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Cadmium availability in soil and retention in oak roots: Potential for phytostabilization

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ABSTRACT

Afforestation of contaminated land by trees is considered as a feasible strategy for the extensive stabilization of contaminants. In this work, we studied the patterns of metal availability (Cd, Cu, Pb and Zn) in a contaminated and afforested area. Specifically, we observed the response of Holm oak (*Quercus ilex* subsp. *ballota*) leaves to changes in the availability of metals under field conditions, focusing on Cd. Under controlled conditions we studied the performance of oak seedlings exposed to high levels of Cd, with the aim of analyzing the patterns of translocation and tolerance of the seedlings. Cadmium was the most available metal, in relative terms; 15% of the total Cd in the soil was extracted with NH_4NO_3. The availabilities of Cd, Cu and Zn showed exponential relationships with soil pH (pH values ranged from 2.4 to 8.4). Cadmium accumulation in the leaves was not related to the changes in Cd availability. Greenhouse studies showed that seedlings had a high Cd retention capacity in fine roots (up to 7 g kg^{-1}) and low rates of Cd translocation to the leaves (transfer coefficients below 0.03). Root biomass and thickness was altered by exposure to Cd. In spite of this, the chlorophyll fluorescence measurements (an indicator of plant stress) only differed slightly from the control treatment at a Cd dose of 200 mg L^{-1}. Due to the relatively high tolerance to Cd and the capacity of roots to retain this metal, Holm oak may be useful for the phytostabilization of soils contaminated by Cd.

1. Introduction

During the last decade, the afforestation of contaminated areas has been accepted as a feasible strategy for the long-term, extensive management of these sites. The use of trees for reclamation of contaminated land can be a low-cost and ecologically sustainable alternative to other techniques, such as washing or containment, especially for large areas (Dickinson, 2000). The main benefit of the use of trees in soil remediation is their high stabilization potential. Phytostabilization potential comprises different processes in the soil that lead to a decrease in the spread of contaminants through the ecosystem (Vangronsveld et al., 1995; Mendez and Maier, 2008). Due to their vast root systems, trees bind the soil, reducing wind and water erosion. In addition, the high transpiration rates of the trees may help to reduce the migration of contaminants to surface and ground waters (Garten, 1999). The pool of available heavy metals may decrease by absorption and accumulation of these metals by tree roots, by adsorption onto roots, or by precipitation within the rhizosphere (Pulford and Watson, 2003; Wong, 2003). Phytostabilization may be a more feasible approach for the management of contaminated sites than other strategies using trees, such as phytoextraction, which is the removal of metals from the soil by tree uptake and translocation into the aboveground biomass of the vegetation (Kumar et al., 1995), because the long-term accumulation of metals in the aboveground biomass of the trees may pose a risk of transfer to the food chain (Mertens et al., 2004), especially in extensive areas where metal fluxes cannot be fully controlled.

Afforestation with native Mediterranean woody plants, such as the Holm oak (*Quercus ilex* subsp. *ballota*), has been encouraged in the management of degraded areas in southern Spain, such as contaminated sites or abandoned croplands. Often, the establishment of trees in these sites is limited by high irradiance, low water availability and weed competition (Rey-Benayas et al., 2003, 2005). The soils in these areas are commonly characterized by low levels of organic matter and poor substrate structure; in addition the soils of mining areas are generally acidified (Conesa et al., 2007). All these soil conditions may enhance metal bioavailability (Greger, 1999).

Mediterranean tree species may have a high potential for phytostabilization of contaminated soil. They have massive root systems that allow them to survive in low-water and low-nutrient conditions, which are typical of Mediterranean environments (Canadell et al., 1996). It has been found that Mediterranean sclerophyllous species growing on substrates with a high availability of metals normally show a low transfer of metals to the leaves (Arduini et al., 1996; Fuentes et al., 2007), so these species may have important mechanisms of metal tolerance in the roots.

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For a tree species to be suitable for phytostabilization, the root system should be able to both retain and tolerate high concentrations of metals. Inhibition of root growth is one of the first symptoms of toxicity observed in tree seedlings (Kahle, 1993; Wisniewski and Dickinson, 2003). This reduction in root growth could reduce the resistance of plants to summer drought, which is one of the main causes of mortality in young plants in the Mediterranean climate (Pausas et al., 2004), and could thus reduce the success of afforestation programs.

