mines still operating in Portugal in the twenty-first century. This mine has been operating continuously since

1896, apart from a brief period at the end of the World War II and in the mid-1990s. The ore exploitation has generated large amounts of waste materials, which were disposed on the landscape constituting huge tailings, giving rise to a strong visual impact. Active waste heaps steadily increasing every day are bared. Older (30–80 years old) and relatively recent (5–10 years old) tailings, where soils have been developed, present vegetation cover, mainly composed of pine trees (*Pinus pinaster* Aiton), arbutus trees, also known as strawberry trees (*Arbutus unedo* L.) and several shrubs belonging to the genera *Calluna*, *Cistus*, *Cytisus*, *Erica*, etc. (Abreu and Magalhães 2009). The waste materials contain high concentrations of hazardous elements, namely As (466–12,000 mg/kg), Cd (2.6–87 mg/kg), Cu (214–3,741 mg/kg), W (40–12,000 mg/kg) and Zn (340–4,224 mg/kg) (Ávila et al. 2008; Godinho 2008).

Arbutus unedo that belongs to the Ericaceae family is a perennial plant, which usually presents small dimensions, but it can occasionally reach 9 m tall and 8 m wide (Noronha 2001), as is the case in the Panasqueira mine area. This Mediterranean shrub is known by its ornamental, meliferous and medical (laxative effects, antiseptic and diuretic properties, to treat cardiovascular pathologies, diabetes and inflammatory conditions) importance (Afkir et al. 2008; El Haouari et al. 2007; Mekhfi et al. 2006; Oliveira et al. 2011; Silva 2007). According to Malheiro et al. (2012), *A. unedo* leaves are a potential source for natural compounds, mainly polyphenolic compounds, which have significant bioactive properties that can be explored by the pharmaceutical, chemical and food industries. The mature arbutus berries are extremely sweet (high fructose content, Barros et al. 2010), being edible and frequently used to produce sweets, jam, jellies and brandy by their fermentation and distillation. It is common knowledge that eating a few arbutus berries causes signs of drunkenness, and owing to this fact, they are considered rich in ethanol. As noted by Molina et al. (2011), there is no reference, in the scientific literature, to the presence of ethanol in these fruits. Fruits are a very good source of health promoter compounds having a wide range of antioxidants, including vitamin C and E, niacin, carotenoids, polyphenolic compounds, dietary fibre and omega-3 polyunsaturated fatty acids (Alarcão-e-Silva et al. 2001; Barros et al. 2010; Oliveira et al. 2011; Pallauf et al. 2008; Ruiz-Rodríguez et al. 2011).

Despite all the referred possible uses of this plant species, the brandy is, nowadays, an economic value-added product (DGRF 2005). The brandy is a traditional beverage known in Portugal as *Aguardente de medronho* produced in small- or industrial-scale units, being an important social agro-sustainable business (Alarcão-e-Silva et al. 2001; Santo et al. 2012; Soufleros et al. 2005).

In the Panasqueira mine area, over and above the social and economic interest of the *A. unedo*, this shrub may also have a potential to be used in the phytostabilization of degraded soils and waste heaps due to the observed good vegetative development. Moreover, in order to be used in phytostabilization programs, this shrub cannot be an accumulator of hazardous elements in shoot tissues (leaves, twigs, fruits and seeds), once it can be consumed by humans and/or animals (Mendez and Maier 2008; Prasad and Freitas 2003).

Phytostabilization is a soil/waste remediation technology whose strategy is to create a vegetative cover for long-term stabilization and contaminant containment, aiming to reduce risks to human health and environment. The plant canopy reduces aeolian dispersion of particles, and roots prevent water erosion, immobilize hazardous elements and provide a rhizosphere environment where elements can be stabilized (Abreu and Magalhães 2009; Mendez and Maier 2008). This is achieved using specific tolerant and well-adapted plants, as it appears to be the case of arbutus tree.

Considering that the waste materials in the Panasqueira mine area have high concentrations of hazardous elements, attention should be paid to the possible risks to the human health arising from a potential soil-to-plant transfer of those chemical elements and their translocation from the roots to the shoots, with particular regard to the fruits. The aims of this study were to evaluate the biogeochemical impact of the mining activity on soils from the Panasqueira mine area, mainly those developed on waste materials, as well as on *A. unedo* growing on these soils; to access the possible risks for human health linked to the consumption of the leaves and twigs, arbutus berries and the derived brandy; and also to evaluate the potential of arbutus tree in the phytostabilization of the contaminated soils and tailings of Panasqueira.

2 Materials and methods

2.1 Site description

The Panasqueira mine is located in Central Portugal, Beira Interior region, at approximately 300 km north-east of Lisbon and 200 km south-east of Porto City. The Panasqueira tungsten and tin deposit is reported to be the largest quartz vein deposit in Europe related to hydrothermal mineralization associated with Hercynian plutonism (Cavey and Gunning 2006). This deposit is located in a folded metasedimentary sequence, the Beira-Schist Formation of the upper Precambrian-Cambrian age. The Panasqueira ore deposit consists of a series of stacked, subhorizontal, hydrothermal quartz veins intruding into the Beira-Schist Formation containing mainly wolframite, arsenopyrite, chalcopyrite and cassiterite (Corrêa de Sá et al. 1999). Intrusive is an important component to the mineralizing events at Panasqueira; a granite intrusion is thought to be the principal source of the mineralizing fluids responsible for the economic wolframite vein system (Thadeu 1951). The mineralized zone has a dimension of around 2,500 m in length, and the width varies from 400 to 2,200 m and reaches to at least 500 m in depth (Cavey and Gunning 2006; Corrêa de Sá et al. 1999). Wolframite mineralization occurs as large aggregates of crystals or very large crystals usually concentrated towards the margins of the quartz veins or, occasionally, close to the central portion of the quartz veins (Corrêa de Sá et al. 1999).

The Panasqueira mine area includes three subareas: Cabeço do Peão and Panasqueira, which are nowadays inactivated, and Barroca Grande where the present and active underground mine processing plant is located. The Barroca Grande site includes underground mine and portals, the active processing plant, mine offices and employees' housing, in addition to the active tailings' disposal areas, two mud dams and the Salgueira water treatment plant (Cavey and Gunning 2006).

The active heaps extend along more than 1 km south-east from the mine portals in the north river side of the Bodelhão stream that drains the Barroca Grande subarea to the Zêzere River. These heaps present steep slopes; they are bared and composed of gravel, stones and sand materials containing high concentrations of hazardous chemical elements associated with arsenopyrite and other ore minerals (Ávila et al. 2008). On the surface of the inactive tailings (30–80 years old or even younger (>5 years old)), a thin layer of soil (Spolic Technosol Toxic, IUSS Working Group WRB 2007) was developed supporting a vegetation cover. Part of the vegetation was planted to feed the paper industry (pine trees), but spontaneous plants, like arbutus tree, heathers, rockrose and brooms, also colonized those tailings.

