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Full Length Research Paper

Transfer and accumulation of lead, zinc, cadmium and copper in plants growing in abandoned mining-district area

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The analysis of the Jebel Hallouf-Sidi Bouaouane mining-district soil has shown that the surface horizons are strongly contaminated by heavy metals, especially during floods or in lee-side areas. The contents of metallic-trace elements (MTE), in the soil and two cultivated plant species, have been determined by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). The results show that the concentration of metals in the soil are up to 39 g. kg^{-1}, 6.3 g. kg^{-1}, 56 mg. kg^{-1} and 131 mg. kg^{-1}, for lead (Pb), zinc (Zn), cadmium (Cd) and copper (Cu), respectively. The chemical analysis of plants: broad beans [*Vicia faba* (L.)] and barley [*Hordeum vulgare* (L.)], sampled inside the mining district, show that the roots and the upper part (leaves and stem) are enriched for Pb (up to 508 and 220 mg.kg^{-1}, respectively), Cd (up to 8 and 5 mg.kg^{-1}, respectively), and Zn (up to 491 and 468 mg.kg^{-1}, respectively) when compared with similar species collected far from the contaminated site (up to 9.6 and 0.8 mg.kg^{-1} for Pb, up to 0.04 and 0.04 mg.kg^{-1} for Cd, up to 44 and 15 mg.kg^{-1} for Zn, respectively). Statistically, the concentration of MTE in the soils and plants of the mining area is significantly different from the concentrations of the same elements in the soils and plants of the control ones ($p < 0.01$). This is expressed by a strong Enrichment Coefficient (EC), which is given by the ratio between the concentration of the metal in the contaminated plant and the concentration of the same element in the similar control one. The results (up to 405, 342 and 32, respectively), indicate that the contamination has reached the first link of the food chain. However, the calculation of the translocation factor (TF), deduced from the ratio between the concentration of the metal in the outer part of the plant and the concentration of the same element in the roots, shows that, the broad beans accumulates metals in the roots (TF < 1); whereas for barley, metals are fairly distributed between the roots and the outer part (TF ≈ 1). These results suggest that the cultivated areas inside the mining district constitute a serious source of contamination of the food chain. Therefore, actions have to be taken in order to remedy this problem.

Key words: MTE, contamination, transfer, soil, plant.

INTRODUCTION

The development of mining industries has generated a great number of risks and hazards that jeopardize eco-systems throughout the world (Gupta and Sinha, 2006). Ninety percent of the mining wastes come from the extraction of metals as sulfides (Moore and Luoma, 1990). High concentrations of toxic metals (e.g. Cu, Zn, Cd and Pb) are usually contained in these wastes (Levy et al., 1997). These metals can cause widespread contamination of soils and sediments in the vicinity of the mining areas (Clement et al., 2007; Zhou et al., 2007). At very high concentration rates in the soil, and when

