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Accumulation and tolerance characteristics of cadmium in a halophytic Cd-hyperaccumulator, *Arthrocnemum macrostachyum*

Susana Redondo-Gómez*, Enrique Mateos-Naranjo, Luis Andrades-Moreno

Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, Apartado 1095, 41080 Sevilla, Spain

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ABSTRACT

The potential of the extreme halophyte *Arthrocnemum macrostachyum* was examined to determine its tolerance and ability to accumulate cadmium for phytoremediation purposes. A glasshouse experiment was designed to investigate the effect of cadmium from 0 to 1.35 mmol l^{-1} on the growth and the photosynthetic apparatus of *A. macrostachyum* by measuring chlorophyll fluorescence parameters, gas exchange and photosynthetic pigment concentrations. We also determined ash, cadmium, calcium, copper, iron, manganese, magnesium, phosphorous, sodium, and zinc concentrations, and C/N ratio. *A. macrostachyum* demonstrated hypertolerance to cadmium stress; it did not show phytotoxicity at shoot concentration as high as 70 mg kg^{-1}. The bioaccumulator factors exceeded the critical value (1.0) for all Cd treatments, and the transport factors indicated that this species has higher ability to transfer Cd from roots to shoots at lower Cd concentrations. At 1.35 mmol l^{-1} Cd *A. macrostachyum* showed 25% biomass reduction after a month of treatment. Long-term effects of cadmium on the growth were mainly determined by variations in net photosynthetic rate (P_N). Reductions in P_N could be accounted by higher dark respiration and lower pigment concentrations. Finally, *A. macrostachyum* has the basic characteristics of a Cd-hyperaccumulator and may be useful for restoring Cd-contaminated sites.

1. Introduction

Cadmium is one of the most hazardous and ubiquitous contaminants in soil and water generated from industrial and agricultural activities such as mining and smelting of metalliferous ores, wastewater irrigation, and abuse of chemical fertilizers and pesticides [1,2]. Compared with other heavy metals, cadmium is not an essential nutrient in higher plants, and the exposure to relatively low concentrations results in high toxicity to plant and animal [2,3]. Therefore, it is important and urgent to develop methods to cleanup Cd-contaminated soils.

Phytoremediation has become a promising soil remediation technique, whose success is dependent on several factors such as the ability of the selected plant species for accumulating high concentrations of metal in the shoots and to produce high biomass [4]. Thus, it is very important to indentify new feasible hyperaccumulators or accumulators of Cd as the groundwork for the successful phytoremediation of Cd-contaminated soils [5].

In the plants, cadmium can have multiple effects including chlorosis, lower carbon, and nitrogen metabolic activities, and growth reduction [6]. Cd also interacts with the plant water balance [7], inhibits stomatal opening [8], provokes damages to the photosystems I and II [9], and inhibits some of the enzymes of the Calvin cycle [10]. The normal range of Cd concentration in leaf tissue (dry weight) of some species is 0.05–0.2 μg g^{-1}, however, hyperaccumulators have been known to accumulate Cd above 0.01% dry tissue (100 μg g^{-1}) [5].

Arthrocnemum macrostachyum (Moric) C. Koch is such a C3 shrub, distributed on coasts from S.W. Iberia, through Mediterranean region, to the Middle East and Asia. In the marshes of the south-west of Spain, it occurs in the middle to high elevations in the tidal range, where it is subject to occasional tidal inundations and seasonal hypersalinity [11]. This species has been previously used in phytoremediation studies; Conesa and Schulin [12] studied Cu, Pb and Zn uptake by *A. macrostachyum* in unstabilized mining wastes. Madejón et al. [13] analyzed the above-ground trace element accumulation in plants of this species growing on a highly contaminated estuary in order to asses the risk of wider dispersal pollutants in biota. As a result of field investigation in the SE of Spain, where only Cd content was determined, this species has

Abbreviations: Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; C_i, intercellular CO_2 concentration; C_x+c, carotenoids; F_0, minimal fluorescence level in the dark-adapted state; F_m, maximal fluorescence level in the dark-adapted state; F_v, variable fluorescence level in the dark-adapted state; F_v/F_m, maximum quantum efficiency of PSII photochemistry; Φ_{PSII}, quantum efficiency of PSII; G_s, stomatal conductance; NPQ, non-photochemical quenching; P_N, net photosynthetic rate; RGR, relative growth rate; WUE, water-use efficiency.

* Corresponding author. Tel.: +34 95 4557165; fax: +34 95 4615780.
E-mail address: susana@us.es (S. Redondo-Gómez).

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been described as a Cd-accumulator [14]. Nonetheless, no studies have reported its tolerance and the physiological impact of such bioaccumulation.

The main objectives of the present study were to evaluate the tolerance of *A. macrostachyum* to elevated levels of cadmium and test its ability in Cd extraction. The specific objectives were to: (1) analyze the growth of plants in experimental cadmium treatments ranging from 0 to 1.35 mmol l^{-1} Cd; (2) ascertain the extent to which effects on the photosynthetic apparatus (PSII chemistry), gas exchange characteristics and photosynthetic pigments determine plant performance with cadmium increasing; and (3) examine possible role of concentrations of mineral matter (ash), calcium, cadmium, copper, iron, manganese, magnesium, nitrogen, phosphorous, sodium, and zinc accumulated in response to increasing external Cd in explaining effects on growth.

2. Materials and methods

2.1. Plant material

In the genus *Arthrocnemum*, the leaves are highly reduced and the assimilatory surface is effectively composed of succulent branches. The photosynthetic part of the branch has an articulated structure, formed of succulent cylindrical internodes [15]. At both the proximal and distal ends of the branch, this succulent covering may atrophy, leaving hard, dry, lignified sections of stem.