2.2 Soil sampling and analysis

Thirteen (10–15 cm deep) samples of soils, developed over tailings, over schist that receives the influence of seepage water or treatment plant effluents, and on colluvium-alluvium materials, were collected on April and November 2007 and March 2008 in the surrounding area of Barroca Grande (Fig. 1). These soils, classified as Spolic Technosol Toxic (IUSS Working Group WRB 2007), were considered as representative of the different soils where arbutus trees grow.

Soil samples (fraction <2 mm) were characterised as follows: pH in water suspension (1:2.5 *m*/*v*), particle size distribution by sieving and sedimentation, organic carbon (C_{org}) by dry combustion (Ströhlein method), cation exchange capacity (CEC) and exchangeable cations by 1 mol/L ammonium acetate at pH 7 (Póvoas and Barral 1992), extractable phosphorous and potassium (Égner et al. 1960), mineral nitrogen (Keeney and Nelson 1982), iron from iron oxides (De Endredy 1963) and manganese from manganese oxides (Chao 1972). The same fraction of each soil sample was analysed for elemental total concentrations by inductively coupled plasma atomic emission spectroscopy (ICP) and instrumental neutron activation analysis (INAA) after four-acid digestion (perchloric acid + nitric acid + hydrochloric acid + hydrofluoric acid), in a certified laboratory (Activation Laboratories 2010a, ISO/IEC 17025). Soil available fraction of the same chemical elements was determined by ICP after extraction with diethylenetriaminepentaacetic acid (DTPA) aqueous solution (0.005 mol/L DTPA+0.1 mol/L triethanolamine (TEA)+0.01 mol/L calcium chloride; Lindsay and Norvell 1978).

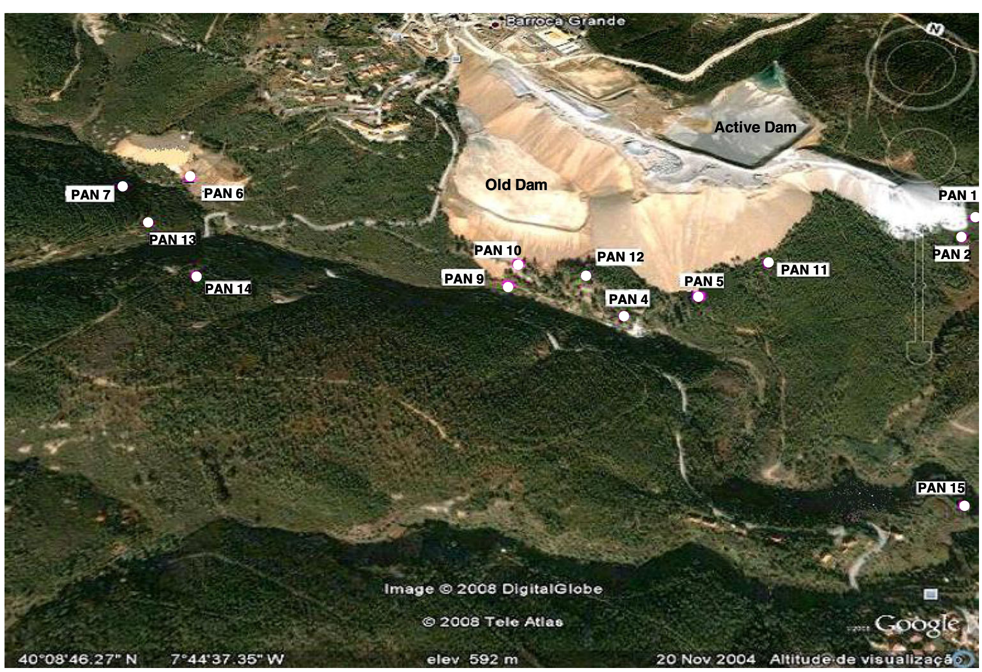

Fig. 1 Location of sample sites (soils and plants) within the mining area of Panasqueira. The present and active underground mine processing plant is located in Barroca Grande

2.3 Plant sampling and analysis

Representative samples of arbutus trees' aerial parts (leaves and twigs—13 samples) and roots (six samples) as well as six samples of fruits (arbutus berries) were collected from plants growing on the above-described soil sampling places (Fig. 1). Leaves and twigs, and roots were washed in abundant tap water and rinsed with deionised water, dried at 40 °C, homogenised and finely ground. Before drying, roots were also cut in small pieces and rewashed with deionised water in an ultrasound-assisted bath for 30 min. In order to copy the fruits' fermentation conditions traditionally used by local farmers, the arbutus berries were not washed before being dried.

In the plant samples, the total concentrations of the same chemical elements that were analysed in soils were determined by ICP/MS, after ashing (480 ºC) and acid digestion (HNO_3 and H_2O_2) (Activation Laboratories 2010b).

2.4 Brandy

Three brandy samples distilled from arbutus berries collected by local brandy producers, from plants growing on contaminated soils (BAL 1) and on soils of the surrounding area (Panasqueira) but with no contaminant influence (BAL 2 and BAL 3), were analysed by ICP. The brandy was produced in small local private distilleries for personal consumption.

2.5 Data analysis

Statistical analysis (regression equations) was performed using the Excel for Windows (Microsoft Office Excel 2003) in order to correlate chemical elements' concentrations in soils and plants. The quality control of the multielementar chemical analysis was done by the Activation Laboratories (international certified laboratory, ISO/IEC 17025).

Soil-to-plant transfer coefficient (TransferC) characterizes the uptake capacity and accumulation of a specific chemical element in the aerial part of the plant. This coefficient was calculated as follows: TransferC = [total leaves and twigs element]/[total soil element]. The plant capacity to absorb a chemical element from the soil, when it occurs in an available form (water soluble or water soluble plus exchangeable) for plants determined after soil chemical extraction using an appropriated solution, can be expressed as the bioconcentration coefficient (BC). This coefficient represents the level of plant tolerance for a potential toxic element, the plant being considered tolerant when BC>1 (Abreu et al. 2008), and it was calculated as follows: BC = [leaves and twigs element]/[element available soil fraction, extracted with DTPA aqueous solution]. The translocation coefficient (TranslC = [total leaves and twigs element]/[total roots element]) evaluates the plant capacity to translocate a chemical element from roots to aerial parts. According to Bu-Olayan and Thomas (2009) and McGrath and Zhao (2003), plants are considered accumulators of a trace element if the calculated soil–plant transfer coefficient is higher than one.

