



Chloride salinity reduces cadmium accumulation by the Mediterranean halophyte species *Atriplex halimus* L.

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ABSTRACT

Soil salinity usually increases bioavailability of Cd on heavy metal polluted soils but its impact on Cd absorption and accumulation by plants remains largely unknown. Plants from the halophyte species *Atriplex halimus* were therefore exposed for 12 and 14 days to nutrient solution containing 50 μM CdCl_2 in the presence of NaCl, KCl or NaNO_3 50 mM. Most Cd present in solution remained as Cd–EDTA and salinity had no impact on Cd speciation. Chloride salinity (NaCl and KCl) reduced Cd accumulation in shoots and roots while NaNO_3 increased Cd accumulation in leaves. More than 30% of accumulated Cd was found at the leaf surface and accumulated in trichomes but all tested salts decreased the proportion of excreted Cd. Cadmium induced a decrease in the leaf water content. External NaCl and KCl mitigated the deleterious impact of Cd by inducing osmotic adjustment while NaNO_3 and synthesis of protecting compounds such as soluble sugars and glycinebetaine. Free polyamines (putrescine, spermidine and spermine) increased in response to Cd, Cd + NaCl and Cd + KCl while only putrescine increased in response to Cd + NaNO_3 . Proline exhibited maximal concentration in the leaves of Cd + NaCl and Cd + KCl-treated plants and was correlated with osmotic adjustment. Our results suggest that chloride salinity improved the resistance of *A. halimus* to Cd toxicity both by decreasing the absorption of heavy metal and by improving tissular tolerance through an increase in the synthesis of osmoprotective compounds.

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1. Introduction

Cadmium is one of the main toxic pollutants generated by mining or industrial activities and constitutes an important threat to human health. Most of Cd present in the human body is ingested through the diet and drinking water and there is consequently an important need to consider the potential food-chain transfer of soil Cd. Several phosphorus fertilizers contain high amounts of Cd which will result in the accumulation of this pollutant in the soil over time (Huang et al., 2004). The use of sewage sludge or poor quality water for irrigation may also contribute to Cd accumulation in the soil (Pascual et al., 2004).

The lack of wastewater treatment facilities in dry areas of developing countries is responsible for considerable loads of both salt and heavy metals on agricultural soils (Helal et al., 1996, 1999). Previous studies demonstrated that chloride salinity, mainly NaCl, increased Cd concentration in harvestable parts of cultivated plants (McLaughlin et al., 1994; Li et al., 1994; Smolders et al., 1998; Helal

et al., 1999; Weggler-Beaton et al., 2000; Mühling and Läuchli, 2003). This effect has been attributed to an increase of Cd availability in the soil as a consequence of a chloride-induced decrease of Cd sorption on clay particles and concomitant formation of soluble mobile CdCl^+ ion (García-Miragaya and Page, 1976; Bingham et al., 1983, 1984; Stevens et al., 2003). Although numerous data concerning the impact of salt on Cd availability in the soil are available, only few studies until now considered the precise effect of salinity on cadmium absorption and translocation by the roots independently of the surrounding soil properties and desorption processes from clay particles. Such an effect, however, is of crucial importance not only for cultivated crops but also for plant species which may be used for phytoextraction purposes on marginal lands and which are frequently exposed to several types of ion toxicities. In this respect, hydroponic cultures may constitute a convenient tool to focus on the interaction between Cd and salt on the root absorption steps.

Halophyte species belonging to the genus *Atriplex* have been recommended for remediation of former mining areas and industrial sites (Salo et al., 1996; Glenn et al., 2001) especially in coastal or desertic areas where high concentrations of soluble salts are also present in the soil (de Villiers et al., 1995). This might be related to the fact that those species are naturally tolerant to salt and drought

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stress and that most of them behave as typical “includer” species (Reimann and Breckle, 1993). Some data lead us to hypothesize that tolerance to salt and to heavy metals may, at least partly, rely on common physiological mechanisms (Przymusiński et al., 2004). Tolerance of halophytes to the ionic and the osmotic components of salt stress are linked to their ability to synthesize osmoprotectants in order to maintain a favourable water potential gradient and to protect cellular structures. Proline may accumulate in response to Cd and other heavy metals (Schat et al., 1997; Sharma and Dietz, 2006), but to the best of our knowledge, no data is available for glycinebetaine which is the most efficient osmoprotectant synthesized by Chenopodiaceae (Rhodes and Hanson, 1993). Similarly, polyamines may be involved in the protection of cellular structures under drought and salt stress conditions (Bouchereau et al., 1999) but their involvement in the plant response to heavy metal is only poorly documented (Weinstein et al., 1986). Since Cd stress induces both a secondary water stress (Poschenrieder et al., 1989) and oxidative damages to cellular structures (Romero-Puertas et al., 2004), the ability of halophytes to synthesize those osmoprotectants may be involved in their ability to cope with heavy metals both in the absence and in the presence of salinity. Besides tolerance mechanisms allowing the plants to cope with internal accumulated ions, some halophytes also use excretion processes in order to remove the excess of deleterious toxic ions from photosynthetically active tissues. In the *Atriplex* genus, leaves are covered by trichomes which accumulate high concentration of Na⁺ and Cl⁻ and then burst, leaving salt crystals at the leaf surface. Trichomes are produced continuously during the life of the leaf. Although the presence of Cd has been reported in trichomes of non-halophytes such as tobacco (Choi et al., 2001) or the hyperaccumulating species *Arabidopsis halleri* (Küpper et al., 2000), only few data are available in this respect for halophytes species (Hagemeyer and Waisel, 1988).

