Environmental and Experimental Botany xxx (2008) xxx-xxx

Contents lists available at ScienceDirect



Environmental and Experimental Botany



journal homepage: www.elsevier.com/locate/envexpbot

Response of native grasses and *Cicer arietinum* to soil polluted with mining wastes: Implications for the management of land adjacent to mine sites

Héctor M. Conesa^{a,*}, Ahmad B. Moradi^a, Brett H. Robinson^a, Guido Kühne^b, Eberhard Lehmann^b, Rainer Schulin^a

^a Institute of Terrestrial Ecosystems, ETH Zurich, Universitaetstrasse 16, 8092 Zürich, Switzerland ^b Paul Scherrer Institute (PSI), 5232 Villigen, Switzerland

ARTICLE INFO

Article history: Received 25 June 2008 Accepted 11 September 2008

Keywords: Phytostabilisation Phytomanagement Mine tailings Metal uptake Neutron radiography Metal tolerance

ABSTRACT

Mine tailings are an environmental problem in Southern Spain because wind and water erosion of bare surfaces results in the dispersal of toxic metals over nearby urban or agricultural areas. Revegetation with tolerant native species may reduce this risk. We grew two grasses, Lygeum spartum and Piptatherum miliaceum, and the crop species Cicer arietinum (chickpea) under controlled conditions in pots containing a mine tailings mixed into non-polluted soil to give treatments of 0%, 25%, 50%, 75% and 100% mine tailings. We tested a neutral (pH 7.4) mine tailings which contained high concentrations of Cd, Cu, Pb and Zn. Waterextractable metal concentrations increased in proportion to the amount of tailings added. The biomass of the two grasses decreased in proportion to the rate of neutral mine-tailing addition, while the biomass of *C. arietinum* only decreased in relation to the control treatment. Neutron radiography revealed that root development of C. arietinum was perturbed in soil amended with the neutral tailings compared to those of the control treatment, despite a lack of toxicity symptoms in the shoots. In all treatments and for all metals, the plants accumulated higher concentrations in the roots than in shoots. The highest concentrations occurred in the roots of *P. miliaceum* ($2500 \text{ mg kg}^{-1} \text{ Pb}$, 146 mg kg $^{-1} \text{ Cd}$, 185 mg kg $^{-1} \text{ Cu}$, 2700 mg kg $^{-1} \text{ Zn}$). *C. arietinum* seeds had normal concentrations of $Zn (70-90 \text{ mg kg}^{-1})$ and $Cu (6-9 \text{ mg kg}^{-1})$. However, the Cd concentration in this species was $\sim 1 \text{ mg kg}^{-1}$ in the seeds and 14.5 mg kg⁻¹ in shoots. Consumption of these plant species by cattle and wild fauna may present a risk of toxic metals entering the food chain. © 2008 Elsevier B.V. All rights reserved.

1. Introduction

The disposal of mining wastes can have deleterious effects on the environment. Tailings often have low pH, high concentrations of heavy metals, lack of nutrients (Wong, 2003; Liu et al., 2006), low water retention capacity (Ernst, 1996), high electrical conductivity and steep slopes. These factors inhibit plant establishment and, consequently, their surfaces are exposed to wind and water erosion, which may result in the contamination of nearby waters and soils with toxic metals. Although natural revegetation of mine tailings is usually slow, a few species of metal- and salt-tolerant plants can survive in these extreme environments (Al-Farraj and Al-Wabel, 2007). Annual species adapt faster to these conditions than perennial plants because their shorter life cycles allow them

* Corresponding author at: Befesa Gestión de Residuos Industriales S.L., Soil Management Department, Calle Benito Mas y Prat 5, 1ª Planta, Oficinas 10-13, 41005 Seville, Spain. Tel.: +34 954980672; fax: +34 954980885.

E-mail addresses: hector.conesa@befesa.abengoa.com, hectolines2001@yahoo.es (H.M. Conesa).

to produce a larger variety of genotypes in a shorter time (Wei et al., 2005).

When grown in soil with elevated metal concentrations, some plants accumulate metals without showing visible signs of toxicity. This may facilitate the entry of heavy metals into the food chain via herbivory or the consumption of contaminated crops or livestock products by humans. Some heavy metals, such as Zn and Cu, are beneficial to human health at low to moderate concentrations. Here, in fact increased plant uptake may actually enhance the nutritive value of the crop. Phytotoxicity of crop plants may reduce the entry of these essential nutrients into human food at concentrations which could present a health risk (Murillo et al., 1999). However, despite physiological barriers that reduce plant metal uptake, plants may be contaminated by dust and rain splash deposition of metals on external surfaces. Moreover, non-essential metals such as Pb and Cd are toxic to humans at low concentrations. Plant uptake of these elements may pose a risk to humans and animals at soil concentration that do not cause phytotoxicity.

