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Accumulation and tolerance characteristics of chromium in a cordgrass Cr-hyperaccumulator, *Spartina argentinensis*

Susana Redondo-Gómez[a,*], Enrique Mateos-Naranjo[a], Inmaculada Vecino-Bueno[a], Susana R. Feldman[b]

[a] *Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, Apartado 1095, 41080 Sevilla, Spain*
[b] *Biología, Facultad de Ciencias Agrarias y CIUNR, Universidad Nacional de Rosario, Spain*

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ABSTRACT

The cordgrass *Spartina argentinensis*, which occurs in inland marshes of the Chaco-Pampean regions of Argentina, has been found to be a new chromium hyperaccumulator. A glasshouse experiment was designed to investigate the effect of Cr^{6+} from 0 to 20 mmol l^{-1} on growth and photosynthetic apparatus of *S. argentinensis* by measuring chlorophyll fluorescence parameters, gas exchange and photosynthetic pigment concentrations. Boron, calcium, chromium, copper, iron, manganese, magnesium, potassium and phosphorous concentrations were also determined. *S. argentinensis* showed phytotoxicity at tiller concentration of 4 mg g^{-1} Cr, and symptoms of stress at tiller concentration of 1.5 mg g^{-1} Cr, as well as reductions in leaf gas exchange, in chlorophyll *a* fluorescence parameters, in photosynthetic pigment contents and in the uptake of essential nutrients. Reductions in net photosynthetic rate could be accounted for by non-stomatal limitations. Moreover, the bioaccumulator factors exceeded greatly the critical value (1.0) for all Cr treatments, and the transport factors indicated that this species has a higher ability to transfer Cr from roots to tillers at higher Cr concentrations. These results confirmed that *S. argentinensis* is a chromium hyperaccumulator and that it may be useful for restoring Cr-contaminated sites.

1. Introduction

Man has severely impacted on the environment since the invention of agriculture, but industrial processes increase harmful side effects, including the accumulation of heavy metals. Therefore, environmental pollution has become a major concern nowadays. Anthropogenic sources of Cr are responsible for the elevated content of this metal in plants. Elevated contents of Cr (up to 600 ppm) in some phosphate fertilizers may be a significant source of this metal in soils, although the most hazardous addition of Cr to a soil is related to tannery sludges, which can contain up to 2.8% of this metal [1]. A special environment concern, however, is the oxidation stage of Cr in tannery sludges, as Cr^{6+} is a highly reactive form that influences both plants and animals [2]. It has been demonstrated that, at high concentrations, chromium is mutagen, teratogen and carcinogen [3]. Cr also causes deleterious effects on plant physiological processes such as photosynthesis, water relations, and mineral nutrition [4]. Thus, there is an urgent and imperative need to develop efficient techniques for chromium removal from the environment.

Phytoremediation has become a promising soil remediation technique, its success being dependent on the ability of the selected plant species for accumulating high concentrations of metal in the shoots and to produce high biomass [5]. Among the species used for chromium phytoremediation we find *Pluchea indica* and *Cynodon dactylon* [6], *Phragmites australis*, *Typha angustifolia* [7,8], *Pterocarpus indicus* and *Jatropha curcas* [9]. However, it is very important to indentify new feasible hyperaccumulators or accumulators of Cr as the groundwork for the successful phytoremediation of Cr-contaminated soils.

The genus *Spartina* comprise C_4 grasses that thrive on severe, saline, frequently flooded areas and have been shown to be useful for bioremediation [10,11]. Cambrollé et al. [12] found that heavy metals (As, Cu, Fe, Mn, Pb and Zn) accumulated at different rates

Abbreviations: A, net photosynthetic rate; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; Ci, 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; Gs, stomatal conductance; NPQ, non-photochemical quenching; RGR, relative growth rate; WUE, water use efficiency.

* Corresponding author at: Dpto. Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, Avda. Reina Mercedes s/n, 41012 Sevilla, Spain.
Tel.: +34 95 4557165; fax: +34 95 4615780.
E-mail address: susana@us.es (S. Redondo-Gómez).

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in *S. densiflora* and *S. maritima* tissues and around their roots, concluding that these species could be used for phytoremediation and phytostabilization of estuarine sediments.