Health risk assessment methods can be based on the hazard quotient (HQ), a ratio between the estimated exposure dose of a specific contaminant and its oral reference dose (R_fD), defined by U.S. EPA as the maximum acceptable oral dose of a toxic substance (U.S. EPA 2000). The HQ assumes that there is a level of exposure below which it is unlikely, even for sensitive populations, to experience adverse health effects during lifetime. If the HQ exceeds unity, there may be a concern for potential non-carcinogenic health effects. But if HQ does not exceed unity, it is assumed that no chronic risks are likely to occur. To calculate the HQ, it is necessary to estimate the element exposure dose from fruits or leaves and twigs ingestion pathway (ED_{ing}). For each chemical element, the exposure dose (milligrams per (kilogram-day)) and the HQ value was defined as follows (U.S. EPA 1989, 2000):

$$\text{Exposure Dose} \quad ED_{ing} = (C \times F_i \times E_d \times E_f)/(W \times T_e) \tag{1}$$

$$\text{Hazard Quotient} \quad HQ = ED_{ing}/R_fD \tag{2}$$

where C is the mean element concentration in fruit or leaves and twigs samples (milligrams per kilogram), F_i is the food ingestion rate per person (kilograms per day), E_d is the exposure duration (in this study, equivalent to the average adult lifetime, years), E_f is the exposure frequency (days per year), W is the average body weight (kilograms), T_e is the average exposure time for non-cancer risk ($E_d \times 365$ days) and R_fD is the oral reference dose (milligrams per kilogram per day).

The potential non-cancer risk of the populations for exposure to As, Cd, Cu and Zn, on using arbutus leaves and twigs for the preparation of infusions or consuming the arbutus berries from the Panasqueira mine area, was evaluated. The HQ for W and Pb was not calculated once there is no known oral reference dose for these chemical elements (ASTSWMO 2011; U.S. EPA - IRIS 2004).

3 Results and discussion

3.1 Soils

Soil characteristics (pH, particle size distribution, organic carbon, iron and manganese in their oxides, cation exchange capacity, extractable phosphorous and potassium and mineral nitrogen) are presented in Table 1.

Table 1 Physical and chemical characteristics of the soils, pH, clay (<2 μm), silt (2–20 μm), sand (20–2,000 μm), organic carbon (C_{org}), Fe in iron oxides, Mn in manganese oxides, mineral nitrogen and extractable P and K

	pH	Clay	Silt	Sand	C_{org}	CEC	Fe in iron oxides	Mn in Mn oxides	N mineral	P extractable	K extractable
	H_2O	g kg^{-1}				$cmol_c kg^{-1}$	g kg^{-1}	mg kg^{-1}			
PAN 1S (A)	5.84	171	490	339	88.34	32.69	15.22	38.13	13.15	13.56	>166
PAN 2S (B1)	5.25	110	367	524	64.04	23.03	13.12	42.40	26.34	9.97	>166
PAN 4S (C1)	5.49	50	312	638	32.6	12.91	15.15	1071.28	7.98	39.97	96.3
PAN 5S (B2)	4.91	133	448	419	58.64	19.16	18.46	70.84	14.99	13.82	149.4
PAN 6S (D)	3.83	137	367	339	31.03	16.77	45.67	398.58	14.96	2.28	67.2
PAN 7S (E)	5.56	115	321	524	32.77	12.88	18.09	826.09	9.05	12.02	150.5
PAN 9S (C2)	8.23	29	639	638	93.39	43.02	9.23	4840.09	9.31	54.27	>166
PAN 10 S (C3)	4.19	117	380	419	81.09	48.46	18.47	59.54	9.60	166.27	116.2
PAN 11S (B2)	4.70	105	390	339	68.68	33.86	11.58	14.34	7.40	14.06	99.6
PAN 12S (B2)	4.61	107	394	524	57.42	27.57	11.65	36.62	19.70	15.13	116.2
PAN 13S (F)	5.21	140	458	638	41.18	19.73	9.77	78.97	3.43	3.26	116.2
PAN 14S (F)	5.90	97	423	419	48.55	21.41	19.01	240.95	7.24	4.05	149.4
PAN 15S (G)	4.17	62	227	339	29.52	17.95	16.56	1160.23	6.17	131.15	58.1

(A) soil developed on schists, *(B1)* soil developed on schists and under the influence of seepage water from active waste heap, nowadays the soil is buried, *(B2)* soil developed on schists and under the influence of seepage water from tailings with 5–10 years old, *(C1)* soil developed on tailings (5–10 years old), *(C2)* soil developed on tailings (5–10 years old) and covered by a yellow-orange mud, from the rupture of the pipe conducting effluent from the mine water treatment plant to the active dam, *(C3)* soil developed on tailings (5– years old) and under the influence of seepage water from active waste heap, *(D)* soil developed on tailings (~30 years old), *(E)* soil developed on tailings (~60 years old), *(F)* soil developed on tailings (~80 years old), *(G)* soil developed on colluvium-alluvium materials

Panasqueira mine soils are mainly acidic (3.83≤pH≤5.90), except soil PAN 9S with pH=8.30, which was developed on waste materials covered by a yellow-orange mud, from the rupture of the pipe conducting effluent from the mine water treatment plant (where water was treated with lime) to a pond. From the values of the particle size distribution, the soils' texture can be classified mainly as silty loam. The concentration of organic matter ($OM=C_{org}\times 1.724$) in the soils is considered high (41≤OM≤60 g/kg) or very high (OM≥61 g/kg) (INIA - LQARS 2000). The soils present cation exchange capacity values ranging from 12.9 to 48.5 $cmol_c kg^{-1}$ that are probably a consequence of their organic matter richness. In spite of the low concentrations of Ca (0.11–6.96 $cmol_c kg^{-1}$) and Mg (0.07–3.47 $cmol_c kg^{-1}$) in the exchangeable complex, these are the dominant exchangeable cations in the studied soils. The soil PAN 9S has very high values for the concentrations of exchangeable Ca (30.32 $cmol_c kg^{-1}$) and Mg (27.67 $cmol_c kg^{-1}$) as a result of the composition of the mud from the water treatment plant.

Panasqueira mine soils are rich in extractable K, with the sample PAN 15S showing the lowest value (Table 1), which according to INIA - LQARS (2000) is classified as a soil having medium fertility (41–85 mg K kg^{-1}). The soils have variable concentrations of extractable P and mineral N (11.5± 6.3 mg N kg^{-1}). Regarding the extractable P, soils PAN 10S and PAN 15S have the highest fertility rate (>90 mg P kg^{-1}), the soils PAN 4S and PAN 9S have medium and high fertility, respectively (21–45 and 46–90 mg P kg^{-1}), whereas the remaining soil samples can be classified as having low (<20 mg P kg^{-1}) and very low (≤10 mg P kg^{-1}) fertility (INIA - LQARS 2000).

The soils contain relatively high amounts of iron oxides (Table 1), reflecting the mineralogy and chemical composition of the wastes. Contrasting with iron oxides, the manganese oxides are less abundant.

All soil samples are considered to be contaminated with As (Table 2) once its total concentration exceeds largely the maximum allowed value (MAV) for soils and for agriculture use (12 mg As kg^{-1}, CCME 2007). Ávila et al. (2008) identified arsenopyrite as the main sulfide mineral that has been rejected after ore processing, being part of the waste materials. Arsenopyrite (FeAsS), similarly to what occurs with other minerals containing Fe(II), is unstable when subjected to oxidizing environments in the weathering crust, giving rise to iron oxides and the release of several chemical elements, which can be spread to the surrounding environments.