Seeds of *A. macrostachyum* were collected in October 2008 from Odiel Marshes (37°15′N, 6°58′W; SW Iberian Peninsula). Physicochemical properties and metals concentrations of the sampling site have been previously described by Cambrollé et al. [16] (site 1). Seeds were placed in a germinator (ASL Aparatos Científicos M-92004, Spain), and subjected to an alternating diurnal regime of 10 h of light (photon flux rate, 400–700 nm, 35 μmol m^{-2} s^{-1}) at 20 °C/14 h of darkness at 5 °C, for 30 days. This temperature regime was chosen to mimic the autumn conditions in the Odiel marshes when this species germinates [17]. Seedlings were planted in individual plastic pots filled with perlite and placed in a glasshouse (37°23′N, 5°59′W; S.W. Iberian Peninsula) with controlled temperature of 21–25 °C, 40–60% relative humidity and natural daylight (maximum light flux: 1000 μmol m^{-2} s^{-1}). Immediately, the pots were allocated in shallow trays with 171 mM NaCl, since growth of *A. macrostachyum* has an optimum at this external salinity concentration [11]. Salinity treatment was established by combining 20% Hoagland's solution [18] and NaCl of the appropriate concentration. Thus, 3 l of the solution was placed in each of the trays (to a depth of 1 cm). The levels in the trays were monitored and they were topped up to the marked level with 20% Hoagland's solution (without NaCl) whenever necessary to maintain the salt concentration. In addition, the entire solution (including NaCl) was changed every 2 weeks.

2.2. Stress treatments

When seedlings were between 10 and 15 cm in height (after 12 months), the pots were allocated to five Cd treatments in shallow trays (seven pots per tray, with one tray per Cd treatment): 0, 0.05, 0.20, 0.65 and 1.35 mmol l^{-1} Cd, in the same glasshouse. Cd treatments were established by combining 20% Hoagland's solution and $CdCl_2$ of the appropriate concentration. Cd concentrations were chosen to cover variations recorded by Perez-Sirvent et al. [14] and Redondo-Gómez et al. [19] in the salt marshes of southern Iberian Peninsula.

At the beginning of the experiment 3 l of the appropriate solution were placed in each of the trays down to a depth of 1 cm. During the experiment, the levels in the trays were monitored and they were topped up to the marked level with 20% Hoagland's solution (without additional $CdCl_2$ or NaCl) as a way to limit the change of Cd and NaCl concentrations due to water evaporation of the nutritive solution. In addition, the entire solution (including $CdCl_2$ and NaCl) was changed on a weekly basis.

2.3. Growth analysis

Five plants were harvested at the beginning of the experiment (one plant per treatment), and six more were harvested from each treatment at the end (after 30 days of treatment); plants were dried at 80 °C for 48 h (enough for dry procedure) and weighed. The dry mass of shoot and root samples was determined.

The relative growth rate (RGR) in ash-free dry mass of whole plants was calculated by using the formula:

$$\mathrm{RGR} = (\ln B_f - \ln B_i) \cdot D^{-1} (\mathrm{g\ g^{-1}\ day^{-1}})$$

where B_f is the final dry mass, B_i is the initial dry mass (an average of the five plants dried at the beginning of the experiment) and D is the duration of experiment (days).

2.4. Chemical analysis of plant samples

In accordance with protocols of Redondo-Gómez et al. [17], at the end of the experiment, shoot and root samples were dried at 80 °C for 48 h and ground. Shoots and roots were carefully washed with distilled water before any further analysis. Then 0.5 g samples, taken from a mixture of the shoots or the roots belonging to the six plants used for each treatment, were tripicately digested with 6 ml HNO_3, 0.5 ml HF and 1 ml H_2O_2. Ca, Cd, Cu, Fe, Mg, Mn, Na, P and Zn were measured by inductively coupled plasma (ICP) spectroscopy (ARL-Fison 3410, USA). Total N and C concentrations were determined for undigested dry samples with an elemental analyzer (Leco CHNS-932, Spain).

2.5. Measurement of chlorophyll fluorescence

Chlorophyll fluorescence was measured ($n = 12$, two measurements per plant) using a portable modulated fluorimeter (FMS-2, Hansatech Instruments Ltd., England) after 24 h, and 7 and 30 days of treatment. Light and dark-adapted fluorescence parameters were measured at dawn (stable 50 μmol m^{-2} s^{-1} ambient light) and at mid-day (1600 μmol m^{-2} s^{-1}) to investigate whether Cd concentration affected the sensitivity of plants to photoinhibition.

Plants were dark-adapted for 30 min, using leaf-clips exclusively designed for this purpose. The minimal fluorescence level in the dark-adapted state (F_0) was measured using a modulated pulse (<0.05 μmol m^{-2} s^{-1} for 1.8 μs) which was too small to induce significant physiological changes in the plant. The data stored were an average taken over a 1.6 s period. Maximal fluorescence in this state (F_m) was measured after applying a saturating actinic light pulse of 15,000 μmol m^{-2} s^{-1} for 0.7 s. Values of the variable fluorescence ($F_v = F_m - F_0$) and maximum quantum efficiency of PSII photochemistry (F_v/F_m) were calculated from F_0 and F_m. This ratio of variable to maximal fluorescence correlates with the number of functional PSII reaction centers, and dark adapted values of F_v/F_m can be used to quantify photoinhibition [20].

The same internode section (the fifth internode from the distal end of the stem) of each plant was used to measure light-adapted parameters. Steady state fluorescence yield (F_s) was recorded after adapting plants to ambient light conditions for 30 min. A saturating actinic light pulse of 15,000 μmol m^{-2} s^{-1} for 0.7 s was then used to produce the maximum fluorescence yield (F'_m) by temporarily inhibiting PSII photochemistry.