In this work, we studied the patterns of metal availability (Cd, Cu, Pb and Zn) in the Guadiamar Valley in southwest Spain, an area that was contaminated by a mine spill in 1998 and later afforested with woody plants. In a previous paper, we analyzed the accumulation of trace elements in the leaves of the tree and shrub species used in the remediation program (Domínguez et al., 2008). In this paper, we focus on the response of the foliar chemistry of the Holm oak, one of the most planted species, to changes in the availability of metals under field conditions. As Cd was the element with the highest potential soil-plant transfer, we studied the response of Holm oak seedlings to exposure to different amounts of Cd under controlled conditions. Specifically, we monitored biomass production, root growth and chlorophyll fluorescence, as well as the Cd accumulation patterns. We addressed four specific questions: (1) How is the availability of Cd and other metals affected by soil conditions seven years after soil remediation? (2) What is the relationship between available Cd in the soil and Cd in the leaves under field conditions? (3) What is the process of Cd translocation from the substrate to oak roots and leaves? (4) Is the Holm oak tolerant to high Cd levels in the soil, thus suggesting that it could be used for phytostabilization of contaminated soils?

2. Materials and methods

2.1. Study area and study species

The field study was conducted in the Guadiamar River Valley in southwest Spain, which has a semi-arid Mediterranean climate with mild, rainy winters and warm, dry summers. This area was affected by a toxic flood that inundated 4286 ha of the river basin with trace element-polluted sludge and acidic water after the failure of a mine-tailing dam (details of the accident in Grimalt et al. (1999)). After the accident, a large remediation program was implemented, which included removal of the polluted topsoil, the addition of soil amendments and the revegetation of around 2700 ha with native Mediterranean tree and shrub species. In spite of these efforts, the underlying soil still contained elevated amounts of trace elements, specifically As, Cd, Cu, Pb, Tl and Zn (Cabrera et al., 2008).

The Holm oak (*Q. ilex* subsp. *ballota* (Desf.) Samp.) is one of the most abundant trees in the study area. Currently, this species is present in scattered and fragmented savannah-like woodlands in the terraces of the river, since most of the land was used for agriculture before the accident. Holm oak saplings were widely used during the afforestation of the polluted sites, although survival of the saplings during the first several years after planting was low (Domínguez et al., in press).

2.2. Soil and plant sampling

Sampling was conducted in autumn 2005. The Guadiamar River Valley was divided into four zones, 40 km along the river in the north-south direction, with different soil characteristics and contamination levels. Zone 1 was located in the surroundings of the mine tailing, and soils were mostly acidic sandy loam. Zone 2, which had similar soil properties to Zone 1, was one the most contaminated areas along the Guadiamar Basin because the sludge was temporally stored in this area during the soil clean-up operations. Zone 3 also contained highly contaminated areas, but the soils were mostly neutral and basic loam. Finally, the soils in Zone 4 were mostly clay loam, due to the vicinity of the salt marshes in the south of the basin.

Along the first three zones, we selected 18 Holm oak trees (10 adult trees and 8 saplings). Around each tree, the leaf litter was removed and two soil cores were taken from the root zone at two depths (0–25 cm and 25–40 cm) to make a composite soil sample for each tree. A composite leaf sample was taken from the outer canopy of each tree. To increase the data set for the study of trace element availability in the soil (objective 1), additional soil samples were taken underneath 18 white poplar trees (*Populus alba*) in Zones 3 and 4, where this species is more abundant than the Holm oak. Thus, we got a broad range of soil types, especially in relation to pH and clay content. The total number of soil samples was 72 (36 for each depth). We also took leaf samples from the 18 white poplars, but the corresponding leaf data have not been included in this work.

2.3. Soil and plant analyses

The soil samples were oven-dried at 40 °C, then sieved to <2 mm for the analysis of their general properties. A fraction of each sample was then ground to <1 mm for metal analysis.

The soil texture was determined by the hydrometer method (Gee and Bauder, 1979). The pH was determined potentiometrically in a 1:2.5 soil-water suspension. The organic matter content was analyzed according the method of Walkley and Black (1934). The cation exchange capacity was determined by the ammonium acetate method (Peech et al., 1947). To determine the total metal concentrations (Cd, Cu, Pb and Zn), one replicate of each soil was digested using concentrated HNO_3 and HCl (1:3 v/v, aqua regia) and analyzed by ICP-MS (Inductively Coupled Plasma Mass Spectroscopy; Perkin Elemer, Sciex-Elan 5000). The available concentrations of these elements were analyzed by extraction with NH_4NO_3, according to the Deutsches Institut für Normung protocol (1993), and determination by atomic absorption spectrophotometry (Perkin Elmer 1100B). In this method, the pH of the extracting solution is not adjusted; therefore, the extraction takes place under pH conditions similar to those for the native soil solution (Gryschko et al., 2005). Three replicates per sample were analyzed.