The soils can also be considered contaminated with Cd, Cu, Pb and Zn, as more than 50 % of the soils (Table 2) have total concentrations of those elements above the MAV according to the Canadian legislation (CCME 2007; 1.4 mg Cd kg^{-1}, 63 mg Cu kg^{-1}, 70 mg P kg^{-1} and 200 mg Zn kg^{-1}). Cadmium is found in most zinc ores (Greenwood and Earnshaw 1995),

Table 2 Chemical elements' concentrations in soils total fraction and available fraction extracted with DTPA

	Al	As	Cd	Cu	Fe	Mn	Pb	W	Zn
Total fraction									
PAN 1S	47,600	*160*	0.7	*75*	41.0	255	*79*	*19*	160
PAN 2S	43,300	*158*	0.6	51	41.1	239	29	*20*	142
PAN 4S	41,700	*1,230*	*3.4*	*552*	42.3	1,310	68	*338*	*374*
PAN 5S	56,500	*507*	*2.2*	*183*	38.5	231	35	*94*	*235*
PAN 6S	40,800	*3,680*	*1.9*	*281*	118.0	1,240	*205*	*284*	*245*
PAN 7S	53,500	*499*	*1.45*	*161.5*	41.7	1,190	*79*	*152*	*240*
PAN 9S	71,800	*1,920*	*79*	*4,080*	36.3	8,900	*75*	*587*	*12,300*
PAN 10S	70,700	*3,900*	*8.3*	*1,460*	44.5	318	*80*	*799*	*452*
PAN 11S	71,400	*360*	0.8	*88*	33.3	151	28	*36*	161
PAN 12S	107,000	*311*	0.6	*81*	37.6	214	32	*30*	163
PAN 13S	141,000	*215*	0.7	*83*	48.0	458	38	*162*	179
PAN 14S	102,000	*261*	0.9	*115*	47.0	697	41	*74*	*253*
PAN 15S	80,500	*7,790*	6.7	*1,200*	63.9	2,820	*106*	*1,450*	*566*
Available fraction									
PAN 1S	491.95	0.65	nd	2.87	153.70	6.26	3.72	1.39	8.92
PAN 2S	478.42	0.66	nd	2.47	124.04	12.41	1.80	1.13	7.90
PAN 4S	99.90	18.44	nd	90.87	262.83	38.93	2.27	1.13	66.17
PAN 5S	330.26	6.89	nd	25.79	511.45	20.87	4.04	0.85	42.20
PAN 6S	4.26	1.45	nd	6.44	631.18	7.95	1.76	2.45	3.63
PAN 7S	257.99	<dl	nd	4.01	53.13	11.93	9.51	2.02	5.40
PAN 9S	72.54	<dl	41.88	1,591.87	783.10	1,243.45	5.26	71.17	6,470.76
PAN 10S	667.45	154.68	6.33	539.90	2,140.97	53.87	14.99	<dl	272.45
PAN 11S	652.87	<dl	<dl	19.56	1,140.90	24.68	6.17	<dl	68.45
PAN 12S	699.76	<dl	<dl	17.61	1,080.48	46.41	8.45	<dl	46.77
PAN 13S	799.01	<dl	<dl	4.17	239.63	25.97	<dl	<dl	<dl
PAN 14S	581.77	<dl	<dl	6.62	612.14	40.99	<dl	<dl	13.13
PAN 15S	213.64	114.34	<dl	279.56	1,951.88	89.71	<dl	<dl	25.47

Concentrations are in milligrams per kilogram, except for Fe which is in grams per kilogram. Values in italics are above the values referred by the Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health (CCME 2007), except for W, which limit the value to <5.0 mg/kg (mean value for top soils of Europe, after FOREGS (2005)

dl detection limit (mg, As=1.3, Cd=0.26, Pb=0.52, W=0.52, Zn=0.26), *nd* not determined

and for the analysed soils, a strong positive correlation was found between the total concentrations of Zn and Cd (R^2= 0.8495, Table 3). The values found suggest that, in the Panasqueira mine, Zn and Cd should be found in the same ore, similarly to the most occurrences.

The soil sample PAN 9S, owing to its elemental composition (Table 2), can be considered not a representative of the soils in the area because of the particular influence of the lime-treated sewage from the leaking pipe on the tailing materials where the soil was developed, as was explained before. This soil presents high total concentrations of Cd, Cu and Zn (Table 2), and the available fraction (DTPA extracted) attained 53 % for Cd and Zn and 39 % for Cu, of their total concentrations. Although the total concentrations of Cd, Cu and Zn in the soil sample PAN 10S are lower than those in the sample PAN 9S, the soil PAN 10S has higher Cd and Zn soil available fractions (76 and 60 % of the total, respectively), but Cu has similar values (~38 % of the total) in both soils. In spite of the geographical proximity of the above-referred soils, PAN 10S is only over the influence of direct drainage from the waste dump (Fig. 1). In opposition, the soil PAN 15S, which is developed on colluvium-alluvium materials, has much lower soil available fractions for the same chemical elements (Cd <4 %, Cu=23 % and Zn=4.5 % of the total) despite the similar total concentrations of those elements.

The majority of the soil samples had total concentrations of Pb which were within the allowed values for soils and agriculture use (70 mg Pb kg^{-1}, CCME 2007). In spite of the higher concentrations of Pb in the soils PAN 6S and PAN 15S, the concentrations of this element in the soil available fraction

Table 3 Correlation values (R^2) and the respective regression equations between elements' concentrations in soils (total and available fraction) and in *A. unedo* shoots (leaves and twigs) and berries and between Zn and Cd concentrations in plant shoots

	[Element] in soil available fraction	Total [Cd] in soil	[Element] in shoots	[Element] in berries	[Zn] in shoots
Total [element] in soil					
As	R^2=0.581 Y=0.0169X−5.2524				
Cd	R^2=0.989 Y=0.538X−0.642		R^2=0.878 Y=0.1524X+0.9663	R^2=0.754 Y=0.0206X+0.0131	
Cu	R^2=0.986 Y=0.5324X−105.82				
Mn	R^2=0.905 Y=0.1338X−69.702				
Zn	R^2=0.998 Y=0.5324X−105.82	R^2=0.8495 Y=47.699X+151.87			
[Element] in soil available fraction					
Cd			R^2=0.962 Y=3.3543X−2.4889		
[Cd] in shoots					R^2=0.884 Y=112.73X−0.9107

R^2 values ≤0.5 are not shown

are the lowest (Table 2). One possible explanation arises from the fact that these soils also have high concentrations of As and Fe, which can originate solid phases with very low solubilities as was also observed in soils from São Domingos mine (Santos et al. 2012). Although the Canadian legislation (CCME 2007) does not refer to the maximum allowed value for W, its total concentration in the majority of the soil samples (Table 2) exceeds the normal range in soils (0.5–83 mg W kg^{-1}, Agency for Toxic Substances and Disease Registry 2005) and even the average concentration reported by Pyatt and Pyatt (2004) for soils in the vicinity of mining/smelting sites in North Queensland, Australia (56 and 78.4 mg W kg^{-1} for top and deeper soil, respectively). The soil available fraction of W is, for the majority of the samples, <1 % of the total concentration, which is probably a result of the low values of the soil pH. The exceptions are the soil samples PAN 1S, PAN 2S and PAN 9S, especially the latter one (pH=8.3) where the available fraction attained 12 % of the total concentration of the element. This can be a consequence of the anion monomer species of W formation that occurs for pH>6.2 (Koutsospyros et al. 2006).