Using fluorescence parameters determined in both light- and dark-adapted states, the following were calculated: quantum efficiency of PSII ($\Phi_{PSII} = (F'_m - F_s)/F'_m$) and non-photochemical quenching (NPQ = $(F_m - F'_m)/F_m$) [21].

2.6. Gas exchange

Measurements were taken from one primary branch of each of the plants in the five Cd treatments ($n = 6$) using an infrared gas analyzer in an open system (LI-6400, LI-COR Inc., Neb., USA) after 24 h, and 7 and 30 days of treatment. Net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i) and stomatal conductance to CO_2 (G_s) were all determined at an ambient CO_2 concentration of 360 μmol mol^{-1}, temperature of 25/28 °C, 50 ± 5% relative humidity and a photon flux density of 1000 μmol m^{-2} s^{-1}. Dark respiration was measured with the same equipment and under the same temperature and relative humidity conditions.

P_N, C_i and G_s were calculated using standard formulae from Von Caemmerer and Farquhar [22]. Photosynthetic area was approximated as half the area of the cylindrical branches, as only the upper half received the unilateral illumination in the leaf chamber. The water-use efficiency (WUE) was calculated as the ratio between P_N and transpiration rate [mmol (CO_2 assimilated) mol^{-1} (H_2O transpired)].

2.7. Photosynthetic pigments

Photosynthetic pigments of five shoots per treatment were extracted using 0.05 g of fresh material in 5 ml of 80% aqueous acetone. After filtering, 1 ml of the suspension was diluted with a further 2 ml of acetone and chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoid ($C_x + c$) contents were determined with a Hitachi U-2001 spectrophotometer (Hitachi Ltd., Japan), using three wavelengths (663.2, 646.8 and 470.0 nm). Concentrations of pigments (μg g fwt^{-1}) were obtained through calculation [23].

2.8. Statistical analysis

Statistical analysis was carried out using Statistica v. 6.0 (Statsoft Inc.). Pearson coefficients were calculated to assess correlation between different variables. Data were analyzed using one-way analysis of variance (*F*-test). Data were first tested for normality with the Kolmogorov–Smirnov test and for homogeneity of variance with the Brown–Forsythe test. Significant test results were followed by LSD test for identification of important contrasts. Differences between measurements of fluorescence at dawn and mid-day were compared by the Student's test (*t*-test). In all cases, a significance level of $p < 0.05$ was used.

3. Results

3.1. Growth

Mean relative growth rate (RGR) declined with increasing Cd concentration ($r = -0.89$, $P < 0.05$; Fig. 1). RGR was inhibited by Cd excess, since the lowest values were recorded at 0.65 and 1.35 mmol l^{-1} Cd (ANOVA, $P < 0.01$). However, Cd treated plants did not show chlorosis.

3.2. Chemical analysis of plant samples and ash concentrations

The mineral (ash) content of both shoots and roots were little affected by external Cd concentration (Fig. 2A). By the end of the experiment, ash contents were greater in the shoots than in roots, and this trend was entirely reflected in tissue Na concentrations (Fig. 2B).

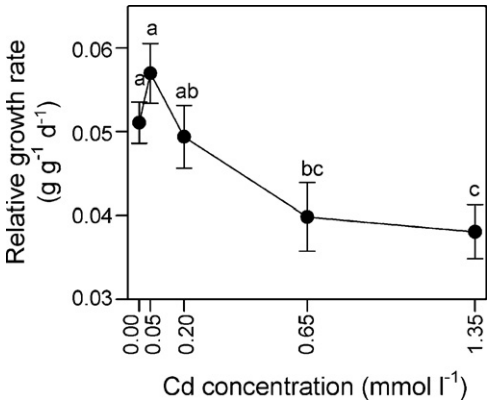

Fig. 1. Relative growth rate of *Arthrocnemum macrostachyum* in response to treatment with a range of Cd concentrations over 30 days. Values represent mean ± SE, $n = 6$. The analysis was carried out on an ash-free dry mass basis. Different letters indicate means that are significantly different from each other (LSD test, $P < 0.05$).

Overall, tissue Cd concentrations were greater in the roots than in leaves (ANOVA, $P < 0.0001$), and increased with external Cd concentration ($r = 0.98$, $P < 0.01$; $r = 0.93$, $P < 0.05$, for shoot and root, respectively; Fig. 2C). In contrast, shoot and root P and Ca, and root Mg concentrations showed no significant overall response to Cd concentration (Fig. 2D–F), although shoot Ca and root Mg concentrations were lower in absence of cadmium (ANOVA, $P < 0.0001$). Contrary, root Ca and Cu concentrations were higher at 0 mmol l^{-1} Cd ($P < 0.0001$; Fig. 2G).

On the other hand, shoot tissue magnesium concentration increased with external Cd concentration ($r = 0.94$, $P < 0.05$; Fig. 2F). Finally, tissue Fe, Mn and Zn concentrations in shoots (ca. 0.04 mg g^{-1}, for all Fe, Mn and Zn concentrations) and roots (ca. 0.5, 0.02 and 0.04 mg g^{-1}, for Fe, Mn and Zn concentrations, respectively) were similar for all Cd concentrations ($P > 0.05$, data not presented).

C/N ratio was considerably higher in the roots than in the shoots, and this ratio was little affected by external Cd concentration for both tissues (Fig. 2H).

