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Heavy Metal Content of Spoil Heaps from an Abandoned Iron- and Copper-Mine and Metal Accumulation in *Armeria linkiana* Nieto Feliner

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Portugal faces serious environmental problems resulting from past mining activities (Fernandes and Henriques 1990) that are currently being addressed. Mine da Tinoca, situated in the north-eastern part of the Alentejo Province, was exploited for its pyrite and chalcopyrite deposits since Roman occupation of the Iberian Peninsula. The mine was shut down five decades ago, leaving behind fifty acres of spoil heaps accumulated during the last years of mining activity. These spoil heaps remain essentially barren, as does most of the surrounding land, because plants are not able to thrive in the acid and metal-enriched substrate. Recently, Feio (1993) carried out an extensive survey of the flora of the whole mine area and found that *Armeria linkiana* (Plumbaginaceae) colonized the spoil heaps with no visible signs of injury, although at a frequency of only a few individuals per acre.

Two objectives of this work were: (i) to assess the potential and actual phytotoxicity of the spoil heaps by measuring total- and extractable -metal concentrations, and (ii) to understand how *A. linkiana* copes with the excessive metal availability in its environment. It is shown that extractable Cu levels, in particular, are manifold higher than in normal soils, and that roots and shoots of *A. linkiana* accumulate this metal to concentrations that greatly exceed its toxicity threshold, suggesting the presence of some internal detoxification mechanism.

MATERIALS AND METHODS

The Mine da Tinoca is located in Alto Alentejo, Portugal, 30 miles south of the city of Portalegre, at the border with Spain (Fig.1). The mine, exploited for iron and copper extracted from extensive pyrite and chalcopyrite deposits, was closed ca. 50 years ago. The mine's accumulated debris form a set of large heaps covering an area of 50 acres, both south and north of the mine´s wells. *Armeria linkiana* Nieto Feliner specimens were collected on the spoil heaps south of the mine wells; plants of *A. linkiana* from non-contaminated sites within the same geographic region were also collected to serve as controls.
Whole plants were washed thorougly with running tap water to remove adhering substrate materials, rinsed twice for 30 seconds with distilled water and blotted dry. Plants were then separated into shoot (leaves only) and root parts, and the

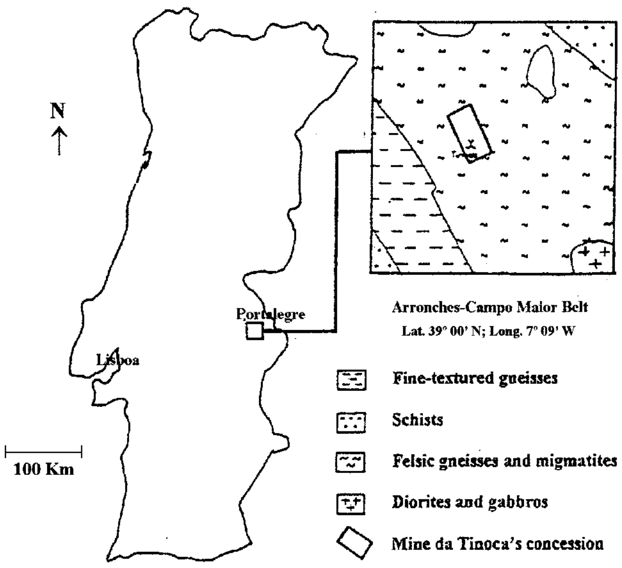

Figure 1. Location and geology of Mine da Tinoca.

latter further divided into fine, lateral roots and the primary root. Plant parts were oven-dried, dry-ashed at 500 °C for 4 hours in a muffle furnace, dissolved in 3N HCl and cations determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, TJA, Iris-Advantage). Soil samples were oven-dried, sieved (2mm) and fine-earth subsamples extracted overnight either with aqua regia (HCl/HNO_3 3:1,v/v) for total metals determination or with 0,5 M ammonium acetate, 0,5 M acetic acid and 0,02 M EDTA (pH 4.65) for extractable metals determination. The metals in the extracts were quantified by atomic absorption spectroscopy, using a model 3030 Perkin-Elmer spectrophotometer.