The Cartagena-La Union Mining District (0–400 m above sea level; $37^{\circ}37'20''N$, $0^{\circ}50'55''W-37^{\circ}40'03''N$, $0^{\circ}48'12''W$) is at the Southeast of the Iberian Peninsula and covers an area of 50 km^2 .

^{0098-8472/\$ -} see front matter © 2008 Elsevier B.V. All rights reserved. doi:10.1016/j.envexpbot.2008.09.004

H.M. Conesa et al. / Environmental and Experimental Botany xxx (2008) xxx-xxx

2 Table 1

Mean values of pH, Electrical Conductivity (EC) and concentrations of Cu, Zn, Cd and Pb in the mine tailing/soil mixtures (treatments) at the beginning of the experiments. Values in brackets are standard deviation of three replicate treatments.

Treatment	pHª	EC 1:5	Total metals	Total metals			
			Cu	Zn	Cd	Pb	
		dSm^{-1}	mg kg ⁻¹				
100% neutral tailing	7.4	3.25 (0.05)	81(1)	9130 (60)	33(0.6)	5310 (90)	
75% neutral tailing	7.2	2.91 (0.01)	69(3)	6910 (100)	25(0.6)	4130 (40)	
50% neutral tailing	7.0	2.67 (0.02)	45(1)	4550 (70)	17(0.6)	2770 (30)	
25% neutral tailing	7.0	2.42 (0.02)	26(2)	2160 (10)	7.7 (0.3)	1310 (10)	
Control soil	7.0	0.12 (0.01)	9(1)	35(1)	<1.0	12(1)	

^a Geometric mean. The differences between the samples were <0.1, the detection limit of our apparatus.

Most of it lies in the region known as the Sierra of Cartagena-La Unión. The semiarid climate of the zone is typically Mediterranean with an annual rainfall of 250–300 mm, concentrated during spring and autumn. The annual average temperature is 18 °C. Mining occurred in this area for more than 2500 years until it ceased in 1991. Base metals were smelted from sulphide minerals that included galena and sphaelerite. Until 1955, mine wastes were dumped into local streams. Thereafter, the wastes were stockpiled into tailings. These tailings pose a risk to local ecosystems and human health, due to the dispersal of dust and sediments by erosion (Conesa et al., 2006). At present, there are 48 tailings dump sites that cover some 160 ha in this zone. Some are near urban and agricultural areas, increasing the risk of human exposure to metals via wind-borne dust or consumption of contaminated garden and agricultural crops.

Pérez-Chacón (2002) reported high metal concentrations in soils and lettuce in an agricultural area near mine tailings in La Unión. However, there is a lacuna of information on the risk of metal uptake by other crops and on metal transfer to livestock via pasture. Nor is it clear whether the high plant metal concentrations that previous studies measured *in situ* were the result of metal uptake through roots or through particle deposition onto leaves. This may have important implications for site management, since stabilisation of the tailings using vegetation or other means would reduce both wind and water erosion.

The aims of this work were to investigate the effect of increasing tailings dosages on metal solubility in an agricultural soil as well as the growth and metal uptake by chickpea and two native pasture grasses from the Cartagena-La Unión mining District.

2. Materials and methods

2.1. Soil samples

The "El Gorguel" mine tailing lies along the dry "Gorguel" river (U.T.M. X 687 480 m, Y 4 162 800 m, Z 135 m). Conesa et al. (2006, 2007a) describes the sampling locations and local vegetation. Tailing samples were taken from the upper 40 cm of 15 separate soil pits that were dug at regular intervals, at least 8 m apart, on each tailing pile. All samples of a tailing were mixed to give one homogenized composite sample per mine tailing. The composite samples were air dried, sieved to <2 mm and stored in plastic bags prior to laboratory analysis and the pot experiment. Control soil (pH 7; 87% sand; 12 cmol(+) kg⁻¹; 3.2% Organic Carbon; <0.03% Total Nitrogen) was taken from a forest site near Zürich (Switzerland) (Menon et al. (2005). More information about tailing soil used in the experiment is given in Conesa et al. (2006) (pH 7.4; 73% sand; 9 cmol(+) kg⁻¹; 0.4% Organic Carbon; <0.03% Total Nitrogen).