3.3. Chlorophyll fluorescence

Values of F_v/F_m diminished, either at mid-day or dawn, with increasing Cd concentration (mid-day: 24 h, $r = -0.40$, $P < 0.01$; 7 days, $r = -0.39$, $P < 0.01$; and 30 days, $r = -0.56$, $P < 0.0001$; dawn: 24 h, $r = -0.34$, $P < 0.01$; 30 days, $r = -0.50$, $P < 0.0001$; Fig. 3). Furthermore, F_v/F_m values were significantly higher for the control at both mid-day and dawn after 30 days of treatment (ANOVA, $P < 0.0001$, for mid-day and dawn) because of higher values of F_m (data not presented). F_v/F_m was always lower at mid-day and the reductions resulted again mainly from the fact that values of F_m were lower at mid-day than at dawn (*t*-test, $P < 0.05$; Fig. 3).

The quantum efficiency of PSII (Φ_{PSII}) at mid-day did not show a significant relationship with Cd concentration at any time (Fig. 4A–C). By 7 days Φ_{PSII} values were significantly higher at 0 and 0.05 mmol l^{-1} Cd (ANOVA, $P < 0.01$). However, this effect disappeared after 30 days of treatment; there were no significant differences of Φ_{PSII} among Cd concentrations ($P > 0.05$; Fig. 4C). Finally, NPQ at mid-day did not show a significant relationship with Cd concentration at any time ($P > 0.05$; Fig. 4D–F).

3.4. Gas exchange

There was not relationship between net photosynthetic rate (P_N) and Cd concentration after 24 h and 7 days of treatment. Nonetheless, P_N declined significantly with increasing Cd concentration

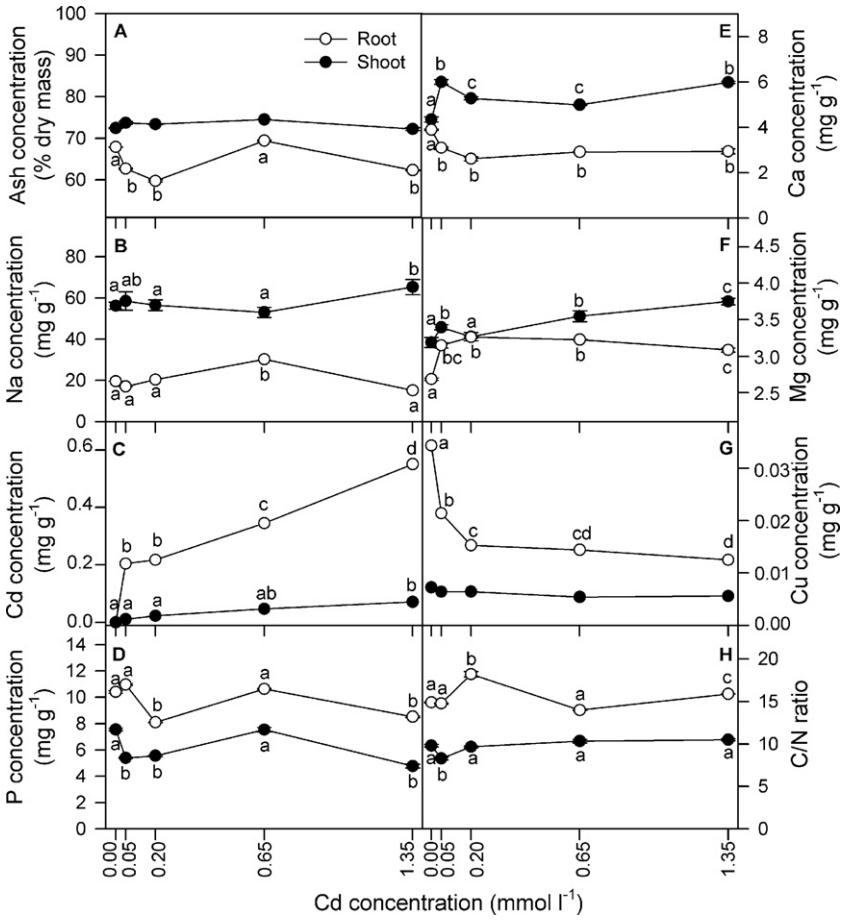

Fig. 2. Ash concentration (A), total sodium (B), total cadmium (C), total phosphorous (D), total calcium (E), total magnesium (F) and total copper (G) concentrations and C/N ratio (H) for shoot and root dry masses of *A. macrostachyum* in response to treatment with a range of Cd concentrations after 30 days. Values represent mean ± SE, $n = 3$. Different letters indicate means that are significantly different from each other (LSD test, $P < 0.05$).

after 30 days ($r = -0.93$, $P < 0.05$; Fig. 5A–C). Furthermore, there was a strong linear relationship between P_N and RGR after 30 days ($r = 0.96$, $P < 0.01$).

Moreover, there were no significant relationships between both stomatal conductance (G_s) and intercellular CO_2 concentration (C_i) and external Cd concentration, at 24 h or 30 days of treatment (Fig. 5D–I). By 7 days, however, G_s and C_i both declined significantly with increasing Cd concentration ($r = -0.41$, $P < 0.05$ and $r = -0.63$, $P < 0.01$, respectively; Fig. 5E and H). The G_s values recorded at 0 and 0.05 mmol l^{-1} Cd after 7 days were significantly higher than at other concentrations (ANOVA, $P < 0.05$).

Water-use efficiency (WUE) responded differently to cadmium at the early stage of the experiment than at later stages: it decreased significantly with Cd concentration after 24 h of treatment ($r = -0.52$, $P < 0.01$) but did not show relationship with Cd concentration after 7 and 30 days (Fig. 6A–C). Plants treated with Cd showed lower WUE values than the control after 24 h of treatment (ANOVA, $P < 0.01$). Dark respiration rate did not show a significant relationship with Cd concentration at any time, although it tended to increase with increasing Cd concentration after 30 days of treatment (Fig. 6D–F).