Leaf samples were washed thoroughly with distilled water, dried at 60 °C for at least 48 h and ground. One replicate of each sample was digested using concentrated HNO_3 in a microwave digester (ETHOS D, Milestone, Italy), and Cd, Cu, Pb and Zn concentrations were determined by ICP-MS.

The quality of the trace element analyses was assessed by analyzing different reference materials: NCS DC 73350 (white poplar leaves, China National Analysis Center for Iron and Steel), BCR-62 (olive tree leaves, European Community Bureau of Reference) and CRM 141R (calcareous loam soil, European Community Bureau of Reference). Our experimental values showed recoveries of 81–105% for the plant samples and 83–91% for the soils samples when compared to the certified values.

2.4. Greenhouse experimental design

Under controlled conditions in a greenhouse (University of Seville, Spain), Holm oak seedlings were exposed to different concentrations of Cd. Acorns were germinated in forestry trays of twenty 8 × 20 cm cells, each containing 550 g of pure silica sand. One hundred twenty seedlings with similar heights were selected. Then, the seedlings were exposed to four different treatments (two trays of 15 plants for each treatment, $N = 30$ per treatment). The control treatment trays received a nutritive solution of 1 M KNO_3, 1 M $Ca(NO_3)_2$, 1 M KH_2PO_4, 1 M $MgSO_4$ and 0.01 M Fe-EDTA (pH = 7), which was renewed weekly. The other three treatments consisted of different levels of Cd, which was provided as $CdCl_2$ added to the nutritive solution (20, 80 and 200 mg L^{-1}, respectively). The exposure to Cd for the 20 mg L^{-1} and the 80 mg L^{-1} treatments was increased gradually. During the first week, both treatments received the lowest dose (20 mg L^{-1}), and then the dose was increased to 80 mg L^{-1} during the second week. The dose was increased again during the third week to 200 mg L^{-1} for the last treatment. Plants grew in the greenhouse over a period of six months, with a photoperiod of 16 h and a maximum radiation of 1000 μmol m^{-2} s^{-1}.

2.5. Chlorophyll fluorescence and plant analysis

After six months, six plants per treatment were selected. The maximum photochemical efficiency of photosystem II (Fv/Fm) was calculated from the minimum (Fo) and the maximum (Fm) fluorescence values measured in three leaves per plant. These measurements were taken at midday with an FM2 fluorimeter (Hansatech, Norwolk, UK) after adaptation of the leaves to darkness for at least 20 min.

After the chlorophyll measurements, ten plants per treatment were harvested and separated into leaves, stems and roots. Morphometrical measurements of the taproot were taken (total length and diameter of four sections of the root). Each part was washed, dried and weighed separately. In a subsample of the harvested plants (five per treatment), the roots were separated into taproots and lateral fine roots ($\leqslant$2 mm diameter), and each type of root was weighed separately. This subsample was used for chemical analysis. Fine roots, taproots and leaves were ground and digested as described above. The Cd concentration in each sample was determined by ICP-MS, and the concentrations of other elements (Ca, K, Mg, Mn, S and P) were determined by ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy; Thermo Jarrell Iris Advantage).

2.6. Field and greenhouse data analyses

Linear regression models were used to analyze the influence of pH, organic matter content and cation exchange capacity on the availability of the trace metals. The corresponding total concentrations of metals in soils were also considered as predictors in the models. The best subset of models for each studied metal was selected as those showing the minimum value of *Cp* coefficients (Mallow, 1973). Log-transformations of the variables were applied previously.

Bioaccumulation coefficients (BC) were calculated using the field data. The bioaccumulation coefficient is defined as the ratio of the Cd concentration in the plant to the total Cd concentration in the soil (Adriano, 2001).

Using the greenhouse data, one way ANOVAs were used to compare the different morphological variables among treatments. Previously, normality and homoskedasticity of the data were checked. The translocation coefficient (TC), which is the ratio of concentrations between different plant organs, was used to quantify the transfer of Cd within the plant. All statistical analyses were performed with STATISTICA v. 6.0. (StatSoft Inc., Tulsa, USA).

