Water Air Soil Pollut (2011) 216:485–504
DOI 10.1007/s11270-010-0547-1

Heavy Metal Concentration Survey in Soils and Plants of the Les Malines Mining District (Southern France): Implications for Soil Restoration

Jose Escarré · Claude Lefèbvre · Stephan Raboyeau · Anabelle Dossantos · Wolf Gruber · Jean Claude Cleyet Marel · Helene Frérot · Nausicaa Noret · Stephanie Mahieu · Christian Collin · Folkert van Oort

Received: 11 March 2010 / Accepted: 7 July 2010 / Published online: 5 August 2010

Abstract Mining activities generate spoils and effluents with extremely high metal concentrations of heavy metals that might have adverse effects on ecosystems and human health. Therefore, information on soil and plant metal concentrations is needed to assess the severity of the pollution and develop a strategy for soil reclamation such as phytoremediation. Here, we studied soils and vegetation in three heavily contaminated sites with potential toxic metals and metalloids (Zn, Pb, Cd, As, TI) in the mining district of Les Malines in the Languedoc region (southern France). Extremely high concentrations were found at different places such as the Les Aviniéres tailing basins (up to 160,000 mg kg^{-1} Zn, 90,000 mg kg^{-1} Pb, 9,700 mg kg^{-1} of As and 245 mg kg^{-1} of Tl) near a former furnace. Metal contamination extended several kilometres away from the mine sites probably because of the transport of toxic mining residues by wind and water. Spontaneous vegetation growing on the three mine sites was highly diversified and included 116 plant species. The vegetation cover consisted of species also found in non-contaminated soils, some of which have been shown to be metal-tolerant ecotypes (*Festuca arvernensis, Koeleria vallesiana* and *Armeria arenaria*) and several Zn, Cd and Tl hyperaccumulators such as *Anthyllis vulneraria, Thlaspi caerulescens, Iberis intermedia* and *Silene latifolia*. This latter species was highlighted as a new thallium hyperaccumulator, accumulating nearly 1,500 mg kg^{-1}. These species represent a patrimonial interest for their potential use for the phytoremediation of toxic metal-polluted areas.

J. Escarré (✉) · S. Raboyeau · A. Dossantos · C. Collin
Centre d'Ecologie Fonctionnelle et Evolutive,
UMR CNRS 5175,
Route de Mende 1919,
34293 Montpellier Cedex 05,
France
e-mail: jose.escarre@cefe.cnrs.fr

C. Lefèbvre · W. Gruber · N. Noret
Laboratoire d'Ecologie végétale et
de Biogéochimie,
Université Libre de Bruxelles,
ULB-Campus de la plaine, C5,
CP244. Boulevard du
Triomphe,
1050 Bruxelles, Belgium

J. C. Cleyet Marel · S. Mahieu
INRA, USC1242, Laboratoire des Symbioses Tropicales
et Méditerranéennes,
Campus International de Baillarguet TA A-82/J,
34398 Montpellier Cedex 05, France

H. Frérot
Laboratoire de Génétique et Evolution des Populations
Végétales, UMR CNRS 8016,
Université Lille 1, Bâtiment SN2,
59655 Villeneuve d'Ascq Cedex, France

F. van Oort
INRA, UR 251 Physicochimie et Ecotoxicologie des Sols
d'Agrosystèmes Contaminés (Pessac), RD-10,
78026 Versailles Cedex, France

Keywords Soil pollution · Hyperaccumulation · Phytostabilisation · Mine tailings · Metal-tolerant species

1 Introduction

Environmental disturbances resulting from human activities, such as deforestation, land clearing or mining, deeply modify the dynamics and functioning of ecosystems (Vitousek et al. 1997). The cessation of such disturbances generally leads to the long-term natural reconstitution of the ecosystem (Holl 2002). However, for major disturbances such as soil destruction, the return to the former natural ecosystem is very difficult (Retana et al. 2002) or even hopeless. This is the case for many sites contaminated by mining activities that generated spoils, effluents and dust with outstandingly large concentrations of metal elements (Zn, Pb, Cd, Cu) or metalloids (As), which might have adverse effects on biological receptors and ecosystems (Wiegleb and Felinks 2001).[1] The ecotoxicity and mobility of these elements in soils depend, among other intrinsic and external soil factors, mainly on their chemical speciation. The spatial distribution and degree of contamination is mainly governed by the ways of dissemination of metal pollutants from the source into the environment, such as gully erosion by surface drainage of the waste heap, groundwater contamination by vertical drainage (Dère et al. 2006), water transport by rivers (Audry et al. 2004; Gabler and Schneider 2000), wind erosion or industrial dust fallout from smelters (Douay et al. 2008; Fernandez et al. 2007; van Oort et al. 2009) or by the breaking of a mine tailing dike, as that which occurred in Aznalcollar, Spain (Grimalt et al. 1999).

The decontamination of highly metal-contaminated soils by physical or chemical methods is very difficult and costly (Cunningham and Berti 2000). Phytostabilisation, i.e. the use of plants to cover and stabilise contaminated soils (Terry and Bañuelos 2000), represents a low-cost technology. For this, detailed studies of the soils and vegetation of such strongly metal-contaminated sites and their peripheries are essential for an accurate assessment of the metal toxicity of soils and aerial plant parts with regard to the possible toxic impacts on herbivorous consumers and human health. Such studies are also necessary to provide valuable information for reasoning strategies of land reclamation in metalliferous sites.

Only a restricted number of plants from the local flora are able to grow in metalliferous soils. In several study cases, these plants have physiological adaptations such as metal tolerance that are different from non-tolerant plants of the same species that grow in uncontaminated soils (Antonovics et al. 1971). They can even be considered different ecotypes (Lefèbvre and Vernet 1990). The reclamation of metal-contaminated sites by plants native to toxic areas aims to stabilise the soil, immobilising trace elements in the rhizosphere and thereby reducing the risks of the dissemination of metalliferous dust by wind, water erosion or by downward water percolation from the root zone.

The aim of this study was to assess the diversity of plant species occurring in strongly contaminated mine sites in relation to the nature, composition and metal concentrations of mine topsoils. For this, we studied several strongly Zn- and Pb-contaminated sites of the Les Malines mining district in the French Languedoc region, where extraction activities took place from Roman times (200–100 BC) until the end of the twentieth century. We sampled the surface layers of soils in three locations of the mining sites (2 km apart from each other), selected according to a decreasing age of abandonment of mining activity, as well as soils up to about 4.5 km from the mining sites to assess the spatial extension of metal contamination. All plant species occurring in these mining sites were identified, and their abundances and metal uptake capacities were quantified and related to the amounts of heavy metals in the soils with the objective of assessing their potential use in phytostabilisation and phytoextraction tasks.

2 Materials and Methods

2.1 Site Characteristics and Sampling Conditions

The studied mine sites are located in the mining district of Les Malines in the vicinity of Ganges, near the village of Saint-Laurent-le-Minier (N 43°55′55″ E 003°39′19″), 40 km north of Montpellier, France. Mean monthly temperatures vary between 5.7–16.7°C

[1] For ease and convenience, both metals and metalloids are hereafter referred to as metals.

from January to May, 23–25°C from June to September and 6.3–15.0°C from October to December. The average annual rainfall reaches 990 mm (2001–2006). Metal deposits mined in the Les Malines district are very rich in Zn and Pb (Bouladon 1977), associated with Fe-S (pyrite) components.

We sampled three major mining sites (Fig. 1):

1. The Petra Alba site (PA) was mined approximately between the eleventh and fifteenth centuries (Bailly-Maître 1997). The site is located on a Cambrian calcschist substrate and its residual spoils differed from the waste material generated by the two other mines, where lenticular metal ore veins occurred in dolomite rock (Leach et al. 2006). The PA mine is located at the beginning of the Crenze valley, upstream from the village of Saint-Laurent-le-Minier. Samples were taken from three waste heaps of about 300 m^2 that were 50 m apart from each other and located on steep slopes covered with herbaceous vegetation, clearly contrasting with the surrounding chestnut (*Castanea sativa*) forest.
2. The Les Avinières site, located 1 km downstream from Saint-Laurent-le-Minier and 3.5 km from Ganges, opened in 1870 and became one of the major zinc mines in France (Vincent 2006). The site closed in 1914 and was never reclaimed. The site of Les Avinières includes two distinct parts of approximately 2 and 3 ha, respectively:
   a. the tailing ponds along the Vis River valley (hereafter 'AT' for Les Avinières Tailings), which were used during the twentieth century for processing ore from the Les Malines mine. We sampled three different types of soils: (1) bare soils of the tailing ponds, (2) soils from several formerly cultivated terraces above the contaminated tailing ponds and (3) pellets of soil with a characteristic orange colour close to an old furnace and under several individuals of *Iberis intermedia*, a species that hyperaccumulates thallium (Leblanc et al. 1999).
   b. the waste heaps (steep tailing mounds) of the old zinc and lead mine above the tailing ponds on the slope of the hill (hereafter 'AM' for Les Avinières mine). Soil sampling was performed in three topographic situations: (1) the rocky slopes with very sparse vegetation, (2) the vegetated slopes covering the mining waste heaps and (3) the flat surfaces along the extraction pits covered with sparse vegetation.
3. The Les Malines site (MAL), located 2.5 km upstream of Saint-Laurent-le-Minier and 6 km from Ganges, was the most important mine of the region. Its exploitation started in 1885 and closed in 1991, representing the last Zn and Pb mining activity in France (Rolley 2002). The upper part of the site contains many old spoil heaps of

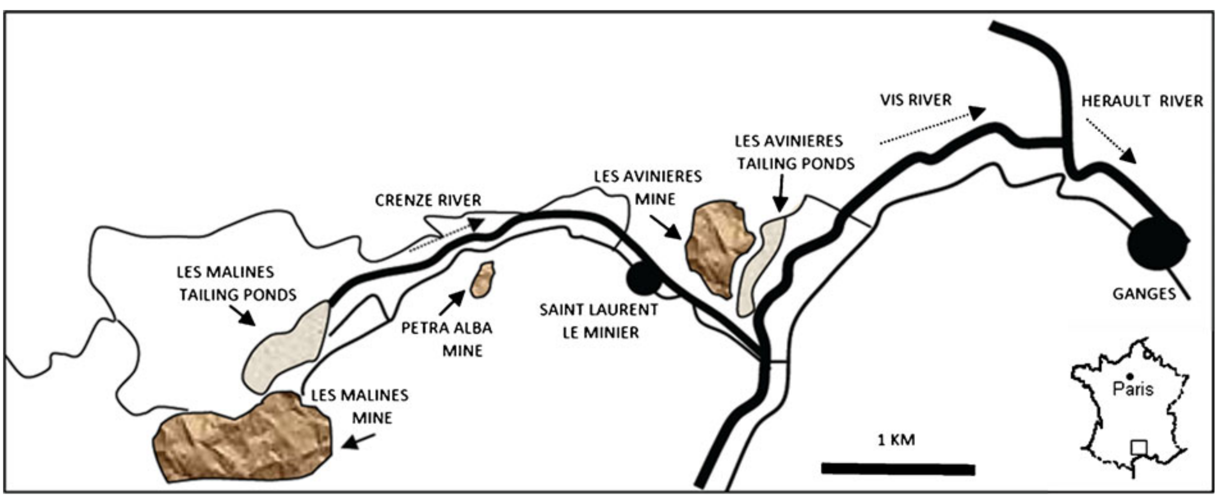

Fig. 1 Map of the mining district of Les Malines, 40 km north of the Montpellier region, southern France, with the locations of the three sampling sites

variable surfaces from 100 to 1,000 m^2 in size. The lower part corresponds to the washing ore basin of about 1 ha in size that was used during the most recent mining activity. The site was reclaimed in the 1990s by banking work and by the revegetalisation of the tailing pond (Amphoux and Laroche 1989) and since then the original soil surface has been consistently modified. Samples were taken in formerly strongly polluted plots, identified by old photographs, and corresponding in the field by locations with characteristic metallicolous herbaceous vegetation.