2.2. Metal extractability studies

Tailings and agricultural soil were mixed at different proportions to give treatments of 0% (uncontaminated soil), 25%, 50%, 75% and

100% (pure mine tailings) (Table 1). For each mixture, we measured pH at a soil:water ratio of 1:2.5 using a Metrohm (Omega) pH Meter. Total metal concentrations were determined by X-ray fluorescence spectroscopy (Spectro X-Lab 2000). Soluble metals were extracted with H₂O at a 1:5 soil:water ratio (Ernst, 1996). The samples were shaken for 2 h, centrifuged for 10 min at 4000 rpm, and filtered through Macherey-Nagel (MN 640d) paper. The filtered extracts were analyzed for Cu, Cd, Pb, Zn, K, Mg, Ca and Na by flame atomic absorption spectrometry (SpectraAA 220/FS, Varian). The detection limits in the extracts were 0.05 mg L⁻¹ for all the elements except for Cd and Zn, where the detection limit was 0.01 mg L⁻¹. Electrical conductivity was measured in the water extract (1:5 soil:water ratio) using a conductivity meter WTW LF318/SET.

2.3. Pot experiment

The pot experiment was performed with *Piptatherum miliaceum*, *Lygeum Spartum* and *Cicer arietinum* (chickpea). *L. spartum* L. (Family *Poaceae*) is a widespread grass in the Mediterranean zone that tolerates extreme conditions of aridity, salinity and high temperatures (Diaz and Honrubia, 1993). This species occurs on acid mine tailings (pH < 5) in Southern Spain (Conesa et al., 2006, 2007a), but does not colonise neutral mine tailings in the same zone. *P. miliaceum* (L.) (Cosson) (Family *Poaceae*) also occurs naturally in these polluted ecosystems (Conesa et al., 2006). It grows in pastures and open grassy places throughout the Mediterranean region and young plants are eaten by cattle. Both, *L. spartum* and *P. miliaceum*, have a strong rhizomatic root system that binds soil, thus reducing erosion. *C. arietinum* L. (Family *Fabaceae*) is a traditional crop in this area.

P. miliaceum, *L. Spartum* and *C. arietinum* were grown from seed in plastic pots containing approximately 0.4 kg of the aforementioned mine tailings–soil mixtures. We used seeds of *L. spartum* and *P. miliaceum* collected from the mining area of Cartagena-La Unión and commercial seeds (*Gourmet BlOengagement*) of *C. arietinum*. Two seeds of each species were planted in each pot. Dead plants were replaced within the first two weeks of the experiment. Pots were irrigated with nutrient solution containing 400 mM Ca(NO₃)₃.4H₂O, 200 mM MgSO₄.7H₂O, 100 mM KH₂PO₄, 500 mM KNO₃ (all reagents from Merck, Germany). Soils were maintained near field capacity by adding 100–150 mL of solution per week. The plants were grown for 10 weeks in a climate chamber with a light cycle of 16 h light/8 h darkness under controlled humidity (50%/90%) and temperature (16 °C/23 °C night/day).

The plants were harvested in the 11th week of the experiment, washed with deionised water, and dried at 65 °C for 72 h. Shoots, roots and seeds (in the case of *C. arietinum*) were separated. Dry weights were determined for individual plants. Samples (0.050–0.200 g) were digested in Teflon tubes with 15 mL HNO₃ (65%) at 150 °C for 1 h in a heating block DigiPREP MS (SCP Science). The digests were diluted to 20 mL with nanopure water. Copper, Zn, Pb and Cd were measured by means of ICP-OES (Vista-

H.M. Conesa et al. / Environmental and Experimental Botany xxx (2008) xxx-xxx

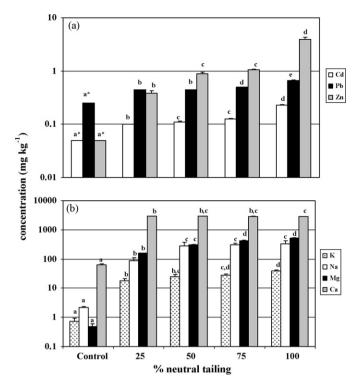


Fig. 1. Metals extracted with water from the different combinations of neutral tailing material with control soil. Error bars represent standard errors (N = 3). Cu concentrations were below detection limit ($0.1 \text{ mg} \text{ L}^{-1}$ in water extracts; $0.25 \text{ mg} \text{ kg}^{-1}$ in soil). Different letters indicated significant differences among treatments for the corresponding element (P < 0.05). a* means that the detection limit is given, as the measurements did not exceed the respective detection limits that were: 0.25 mg kg⁻¹ Pb and 0.05 mg kg⁻¹ for Cd and Zn.

MPX Varian). For quality assurance we analyzed Certified Reference Material from the Community Bureau of Reference BCR No. 62 (*Olea europaea*). We obtained recoveries of 80% for Pb and Cu and 90% for Zn. The detection limit in the extracts was 1 μ g L⁻¹. The Cd concentrations of the reference materials (0.1 mg kg⁻¹) were under our detection limit.

We used SPSS 14.0.0 (SPSS, Chicago, IL, USA) for all statistical analysis (ANOVA with LSD). Data that were log-normally distributed according to the Levene test were log-transformed before analysis. Differences at P < 0.05 level were considered significant.