3. Results and discussion

3.1. Influence of soil conditions on metal availability

The total and available metal concentrations were highly variable within and among zones, as indicated by the high standard deviations (Table 1). Under field conditions, Cd was the most available element, in relative terms. On average, 15% of the total soil Cd was extracted by NH_4NO_3. The so-extracted Cd comprises the water-soluble and easily exchangeable fraction in the soil. Mean available fractions of Zn, Cu and Pb were 5.6%, 2.6% and 0.035%, respectively. In all of the zones, the NH_4NO_3-extracted concentrations of Cd were higher than 0.04 mg kg^{-1}, which is the action value for NH_4NO_3-extracted Cd in Germany (BBodSchV, 1999). The maximum concentration of available Cd was 1.86 mg kg^{-1}, which was found in some subsoil samples (25–40 cm depth) from Zone 3. Likewise, the available concentrations of Pb and Zn exceeded the German action values (0.1 and 2 mg kg^{-1}, respectively) in all of the zones. However, the available Cd concentrations found in this work were not much higher than the NH_4NO_3-extracted Cd concentrations in some unpolluted reference soils (average of 0.049 mg kg^{-1}, Mèers et al., 2007).

The highest total concentrations of Cd and Zn were found in the southern zone (Zone 4), while Cu and Pb showed the highest concentrations in the central areas (Zone 2 and 3). This pattern can be related to the different solubility of each metal. The more labile elements (Cd and Zn) would have been more easily transported through the river basin to Zone 4, where the soils are predominantly calcareous clay loams and had a higher capacity for metal retention than soils in Zones 2 and 3. Cu and Pb, on the other hand, would have been previously deposited upstream nearer the spill source due to their higher affinities for the soil solid phase. These results are consistent with other investigations in the study area (Cabrera et al., 2008).

The high variations in the metal availability may be due to differences in the soil properties. The lowest soil pH values were observed in Zones 2 and 3, which also had the highest sand content. In these sites, extremely acid soils (pH < 4) were observed. In general, organic matter content was moderately low in the whole area and was similar among zones (Table 2). Soil pH was the most important factor explaining the patterns of solubility of the studied metals. In the regression models, the β coefficient (which indicates the relative importance of each predictor in the variance of the dependent variable) for soil pH was higher than the β coefficients for the rest of the predictors (Table 3). Therefore, the availability of Cd, Cu and Zn could be well predicted from the soil pH values (see example for surface soils, Fig. 1). For Cd, the organic matter content and the cation exchange capacity had also significant influences on the metal availability in the surface soils (Table 3). Finally, Pb availability showed a non-linear relationship to soil pH, with large increases in availability in acid soils with pH < 3.

The different fractions of organic carbon in the soil play an important role in metal mobility. For example, Cu and Pb form complexes with dissolved organic carbon, while Cd and Zn usually interact with the electrostatic binding sites of organic matter (McBride, 1989). In our study area, the structure of the soil in many sites was severely affected by the clean-up procedure after the accident, which removed the contaminated topsoil (10–20 cm depth). This could have enhanced the penetration of the remnants of sludge into deeper depths of the soils, as reflected by similar metal concentrations at 0–25 cm and at 25–40 cm depth. In general, the organic matter content of the soil was drastically reduced, so the effect of organic matter content is expected to be low. However, the afforested woody species act as sources of organic matter via litterfall. Therefore, the organic matter content in the soil may increase due to litter deposition, and this edaphic factor may have a greater impact on metal availability in the future.

The long-term effect of tree growth on metal mobility is an issue under discussion. On one hand, metal retention in the rhizosphere can reduce metal mobility, but, on the other hand, the production of organic acids by root exudation and by litter decomposition may enhance metal solubility depending on the types of

Table 1
Trace element concentrations (total and NH_4NO_3-extracted, mg kg^{-1}) in the studied soils. Mean ± standard deviation. $N = 72$ (36 for each depth).