3.5. Photosynthetic pigment concentration

The control and plants treated with 0.05 mmol l^{-1} Cd showed higher pigment concentrations (Chl *a*, Chl *b* and $C_x + c$, all in μg g fwt^{-1}) than under the other treatments (ANOVA, $P < 0.05$; Fig. 7). Compared to the control, the reductions in both Chl *a* and *b* were ca. 30%, for plants treated with 0.20 and 1.35 mmol l^{-1} Cd, and 18% for 0.65 mmol l^{-1} Cd. While carotenoids diminished 25, 10 and 20% for 0.20, 0.65 and 1.35 mmol l^{-1} Cd, respectively.

4. Discussion

A. macrostachyum demonstrated hypertolerance to cadmium stress, since all plants survived and they did not show visible Cd toxicity symptoms such as necrosis or chlorosis; C/N ratio remained unaltered at elevated Cd concentrations. Previous studies have documented that plants can suffer toxic effects when the tissue cadmium concentration reaches 3–10 mg kg^{-1} dry weight [24]. However, *A. macrostachyum* did not show phytotoxicity at shoot concentration as high as 70 mg kg^{-1}. Relative growth rate of plants exposed to 0.05 and 0.20 mmol l^{-1} Cd had no significant differences compared with the control, in fact RGR was similar to optimal values described by Redondo-Gómez et al. [11] for *A. macrostachyum*. With the increase of Cd concentration in the medium growth decreased gradually, compared to the control, the reductions in RGR with 0.65 and 1.35 mmol l^{-1} Cd were 22 and 25%, respectively. Reduced RGR at high Cd concentrations can be attributed to the decline recorded in the photosynthetic carbon assimilation (P_N). Overt symptoms induced by elevated Cd contents of plants are growth retardation and inhibitory effects on photosynthesis; the excess Cd has a complex inhibitory impact on the Calvin cycle, and especially disturbs a function of the key enzyme, ribulose diphosphate carboxyhydrazine [10]. Nevertheless, effective concentration of *A.*

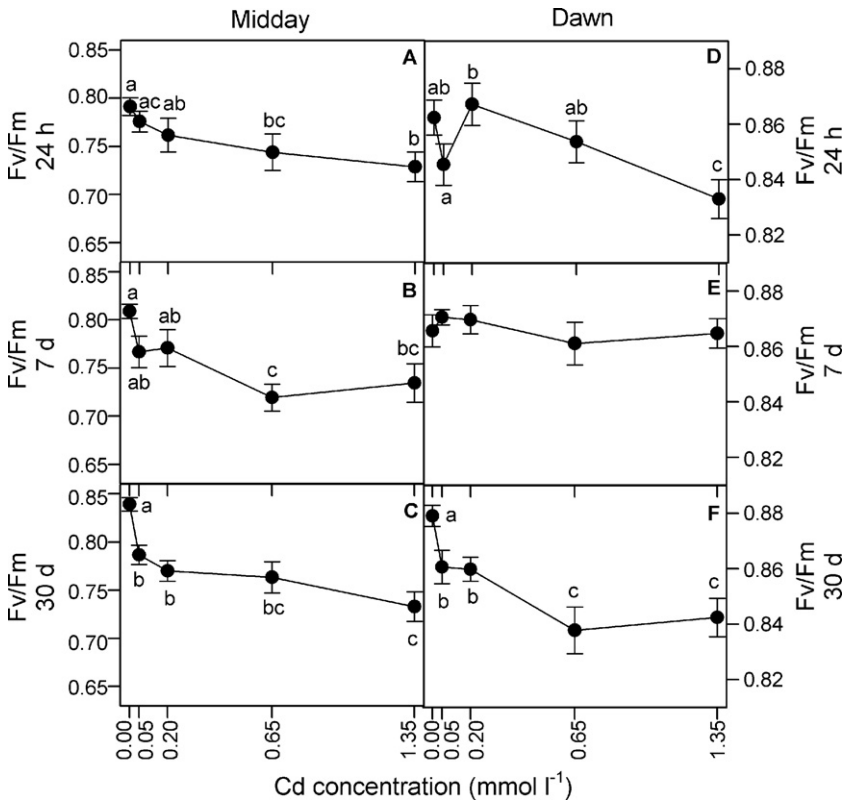

Fig. 3. Maximum quantum efficiency of PSII photochemistry (F_v/F_m) at mid-day (A–C) and at dawn (D–F) of *A. macrostachyum* in response to treatment with a range of Cd concentrations after 24 h (A, D), 7 days (B, E) and 30 days (C, F). Values represent mean ± SE, $n = 12$. Different letters indicate means that are significantly different from each other (LSD test, $P < 0.05$).

