PHYTOEXTRACTION OF CADMIUM BY *ATRIPLEX HALIMUS*

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ABSTRACT

The possibility of remediating contaminated soils through the use of high biomass-generating, native plant species capable of removing heavy metals is receiving increased attention. The capacity of accumulation cadmium (Cd) of *Atriplex halimus* was tested by growing transplanted specie in a pine bark compost substrate (pH 5.6) contaminated with 100 mg Cd kg^{-1}. After 70 days, the increase in biomass in the plant enhanced the phytoextraction of Cd. The leaf Cd concentration reached 35 mg Cd kg^{-1}. The normal growth shown by this specie in the presence of high concentration of Cd and under conditions of high temperature and acidic pH, suggests that this specie may be used to generate a green cover on soils contaminated by metals and may contribute to their remediation.

Keywords: Cd-uptake, bioconcentration factor, metal translocation, soil remediation.

INTRODUCTION

Phytoextraction is a relatively new remediation technology that uses plants to extract heavy metals from contaminated soils. These metals are accumulated in the aboveground parts of the plants, which can then be harvested by conventional methods and taken away. The trend in phytoextraction research is to study species showing high biomass production, that are native to the area requiring remediation, that are easily cropped, or that can be used in reforestation (Archer and Caldwell, 2004; Marchiol et al., 2004). These properties can compensate for a modest metal accumulation capacity (Zhuang et al., 2007; Hernández-Allica et al., 2008).

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Cadmium (Cd), which is more mobile and more soluble than other heavy metals, is one of the most toxic to enter the food chain (Chen et al., 2000; Wu et al., 2004). Human activity associated with mining, the use of incinerators, urban traffic, the paint industry, the application of fertilizers, and the generation of sewage sludge etc., all cause soil Cd contamination. High soil Cd concentrations are deemed present when they exceed 1 mg kg^{-1} (Alloway, 1995). In general, most plants accumulate metals more strongly in their roots than in their aerial parts (Gupta et al., 2007; Simonová et al., 2007). The normal Cd concentration of the leaf is usually less than 1 mg kg^{-1} (Chaney, 1997).

The aims of the present study were 1) to assess Cd accumulation in Atriplex halimus grown in a substrate with high Cd bioavailability, and 2) to determine the phytoextraction capacity of this specie.

MATERIAL AND METHODS

The present work was conducted in the research greenhouses of the Universidad Autonóma de Madrid (UAM, Spain). Experiments were performed during the summer months (2006) in order to encourage biomass production.

Commercial pine bark compost was used as the growth substrate for the plants. The pH and electrical conductivity (EC) of the substrate used were determined in an aqueous extract (1:5 v/v) using an Orion electrode 720A and Crison conductivity meter CM 2200 respectively. To determine the apparent bulk density, fresh samples were weighed in 1 L standard stainless steel cylinders. Percentage dry matter was determined by drying samples at 103º ± 2º C until a constant weight was reached. Total organic matter (MOT) was determined by combustion in a furnace at 450ºC (Select-Horn, P Selecta). The total concentration of heavy metals (Cd, Cu, Fe, Mn, Pb, Zn) was determined by atomic absorption spectrophotometry (AAS) (Perkin Elmer Analist 800) after the digestion of dry samples (55ºC) and their grinding to <63 μm in aqua regia (HNO3-HCl, 1:3). All analyses were performed in quadruplicate.

The experimental substrate was artificially contaminated with 100 mg Cd kg^{-1} dry matter via the addition of $CdSO_4$ in aqueous solution; no Cd was added to the control substrate. Both substrates were incubated for four weeks in the laboratory at ~15ºC and 37% relative humidity (RH). The moisture level of these composts during incubation was maintained at approximately 70% of container capacity.

Atriplex halimus was obtained in alveoli from a commercial greenhouse of height 42.1± 10.5 cm and 6.7± 2.7 g dry weight [DW] of aerial parts. The plant was transplanted

into 1.6 L pots containing either the control or the Cd-contaminated substrate. The pots were watered with Hoagland and Arnon fertilizer solution diluted to 25% and without micronutrients, pH 5.6, to encourage biomass production. The daily irrigation volume was ~200 -220 ml per pot, applied through a drip irrigation system. Growth was allowed for up to 70 days, during July-August and part of September. The average daytime temperature was 32± 8°C and the RH 32±11%. After 35 and 70 days of growth, the height, DW and total Cd concentration of the plants were determined. Plant (n=12) was separated into shoot (leaf and stem) and root. All samples were carefully washed with distilled water, dried at 65± 5°C for 48 h and their DW determined. These dried samples and digested in HNO_3 and H_2O_2 using a microwave digester (CEM, Mars Xpress) following the method of Rossini et al. (2003). The total Cd concentration was then determined by AAS.

