# Chapter 9
# Heavy Metals in Native Mediterranean Grassland Species Growing at Abandoned Mine Sites: Ecotoxicological Assessment and Phytoremediation of Polluted Soils

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## 9.1 Introduction

The closing of several mines in the central Iberian Peninsula has left behind a bleak scenario of areas of soils highly polluted with heavy metals and trace elements. The plant communities that thrive at these sites are mainly comprised of grassland species, and it is of major concern that these plants are consumed by livestock or wild animals. These grasslands have been the focus of several years of study by our research group, both because of the impacts of their polluted soils on ecosystems and because of their possible remediation role (Pastor and Hernández 2008).

Based on the results of numerous plant surveys, the dynamics of these communities exposed to elevated levels of trace elements (Hernández and Pastor 2008a) can be summarised as follows. The most evident impact of pollutants on plant communities is a loss of species diversity. The presence of a pollutant in a habitat affects either the area occupied by each species or the resources they use. These effects depend on the tolerance level of species or their sensitivity to a given pollutant (Pastor and Hernández 2007). As a consequence, effects of pollutants on competition may upset the balance among the community's components, which could wipe out some of the more sensitive populations. This may be observed at the sites examined here; although the dominance of grassland species is evident, legume species are hardly present, probably due to the heavy metals in their soils (Hernández and Pastor 2005). Thus, species frequencies in an ecosystem will vary along a gradient of chronic pollution.

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Knowledge of soil-plant processes and interrelations in terrestrial ecosystems is essential to understand how these systems work and how they are affected by any perturbation. Processes such as those controlling heavy metal dynamics are slow and complex. When toxic elements are incorporated into food webs, they may have direct effects on ecosystem health, human health or population welfare (Hernández et al. 2009). These problems need to be understood in detail to avoid their consequences through the design of remediation measures.

This study is a part of a research line that focuses on the ecological restoration of sites with heavy metal-polluted soils (Hernández and Pastor 2008b). Based on our knowledge of the behaviour of plant communities at these abandoned mine sites, the present study was designed to determine the heavy metal, trace element and Al contents of both the topsoil layers and plant species growing at these sites with the objectives: (1) to assess the remediation capacity of the native species forming these communities and, perhaps more importantly, (2) to assess the ecological risks of the accumulating behaviour of these plants given that the grasslands are grazed by wild and domestic animals.

## 9.2 Materials and Methods

### *9.2.1 Mine Sites and Sampling*

The eight abandoned mine sites (Fig 9.1) examined are named according to their location (town or village) in central Spain; the main element mined and Universal Transverse Mercator (UTM) coordinates for the site are indicated in parentheses. Five sites are found in the Comunidad de Madrid: Garganta de los Montes (Cu, $X = 443297$, $Y = 4529867$, 30 T, henceforth *Garganta*), Bustarviejo (Ag, $X = 438423$, $Y = 4524252$, 30 T), Navas del Rey (Ba, $X = 393455$, $Y = 4474405$, 30 T, henceforth *Navas*), Colmenar del Arroyo (Pb, $X = 395182$, $Y = 4474723$, 30 T, henceforth *Colmenar*) and Colmenarejo (Cu, $X = 415799$, $Y = 4486912$, 30 T). Their main characteristics are described in Fernández-Rubio (2007), Jiménez et al. (2004) and Jiménez-Ballesta et al. (1990). The other three sites are located in the Comunidad de Castilla-La Mancha; two in the Toledo province, Mazarambroz (Ag, $X = 406730$, $Y = 4397755$, 30 S) and Buenasbodas (Au, $X = 332410$, $Y = 4392147$, 30 S); and the other in the Guadalajara province, Hiendelaencina (Ag, $X = 500459$, $Y = 4548926$, 30 T) (Libro Blanco de la Minería de Castilla-La Mancha 2004).

Soil and plant sampling was conducted in different geomorphologic units at each site (tailings, slopes and valleys affected by mining activities) since heavy metals spread beyond tailings (Encabo et al. 1987; Gutiérrez-Maroto et al. 1989; Lacal et al. 1995). In each geomorphologic unit, sampling was random. The number of points sampled depended on the unit size and its visual heterogeneity. At each point, an average soil sample was collected of the topsoil layer (0–10 cm) using a hoe. Plant sampling was performed across 1-m$^2$ squares to include the above-

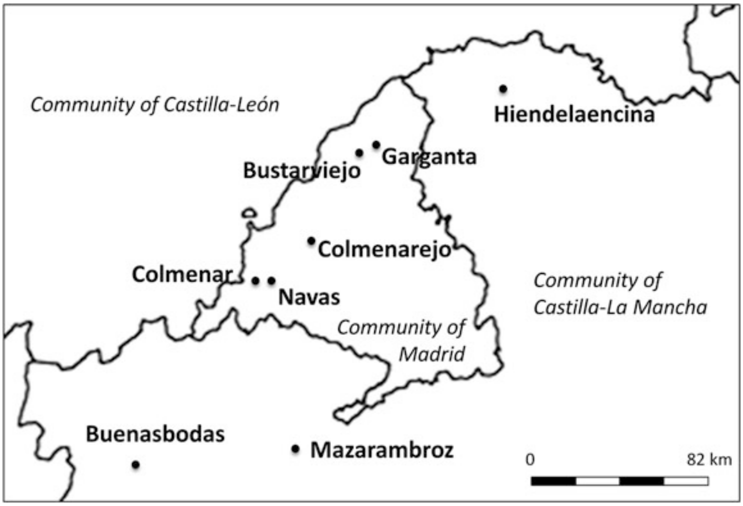


**Fig. 9.1** Localization of the abandoned mine sites

ground mass of dominant species or species contributing to the food web (171 grass species total). Additionally, we collected samples of 15 woody species because of their structural role. Species were identified according to the Flora de Andalucía and Flora Ibérica although the nomenclature used is that accepted in January 2013 by Anthos (Sistema de Información sobre las plantas de España, www.anthos.es). The name of the author describing each species is not included in the result tables. Species are classified into the three most abundant Mediterranean grassland families (Poaceae or grasses, Fabaceae or legumes and Asteraceae or composites).