Measurements of pH were made on a slurried (1:2.5, soil/water) soil sample, using a Radiometer 61 M potentiometer. Organic matter content was determined by oxidation with sodium dichromate, according to Tinsley (1950).

RESULTS AND DISCUSSION

Total and ammonium acetate-EDTA extractable Fe, Mn, Zn and Cu concentrations in the fine-earth fraction of the spoil heaps are shown in Table 1. Compared to control soil, the contaminated substrate contains levels of total Cu, Zn, Fe and Mn that are ca. 100-fold, 10-fold, 5-fold and 1.5-fold higher, respectively. Metal values reported in contamination studies often refer to total concentrations, but these do not necessarily reflect their availability and, consequently, their actual phytotoxicity. To assess metal bioavailability, measurement of extractable fractions are more relevant and our data on ammonium acetate-EDTA extractable metal contents (Table 1) show that Cu is "available" at very high concentration, Mn and Zn are "available" at relatively high concentrations and Fe is the least "available" cation. Fe is the metal present at the highest concentration in the mine's debris, but it is mostly in amorphous and even crystalline forms that are unavailable for plant uptake. The "available" Cu level is particularly high, even compared with other values reported for mine dumps (Henriques and Fernandes 1991; McLaughlin and Crowder 1988; Menzies and Mulligan 2000; Neumann et al. 1995). The spoil heap substrate is acidic (pH=5.35) and contains low levels of organic matter (0.5%); also, its relatively recent origin did not permit formation of any significant amounts of clay material and, thus, the metal binding capacity of the mine's substrate is very low. Both the pH and the colloidal fraction of the spoil heaps should be increased during implementation of restoration schemes to alleviate metal toxicity (see below).

Table 1. Total* and extractable** heavy metal contents from spoil heaps substrate and control soil***.

Heavy metal	Spoil heaps		Control soil	
	Total	Extractable	Total	Extractable
	mg.Kg^{-1} dry soil			
Fe	72 000±10 500	24±3	15 262±2 550	191±29
Mn	750±82	52±7	490±70	21±3
Zn	525±67	7.5±1	49.5±8	1.3±0.4
Cu	1 550±290	97±11	14.5±3	1.0 ±0.3

*Total metals were determined from substrate samples digested in HCl/HNO_3

**Extractable metals were determined from 0.5 M ammonium acetate, 0.5 M acetic acid, 0.02 M EDTA (pH 4.65) extracts of substrate

***Results are mean values±SD of three replicates

Table 2 presents the Fe, Mn, Zn and Cu concentrations in roots and shoots of *A.linkiana* grown on the spoilage heap and on uncontaminated soil. Data in this table show that plants grown on the metal-enriched substrate contain much higher metal concentrations than the control plants, both in its roots and in its shoots. Specifically, shoot Cu concentrations are ca. 60 times higher in contaminated plants, Fe and Zn are ca. 8 times higher and Mn is roughly doubled. Note that excessive Fe preferentially accumulates in the shoot, whereas Cu is most retained in the root. It could be argued that part of the Cu found associated with the root fraction corresponds to metal adsorbed to its surface, that was not removed during the rinsing procedure, but the distribution found for Fe strongly argues against such a view. In plants grown on contaminated substrate, Mn is mostly found in the shoots, which are also slightly enriched in Zn relatively to the roots. Finally, it should be noted that the thick, lignified, primary root shows the lowest metal contents of all examined plant parts, suggesting it does not play an important role in metal accumulation and detoxification.

