Soil-plant system and potential human health risk of chinese cabbage and oregano growing in soils from Mn- and Fe-abandoned mines: Microcosm assay

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Abstract

In Portugal many abandoned mines are often close to agricultural areas and might be used for plant food cultivation. Soils in the vicinity of two Mn- and Fe- abandoned mines (Ferragudo and Rosalgar, SW of Portugal) were collected to cultivate two different food species (*Brassica rapa* subsp. *pekinensis* (Lour.) Hanelt and *Origanum vulgare* L.). Chemical characterization of the soil-plant system and potential risk of adverse effects for human health posed by plants associated with soil contamination, based on the estimation of hazard quotient (HQ), were assessed in a microcosm assay under greenhouse conditions. In both soils, the average total concentrations of Fe and Mn were above the normal values for soils in the region and their concentration in shoots of both species was very high. *Brassica rapa* subsp. *pekinensis* grew better in Ferragudo than in Rosalgar soils and it behaved as an excluder of Cu, Mn, Fe, S and Zn in both soils. The HQ for Cu, Fe, Mn and Zn in the studied species grown on both soils was lower than unit indicating that its consumption is safe. The high Mn tolerance found in both species might be due in part to the high contents of Fe in the soil available fraction that might contribute to an antagonism effect in the uptake and translocation of Mn. The obtained results emphasize the need of further studies with different food crops before cultivation in the studied soils to assess health risks associated with high metal intake.

Keywords: Mining, Human health risks, Chinese cabbage, Oregano

Introduction

Mining activities can be responsible for the release of several potentially toxic elements in soil, water and air. Nonetheless, the abandoned mines often cause more environmental impacts than active mines due to the inexistence of a legislation requiring rehabilitation of mining areas after closure and/or Government inspection (Kim et al., 2012; Santos et al., 2018).

Soils adjacent to the mining areas and/or developed on contaminated materials (*e.g.* mine wastes) can be a source of potentially toxic elements for plants (Kabata-Pendias 2004; Abreu et al. 2014a). Although plants only take up elements from the available soil fraction, which corresponds to the amount of the chemical elements in the soil solution and associated to the exchangeable complex of inorganic and organic soil colloids, several characteristics related to plant species, climate and soils can affect this fraction (Adriano 2001; Kabata-Pendias 2004). After uptake, the distribution and accumulation of the elements in the different plant parts at different concentrations can vary, for instance, with the element and plant species (Abreu et al. 2014a). The elements accumulation in the edible part of plants can represent the principal entry for food chain (van Rijn et al. 2002). Therefore, the soil enrichment with elements due to mining activities, namely in kitchen gardens, increases the possibilities of these elements getting into the food chain at levels that may be dangerous to human health (Abreu et al. 2014a; Hough et al. 2004; Zhuang et al. 2009). In fact, the consumption of contaminated vegetables can be an important route to metal exposure (Hough et al. 2004; Zhuang et al. 2009; Abreu et al. 2014a). Consumption of food with high concentrations of potentially toxic elements is a major contributor (more than 90%) to human exposure to elements toxicity (Loutfy et al. 2006). This is the reason why research on environmental health risks has increased in recent years (Árvay et al. 2017; Chen et al. 2019) especially in the areas affected by mining activities.

El Hamiani et al. (2010, 2015) studied the effects on consumer's health after eating food crops and/or vegetables produced in the vicinity of Southern Morocco mines pointing out the risks, especially due to high concentrations of Mn, in vegetables

(*Lactuca sativa* L. and *Lolium multiflorum* L.) even if the consumption of the edible part is relatively free of risks. In kitchen gardens closed to abandoned pyrite mines from South of Portugal, the potential risk for human health depended on the plant species and element. Thus, the accumulation of Cu and Zn in lettuce (*Lactuca sativa* L.), coriander (*Coriandrum sativum* L.) and cabbage (*Brassica oleracea* L.) from Aljustrel, Lousal and São Domingos did not represent risks for human health (Alvarenga et al. 2014). However, coriander and cabbage cultivated in kitchen gardens near São Domingos mine exceeded the maximum allowed values for Pb and As indicating a possible health risk (Gonzalez-Fernandez et al. 2011). Studies carried out by Neves et al. (2012) in a uranium contaminated area from North-Centre Portugal showed that the estimated level of U exposure through the ingestion of several vegetable foodstuffs (*Lactuca sativa* L., *Solanum tuberosum* L., *Phaseolus vulgaris* L., *Daucus carota* L., *Brassica oleracea* L., *Malus domestica* Borkh and *Zea mays* L.) growing in the area was low, suggesting no chemical health risk for local residents during their lifetime. Another study on the level of toxic elements in foodstuffs from soils closed to abandoned mines in Korea concluded that the estimated daily metal intake was below the provisional tolerable daily intake limits (Ji et al. 2013) and in Uganda the consumption of vegetables growing in farmer gardens around Kampala City was also safe (Nabulo et al. 2010). However, residents in a village close to abandoned Cu mines (Goseong, Korea) denoted high Cd concentrations in the blood and urine (Kim et al. 2008).