### *9.2.2 Chemical and Data Analysis*

Soil samples were dried at room temperature for 1 week and sieved through a 2 mm mesh. Tests conducted were pH in slurry, organic matter by potassium dichromate reduction (according to protocols described in Hernández and Pastor 1989), pseudo-total metal contents by inductively coupled plasma-optical emission spectroscopy (ICP-OES, PerkinElmer Optima 4300DV), following $HNO_3$ and $HClO_4$ digestion (Walsh and SSSA 1971), and As and Ba contents by X-ray fluorescence (Siemens SRS 300). All plant samples of above-ground parts were washed with tap water and rinsed twice in deionised water, oven-dried at 70 °C for 48 h and ground in an IKA Werke Yellow Line A10 grinder. The procedures used to determine metals were those described for the soil samples. The metals determined in plants were those that may enter the food chain (Cu, Zn, Pb, Cd, Ni, Cr, Al and Mn).

Results are provided as minimum and maximum concentrations.

The index Relative Deviation to Background (RDB) described by Kabata-Pendias and Mukherjee (2007), relating the average heavy metal contents of all samples of the same species to a reference value, was used as a measure of the accumulation capacity of native plants.

## 9.3 Results and Discussion

### *9.3.1 Concentrations of Heavy Metals and Trace Elements in the Mine Soils*

In total, 191 soil samples from the eight abandoned mine sites were analysed (see Table 9.1). At the Garganta and Mazarambroz sites, two sampling sessions were needed due to their large size and widely varying metal concentrations.

The prevailing soil pH of the eight sites is acidic, determining a risk of high metal bioavailability. Soils from valleys and slope breaks showed the higher organic matter (OM) contents. In contrast, tailing samples had the least OM. Since it is difficult to find permissible reference values for Al and Mn, values obtained for these elements were compared to the mean for the Earth's crust and light sandy soils (as are most of the soils examined) as described in Kabata-Pendias and Mukherjee (2007). Soil Cr levels were always under their reference values. Ni was detected in a concentration higher than the reference just in two sampling points (one in Navas and one in Mazarambroz). Ba was only determined at the mine where this element was exploited (Navas). The following is a summary of the different sites in terms of the metals that mostly contribute to their polluted soils.

Practically all the mine soils were found to contain several metals at higher concentrations than reference levels. The Garganta mine is mainly polluted by Cu. Although one of the samples examined showed substantial Pb and Cd concentrations (3,750 mg $kg^{-1}$ and 340 mg $kg^{-1}$, respectively), these metals normally appeared at concentrations of around 100 mg $kg^{-1}$ and $<10$ mg $kg^{-1}$, respectively. Arsenic is the main pollutant of the Bustarviejo mine, but in some areas Cu, Zn, Pb and Cd levels were also worrying. Only two soil samples from the Navas site featured higher element levels than reference values. Similarly, only one soil sample from the Colmenar mine contained high Cu, Zn and Pb concentrations. Mazarambroz was the mine with the largest area affected by heavy metals, mainly Zn and Pb, but also As, Cu and Cd at several points. None of the samples collected from the Hiendelaencina mine showed polluting levels of metals as determined by XRF; thus, these samples were not subjected to ICP-OES tests.

**Table 9.1** Number of soil samples (*N*) collected from each mine site and soil pH, organic matter (OM %), pseudo-total contents of Al (%) and metals (mg kg$^{-1}$) and total content (XRF) of As (mg kg$^{-1}$)

| Mine | *N* | pH | OM | Ba | Al | Mn | Cu | Zn | Pb | Cd | As | Cr | Ni |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Garganta | 50 | 4.6–7.6 | 0.3–17.9 | – | 1.6–5.4 | 70–1,450 | 30–3,500 | 40–540 | 5–3,750 | 0.1–340 | 21–45 | 2.0–35 | 8.0–26 |
| Bustarviejo | 15 | 4.0–6.2 | 0.3–9.0 | – | 1.2–4.1 | 200–690 | 45–2,070 | 140–11,010 | 40–3,953 | 4.5–263 | 205–15,436 | 2.0–12 | 1.0–6.0 |
| Colmenarejo | 12 | 5.7–7.0 | 0.9–8.0 | – | 1.2–3.0 | 266–449 | 22–10,909 | 87–248 | 11–178 | 0.0–6.0 | 9.0–224 | 0.0–10 | 1.0–5.1 |
| Navas | 11 | 4.7–6.7 | 2.0–7.1 | 42–1,630 | 1.7–2.9 | 135–605 | 4.5–888 | 69–1,130 | 10–249 | 4.0–5.5 | – | 5.6–21 | 3.5–32 |
| Colmenar | 4 | 6.2–7.6 | 3.7–7.5 | – | 2.0–3.8 | 58–609 | 1.5–435 | 131–2,641 | 209–8,584 | 0.0–16 | n.d. | 1.0–10 | 1.5–15 |
| Mazarambroz | 79 | 1.6–8.0 | 0.3–9.7 | – | 0.4–5.2 | 45–1,625 | 8.5–201 | 62–10,430 | 20–6,542 | 0.0–35 | 36–531 | 0.0–54 | 3.0–41 |
| Buenasbodas | 12 | 5.0–6.9 | 1.4–10.0 | – | 1.6–5.2 | 41–1,315 | 5–25 | 16–69 | 1–25 | 0.0–0.5 | – | 2.5–10 | 2.0–24 |
| Hiendelaencina[a] | 8 | – | – | – | | | 26–63 | 91–420 | 18–516 | – | – | | |
| Reference level | pH < 7[b] | | | 160[c] | 8, 4[d] | 1,200, 2,000[d] | 50 | 150 | 50 | 1 | 29[c] | 100 | 30 |
| | pH > 7[b] | | | | | | 210 | 450 | 300 | 3 | | 150 | 112 |

[a]Samples only subjected to XRF due to their low pollutant levels
[b]Reference according to Spanish law RD1310/1990 (BOE 1990)
[c]Dutch reference
[d]Concentration in Earth's crust—concentration in light sandy soils, Kabata-Pendias and Mukherjee (2007)
n.d.: not detected
–: not analysed

### 9.3.2 Heavy Metals, Trace Elements and Al in the Shoots of Grass Species Growing at the Mine Sites

One hundred and seventy-one native grass species of the eight mine sites plus 16 woody species were tested. Most species had metals and trace elements at higher concentrations than considered normal. Tables 9.2, 9.3 and 9.4 show the most relevant species according to their accumulation capacity. The chosen ones were those with concentrations higher than that considered toxic for plants (Table 9.5) of at least two metals.