Zones		Upper soils (0–25 cm)				Subsoils (25–40 cm)			
		1	2	3	4	1	2	3	4
Total	Cd	0.59 ± 0.42	1.3 ± 0.8	1.3 ± 0.9	2.4 ± 1.4	0.46 ± 0.27	1.1 ± 1.2	2.0 ± 1.8	2.5 ± 0.6
	Cu	74 ± 36	150 ± 68	173 ± 52	128 ± 51	70 ± 45	140 ± 97	189 ± 96	138 ± 23
	Pb	173 ± 131	268 ± 157	511 ± 625	97 ± 28	185 ± 130	244 ± 189	293 ± 320	252 ± 165
	Zn	239 ± 141	425 ± 236	440 ± 267	768 ± 395	170 ± 85	361 ± 325	555 ± 550	718 ± 206
Available	Cd	0.06 ± 0.03	0.17 ± 0.23	0.18 ± 0.12	0.08 ± 0.05	0.07 ± 0.03	0.17 ± 0.14	0.34 ± 0.55	0.12 ± 0.05
	Cu	0.29 ± 0.21	5.5 ± 11.3	10 ± 15	0.45 ± 0.17	0.91 ± 2.48	8.2 ± 11.7	4.5 ± 8.8	0.37 ± 0.14
	Pb	0.39 ± 0.25	0.37 ± 0.18	5.0 ± 13.7	0.47 ± 0.12	0.47 ± 0.35	0.90 ± 0.78	1.4 ± 2.2	0.75 ± 0.17
	Zn	4.3 ± 4.8	29 ± 60	30 ± 36	0.92 ± 0.98	2.3 ± 3.0	29.9 ± 42.5	53 ± 103	1.5 ± 2.7

Table 2
Soil properties in the studied zones. OM = organic matter content; CEC = cation exchange capacity. Mean ± standard deviation. $N = 72$ (36 for each depth).

Depth (cm)	Variable	Zone			
		1	2	3	4
0–25	pH	6.6 ± 1.1	6.4 ± 1.8	5.5 ± 2.4	7.7 ± 0.5
	OM (g kg^{-1})	22 ± 10	21 ± 9	24 ± 8	19 ± 8
	CEC (cmol kg^{-1})	21 ± 19	21 ± 15	17 ± 10	16 ± 11
	Sand (%)	43 ± 16	52 ± 18	46 ± 17	44 ± 9
	Silt (%)	28 ± 4	25 ± 11	32 ± 11	31 ± 11
	Clay (%)	29 ± 13	22 ± 8	23 ± 7	25 ± 8
25–40	pH	6.3 ± 0.8	5.4 ± 1.9	5.9 ± 2.0	7.3 ± 0.6
	OM (g kg^{-1})	16 ± 7	19 ± 7	26 ± 19	21 ± 15
	CEC (cmol kg^{-1})	25 ± 21	22 ± 15	20 ± 17	15 ± 6
	Sand (%)	45 ± 18	55 ± 20	52 ± 14	39 ± 10
	Silt (%)	26 ± 6	29 ± 16	28 ± 3	30 ± 17
	Clay (%)	29 ± 13	19 ± 8	23 ± 16	31 ± 14

Table 3
Results of the regression models, explaining the influence of the studied soil properties on metal availability. $N = 72$ (36 for each depth).

Depth (cm)	Variable	Significant predictors	β Coefficient, *p*-value	Model r^2
0–25	Cd	pH	−0.89, <0.0001	0.69
		OM	−0.25, 0.028	
		CEC	0.25, 0.023	
		Cd_{total}	0.50, <0.0001	
	Cu	pH	−0.67, <0.0001	0.57
		CEC	0.28, 0.034	
		Cu_{total}	0.38, 0.009	
	Pb	pH	−0.68, <0.0001	0.73
		Pb_{total}	0.27, 0.014	
	Zn	pH	−0.9, 0.004	0.33
25–40	Cd	pH	−0.73, <0.0001	0.63
		Cd_{total}	0.58, <0.0001	
	Cu	pH	−0.58, <0.0001	0.79
		Cu_{total}	0.57, <0.0001	
	Pb	Pb_{total}	0.74, <0.0001	0.54
	Zn	pH	−0.85, <0.0001	0.67
		Zn_{total}	0.34, 0.002	

acid produced (Jones and Darrah, 1994; Marschner, 1995). The afforestation of grasslands or former agricultural lands normally results in the acidification of the soil (Jobbágy and Jackson, 2003; Mertens et al., 2007), which could influence the metal solubility (Strobel et al., 2001; Andersen et al., 2004). The effect of trees on the soil properties can be very different depending on the species planted (Mertens et al., 2007). In the Guadiamar Valley, different tree species, with different foliar chemistries, were planted. In the future, it would be interesting to analyze the different footprint of each species on the soil properties. The possible soil acidification after afforestation should be monitored, since there are already some extremely acid soils in the area where metals have a higher mobility.