Although the micronutrients (like Mn) present low toxicity, the continuous intake of edible plants with high concentrations of chemical elements can lead to an excess of the elements in the organism and, consequently a significant risk for human health. Manganese uptake by humans is primarily through foodstuffs and its toxic effects occur in the respiratory tract and the brain (Tongesayi et al. 2013). It is also responsible for Parkinson’s disease (Powers et al. 2003; Lucchini et al. 2007; Sherzai et al. 2016).

The aims of this work were to evaluate: i) the soil–plant system of two species of food crops growing, in greenhouse and microcosm conditions, on soils from the abandoned Mn and Fe mines (Rosalgar and Ferragudo), and ii) the concentration of some potentially toxic elements in the same food crops growing in these soils and the consequent potential human health risk through their consumption. Two different plant species were selected, *Origanum vulgare* (oregano) and *Brassica rapa* subsp. *pekinensis* (chinese cabbage), due to their short growth cycle. Moreover, oregano is an herb that grows spontaneously in these mine areas, and its use as culinary and medical herbs in South Portugal is very common, while the chinese cabbage is also cultivated in some kitchen gardens.

Material and Methods

Site description

Two soils collected in the abandoned Fe-Mn mines located in SW of Portugal (Cercal-Odemira region) were studied: Ferragudo (Beja district) and Rosalgar (Setubal district). Both mine areas are included in the Portuguese sector of the Iberian Pyrite Belt (Matos and Martins 2006). The orebody forming vein structures was composed of Fe and Mn oxides in Rosalgar and Fe and Mn oxides as well as Mn carbonates in Ferragudo. The mining activity in Rosalgar began 1867 but the intensive ore exploitation occurred between 1959 and 2001. In Ferragudo, the ore exploitation occurred between 1875 and 1975 in both galleries and open pit, being intensively exploited in the forties and fifties of the 20th century. The ending of the mining activity was mainly related to the closure of the company where the ore was processed (Rosa et al. 2013).

In the Portuguese sector of the Iberian Pyrite Belt context, Rosalgar and Ferragudo are considered with intermediate level of environmental hazard impact (level number two and three, respectively, where level five is considered with extreme impact) due to the small volumes of mine wastes with total concentrations of potentially toxic elements relatively low, except for Mn (Matos et al. 2008).

Nowadays, in Rosalgar mine a cork oak tree system is naturally colonized by several autochthones plant species, some of them aromatic species consumed by local population (like *Origanum vulgare* subsp. *virens* (Hoffmanns. & Link) Bonnier & Layens, *Lavandula stoechas* subsp. *luisieri* (Rozeira) Rozeira, *Calamintha nepeta* (L.) Savi subsp. *nepeta*) (Rossini-Oliva et al. 2019), while in Ferragudo mine a holm oak woodland was implemented with small tree density and cultivated grass species. The soil interventions leaded to the actual existence of soils developed on different mine wastes and host rocks being classified as Spolic Technosol (IUSS Working Group WRB 2015).

Pot experiment

Composite soils samples, up to 20 cm depth, were collected during spring 2017 in the two abandoned Mn–Fe mines (Rosalgar: 37°46'25.0"N 8°41'59.7"W; Ferragudo: 37°38'01.0"N 8°03'50.4"W). These samples were air dried, sieved and the fraction <5 mm was used to a pot experiment. Randomized microcosm experiment, using 1.5 kg of each soil per pot, was carried out in greenhouse and under controlled conditions to evaluate plant growth, nutrients content and their accumulation patterns in chinese cabbage and oregano.