Interestingly, the Poaceae family (Table 9.2) contained a great number of species with high metal concentrations. Among all those tested, *Agrostis castellana* was the species showing the highest levels of Cu, Zn and Pb. This species was also broadly distributed across all sites. Many species of this family featured outstanding concentrations of Zn, but species with high levels of Cu, Pb, Cd or As were not that frequent. Of interest, Zn, Pb, Cd and As concentrations were high in *Arrhenatherum elatius* subsp. *bulbosum*, *Holcus annuus* subsp. *setiglumis* and *Vulpia myuros*. Apart from *Agrostis castellana*, *A. stolonifera*, *Corynephorus canescens* and *Lolium multiflorum* showed the greatest Cu contents.

The metal contents of the legumes were unremarkable (Table 9.3) and always lower than the concentrations detected in the grasses, as also reported by Kabata-Pendias and Mukherjee (2007). However, metal levels were high in some species such as *Trifolium scabrum* (maximum 496 mg Zn kg$^{-1}$, 100 mg Pb kg$^{-1}$) or *T. campestre* (maximum 8 mg Cd kg$^{-1}$). None of the legumes were found to contain As in their shoots, so this element is not shown in the table. In contrast, Jana et al. (2012) detected an As concentration of 208 mg kg$^{-1}$ in the leaves of *Trifolium pratense*.

Species of the Asteraceae family (Table 9.3) are well known for their high element accumulation capacity. However, although some of the species showed significant metal concentrations, these were never comparable to those detected in the grasses. The highest Zn concentration (1,087 mg Zn kg$^{-1}$) was observed in *Leontodon saxatilis*. *Carduus pycnocephalus* and *Crepis vesicaria* featured high Pb concentrations, and marked Cd concentrations were observed in all of this family's species (up to 44 mg kg$^{-1}$ in *Andryala ragusina*), even exceeding those of the grasses. Once again, Arsenic was not detected in any species.

In the remaining families (Table 9.4), several species showed a notable accumulation capacity. *Scirpoides holoschoenus* was the only species containing As (up to 57 mg kg$^{-1}$), although similar species have been described by Jana et al. (2012) to take up this metal: *Juncus effusus* ($29 \pm 0.3$ mg As kg$^{-1}$) and *Plantago major* (7 mg kg$^{-1}$). Species of the *Rumex* genus featured marked overall contents of Cu, Zn, Pb and Cd. *Spergularia rubra*, species of the genus *Plantago* and those of the Brassicaceae family are also interesting accumulators, though their Cu accumulation capacity is limited. The maximum Cd concentration in all the tested plants was found in *Jasione montana* (64 mg Cd kg$^{-1}$), but species such as *Andryala ragusina* or *Corrigiola telephiifolia* were also observed to accumulate

**Table 9.2** Heavy metal and As concentrations (mg kg$^{-1}$) detected in the shoots of native Poaceae species collected from the mine sites

| Poaceae species | | Cu | Zn | Pb | Cd | As | Al | Mn |
|---|---|---|---|---|---|---|---|---|
| *Agrostis* | ***A. castellana*** | 0.0–424 | 17–9,065 | 0.0–1,572 | 0.0–12 | 0.0–135 | 11–3,059 | 40–2,465 |
| | ***A. stolonifera*** | 2.1–941 | 3.6–123 | 0.0–12 | 0.0–2.8 | 0.0 | 1,247 | 9.5–118 |
| *Aira* | *A. caryophyllea* | 0.8–93 | 39–140 | 0.0 | 0.0–2.0 | 0.0 | 189–626 | 49–231 |
| *Arrhenatherum* | *A. album* | 0.0–39 | 43–268 | 0.0–8.3 | 0.0 | – | 40–171 | 0.0–218 |
| | ***A. elatius*** | 0.0–98 | 20–790 | 0.0–43 | 0.0–16 | – | 139–1,078 | 20–373 |
| | ***A. elatius* subsp. *bulbosum*** | 0.5–16 | 0.6–1,740 | 0–198 | 0–10 | 0.0–51 | 1.8–325 | 1.1–848 |
| *Avena* | ***A. barbata*** | 0.7–14 | 30–423 | 0.0–249 | 0.0–5.5 | – | 54–2,511 | 32–442 |
| | *A. sterilis* | 4.8–11 | 14–320 | 0.0–44 | 0.0–2.6 | 0.0 | 2.1–77 | 28–484 |
| *Bromus* | *B. diandrus* | 2.3–45 | 0.0–194 | 0.0 | 0.0 | – | 28–362 | 10–130 |
| | *B. hordeaceus* | 2.0–64 | 14–210 | 0.0–18 | 0.0–1.2 | – | 31–150 | 25–253 |
| | ***B. madritensis*** | 1.3–26 | 131–720 | 3–74 | 0.0–3.1 | – | 61–335 | 8.2–78 |
| | ***B. rubens*** | 1.2–19 | 17–677 | 0.0–309 | 0.0–1.9 | 0.0 | 44–2,205 | 5.1–97 |
| | *B. tectorum* | 0.0–47 | 33–487 | 0.0–78 | 0.0–4.7 | 0.0 | 25–389 | 1.9–278 |
| *Corynephorus* | ***C. canescens*** | 2.2–659 | 54–108 | 0.0 | 0.0–0.4 | 0.0 | 249–2,419 | 30–198 |
| *Cynodon* | ***C. dactylon*** | 7.1–86 | 24–462 | 0.0–98 | 0.0–6.0 | 0.0 | 53–571 | 13–834 |
| *Dactylis* | *D. glomerata* | 0.0–24 | 21–424 | 0.0–50 | 0.0–0.8 | – | 12–362 | 7.2–327 |
| *Holcus* | ***H. annuus* subsp. *setiglumis*** | 0.4–34 | 24–1,220 | 0.0–262 | 0.0–4.6 | 0.0–51 | 18–456 | 4.1–695 |
| | *H. lanatus* | 0.0–84 | 20–840 | 0.0–3.5 | 0.0–8.6 | 0.0 | 24–2,531 | 72–1,140 |
| *Lolium* | ***L. multiflorum*** | 0.3–662 | 2.6–209 | 0.0 | 0.0–4.9 | 0.0 | 0.0–133 | 1.5–117 |
| | ***L. rigidum*** | 4.3–38 | 48–147 | 0.0–6.0 | 0.0 | 0.0–195 | 41–1,510 | 135–295 |
| *Melica* | *M. ciliata* | 1.9–10 | 24–206 | 0.0–4.6 | 0.0–4.1 | – | 79–94 | 6.2–35 |
| *Micropyrum* | ***M. tenellum*** | 2.0–21 | 78–781 | 0.0–104 | 0.0–2.6 | – | 13–1,171 | 32–206 |
| *Molineriella* | *M. laevis* | 5.2–89 | 33–748 | 0.0 | 0.0–7.8 | 0.0 | 31–431 | 74–524 |
| *Periballia* | ***P. involucrata*** | 3.2–33 | 111–304 | 0.0–21 | 0.0–1.4 | 0.0–200 | 37–716 | 82–147 |
| *Poa* | *P. trivialis* | 1.5–46 | 12–339 | 0.0 | 0.0–2.0 | – | – | 29–82 |