In summer 2005, we sampled soils and vegetation in the AM, AT, MAL and PA mining sites. At each sampling point three samples from the 0–20 cm surface layer were collected, at least 3 m apart from one another, and mixed to produce a pooled sample. Such an average value of metal concentration determined for a 20 cm soil depth is useful for a global comparison of strongly metal-polluted sites (Balabane et al. 1999; Dahmani-Muller et al. 2000). However, it does not well account for the often strong differentiation between organic and mineral soil layers and variable metal concentrations over a short distance at the soil's surface. Therefore, we additionally examined small pits in the mine soils for the sites PA, AM and AT, from 20 to 50 cm deep, to study the nature and chemical composition of successive mine-waste layers and the vertical distribution of metal concentrations and to assess evidence of initial soil formation. This work was not undertaken at the MAL site because it had been reclaimed. At the same sampling points, all the plant species occurring in an area of 100 m^2, with the exception of some sampling points without vegetation (e.g. the centres of tailing ponds and some rocky slopes), were sampled and identified using Tutin et al. (1964–1993). We sampled only healthy adult plants but in species with few individuals we collected only some leaves. The percentage of soil coverage by plants in each sample was estimated visually by two independent observers. In summer 2006 and 2007, we took additional soil samples in waste heaps devoid of vegetation in the Les Avinières mine, as well as some individuals of plant species collected in few numbers in preceding years or that showed an extraordinary concentration for some metals.

To determine the soil metal contents in the vicinity of the mining sites, we carried out a sampling campaign designed according to the distance from the Les Malines and Les Avinières mines. First, soil samples were taken following road axes likely to be polluted by the transport of ore. Second, soils were sampled along the banks of the Hérault River at locations where human activities regularly occur (river beaches, kitchen gardens). Third, one soil site was sampled in a *Quercus ilex* forest, 700 m away from any mine site, and used as a reference for non-polluted soil. Finally, data from the formerly cultivated terraces were also used, located at increasing distances from the tailing ponds.

2.2 Soil and Plant Analyses

Soil bulk samples were air-dried, ground and the <2 mm fractions were separated from coarse rock fragments by sieving. All chemical analyses were performed on the <2 mm soil fractions. Mineral elements were extracted with ammonium acetate-EDTA 1 N (pH 4.65) for 30 min (10 g dry soil in 50 ml of extractant) (Cottenie et al. 1982). The supernatant was filtered and analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX). This method is known to extract the 'labile' and 'less labile' pools of trace elements (Fangueiro et al. 2005; Labanowski et al. 2008), i.e. the mineral fraction potentially available to plants via root absorption). In previous experiments, this fraction showed a good correlation with the concentrations of Zn and Cd in aerial tissues of *Thlaspi caerulescens*, a hyperaccumulating plant species present in the region (Robinson et al. 1998). In addition, a selection of soil samples were analysed for total metal concentrations using a mixture of concentrated hydrochloric (3 vol.) and nitric (1 vol.) acids (*aqua regia*) and compared with the data from the EDTA extraction. The *aqua regia* method is used for the extraction of the pseudo-total soil content, including metal fractions not available to plants, and represents the long-range potential soil toxicity (Ernst 1996). The precision and accuracy of analysis for element concentrations were monitored using BCR-100 (Community Bureau of Reference) as well as house standards. Analyses agreed with certified values to within ±5%. For samples collected in the pits, analyses of particle size distribution and soil physicochemical parameters, i.e. pH_{water}, $CaCO_3$ content and cation exchange capacity (CEC using the cobaltihexamine method), were carried out according to French standard methods (AFNOR 1996). Total C and N contents were analysed by dry combustion using a C/H/N analyser (Thermo Finnigan, Milan, Italy).

Metal concentrations in plants were measured in species belonging to genera that included the following metal-tolerant or metal-accumulating species *Silene* (Searcy and Mulcahy 1985), *Thlaspi* (Robinson et al. 1998), *Iberis* (Leblanc et al. 1999), *Armeria* (Lefebvre 1974), *Biscutella* (Wierzbicka and Pielichowska 2004) and *Genista* and *Reseda* (Ernst 1974), as well as species we had studied in previous phytoremediation experiments conducted at the Les Avinières site: *Festuca arvernensis*, *Koeleria vallesiana* and *Anthyllis vulneraria* (Frérot et al. 2006). Plants collected at each site were carefully and repeatedly hand-washed and rinsed in deionised water to eliminate the dust that had accumulated on the aerial parts. Plant parts were then oven-dried at 60°C for 3 days and mineralised in a mixture of nitric (69.2%) and perchloric acid (60%) with a Tecator digester according the method of Comité Inter-Instituts d'Etudes Des Techniques Analytiques (C.I.I).

Analyses were performed by ICP-OES for the same elements as for soils. The number of samples per species varied depending on their rarity at each site. Four metals were studied in particular (Zn, Pb, Cd and Tl) because previous studies (Escarré et al. 2000; Leblanc et al. 1999; Robinson et al. 1998) indicated that some plants from the Les Malines area were able to accumulate or hyperaccumulate these metals.

In addition, we carefully checked that the Zn and Pb accumulations demonstrated for *T. caerulescens* (see Section 3) were not because of contamination by the residual dust remaining after washing from the tailing basins. Therefore, at the CEFE-CNRS in Montpellier we conducted pot experiments with seeds of *T. caerulescens* species collected at the three mine sites, where samples were cultivated for 1 year in 1 L pots with soil from the most contaminated AT site. Collected plant material was washed and analysed using the same methods as for field plants.

Finally, we calculated the relationship between the metal in dry plants and the available metal in soils to test whether variation in element concentrations within species among sites could be accounted by metal concentrations in the soil.

2.3 Statistical Analysis

The differences in soil metal concentrations were analysed by one-way ANOVA using the general linear models procedure (SAS 2004). The comparisons of means were performed using least square means tests. We analysed the relationships between plant species and the concentrations of trace and major elements in topsoils (11 soil variables: Zn, Pb, Cd, As, Tl, Mn, Fe, Ca, K, Mg and P concentrations) with multivariate correspondence analysis using the Vegana package (De Cáceres et al. 2003).

3 Results

3.1 Physical and Chemical Characteristics of Soils on Mine-Waste Sites

The general morphological aspects of the soil pits at the PA, AM and AT mine sites are presented in Fig. 2 and the results of the physical and chemical data obtained on selected horizons and layers are presented in Table 1. Analyses were performed on the soil fractions <2 mm; the proportion of these fractions with respect to the collected bulk samples is indicated in Table 1. The composition of mine spoils from Les Avinières (AM), collected from a vegetated flat surface was dominated by dolomite rock residues, conferring a characteristic sandy texture and a high pH_{water} of between 7 and 8 for the different soil layers (Table 1), and exchangeable Ca and Mg dominated the soil exchange complex. Yet, the highly variable composition, both laterally (AC1-C2 horizon) as well as vertically, gave evidence of an anthropogenic origin of the mineral substrate. In the upper 10 to 15 cm, the CEC values were highly related to the presence of approximately 20% clay and large organic matter contents (~20% in O+Ah, ~5% in the AC1 horizon). By contrast, the spoils of the tailing ponds from Les Avinières (AT) were rich in clay below 10 cm (>50%), indicative of the washing and purification activities of metal ores that generate predominantly fine material. The superficial AT-1 layer (0–10 cm) was more sandy textured than the underlying AT-2 layer, which contained more carbonate and

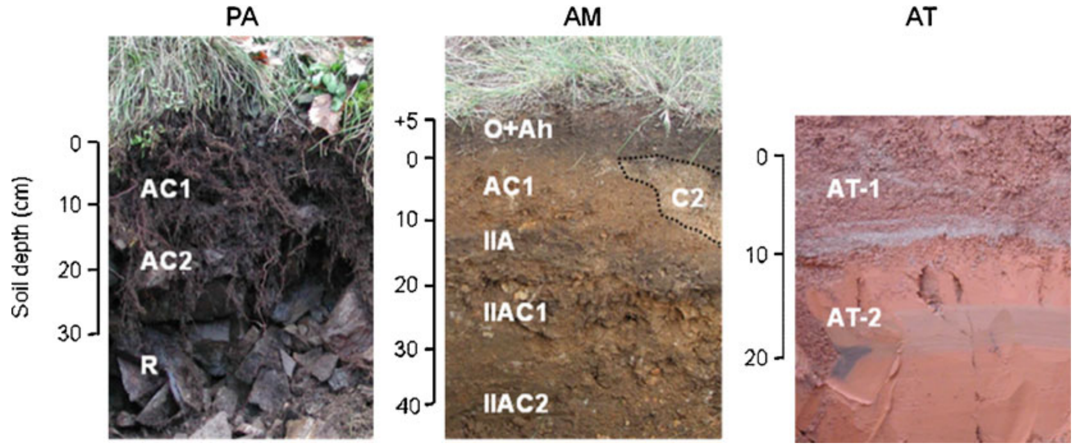

Fig. 2 Morphological aspects of Technosols on mine waste in Les Avinières mining site (*AM*), Les Avinières tailing ponds (*AT*) and the Petra Alba site (*PA*), with an indication of soil horizons and layers

showed a much higher organic carbon content, ascribed to the accumulation of colluviated material from neighbouring slopes. In the soil of the oldest mining site (PA), clay and silt (<20 µm) were the most important fractions and only contained traces of carbonate (<0.2%), which is in agreement with the schistose nature of the parent material. The soil of PA was slightly acidic (pH 6.3–6.4). The values of C-to-N ratios were approximately 15 for the AC1 and AC2 horizons, consistently lower than the

Table 1 Characteristic physical and chemical data determined on the fine earth fraction (<2 mm) from selected soil horizons and layers from the Petra Alba (PA), Les Avinières mine (AM) and Les Avinières tailing ponds (AT) mine sites