Ten commercial seeds of each plant species were sown in each pot and thinned to leave 2–3 plants per pot. Five replicates for each soil and species were conducted. All pots were kept at 70% of the maximum water-holding capacity during plants growth.

Some parameters associated to the plant growth (Photochemical Reflectance Index (PRI), Normalized Difference Vegetation Index (NDVI), plant height and plant fresh weight) were measure in both species at harvest, and soils and plants were collected for chemical analysis. The PRI and NDVI were measured using a PlantPen model PRI 200 and PlantPen model NDVI 300 (Photon Systems Instruments), respectively. The NDVI is an important indicator of chlorophyll content in plants. The PRI is sensitive to changes in the carotenoid pigments, and it is related to the photosynthetic light use efficiency and the rate of carbon dioxide fixation, being used as a reliable stress index in studies of vegetation productivity (Garbulsky et al. 2011).

The chinese cabbage plants were harvested after two months and the oregano plants after four months (because its much slower growth rate). Roots and shoots (leaves + stems) of both plant species were collected separately. For oregano, leaves were separated from stems since the last one are not edible. Shoots/leaves were washed with distilled water and roots were washed with tap water and then with distilled water using an ultrasound-assisted bath for 20 minutes (to eliminate the fine soil particles more strongly adhered). The plant material was dried at 60 ºC during 48 h and milled to powder homogenously. Plant samples were digested with pure HNO_3 in a DigiPrep digestor and concentrations of Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn were analyzed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES Thermo Scientific Mod. CAP 7000 Series).

Initial soil samples (fraction <2 mm) were analyzed with standard soil methodologies (Póvoas and Barral 1992): pH and electrical conductivity (EC) in soil:water suspension (1/2.5, *m*/*V*); exchangeable cations and cation exchange capacity (CEC) at 1 mol L^{-1} ammonium acetate (pH 7); extractable P and K (Egner-Riehm method) and total nitrogen (Kjeldahl method). Total organic carbon concentration was determined using an Analytikjena analyser (MultiEA 4000 model) after sample combustion at 550 °C. Iron content in both non-crystalline Fe oxides and total Fe oxides (*i.e.* non-crystalline and crystalline phases) was extracted by a single step extraction using Tamm reagent (0.1 mol L^{-1} oxalic acid and 0.175 mol L^{-1} ammonium oxalate at pH 3.2) in dark conditions (Schwertmann 1964) and under UV radiation (De Endredy 1963), respectively, and determined by ICP-OES. Manganese in manganese oxides was also determined by ICP-OES after extraction with acidified hydroxylamine hydrochloride at 0.1 mol L^{-1} (Chao 1972).

Total soil (fraction <2 mm) concentration of the same elements than in plants were determined using ICP-OES (Thermo Scientific Mod. CAP 7000 Series) after microwave digestion with a two-acid mixture (HNO_3–HCl 1:3 *V*/*V*) + H_2O_2 addition. The same elements were also analyzed in a soil extractable aqueous solution, composed of a mixture of organic acids (acetic acid + lactic acid + citric acid + malic acid + formic acid at 10 mmol L^{-1}) which simulates the rhizosphere conditions (Feng et al. 2005), and therefore considered as the available fraction of the elements for plants in the soils. This available fraction of the elements was determined in the initial soil and after the plant growth (fraction <2 mm). These extractable soil solutions were analysed by ICP-OES.

Quality assurance of chemical analysis in plants was performed using analytical blanks and certified reference material (NCSDC73348). The results obtained for certified materials showed a recovery range from 86 to 103% while procedural blanks were usually below the detection limit. All analyses performed were done in duplicate and all results for plants and soils were calculated on a dry weight basis.