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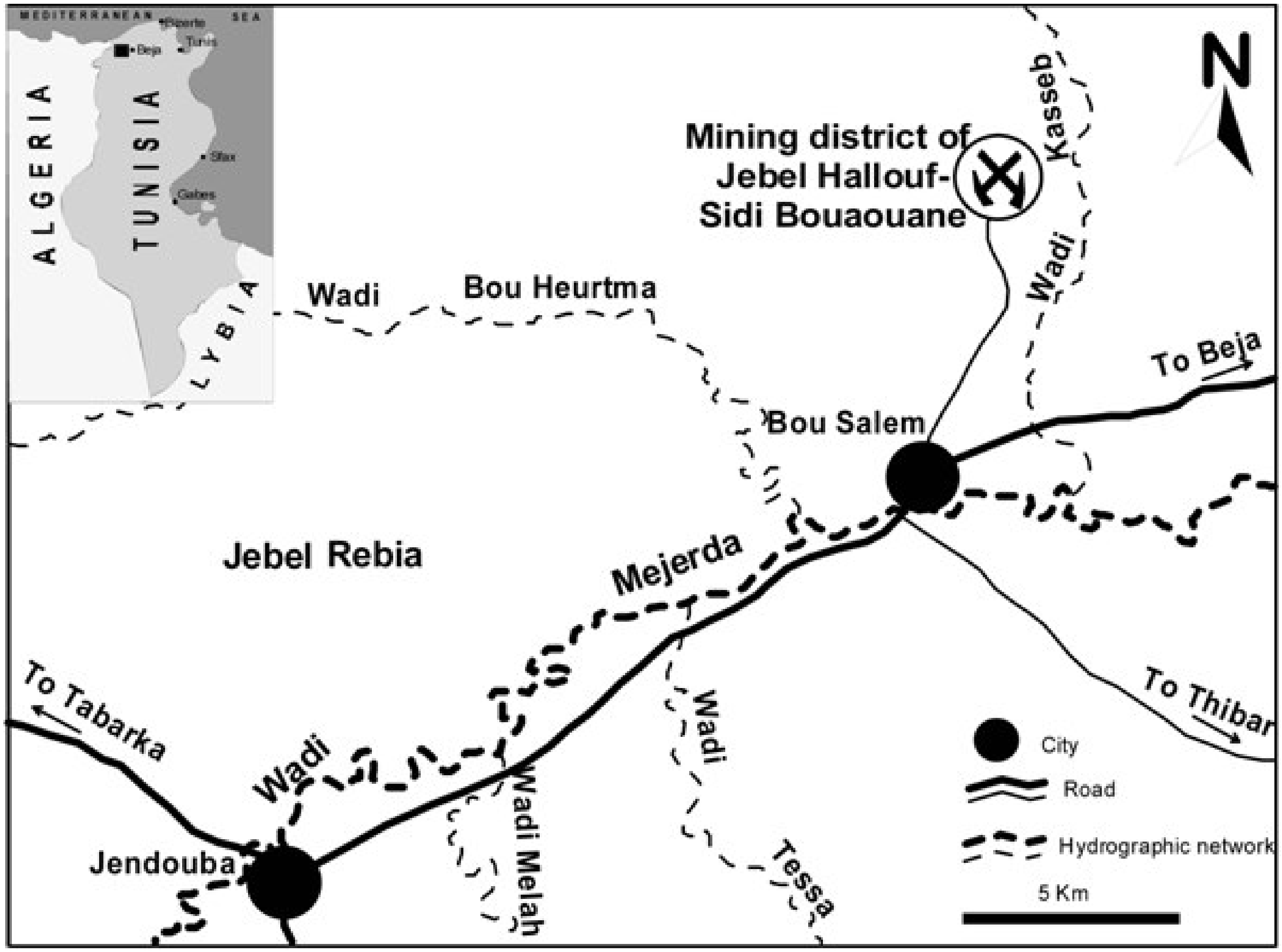

Figure 1. Location map of the Jebel Hallouf-Sidi Bouaouane mining district.

introduced in the food chain, these elements can adversely affect plant growth and human health (Tiryakioglu et al., 2006). In order to evaluate the potential health or the ecological hazards of a contaminated soil, the first step consists in determining the total MTE concentration rate in the soil (Cook et al., 2000), and the containment of MTE dispersion. The second step of the study is to assess the mobility of the elements and their bioavailability.

Lead and cadmium are considered as toxic elements with respect to biosphere (Pereira and Sonnet, 2007), especially in arable lands on the outskirts of mining areas (Das et al., 1997; Sanita and Gabbrielli, 1999). Copper, which occurs in a great number of proteins (Clemens, 2001; Loue, 1993; Marschner, 1995) and zinc are necessary to the metabolism of plants (Marcic, 2005). The latter becomes toxic if available in high concentration.

Plant contamination is mainly caused by the more mobile MTE chemical species, as long as they are easily absorbed by plants (Cook et al., 2000; Parida et al., 2003); so, it is important to predict the likely mobility of metals in the soil (Campos et al., 1998). Previous studies (Ahumada et al., 1999; Hatira, 2004) have shown that Cd and Zn are very mobile and easily absorbed by plants, while under reduced conditions Pb is less mobile and very toxic.

In this work, the authors aim to evaluate the heavy-metal (Pb, Zn, Cd, Cu) concentrations in soils contaminated by particulate material transported by running waters, along with the assessment of the food-chain contamination level through the analysis of plants cultivated inside the mining area.