		Particle size distribution					Organic matter			Iron		Exchange capacity			Total metal concentrations			
Horizon	Soil depth	Clay	Silt	Sand	<2 mm/bulk	$CaCO_3$	C	C/N	pH	Fe_T	Fe_A/Fe_T	CEC[a]	Ca^{2+}	Mg^{2+}	Zn	Pb	Cd	Tl
	cm	$g\ kg^{-1}$			%	$g\ kg^{-}$				$g\ kg^{-1}$	%	$cmol^{+}\ kg^{-1}$			$mg\ kg^{-1}$			
PA																		
AC1	0–10	203	333	464	34	1.6	81.8	14.2	6.3	5.9	23	20.4	15.5	4.5	4,690	3,967	37.7	1.16
AC2	10–20	189	357	454	14	2.1	45.6	14.9	6.4	7.9	24	13.9	10.5	3.1	6,107	4,415	37.9	0.82
AM																		
Ol+Ah	+5–0	153	255	592	87	230	129	20.4	7.1	4.5	5	13.7	9.8	3.5	32,513	18,770	108	16.8
AC1	0–12	290	115	595	70	69.3	31.1	41.3	7.2	13.8	3	5.2	3.0	1.9	91,454	84,130	311	46.8
C2	–	61	96	843	90	749	4.05	14.0	7.5	3.3	3	1.4	0.8	0.5	22,088	12,650	78.6	8.0
IIA	12–16	331	314	355	96	29.9	11.2	15.3	7.5	4.7	7	9.9	6.1	3.4	9,980	4,401	39.6	7.4
IIAC1	16–40	269	285	446	65	188	24.6	44.2	7.9	4.1	6	8.1	4.9	2.9	12,841	4,135	45.4	7.2
IIAC2	>40	283	324	393	93	136	15.2	25.3	8.0	3.9	7	9.7	6.0	3.4	9,929	4,433	35.2	6.4
AT																		
C1	0–10	89	500	411	99.6	108	51.6	90	7.1	5.8	8	0.72	0.54	0.09	129,032	38,305	745	27.0
C2	10–20	563	432	5	100	89	12.8	31.5	7.0	13.4	5	7.0	5.5	1.18	131,365	36,354	700	115.1

The proportion of the <2 mm soil fraction to the total collected bulk samples (5–10 kg) is presented as <2 mm/bulk. Fe_A/Fe_T represents the percentage of free amorphous iron (ammonium oxalate extraction) (Tamm 1922) to the total iron content

[a] Cation exchange capacity by cobaltihexamine method

values of 20–45 that were observed in the upper layers of AM.

This diverging nature and origin of the mining waste also influenced the concentrations of trace metal and iron in the different soil layers and horizons (Table 1). Strongly contrasting but very high concentrations were found for Zn, Pb and Cd between the first 10 to 15 cm and deeper layers in AM and also for Tl in the AT-2 layer. Such variability in trace metal concentrations supports a strategy for considering an average sampling depth of 20 cm, being predominantly explored by the root system in the different stations of the mine sites to assess relationships between metal concentrations in the soils and the composition of vegetation.

3.2 Metal Concentrations in 0–20 cm Topsoils

The average values of trace element concentrations (Table 2) showed that Zn values were highest for the Les Avinières plots and lowest for Petra Alba. Extremely high values of Zn were found in the Les Avinières tailing ponds with concentrations up to more than 12.5%. In general, the mean values showed high standard errors because of the large heterogeneity between sampling plots. For this reason the mean values for Pb were not significantly different between stations (Table 2). As found for Zn, the highest Pb concentrations were found in AT (>5%) but high Pb concentrations were also observed for the flats of AM. Significant differences among stations were found for Cd, with the highest values for the Les Avinières basins. The As and Tl concentrations did not show significant differences among stations. Also, the stations within the AM mine site did not show significant differences in metal contents despite the contrasting topographical situations and vegetation cover, particularly between the bare rocky slopes without vegetation and the vegetated slopes. Soil samples taken near the furnace showed the highest As and Tl total concentrations (9,700 mg kg^{-1} and 245 mg kg^{-1}, respectively) found in the three sites (Table 2). Soil from under *I. intermedia* individuals (Tl hyperaccumulators) also showed high values for Cd, As and Tl.

In general, the mean EDTA/total (pseudo-total) metal content ratios were 34±5% for Zn, 63±4% for Pb, 52±5% for Cd and 18±6% for As. The relationships between pseudo-total and EDTA metal contents were consistently highly significant (Table 4), particularly for Pb and Cd, where a correlation coefficient of >0.95 was found. The lowest (but significant) correlations were between Pb and Zn and Pb and Cd, especially for values obtained with an EDTA extraction.

3.3 Metal Concentrations in Topsoils at Increasing Distances from the Mine Sites

Soil samples collected from up to 1.5 km along the roadsides in the vicinity of the Les Malines mine showed very high metal concentrations (Table 3). The same situation was found along the banks of the Vis and Hérault rivers and in the old field terraces above the Les Avinières basins. Metal concentrations in the soils decreased with increasing distance from the mine sites (Fig. 3, Table 3): soil samples taken 1.5 km from the Les Malines mine showed clearly lower values, but they were consistently higher than the median values found for rural soils in France and in the USA (Table 3). The soils under the undisturbed Holm oak coppice, 700 m away from Les Avinières basins, showed the lowest metal concentrations but Pb concentrations clearly exceeded the current values cited for soils in France (Baize 1997, cf. Table 3).

3.4 Relationships Among Mine Sites, Plant Species and Soil Characteristics

Spontaneous vegetation growing on the three mine sites was highly diversified and included 116 plant species. Table 5 shows the occurrence of the most abundant species (species with a frequency higher than 10% of the samples) at the stations and Table 6 the Raunkiaer's life form for each site. At the PA mine stations, there was a relatively lower number of species per sample (19) but plant coverage (53%), mostly because of *F. arvernensis*, was substantial despite the shallow soil depth and very steep rocky slopes. On the Les Avinières rocky slopes, plant cover was very low, at 10%, and the number of species per sample was the smallest (5.5). The AT site showed the highest plant cover in the peripheral areas, strongly contrasting with that in the central parts, which were totally devoid of vegetation. Finally, the old fields near the tailing ponds had the highest number of species. The soils of these fields, despite their metal levels, had undergone little anthropogenic disturbance and were contaminated mainly by atmospheric dust

Table 2 Total and pseudo-total EDTA-extracted concentrations (mg kg^{-1}) of Zn, Pb, Cd, As and Tl at the mine sites of Les Malines, Petra Alba and Les Avinières; in italics: standard errors

Station	Method	Zn	Pb	Cd	As	Tl
Malines mine	Total	39,364	34,289	225	338	3.5
	n=2	*3,490*	*7,992*	*3*	*70*	*2*
	EDTA	6,144 a, b	12,798 a	50 a, b	17 a	0.6 a
	n=8	*1,583*	*4,731*	*14*	*5*	*0.1*
Avinières mine rocky slopes	Total	–	–	–	–	–
	EDTA	22,985 a	15,639 a	58 a, b	13 a	2 a
	n=14	*4,715*	*3,899*	*8*	*2*	*0.8*
Avinières mine flats	Total *n*=1	61,983	51,450	210	0	32
	EDTA	17,905 a	15,615 a	41 a, b	0 a	1 a
	n=11	*4,805*	*3,961*	*7*		*1*
Avinières mine slopes	Total *n*=1	76,162	35,421	315	994	8.4
	EDTA	12,319 a, b	1,4795 a	30 a, b	15 a	1 a
	n=8	*7,077*	*4,966*	*5*	*2*	*1*
Avinières tailing ponds	Total	126,126	51,260	899	513	28
	n=3	*17,620*	*19,580*	*200*	*220*	*9.8*
	EDTA	14,563 a	28,838 a	75 a	11 a	6 a
	n=4	*2,373*	*6,346*	*10*	*2*	*5*
Petra Alba mine	Total	3,871	5,997	22.5	364	0.0
	n=2	*1,080*	*1,277*	*10.8*	(*n=1*)	
	EDTA	807 b	1,881 a	10 b	15 a	0 a
	n=3	*207*	*658*	*4*	*5*	
ANOVA among stations	EDTA	F=4.0**	F=1.5 n.s.	F=2.7*	F=0.7 n.s.	F=1.4 n.s.
Soil near furnaces	Total *n*=1	45,470	56,710	149.2	9,725	244.9
Soil below *Iberis intermedia*	Total *n*=1	40,830	25,013	310	202	46.5

For each metal, means for EDTA with the same letter were not statistically significant ($p<0.05$) between sampling places with a least square means test. The values for the old fields above the tailing ponds of Les Avinières are given in Table 3

generated by mining activities. Among the most frequent species, *F. arvernensis*, *Galium mollugo, Galium jordanii, Silene latifolia, Hieracium murorum* and *T. caerulescens* were found in all of the mine sites regardless of their metal contents. Some species, such as *A. vulneraria, K. vallesiana* and *Arenaria aggregata,* only occurred at the Les Avinières mine. *Sedum reflexum* and *Centaurea pectinata* were only found in Les Malines and Petra Alba. Finally, a few rare species were found to occur as scattered individuals, such as *I. intermedia* or *Genista pilosa,* or in a single clump, such as *Alyssum serpyllifolium* (not present in Fig. 4) at Les Avinières. Raunkiaer's life form (Table 6) showed a very low occurrence of therophytes and cryptophytes and a large dominance of hemicryptophytes with more than half of species and chamaephytes and phanerophytes (between 13% and 21%).

There was no link between the time since abandonment of the mines and the plant species number. Only some species from open spaces, such as *Biscutella laevigata*, *I. intermedia*, *Armeria hallerii* and *Scabiosa maritima*, were missing at Petra Alba, the oldest mine. Furthermore, despite the long time since its abandonment, there was no colonisation at Petra Alba by tree species from the *C. sativa* forests surrounding the wastes.

Similarly, the heavy metal soil concentrations had no effect on the vegetal cover: the rocky slopes poorly covered with vegetation showed the same metal

Table 3 Total and EDTA extractable Zn, Pb, Cd and As in $mg kg^{-1}$ in several places from the mine sites of Les Malines and Les Avinières

		Zn	Pb	Cd	As
Soil samples near malines mine—roadside					
Road, 0.2 km from Malines mine	Total	20,521	10,611	82.5	105
	EDTA	10,003	52,626	46.0	5.7
Road, 0.5 km from Malines mine	Total	9,410	14,165	34.7	91.2
	EDTA	7,323	6,649	35.2	<5
Road, 1 km from Malines mine	Total	179	143	2.7	20.6
	EDTA	27.6	54.1	<1	<5
Soil samples near Avinières tailing ponds—Vis and Herault river banks					
Vis river bank, 0.2 km from Les Avinières tailing ponds	Total	59,040	62,051	358	436
	EDTA	14,075	31,509	66.4	18.2
Hérault river bank near Ganges city, 2 km from Les Avinières tailing ponds	Total	4,618	3,541	22.9	93.3
	EDTA	835	2,304	10.7	<5
Hérault river bank, 4.5 km from Les Avinières tailing ponds	Total	1,280	1,090	6.5	55.3
	EDTA	414	668	3.0	<5
Old fields above tailing ponds of Les Avinieres					
Old field, 60 m from Les Avinières ponds	Total	10,555	5,324	77.2	82.4
	EDTA	5,952	6,894	55.0	16.4
Old field, 150 m from Les Avinières ponds	Total	2,979	1,673	24.3	41.4
	EDTA	911	760	9.0	0
Old field, 240 m from Les Avinières ponds	Total	938	406	3.5	35.8
	EDTA	214	217	0	18.4
Downy Oak coppice, 700 m from Les Avinières ponds	Total	157	145	0.6	16.6
	EDTA	19.8	68.4	0.2	0
Current values observed in "ordinary" French soils[a]	Total	10–100	9–50	0.05–0.45	1.0–25
Median values for ploughed horizons, French rural soils[a]		68	30.4	0.22	n.a.
Median values for rural soils USA[b]		53	11.0	0.20	n.a.