The total concentration of Mn was, in general, in the normal range for soils (200–300 mg Mn kg^{-1}, Srivastava and Gupta 1996), with a maximum value (8.9 g Mn kg^{-1}) in the PAN 9S sample. The overall percentage of Mn in the available fraction is very small (<0.05 %), when compared with its total concentration.

The concentrations of the metals in the DTPA solutions used for the soil available fraction extraction are positively correlated to their total concentrations in the soil samples, laying the R^2 values in the range of 0.905–0.998 (Table 3). The correlation value for As was considerably lower (R^2=0.581).

3.2 Plants

Chemical elements' concentrations in roots, shoots (leaves and twigs) and fruits of *A. unedo* are shown in Table 4. Concentrations of Al (143.8±63.5 mg kg^{-1} dry matter) and Fe (<100 mg kg^{-1} dry matter for the majority of the samples) in leaves and twigs of arbutus trees are in the range considered normal for plants (50–200 mg Al kg^{-1} and 50–250 mg Fe kg^{-1}, Srivastava and Gupta 1996) despite the high concentrations of these elements in the soil available fraction extracted with DTPA (Table 2). These elements are mostly accumulated in the roots of the plant as the calculated translocation factor from roots to shoots, for the majority of the plants, is lower than unity (Table 5).

The As concentrations in total and available fractions of the studied soils (Table 2) exceeded the toxic values for many plant species according to Srivastava and Gupta (1996; 25–100 and 2 mg As kg^{-1} for total and available fractions, respectively), but the concentrations in plant shoots (Table 3) are lower than the toxic range limit for the majority of plants (5–20 mg As kg^{-1}, Kabata-Pendias 2011). Plants mainly accumulate As in roots (Tables 4 and 5). Arsenic concentrations in the studied arbutus trees' shoots (leaves and twigs) are similar to those obtained by Moreno-Jiménez et al. (2008) for the same species growing under controlled conditions, while As concentration in roots is lower than that obtained by the above-referred authors.

Table 4 Chemical elements' concentrations in plants (*A. unedo* L.): roots, shoots (leaves and twigs) and fruits

	Al	As	Cd	Cu	Fe	Mn	Pb	W	Zn
Roots									
PAN 9R	77	9	2.21	18.8	100	77.1	0.8	1.6	74
PAN 10R	240	11	2.11	18	100	84.7	1.2	1.1	62
PAN 11R	516	7	0.42	2.7	300	47.5	1.4	<dl	24
PAN 12R	415	3	0.56	5.3	200	76.6	2.6	<dl	50
PAN 13R	374	<dl	0.14	2	200	149	1.3	<dl	6
PAN 14R	178	<dl	0.28	2	100	16.2	1.3	<dl	8
Aerial part (leaves and twigs)									
PAN 1P	52	<dl	0.26	2.6	<dl	12.6	0.2	<dl	26
PAN 2P	110	2	0.28	2.7	<dl	33.3	0.2	<dl	49
PAN 4P	77	<dl	1.03	2.3	<dl	60	0.2	<dl	121
PAN 5P	223	<dl	*4.94*	3.1	<dl	144	0.2	<dl	*570*
PAN 6P	88	<dl	0.78	2.3	<dl	111	0.2	<dl	107
PAN 7P	148	<dl	1.86	2.8	<dl	90.4	0.3	<dl	58
PAN 9P	162	4	*12.9*	5.9	100	115	0.7	3.4	*416*
PAN 10P	243	*5*	2.16	12.9	100	*673*	0.6	2.3	*328*
PAN 11P	236	*5*	0.29	5.1	200	74.1	1.3	2	64
PAN 12P	156	3	0.74	3.9	100	96.3	0.8	1.7	86
PAN 13P	111	2	0.39	2.7	<dl	121	0.5	<dl	32
PAN 14P	83	<dl	0.47	2.1	<dl	87.1	0.8	<dl	37
PAN 15P	180	3	2.81	4.8	<dl	28.4	0.5	<dl	*203*
Arbutus berries									
PAN 1F	39	1.1	0.03	3	<dl	28.8	<dl	<dl	9
PAN 2F	20	2	0.03	2.5	<dl	3.2	<dl	<dl	7
PAN 4F	124	<dl	0.08	2.4	<dl	3.4	<dl	0.7	10
PAN 5F	46	1.1	0.07	2.7	<dl	3.5	<dl	<dl	9
PAN 6F	15	<dl	0.06	3.2	<dl	4.8	<dl	<dl	14
PAN 7F	107	<dl	0.02	2.6	<dl	2.9	<dl	<dl	7

Concentrations in milligrams per kilogram dry weight. Values in italics are within the toxic range limit for the majority of plants (Kabata-Pendias 2011)

dl detection limit (milligrams): As=1, Fe=100, Pb=0.1, W=0.5

Cadmium concentrations in leaves and twigs of 62 % of the collected plants (Table 4) are above the level considered tolerable for crops (0.05–0.2 mg Cd kg^{-1}, Kabata-Pendias 2011). Plants growing on the soil PAN S9, which contains Cd above the critical toxic level (10–30 mg Cd kg^{-1} of soil, Srivastava and Gupta 1996), presented the highest Cd concentration (12.9 mg Cd kg^{-1} dry mass), which is already in the range of the excessive or toxic value for plants in general (Kabata-Pendias 2011). Concentration of Cd in the shoots is strongly correlated to the concentration of the element in soil (total and available fraction, R^2=0.878 and R^2=0.962, respectively; Table 3). Arbutus tree translocates Cd from roots to shoots (Table 5, median of the TranslC=1.5), but this element was not translocated to the fruits (median of the TranslC shoots to fruits=0.08).