Spartina argentinensis Parodi is the dominant species of temporally flooded inland marsh communities of Argentina, growing in hydro-halomorphic soils with high Na concentration in the upper layers. These communities cover approximately three millions hectares in the Chaco-Pampean regions of central Argentina [13]. Considering that this species has high photosynthetic and growth rates, resprouts after disturbance even under severe drought conditions [14,15], can grow on contaminated substrates [16], and that the closely related species, *S. densiflora* can accumulate several metals [12], it was hypothesized that *S. argentinensis* could be used for chromium phytoremediation. Consequently, the main objectives of the present study were to evaluate the tolerance of *S. argentinensis* to elevated levels of chromium and test its ability in Cr extraction. The specific objectives were to: (1) analyze the growth of plants in experimental chromium treatments ranging from 0 to 20 mmol l^{-1} Cr; (2) ascertain the extent to which effects on the photosynthetic apparatus (PSII chemistry), gas exchange characteristics and photosynthetic pigments determine plant performance with chromium increasing; and (3) examine possible role of concentrations of boron, calcium, chromium, copper, iron, manganese, magnesium, potassium and phosphorous accumulated in response to increasing external Cr in explaining effects on growth.

2. Materials and methods

2.1. Plant material

Seeds of *S. argentinensis* were collected in January 2009 from an inland marsh at the reserve Federico Wildermuth (Colonia Bel Grano, Dto. San Martín, 31°57′S, 61°23′W; Santa Fe, Argentina). 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. Seedlings were planted in individual plastic pots (11 cm of diameter) filled with an inert substrate, pearlite, 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}). Pots were carefully irrigated with 20% Hoagland's solution [17] as necessary. The possibility of adding NaCl to the culture medium was disregarded because salt does not affect either the photosynthetic functions or the growth of *S. argentinensis*.

2.2. Stress treatments

In June 2009, when plants had between 10 and 15 tillers (after six months), the pots were allocated to six Cr treatments in shallow trays (seven pots per tray, with one tray per Cr treatment): 0, 1, 2, 5, 10 and 20 mmol l^{-1} Cr, in the same glasshouse. Cr treatments were established by combining 20% Hoagland's solution and $K_2Cr_2O_7$ (Cr^{6+}) of the appropriate concentration. Plants were grown for 15 days under the previously described conditions, long enough to assess the accumulation capacity and tolerance in Cr-treated plants [18–20].

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 $K_2Cr_2O_7$) as a way to limit the change of Cr concentrations due to water evaporation of the nutritive solution. In addition, the entire solution (including $K_2Cr_2O_7$) was changed on a weekly basis to avoid excessive changes in Cr concentration. The pH of Cr solutions was ca. 6.8 during the experiment period and it was weekly measured using a portable meter and electrode system (Crison pH/mV p-506, Spain). At this pH value, spontaneous reduction of Cr^{6+} to Cr^{3+} and/or Cr^{3+} precipitation were not detected [18].

2.3. Growth analysis

Six plants were harvested at the beginning of the experiment (one plant per treatment), and six more were harvested from each treatment at the end (i.e. after 15 days of treatment); plants were dried at 80 °C for 48 h and weighed. The dry mass of shoot and root samples was determined.

The relative growth rate (RGR) of whole plants was calculated by using the formula:

$$\mathrm{RGR} = (\ln \mathrm{Bf} - \ln \mathrm{Bi})D^{-1} (\mathrm{g\,g^{-1}\,day^{-1}})$$

where Bf = final dry mass, Bi = initial dry mass (an average of the six plants dried at the beginning of the experiment) and D = duration of experiment (days).

2.4. Chemical analysis of plant samples

In accordance with protocols of Redondo-Gómez et al. [21], at the end of the experiment, tiller and root samples were dried at 80 °C for 48 h and ground. Tillers and roots were carefully washed with distilled water before any further analysis. Then 0.5 g samples, taken from a mixture of the tillers or the roots belonging to the six plants used for each treatment, were digested with 6 ml HNO_3, 0.5 ml HF and 1 ml H_2O_2. B, Ca, Cu, Cr, Fe, K, Mg, Mn, Na and S were measured by inductively coupled plasma (ICP-AES) spectroscopy (ARL-Fisons 3410, USA). Reference materials from Fisons certified for B, Ca, Cu, Cr, Fe, K, Mg, Mn, Na and S were used to check accuracy and precision in the analysis of total elements. The average uncertainty of elements determination was in all cases <2% (see Camrbollé et al. [12] for more details).

2.5. Measurement of chlorophyll fluorescence

Chlorophyll fluorescence was measured in random, fully developed penultimate leaves (n = 12, two measurements per plant) using a portable modulated fluorimeter (FMS-2, Hansatech Instruments Ltd., England) after 2, 9 and 15 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 Cr 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 centres, and dark adapted values of F_v/F_m can be used to quantify photoinhibition [22].