[a] Values for French ordinary (unpolluted excluding geochemical anomalies) and rural soils (Baize 1997)

[b] Values for agricultural soils of the USA (Holmgren et al. 1993) are at the bottom of the table

contents as the densely covered slopes. Other non-studied factors such as soil erosion, water stress and nutrient availability can play a very important role in the absence of vegetation in these sites. Figure 4 shows the results of a multivariate analysis on the species, sampling points and soil variables. From right to left, the sampling points showed an increasing metal content. Thus, highly polluted soils such as three of the four samples from the Les Avinières tailing ponds are in the left part of the figure, together with the most frequent species present in highly contaminated soils such as *F. arvernensis*, *K. vallesiana*, *T. caerulescens* and *Armeria arenaria*. At the opposite, we found at the right part of the figure the four samples from old fields near Les Avinières which are among the less polluted soils where *Clematis flammula, Aphyllanthes monspeliensis* and *Rubus ulmifolius* occur. The other sampling points did not show any pattern; for instance, the points from the Les Avinières rocky slopes were mixed with points from Les Malines and Petra Alba.

3.5 Heavy Metal Concentrations in Leaves

Most of the plant species accumulated Zn and Cd (Fig. 5) but much less Pb and Tl. Because none of the studied species accumulated As, these data were not included in Fig. 5. Only some individuals of *T. caerulescens* and *A. vulneraria* reached the threshold values for the hyperaccumulation of Zn, fixed at 10,000 $mg kg^{-1}$ (Baker and Walker 1990). *T.*

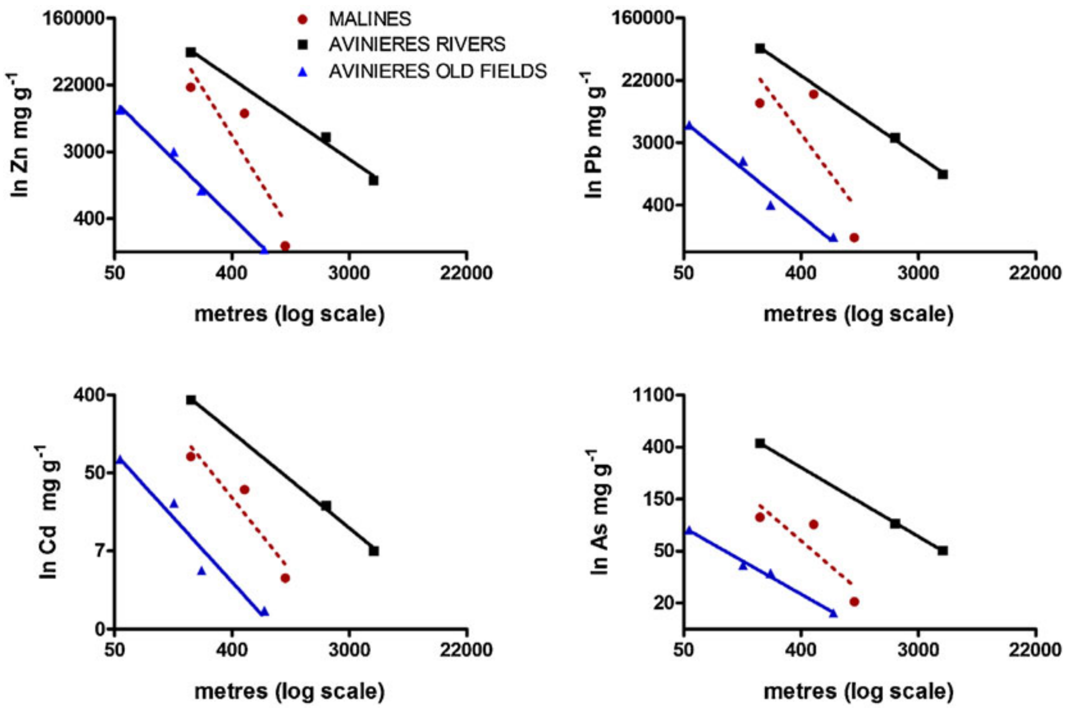

Fig. 3 Zn, Pb, Cd and As concentrations in soils according to the distance from the Les Malines and Les Avinières mines. Each point represents a single observation. Both axes are in log scale. *Solid lines* are statistically significant ($p<0.05$)

caerulescens cultivated under controlled conditions displayed the same range of Zn concentrations as field plants. *A. aggregata* and *B. laevigata* also showed high Zn concentrations but with mean values largely below the threshold (Fig. 5). The grasses *K. vallesiana* and *F. arvernensis* and the legume *G. pilosa* did not accumulate consistent amounts of metals in their aerial parts.

Generally, lead is not accumulated in plant aerial parts (Baker et al. 2000). In our study, however, three species occasionally exceeded the threshold values for the hyperaccumulation of Pb, fixed at 1,000 $mg kg^{-1}$: *A. vulneraria, T. caerulescens* and *A. aggregata*. As observed for Zn, however, these mean values were below the threshold, except for *T. caerulescens* individuals cultivated under controlled conditions (Fig. 5). Great difficulties were encountered when trying to accurately wash *A. aggregata* specimens because of their dense foliage close to the soil; hence, the results for this species must be taken with caution. Concentrations of Cd were high in *A. vulneraria* and *B. laevigata*, yet they were much lower than values measured for *T. caerulescens* both in the field and in controlled conditions.

Thallium, a very toxic metal, was accumulated in large concentrations by some individuals of *I. intermedia, B. laevigata, Silene vulgaris* and *S. latifolia.* This last species showed exceptionally high, although widely variable, Tl concentrations among individuals, with an extreme value reaching 1,489 $mg kg^{-1}$ (Fig. 5).

Despite the high metal concentrations found in the aerial parts of the plants, only a few species showed a

Table 4 Correlation coefficients among the Zn, Pb and Cd concentrations in the soils

	Zn	Pb	Cd
Zn	0.8938***	0.8484***	0.9835***
Pb	*0.3817***	0.9557***	0.8301***
Cd	*0.7039****	*0.4956****	0.9619***

Total metal content above the diagonal (n=31), EDTA metal content below the diagonal (italics, n=48). Correlation between total and EDTA metal content along the diagonal, (n=31)

**$p<0.01$; ***$p<0.001$

Table 5 Vegetation cover (%), number of species per sample, Raunkiaer's life form and frequency of occurrence of species in mine sites from Les Malines, Les Avinières and Petra Alba (species present in less than 10% of samples were not included)

	Life form	Malines *n*=7	Avinieres rocky slopes *n*=14	Avinieres slopes *n*=8	Avinieres flats *n*=11	Avinieres tailing ponds *n*=4	Old fields near Avinieres *n*=4	Petra Alba *n*=3	% samples
Vegetation cover%		58 (±9)	10 (±4)	63(±9)	43(±7)	69 (±8)	58(±12)	53(±9)	
Number of species per sample		20(±2)	5.5(±2)	21(±1.5)	12.4 (±2.5)	19(±2)	26(±1.3)	19(±1)	
Festuca arvernensis	H	71.4	57.1	100.0	72.7	75.0	0.0	100.0	68.6
Arenaria aggregata	CH	0.0	78.6	75.0	90.9	100.0	0.0	0.0	64.6
Galium mollugo	H	100.0	7.1	87.5	45.5	100.0	100.0	100.0	60.8
Biscutella laevigata	H	100.0	35.7	75.0	54.5	100.0	25.0	0.0	60.4
Thlaspi caerulescens	H	71.4	7.1	100.0	45.5	100.0	75.0	100.0	56.9
Koeleria valesiana	H	0.0	35.7	100.0	72.7	75.0	50.0	0.0	54.2
Jasione montana	H	0.0	14.3	100.0	63.6	100.0	75.0	100.0	52.9
Bupleurum rigidum	H	0.0	21.4	87.5	45.5	100.0	100.0	0.0	47.9
Anthyllis vulneraria	H	0.0	50.0	62.5	72.7	75.0	0.0	0.0	47.9
Hieracium murorum	H	57.1	28.6	62.5	27.3	25.0	75.0	33.3	42.9
Silene latifolia	H	71.4	0.0	62.5	9.1	75.0	75.0	100.0	39.2
Iberis intermedia	H	57.1	28.6	37.5	27.3	50.0	50.0	0.0	37.5
Armeria arenaria	H	28.6	14.3	50.0	54.5	75.0	25.0	0.0	37.5
Silene vulgaris	H	57.1	0.0	62.5	18.2	100.0	50.0	0.0	35.4
Galium jordanii	H	14.3	7.1	50.0	54.5	25.0	50.0	66.7	34.0
Dactylis glomerata	H	71.4	0.0	37.5	0.0	100.0	75.0	0.0	31.3
Thymus vulgaris	CH	0.0	21.4	50.0	45.5	0.0	50.0	0.0	29.2
Scabiosa maritima	H	57.1	7.1	25.0	18.2	75.0	50.0	0.0	29.2
Helianthemum appeninum	CH	0.0	14.3	62.5	63.6	0.0	0.0	0.0	29.2
Reseda lutea	H	28.6	35.7	37.5	27.3	0.0	0.0	0.0	27.1
Euphorbia nicaensis	CH	0.0	21.4	50.0	27.3	0.0	50.0	0.0	25.0
Echium vulgare	H	14.3	0.0	37.5	18.2	50.0	75.0	0.0	22.9
Daucus carota	H	85.7	0.0	12.5	0.0	75.0	25.0	0.0	22.9
Asparagus acutifolius	CH	0.0	0.0	62.5	9.1	25.0	100.0	0.0	22.9
Clematis flammula	P	71.4	0.0	12.5	0.0	0.0	100.0	0.0	20.8
Picris hieracioides	H	71.4	0.0	0.0	0.0	50.0	50.0	33.3	20.4
Rubus ulmifolius	P	28.6	0.0	37.5	0.0	0.0	75.0	66.7	20.0
Asperula tinctoria	H	0.0	21.4	25.0	27.3	0.0	25.0	0.0	18.8
Reichardia picroides	T	0.0	0.0	50.0	27.3	0.0	25.0	0.0	16.7
Urospermum daleschampii	H	0.0	7.1	37.5	27.3	0.0	0.0	0.0	14.6
Plantago lanceolata	H	85.7	0.0	12.5	0.0	0.0	0.0	0.0	14.6
Scrophularia canina	H	14.3	0.0	37.5	18.2	0.0	0.0	33.3	14.3
Centaurea pectinata	H	28.6	0.0	37.5	0.0	0.0	0.0	66.7	14.0
Sanguisorba minor	H	0.0	14.3	37.5	9.1	0.0	0.0	0.0	12.5
Rumex acetosa	H	28.6	0.0	0.0	0.0	50.0	50.0	0.0	12.5
Genista pilosa	CH	14.3	0.0	25.0	9.1	0.0	0.0	66.7	12.0
Campanula rapunculus	H	0.0	0.0	12.5	0.0	0.0	75.0	66.7	12.0

n sample number

Table 6 Raunkiaer's life forms (%),per sample and frequency of occurrence of species in mine sites from Les Malines, Les Avinières and Petra Alba