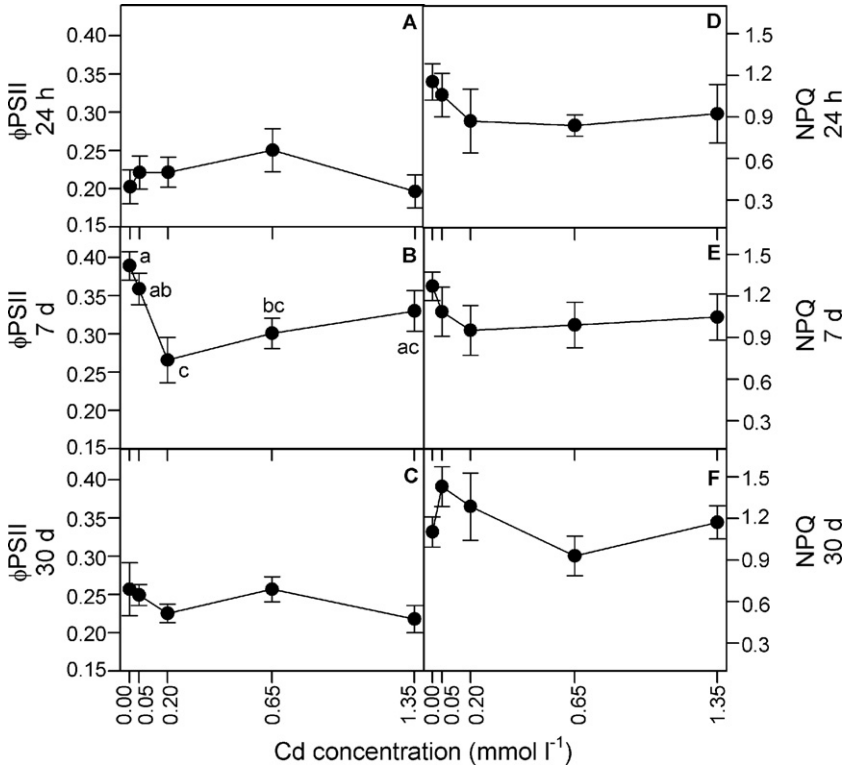

Fig. 4. (A–C) Quantum efficiency of PSII (Φ_{PSII}) and (D–F) non-photochemical quenching (NPQ) at mid-day of *A. macrostachyum* in response to treatment with a range of Cd concentrations after 24 h (A, D), 7 days (B, E) and 30 days (C, F). Values represent mean ± SE, $n = 12$. Different letters indicate means that are significantly different from each other (LSD test, $P < 0.05$).

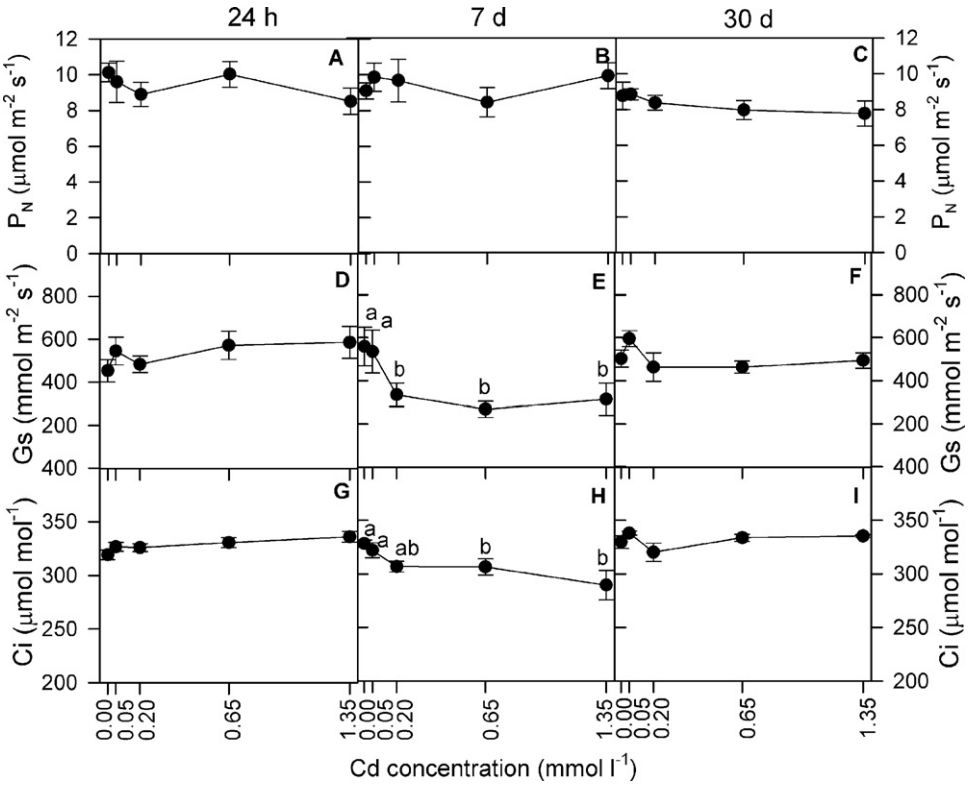

Fig. 5. (A–C) Net photosynthetic rate (P_N), (D–F) stomatal conductance (G_s), (G–I) intercellular CO_2 concentration (C_i) in *A. macrostachyum* in response to treatment with a range of Cd concentrations after 24 h (A, D, G), 7 days (B, E, H) and 30 days (C, F, I). Values represent mean ± SE, $n = 6$. Different letters indicate means that are significantly different from each other (LSD test, $P < 0.05$).

macrostachyum (EC50, substrate cadmium concentration resulting in 50% biomass reduction [25]) was higher than 1.35 mmol l^{-1} Cd. Zhang et al. [26] found that the measures of EC50 for the Cd-accumulator *Malva sinensis* ranged from 1.12 to 1.35 mmol l^{-1} Cd.

Bioaccumulator factor (BF) index, which is defined as the ratio of the metal concentration in the plant tissue to that in the soil [27], is used to evaluate metal accumulation efficiency in plants. The BF values of *A. macrostachyum* were 43, 12 and 5 for 0.05, 0.20 and 0.65 and 1.35 mmol l^{-1} Cd, respectively. Hence the BF values decreased with increasing Cd concentrations, which indicated a diminishing efficiency of Cd accumulation with increasing Cd concentrations. Accordingly, this may suggest that *A. macrostachyum* has a steady of Cd accumulation. Sun et al. [2] found similar result for Cd-hyperaccumulator *Bidens pilosa*. Nevertheless, the BF values of *A. macrostachyum* exceeded the critical value (1.0) under different Cd treatments. The BF values are more important than shoot concentration *per se* when one considers the potential of phytoextraction for a given species [28].