(continued)

**Table 9.2** (continued)

| Poaceae species | | Cu | Zn | Pb | Cd | As | Al | Mn |
|---|---|---|---|---|---|---|---|---|
| *Stipa* | *S. lagascae* | 0.7–16 | 15–301 | 0.0–75 | 0.0–1.1 | 0.0 | 14–286 | 10–129 |
| *Taeniatherum* | *T. caput-medusae* | 1.2–152 | 0.0–21 | 0.0 | 0.0 | 0.0–57 | 41–100 | 18–26 |
| *Trisetum* | *T. ovatum* | 1.9–7.2 | 25–109 | 0.0 | 0.0–0.3 | – | – | 47–400 |
| *Vulpia* | ***V. bromoides*** | 3.6–49 | 88–892 | 0.0–58 | 0.0–2.4 | 0.0 | 56–1,998 | 63–394 |
| | ***V. ciliata*** | 4.3–8.1 | 133–254 | 0.0–107 | 0.0–0.7 | 0.0 | 480–3,608 | 0.0–398 |
| | ***V. myuros*** | 0.0–47 | 6.3–3,165 | 0.0–771 | 0.0–20 | 0.0–139 | 22–2,118 | 2.5–602 |

**Table 9.3** Heavy metal and As concentrations (mg $kg^{-1}$) detected in the shoots of native Fabaceae and Asteraceae species collected from the mine sites

| Fabaceae species | | Cu | Zn | Pb | Cd | Al | Mn |
|---|---|---|---|---|---|---|---|
| *Lotus* | *L. corniculatus* | 2.0–6.9 | 34–324 | 0.0 | 0.0–1.3 | – | 63–91 |
| | *L. pedunculatus* | 2.1–7.6 | 1.0–80 | 0.0 | 0.0–2.7 | – | 21–236 |
| *Ornithopus* | *O. compressus* | 4.4–13 | 27–209 | 0.0 | 0.0 | 32–275 | 138–353 |
| | ***O. perpusillus*** | 1.5–6.8 | 2.1–234 | 0.0 | 0.0–3.6 | – | 20–113 |
| *Trifolium* | *T. campestre* | 7.0–38 | 84–214 | 0.0 | 0.0–8.1 | 111–3,294 | 43–171 |
| | *T. gemellum* | 3.0–5.0 | 62–348 | 0.0–32 | 0.0 | 78–311 | 65–151 |
| | *T.ochroleucon* | 8.6–23 | 50–220 | 0.0 | 0.0–0.5 | 18–205 | 7.0–69 |
| | ***T. scabrum*** | 13–17 | 114–496 | 0.0–100 | 0.0–0.1 | 154–308 | 45–223 |
| | *T. strictum* | 4.0–79 | 34–120 | 0.0 | 0.0–1.5 | 50–114 | 22–69 |
| | *T. subterraneum* | 7.0– 21 | 93–214 | 0.0 | 0.0–1.2 | 66–168 | 131–198 |
| | ***T. tomentosum*** | 2.6–15 | 21–295 | 0.0–76 | 0.0–2.4 | 65–552 | 14–29 |
| Asteraceae species | | | | | | | |
| *Anacyclus* | *A. clavatus* | 5.9–66 | 43–79 | 0.0 | 0.3–4.6 | 25–432 | 155–334 |
| ***Andryala*** | ***A. integrifolia*** | 2.2–51 | 12–172 | 0.0 | 0.0–20 | 26–637 | 7.5–63 |
| | *A. laxiflora* | 7.4–15 | 191–285 | 2.1–8.2 | 0.0–10 | 48–96 | 71–206 |
| | ***A. ragusina*** | 6.3–27 | 167–824 | 0.0–57 | 4.8–44 | 158–811 | 32–60 |
| *Artemisia* | *A.* sp. | 0.8 – 1.0 | 12–206 | 0.0 | 0.0–2.0 | – | 9.4–14 |
| ***Carduus*** | ***C. pycnocephalus*** | 3.7–20 | 27–472 | 0.0–115 | 0.0–5.1 | 21–75 | 0.0–44 |
| *Centaurea* | *C. paniculata* | 8.9–13 | 14–94 | 0.0 | 0.0–2.5 | 20–57 | 4.8–14 |
| | *C. melitensis* | 1.3–8.0 | 71–224 | 0.0–52 | 0.0–6.1 | 41–128 | 0.0–96 |
| ***Chondrilla*** | ***C. juncea*** | 1.0–19 | 0.4–294 | 0.0–6.4 | 0.0–14 | 48–1,019 | 0.0–1,312 |
| ***Cirsium*** | ***C. sp.*** | 4.5–19 | 14–82 | 0.0 | 0.0–6.5 | 28–239 | 2.3–9.1 |
| *Crepis* | *C. capillaris* | 6.2–39 | 48–264 | 0.0–8.6 | 0.0–8.7 | 74–1,060 | 25–248 |
| | ***C. vesicaria*** | 2.4–25 | 34–603 | 0.0–176 | 0.0–20 | 23–534 | 3.9–346 |
| *Filago* | *F. arvensis* | 1.5–74 | 12–459 | 0.0 | 0.0–4.0 | – | 21–281 |
| | ***F. gallica*** | 11–25 | 85–461 | 9.0–97 | 1.7–13 | 62–425 | 37–51 |