Data analysis

A statistical package (Statsoft package v6.12) was used for statistical data analysis. In the pot assay, comparisons between plant and soils characteristics were performed with the t–test. Relations between plant and soil variables were determined by Pearson correlation analysis. In all cases, the values for $p< 0.05$ were considered as significant. The translocation coefficient (TC, *i.e* the ratio between element concentrations in aerial part and roots) was calculated for both plant species and assays in order to evaluate the plant capacity to translocate a chemical element from roots to aerial parts. The bioaccumulation coefficient (BC, *i.e.* the ratio between element concentrations in shoots/leaves and the available content in soil) was also calculated to determine the ability of the studied species to uptake and accumulate elements in the aerial part. According to Bu-Olayan and Thomas (2009), the plants may be considered accumulators of an element if TF is >1 and BC >1. In order to evaluate the potential risk of adverse effects for human health posed by both plant species, the hazard quotient (HQ) for Cu, Fe, Mn and Zn was calculated as follows (USEPA 2000):

$$HQ = ED/RfD$$

ED (Exposure Dose) was calculated by the equation:

$$ED = MC \times D_{food} \times E_{d} \times E_{f} / B_{average\ weight} \times T_{e}$$

where, MC is the measured concentration of metal in the sample (mg kg^{-1} of dry weight), D_{food} is the mean mass of oregano and chinese cabbage being consumed daily by an individual (kg/person/d FW), E_{d} is the exposure duration (in this study 70 years), E_{f} is the exposure frequency (day for year), $B_{average\ body\ weight}$ is the body weight (65 kg),

T_e is the average exposure time for non cancer risk (E_d ×365 days) and RfD is the oral reference dose (mg/kg/day) defined as the maximum tolerable daily intake of a specific metal that has not adverse affect The RfD of Cu, Fe, Mn and Zn was reported by USEPA (2011, 2012) . The E_f for chinese cabbage was calculated considering that local population consume this vegetable at a maximum of 150 day/years while for oregano was 180 day/year. Values of HQ>1 implies a potential risk to consumers (USEPA 2002).

It was assumed, according to the local population use, that the local adult inhabitants consumed an average of 1 g oregano/day and an average of 28 g chinese cabbage/day. The MC metal of plant was converted with a factor of 0.20, because both species commonly contain approximately 80% water content in the aerial part. The risk to human health for a metal was only associated with the direct ingestion of the edible part of the species.

Results and discussion

Soil properties and chemical composition

The two soils properties are given in Tables 1 and 2. The results showed that the soils were different regarding chemical characteristics except for organic C contents (Table 1) that, according to their medium texture, can be considered as very high for both soils (INIA-LQARS 2000). Both soils had almost neutral pH, although Ferragudo had a significantly higher value (6.9), and can be classified as non-saline considering the conductivity values that inhibit seed germination and plant growth (4 dS m^{-1}) (Ye et al. 2002). The cation exchange capacity (CEC) also varied between soils (Table 1), although both values can be considered low (INIA-LQARS 2000). However, the soil from Rosalgar presented lower CEC than Ferragudo soil, which can be related to lower Mn oxides concentrations (Table 1) and quantity and type of clay minerals, and therefore a smallest capacity to supply nutrients. In fact, the soil from Rosalgar showed a lower content of exchangeable cations than those from Ferragudo (Table 1).

Ferragudo soil presented higher extractable P and K contents (Table 1), compared to Rosalgar soil, but the opposite was obtained for total N concentration. It is important to underline the very low value of extractable P and medium values of extractable K ($\leq$10 mg P kg^{-1} and 41–85 mg K kg^{-1}; INIA-LQARS 2000) in the Rosalgar soil. Also, the total and available concentration of nutrients, such as Ca, K, P and Mg (Tables 2 and 3) was the lowest in Rosalgar soil. This variation at available nutrients level can be associated to the intervention realized in the soil of each area (namely soil fertilization) due to their use. In Rosalgar mine was stablished a cork oak tree system naturally colonized by several autochthonous plant species while in Ferragudo mine was implemented a silvopastoril system (especially with cultivated grass species) in the holm oak woodland. In general, and considering the available concentrations of the majority of nutrients (Tables 1 and 3), Ferragudo soil could be considered more fertile than Rosalgar.

Iron in total Fe oxides was higher in Rosalgar soil than Ferragudo soil while the opposite was obtained for Fe in non-crystalline Fe oxides. Moreover, Fe was mainly associated to the crystalline Fe oxides fraction of both soils. Maganese concentrations associated to Mn oxides were very high in both soils, especially in Ferragudo soil where it reached ~31 g kg^{-1} (Table 1). This Mn oxides concentration in Ferragudo can also justify the highest CEC values obtained in this soil (Brady and Weil 2008).

