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Alleviation of arsenic stress in cardoon plants via the supply of a low cadmium concentration

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ABSTRACT

The effect of As (0–80 μM) and of As+Cd (0–80 μM + 5 μM) combinations on plant growth, toxicological variables and As and Cd bioaccumulation was studied in cardoon plants under controlled conditions. Plants grown in the presence of As alone showed less reduction in overall root and shoot development than those exposed to As+Cd, although the main root was shorter than in the latter plants. The effective added concentrations of As that reduced shoot or root dry weight by 50% (EC_{50}) and the critical toxic concentration that caused a 10% reduction in plant growth ($CTC_{10\%}$) were higher in plants grown with As alone. In both treatments (As and As+Cd), the $CTC_{10\%}$ was higher in the roots, but the root EC_{50} was lower than the shoot EC_{50}. The presence of Cd increased the accumulation of As in the shoot, but ≥20 μM As reduced the shoot bioaccumulation of Cd. Thus, the presence of 5 μM Cd with As appears to reduce the tolerance of cardoon plants to the latter element, but it increases their As phytoextraction capacity. Cardoon plants could be used as excluders in As-contaminated sites and as accumulators in those co-contaminated with As and Cd.

1. Introduction

Trace element pollution is a major concern given its food chain-associated impact on human health and its persistence in soils. Cadmium (Cd), which has no biological function, is very toxic to both plants and animals (Sanità di Toppi and Gabbrielli, 1999); indeed, some authors refer to its toxicity as being 2–20 times greater than that of other heavy metals (Kabata-Pendias and Pendias, 2001). Major anthropogenic sources of Cd include field-applied sewage sludge, pesticides and phosphate fertilisers (Kabata-Pendias and Pendias, 2001). Cadmium is easily taken up by plant roots, and leaf concentrations greater than 5–10 mg Cd kg^{-1} dry weight (DW) are toxic to most plants (White and Brown, 2010). Those affected usually show stunted growth, chlorosis, necrosis, leaf curling and epinasty, brown and stunted roots, and alterations of cell division and chloroplast ultrastructure (Sanità di Toppi and Gabbrielli, 1999; White and Brown, 2010). These problems are the result of induced problems in photosynthesis and transport across membranes, the inactivation of enzymes involved in CO_2 fixation, disturbances in the uptake and distribution of water and nutrients, and homeostatic abnormalities (Sanità di Toppi and Gabbrielli, 1999).

Arsenic (As) is a non-essential toxic element commonly found in the environment and in organisms (Adriano, 2001). Substantial amounts of As are released by geological processes, but mining, coal burning and agriculture can all increase soil As concentrations (Adriano, 2001). Arsenite [As(III)] and arsenate [As(V)] are the phytoavailable forms of inorganic As in soil solution. Arsenate is taken up by plants via phosphate transporters in the plasma membrane of root cells, and is rapidly reduced to arsenite once inside the cytoplasm. Since arsenate and phosphate behave as analogues with respect to their uptake, arsenate toxicity is linked to phosphorus nutrition, and high levels of phosphate can mitigate arsenate toxicity (Esteban et al., 2003). Exposure to As causes reduced root elongation and branching, leaf chlorosis, and the shrinking and even necrosis of the aerial parts of plants (Carbonell-Barrachina et al., 1998). Arsenic can also induce the production of reactive oxygen species (ROS) in plants, increasing lipid peroxidation and oxidative stress (Srivastava et al., 2005).

Soil contamination by a single metal(oid) is rare; where one is highly concentrated, there are usually others, perhaps up to five depending on the source of contamination (Spurgeon et al., 1994). The environmental effects of these combinations may be quite different to those induced by individual pollutants, the

Abbreviations: As, arsenic; BAF, bioaccumulation factor; $CTC_{10\%}$, critical toxic concentration of As that causes a 10% reduction in plant growth; Cd, cadmium; DW, dry weight; EC_{50}, the effective added concentration of As that reduces shoot or root dry weight by 50%; FW, fresh weight.

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result of interactions between them that can be difficult to predict (MacFarlane and Burchett, 2002). For example, Cd and Zn have antagonistic effects in the hyperaccumulator species *Potentilla griffthii* (Qiu et al., 2011) and *Sedum alfredii* (Li et al., 2009). The same is reported for Cd and Mn in white lupin (Zornoza et al., 2010), and for Cd and Hg in rice (Meharg and Jardine, 2003). However, Cd and As have synergistic effects in alfalfa (Zhou and Gao, 1994), as do Cu, Cd and Pb in cucumber (An et al., 2004b).

Cardoon (*Cynara cardunculus* L.) plants grow large but require less irrigation and fertiliser than other crops grown in Mediterranean countries (Gominho et al., 2011), and there is growing interest in their use as an energy/biofuel crop (Gominho et al., 2011). They also show considerable tolerance to As (Llugany et al., 2012), Cd (Papazoglou, 2011; Llugany et al., 2012) and Ni (Papazoglou, 2011), and might therefore be grown in marginal areas or be used to recover contaminated soils. However, the effects of only one combination of pollutant trace elements – Cd—Ni – have been investigated in cardoon plants (Papazoglou, 2011). The present work studies the response of cardoon plants to As alone and of As+Cd to determine: (i) whether the supply of 5 μM of Cd increases or reduces tolerance to As; (ii) and whether cardoon plants might be used to recover sites co-contaminated by As or As+Cd. The specific aims of this work were therefore: (i) to study the phytotoxic effects of As alone and in combination with Cd, (ii) to determine the toxic As concentration in the absence and presence of Cd, and (iii) to compare tolerance to As and As+Cd in this species. For this, differences in growth, As and Cd bioaccumulation, and toxicological variables, were assessed in plants grown under controlled conditions.