MATERIALS AND METHODS

Site characteristics

The district of Jebel Hallouf Sidi Bouaouane, a Pb-Zn-mining area abandoned since 1986, is located in the North-West of Tunisia (Figure 1), 10 Km to the North of Bou Salem (8°59'42'' - 9°15' E and 36°42'18''-36°41'24'' N). Millions of tons of solid wastes have

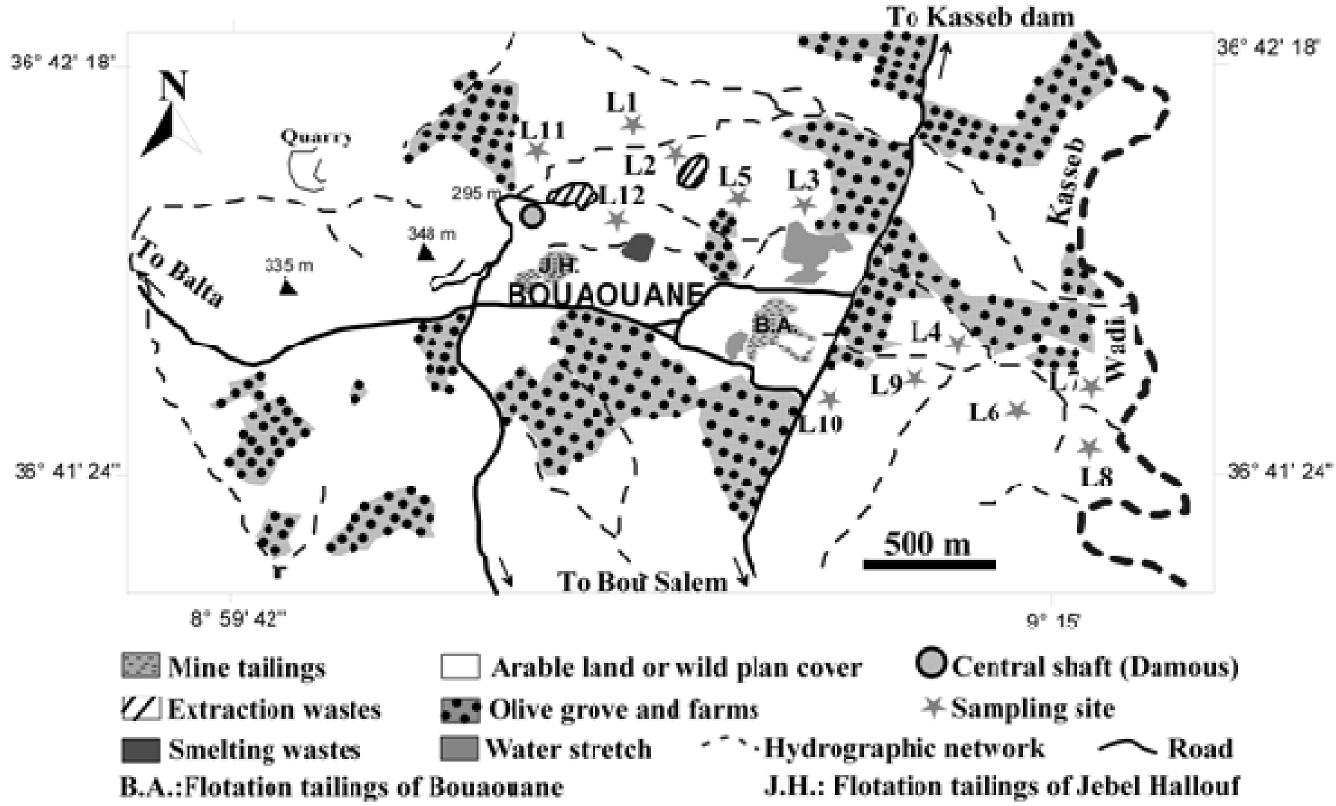

Figure 2. Location map of the plant and their rhizosphere sampling sites in the study area.