(continued)

**Table 9.3** (continued)

| Fabaceae species | | Cu | Zn | Pb | Cd | Al | Mn |
|---|---|---|---|---|---|---|---|
| ***Hypochaeris*** | ***H. radicata*** | 2.4–73 | 36–724 | 0.0–0.2 | 0.0–17 | 90–1,734 | 20–327 |
| ***Leontodon*** | ***L. saxatilis*** | 4.8–54 | 91–1,087 | 0.0–45 | 0.0–24 | 70–199 | 9.3–433 |
| ***Pilosella*** | ***P. officinarum*** | 5.0–25 | 15–84 | 0.0–1.9 | 0.0–16 | 99–1,144 | 10–75 |
| ***Santolina*** | ***S. rosmarinifolia*** | 5.0–52 | 15–193 | 0.0 | 0.0–9.3 | 85–1,537 | 0.5–107 |
| *Scolymus* | *S. hispanicus* | 0.0–23 | 40–579 | 0.0–23 | 0.0–8.5 | 41–173 | 0.0–86 |
| ***Sonchus*** | ***S. asper*** | 0.9–3.5 | 12–380 | 0.0–43 | 0.0–13 | – | 1.6–7.9 |

large quantities of this metal. These species, however, do not show this metal in their shoots as frequently as *J. montana*, which took up this metal in all the samples tested. Despite being highly bioavailable (Kabata-Pendias and Mukherjee 2007), Cd concentrations in shoots are not usually high. In a study examining Cd accumulation in native species from mine sites, Zhang et al. (2012) also detected scarce amounts of Cd in vascular plants. In general, the metal concentrations detected here in native species from eight mine sites in central Spain were higher than those reported by other authors (Conesa et al. 2006, 2007; García-Salgado et al. 2012; Jana et al. 2012; Massa et al. 2010; Moreno-Jiménez et al. 2009; Pratas et al. 2013; Zhang et al. 2012), though for different plant communities.

The main woody species growing at the 8 mine sites were also tested (Table 9.4) since they are often consumed by goats. However, appreciable metal concentrations were only detected in the leaves of *Thymus zygis*.

Other analysed species that did not show very relevant concentration of metals and As were *Aegilops geniculata*, *A. triuncialis*, *Anthoxanthum aristatum*, *Avena sativa*, *Bromus sterilis*, *Cynosurus cristatus*, *C. echinatus*, *Festuca rothmaleri*, *Hordeum murinum*, *Koeleria vallesiana*, *Phalaris canariensis*, *P. minor*, *Phleum pratense*, *Poa annua*, *P. bulbosa*, *Stipa parviflora*, *Vulpia membranacea* and *V. unilateralis* of Poaceae family; *Anthyllis vulneraria*, *Lathyrus angulatus*, *Medicago minima*, *M. sativa*, *M. turbinate*, *Melilotus officinalis*, *Ononis pusilla*, *O. spinosa*, *Trifolium angustifolium*, *T. arvense*, *T. cherleri*, *T. dubium*, *T. glomeratum*, *T. repens*, *T. striatum*, *T. suffocatum*, *T. sylvaticum*, *Vicia angustifolia*, *V. benghalensis* and *V. hirsuta* of Fabaceae family; *Carthamus lanatus*, *Centaurea alba*, *C. calcitrapa*, *Chamaemelum mixtum*, *Cichorium intybus*, *Mantisalca salmantica*, *Pallenis spinosa*, *Picnomon acarna*, *Podospermum laciniatum and Tragopogon crocifolius* of Asteraceae family; *Anarrhinum bellidifolium*, *Campanula rapunculus*, *Carum verticillatum*, *Centranthus calcitrapae*, *Clinopodium vulgare*, *Crucianella angustifolia*, *Eryngium campestre*, *Foeniculum vulgare*, *Juncus effusus*, *Lomelosia simplex*, *Malva neglecta*, *Marrubium vulgare*, *Papaver rhoeas*, *Parentucellia latifolia*, *Paronychia argentea*, *Plantago holosteum*, *Rumex induratus*, *R. pulcher*, *Salvia verbenaca*, *Sanguisorba minor*, *Sesamoides purpurascens*, *Silene colorata*, *Torilis nodosa* and *Veronica arvensis* of other herb families; and *Cistus ladanifer*, *Crataegus* sp., *Daphne gnidium*, *Genista cinerascens*, *Helianthemum caput-felis*, *Lavandula pedunculata*, *Olea europaea*, *Quercus ilex* ssp. *ballota*, *Salvia verbenaca* and *Sambucus nigra* of woody species.