The same leaf section 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

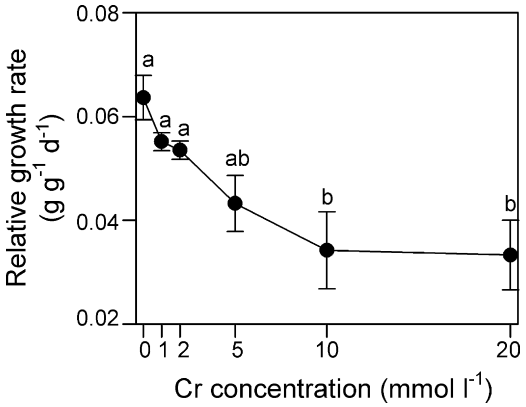

Fig. 1. Relative growth rate of *Spartina argentinensis* in response to treatment with a range of Cr concentrations over 15 days. Values represent mean ± SE, $n = 6$. Different letters indicate means that are significantly different from each other (LSD test, $P < 0.05$).

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 ($NPQ = (F_m - F_m')/F_m'$) [23].

2.6. Gas exchange

Measurements were taken on random, fully expanded leaves ($n = 6$) using an infrared gas analyzer in an open system (LI-6400, LI-COR Inc., Neb., USA) after 2, 9 and 15 days of treatment. Net photosynthetic rate (A), intercellular CO_2 concentration (Ci) and stomatal conductance to CO_2 (Gs) 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.

A, Ci and Gs were calculated using standard formulae from Von Caemmerer and Farquhar [24]. Photosynthetic area was approximated as the area of a trapezium. The water-use efficiency (WUE) was calculated as the ratio between A and transpiration rate [mmol (CO_2 assimilated) mol^{-1} (H_2O transpired)].

2.7. Photosynthetic pigments

Photosynthetic pigments of five tillers 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 [25].

2.8. Statistical analysis

Statistical analysis was carried out using Statistica v. 6.0 (Statsoft Inc.). Pearson coefficients were calculated to assess correlation between Cr concentration and different variables, and between relative growth rate and net photosynthetic rate. Data were analyzed using a one-way analysis of variance (*F*-test). Data were first tested

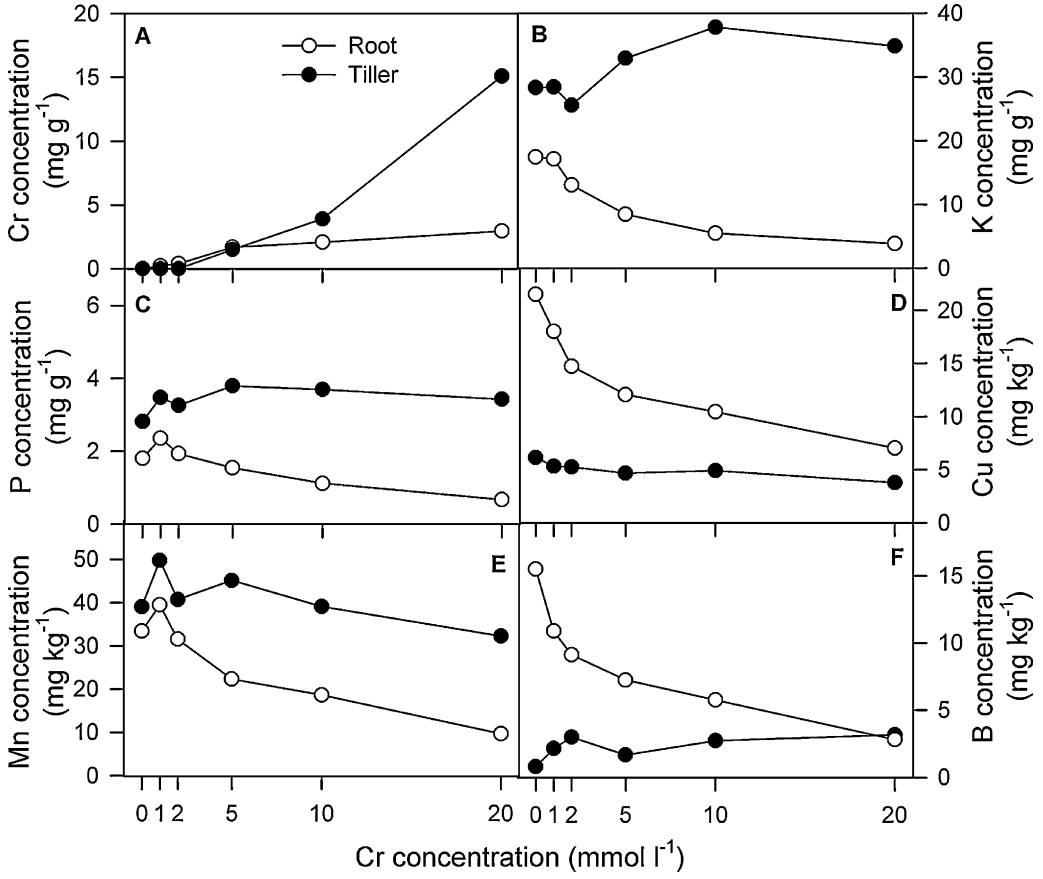

Fig. 2. Total chromium (A), total potassium (B), total phosphorous (C), total copper (D), total manganese (E) and total boron (F) concentrations for tiller and root dry masses of *Spartina argentinensis* in response to treatment with a range of Cr concentrations after 15 days.