	Malines n=7	Avinieres rocky slopes n=14	Avinieres slopes n=8	Avinieres flats n=11	Avinieres tailing ponds n=4	Old fields near avinieres n=4	Petra Alba n=3	% samples
Therophytes (T)	2.0	0.0	4.4	4.9	6.7	8.9	6.3	7.0
Hemicryptophytes (H)	68.4	58.6	60.0	65.8	70.0	53.6	68.7	52.2
Cryptophytes (CR)	3.6	0.0	4.4	2.4	3.3	5.4	9.4	7.0
Chamaephytes (CH)	5.6	17.2	20.0	17.1	6.7	8.9	12.5	13.0
Phanerophytes (P)	20.4	24.2	11.2	9.8	13.3	23.2	3.1	20.8

n sample number, *T* annual plants, *H* buds near soil surface (grasses or rosette plants), *CR* buds beneath the surface of the ground (rhizomes, bulbs), *CH* buds on persistent shoots near the ground (shrubs), *P* buds more than 25 cm above soil level (woody perennials)

relationship between metal in dry plant/available metal in soil higher than 1. For Zn and Cd, only *T. caerulescens* averaged at 2.6 (SE±0.49; n=24, p< 0.01) for Zn and 73.7 (SE±15; n=24, p<0.001) for Cd. No species exceeded a coefficient value of 1 for Pb. Four species with high Tl concentrations showed a coefficient >1: *B. laevigata* (19.5±7.7, n=9, p< 0.02), *I. intermedia* (24.1±9.4, n=6, p=0.03), *S. latifolia* (162.0±113, n=7, p=0.09) and *S. vulgaris* (15.3±6.6, n=6, p=0.04).

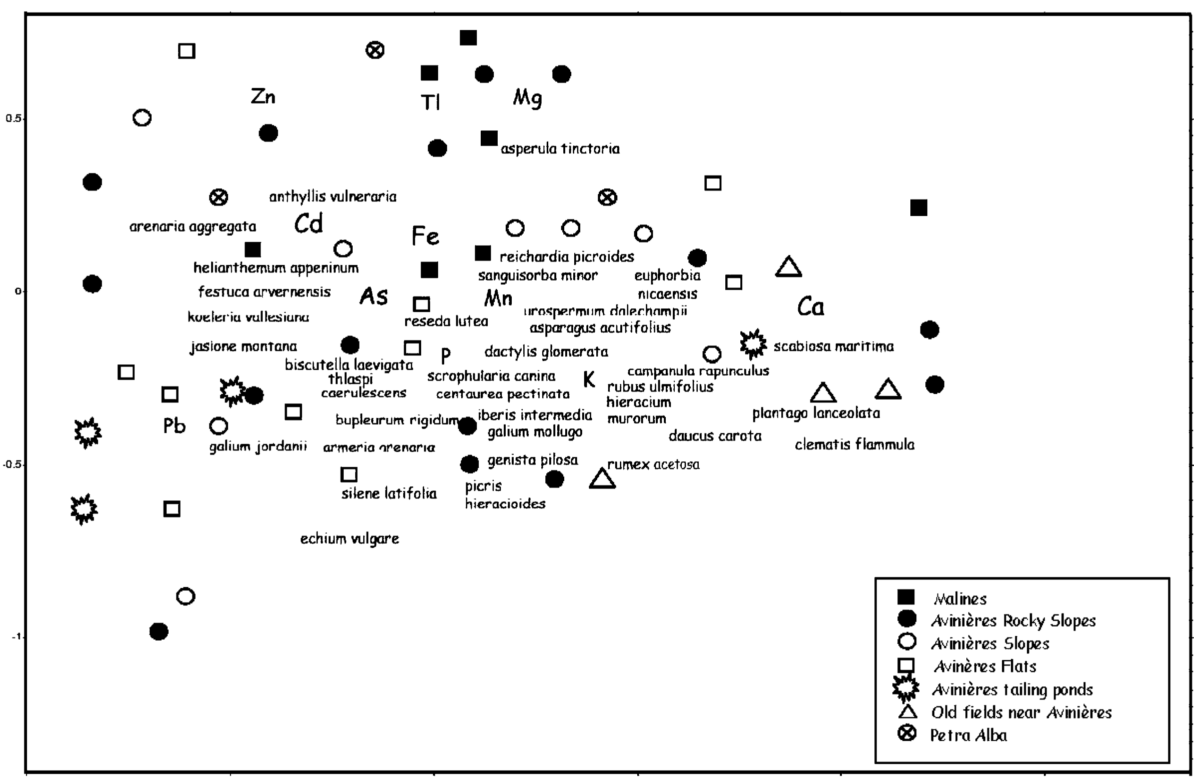

Fig. 4 Results of multivariate correspondence analysis of plant species and soil metal concentrations for the sites of Les Malines, Les Avinières and Petra Alba. For the sake of clarity only plants present in Table 5 were included in the figure

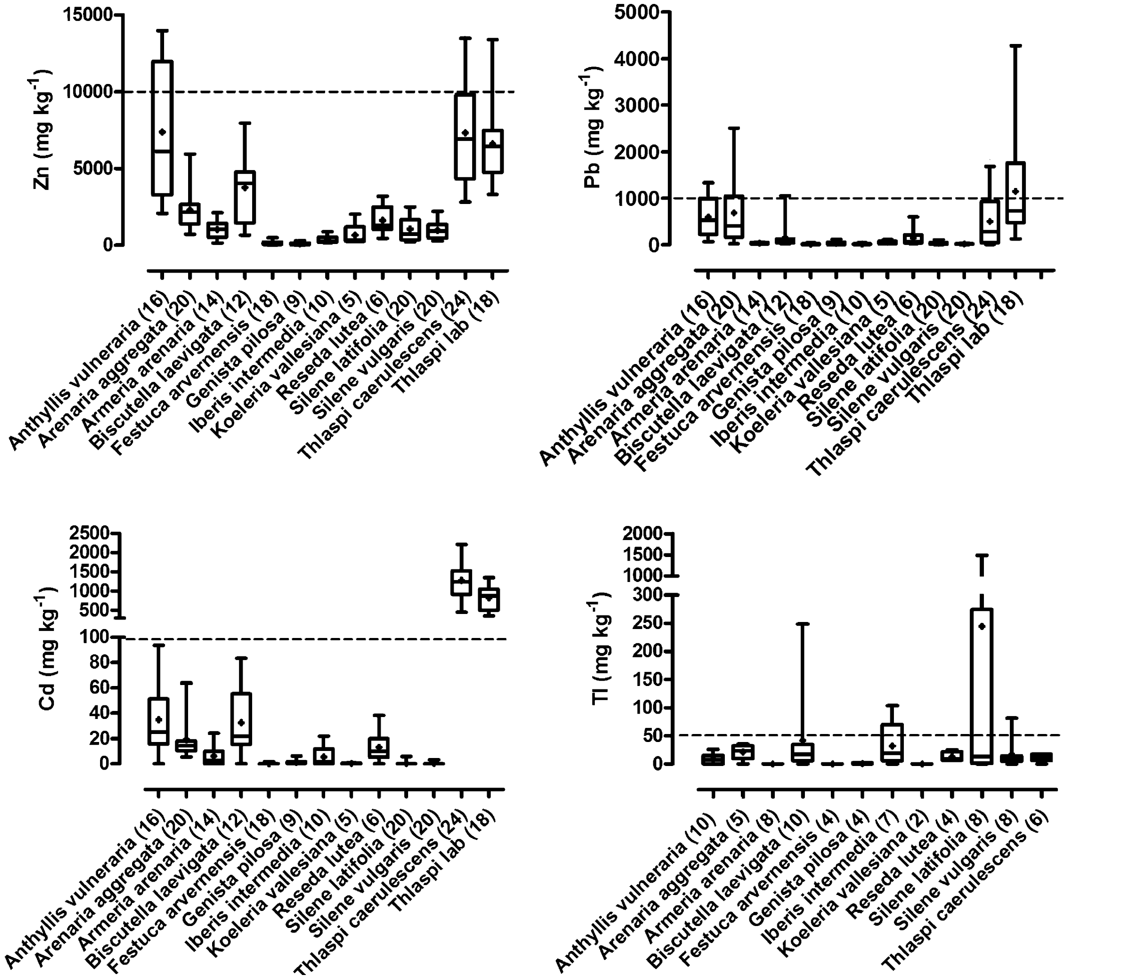

Fig. 5 Zn, Pb, Cd and Tl contents in aerial parts of selected plant species from Les Avinières, Les Malines and Petra Alba. Hyperaccumulation thresholds are indicated with a dotted horizontal line. The *vertical rectangles* correspond to the 25% and 75% percentiles; (*plus sign*) is the mean, the *horizontal* trait in the *rectangles* is the median and *vertical lines* the maximal and minimal values. The sampling number is between parentheses after the name of species in each graph. The number of samples for Tl was reduced because some soils did not contain this metal. *Thlaspi* lab: individuals cultivated in controlled conditions with the Les Avinières soil

4 Discussion

4.1 Mine Soils and Metal Concentrations

Soil development in the studied mine sites occurs on mineral waste resulting from ancient and recent ore extraction and processing activities. Such stratified substrates are heterogeneous with respect to their mineral composition, texture and stage and degree of alteration, but they all exhibit high metal contents. From a pedogenetic viewpoint, soil formation is strongly limited in the Les Avinières mine sites, and the soils relate to Technosols (FAO 2006). This is the case for the bare, finely textured, stratified surface layer occurring in the central parts of the tailing ponds (AT, Fig. 2). The absence of soil formation was corroborated by very high C-to-N ratios (30–90, Table 1), which indicate the limited decomposition of organic matter from a predominantly colluvial origin. Similarly high C-to-N ratios (40–93) were found in a former metallurgical landfill (Remon et al. 2005). On the flats of the mining sites of AM, under a cover of metal-tolerant species, a 5 cm thick litter layer developed on the top of the mine waste. In the underlying AC1 horizon, small organic debris occurred juxtaposed to the mineral compounds.

Together with the fairly high C-to-N ratios (20–40), these findings suggest that some organic matter degradation by living organisms is occurring, but that its incorporation down into the depths is strongly reduced. Such soil morphologies and characteristics show striking similarities with the surface layers of soils under metallicolous grassland developing on strongly polluted areas close to zinc and lead smelters in northern France. In these latter ecosystems, it was found that the reduced decomposition of highly metal-enriched plant residues with high C-to-N ratios (Balabane et al. 1999), lacking faunal homogenisation activity (van Oort et al. 2009), led to an accumulation of *Armeria maritima* and *Arabidopsis halleri* plant fragments in the form of a 5 to 8 cm thick litter layer (Dahmani-Muller et al. 2000).