been generated (Direction des Mines et de la Géologie, 2003; Sainfeld, 1952), and are dumped on the eastern side of Jebel Hallouf, situated in the watershed of Wadi Kasseb, a tributary of Wadi Mejerda (river) (Figure 2). This one constitutes an important water-reserve which provides the North of Tunisia with household and irrigation water. The soil of the mining district and the surrounding areas is of fersiallitic type, developed on carbonate rock material. From the climatological point of view, the study area is characterized by humid conditions in winter and dry conditions in summer. Mean rainfall ranges from 300 to 600 mm/year, and may go up to 900 mm during the rain seasons. Permanent winds blow from the West to North-West at a mean speed of 19 m/s. Evaporation is around 1585 mm/year (Institut National de Météorologie (I.N.M, 2003).

Sampling, preparation and chemical analysis

Two species, representing the locally-cultivated green layer, are considered in this study. Samples are selected from twelve different lots, each one consisting of several plants of the same species collected from the same lot: broad beans (*Vicia faba* (L.)), being leguminous (four samples selected) and barley (*Hordeum vulgare* (L.)), being graminous (eight samples selected). Here, the plants and their rhizospheres have been collected (Figure 2). The same species and their rhizosphere have been sampled at a 3 and 7 km distance (two samples), from the mining area, and that will be used as bio- and geochemical control samples.

In the laboratory, plant samples are washed with distilled water and ultrasonic batch to remove all the dust and earth left. The upper part (leaves and stem) and the roots are separated then dried (at 50°C for 48 h), then finely grounded (< 2 mm) to be readily soluble in 1% nitric solution.

The soil samples were collected in a 0 to 20 cm depth interval below surface, then dried (at 50°C for 48 h) and sieved. The fraction below 2 mm has been dissolved in triacid solution ($HClO_4$, HF and HNO_3). The solubilisation of all samples and their analysis by ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy: Activa of HORIBA-JOBIN YVON) for the determination of Pb, Zn, Cd and Cu, have been conducted in the Laboratory of Applied Ecophysiology, at the Faculty of Sciences, University Jean Monnet and in the GENERIC Department of the National Mining College at Saint Etienne. One plant and two rock standards (tobacco, ACE and GSN, respectively) have been used for calibration. Measurement reproducibility was better than 5% for all elements.

Two parameters are calculated for soil and plants: the first one is the enrichment coefficient (EC) calculated for soil: $EC_{soil} = [Me]_{site\ soil} / [Me]_{control\ soil}$ and plant (EC_{plant}) : $EC_{plant} = [Me]_{site\ plant} / [Me]_{control\ plant}$. This parameter is very important to deduce the degree of soil and plant contamination.

The second parameter is the translocation factor (TF): $TF = [Me]_{outer\ part} / [Me]_{root}$. This one gives indicators about the capacity of the plant to accumulate or to transfer the toxic elements from one organ to another and explain the adjustment capacity of a plant absorbing a solution enriched with MTE from the rhizosphere.

Statistical analysis

One-way ANOVA was used to compare mean concentrations of MTE in the soil and plants of the study area and the control ones. The calculated probability (p) is considered as statistically significant when $p < 0.05$. Statistical analyses were made using XL

Table 1. Concentrations of the MTE in the upper part (leaves and stem), roots and soil of the broad bean (*Vicia faba*).

		Concentration (mg.kg^{-1})			
		Cd	**Cu**	**Pb**	**Zn**
	Leaves+stem	0.04	9.5	0.5	15
Control	Root	0.04	10.2	1.02	16
	Soil	1.1	35	153	170
	Leaves+stem	0.2	8.7	14	77
L1	Root	3.8	31.7	459	388
	Soil	15.6	123	2341	1812
	Leaves+stem	0.1	10.2	18	51
L2	Root	2.1	52.6	313	179
	Soil	18.8	135	3421	1849
	Leaves+stem	0.7	1.8	19	155
L3	Root	12.9	16.1	535	903
	Soil	49.1	155	7209	3257
	Leaves+stem	2.3	9.2	48	104
L4	Root	13.2	23.2	728	496
	Soil	38.5	139	5686	3615

stat software.