2.4. Neutron Imaging

Plant root growth patterns in the control and neutral tailings treatments were imaged with the Image with Cold Neutrons (ICON) facility (Kühne et al., 2005) at the Paul Scherrer Institute (PSI), Villigen, Switzerland. *C. arietinum* were grown in control soil and neutral tailings inside aluminium containers with inner dimensions of $170 \text{ mm} \times 150 \text{ mm} \times 13 \text{ mm}$ as is described in Menon et al. (2007). Plants were kept under the same climatic conditions as the plants in the pot experiment and were given same irrigation and fertilization. Images were taken in the first, third and eighth weeks of the pot experiment. We used ImageJ 1.63b (Wayne Rasband National Institutes of Health, USA) for image processing.

3. Results

3.1. Soil parameters

In all treatments with tailings, the water-extractable Cu concentrations were below the detection limits (<0.25 mg kg⁻¹) (Fig. 1).

The percentages of water-extractable Zn, Pb and Cd never exceeded 1% of the total concentration, but they were in all tailings treatments higher than in the control soil. Water-extractable Ca, Mg, K and Na (Fig. 1b) also increased with the neutral tailing addition.

3.2. Plant growth and metal uptake

Plants showed no chlorosis. Nevertheless, biomass production significantly decreased with increasing tailings addition (data not shown). Compared to the control, the presence of tailings material led to a decrease in the biomass of *C. arietinum* and *P. miliaceum*. However, in *C. arietinum* there were no significant differences between the 25%, 50% and 100% treatments. The biomass production of *P. miliaceum*, continued to decrease significantly with increasing percentage of tailing material indicating that the tailings were phytotoxic for this species. The biomass of the other grass, *L. spartum* did not show a clear trend.

The roots of chickpeas growing in tailings were stunted (Fig. 2a1, 2a2 and 2a3) compared to the plants that grew in the control soil (Fig. 2b1, 2b2 and 2b3). However, despite of the perturbed root system in tailings treatments, there were no visible signs of toxicity in the above ground portions (apart of the aforementioned lower biomass). Flowering and fructification occurred at the same time in both control and tailings treatments.

Here, we use shoot:root biomass ratios to evaluate the health and vigor of plants and to determine the success of plant establishment. Often, shoot:root biomass ratios decrease under conditions of mineral nutrient deficiency and increase at toxic levels of mineral supply (Roosens et al., 2003). Tailings addition affected the shoot:root ratio of *C. arietinum*. The highest values for this plant were found in the 100% tailing treatment (0.85), followed by the control (0.80). The intermediate tailing treatments gave values around 0.6. In the two grasses species, the highest shoot:root ratios occurred in *L. spartum* s of the control treatments (1.31). For the other treatments, this ratio was between 0.83 and 1.03 but the decrease was not correlated with the amount of tailing addition. In *P. miliaceum*, this ratio was lowest in the control treatment (0.38) and increased in proportion to the tailing addition to 0.64 in the 100% treatment.

Plant metal concentrations increased in proportion to the amount of mine tailings added (Figs. 3, 4 and 5). In most cases, already the 25% neutral tailing treatment caused significant increases in plant metal uptake (Figs. 3, 4 and 5).

The shoot Zn concentrations (Figs. 3b, 4b and 5b) were below 500 mg kg^{-1} in all three species except in the 100% treatment where they reached 700 mg kg⁻¹ in *P. miliaceum*. The differences between the control and the 25% treatment (*P*<0.05) were higher in *P. miliaceum* (7 fold), than in *L. spartum* (5 fold) or *C. arietinum* (6 fold). The behaviour in roots was similar to that in the shoots but the Zn concentrations were higher: 8000 mg kg⁻¹ in *P. miliaceum*, 700 mg kg⁻¹ in *L. spartum* and 1700 mg kg⁻¹ in *C. arietinum*.

The highest Pb values occurred in *P. miliaceum* (190 mg kg⁻¹ in shoots and 2500 mg kg⁻¹ in roots) (Fig. 3d). Shoots of *C. arietinum* contained less than 10 mg Pb kg^{-1} while root Pb concentrations reached 1100 mg Pb kg⁻¹ in the 100% treatment (Fig. 5d).

Cadmium uptake by shoots (Figs. 3c, 4c and 5c) was in the range of $5-7 \text{ mg kg}^{-1}$ for *C. arietinum*, $1-15 \text{ mg kg}^{-1}$ for *P. miliaceum* and $8-17 \text{ mg kg}^{-1}$ for *L. spartum* in treatment with mine tailings. In all cases, the treatments had significantly higher Cd concentrations compared to the controls.