### *9.3.3 Ecotoxicological Assessment and Implications for Phytoremediation*

For an ecotoxicological assessment, it is necessary to know which species can tolerate high soil metal concentrations since these could enter the food webs of the

**Table 9.4** Heavy metal and As concentrations (mg kg$^{-1}$) in the shoots of native herb species of other families and woody species collected from the mine sites

| Species of other families | | Cu | Zn | Pb | Cd | Al | Mn |
|---|---|---|---|---|---|---|---|
| ***Armeria*** | ***A. arenaria*** | 10–156 | 34–219 | 0.0 | 0.0–2.2 | 125–3,831 | 15–74 |
| *Bartsia* | *B. trixago* | 2.8–21 | 39–385 | 0.0–102 | 0.0–2.0 | 59–1,986 | 15–99 |
| *Cerastium* | *C. glomeratum* | 2.0–4.4 | 22–185 | 0.0–3.2 | 0.0–0.3 | 41–207 | 34–122 |
| *Convolvulus* | *C. arvensis* | 10–122 | 56–106 | 0.0 | 0.0–2.3 | 138–814 | 18–85 |
| ***Coronopus*** | ***C.* sp.** | 10–125 | 21–100 | 0.0 | 0.0 | – | 21–73 |
| ***Corrigiola*** | ***C. telephiifolia*** | 4.6–465 | 31–554 | 0.0 | 0.0–57 | 53–630 | 10–575 |
| *Daucus* | *D. carota* | 1.0–20 | 22–179 | 0.0–7.3 | 0.0–6.2 | 54–1,253 | 9.0–200 |
| ***Digitalis*** | ***D. purpurea*** | 6.9–17 | 211–276 | 0.0–8.5 | 5.4–12 | – | 19–303 |
| *Diplotaxis* | *D. catholica* | 3.0–14 | 63–1,375 | 0.0–96 | 0.0–10 | 39–115 | 50–88 |
| *Echium* | *E. vulgare* | 2.8–25 | 59–581 | 0.0–57 | 0.0–5.1 | 35–653 | 20–520 |
| ***Galium*** | ***G. aparine*** | 7.0–8.0 | 134–481 | 10–66 | 0.0–1.9 | 94–186 | 33–459 |
| ***Geranium*** | ***G. molle*** | 5.5–29 | 84–216 | 0.0 | 0.0–18 | 349–558 | 3.9–130 |
| *Heliotropium* | *H. europaeum* | 9.0–13 | 171–248 | 20–52 | 0.0 | 26–107 | 33–133 |
| ***Hirschfeldia*** | ***H. incana*** | 0.0–6.6 | 29–827 | 0.0–142 | 0.0–11 | 31–1,094 | 0.0–34 |
| ***Hypericum*** | ***H. humifusum*** | 3.6–15 | 14–242 | 0.0–0.1 | 0.0–15 | 92–118 | 31–651 |
| | ***H. perforatum*** | 2.1–12 | 12–94 | 0.0 | 0.0–25 | 39–312 | 28–71 |
| ***Jasione*** | ***J. montana*** | 1.7–26 | 35–1,549 | 0.0–43 | 0.0–64 | 51–508 | 43–654 |
| *Marrubium* | *M. supinum* | 1.9–11 | 74–352 | 0.0–80 | 0.0–0.7 | 49–320 | 0.0–32 |
| *Mentha* | *M. pulegium* | 4.1–18 | 70–354 | 0.0–24 | 0.0–15 | 32–393 | 5.0–91 |
| ***Petrorhagia*** | ***P. nanteuilii*** | 6.2–31 | 12–305 | 0.0 | 0.0–7.1 | 98–687 | 25–111 |
| | *P. prolifera* | 1.0–37 | 38–184 | 0.0 | 0.0–3.2 | 106–261 | 12–172 |
| ***Plantago*** | ***P. afra*** | 3.7–15 | 294–1,105 | 2.1–256 | 0.0–12 | 23–164 | 15–410 |
| | ***P. coronopus*** | 5.4–31 | 72–799 | 0.0–239 | 0.0–8.0 | 43–420 | 8.9–423 |
| | ***P. lagopus*** | 1.8–97 | 21–1,622 | 0.0–292 | 0.0–14 | 46–3,037 | 3.6–985 |
| | *P. lanceolata* | 3.1–39 | 10–417 | 0.0–41 | 0.0–4.5 | 49–824 | 7.5–322 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | ***P. ovata*** | 4.0–11 | 27–400 | 0.0–31 | 0.0–3.5 | – | 13–45 |
| *Reseda* | *R. lutea* | 3.3–20 | 60–173 | 0.0–7.9 | 0.0–1.3 | – | 14–62 |
| ***Rumex*** | ***R. acetosella*** | 0.5–447 | 5.3–1,114 | 0.0–108 | 0.0–11 | 65–1,036 | 0.0–998 |
| | ***R. bucephalophorus*** | 2.6–177 | 17–3,077 | 0.0–525 | 0.0–25 | 54–664 | 0.0–564 |
| | ***R. papillaris*** | 6.3–597 | 12–242 | 0.0 | 0.0–6.4 | 61–322 | 1.2–89 |
| ***Scandix*** | ***S. pecten-veneris*** | 3.0–6.3 | 180–461 | 37–78 | 0.0 | 86–194 | 58–389 |
| ***Scirpoides*** | ***S. holoschoenus*** | 0.0–36 | 11–1,747 | 0.0–231 | 0–20 | 6.4–401 | 4.5–2,901 |
| *Silene* | *S. vulgaris* | 5.4–18 | 24–127 | 0.0 | 0.0–4.3 | 84–681 | 1.6–302 |
| *Sinapis* | *S. arvensis* | 2.4–7.3 | 17–1,656 | 0.0–83 | 0.0–8.8 | 10–166 | 20–59 |
| *Spergularia* | *S. rubra* | 3.0–82 | 69–2,775 | 0.0–805 | 0.0–11 | 51–1,914 | 22–590 |
| *Verbascum* | *V. pulverulentum* | 5.8–12 | 669–726 | 26–38 | 0.4–2.0 | 109–145 | 11–42 |
| *Xolantha* | *X. guttata* | 14–20 | 68–69 | 0.0 | 3.2–5.5 | 105–2,518 | 140–426 |
| Woody species | | | | | | | |
| *Adenocarpus* | *A. complicatus* | 15 | 103 | 0.0 | 2.4 | 161 | 100 |
| *Cytisus* | *C. scoparius* | 15 | 225 | 0.0 | 2.7 | – | 234 |
| ***Helichrysum*** | ***H. stoechas*** | 31 | 64 | 0.0 | 13 | 2,189 | 143 |
| *Lavandula* | *L. stoechas* | 6.9 | 192 | 0.0 | 1.7 | – | 245 |
| *Thymus* | *T. zygis* | 40 | 431 | 275 | 1.5 | 625 | 166 |
| | *T. mastichina* | 37 | 73 | 0.0 | 2.6 | 999 | 66 |