Table 3 shows the range of Fe, Mn, Zn and Cu contents in leaves of several agricultural crops and the values at which symptoms of toxicity begin to develop. Levels of Cu in the shoots of *A. linkiana* grown on the spoil heaps are

Table 2. Fe, Mn, Zn and Cu contents in *A. linkiana* grown on spoil heaps (A) and on uncontaminated soil (B)*.

Metal	A			B	
	Root		Shoot	Root	Shoot
	Fine	Coarse			
			mg.kg^{-1}		
Fe	669±51	467±34	867±48	137±13	107±11
Mn	65±11	44±5	164±17	71±8	67±5
Zn	151±15	139±12	202±14	31±5	24±4
Cu	572±49	291±21	382±29	7±2	6±1

*Results are mean values±SD of three replicates

ca. 15 times above this toxicity threshold; Fe and Zn concentrations exceed twice the minimum critical value whereas the Mn concentration, although elevated, is below the critical value reported to produce significant growth impairment. Cu is

Table 3. Average range and toxicity thresholds of Fe, Mn, Zn and Cu in leaves of several crop species (Howeler 1983).

Heavy Metal	Range	Toxicity threshold
	mg.kg^{-1}	
Fe	130-400	>400
Mn	80-140	200-2500
Zn	45-50	100-1500
Cu	8-20	15-40

an essential micronutrient but it is known to cause severe growth disturbances at internal concentrations only slightly above its physiological optimum level (Fernandes and Henriques 1991). However, the *A. linkiana* plants from the spoil heaps show no visible symptoms of injury, which implies the presence of some internal metal detoxification (tolerance) mechanism. In the specific case of Cu, the processes responsible for such tolerance could include either complexation of the metal by several ligands (Cobbett 2000; Fernandes and Henriques 1991; Lolkema et al. 1984; Salt et al. 1989) or its sequestration on cell walls and in vacuoles (Fernandes and Henriques 1991; Lidon and Henriques 1994; Neumann et al. 1995), the relative importance of each process apparently depending on the plant species, stage of development, metal concentration and time of exposure, among other factors (Sanità di Toppi and Gabbrielli 1999). In *A. maritime* grown on soil derived from a Cu-mine dump, it was shown (Neumann et al. 1995) that copper tolerance involves both biochemical and anatomical adaptations. Most of the Cu accumulated was sequestred in vacuoles of idioblasts and a smaller fraction was excreted by salt glands present on both leaf surfaces. Many important Cu resistant species, comprising the well-known "copper flowers" *Haumaniastrum spp*. (Brooks 1977), rely on tolerance mechanisms to avoid toxic effects of excessive metal, but Cu exclusion mechanisms have also been reported for cuprophytes, such as in *Silene* spp. and other caryophyllaceae (Baker et al. 1983; Fernandes and Henriques 1991).

In conclusion, our data show that extractable concentrations of Mn and Zn in the spoil heaps are high and those of Cu are exceedingly high, posing significant risks of phytotoxicity as well as of surface and ground water contamination. Indeed, leaching of metal ions through the spoil heap profile pollutes the streams in the dump site catchment area and has already caused several incidents of cattle poisoning (Feio 1993). This situation may become worse if the spoil heaps are leveled for land reclamation, as this will expose layers of less oxidized debris where metals have accumulated for decades by leaching from the upper horizons. Most of the metal sulphides still present in the lower layers of the spoil heaps will be oxidized and will release associated heavy metals, thereby increasing their bioavailability. To attenuate problems of phytotoxicity and to create a physical/chemical environment more propitious for plant establishment, it is suggested that the spoilage's pH and cation exchange capacity be enhanced, by liming and addition of organic matter-enriched materials. Biosolids from a variety of sources have been widely used as both an amendment and a fertilizer in mine-land reclamation to help improve soil structure and water-holding capacity and to provide essential nutrients; field experiments are being planned to test the usefulness of such applications in the revegetation of mine da Tinoca spoil sites. *A. linkiana* could serve as a foundation plant for long term reclamation of the land, but other plant species will be tested for that purpose.

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