By contrast, in the Petra Alba site evidence for initial soil formation was given by the absence of litter accumulation, a C-to-N ratio of approximately 15 in the AC1 and AC2 horizons and field observations of a dense rooting system with the presence of organo-mineral microaggregates (Fig. 2). An additional indication was the relatively high proportion of free amorphous iron (Table 1), pointing to a partial weathering of the schist rock fragments, the liberation of iron and its association with the clay-humus complex in a low or non-crystalline form. This soil is related to a Technic Leptosol (humic), according to the FAO (2006).

The amount of heavy metal in the topsoils, expressed either as total or EDTA-extracted concentrations, widely exceeded current values in French soils (Table 3). To the best of our knowledge, Zn, Pb and Cd concentrations determined in samples collected at local spots of the Les Avinières basins or near the old furnaces for As were among the largest concentrations published for mining sites in Europe. The highest concentrations found were in a sample from the Les Avinières tailing ponds (Zn: 161,000 $mgkg^{-1}$, Pb: 92,700 $mgkg^{-1}$, Cd: 1,382 $mgkg^{-1}$). The two other sites showed lower metal concentrations, which can be explained by the reclamation work for the Les Malines mine and by the different nature of the ore envelope at Petra Alba.

Some other European mining sites have also shown such outstandingly high concentrations of total Zn, Cd and Pb, for instance the Trelogan mine (UK) where soil metal concentrations reached 95,000 mg kg^{-1} Zn, 40,000 mg kg^{-1} Pb and 267 mg kg^{-1} Cd (Bradshaw and Chadwick 1980) or in Belgium where mine deposits at Plombières and La Calamine are also highly contaminated with zinc (19,000 and 101,000 mg kg^{-1}, respectively) and lead (35,000 and 9,000 mg kg^{-1}, respectively) (Assuncao et al. 2003; Simon 1978).

Soils around smelters can also contain large amounts of heavy metals. This was the case at Le Bois des Asturies, Auby (northern France), near a smelter factory where 50,000 mg kg^{-1} Zn, 7,700 mg kg^{-1} Pb and 300 mg kg^{-1} Cd were found (Bert et al. 2000). In the topsoils under the cover of *A. maritima* ssp. *halleri* and *A. halleri* at Mortagne-du-Nord, metal concentrations up to 49,100 mg kg^{-1} Zn, 16,900 mg kg^{-1} Pb, 200 mg kg^{-1} Cd (van Oort et al. 2009) and 340 mg kg^{-1} As (van Oort et al. 2000) were reported.

Soils at a distance of less than 1.5 km from the Les Malines mine sites were still contaminated, but the concentration decreased exponentially with the distance. For metal smelters in northern France, such non-linear decreasing Pb concentrations in agricultural topsoils with increasing distance to the emission source have also been shown (Sterckeman et al. 2002) for the Auby site (Metaleurop, Noyelles-Godault) and aspects of heterogeneity of such non-linear decreases were highlighted for the Mortagne-du-Nord site (van Oort et al. 2009). In the USA, the emissions of Zn smelters at Palmerton, Pennsylvania contaminated all the ecosystems in the vicinity. One kilometre away from the factory, the upper soil horizons still contained 35,000 mg Zn kg^{-1}, 1,300 mg Cd kg^{-1} and 3,200 mg Pb kg^{-1}. These values only decreased to 470 mg Zn kg^{-1}, 5 mg Cd kg^{-1} and 260 mg Pb kg^{-1} 12 km from the factory (Beyer 1988).

Fortunately, the areas of highly contaminated soils in the Les Malines region are limited to only a few hectares. This is in contrast to other mining sites, for instance in the Sierra of Cartagena, where soil contamination resulting from mining activities covers an area of approximately 1,000 km^2. The metal concentrations in sediments reached up to between 19,000 and 85,000 mg kg^{-1} for Pb and between 100 and 10,000 mg kg^{-1} for Zn (Robles-Arenas et al. 2006). Heavy metal pollution around mine sites can cause health problems; for instance, two children living in the area surrounding the mine at Les Avinières had a Pb blood concentration higher than 100 $\mu g\ L^{-1}$: a critical level for children (Cicchelero

2006). In contrast to the soils, the water from the Vis River in the vicinity of Les Avinières is not polluted. For instance, the Pb concentration was 4 nmol L^{-1} (Petelet-Giraud et al. 2004), which was well below the 48 nmol L^{-1} threshold fixed by the French Ministry of Health as the limit of toxicity for drinking water (Ministère de la Santé 2004).

4.2 Plant Species, Soil Toxicity and Vegetation Dynamics

Most of the plant species present on the contaminated mine sites were also observed on the surrounding non-polluted habitats. Even *T. caerulescens,* a well-known and extensively studied hyperaccumulator of Zn and Cd, was found on calcareous bedrocks in the Causses region (Escarré et al. 2000). In our study area, there were no morphological differences between *T. caerulescens* plants in polluted and non-polluted places. However, in previous studies we showed physiological differences because the populations from mine sites developed a higher heavy metal tolerance and a lower hyperaccumulation level in comparison to plants from non-contaminated sites (Escarré et al. 2000; Frérot et al. 2003, 2005). Similar differences of tolerance between metallicolous and non-metallicolous populations of *F. arvernensis, K. vallesiana* and *A. arenaria* have also been shown (Frérot et al. 2006, 2009) as well as in other species such as *I. intermedia* and *B. laevigata* (J. Escarré, unpublished results). However, some other species present in the contaminated soils were rare or absent in soils surrounding the mine sites. For instance, the population of *A. vulneraria* from Les Avinières belong to subsp. *carpatica* only found in this mine. Similarly, *A. aggregata*, *G. pilosa* or *I. intermedia* were rare or absent in uncontaminated soils of the region.

Our data failed to show any relationships between the time of mine abandonment, soil concentration and the identity of species colonising these soils. In other perturbed ecosystems of the region, such as abandoned vineyards, bare soil is initially colonised by annual species and trees such as *Quercus pubescens*, *Q. ilex* or *Pinus halepensis* after one century of abandonment (Escarré et al. 1983). In the mine studied, annual species did not colonise bare soil, and hemicryptophytes were the life forms largely dominant in all sites. In addition, in the mine sites such as Petra Alba that have been abandoned for many centuries, no trees colonised the waste heaps despite a chestnut forest surrounding the site. Therefore, there were no apparent dynamic processes of plant colonisation in toxic areas: the vegetation cover remained unchanged through time. The same situation was documented by Lefèbvre and Simon (1979) for an open lawn near a zinc and lead mine in Belgium, where no change was observed for decades. Similarly, the colonisation of waste was not linked to the metal soil concentration. Other factors such as the type of waste, steepness of the heap, instability of soils in the tailing basins or lack of soil in some wastes could explain the differences of plant cover.

4.3 Metal Concentrations in Plant Species

A prerequisite for the reclamation of heavy metal-polluted sites using the phytoextraction process is the knowledge of the ability of tolerant species to accumulate metals in their aerial biomass in situ, because the soil buffering capacity affects nutrient availability to plants (Robinson et al. 1998). Our data revealed that hyperaccumulation was found in some individuals of *A. vulneraria* for Zn and Pb, in *T. caerulescens for* Zn, Pb and Cd, and in *B. laevigata*, *I. intermedia*, *S. latifolia* and *S. vulgaris* for Tl. However, the average metal content of these species was below the hyperaccumulation thresholds, except for the Cd in *T. caerulescens*.

The genotypic nature of the hyperaccumulation for lead hyperaccumulation in *T. caerulescens* was tested in experimental conditions to avoid contamination by metal dust deposits. The results confirmed the field data. The hyperaccumulation of Pb was previously noted in *T. caerulescens* growing in a lead mine district in the Pennine Mountains, England (Shimwell and Laurie 1972). Previous research under controlled conditions with *T. caerulescens* from the Les Malines district also showed that the mean Zn concentration was below the hyperaccumulation threshold. Nevertheless, *T. caerulescens* from non-metallicolous soils cultivated on the Les Avinières soils showed Zn values above 10,000 mg kg^{-1} (Escarré et al. 2000). Similar results were obtained with non-metallicolous *T. caerulescens* plants cultivated under controlled conditions on metal-contaminated soils (Meerts and Van Isacker 1997) and with field plants growing on road banks in Luxembourg (Molitor et al. 2005).

Recent experiments with *A. vulneraria* from the Les Avinières mine cultivated on tailing basins soils (Mahieu et al. unpublished) showed average Zn and Pb values ($Zn = 5060 \pm 1290$; $Pb = 776 \pm 178$) slightly lower than the mean field results (Fig. 5).

The population of *T. caerulescens* from Les Avinières, designated the "Ganges ecotype" (Lombi et al. 2001), was shown to accumulate Cd at very high concentrations, but this is a general feature shared by other metallicolous or non-metallicolous populations of this species in the region (Escarré et al. 2000; Roosens et al. 2003). Nevertheless, Cd concentrations in plants sampled in the tailing ponds or cultivated under controlled conditions within the Les Avinières soil did not exceed 2,200 mg kg^{-1} (Escarré et al. 2000), much lower than the 14,000 (Lombi et al. 2001) and 6,000 mg kg^{-1} (Liu et al. 2008) obtained in hydroponics.

Similar contrasting results were obtained for Zn concentration with *T. caerulescens* in Prayon (Belgium). Plants cultivated in soil from this site had a Zn concentration less than 10,000 mg kg^{-1} (Meerts and Van Isacker 1997) compared with 30,000 mg kg^{-1} (Brown et al. 1995; Shen et al. 1997) in hydroponic studies of the same population. Therefore, the utilisation of the metal concentrations obtained in controlled hydroponic conditions to evaluate the potential of phytoextraction of hyperaccumulator plant species in the field must be taken with caution (notably owing to the differences in root structure between the two treatments).

I. intermedia has been considered an exceptional case of a thallium-hyperaccumulating species (Leblanc et al. 1999). However, our work has revealed the existence of other Tl-hyperaccumulating plant species, i.e. *B. laevigata*, *S. vulgaris* and *S. latifolia*. *S. latifolia* deserves particular attention as some individuals of this species accumulate extremely high levels of Tl (up to 1,489 mg kg^{-1}). Nevertheless, the results were extremely variable from one individual to another because some samples showed no Tl content. However, this was not linked to the variation of Tl concentrations in the soils, suggesting a genetic variation between individuals. Since the Tl concentrations in soils were largely lower than those in the plants, the high Tl concentrations found in some species might be the result of an active uptake.

Such high metal concentrations in plant aerial parts might be detrimental for the local herbivorous fauna, which could be unable to detect the presence of toxic metals in plant tissues as shown for snails (Noret et al. 2007). Nevertheless, in several other studies animals could discriminate between toxic metals (Vesk and Reichman 2009). As a reference value, Tl concentrations higher than 200 mg kg^{-1} of dry weight in the diet are lethal for cattle (Tremel et al. 1997). In comparison, Tl concentrations in plants not exposed to industrial pollution are only up to 0.05 mg kg^{-1} of dry weight (Wierzbicka et al. 2004).