In general, total concentrations of the studied elements in Ferragudo soil reached the highest values (Table 2). Exceptions were obtained for Fe, S and Zn where Rosalgar soil had higher concentrations than Ferragudo soil. As expected, both soils contained high total content of Mn being higher in Ferragudo soil. The high Fe content obtained in Rosalgar soil (Table 2) is a consequence of the highest richness in Fe in the Rosalgar Fe–Mn mineralization (mainly composed of Fe and Mn oxides containing 43% of Fe and 8% of Mn; Matos et al. 2013) than in Ferragudo where the mineralization rich in carbonates and oxides of Mn produced 30% and 50% of Mn, respectively (Almeida and Fernandes 1948; Matos and Rosa 2001).

The total concentrations of Cu, Mn, S and Zn in Rosalgar soil as well as Fe in Ferragudo soil exceeded the European topsoils baseline values (Salminen et al. 2005), as well as the average concentrations in non-contaminated soils from the region (Abreu et al. 2012) indicating an enrichment of these elements in the soils by mining activity. Considering agricultural purposes and total concentrations, both soils also exceeded the Canadian Soil Quality Guideline values for Cu and Zn (values should be lower than 63 mg Cu kg^{-1} and 200 mg Zn kg^{-1}; CCME, 2006) and the maximum allowable concentration for Mn (1500–3000 mg kg^{-1}; Kabata-Pendias 2011).

The elements concentrations (except for Ca) in the available fraction of both soils were small representing lower than 5% of the total concentrations (Table 3). Nonetheless, the availability of Ca was significant representing 15 and 45% of the total concentration for Ferragudo and Rosalgar soil, respectively. The available concentrations of Ca, P and Mg were the highest in Ferragudo soil, agreeing with the tendency obtained for total concentration of the same elements. However, higher concentration of Fe, K, Mn and Zn in the available fraction was obtained in Rosalgar soil (Table 3). It should be noted the great concentration of Mn in the available fraction of both soils (Table 3), being higher in Rosalgar than Ferragudo, whereas the opposite was obtained for total Mn concentrations. Total Mn contents in soils worldwide vary from 411 to 550 mg kg^{-1} (Kabata-Pendias 2011), which are lower than the Mn concentration in the available fraction of Rosalgar and Ferragudo soils (1053 mg kg^{-1} and 873 mg kg^{-1}, respectively). The ability of Mn to form anionic complexes and complexes with organic ligands may contribute to increase Mn concentration in the soil solution (Kabata-Pendias 2011). The available Fe content was very low in Ferragudo (Table 3, 6.88 mg kg^{-1}) but high in Rosalgar (75.8 mg kg^{-1}).

Concentration of the elements in soil available fraction before and after plant growth under pot experiment

In Table 3, the concentration of the elements in the available fraction of the soils before and after the plant cultivation are presented. Each plant species affected differently the pH and the final chemical composition of the soils. Oregano growth leaded to a decrease of the pH in both soils, while chinese cabbage did not change the soil pH.

Nonetheless, elements availability varied with plant species, soil and element. In fact, the available concentrations of most elements were the lowest after chinese cabbage growth (Ferragudo: Ca, K, Mn, P and S; Rosalgar: Fe, K, Mn and Zn). This variation can be associated to the rhizosphere conditions created by each plant species and/or behaviour and nutritional needs of the plants (Dakora and Phillips 2002; Cesco et al. 2010). In general, oregano growth contributed to an increase of the availability of most of the elements in both soils (Ferragudo and Rosalgar: Cu, K, Mg, P and S; Ferragudo: Zn). Manganese contents decreased after both species growth and this pattern was also observed for Fe in Rosalgar and for Ca in Ferragudo. It is interesting to underline the pH change after oregano cultivation in both mine soils (Table 3). This species induced a slightly decrease of soil pH, which can explain the increase of the availability of most of the studied elements (Table 3). Changes in soil pH induced by plant roots may be produced by differential cation/anion uptake, root respiration, H^+ release (carboxylates) or redox processes at the rhizosphere (Hinsinger et al. 2003) and, consequently, affect the elements availability (Kabata-Pendias 2011). Plant root exudates, whose composition varies with plant species or varieties, associated to microorganisms' action and the soil conditions in which the plant grows can also contribute to the availability of the elements to the plants (Kabata-Pendias 1993; Hinsinger et al. 2003).