Copper and Zn are essential trace elements for plants' development, but if in excess, they cause toxicity disorders. Despite the high concentrations of Cu and Zn (ranging from 2.5 to 1,592 mg Cu kg^{-1} and from 3.6 to 6,471 mg Zn kg^{-1}) in the soil available fraction of the soils from the Panasqueira mine area, *A. unedo* presented in the above-ground part of the plant low Cu concentrations, but above the lowest limit for deficiency (2 mg kg^{-1}, Kabata Pendias 2011). Zinc concentrations in the shoots were, in general, within the range considered sufficient or normal for plants, but some samples showed concentrations within the excessive or toxic range (150–400 mg Zn kg^{-1}, Kabata Pendias 2011) as is the case of the samples PAN 5, PAN 9, PAN 10 and PAN 15. In spite of the low concentrations of Cu or high concentrations of Zn in the plants, they did not present visual symptoms of disorders related to deficiency (Cu) or toxicity (Zn). This species translocates Zn to the aerial part whereas Cu has a variable behaviour depending on the sample (Table 5); plants growing on soils with high Cu concentration in the available fraction retain more Cu in the roots (PAN 9 and PAN 10, Tables 2 and 4).

Zinc and Cd ions are chemically similar, for both belong to the same group of the periodic table. Both are mostly found in the same ores being found together in the same environments (Greenwood and Earnshaw 1995). Srivastava and Gupta (1996) mentioned that in soils where the Zn/Cd ratio is high, the translocation of Cd to the plant shoots can occur, as a result of Zn effective competition for the sites of Cd fixation in the soils. Since in the analysed soils there is high Zn/Cd ratios and strong correlation between the total concentrations of Zn and Cd (Table 3), the same phenomenon could be expected to occur in the plants. In fact, for the plant shoots, a strong correlation was found between the total concentrations of Zn and Cd (R^2=0.884 considering all plant samples except PAN 9 that was an outlier, Table 3). It is well known that Cd^{2+} is able to substitute Zn^{2+} in many Zn0containing enzymes, this being one of the reasons why the ion Cd^{2+} is toxic (Lippard and Berg 1994). In spite of the high concentrations of Cd in the plants, they did not present visual symptoms of disorders related to the toxicity of Cd.

Lead concentrations (0.2–1.3 mg kg^{-1}, Table 3) in the plant leaves and twigs samples are relatively low when compared to the values presented by Kabata-Pendias (2011) for various species (5–10 mg kg^{-1}). The translocation of this element from roots to shoots in the studied species is low to very low (Table 5) as was also observed in other shrub species of the same Ericaceae family or the Cistaceae family (Abreu et al. 2008, 2012).

Manganese concentration in the above-ground part (leaves and twigs) of the plants was in the normal range found in the mature leaf tissues (30–300 mg kg^{-1}, Kabata-Pendias 2011), except for the sample PAN 10 whose Mn concentration (673 mg kg^{-1}) lies within the range considered excessive or

Table 5 Calculated translocation coefficient roots to shoots (leaves and twigs) and shoots to fruits (TranslC = [total leaves and twigs element]/[total roots element] and [total fruits element]/[total shoots element]), soil-to-plant transfer coefficient (TransferC = [total leaves and twigs element]/[total soil element]) for *A. unedo* growing in the Panasqueira mining area and bioconcentration coefficient (BC = [total leaves and twigs element]/[element available soil fraction, extracted with DTPA aqueous solution])

	Al	As	Cd	Cu	Fe	Mn	Pb	W	Zn
Translocation coefficient, roots to shoots (leaves and twigs) (TranslC)									
PAN 9P	*2.104*	0.444	*5.837*	0.314	*1.00*	*1.492*	0.875	*2.125*	*5.622*
PAN 10P	1.013	0.455	*1.024*	0.717	*1.00*	*7.946*	0.500	*2.091*	*5.290*
PAN 11P	0.457	0.714	0.690	*1.889*	0.667	*1.560*	0.929	nc	*2.667*
PAN 12P	0.376	*1.0*	*1.321*	0.736	0.50	*1.257*	0.308	nc	*1.720*
PAN 13P	0.297	nc	*2.786*	*1.350*	nc	0.812	0.385	nc	*5.333*
PAN 14P	0.466	nc	*1.679*	*1.050*	nc	*5.377*	0.615	nc	*4.625*
Translocation coefficient, leaves and twigs to fruits (TranslC)									
PAN 1P	0.750	nc	0.115	*1.154*	nc	*2.286*	nc	nc	0.346
PAN 2P	0.182	nc	0.107	0.926	nc	0.096	nc	nc	0.143
PAN 4P	*1.610*	nc	0.078	*1.043*	nc	0.057	nc	nc	0.083
PAN 5P	0.206	nc	0.014	0.871	nc	0.024	nc	nc	0.016
PAN 6P	0.170	nc	0.077	*1.391*	nc	0.043	nc	nc	0.131
PAN 7P	0.723	nc	0.011	0.929	nc	0.032	nc	nc	0.121
Transfer coefficient (TransferC)									
PAN 1P	0.001	nc	0.371	0.035	nc	0.049	0.003	nc	0.163
PAN 2P	0.003	0.0127	0.467	0.053	nc	0.139	0.007	nc	0.345
PAN 4P	0.002	nc	0.303	0.004	nc	0.046	0.003	nc	0.324
PAN 5P	0.004	nc	*2.245*	0.017	nc	0.623	0.006	nc	*2.426*
PAN 6P	0.002	nc	0.411	0.008	nc	0.090	0.001	nc	0.437
PAN 7P	0.003	nc	*1.283*	0.017	nc	0.076	0.004	nc	0.242
PAN 9P	0.002	0.0021	0.163	0.001	0.003	0.013	0.009	0.006	0.034
PAN 10P	0.003	0.0013	0.260	0.009	0.002	*2.116*	0.008	0.003	0.726
PAN 11P	0.003	0.0139	0.363	0.058	0.006	0.491	0.046	0.056	0.398
PAN 12P	0.001	0.0096	*1.233*	0.048	0.003	0.450	0.025	0.057	0.528
PAN 13P	0.001	0.0093	0.557	0.033	nc	0.264	0.013	nc	0.179
PAN 14P	0.001	nc	0.522	0.018	nc	0.125	0.020	nc	0.146
PAN 15P	0.002	0.0004	0.419	0.004	nc	0.010	0.005	nc	0.359
Bioconcentration coefficient (BC)									
PAN 1P	0.106	nc	nc	0.907	nc	*2.014*	0.054	nc	*2.915*
PAN 2P	0.230	nc	nc	*1.095*	nc	*2.684*	0.111	nc	*6.206*
PAN 4P	0.771	nc	nc	0.025	nc	*1.541*	0.088	nc	*1.828*
PAN 5P	0.675	nc	nc	0.120	nc	*6.899*	0.050	nc	*13.506*
PAN 6P	*20.66*	nc	nc	0.357	nc	*13.958*	0.113	nc	*29.497*
PAN 7P	0.574	nc	nc	0.698	nc	*7.580*	0.032	nc	*10.741*
PAN 9P	2.23	nc	0.308	0.004	0.128	0.092	0.133	0.048	0.064
PAN 10P	0.364	0.032	0.341	0.024	0.047	*12.493*	0.040	nc	*1.204*
PAN 11P	0.361	nc	nc	0.261	0.175	*3.003*	0.211	nc	0.935
PAN 12P	0.223	nc	nc	0.222	0.093	*2.075*	0.095	nc	*1.839*
PAN 13P	0.139	nc	nc	0.647	nc	*4.659*	nc	nc	–
PAN 14P	0.143	nc	nc	0.317	nc	*2.125*	nc	nc	*2.819*
PAN 15P	0.843	0.026	nc	0.017	nc	0.317	nc	nc	*7.970*

Values in italics are above unity

nc not calculated because values were below the detection limit toxic by Kabata-Pendias (2011). However, different species show different threshold levels for Mn toxicity (Srivastava and Gupta 1996). As other shrub species, *A. unedo* also translocates manganese from roots to shoots (Table 5, Abreu et al. 2012; Monaci et al. 2011).