PHYTOEXTRACTION CAPACITY

The phytoextraction capacities of the different species were determined via their bioconcentration factor (BCF), the index of transport to the leaf (Ti), and the percentage phytoextraction of Cd, calculated using the following equations (Ghosh and Singh, 2005, Wei et al., 2008):

$$BCF = \frac{Metal\ \ concentration\ in\ shoot\ (mg\ kg^{-1})}{Metal\ \ concentration\ added\ in\ substrate\ (mg\,kg^{-1})}$$

$$T_i = \frac{Metal\ \ concentration\ in\ leaves\ \ (mg\ kg^{-1})}{Metal\ \ concentration\ in\ roots\ (mg\,kg^{-1})}$$

$$Phytoextraction\ \ (\%) = \frac{Metal\ \ content\ \ in\ shoot\ \ (mg)}{Initial\ \ metal\ content\ added\ \ in\ substrate\ \ (mg)}\ x100$$

STATISTICAL ANALYSIS

The Duncan test was used to examine the differences in the DW and height of the plants grown in the control and Cd-contaminated substrates. Significance was set at $p \leq 0.05$. All calculations were performed using SPSS v.13.00 software (Statistical Product and Service Solutions).

RESULTS AND DISCUSSION

The substrate pH and EC values were within the recommended range for the growth of most plants (Table 1). The total heavy metals concentration (Cd, Cu, Mn, Pb and Zn) of the substrate was within the limits allowed by Spanish legislation (RD 824/2005).

Table 2 and 3 shows the leaf, stem and root DW and height of plants after 70 days of growth. *A. halimus* grown in the Cd-contaminated and control substrates showed no significant differences in stem, root or leaf DW ($p \leq 0.05$). *A. halimus* not showed visual symptoms of toxicity, despite the high bioavailability of Cd. This specie was therefore tolerant to the dose of 100 mg Cd kg^{-1}, and though this plant normally grow in alkaline soils (Lopez, 2002) this plant adapted to the pH of the substrate. Tolerance to high Cd concentrations has been reported in similar experiments involving potted plants, e.g., in *Calendula officinalis* and *Althaea rosea* growing in pots containing natural soil contaminated with 100 mg Cd kg^{-1} (Liu et al., 2008), and *Arrhenatherum elatius* growing in a peat and perlite substrate contaminated with 300 mg Cd kg^{-1} (Deram et al., 2007). However growth was reduced in *Brassica juncea, Brassica campestris, Dhatura innoxia, Ipomea carnea, Phragmytes karka* cultivated in soil contaminated with 100 mg Cd kg^{-1} (Ghosh and Shing, 2005; Cao et al., 2008).

After 35 days *A. halimus* showed a uniform distribution of Cd, indicating its great capacity for metal translocation (Table 4). *A. halimus* showed high concentration of Cd in the leaf and stem (31.3 and 25.5 mg Cd kg^{-1} respectively) and maintained this high concentration after 70 days. The fact that no significant difference was seen between the leaf Cd concentration on day 35 and day 70 in *A. halimus* illustrates the capacity of this species to accumulate Cd in relative short time. However the hyperaccumulators species can reach 100 mg Cd kg^{-1} (Chaney et al., 1997). *Atriplex halimus* showed high percentage phytoextraction of Cd after 35 days (0.494%) (Table 5). Hernández-Allica et al. (2008) recorded 0.3%, 3.0% and 0.7% Cd phytoextraction for *Zea mays*, *Brassica napus* and *Cynara cardunculus* respectively after 40 days in a substrate (pH 6.8) contaminated with 100 mg Cd kg^{-1}. The figure for *A. halimus* after 35 days, in the present work, was similar to that reported for *Brassica juncea* (0.432%) grown for 90 days in soil (pH 7.8) contaminated with 100 mg Cd kg^{-1} (Ghosh and Singh 2005). *Brassica juncea*, which is considered a Cd indicator species, showed an increase in DW of 1.73 g in the shoot after 90 days, whereas *A. halimus* generated about 10-12 g after 70 days. Cd phytoextraction after 70 days increased, indicating that biomass generation favored the extraction of Cd.