The Cu concentrations in *C. arietinum* shoots did not differ significantly from the control treatment (Fig. 5a). Similarly, the Cu concentration in *L. spartum* roots (Fig. 4a) was $3.5-4.1 \text{ mg kg}^{-1}$. All shoot Cu concentrations were below 15 mg kg^{-1} , except in the case of *P. miliaceum* in the 100% treatment (38 mg kg^{-1}). The roots of *P.*

3

4

ARTICLE IN PRESS

H.M. Conesa et al. / Environmental and Experimental Botany xxx (2008) xxx-xxx

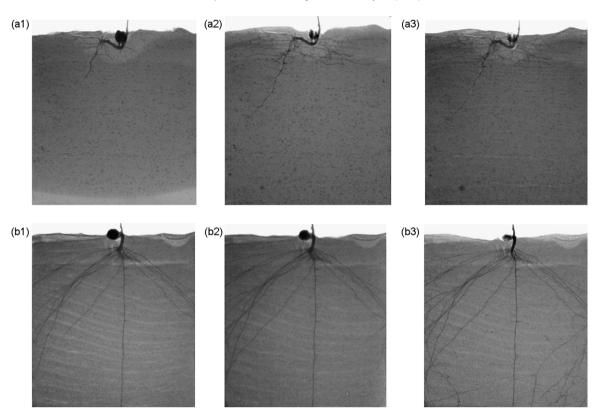


Fig. 2. Roots development for C. arietinum growing in neutral tailing soil (a) and in control soil (b) in the 2nd (1), 4th (2) and 7th (3) weeks of experiment.

miliaceum had the highest Cu concentrations (185 mg kg^{-1} in 100% tailing treatment) (Fig. 3a).

C. arietinum produced seeds in all treatments, although the number of seeds were higher in the control treatment (3.6 per plant) than in the tailing treatments (between 1 and 2 per plant). However, the seeds produced on control soil weighed less (on average of 0.017 g dry weight per seed) than seeds from the tailings

treatments (between 0.1 and 0.2 g per seed). None of the treatments had a significant effect on the Cu concentrations $(6-9 \text{ mg kg}^{-1})$ of the seeds. However, Zn concentrations of seeds from treatments with tailings material were higher than in seeds from the control treatment (55 mg kg⁻¹) but there were no significant differences among the four tailing treatments (values between 80 and 90 mg kg⁻¹). The Cd concentrations were between 0.5 and

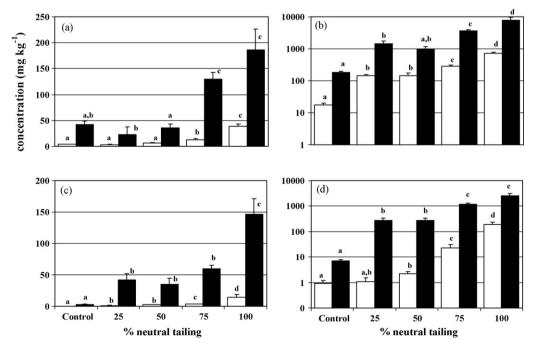


Fig. 3. Metal uptake in *P. miliaceum* for (a) Cu; (b) Zn; (c) Cd; (d) Pb. 3 < *N* < 6. White columns are shoots and black columns are roots. Error bars represent standard errors. Different letters indicated significant differences among treatments for the shoots or roots (*P* < 0.05).

H.M. Conesa et al. / Environmental and Experimental Botany xxx (2008) xxx-xxx

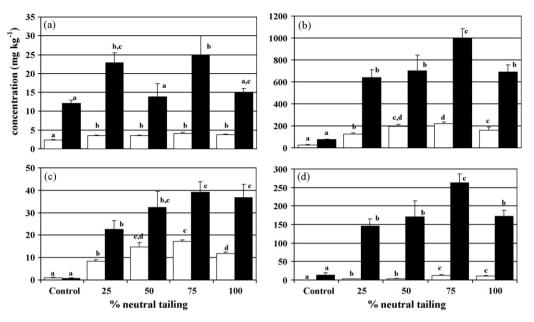


Fig. 4. Metal uptake in L. spartum for (a) Cu; (b) Zn; (c) Cd; (d) Pb. 3 < N < 6. White columns are shoots and black columns are roots. Error bars represent standard errors. Different letters indicated significant differences among treatments for the shoots or roots (P < 0.05). 5 < N < 6.

0.9 mg kg⁻¹ except for the control treatment when the concentration in the extract was below the detection limit $(1 \mu g L^{-1})$. Translated into concentration of metals in the seed biomass, the detection limit for Cd was 1.33 mg kg⁻¹ for the highest deeds of the control treatment. Similarly, seed Pb concentrations were below the detection limit in all the treatments ($<1.33 \text{ mg kg}^{-1}$ for the control treatment and $<0.6 \text{ mg kg}^{-1}$ for the rest of treatments).

the metal concentrations exceeded limit values (>4000 mg kg⁻¹ Zn; $>1200 \text{ mg kg}^{-1} \text{ Pb}$).