Soil-plant system, growth and elements accumulation in food crops under pot experiment

Oregano plants presented good development and without visual symptoms of elements deficiency or toxicity in both soils, while chinese cabbage showed reddish coloured leaves when grown in Rosalgar soils, that is a common symptom of P deficiency (Lee et al. 1996) (Figure 1). Differences in the plant growth (measured through fresh biomass weight) in the two studied soils were obtained only in chinese cabbage plants (Table 4). Moreover, the cabbage plants growing in Ferragudo soil exhibited the best growth, in terms of plant weight and height (Table 4). This fact can be associated to the better fertility of Ferragudo soil than Rosalgar soil (Table 1) namely higher contents of available cations, as Ca, Mg and P (Table 3).

The photosynthetic performance indexes PRI and NDVI measured on cabbage leaves were not statistically different between plants growing in the two soils (Table 4). No correlations were found between PRI and NDVI, plant biomass or plant height. Similar results were observed for oregano plants (Table 4). In both soils, the two species showed a mean value of NDVI that can be considered as normal for healthy plants (0.2–0.8; Rouse et al. 1974). The NDVI is a good estimator of the spatial variability of carbon uptake (Garbulsky and Paruelo 2004) and PRI is a good index to estimate photosynthetic efficiency (Gamon et al. 1992; Garbulsky et al. 2011). Thus, the present results indicated that cabbage and oregano plants growing in both soils had similar photosynthetic efficiency.

The plant analysis (Table 5) indicate that both species growing in metal enriched soils have high element concentrations, mainly Fe, Mn, S and Zn, in the leaves even if elements uptake and translocation change according to plant species and ecotype (Abreu et al. 2014a; He et al. 2015). Regardless the high concentrations of those elements in above ground part of both species, their levels were different. Significant differences in metal accumulation (Cd, Hg, Pb, Zn) have also been found in different vegetable gardens (e.g. tomato, been, carrot) near an abandoned lead/zinc mine (Sipter et al. 2008). Manganese concentrations in the leaves of oregano and shoots of chinese cabbage grown in both soils (Table 5) were greater than the normal range for mature leaf tissues (30–300 mg kg^{-1}; Kabata-Pendias 2011) but non-toxic for cattle and other domestic animals (400-2000 mg kg^{-1}, Chaney 1989; National Research Council 2005). Generally, Mn is rapidly taken up, when it occurs in soils in available forms, and translocated within plants (Kabata-Pendias 2011). Nonetheless, the highest Mn concentrations in the available fraction in Rosalgar soil (Table 3) were not reflected in the Mn concentration of the plants shoots. In fact, elements concentrations in plants cannot be the direct result of the available soil fraction of the element since other processes can occur. These processes can be related to the interaction between elements (antagonism and/or synergism) from available soil fraction and/or potential uptake restriction and low extent uptake of elements by plants when their concentrations in soil are high (Abreu et al. 2014a**).**

Iron concentration in the aerial part of both species (Table 5) was higher in plants growing in Rosalgar soil than in Ferragudo soil, and it was elevated considering the critical value referred by Markert (1996; 500 mg Fe kg^{-1}), reflecting the higher total and available Fe content in Rosalgar soil (Tables 2 and 3). The Fe concentration measured in aerial part of both species grown in Rosalgar soil was much higher than the maximum tolerable value for some domestic animals like cattle, sheep and poultry (500 mg kg^{-1}, National Research Council 2005) and higher than the range considered normal for plants (50–250 mg Fe kg^{-1}, Srivastava and Gupta 1996). Nonetheless, both species tolerated well this high Fe concentration and visual toxicity symptoms were not displayed. Plants tolerate Fe excess by different mechanisms, which include the oxidation and immobilization and/or exclusion of soluble Fe by roots (Kabata-Pendias 2011).

The P concentration in cabbage shoots grown in Rosalgar soil was significantly lower than when grown in Ferragudo soils explaining the visual deficiency symptoms already referred (Fig. 1). The low P content in the shoots of cabbage (Table 5) might be due to the high level of Fe, element that may interfere with P uptake (Zheng et al. 2009; Kabata-Pendias 2011; Rai et al. 2015), and/or the low contents of available P in Rosalgar soil (Table 1). The symptoms of P deficiency were not observed in oregano probably because its roots were able to acidify the rhizosphere soil (Table 3), increasing de availability of P in the soil, which can be taken up by the plants.