The bioconcentration factor (BCF) for Cd increased in A. halimus after 35 days (Fig 1.). This indicates that the BCF increased with increasing biomass. In heavy metal hyperaccumulator species an increase in biomass is not related to an increase in plant heavy metal concentration (Deram et al., 2006). *Atriplex halimus* showed the highest BCF=0.35. Phytoextraction is feasible when the BCF is greater than 1 (McGrath and Zhao, 2003), as seen in hyperaccumulator species. However there is debate about whether high biomass generation can compensate for the low phytoextraction capacity of non-hyperaccumulator species (McGrath and Zhao, 2003; Marchiol et al., 2004, Hernández-Allica et al., 2008). The Cd transport index of the shoot (Ti) also increased over time (Fig. 2). The BCF (0.35) and Ti (1.68) of *A. halimus* are comparable to those reported for herbaceous species in other assays. For *Chenopodium album*, of the same family as *A. halimus*, the BCF was 0.55 and the Ti 0.63 in pots containing soil (pH 6.6) contaminated with 10 mg Cd kg^{-1} (Wei et al., 2008). For *Brassica juncea* the BCF was 0.49 and Ti of 0.53 after 90 days of cultivation in pots containing soil (pH 7.8) contaminated with 100 mg Cd kg^{-1} (Ghosh and Singh, 2005). Finally, in a hydroponic assay, *A. halimus* reached extreme Cd concentrations in the leaf (833 mg kg^{-1}) (Lutts et al., 2004); in addition this species has been grown in soils with high selenium levels (Moreno et al., 2005).

The notable ability of *A. halimus* to translocate Cd to the shoot could be related to the halophyte character of this species (Martinez et al., 2005). Halophyte species excrete salts as crystals through a system of glands, a mechanism that increases their resistance to salinity. Cd uptake increases with salinity in the halophyte *Tamarix smyrnensis*, the plant excreting Cd onto the surface of its leaves (Manousaki et al., 2008). The resistance of *A. halimus* to high concentrations of Cd has also been linked to the precipitation of Cd as Cd oxalate crystals in the stem (Lutts et al., 2004).

CONCLUSIONS

Atriplex halimus not showed visual signs of toxicity when grown substrate contaminated with 100 mg Cd kg^{-1}. *A. halimus* behaves as an indicator plant. This species showed high Cd phytoextraction capacity, with leaf concentrations of 35 mg Cd kg^{-1}. The normal growth shown by this specie in the presence of high concentrations of Cd and under conditions of high temperature and acidic pH, suggests that this specie may be used to generate a green cover on soils contaminated by metals and may contribute to their remediation.

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Table 1: Physico-chemical properties of the pine bark substrate (values are means±standard deviations, n=4)

pH (1:5 extract)	5.6±0.1
Electrical conductivity (dS m^{-1}) (1:5 extract)	1.07±0.02
Density (kg m^{-3})	354±3.5
Dry matter (%)	55
Cd (mg kg^{-1})	0.45±0.01
Cu (mg kg^{-1})	7.1±0.4
Mn (mg kg^{-1})	93.3±6.0
Pb (mg kg^{-1})	12.1±1.7
Zn (mg kg^{-1})	24.6±1.0

Table 2: Dry weight (g) of leaf, stem and root of *Atriplex halimus* after 70 days growth in the control and Cd-contaminated (100 mg Cd kg^{-1}) substrates (values are mean ±standard deviations; n=12)

Plant Organ	Treatment	Dry weight (g)
Leaf	control	9.0±0.9a
	100 Cd	7.5±1.9a
Stem	control	11.4±2.9a
	100 Cd	8.4±3.2a
Root	control	5.4±0.3a
	100 Cd	4.4±1.3a

Different letters in the same column indicate significant differences among treatments according to the Duncan test $p \leq 0.05$.

Table 3: Height (cm) after 70 days growth of *Atriplex halimus* in the control and Cd-contaminated (100 mg Cd kg^{-1}) substrates (values are mean ±standard deviations; n=12)

Treatment	Height (cm)
Control	74.5±16.4a
100 Cd	68.3±20.3a

Different letters in the same column indicate significant differences among treatments according to the Duncan test $p \leq 0.05$.