RESULTS AND DISCUSSION

Analysis of MTE in the soil

The study of the distribution of the MTE, in soil samples all around the tailings and rock dumps, has shown high concentration rates in Pb, Zn, and Cd, and notably (Tables 1 and 2), in terms of the corresponding amounts in control soil samples. These areas are the most contaminated by metals in mining districts (Lee et al., 2001; Xenidis et al., 2003). The probability calculation (p = 0.0038 for the soil cultivated for broad bean and p = 0.0018 for the soil cultivated for barley), shows that the concentration of MTE in the considered soils is significantly different from the concentrations of the same elements in the control ones.

To assess the degree of soil contamination, EC_{soil}, characterizing the soils in the mining area (Table 3), used for the cultivation of broad beans or barley, ranges from 92 to 111, for Pb, 35 to 50, for Cd and 16 to 21 for Zn. These factors show that the soils are highly contaminated by Pb and Cd and, at a lesser extent, by Zn. This enrichment in MTE is mainly caused by the propagation of the contaminants in the soils due to the mobilization of the mining wastes as particulate materials by running waters during the flood periods. For copper, however, the EC_{soil} that ranges from 2 to 3 shows that the soil of the district is not contaminated by this element.

Analysis of MTE in plants

The analysis of MTE (Pb, Zn, Cd and Cu) has been conducted on four broad-bean samples and eight barley ones, all collected inside the mining area, as well as on two control samples of the two species under consideration. The charts (Figure 3) highlight the high mean concentrations in MTE in the different physiologic parts (roots and upper part) of the cultivated plants inside the district for the corresponding mean concentrations in the reference plants. Such variations are due to the high amounts of MTE in the rhizosphere of these plants.

The analyses of the roots of these plants show that, in broad beans, the concentration rates may go up to 508 mg.kg^{-1} for Pb, 491 mg.kg^{-1} for Zn, 8 mg.kg^{-1} for Cd and 31 mg.kg^{-1} for Cu, whereas in barley the highest amount of concentration may go up to 237 mg.kg^{-1}, 440 mg.kg^{-1}, 6 mg.kg^{-1} and 14 mg.kg^{-1}, respectively for the same elements. The probability calculation (p = 0.0013 for broad bean and p = 0.00016 for barley), shows that the concentration of MTE in the roots of plants is significantly different from the concentrations of the same elements in the roots of the control ones. Wierzbicka (1998) and Chaignon (2001), who take specific interest in Pb and Cu, have stated that the major part of these elements is

Table 2. Concentrations of the MTE in the upper part (leaves and stem), roots and soil of barley (*Hordeum vulgare*).

		Concentration ($mg.kg^{-1}$)			
		Cd	**Cu**	**Pb**	**Zn**
	Leaves+stem	0.04	3.6	0.8	15.4
Control	Root	0.04	10	9.6	44.4
	Soil	0.36	15.5	26	68
	Leaves+stem	0.8	8.2	50	402
L5	Root	3.1	13.7	188	177
	Soil	46.8	157	7160	3299
	Leaves+stem	0.2	4.9	6	59
L6	Root	1.8	11.5	48	174
	Soil	8.3	75	968	1200
	Leaves+stem	0.4	6.9	3	118
L7	Root	1.8	11.4	67	292
	Soil	6.5	57	869	1088
	Leaves+stem	2.3	11.2	62	278
L8	Root	7.6	28.1	471	446
	Soil	18.5	110	3806	2036
	Leaves+stem	3.6	11.8	61	757
L9	Root	14.8	13.6	288	1028
	Soil	39.9	104	4674	3633
	Leaves+stem	0.7	7.4	18	201
L10	Root	4.5	12.3	160	478
	Soil	17.7	75	1789	1938
	Leaves+stem	0.2	5.7	4	89
L11	Root	1.9	10.2	63	241
	Soil	5.2	42	464	640
	Leaves+stem	3.7	7.6	99	448
L12	Root	10.3	14.4	613	686
	Soil	31.9	157	11139	2736

Table 3. Mean concentration [M_{SP}] and enrichment coefficient (EC_{soil}) of the MTE in soils (n=number of lots).