All the *C. arietinum* plants showed significantly reduced biomass in treatments with neutral tailing in relation to the control, but were unaffected by the dosage of tailing (and the corresponding higher metal uptake). In this case, salinity may play a more important role in plant toxicity than heavy metal concentration since grain legumes are sensitive to salinity (Rao et al., 2002). This implies that the elevated metal concentration did not affect the biomass of this species. Thus, plants may have elevated concentrations of toxic heavy metals with showing visible signs of toxicity.

4. Discussion

According to European and Spanish legislation (European Communities Council, 1986) the addition of the tailings materials used in this study to agricultural soils would be forbidden, because

For most treatments, the metal concentrations in shoots were within the normal ranges for plants (Table 2). However, P. miliaceum had toxic levels of metals in the highest treatments. Most

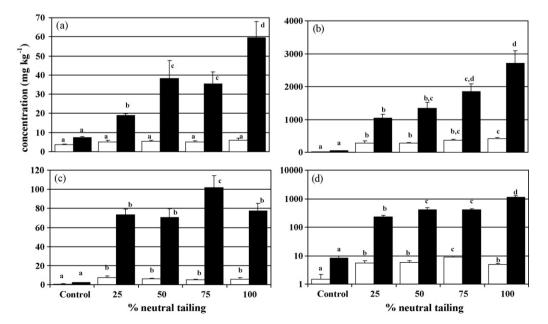


Fig. 5. Metal uptake in C. arietinum for (a) Cu; (b) Zn; (c) Cd; (d) Pb. 3 < N < 6. White columns are shoots and black columns are roots. Error bars represent standard errors. Different letters indicated significant differences among treatments for the shoots or roots (P < 0.05). 4 < N < 6.

6

ARTICLE IN PRESS

H.M. Conesa et al. / Environmental and Experimental Botany xxx (2008) xxx-xxx

e 2

Normal ranges and phytotoxic metal concentrations in vegetables given by some authors. Cells in blank mean not available data.

Metal	Normal levels dry foliage ^a mg kg ⁻¹	Normal concentration in shoots ^b mg kg ⁻¹	Phytotoxic levels dry foliage ^a mg kg ⁻¹	Phytotoxic levels in shoots ^b mg kg ⁻¹	Hyperaccumulator plant ^c mg kg ⁻¹	Maximum levels tolerated by live stock ^a mg kg ⁻¹ diet
Cu	3-20	5-20	25-40	20-100	5000	25-300
Zn	15-150	25-150	500-1500	100-400	10000	300-1000
Cd	0.1-1	0.05-0.7	5-700	5-30	100	0.5
Pb	2–5	5-10	-	30-300	-	30

^a Values according to Chaney (1989).

^b Values according to Barceló and Poschenrieder (1992).

^c Values according to Brooks (1998).

treatments reached phytotoxic concentrations of Cd. The metal concentrations in *L. spartum* in the acid tailings treatments were higher than those considered normal for Pb, Zn and Cd. Conesa et al. (2006), reported levels ~150 mg kg⁻¹ for Zn, ~50 mg kg⁻¹ for Pb and ~5 mg kg⁻¹ for Cu in *P. miliaceum* and *L. spartum* plants growing *in situ* in tailings from the Cartagena-La Unión mining area. These concentrations were lower than the values obtained in our study (even in relation to the lowest polluted treatment). Conesa et al. (2007b,c) showed how the growth of certain plant species under controlled conditions resulted in significantly higher metal uptake compared to plants sampled in the field due to the changes in growing conditions. These changes were especially important when plants from arid environments are oversupplied with water.