3.2. Accumulation of Cd and other metals in Holm oak leaves

Under field conditions, the mean leaf concentrations of Cd, Cu, Pb and Zn were 0.20, 10.8, 2.66 and 75.7 mg kg^{-1}, respectively. These values are within the normal ranges for higher plants (Chaney, 1989). The highest BC were found for Cd and Zn, with mean values around 0.30, followed by Cu (0.14) and Pb (0.022). In the case of Cd, the maximum BC was 1.1, but for most of the trees (78%), the BC was below 0.6. Neither the leaf concentrations nor the bioaccumulation coefficients were related to the available concentration of metals in the soils (see example for Cd, Fig. 2). No correlation was neither found with soil pH, organic matter content, nor CEC. Thus, oaks growing on acidic soils did not significantly

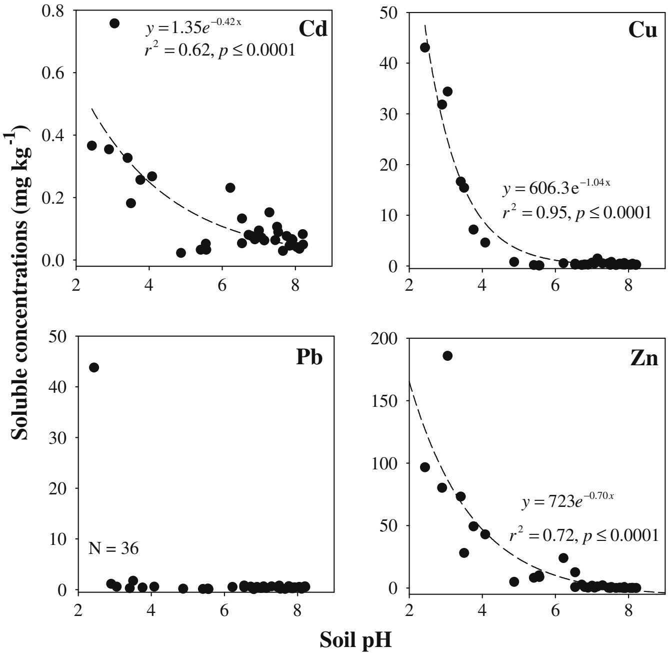

Fig. 1. Prediction of available metal concentrations from the soil pH values (0–25 cm depth). Linear regression equations and parameters are indicated. Data for Pb did not fit linear distribution.

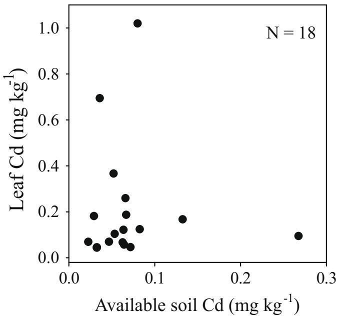

Fig. 2. Cadmium availability in soils and concentrations in Holm oak leaves.

accumulate more metals than those growing on neutral or basic soils (data not shown).

Some studies have shown that the concentrations of metals extracted from soil with neutral salts, in particular with ammonium nitrate, are well correlated with the metal concentrations in plant shoots or plant yield, and the use of these salts has been proposed to measure metal phytoavailability (Bhogal et al., 2003; Menzies et al., 2007). In contrast, other studies have shown that ammonium nitrate is not the best extractant for the prediction of metal uptake (Hall et al., 1998; Grønflaten and Steinnes, 2005). Plant species differ in their mechanisms of uptake and translocation of metals, and these differences may result in variable tissue concentrations, which could be much different from the potentially available metal concentrations in the soil. For example, leaves of *P. alba* have been shown to be good indicators of the available Cd and Zn (but not Pb) levels in soil under the conditions of the Guadiamar River Valley (Madejón et al., 2004). In contrast, oak leaves do not reflect such levels, probably due to important mechanisms of avoidance of metal uptake by the roots, or to low translocation rates to the leaves (Dickinson et al., 1991). In soils with a heterogeneous distribution of contaminants, root proliferation is promoted in the less contaminated spots (Turner and Dickinson, 1993; Menon et al., 2007). The roots of trees can also exudate organic acids, which have been suggested to prevent metal uptake (Heim et al., 1999; Ahonen-Jonnarth et al., 2000). Immobilization of the metals in the cell walls of the roots is one of the most common mechanisms of metal detoxification in trees (Kahle, 1993; Brunner et al., 2008).