2. Materials and methods

2.1. Growth conditions and treatments

Seeds of cardoon (*C. cardunculus* L.) CLON N° 1 were germinated on a mixture of water-moistened perlite and peat (50%) in the dark for 7 days at 28 °C. The seedlings obtained were placed in plastic containers (3 L) containing a continuously aerated nutrient solution: 1.5 mM $Ca(NO_3)_2$•$4H_2O$, 4.0 mM KNO_3, 1.5 mM KH_2PO_4, 1.0 mM $MgSO_4$•$7H_2O$, 36 μM Fe-EDDHA, 33 μM $MnSO_4$•H_2O, 1.6 μM $ZnSO_4$•$7H_2O$, 1.6 μM $CuSO_4$•$5H_2O$, 46 μM H_3BO_3, 0.1 μM $(NH_4)_6Mo_7O_{24}$•$4H_2O$ (pH 5.5–6.0). All plants were grown in a controlled environment chamber under the following night/day conditions: temperature 20/25 °C, photoperiod 11/13 h, and relative humidity 60/40%. The photon flux density during the light period was 520 μmol m^{-2} s^{-1}. The plants were supplied with one of six As doses (0, 5, 10, 20, 40 and 80 μM Na_2HAsO_4•$7H_2O$) in the presence or absence of 5 μM Cd ($CdSO_4$). This relatively low Cd concentration was selected (a) for fear of killing the plants when in combination with high As concentrations, (b) because it is a realistic concentration for Cd-contaminated sites, and (c) because others authors (Llugany et al., 2012) report the tolerance of this species to this concentration. Experiments were performed with three independent replicates, following a randomised block design.

Plants were harvested 28 days after sowing, separated into shoots and roots, their fresh weight (FW) recorded, and the main root length measured. They were then washed thoroughly with Tween 80 (0.1%, v/v), and a further three times with deionised water. This plant material was then dried at 80 °C for 3 days until a constant DW was achieved.

2.2. Determination of As and Cd

The concentration of As and Cd in the shoots (leaves plus stems) and roots was determined by digesting 200 mg DW of homogenised samples with a mixture of HNO_3:H_2O_2:H_2O (3:2:10, v:v:v) for 30 min at 125 °C under a pressure of 1.5 kPa (Lozano-Rodríguez et al., 1995). The As and Cd concentrations were determined by atomic fluorescence (Millennium Excalibur System, PSAnalytical) and atomic absorption spectrometry (Perkin-Elmer Analyst 800) respectively.

The bioaccumulation factor (BAF) for As and Cd in all plants was calculated as the ratio between the As or Cd concentration in each plant organ and the total As or Cd concentration added per plant to the nutrient solution.

$$\text{BAF} = \frac{\text{Trace element concentration in plant organ } (\mu g\ plant^{-1})}{\text{Trace element concentration in nutrient solution } (\mu g\ plant^{-1})}$$

2.3. Toxicological variables and statistical analyses

Relative shoot and root growth rates were expressed as a percentage of the growth of the plants (based on FW) compared to the corresponding control treatment (An, 2004a). The calculation of the bioaccumulation factor ($CTC_{10\%}$) and the effective added concentration of As that reduced shoot or root dry weight by 50% (EC_{50}) was performed by regression analysis using SigmaPlot 9.0 software (SPSS Inc., Chicago, IL). EC_{50} and $CTC_{10\%}$ values were calculated using a one-variable logarithmic curve.

The data presented are the means ± standard errors (S.E.) of three independent replicates. To ensure that the assumptions for statistical analysis were fulfilled, the equality of variances and the normality of the data were tested. Differences between means for each variable were tested for significance by one-way analysis of variance (ANOVA). Significant differences ($P < 0.05$) between treatments were sought using the least significant difference test or Duncan's test as appropriate.

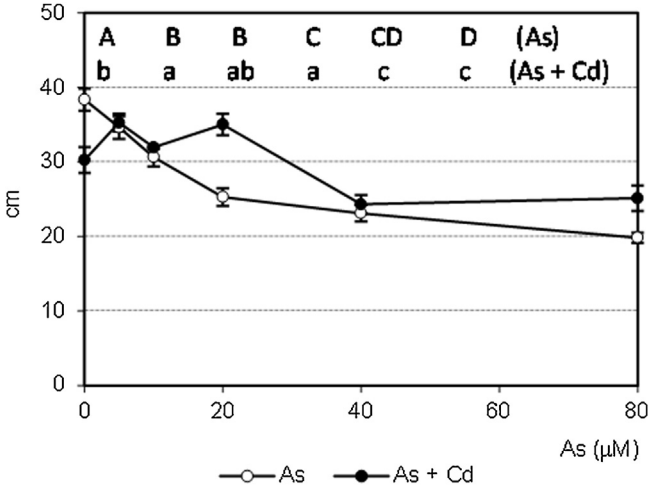

Fig. 1. Effect of different As concentrations alone and different As concentrations+Cd (5 μM) on root length. Data are means ± S.E. ($n = 3$). Different letters above the bars indicate significant differences ($P < 0.05$) between different doses of As (capital letters) and As+Cd (lower case letters).

3. Results

3.1. Root length and plant growth

Fig. 1 shows the effect of the different doses of As and As + 5 μM Cd on the root length of the plants. Root length was reduced in plants exposed to As in both the presence and absence of Cd, but especially under the latter condition. In plants exposed to As alone, the root length was reduced significantly in the presence of just 5 μM of this element, while in the plants exposed to As + 5 μM Cd,