A. serpyllifolium, a rare species in the area, occurs sporadically as a few dozen individuals at Les Avinières, where it is a moderate accumulator of Zn (3,500 mg kg^{-1}). This species, known as a hyperaccumulator of Ni, currently occurs on serpentine rock outcrops (Freitas et al. 2004).

Whatever the hyperaccumulation potentialities of the species cited above, it is evident that, considering the huge amounts of metals in the three sites, these plant species cannot be efficient in cleaning the soils by phytoextraction. Instead, phytostabilisation is a more suitable solution. Only soils with low metal concentrations can be reclaimed through a phytoextraction process (see Robinson et al. 1998 for an evaluation of the Zn and Cd phytoextraction capacity of *T. caerulescens*).

4.4 Tolerant Plant Species and Phytoremediation

Several species from the three mining sites could be efficient candidates for revegetation processes in the view of their ability to grow in soil highly polluted with toxic heavy metals. In this respect, *F. arvernensis*, *K. vallesiana* and *A. vulneraria* have been successfully tested in revegetation trials under the highly toxic conditions of bare soil in the tailing ponds at Les Avinières (Frérot et al. 2006). These three species showed a higher tolerance to heavy metals compared with populations of the same species from non-contaminated soils (Frérot et al. 2006). Moreover, the legume *A. vulneraria* worked as a facilitator for the growth of co-occurring species in trial plots by supplying 400 kg N ha^{-1} during the 4 years of the experiment (Frérot et al. 2006; Mahieu et al. unpublished data). The symbiotic N_2-fixing bacterium associated with *A. vulneraria*, a new species named *Mesorhizobium metallidurans*, was discovered to be tolerant to heavy metals (Vidal et al. 2009). Despite *A. vulneraria* accumulated Zn at high concentrations, it can be used at the start of the phytoremediation process because the species disappears after flowering since it is less competitive that the grass species such as *F. arvernensis* or *K. vallesiana*.

Although several species have evolved to metal-tolerant ecotypes in response to mine habitats, extended areas remain uncovered by vegetation because of the Mediterranean summer drought, the instability of soils and strong erosion by wind and water. Whatever the other additional environmental constraints, the use of plant materials other than locally adapted flora (commercial varieties, natural populations from non-polluted origins) is doomed to failure. We tested the introduction of non-tolerant ecotypes of corresponding species collected from unpolluted soils. They did not survive more than 1 year in the trial experiment we performed at Les Avinières (Frérot et al. 2009). Moreover, metal-tolerant ecotypes from the temperate climates might be not successful either because they are unlikely to cope with the semi-arid conditions of the Mediterranean climate (Wali 1999). Regional heavy metal-tolerant populations are the most reasonable choice to succeed in phytostabilisation experiments.

To conclude, the three study sites are heavily polluted by various toxic heavy metals at different concentrations. At Les Avinières, the soil concentrations of metal elements are in some places among the highest in Europe. The contamination extends several kilometres away from the mine sites, most probably because of the transport of metalliferous particles by wind and water. The vegetation cover consists of heavy metal-tolerant ecotypes of species and plastic species also present in non-contaminated soils. A few of these metal-tolerant species were heavy metal hyperaccumulators that have a scientific interest for studies on metal transport mechanisms in plants. Tolerant species can be used as efficient tools for phytostabilisation.

Acknowledgements The authors thank Guy Delmot for authorising the work at the Les Avinières site and for his kind hospitality. We also thank Perrine Gauthier, Violette Sarda and David Degueldre for their technical assistance and Cécile Grand (Ademe) for her scientific and administrative help. This research was fully supported by a contract with the Agence de l'Environnement et de la Maîtrise de l'Energie (Ademe contract 04.72.C.0037).

References

AFNOR. (1996). *Qualité des sols. Recueil de normes françaises*. Paris: AFNOR.

Amphoux, G., & Laroche, D. (1989). La mine des Malines. *Paysage-Actualités, 122*, 115–121.

Antonovics, J., Bradshaw, A. D., & Turner, R. G. (1971). Heavy metal tolerance in plants. *Advances in Ecological Research, 7*, 1–85.

Assuncao, A. G. L., Bookum, W. M., Nelissen, H. J. M., Vooijs, R., Schat, H., & Ernst, W. H. O. (2003). Differential metal-specific tolerance and accumulation patterns among *Thlaspi caerulescens* populations originating from different soil types. *The New Phytologist, 159*, 411–419.

Audry, S., Blanc, G., & Schafer, J. (2004). Cadmium transport in the Lot-Garonne River system (France) – temporal variability and a model for flux estimation. *The Science of the Total Environment, 319*, 197–213.

Bailly-Maître, M. C. (1997). La mine au Moyen Age. La gestion de l'espace souterrain, Mélange Cl. Domergue. *Pallas, 46*, 287–295.

Baize, D. (1997). *Teneurs totales en éléments traces métalliques dans les sols (France). Références et stratégies d'interprétation* (p. 410 p). Paris: INRA Éditions.

Baker, A. J. M., & Walker, P. L. (1990). Ecophysiology of metal uptake by tolerant plants. In A. J. Shaw (Ed.), *Heavy metal tolerance in plants: evolutionary aspects* (pp. 155–177). Boca Raton: CRC Press.

Baker, A. J. M., McGrath, S. P., Reeves, R. D., & Smith, J. A. C. (2000). Metal hyperaccumulator plants: a review of the ecology and physiology of a biochemical resource for phytoremediation of metal-polluted soils. In N. Terry & G. Bañuelos (Eds.), *Phytoremediation of Contaminated Soil and Water* (pp. 85–107). Boca Raton: Lewis Publishers.

Balabane, M., Faivre, D., van Oort, F., & Dahmani-Muller, H. (1999). Mutual effects of organic matter dynamics and heavy metal fate in a metallophyte grassland. *Environmental Pollution, 105*, 45–54.

Bert, V., MacNair, M. R., DeLaguerie, P., SaumitouLaprade, P., & Petit, D. (2000). Zinc tolerance and accumulation in metallicolous and nonmetallicolous populations of *Arabidopsis halleri* (Brassicaceae). *The New Phytologist, 146*, 225–233.

Beyer, W. N. (1988). Damage to the forest ecosystem on Blue Mountain from zinc smelting. *Trace Substances in Environmental Health, 22*, 249–262.

Bouladon, J. (1977). Les gisements de plomb-zinc-argent du Massif Central. *Bulletin du BRGM*, Section II, 67–90.

Bradshaw, A. D., & Chadwick, M. J. (1980). *The restoration of land* (p. 317). Oxford: Blackwell.

Brown, S. L., Chaney, R. L., Angle, J. S., & Baker, A. J. M. (1995). Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution. *Soil Science Society of America Journal, 59*, 125.

Cicchelero, V. (2006) Dépistage du saturnisme dans la commune de Saint-Laurent-le-Minier (Gard), mai 2005. Unpublished report with the participation of: Institut de Veille Sanitaire, Cellule interrégionale d'épidémiologie Languedoc-Roussillon, Préfecture de Région de Midi

Pyrénées, Direction Départementale des Affaires Sanitaires et Sociales de Gard.

Cottenie, A., Verloo, M., Kiekens, L., Velghe, G., Camerlynck, R. (1982). Chemical analysis of plants and soils. Laboratory of analytical and agrochemistry, State University Ghent, Belgium. p.63

Cunningham, S. D., & Berti, W. R. (2000). Phytoextraction and phytostabilization: technical, economic, and regulatory considerations of the soil-lead issue. In N. Terry & G. Bañuelos (Eds.), *Phytoremediation of Contaminated Soil and Water* (pp. 359–376). Boca Raton: Lewis Publishers.

Dahmani-Muller, H., van Oort, F., Gelie, B., & Balabane, M. (2000). Strategies of heavy metal uptake by three plant species growing near a metal smelter. *Environmental Pollution, 109*, 231–238.

De Cáceres, M., Font, X., García, R., Oliva, F. (2003). Vegana, un paquete de programas para la gestión y análisis de datos ecológicos. VII Congreso Nacional de la Asociación Española de Ecología Terrestre. Barcelona, Spain. pp. 1481–1497

Dère, C., Lamy, I., van Oort, F., Baize, D., & Cornu, S. (2006). Reconstitution des apports en éléments traces métalliques et bilan de leur migration dans un Luvisol sableux soumis à 100 ans d'irrigation massive par des eaux usées brutes. *Comptes Rendus Geosciences, 338*, 565–573.

Douay, F., Pruvot, C., Roussel, H., Ciesielski, H., Fourrier, H., Proix, N., et al. (2008). Contamination of urban soils in an area of Northern France polluted by dust emissions of two smelters. *Water, Air, and Soil Pollution, 188*, 247–260.

Ernst, W. H. O. (1974). *Schwermetallvegetation der Erde*. Stuttgart: Gustav Fischer Verlag.

Ernst, W. H. O. (1996). Bioavailability of heavy metals and decontamination of soils by plants. *Applied Geochemistry, 11*, 163–167.

Escarré, J., Houssard, C., Debussche, M., & Lepart, J. (1983). Evolution de la végétation et du sol après abandon cultural en région méditerranéenne: étude de succession dans la garrigues du Montpelliérais (France). *Acta Oecologica, Oecologia Plantarum, 4*, 221–239.

Escarré, J., Lefèbvre, C., Gruber, W., Leblanc, M., Lepart, J., Rivière, Y., et al. (2000). Zinc and cadmium hyperaccumulation by *Thlaspi caerulescens* from metalliferous and nonmetalliferous sites in the Mediterranean area: implications for phytoremediation. *The New Phytologist, 145*, 429–437.

Fangueiro, D., Bermond, A., Santos, E., Carapuça, H., & Duarte, A. (2005). Kinetic approach to heavy metal mobilization assessment in sediments: choose of kinetic equations and models to achieve maximum information. *Talanta, 66*, 844–857.

FAO. (2006). *Guidelines for soil profile description* (4th ed.). Rome: F.A.O.

Fernandez, C., Labanowski, J., Cambier, P., Jongmans, A. G., & van Oort, F. (2007). Fate of airborne metal pollution in soils as related to agricultural management. 1. Zn and Pb distribution in soil profiles. *European Journal of Soil Science, 58*, 547–559.

Freitas, H., Prasad, M. N. V., & Pratas, J. (2004). Analysis of serpentinophytes from north-east of Portugal for trace metal accumulation - relevance to the management of mine environment. *Chemosphere, 54*, 1625–1642.

Frérot, H., Lefèbvre, C., Gruber, W., Collin, C., Dos Santos, A., & Escarré, J. (2006). Specific interactions between local metallicolous plants improve the phytostabilization of mine soils. *Plant and Soil, 282*, 53–65.