In the cabbage a positive correlation was found between plant P and fresh biomass ($r=0.93$) while a negative one was found between fresh biomass with plant Fe, S and Zn concentrations in shoots ($r=-0.84$; $r=-0.95$; $r=-0.93$ respectively).

Shoots of cabbage and leaves of oregano showed normal Cu concentrations (Kabata-Pendias 2011). Cabbage plants grown in Rosalgar soil showed higher Zn and S concentrations (Table 5) than normal values in edible part of food plants (Kabata-Pendias 2011) and in the reference plant for S (Markert, 1996), but not reaching Zn toxicity levels for cattle, sheep and chicken (300-1000 mg kg-^{1}, Chaney 1989) or other domestic species (swine and poultry) (500-1000 mg kg-^{1}, National Research Council 2005). Plants growing in Rosalgar soil showed higher Zn concentration than plants growing in Ferragudo, which is in agreement with the highest concentration of this element in the total and available fraction of this soil (Tables 2 and 3).

Potentially toxic elements may be immobilized in the roots or translocated into the shoots (Abreu et al. 2014a; Baker 1981). Root uptake and transport into shoots are influenced by many factors such as the total and available contents of potentially toxic elements, composition of the rhizosphere exudates, environmental conditions and particular plant traits (Kalis et al. 2008; Soriano-Disla et al. 2014). Moreover, some plants have developed different tolerance mechanisms to exclude toxic elements and avoid harmful intracellular levels (Baker et al. 2010; Krämer, 2010; Abreu et al. 2014a; Rossini-Oliva et al. 2018). The studied species stored in their roots most of the chemical elements as the calculated TF was <1 (McGrath and Zhao 2003) (Fig. 2a-b), except for S in cabbage growing in the Rosalgar soil. These species were able to immobilize potentially toxic elements in their roots and limit their translocation to the leaves (*excluders*, according to Baker 1981), this is an important feature since the edible part of the plants is the aerial part in both species.

The bioaccumulation coefficient (BC) was higher than unit for Cu, Fe, S and Zn in both species, except for Mn (Figure 3). Although no significant correlations were found between elements concentrations in the available fraction of the soils and in plants, the BC values seem to indicate that both species were able to uptake elements from the soils when they occur in the available form, except for Mn.

Risk of adverse health effects from human consumption of cabbage and oregano

The transfer of potentially toxic elements from soils to plants is a great concern when the soils are used for cultivation purposes (Chojnacka et al. 2005). The studied species showed high concentrations of Fe and Mn in aerial part (Table 5) and, consequently, their consumption might represent a risk for humans. According to Kabata-Pendias (2011), the Mn content in plant foodstuffs is variable being the highest values obtained in beet roots (36–113 mg kg^{-1}), whereas in edible parts of vegetables the Fe concentration reach mean values from 29 to 130 mg kg^{-1}. The Mn and Fe concentrations found in oregano and cabbage shoots/leaves were much higher than those reported above (Table 5).

According to the calculated values of hazard quotients (Table 6), the contribution of the chinese cabbage and oregano to the daily intake of Cu, Fe, Mn and Zn would not represent risk of adverse effects to consumers health since they were below the unit. Similar results were found in vegetables produced in kitchen gardens located in the vicinity of abandoned pyrite mines from the Portuguese sector of the Iberian Pyrite Belt (Alvarenga et al. 2014) as well as for *Arbutus unedo* leaves collected in Panasqueira mine area of north Portugal (Abreu et al., 2014b).

Conclusions

The past mining activities in Rosalgar and Ferragudo caused soils contamination by Fe and Mn, reaching the total concentration of Mn values up to 122 g kg^{-1} of soil. Soils from both abandoned mines are suitable for the cultivation of cabbage and oregano, since the calculated hazard quotient of Cu, Fe, Mn and Zn were lower than unit and, therefore, its consumption can be considered safe for alimentary uses.

Since the soils are still quite enriched with Mn with high levels in the available fraction, which can be easily uptake by plant food species, attention must be paid by the inhabitants of the region before use these soils for plant food cultivation. More studies using other edible species should be undertaken in both areas. With this information, the best food plant species considering the lower health risk associated to metals excess might be suggested to the local populations in order to produce food plants with lower health risk.

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