	Cd		Cu		Pb		Zn	
	[M_{SP}]*	EC_{Soil}	[M_{SP}]*	EC_{Soil}	[M_{SP}]*	EC_{Soil}	[M_{SP}]*	EC_{Soil}
V. faba (n=4)	31±16	50	138±13	3	4664±2196	111	2633±939	21
H. vulgare (n=8)	22±16	35	97±43	2	3859±3735	92	2071±1081	16

*Mean concentration in soil ($mg.kg^{-1}$) ± SD.

sorbed mainly by the wall cells of the roots.

In the upper parts (stem and leaves) of the plants, the mean amounts of MTE, in broad-bean samples, are equal to 25 $mg.kg^{-1}$ for Pb, 97 $mg.kg^{-1}$ for Zn, 0.8 $mg.kg^{-1}$ for Cd and 7 $mg.kg^{-1}$ for Cu; however, higher mean concentration rates in these elements are observed in

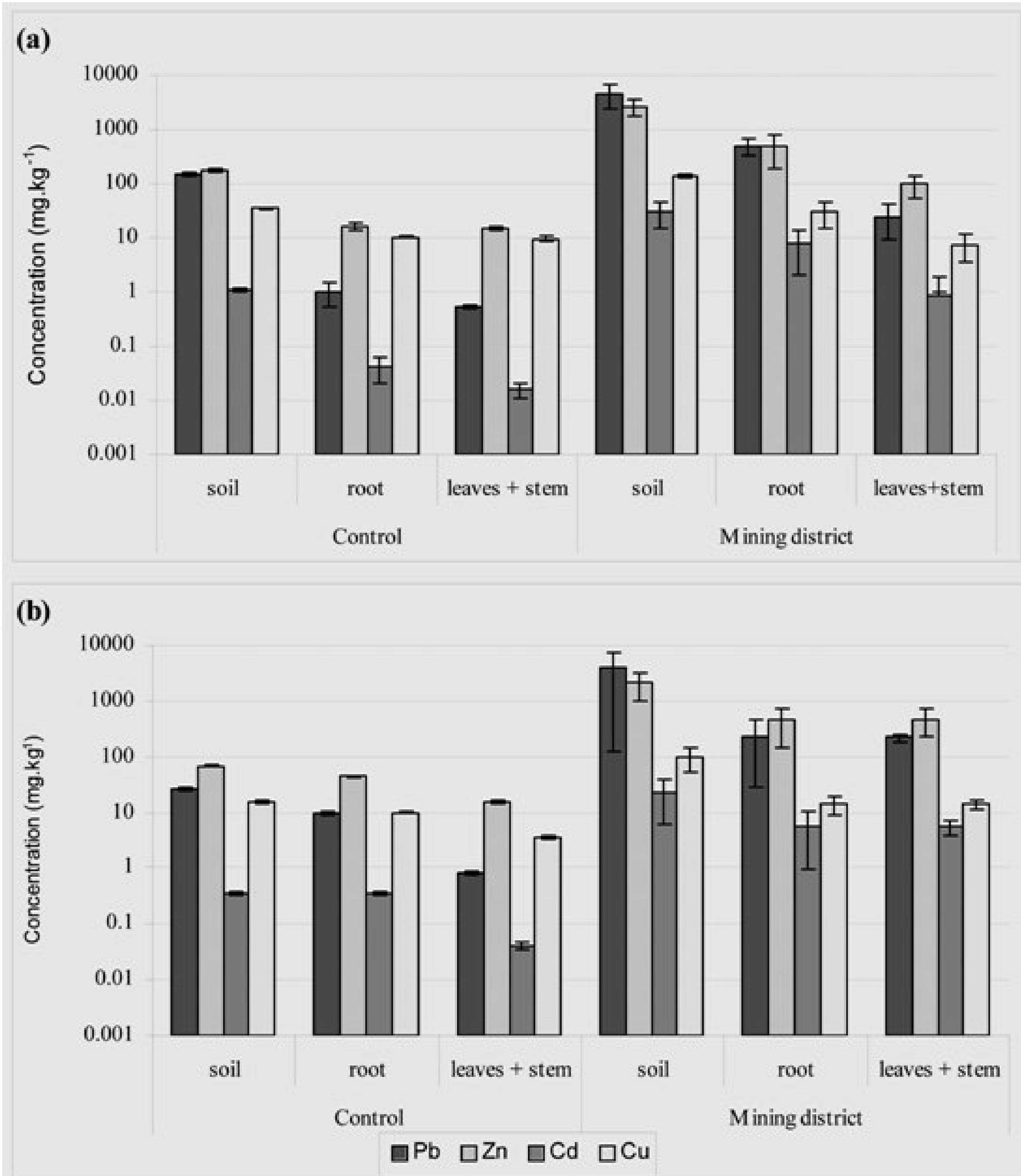

Figure 3. Variations of the MTE mean concentrations in (a) the broad beans (*Vicia faba* (L.)) and (b) barley (*Hordeum vulgare* (L.)) samples.

barley samples, to be in the range of 220 $mg.kg^{-1}$, 468 $mg.kg^{-1}$, 5 $mg.kg^{-1}$ and 14 $mg.kg^{-1}$, respectively. The probability calculation ($p = 0.0013$ for broad-bean and $p = 0.0001$ for barley), shows that the concentration of MTE in the upper parts of the plants is significantly different from the concentrations of the same elements in the upper part of the control ones.

Similar results have been illustrated for Zn by Brune et al. (1994) for barley, to confirm that this element can accumulate in leaves when the plants are exposed to higher metal- concentration levels.

All the high amounts in MTE, recorded for the plants collected in the mining area, should illustrate the high bio-availability of these elements in the soil, by the reactions that may occur at the soil-root interface (Alloway, 1995; Hinsinger, 2001; Mc Laughlin et al., 1998; Mench, 1990).

Table 4. Enrichment Coefficient (EC) of the MTE for the two species considered in this study (*V. faba* and *H. vulgare*).

	V. faba		***H. vulgare***	
	Leaves and stem	**Root**	**Leaves and stem**	**Root**
Pb	405	233	269	25
Cd	342	142	133	16
Zn	32	27.5	30.5	10
Cu	1.4	1.4	4	1.4

Table 5. Translocation factor (TF) of the MTE for the two species considered in this study (*V. faba* and *H. vulgare*).

	V. faba		***H. vulgare***	
	Control	**JH-BA mine**	**Control**	**JH-BA mine**
Pb	0.534	0.049	0.085	0.927
Cd	0.393	0.106	0.116	0.950
Zn	0.921	0.197	0.346	1.064