In our glasshouse experiment we found that Cd levels were much higher in *A. macrostachyum* subterraneous structures than in the aerial structures. Thus, the transfer factor (TF), which is defined as the ratio of the metal concentration in shoots to that in roots [27], were lower than 1.0 for all Cd treatments. Similar result was found by Pérez-Sirvent et al. [14] for *A. macrostachyum* grown in polluted soils. Nonetheless, phytoremediation efficiency depends on plant biomass and the ability of metal to be translocated to the shoots [29]. So, transport factor (TF′) from cadmium accumulation could be better parameter in indicating metal transport efficiency than TF [24]. The TF′ values of *A. macrostachyum* were 0.97, 1.03, 0.68 and 0.39 for 0.05, 0.20, 0.65 and 1.35 mmol l^{-1} Cd, respectively, which indicates that this species has higher ability to transfer Cd from roots to shoots at lower Cd concentrations (0.05 and 0.20 mmol l^{-1} Cd). These results show that *A. macrostachyum* has the basic characteristics of a Cd-hyperaccumulator.

Cd excess is expected to reduce root absorption of essential mineral elements (e.g. Mg and Fe) and consequent dysfunctions and structural changes arising from lack of these and other as well essential elements [30]. In our experiment the presence of Cd affected: slightly the root tissue Ca concentration, and markedly root Cu concentration. However, Cd–Fe, Cd–Mn, Cd–Na, Cd–P and Cd–Zn interactions were not found, despite these interactions have been widely observed and reported [10]; a fact which could explain that the ash content remained unaltered. Furthermore, shoot and root Mg and shoot Ca concentrations were enhanced by the presence of Cd. Synergism may be a secondary effect of the damage to membranes due to the imbalanced proportions of the metals. Thus, Kabata-Pendias and Pendias [10] commented that Cd can alter the permeability of cell membranes.

The maximum quantum efficiency of PSII photochemistry (F_v/F_m) did show a significant reduction at mid-day compared to dawn values. At mid-day, the reduction in F_v/F_m values indicated that *A. macrostachyum* experienced photoinhibition at the higher light flux. This photoinhibition is caused by a lower proportion of open reaction centers (lower values of F_m) resulting from a saturation of photosynthesis by light. Otherwise, F_v/F_m was clearly affected by Cd stress at all stages of the experiment, indicating that cadmium excess enhances photoinhibition induced by light stress. Tukendorf and Baszynki [31] similarly reported that photochemical activities of chloroplasts were significantly reduced at Cd concentrations of 0.050–0.075 mmol l^{-1} Cd in the nutrient medium. Photoinhibition is caused by damage to photosynthetic components, and this effect can be short-term and reversible (dynamic photoinhibition) or long-term and irreversible (chronic photoinhibition) [32]. F_v/F_m values at dawn remained lower than control parameters for unstressed plants [33] after 30 days of treatment,

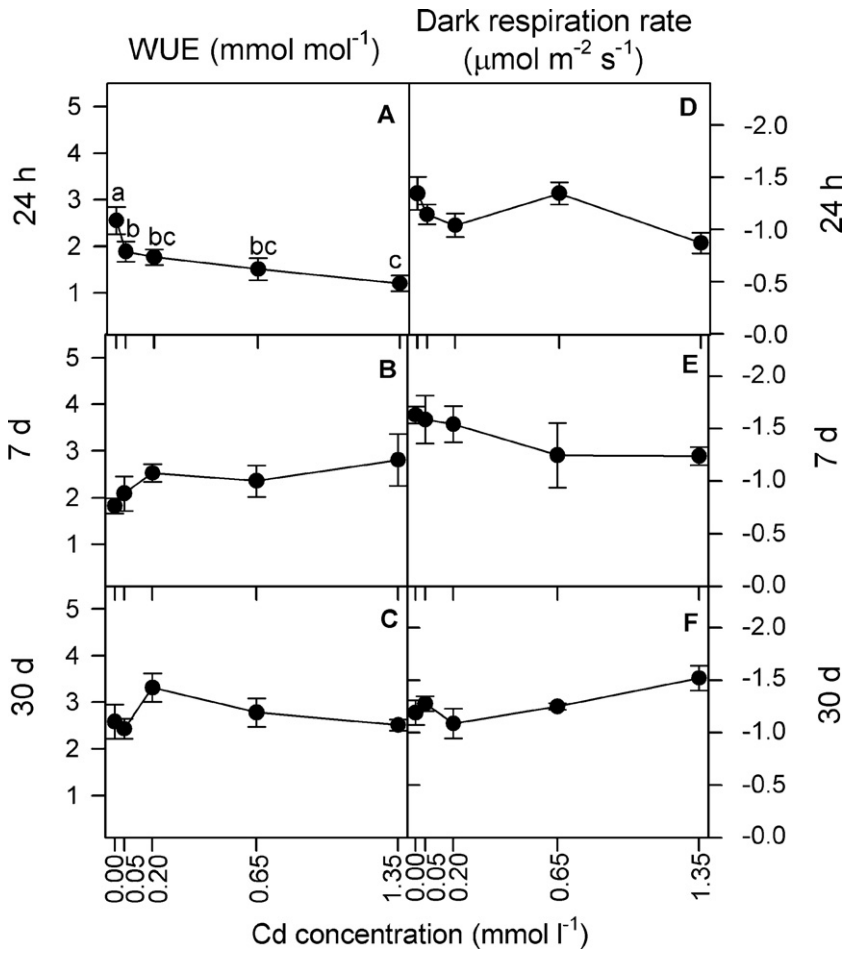

Fig. 6. (A–C) Water-use efficiency (WUE) and (D–F) dark respiration rate in *A. macrostachyum* in response to treatment with a range of Cd concentrations after 24 h (A, D), 7 days (B, E) and 30 days (C, F). Values represent mean ± SE, $n = 6$. Different letters indicate means that are significantly different from each other (LSD test, $P < 0.05$).

revealing a chronic photoinhibition. Moreover, Hormaetxe et al. [34] suggested that chronic photoinhibition would play a photoprotective role, reducing the efficiency of light energy capture.