3.3. Effects of Cd on oak seedling performance

Exposure to Cd produced a decrease in root and shoot biomass of the oak seedlings (Table 4). At a Cd exposure of 200 mg L^{-1}, the shoots of 20% of the plants became dry, and the root biomass decreased to a 30%. The taproot biomass decreased with the increasing Cd exposure. The fine root biomass was reduced at the two highest exposures to Cd, but at 20 mg L^{-1} there were no significant differences compared to the control treatment. Taproot length was not different among treatments, but root thickness was affected by Cd, thus resulting in thinner taproots (same length, but smaller diameter, Table 4). The proportion of plant biomass contributed by the roots was slightly higher in the plants exposed to Cd, as indicated by the Root Mass Ratio (RMR).

In general, the depression of growth parameters such as root elongation, biomass production, root initiation and root hair formation are the first symptoms of heavy metal toxicity in tree species (Kahle, 1993). Different studies with woody plant seedlings exposed to Cd have reported similar or higher inhibition of root elongation (Godbold and Hüttermann, 1985; Arduini et al., 1994) or inhibition of both root elongation and biomass accumulation (Kahle, 1993; Lunackova et al., 2003) at Cd doses much lower than those tested here. For Holm oak seedlings, the rapid establishment of the root system is critical for survival during the first summer periods; any factor that hampers root elongation or thickness could limit the access of the plants to the water in the deep soils during the summer, thus reducing establishment of the seedlings. At low to moderate Cd levels, the effects on root elongation are likely to be low. Other detrimental effects on the plants, such as nutritional deficiencies, may be derived from the inhibition of fine root development. However, the conditions tested in our experiment simulated extremely high contamination and maximum availability of Cd for the plants (due to the inert substrate and the soluble Cd salt

Table 4
Root characteristics in the control and Cd-exposed seedlings, under controlled conditions (mean ± standard deviation). For each variable, significant differences among treatments are indicated by different letters ($p < 0.05$). RMR: Root Mass Ratio (root mass: total plant mass). $N = 5$ for Taproot and Fine root mass; $N = 10$ for the rest of variables.

Variable	Treatment			
	Control	Cd 20 mg L^{-1}	Cd 80 mg L^{-1}	Cd 200 mg L^{-1}
Root mass (g)	4.36 ± 0.41 a	2.83 ± 1.29 b	0.94 ± 0.49 c	1.29 ± 0.73 c
Shoot mass (g)	5.14 ± 1.72 a	1.34 ± 0.32 b	0.70 ± 0.25 b	0.91 ± 0.41 b
RMR	0.46 ± 0.07 a	0.66 ± 0.07 b	0.55 ± 0.12 ab	0.57 ± 0.06 ab
Taproot mass (g)	3.75 ± 0.81 a	1.94 ± 0.92 b	1.04 ± 0.48 b	1.01 ± 0.23 b
Fine root mass (g)	0.49 ± 0.26 a	0.24 ± 0.19 ab	0.09 ± 0.08 b	0.11 ± 0.06 b
Taproot length (cm)	38.2 ± 14.9 a	28.5 ± 7.2 a	31.1 ± 9.4 a	30.3 ± 12.5 a
Taproot diameter (mm)	4.94 ± 0.73 a	3.62 ± 0.78 b	2.17 ± 0.54 b	2.93 ± 0.67 b

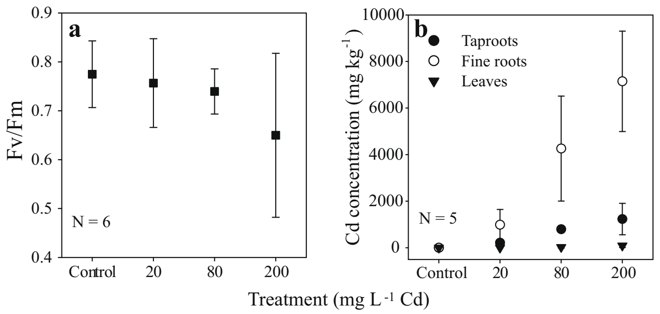

Fig. 3. Chlorophyll fluorescence (a) and Cd accumulation (b) in Holm oak seedlings exposed to different levels of Cd. Fv/Fm: maximum photochemical efficiency of the photosystem II. Mean ± standard deviation.

used). In moderately contaminated soils, the bioavailable levels would probably be lower, especially under semi-arid conditions such as those found in the Guadiamar Valley.