Cu	0.936	0.242	0.355	0.952

Enrichment coefficient (EC)

Results (Table 4) show that the different parts of the plants are rich mainly in cadmium and lead. In the outer parts, the EC_{plant} is equal to 342 and 405, respectively, for broad beans and 133 and 269, respectively, for barley, while for the roots, the EC_{plant} is equal to 142 and 233, respectively, for the broad bean and 16 and 25, respectively, for barley.

The enrichment is less important for zinc either in the root or in the upper parts, with EC_{plant} being equal to 28 and 32, respectively, for broad beans and 10 and 31, respectively, for barley. For copper, the EC_{plant} are almost equal to 1, thus showing that the plants are not contaminated by this element.

In conclusion, it can be stated that the plants, analysed in this study, are contaminated by Pb, Cd and Zn. As a result, these elements might be increasingly taken up by the crop and transferred, at a later stage, to the food chain (Beijer and Jernelöv, 1986, In: Friberg et al., 1986).

Translocation factor (TF)

The TF expresses the capacity of a plant to store the MTE in its upper part. This is defined as the ratio of metal concentration in the upper part to that in the roots (Soltz and Greger, 2002; Yoon et al., 2006).

Results (Table 5) show a contrasting behavior of the plants according to the quality of the soil and when we move from one species to another.

As far as broad beans are concerned, it should be noted that similar results are obtained either with the control sample or the samples taken inside the mining area.

The TFs of Cu and Zn are similar and almost equal to 1 ($TF_{Cu} \approx TF_{Zn} \approx 1$); while the TFs of Pb and Cd are similar (0.534 and 0.393, respectively), although lower than those of Cu and Zn. These results may be justified by the fact that the first two are oligolements, that are necessary for the plant growth (He et al., 2005), whereas the other two are rather harmful from the physiological point of view. So, and as reported by Denaix (2007), the plant keeps the highest quantity of them in the root system, either precipitated in the cells or chelated with an organic compound, thus lowering the trace element translocation to the outer parts.