Frérot, H., Lefèbvre, C., Petit, C., Collin, C., Dos Santos, A., & Escarré, J. (2005). Zinc tolerance and hyperaccumulation in F-1 and F-2 offspring from intra and interecotype crosses of *Thlaspi caerulescens*. *The New Phytologist, 165*, 111–119.

Frérot, H., Lefèbvre, C., Petit, C., Collin, C., DosSantos, A., & Escarre, J. (2009). Adaptation des végétaux aux milieux pollués par des métaux toxiques et phytoremédiation-cas de la région Languedoc-Roussillon. In P. Cambier et al. (Eds.), *Contaminations métalliques des agrosystèmes et écosystèmes péri-industriels* (pp. 287–298). Versailles: Editions Quae.

Frérot, H., Petit, C., Lefèbvre, C., Gruber, W., Collin, C., & Escarré, J. (2003). Zinc and cadmium accumulation in controlled crosses between metallicolous and nonmetallicolous populations of *Thlaspi caerulescens* (Brassicaceae). *The New Phytologist, 157*, 643–648.

Gabler, H. E., & Schneider, J. (2000). Assessment of heavy-metal contamination of floodplain soils due to mining and mineral processing in the Harz Mountains, Germany. *Environmental Geology, 39*, 774–782.

Grimalt, J. O., Ferrer, M., & Macpherson, E. (1999). The mine tailing accident in Aznalcollar. *The Science of the Total Environment, 242*, 3–11.

Holl, K. D. (2002). Effect of shrubs on tree seedling establishment in an abandoned tropical pasture. *Journal of Ecology, 90*, 179–187.

Holmgren, G. G. S., Meyer, M. W., Chaney, R. L., & Daniels, R. B. (1993). Cadmium, lead, zinc, copper, and nickel in agricultural soils of the United States of America. *Journal of Environmental Quality, 22*, 335–348.

Labanowski, J., Monna, F., Bermond, A., Cambier, P., Fernandez, C., Lamy, I., et al. (2008). Kinetic extractions to assess mobilization of Zn, Pb, Cu, and Cd in a metal-contaminated soil: EDTA *vs*. citrate. *Environmental Pollution, 153*, 693–701.

Leach, D., Macquar, J. C., Lagneau, V., Leventhal, J., Emsbo, P., & Premo, W. (2006). Precipitation of lead-zinc ores in the Mississippi Valley-type deposit at Trèves, Cévennes region of southern France. *Geofluids, 6*, 24–44.

Leblanc, M., Petit, D., Deram, A., Robinson, B. H., & Brooks, R. R. (1999). The phytomining and environmental significance of hyperaccumulation of thallium by *Iberis intermedia* from Southern France. *Economic Geology And The Bulletin Of The Society Of Economic Geologists, 94*, 109–113.

Lefebvre, C. (1974). Population variation and taxonomy in Armeria-Maritima with special reference to heavy-metal-tolerant populations. *The New Phytologist, 73*, 209–219.

Lefèbvre, C., & Simon, E. (1979). Plant spacing in open communities from old zinc-lead mines wastes. *Oecologia Plantarum, 14*, 461–474.

Lefèbvre, C., & Vernet, P. (1990). Microevolutionary processes on contaminated deposits. In A. J. Shaw (Ed.), *Heavy metal tolerance in plants: Evolutionary aspects* (pp. 285–300). Boca Raton: CRC Press Inc.

Liu, M. Q., Yanai, J. T., Jiang, R. F., Zhang, F., McGrath, S. P., & Zhao, F. J. (2008). Does cadmium play a physiological role in the hyperaccumulator *Thlaspi caerulescens*? *Chemosphere, 71*, 1276–1283.

Lombi, E., Zhao, F. J., McGrath, S. P., Young, S. D., & Sacchi, G. A. (2001). Physiological evidence for a high-affinity cadmium transporter highly expressed in a *Thlaspi caerulescens* ecotype. *The New Phytologist, 149*, 53–60.

Meerts, P., & Van Isacker, N. (1997). Heavy metal tolerance and accumulation in metallicolous and non-metallicolous populations of *Thlaspi caerulescens* from continental Europe. *Plant Ecology, 133*, 221–231.

Ministère de la Santé. (2004). Circulaire DGS/SD7A no 45 du 5 février 2004 relative au contrôle des paramètres plomb, cuivre et nickel dans les eaux destinées à la consommation humaine.

Molitor, M., Dechamps, C., Gruber, W., & Meerts, P. (2005). *Thlaspi caerulescens* on nonmetalliferous soil in Luxembourg: ecological niche and genetic variation in mineral element composition. *The New Phytologist, 165*, 503–512.

Noret, N., Meerts, P., Vanhaelen, M., Dos Santos, A., & Escarre, J. (2007). Do metal-rich plants deter herbivores? A field test of the defence hypothesis. *Oecologia, 152*, 92–100.

Petelet-Giraud, E., Negrel, P., Luck, J. M., & Ben Othman, D. (2004). Dissolved and particulate heavy metals transport in the Herault watershed: constraints of the origin by lead isotopes. *Houille Blanche-Revue Internationale De L Eau, 2*, 43–48.

Remon, E., Bouchardon, J. L., Cornier, B., Guy, B., Leclerc, J. C., & Faure, O. (2005). Soil characteristics, heavy metal availability and vegetation recovery at a former metallurgical landfill: Implications in risk assessment and site restoration. *Environmental Pollution, 137*, 316–323.

Retana, J., Espelta, J. M., Habrouk, A., Ordonez, J. L., & de Sola Morales, F. (2002). Regeneration patterns of three Mediterranean pines and forest changes after a large wildfire in northeastern Spain. *Ecoscience, 9*, 89–97.

Robinson, B., Leblanc, M., Petit, D., Brooks, R., Kirkman, J., & Gregg, P. (1998). The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. *Plant and Soil, 203*, 47–56.

Robles-Arenas, V. M., Rodriguez, R., Garcia, C., Manteca, J. I., & Candela, L. (2006). Sulphide-mining impacts in the physical environment: Sierra de Cartagena La Union (SE Spain) case study. *Environmental Geology, 51*, 47–64.

Rolley, J. P. (2002). La petite histoire du Plomb et du Zinc en Cévennes. Available at: http://www.ensm-ales.fr/~jprolley/Geologie/Pb-Zn.html. Accessed 25 October 2005.

Roosens, N., Verbruggen, N., Meerts, P., Ximenez-Embun, P., & Smith, J. A. C. (2003). Natural variation in cadmium tolerance and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from western Europe. *Plant, Cell & Environment, 26*, 1657–1672.

SAS. (2004). *SAS-STAT® 9.1 User's guide* (pp. 1731–1846). Cary: SAS Institute Inc.

Searcy, K. B., & Mulcahy, D. L. (1985). The parallel expression of metal tolerance in pollen and sporophytes of Silene-Dioica (L) Clairv, Silene-Alba (Mill) Krause and Mimulus-Guttatus Dc. *Theoretical and Applied Genetics, 69*, 597–602.

Shen, Z. G., Zhao, F. J., & McGrath, S. P. (1997). Uptake and transport of zinc in the hyperaccumulator *Thlaspi caerulescens* and the non-hyperaccumulator *Thlaspi ochroleucum*. *Plant, Cell & Environment, 20*, 898–906.

Shimwell, D. W., & Laurie, A. E. (1972). Lead and zinc contamination of vegetation in the Southern Pennines. *Environmental Pollution, 3*, 291–301.

Simon, E. (1978). Heavy metals in soils, vegetation development and heavy metal tolerance in plant populations from metalliferous areas. *The New Phytologist, 81*, 175–188.

Sterckeman, T., Douay, F., Proix, N., Fourrier, H., & Perdrix, E. (2002). Assessment of the contamination of cultivated soils by eighteen trace elements around smelters in the north of France. *Water, Air, and Soil Pollution, 135*, 173–194.

Tamm, O. (1922). Eine methode zur Bestimmung der anorganischen Komponenten der gelkomplexen in Boden. *Meddelanden fran Statens Skogsförsöksanstalt, 19*, 385–404.

Terry, N., & Bañuelos, G. (Eds.). (2000). *Phytoremediation of contaminated soil and water* (pp. 109–128). Boca Raton, FL: Lewis Publishers.

Tremel, A., Masson, P., Garraud, D., Donard, O. F. X., Baize, D., & Mench, M. (1997). Thallium in French agrosystems. II. Concentration of thallium in field-grown rape and some other plant species. *Environmental Pollution, 97*, 161–168.

Tutin, T. G., Burges, N. A., Chater, A. O., Edmondson, J. R., Heywood, V. H., Moore, D. M., et al. (1964). *Flora Europaea*. Cambridge: Cambridge University Press.

van Oort, F., Dahmani-Muller, H., Balabane, M., Denaix, L., Gélie, B. (2000) Etude de trois espèces végétales métallophytes : quantification, localisation et spéciation du Zn, Pb et Cd à différentes échelles. II. Evaluation de la faisabilité d'application à la réhabilitation de sols pollués. Final Report Convention Ademe. INRA No 98 95 005. p. 78

van Oort, F., Thiry, M., Jongmans, A., Bourennane, H., Cambier, P., Lamy, I., et al. (2009). Les pollutions métalliques d'un site industriel et des sols environnants: Distributions hétérogènes des métaux et relations avec l'usage des sols. In P. Cambier, C. Schvartz & F. van Oort (Eds.), *Contaminations métalliques des agrosystèmes et écosystèmes péri-industriels*. Versailles: Editions Quae. pp. 15–44

Vesk, P. A., & Reichman, S. M. (2009). Hyperaccumulators and herbivores—a Bayesian meta-analysis of feeding choice trials. *Journal of Chemical Ecology, 35*, 289–296.

Vidal, C., Chantreuil, C., Berge, O., Mauré, L., Escarré, J., Béna, G., et al. (2009). Mesorhizobium metallidurans sp. nov., a novel metal-resistant symbiont of *Anthyllis vulneraria* growing on metallicolous soil in Languedoc, France. *International Journal of Systematic and Evolutionary Microbiology, 59*, 850–855.

Vincent, M. (2006). Les mines des Cévennes. Histoire des concessions et des chemins de fer miniers. Association Terre Cévenole.

Vitousek, P., D'Antonio, C., Loope, L., Rejmanek, M., & Westbrooks, R. (1997). Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology, 21*, 1–16.

Wali, M. K. (1999). Ecological succession and the rehabilitation of disturbed terrestrial ecosystems. *Plant and Soil, 213*, 195–220.

Wiegleb, G., & Felinks, B. (2001). Predictability of early stages of primary succession in post-mining landscapes of Lower Lusatia, Germany. *Applied Vegetation Science, 4*, 5–18.

Wierzbicka, M., & Pielichowska, M. (2004). Adaptation of *Biscutella laevigata* L, a metal hyperaccumulator, to growth on a zinc-lead waste heap in southern Poland—I: Differences between waste-heap and mountain populations. *Chemosphere, 54*, 1663–1674.

Wierzbicka, M., Szarek-Lukaszewska, G., & Grodzinska, K. (2004). Highly toxic thallium in plants from the vicinity of Olkusz (Poland). *Ecotoxicology and Environmental Safety, 59*, 84–88.