The common range of W in terrestrial plants is, according to Kabata-Pendias (2011), lower than 0.15 mg kg^{-1}, but some species growing on W-contaminated soils in the vicinity of abandoned mines can accumulate that element presenting highest concentrations of W, which can attain values of 13.6 mg kg^{-1} in *Eucalyptus melanophloia* (Pyatt and Pyatt 2004) and values of 30.7 mg kg^{-1} in *Cistus ladanifer* or 90.8 mg kg^{-1} in *Digitalis purpurea* (Pratas et al. 2005). The W concentrations (≤3.4 mg kg^{-1}) in the shoots of the studied arbutus trees are lower than the values referred by Pratas et al. (2005) for other shrub species (*Calluna vulgaris*—9.74 mg W kg^{-1} and *Erica umbellata*—4.04 mg W kg^{-1}) belonging to the same family (Ericaceae) of *A. unedo*. According to Koutsospyros et al. (2006), it appears that W accumulation by plants is directly related to its concentration in soils, but no significant correlation was found between W concentration in soils (total and available fraction) and *A. unedo*, although the plant sample (PAN 9) with the highest W concentration has been collected in the soil with the maximum concentration of the soil available fraction extracted with DTPA (71.2 mg kg^{-1}, Table 2).

This species is not an accumulator of any of the analysed chemical elements as the calculated coefficient transfer soil to plant (TransferC) for each element is lower than unity (except three samples for Cd and one sample for manganese and Zn, Table 5). However, when this coefficient was calculated considering the concentration of the available fraction of the elements (BC), Mn and Zn showed values >1 (median values of 2.7 and 2.9, respectively; Table 5).

Arbutus unedo is a species with possible use in phytostabilization programs, as the majority of the hazardous chemical elements are mainly accumulated in roots. Although the concentration of Cd in shoots (leaves and twigs) is considered to exceed the normal range in plants, it does not exceed the toxic limit to cattle (10 mg Cd kg^{-1}, National Research Council (NRC) 2005). Only the plant sample PAN 9 (12.9 mg Cd kg^{-1}) exceeds this limit which is a result of the high concentration of the element in soil (total and available fractions). This species was already indicated by Moreno-Jiménez et al. (2008) as a useful species for the phytoremediation of semiarid degraded soils contaminated with As.

3.3 *Arbutus unedo* fruits

Chemical elements' concentrations in arbutus berries are presented in Table 4. Comparing the elemental concentrations of the arbutus berries collected from plants growing on contaminated soils in the Panasqueira mine with the same elements concentrations in the fruits sampled in a non-contaminated area in Turkey (Özcan and Haciseferoğullari 2007), As, Pb and Cd had lower concentrations in the fruits from the mine area than from the non-contaminated area. However, Al, Zn and Cu had, in general, higher concentrations in the fruits from the contaminated mine area (15–124 and 20.11±2.69 mg Al kg^{-1} for Panasqueira and Turkey, respectively; 7–14 and 8.09±0.96 mg Zn kg^{-1} for Panasqueira and Turkey, respectively; and 2.4–3.2 and 1.65±0.41 mg Cu kg^{-1} also for Panasqueira and Turkey, respectively). Also, arbutus berries collected in non-contaminated areas of Spain (Ruiz-Rodríguez et al. 2011) presented concentrations of Cu, Mn and Zn lower than those of the fruits collected both in Turkey and in the Panasqueira mine area.

The calculated shoots (leaves and twigs)-to-fruit translocation coefficient for the analysed chemical elements was low to very low (Table 5), except for Cu with TranslC close to or higher than unity (TranslC—mean value=1.052).

The total concentration of Cd (<0.04 mg kg^{-1} fresh weight) in fruits is lower than the maximum allowed value (0.05 mg kg^{-1} fresh weight, Commission of the European Communities 2001) on vegetables and fruits. Total concentration of Pb in fruit samples (<0.05 mg kg^{-1} fresh weight) was smaller than the European Commission-defined maximum level for berries and small fruits including arbutus tree wild plants (0.2 mg Pb kg^{-1} fresh weight, Commission of the European Communities 2001). Therefore, according to the Commission of the European Communities (2001), the consumption of these fruits does not constitute a public health risk with regard to Pb and Cd concentrations as their concentrations are within the toxicologically acceptable ranges.

3.4 Arbutus berry brandy

Due to the absence of the European or Portuguese legislation that defines the maximum allowed levels of trace elements in arbutus berry brandy, apart from Cu (Decreto-Lei no. 238/2000), the concentrations of the determined chemical elements in the brandy were compared to the mean concentrations of the elements in Portuguese wines (Catarino et al. 2008) and to the maximum allowed values according to the Organization International de le Vigne et du Vin (OIV 2005). The chemical analysis of the three studied samples of arbutus berry brandy (one made with fruits collected on plants growing in the mine area and two made with fruits from plants growing out of the contaminated area) is presented in Table 6.

Although the high concentrations of Al in the majority of the arbutus berries fruits collected in Panasqueira (15–124 mg Al kg^{-}1, Table 4) comparing to fruits sampled by Özcan and Haciseferoğullari (2007; 20.11±2.69 mg Al kg^{-1}) in a non-contaminated area, only one sample of the brandy (BAL 2= 0.097 mg Al L^{-1}, Table 6) exceeded the analytic parameter limit (dl<0.05 mg L^{-1}), which is lower than the mean

Table 6 Chemical elements' concentrations (milligrams per litre) in the arbutus brandy

	Al	As	Cd	Cu	Fe	Mn	Pb	W	Zn
Bal 1	<0.05[a]	0.008	<0.05[a]	3.266	<0.5[a]	0.002	0.342	<0.05[a]	0.097
Bal 2	0.097	0.010	<0.05[a]	1.737	0.593	0.246	0.395	<0.05[a]	6.358
Bal 3	<0.05[a]	<0.005[a]	<0.05[a]	8.417	<0.5[a]	0.004	1.091	<0.05[a]	8.328

BAL 1=brandy from berries collected on arbutus trees growing on contaminated soils collected within the Panasqueira mine area. BAL 2 and BAL 3= brandy from berries collected on arbutus trees growing on non-contaminated soils

[a] Detection limit

concentration of the element in Portuguese wines (0.18–8.6 mg L^{-1}, Catarino et al. 2008). This brandy sample also exceeded the analytic limit of iron (0.593 mg Fe L^{-1}, Table 6), but not the mean content in Portuguese wines (0.24–19.40 mg Fe L^{-1}, Catarino et al. 2008).