The chlorophyll fluorescence data support the conclusion that the seedlings of this species have a relatively high tolerance to Cd. This variable can be a good indicator of the overall performance of the photosynthetic apparatus, and by extension, of the ability of the plants to tolerate environmental stresses (Maxwell and Johnson, 2000). In our case, Fm/Fv for the control treatment and for the exposures to 20 and 80 mg L^{-1} Cd was slightly lower than the theoretical optimum (Fm/Fv = 0.83; Maxwell and Johnson, 2000) (Fig. 3a). At 200 mg L^{-1}, Fm/Fv showed a high variability because two of the six measured plants were dried, and these plants showed Fv/Fm values below 0.5.

3.4. Cadmium accumulation patterns in oak seedlings

The oak seedlings showed a high Cd retention capacity in the roots, especially in the fine roots (Fig. 3b). In the extremely contaminated substrate (200 mg L^{-1} Cd), the fine roots accumulated up to 7 g kg^{-1} of Cd. In the leaves, the lowest Cd dose (20 mg L^{-1}) resulted in a Cd concentration of 2.5 mg kg^{-1}, which is considered by some authors to be the upper limit for normal, non-phytotoxic values for higher plants (Alloway, 1995). For the two highest exposures, the concentrations in the leaves were well above these values (mean ± standard deviation of 28 ± 40 and 92 ± 82 mg kg^{-1}, respectively), and showed a high variability, as indicated by the high standard deviation. However, root-leaf TC were very low. Because of the high level of Cd accumulation in the fine roots, the maximum TC was 0.02.

The fine roots of some tree species have a special capacity for retaining Cd (Unterbrunner et al., 2007; Brunner et al., 2008). The pectins in the cell wall are the main constituents allowing metal binding due to their carboxyl groups, which have a high cation exchange capacity. Between metals, the binding preferences depend on the type of pectin, although, in general, pectins show higher affinities for Al^{3+}, Cu^{2+} and Pb^{2+} than for Cd^{2+}, and a higher affinity for Cd^{2+} than for Ca^{2+} (Franco et al., 2002). In our case, Cd was highly retained in both the fine roots and in the taproots, displacing some divalent ions such as Ca^{2+} and Mg^{2+}. In the fine roots, Cd and Mg concentrations were negatively correlated ($r = -0.72, p = 0.012$). The amount of Cd in the fine roots was also negatively related to the levels of both Ca and Mg in the taproots ($r = -0.78, p = 0.005$, and $r = -0.61$, $p = 0.043$, respectively). Likewise, the Ca concentration in the taproot was negatively correlated with the Cd level in the leaves ($r = -0.74, p = 0.009$).

Under extremely high concentrations of metal in the rhizosphere, saturation of the pectins may lead to random deposition of metals in the cell wall and in the cell lumen (Brunner et al., 2008). This could explain the high variability of the Cd concentrations in the roots of the plants exposed to the highest doses of Cd.

3.5. Potential for phytostabilization of soils contaminated by Cd

For a tree species to be suitable for phytostabilization, the root system should be able to both retain and tolerate high concentrations of available metals. Under the semi-arid conditions of the study area, Cd is potentially the most available metal in the contaminated and remediated soils. The transfer of Cd to the leaves of Holm oaks growing on contaminated soil is also potentially higher than the transfer of other metals, such as Cu and Pb, although the leaves do not reflect the changes in soil availability, probably due to the high Cd retention capacity in the roots. The accumulation patterns of this species (high root retention and very low root-shoot translocation) make it very suitable for phytostabilization (Mendez and Maier, 2008). Root morphology and biomass were altered by exposure to Cd, although the seedlings showed a relatively high tolerance to the extremely high Cd accumulation in the roots and in the leaves, as observed under controlled conditions. Due to the vast root system of the Holm oak, this species may have a high potential to stabilize Cd in low and moderately polluted soils.

However, the overall resistance to simultaneous environmental stresses in the field must be also considered in afforestation programs proposing to use the Holm oak. At low and moderate contamination levels, such as the found here under the field conditions, the effect of Cd alone on plant performance is likely to be small. In our study area, the survival of this species during the first several years after afforestation was much lower than the survival of other sclerophyllous tree species, such as *Olea europaea*, due to a higher sensitivity to summer stress (Domínguez et al., in press). The suitability of this species for phytostabilization would be enhanced if some techniques leading to an increase in summer stress resistance were considered.

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