Atriplex halimus is a Mediterranean xero-halophyte shrub highly resistant to salt (Bajji et al., 1998) and drought (Martínez et al., 2003). It is frequently encountered on marginal soils and degraded lands in South Europe and North Africa. A recent study reported that this plant species is present on heavy metal contaminated mining sites and that it also exhibits a high level of resistance to Cd and Zn (Lutts et al., 2004). Considering that this species is able to produce more than 4 tonnes of dry matter per hectare and per year (Ben Ahmed et al., 1996) and that it may accumulate up to 0.083% Cd on a dry weight basis in the shoots (Lutts et al., 2004), its use for rehabilitation of heavy metals-polluted lands should be considered. Nevertheless, although the plant is frequently present on salt-affected lands and produces numerous trichomes on both abaxial and adaxial faces of its leaves, no data are available on the impact of salt on Cd absorption and distribution processes.

In order to determine the specific impact of salt on cadmium absorption and accumulation by *A. halimus*, the present work was performed on nutritive solution using different types of salt (NaCl, KCl and NaNO₃) in the presence of Cd. The accumulation of this element was analysed in relation to the ability of the plant to synthesize putative osmoprotectants such as soluble sugars, proline, glycinebetaine and polyamines.

2. Materials and methods

2.1. Plant material and culture conditions

Fruits of *A. halimus* were collected during autumn 2000 from plants growing on a mining site located at Llano del Beal, near Cartagena, in the South-East of Spain (37°32'N, 0°56'W). The main minerals extracted from this zone were pyrite (FeS₂), blend (ZnS) and galene (PbS). The residues from ore processing were poured

into the waterbeds and millions of tonnes of wastes actually remain in this zone. Although the soil is heavily contaminated with several heavy metals (Zn, Cd, and Pb), some plants of the Mediterranean saltbush *A. halimus* spontaneously colonize this site and were able to develop to mature fertile plants (Lutts et al., 2004).

After removal of the bracts, seeds were germinated in plastic jars filled with a substrate of silt–clay–loam and sand (1:2:1:2); the jars were incubated in a growth chamber under a 12-h photoperiod (mean light intensity of 110 μmol m⁻² s⁻¹) provided by Sylvania fluorescent tubes (F36W/133-T8/CW) with a day/night temperature of 28/20 °C. Substrate and young seedlings were sprayed daily during a few minutes with sterile deionised water. After 3 weeks, 384 seedlings at the four-leaves stage were transferred in hydroponic cultures in a nutrient solution (5 mM KNO₃, 2 mM MgSO₄, 1 mM KH₂PO₄·3H₂O, 5.5 mM Ca(NO₃)₂·4H₂O, 3 μM MnSO₄·4H₂O, 10 μM H₃BO₃, 1 μM ZnSO₄·7H₂O, 0.3 μM CuSO₄·5H₂O, 1 μM Na₂MoO₄·2H₂O and 157.3 μM FeEDTA (Lefèvre, 2007)). The pH of the solution was adjusted to 5.7. Seedlings were transferred to 2-l vessels (six seedlings per vessel) containing the nutrient solution which was aerated by a permanent stream of air. Plants were placed in phytotrons under a PAR of 275 μmol m⁻² s⁻¹ (supplied by Sylvania tubes (F96T12 CW/VHO) during 16 h day⁻¹), a constant temperature of 26 ± 2 °C and a relative humidity of 75%. Solutions were renewed every 3 days and pH was readjusted daily to 5.7.

After another period of 2 weeks, seedlings were exposed to NaCl, KCl or NaNO₃ 50 mM in the concomitant absence or presence of 50 μM CdCl₂. For each treatment, Cd speciation in the nutritive solution was determined using Visual MINTEQ software (v.2.30). Each treatment was applied to 48 plants (8 vessels per treatment): half of the plant was harvested after 12 days of treatment and the other half after 24 days. No plants died during the time course of the experiment.

2.2. Growth parameters and osmotic potential determination

Ten plants per treatment were randomly collected at the middle of the photoperiod: roots were rinsed in deionised water during 30 s to remove ions from the free space and gently blotted dry. Length of the stem and of the longest root was determined. Stems, leaves and roots were separated and weighed for fresh weight determination. Tissues were then incubated during 48 h in an oven at 70 °C and weighed again.

For osmotic potential (Ψ_s) determination, roots and leaves were quickly collected on eight plants, cut into small segments, then placed in Eppendorf tubes perforated with four small holes and immediately frozen in liquid nitrogen. After being encased individually in a second intact Eppendorf tube, they were allowed to thaw for 30 min and centrifuged at 15,000 × g for 15 min at 4 °C. The collected tissular sap was analysed for Ψ_s estimation. Osmolarity (c) was assessed with a vapour pressure osmometer (Wescor 5500) and converted from mosmoles kg⁻¹ to MPa using the formula: Ψ_s (MPa) = -c (mosmoles kg⁻¹) × 2.58 × 10⁻³ according to the Van't Hoff equation.

2.3. SEM and trichomes removal

For the scanning electron microscopy (SEM Phillips XL20), specimens were flash frozen (-212 °C) in liquid nitrogen under vacuum for cryo-SEM (Oxford CT1500 cryo-system), transferred to the preparation chamber, and then to the SEM chamber where the frozen samples were sublimated (-80 °C) to remove ice particles. Samples were sputter coated with gold in the preparation chamber for 75 s under 1.2 kV at -150 to -170 °C. Specimens were viewed under 2–5 kV at -170 to -190 °C.

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