The three brandy samples had smaller As concentrations than the maximum allowed values for wines (0.2 mg L^{-1}, OIV 2005), and Cd and W concentrations are lower than the analytical detection limit (0.05 mg kg^{-1} for both chemical elements) that is higher than the maximum allowed value for Cd in wines (0.01 mg Cd L^{-1}, OIV 2005) and higher than the concentration of W in Portuguese wines (0.09–10.5 μg W L^{-1}, Catarino et al. 2008). Concerning Cu concentration, the brandy samples did not exceed the limit defined in the Portuguese legislation (Decreto-Lei no. 238/2000) for arbutus berry brandy (15 mg Cu L^{-1}).

Brandy samples distilled from berries collected on arbutus trees growing out of the contaminated area contain Pb and Zn whose concentrations exceeded the maximum allowed values for wines (0.15 mg Pb L^{-1} and 5.0 mg Zn L^{-1}, OIV 2005). This is probably due to the equipment used in the distillation process by local private producers.

The majority of the analysed chemical elements in the arbutus berry brandy distilled from berries collected within the Panasqueira mine area (BAL 1) have concentrations lower than those in the two brandy samples distilled using berries collected on plants growing on non-contaminated soils (BAL 2 and BAL 3), showing that the high concentration of hazardous chemical elements in soils does not appear to have a negative impact on *A. unedo* fruits and on the distilled brandy.

3.5 Health risk from consumption of leaves and twigs, and fruits

Arbutus unedo has been widely used in traditional medicine, throughout the Mediterranean regions (Italy, Morocco and Turkey), with the employment of infusions and decoctions of almost all parts of this plant: leaves, twigs, fruits, barks and roots. Several potential benefits for human health have been reported by several authors for both leaves and fruits (Malheiro et al. 2012; Oliveira et al. 2011; and references therein). Potential uses of arbutus berries in food industry have also been suggested (fruit pieces in yoghurt, pie and pastry filling or cereal products) or as a food colorant, considering their content of β-carotene and anthocyanins (Alarcão-e-Silva et al. 2001).

It is therefore opportune to assess the health risk of *A. unedo* to the inhabitants of the Panasqueira area due to fruit consumption, either fresh or after processing, or to the use of arbutus leaves and twigs to make infusions. The HQs for the

Table 7 Chronic oral reference dose, chemical exposure dose and hazard quotients for arbutus berries and shoots (leaves and twigs) for arsenic, cadmium, copper and zinc

Chemical element	Chronic oral reference dose (R_fD)	Chemical exposure dose (ED_{ing}) (berries)	Chemical exposure dose (ED_{ing}) (shoots)	HQ (berries)	HQ (shoots)
As	3.0E−04[a]	8.65E−05	1.42E−05	2.88E−01	4.74E−02
Cd	1.0E−03[b]	3.46E−06	3.67E−05	3.46E−03	3.67E−02
Cu	5.0E−03[c]	1.38E−04	3.67E−05	2.77E−02	7.34E−03
Zn	3.0E−01[d]	6.06E−04	1.62E−03	2.02E−03	5.40E−03

Chemical exposure dose in milligrams per (kilogram-day). For calculations, the maximum values of the elements' concentrations were used

[a] U.S. EPA (2012)

[b] U.S. EPA (2002)

[c] U.S. EPA (1996)

[d] U.S. EPA (2005)

fruit were calculated for As, Cd, Cu and Zn, using Eq. (2) (Section 2.5), and the chemical exposure dose (ED_{ing}) (Eq. (1)) was estimated from the concentration (C) (maximum values) of the above-referred elements in the fruits (Table 4) together with the following assumptions: a body weight (W) of 76 kg for the average adult (Alves et al. 2006), an adult lifetime of 70 years (E_d), that one person usually does not eat more than 4 kg of berries/year (0,011 kg of fruits a day) (F_i) and that the normal fruits' maturation period is around 4 months (120 days/year) (E_f). The chemical exposure dose for the intake of an infusion made with leaves and twigs of *A. unedo* was calculated considering that 0.3 kg of leaves and twigs per year will be used for infusion, making a maximum of 96 days/year of infusion consumption.

The established value for the oral reference dose (R_fD) of each chemical element as well as the respective calculated chemical exposure dose (ED_{ing}) together with the values of the HQ for the berries and for the leaves and twigs is shown in Table 7. The calculated HQ for each chemical element for an average inhabitant of the Panasqueira areas who uses the berries and the shoots for consumption is lower than unity (Table 7).

The results of this study indicate that the leaves and twigs, and fruits of arbutus tree growing on soils with high total and, in some cases, high available fraction concentrations of As, Cd, Cu and Zn probably do not constitute danger for human health risks during a lifetime. However, more risk assessments and studies have to be done for a more complete exclusion of any such risk.

4 Conclusions

Soils in the Panasqueira mining area, especially those developed on tailings and/or receiving the influence of seepage water from waste heaps or effluent from the mine water treatment plant, were contaminated with trace elements, mainly As, Cu, Pb, W and Zn. The elemental concentrations in the soil available fraction, extracted with DTPA, were quite variable. In the solutions of the soil available fraction, Cd and Zn were the elements that, in general, had the higher percentage of the total soil concentration, reaching a maximum of 76 % for Cd and 60 % for Zn.

The majority of the samples of the *A. unedo* shoots (leaves and twigs) showed concentrations of Al, Cu, Fe, Pb, Mn and Zn in the normal rage for plants in general; however, Cd concentrations exceeded the values considered tolerable for crops, but are lower than the toxic limit for cattle. Cadmium, W and Zn are preferentially translocated from the roots to shoots of *A. unedo* whereas Al, As, Cu, Fe and Pb are accumulated in the roots of this species.

The arbutus tree is part of the Beira Interior's Forest Landscape Planning, as a species with great fire resistance and a good economic profit due to the use of arbutus berry to produce brandy. In fact, the analysis of the fruits collected in the *A. unedo* trees growing on the contaminated soils and the calculated hazard quotient for As, Cd, Cu and Zn allows the authors to conclude that the consumption of the arbutus berries probably does not constitute a risk for human health during a lifetime. Consequently, these fruits can be used to produce brandy and the brandy sample obtained by distillation of the berries collected within the mine area had Cu concentrations below the allowed limit according to the Portuguese legislation for arbutus berry brandy.

Arbutus unedo trees can be used in the phytostabilization programs in the Panasqueira area because it is one of the species that spontaneously colonizes the tailings, belonging to the group of plants of the first stages of vegetation development, promoting waste weathering and pedogenesis and decreasing water and wind erosion. In addition, this species is not an accumulator of trace elements and their concentrations in the above-ground part of the plants will not represent a threat for the biological systems.

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