Table 4: Cd concentration (mg kg^{-1} DW.) in leaf, stem and root of *Atriplex halimus* after 35 and 70 days of growth in the Cd-contaminated (100 mg Cd kg^{-1}) substrate (values are mean ±standard deviations; n=12)

Plant Organ	Days of growth	Cd (mg kg^{-1} DW.)
Leaf	35 days	31.3±5.5a
	70 days	34.7±9.5a
Stem	35 days	25.5±0.6a
	70 days	35.2±12.5a
Root	35 days	35.8±8.9a
	70 days	20.6±1.4b

Different letters in the same column indicate significant differences between sampling days according to the Duncan test $p \leq 0.05$.

Table 5. Percentage phytoextraction of Cd of *Atriplex halimus* after 35 and 70 days of growth in the Cd-contaminated (100 mg Cd kg^{-1}) substrate

Days of growth	% phytoextraction of Cd
35 days	0.494
70 days	1.780

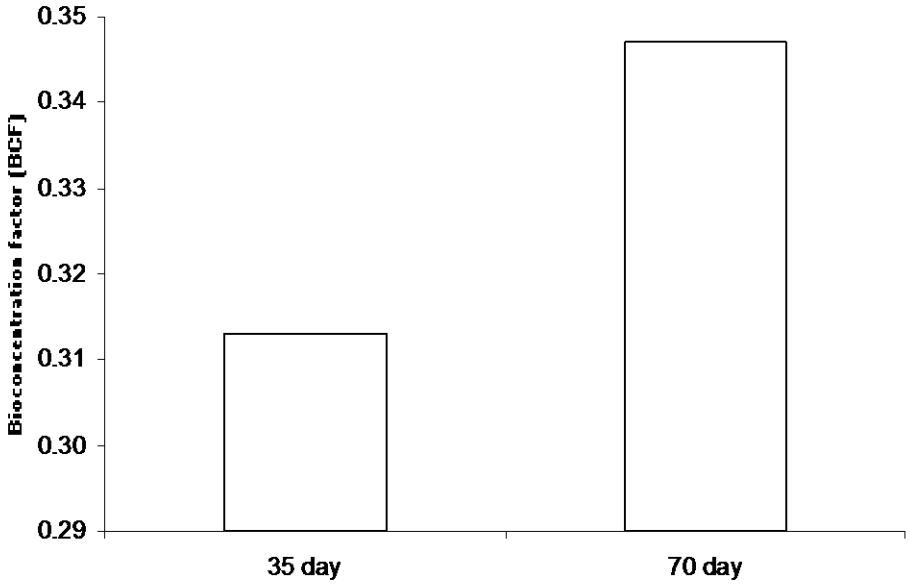

Fig. 1. Bioconcentration factor for Cd ($[Cd]_{shoot}/[Cd]_{substrate}$) in *A. halimus* after 35 and 70 days of growth in the Cd-contaminated (100 mg Cd kg^{-1}) substrate (n=12).

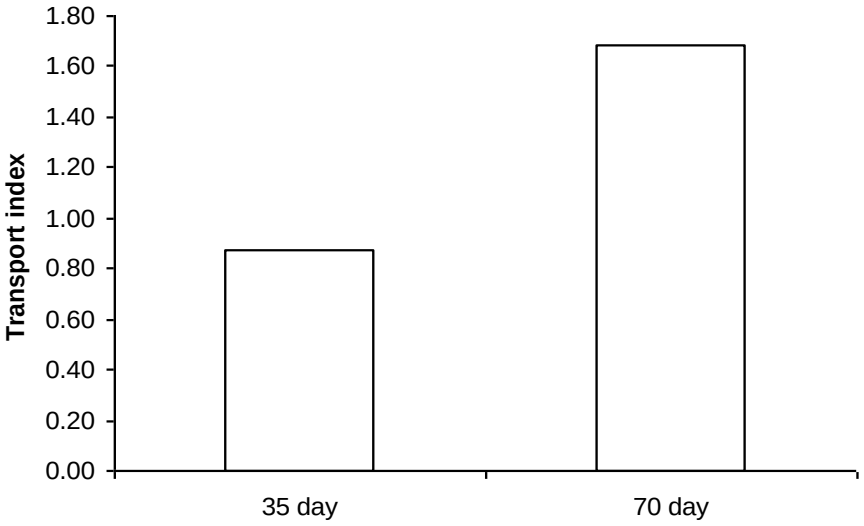

Fig. 2. Transportation index for Cd ($[Cd]_{leaves}/[Cd]_{root}$) in *A. halimus* after 35 and 70 days of growth in the Cd-contaminated (100 mg Cd kg^{-1}) substrate (n=12).