The lower metal concentrations in the seeds compared to the shoots may be attributed to the physiological barrier to metal translocation during seed maturation (Ernst et al., 1992). We found relatively high concentrations of Cd $(0.5-0.9 \text{ mg kg}^{-1})$ in the seeds of *C. arietinum* compared to those reported by Murillo et al. (1999) in seeds of sunflower plants (0.241 mg kg⁻¹) that grew in soils affected by the toxic spill of Aznalcollar. However, our values were in the same range as those that Cobb et al. (2000) obtained for beans (*Phaseolus* sp.) growing in mine tailings (1.06 mg kg⁻¹). Cobb et al. (2000) obtained lower values for Zn in beans (Phaseolus sp.) ($<42 \text{ mg kg}^{-1}$) while Murillo et al. (1999) reported similar Zn (74 mg kg^{-1}) and higher (27 mg kg^{-1}) concentrations for sunflower seeds in relation to the seeds from our study. In our study, seed Pb concentrations were below the detection limits <1.33 mg kg⁻¹ in control treatments and 0.60 mg kg^{-1} in the rest of the treatments. Murillo et al. (1999) reported that sunflower seeds contained 0.423 mg Pb kg⁻¹. The European Union (European Communities Council, 2001) established a maximum of $0.2 \text{ mg Pb kg}^{-1}$ fresh weight for legumes and 0.1–0.2 mg Cd kg⁻¹ fresh weight for various cereals in order to safeguard human health. In our study area, Cd in C. arietinum seeds may be a human health hazard. If consumed as a major part of the diet (chickpeas is a traditional ingredient of the Mediterranean diet) the Provisional Tolerable Weekly Intake (PTWI) of 7 μ g kg⁻¹ of body weight that the World Health Organization proposed (WHO, 2004) could be potentially reached. However, the Zn:Cd concentration ratios in the seeds were between 100 and 150 in the four polluted treatments. This shows that Zn concentrations in the seeds were high in comparison to Cd and that their use as stock fodder would not present a risk for cattle. Underwood and Suttle (1999) showed that diets rich in Zn may be prevent adverse effects of Cd toxicity. Nevertheless, Cd accumulates in animal tissues and pose a risk to consumers of affected animal products. Moreover, using crops from polluted soils as source of cattle fodder may deter consumers.

Although at first view a mixture of 25% or 50% tailing with the surrounding soil may appear unlikely, some previous studies in the area have found that due to deposition of wind-eroded material, more than 50% of tailing materials had been incorporated into soil profiles of lands surrounding the tailings (García-García, 2004). The

fact that the deposition of these sediments occurred in times when there was less environmental conscience is the main reason why no remediation options or monitoring programs are discussed. Strong political initiatives are necessary to inform local populations. In addition, monitoring programs should be implemented in the agricultural areas near the tailings and other locations affected by the deposition of eroded mining wastes. Special emphasis should be given to such as potatoes and root vegetables, since they result in a particularly high metal exposure for humans. The establishment of vegetation is necessary to prevent the erosion and reduce the transport of polluted soils to nearby urban and agricultural areas.

Cattle should be prevented from grazing on *C. arietinum*, *P. mil-iaceum* and *L. spartum* growing on soils affected by deposition of mine tailings material. Although the concentrations of metals taken up through roots and accumulated in plant tissues are low, metal-rich dust particles adhering to the surfaces of plants must also be taken into account. Furthermore, factors such as the direct ingestion of soil by cattle may play an important role for the transfer of the metals into the food chain.

5. Conclusions

Adding mine tailings to non-polluted soil affects soil properties, plant growth and metal accumulation by plants differed. Soils amended with the neutral mine tailings supported plant growth. Here, aboveground plant biomass was reduced, but did not show external symptoms of metal toxicity. However, high metal concentrations perturbed the root development of these plants. Metal accumulation by *C. arietinum* seeds may present a human health risk. The potential ingestion of these plant species by cattle and wild fauna may facilitate the entry of metals into the food chain.

Our experiment indicates that some plants grow in polluted mine-tailing polluted soils and accumulate high concentrations of metals without being showing visible symptoms. Therefore, plant metal uptake may pass unnoticed by farmers and constitute risk for food chain. Revegetating bare mine tailings is important to stabilize the surfaces of these polluted soils and to reduce the migration of polluted materials to the adjacent fields. Future scientific studies could investigate soil amendments that decrease metal bioavailability in polluted areas near tailings.

Acknowledgements

We thank Anna Grünwald and René Saladin for their technical assistance. This work was supported by a postdoctoral grant from the "Fundación Séneca" of the Comunidad Autónoma de la Región de Murcia for Dr. Héctor M. Conesa.

References

Al-Farraj, A.S., Al-Wabel, M.I., 2007. Heavy metals accumulation of some plant species grown on mining area at Mahad AD'Dahab, Saudi Arabia. J. Appl. Sci. 7, 1170–1175.