**Table 9.5** Element concentrations (mg kg$^{-1}$) in plant shoots considered normal versus toxic for the plants themselves, permissible levels for forage plants and maximum levels tolerated by livestock

| Element | Normal for plants[a] | Toxic for plants[a] | Maximum levels permissible for forage[b] | Maximum levels tolerated by livestock[c] | |
|---|---|---|---|---|---|
| | | | | Cattle | Sheep |
| Cu | 5–30 | 20–100 | 2.9 | 100 | 25 |
| Zn | 25–150 | 100–400 | 45 | 500 | 300 |
| Pb | 5–10 | 30–300 | 1.3 | 30 | 30 |
| Cd | 0.01–0.2 | 5–30 | 0.23 | 0.5 | 0.5 |
| As | 1–1.5 | 5–20 | 1 | 50 | 50 |
| Mn | 30–300 | 400–1,000 | 32.5 | 1,000 | 1,000 |

[a]Kabata-Pendias and Mukherjee (2007)
[b]WHO (1992, 1995), FAO (2000)
[c]Adapted from Madejón et al. (2006)

ecosystems where they are found. For phytoremediation purposes, we need to know which species are particularly good at accumulating a trace metal. In ecology, both these issues have been interesting from a perspective of species indicators of polluted sites.

#### 9.3.3.1 Bioaccumulation Capacity

Here, we used the RDB index described by Kabata-Pendias and Mukherjee (2007) as a measure of the average metal accumulation capacity of species that grow in polluted soils. The species found to show a good accumulation capacity (RDBs above 100 % for two or more heavy metals or high indices for highly toxic elements such as Cd or As) are shown in bold at Tables 9.2, 9.3 and 9.4.

The maximum metal concentration found in a species indicates its maximum accumulation capacity. In contrast, the RDB index refers to an average concentration such that it better reflects the general or mean accumulation capacity of a species. As an example, the aforementioned Cd accumulation capacity of *Corrigiola telephiifolia* and *Jasione montana* was revealed by their similar maximum Cd concentrations, yet according to their RDBs, *J. montana* (RDB = 2320) is a better accumulator than *C. telephiifolia* (RDB = 487).

Despite the great metal accumulation capacity of many species collected from the mine sites, only *Agrostis castellana* showed higher or similar Zn and Pb concentrations to the limits generally used to describe a species as a hyperaccumulator (10,000 mg kg$^{-1}$ for Mn and Zn, 1,000 mg kg$^{-1}$ for the rest of the metals, except Cd for which the limit is 100 mg kg$^{-1}$). According to McGrath and Zhao (2003), these limits are rather arbitrary, and these authors argue that hyperaccumulators share the following common characteristics: a bioaccumulation index (BI) > 1 but in some cases as high as 50–100; a transfer factor (shoot/root ratio) > 1, meaning good metal transport to shoots; and a hypertolerance to metals in the soil and inside the plant, indicating good internal detoxification.

Although we consider these criteria reasonable, they are not easy to apply. The accumulation capacity of species (BI) depends on the soil concentration of the pollutant (Gutiérrez-Ginés et al. 2012). In effect, when we calculated the BI for the present species, values > 1 were observed in samples collected from soils with insignificant metal concentrations. Similarly, it was not frequent to obtain values > 1 in plants collected from the most polluted sampling points. Total or pseudo-total soil metal concentrations are generally used to calculate this index, yet bioavailable contents more realistically reflect the amount of metal that in fact can be taken up by plants. For this reason, we did not use this criterion to classify the present species.

Although *Agrostis castellana* was the only species classified as a hyperaccumulator, the following species emerged as able to accumulate large amounts of Zn and Pb together (at the sites Mazarambroz, Colmenar del Arroyo and Bustarviejo): the grasses *Avena barbata*, *Arrhenatherum elatius* subsp. *bulbosum*, *Bromus rubens*, *Holcus annuus* subsp. *setiglumis*, *Micropyrum tenellum*, *Vulpia bromoides* and *V. myuros*; the composite *Crepis vesicaria*; the Brassicaceae species *Diplotaxis catholica*, *Hirschfeldia incana* and *Sinapis arvensis*; and most species of the genera *Plantago* and *Rumex*, as well as *Spergularia rubra* and *Scirpoides holoschoenus*.

Though many species were able to tolerate the extremely high Cu levels found in Colmenarejo and Garganta, few species were good accumulators of this metal. The species showing the highest Cu concentrations were *Agrostis stolonifera*, *Corynephorus canescens*, *Lolium multiflorum*, *Armeria arenaria*, *Convolvulus arvensis*, *Corrigiola telephiifolia* and *S. holoschoenus* and the species of the *Rumex* genus. Among these, *C. telephiifolia* accumulated also the highest concentration of Cd.

#### 9.3.3.2 Tolerance, Resistance and Toxicity

Although mine sites have been ignored for decades, they have aroused some interest regarding plant selection and adaptation over time (Dickinson et al. 1991; Shaw 1990). Thus, the study of metallophytes—plants adapted to heavy metal-enriched soils—has provided knowledge of the natural mechanisms of adaptation to heavy metal stress. Barceló and Poschenrieder (1992) summarised the complexity of stress resistance and behaviour models of plants exposed to metal toxicity: plants can either avoid/exclude the metal or tolerate it.