Under laboratory conditions, there is usually a linear relationship between Φ_{PSII} and net photosynthetic rate [20]. However, in this experiment under stressful conditions that caused P_N to decrease slightly after 30 days of treatment, Φ_{PSII} remained unaltered. This disparity may be explained by changes in dark respiration rate, as this tended to increase progressively with increasing external Cd after 30 days of treatment. The plants could be increasing dark respiration as additional photoprotective mechanisms. This pathway can lead to an additional consumption of reducing equivalents and can thus function as sinks for excessive excitation energy [21,35]. This physiological process could be relevant mechanism to protect *A. macrostachyum* against excess of radiation under high Cd, since the relatively stable NPQ across the cadmium range would indicate that Cd does not produce an increase in thermal dissipation in the PSII antennae. In contrast, Pietrini et al. [36] reported that Cd reduced Φ_{PSII} of poplar clones treated with 0.05 mmol l^{-1} Cd.

There were not clear effects of Cd on stomatal conductance or water-use efficiency in our experiment. In the early stages of the experiment (24 h), where *A. macrostachyum* were probably experiencing a degree of osmotic shock, there was a decline of WUE in plants treated with Cd. Furthermore, at 7 days of the experiment intercellular CO_2 concentration showed a negative association with external Cd, which can be explained by the decrease in G_s, although there was not evidence of a reduction in the later stage of the experiment. Thus, the decrease in chlorophyll and total carotenoid contents of *A. macrostachyum* on a fresh-mass basis, or increase in its degradation, and consequent negative effect on photosynthetic electron transport, could lead as well to a decline in the photosynthetic function. The Cd-induced decrease in the chlorophyll level has been widely reported [5,10]. In fact, the decreased chlorophyll content related to higher Cd concentration could be used to monitor Cd-induced damage [37].

The comparison of growth and photosynthetic responses of *A. macrostachyum* in experimental cadmium treatments, ranging from 0 to 1.35 mmol l^{-1} Cd (0–150 ppm), has provided new insight into Cd tolerance in an extreme halophyte. Unaffected photosynthesis at the early stages of the experiment and the slight decline recorded after 30 days of treatment, as well as, unaffected Φ_{PSII} may indicate that *A. macrostachyum* is not experiencing metal toxicity and, then, that it produces metabolites for further absorption, protection and growth [6]. Furthermore, Cd excess did not affect water relations of this species or absorption of essential mineral elements. Moreover, the lower Cd concentration in shoots of *A. macrostachyum*, compared to that present in roots, could be accounted for by the development of such mechanisms, as compartmentation, which could control the ion transport into leaves, thereby improving its tolerance to Cd. Likewise, increased dark respiration and photoinhibition, as photoprotective mechanisms, might improve tolerance of *A. macrostachyum* to Cd stress. All these results confirm that *A. macrostachyum* is a hypertolerant species to cadmium. Finally, results of hypertolerance, concentration of Cd in roots and shoots, BF and TF′ indicate that *A. macrostachyum* has the

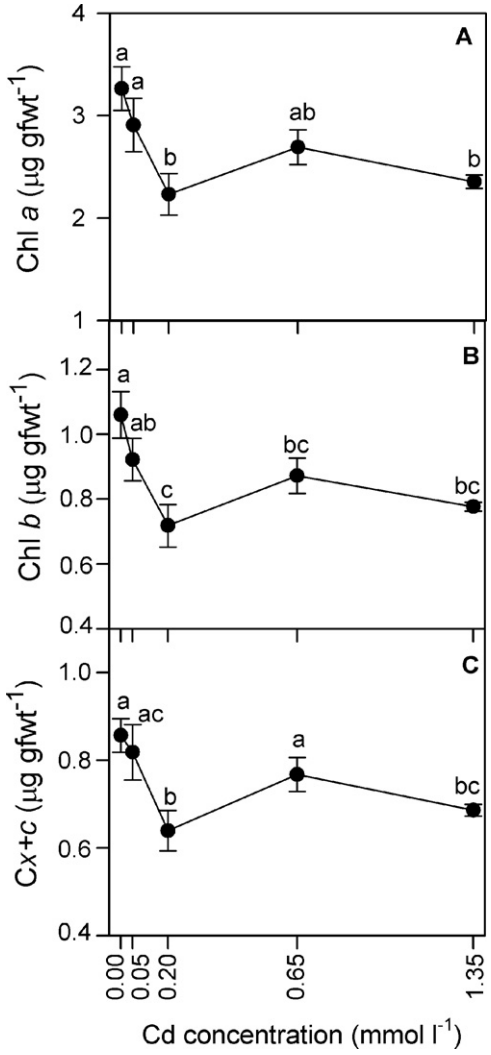

Fig. 7. (A) Chlorophyll *a* (Chl *a*), (B) chlorophyll *b* (Chl *b*) and (C) carotenoid ($C_x + c$) concentrations in *A. macrostachyum* in response to treatment with a range of Cd concentrations after 30 days. Values represent mean ± SE, $n = 5$. Different letters indicate means that are significantly different from each other (LSD test, $P < 0.05$).

basic characteristics of a Cd-hyperaccumulator and may potentially be useful for restoring Cd-contaminated sites.

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