Concerning barley and the control sample, at a preliminary stage, one should note that the TFs of all the elements are lower than one (0.085, 0.116, 0.346 and 0.355, respectively for Pb, Cd, Zn and Cu); which means that the physiological need of the plant for these elements is rather limited. However, similarity in the TFs is observed for Cu and Zn, on the one hand, and for Pb and Cd, on the other, with $TFs_{(Cu,Zn)}$ being superior to $TFs_{(Pb,Cd)}$, showing that the first elements are necessary for the growth of the plant. For the samples collected inside the mining district, an increase in terms of TF compared with the control sample is recorded, for all the elements, all values being close to one (0.927, 0.950, 1.064 and 0.952, respectively, for Pb, Cd, Zn and Cu). This enables us to conclude that within the soil, rich in MTE, the transfer of such elements to the upper part of the plant is enhanced, with no segregation at the level of the different elements.

Some species have the capacity to concentrate these MTE in the epidermic cells of leaves. Indeed, some of these cells have the capacity to accumulate metals

(Denaix, 2007).

In general, the more contaminated plants are the more accumulated heavy metals we notice in root cells, and specifically in the apoplasm. The uptake of these elements is regulated by active (metabolic) and passive (non-metabolic) mechanisms at the soil-root interface (Denaix, 2007).

Trace elements are translocated from roots to shoots via a number of physiological processes, including metal unloading into root xylem cells, long-distance carrying from the xylem to the shoots and metal reabsorption, by leaf mesophyll cells, from the xylem stream. Once the trace metals have been unloaded into the xylem vessels, the metals are carried to the shoots by the transpiration stream (Blaylock and Huang, 2000).

So, the contrasts observed, with respect to the biogeochemical characteristics of the two plant species under consideration, are the illustration of differences in the physiological behavior, either during the acquisition of the element at the soil/root interface, or at the time of the root/upper part transfer (Marschner, 1995).

Conclusion

The geochemical investigation has shown that the soil, in the mining area, is contaminated by lead, zinc and cadmium, due to the relocation of mining wastes in both flood and leeward areas.

The analysis of plants (broad beans and barley) has shown that the species collected in the mining district are highly contaminated with Pb and Cd and, to a lower extent, with Zn. This contamination illustrates the high amounts of these metals in the soil where they should be present as chemical forms that are mobile enough to be bio-available at the soil/root interface.

Taking into consideration the physiologic behavior of the plants, differences have been pointed out between the species sampled in the soil contaminated with heavy metals and the corresponding control samples.

Although the EC values recorded especially for Pb, Zn and Cd in the outer parts of the studied plants, are elevated (up to 405, 32 and 342, respectively), their mean concentration rates (up to 728 $mg.kg^{-1}$, 903 $mg.kg^{-1}$, 13.2 $mg.kg^{-1}$, respectively), are not high enough to allow the use of these plants for phytoremediation. In this technique, only the plants having the capacity to accumulate up to 10000 $mg.kg^{-1}$ in Zn, 1000 $mg.kg^{-1}$ in Pb and 100 $mg.kg^{-1}$ in Cd in their outer parts, are used (Baker and Brooks, 1989; Baker et al., 1994; Wei et al., 2002).

In another respect, in the mining area, broad beans accumulate more metals in their two physiological parts, but the transfer toward the upper part is partial (TF < 1), whereas for barley, metal concentration rates, in the two physiological parts of the plant, are almost equal (TF ≈ 1). According to Baker (1981), this statement shows that the first species, which accumulates metals in the roots behaves as an excluder, while the second one, for which the concentration of metals are in equilibrium between the roots and the outer part, behaves as an indicator.

In conclusion, this study has revealed that the use of arable lands for food production and as pasture lands, and located in the mining district, is hazardous for man and livestock. So, the authors suggest that decontamination actions have to be taken in order to help these areas recover their previous status.

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