Since all the species examined here were collected from mine sites, they may be described as tolerant to the heavy metal concentrations of the soils that sustain them. The fact that legumes do not seem to have evolved as tolerant to heavy metals, as described by Ernst (1996), may be why they were practically absent from our study sites. In contrast, grasses (Poaceae) and composites (Asteraceae) are heavy metal accumulators, indicating the tolerance of these families to the metal conditions of the soils where they grew.

Growth within a plant community can increase the tolerance of some species to pollutants. Some species grow in the most polluted areas, where they would never be found growing alone. Plant interrelations can also reduce the pollutant tolerance of a species when the effort required by one species to tolerate high metal levels reduces its capacity to compete against other species.

Many of the tested species, even those growing in highly polluted soils, did not reflect such high metal concentrations in their shoots. Some plants are excluders and hardly take up metals from soils. The main tolerance mechanism of excluders is thought to be reduced metal transport from roots to shoots (Schat et al. 2000).

In contrast, other species featured such high metal concentrations in their shoots (even higher than those considered toxic) that they must have some sort of internal detoxification mechanism. The main symptom of heavy metal and Al toxicity in plant species growing in polluted soils appears to be their reduced root growth (Barceló and Poschenrieder 1992; Hernández 1986; Hernández et al. 2007).

However, of greater concern is the toxicity produced in animals consuming these plants. Table 9.5 shows some approximate values that may be compared with those in Tables 9.2, 9.3 and 9.4. Many of the species examined here could be harmful to the livestock that graze on these sites (cattle in Garganta or sheep in Mazarambroz or Colmenarejo). Indeed, 70 % of all the analysed species contained at least one metal at a concentration that could cause health problems in these animals.

Many studies have shown toxic element concentrations in animals grazing on polluted soils (Madejón et al. 2006; Morcombe et al. 1994; Petersson et al. 1997; Ronneau and Cara 1984). However, this type of data is still rather scarce (Hapke 1996; Madejón et al. 2009), especially with respect to native species. Accumulating native species are a health risk to both primary consumers and those that feed on them, thus affecting the entire food webs (Kabata-Pendias and Mukherjee 2007). The need to control heavy metals in terrestrial ecosystems prompted the Food and Agriculture Organization of the United Nations (FAO) (2000) to define maximum limits for Pb and Cd levels, and the EU has subsequently adopted such limits.

In prior reports, we presented our results regarding the behaviour of Poaceae species (maize, sorghum, oats and ray grass) when grown in soils obtained from abandoned Cu, Ag and Al mines. Results indicated that the shoots of these species, especially maize, accumulated high quantities of metals (Gutiérrez-Ginés et al. 2010, 2011; Hernández et al. 2007; Pastor and Hernández 2009; Pastor et al. 2012). These species could therefore be candidates for "induced phytorestoration", as their biomass is much greater than that of native accumulator species. However, if they are employed as forage, their use for the phytoremediation of heavy metal-polluted soils is not recommended unless their consumption can be avoided.

#### 9.3.3.3 Phytoremediation Versus Ecotoxicology

Collectively, the results of the present study provide useful information for the phytoremediation of old mine sites. The native grass species of these sites are a valuable resource for this type of planned action. These species are adapted to the environmental conditions in which they grow, which in many cases (such as in the Toledo province with its summer drought) could be too harsh for other introduced species. They are also able to tolerate the high metal concentrations of the soils that sustain them even in situations of competition with other species of the community. Further, by growing on pronounced slopes, such as those of landfills or mountainous regions (such as the Madrid sites), they can thwart erosion, which is among the worse problems facing these sites. These benefits and the stable nature of these communities also translate to economic incentives when designing remediation measures.

As a drawback, we should mention that these native species with the remediation potential do not show a regular distribution within a site itself. This determines a need for more detailed studies at each site before designing any specific remediation protocol for each one. In addition, many of the species showing the greater accumulator capacity are small sized such that the amount of metal they can extract from the soil is limited. In situations in which a site is in urgent need of phytoremediation or in zones where the native vegetation is insufficient, rapid-growth cultivated plants such as maize could be a good complementary option. However, we recommend that the use of cultivated species should not be the main remediation strategy for this type of site. Indeed, conserving the autochthonous communities of abandoned mine sites should be a priority of any form of restoration.

Despite the vast amount of literature focusing on the use of cultivated or allochthonous species, many scientists are starting to appreciate the benefits of native communities adapted to these environments and have centred their studies on this strategy (Conesa et al. 2006, 2007; Jana et al. 2012; Moreno-Jiménez et al. 2009; Zhang et al. 2012).

Finally but not least importantly, we should take into account that many of the native species examined here and those employed in our past trials are forage species or base components of food chains. Thus, their assessment for remediation purposes should also consider this aspect. These consumable populations require monitoring to assess possible risks for human and ecosystem health (Hernández et al. 2009), given that any species that may be used for phytoremediation according to their accumulating capacity can also be a risk factor for introducing metals in food webs.

## 9.4 Conclusions

The heavy metal and trace element pollution of soils (Cu, Zn, Pb, Cd, As, Cr, Ni, Mn, Al and Ba) was examined at eight abandoned mine sites in central Spain. Altogether, six sites showed worrying levels of at least five metals. However, plant communities (Mediterranean grasslands) growing on tailings and surrounding soils seem unaffected by this pollution. After testing the shoots of 171 native species from these communities, our results indicate the plant accumulation capacity for one or more of these metals. Accumulation capacity depends on the potential uptake by each species, as well as the edaphic environment (thus metal bioavailability). As a consequence, classification according to metal tolerance or hyperaccumulation is not easy. The Poaceae family, however, showed the largest number of species with Cu, Zn, Pb and As accumulation capacity. *Agrostis castellana* emerged as a hyperaccumulator of Zn and Pb that can also take up Cu, As, Al and Mn.

Our data reveal the metal accumulation capacity of a large number of grassland species growing at abandoned mine sites. Although they provide important information concerning the candidate species for phytoremediation, we cannot ignore the toxicity risks for the animals that consume these plants.

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