H.M. Conesa et al. / Environmental and Experimental Botany xxx (2008) xxx-xxx

- Barceló, J., Poschenrieder, Ch., 1992. Respuestas de las plantas a la contaminación por metales pesados. Suelo y planta 2, 345–361.
- Brooks, R.R., 1998. Geobotany and hyperaccumulators. In: Brooks, R.R. (Ed.), Plants that Hyperaccumulate Heavy Metals. CAB International, Wallingford, UK, pp. 55–94.
- Chaney, R.L., 1989. Toxic element accumulation in soils and crops: protecting soil fertility and agricultural food-chains. In: Bar-Yosef, B., Barrow, N.J., Goldshmid, J. (Eds.), Inorganic Contaminants in the Vadose Zone. Springer-Verlag, Berlin, pp. 140–158.
- Cobb, G.P., Sands, K., Waters, M., Wixson, B.G., Dorward-King, E., 2000. Accumulation of heavy metals ba vegetables grown in mine wastes. Environ. Toxicol. Chem. 19, 600–607.
- Conesa, H.M., Faz, Á., Arnaldos, R., 2006. Heavy metal accumulation and tolerance in plants from mine tailings of the semiarid Cartagena-La Union mining district (SE Spain). Sci. Total Environ. 366, 1–11.
- Conesa, H.M., García, G., Faz, Á., Arnaldos, R., 2007a. Dynamics of metal tolerant plant communities' development in Mine Tailings from the Cartagena-La Unión Mining District (SE Spain) and their interest for further revegetation purposes. Chemosphere 68, 1180–1185.
- Conesa, H.M., Nowack, B., Schulin, R., 2007b. A laboratory study on revegetation and metal uptake in native plant species from neutral mine tailings. Water Air Soil Pollut. 183, 201–212.
- Conesa, H.M., Robinson, B.H., Schulin, R., Nowack, B., 2007c. Growth of Lygeum spartum in acid mine tailings: response of plants developed from seedlings, rhizomes and at field conditions. Environ. Pollut, 145, 700–707.
- Diaz, G., Honrubia, M., 1993. Respuestas de crecimiento del albardín (*Lygeum spartum L.*) a la inoculación con hongos micorrícicos y a la fertilización fosforada. Cryptogamie Mycologie 14, 117–125.
- Ernst, W.H.O., 1996. Bioavailability of heavy metals and decontamination of soils by plants. Appl. Geochem. 11, 163–167.
- Ernst, W.H.O., Verkleij, J.A.C., Schat, H., 1992. Metal tolerance in plants. Acta Bot. Neerl. 41, 229–248.
- European Communities Council. Commission Regulation 466/2001 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Commun, 2001. L77, 16/03/01, 1–13.
- European Communities Council. Directive (86/278/EEC) on the Protection of the Environment, and in Particular of the Soil, when Sewage Sludge is used in Agriculture, 1986. Off. J. Eur. Commun. L181, 04/07/86, 6–12.

- García-García, C., 2004. Impacto y riesgo medioambiental en los residuos minerometalúrgicos de la Sierra de Cartagena-La Unión. Tesis doctoral. Universidad Politécnica de Cartagena, Cartagena, Spain.
- Kühne, G., Frei, G., Lehmann, E., Vontobel, P., 2005. CNR-the new beamline for cold neutron imaging at the Swiss spallation neutron source SINQ. Nucl. Instrum. Methods A 542, 264–270.
- Liu, Y.-G., Zhang, H.-Z., Zeng, G.-M., Huang, B.-R., Li, X., 2006. Heavy metal accumulation in plants on Mn mine tailings. Pedosphere 16, 131–136.
- Menon, M., Hermle, S., Abbaspour, K.C., Günthardt-Goerg, M.S., Oswald, S.E., Schulin, R., 2005. Water regime of metal-contaminated soil under juvenile forest vegetation. Plant Soil 271, 227–241.
- Menon, M., Robinson, B., Oswald, S.E., Kaestner, A., Abbaspour, K.C., Lehmann, E., Schulin, R., 2007. Visualization of root growth in heterogeneously contaminated soil using neutron radiography. Eur. J. Soil Sci. 58, 802–810.
- Murillo, J.M., Marañón, T., Cabrera, F., López, R., 1999. Accumulation of heavy metals in sunflower and sorghum plants affected by the Guadiamar spill. Sci. Total Environ. 242, 281–292.
- Pérez-Chacón, J.A., 2002. Presencia de Pb, Cu y Zn en suelos próximos a zonas mineras en Roche (Cartagena): Riesgos de bioacumulación en lechuga. Proyecto Fin de Carrera. Universidad Miguel Hernández de Elche, Orihuela, Spain.
- Rao, D.L.N., Giller, K.E., Yeo, E.R., Flowers, T.J., 2002. The effect of salinity and sodicity upon nodulation and nitrogen fixation in chikpea (*Cicer arietinum*). Ann. Bot. 89, 563–570.
- Roosens, N., Verbruggen, N., Meerts, P., Ximénez-Embún, P., Smith, J.A.C., 2003. Natural variation in cadmiun tolerante and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from western Europe. Plant Cell Environ. 26, 1657–1672.
- Underwood, E.J., Suttle, N.F., 1999. The mineral nutrition of livestock, 3rd edition. Cabi Publishing, New york, United States.
- Wei, S., Zhou, Q., Wang, X., 2005. Identification of weed plants excluding the uptake of heavy metals. Environ. Int. 31, 829–834.
- WHO (World Health Organization), 2004. Evaluation of Certain Food Additives and Contaminants. WHO Technical Series Report 922. Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, Helvetic Confederation.
- Wong, M.H., 2003. Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. Chemosphere 50, 775–780.