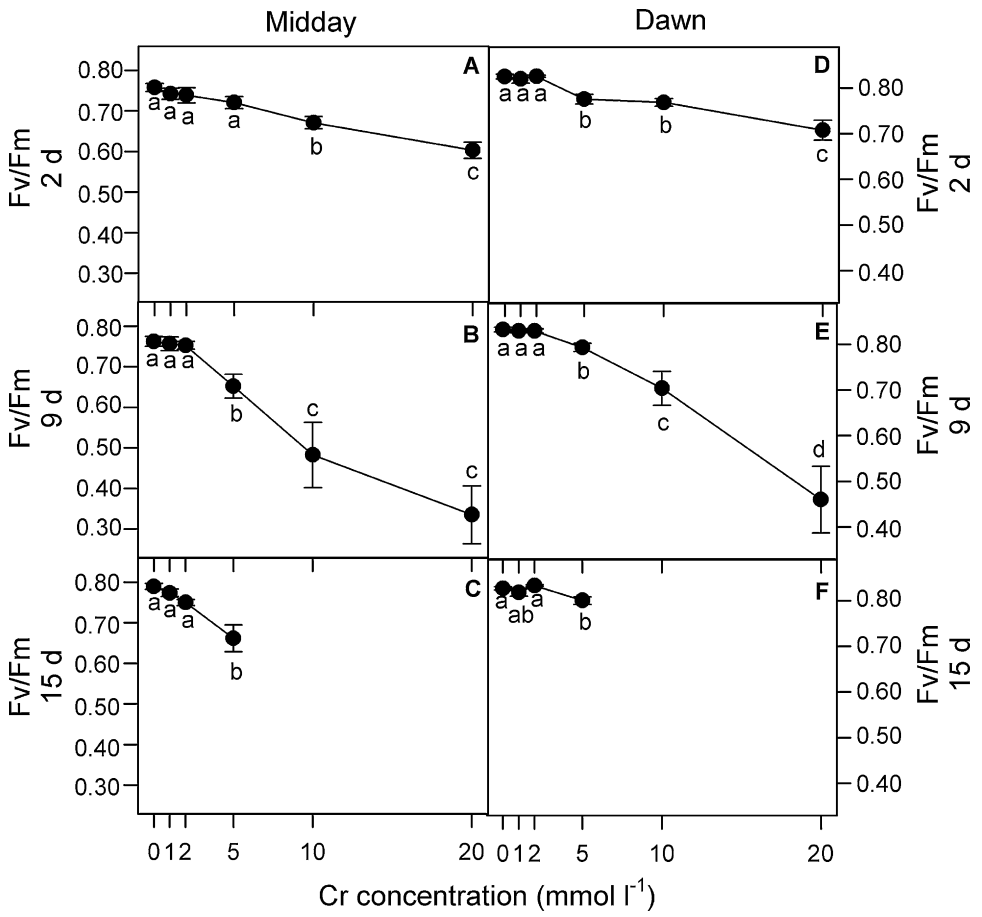

Fig. 3. Maximum quantum efficiency of PSII photochemistry (F_v/F_m) at midday (A–C) and at dawn (D–F) of *Spartina argentinensis* in response to treatment with a range of Cr concentrations after 2 days (A and D), 9 days (B and E) and 15 days (C and F). Values represent mean ± SE, $n = 12$. Different letters indicate means that are significantly different from each other (LSD test, $P < 0.05$).

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 midday were compared by the Student 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 Cr concentration ($r = -0.61$, $P < 0.01$; Fig. 1). Plants grown at 10 and 20 mmol l^{-1} Cr showed a marked chlorosis, especially at the highest Cr concentration.

3.2. Chemical analysis of plant samples

Overall, tissue Cr concentrations were greater in the tillers than in roots, and increased with external Cr concentration ($r = 0.97$, $P < 0.01$; $r = 0.94$, $P < 0.01$, for tiller and root, respectively; Fig. 2A). In contrast, tiller and root Ca, Mg and Fe concentrations showed no significant response to increasing Cr concentration; Ca concentrations ranged between 2 and 4 mg g^{-1} for roots and between 5 and 6 and mg g^{-1} for tillers, Mg concentrations were ca. 1.1 mg g^{-1} for roots and ranged between 3 and 4 mg g^{-1} for tillers, while tissue Fe concentrations were lower in presence of chromium (ca. 0.3 and 0.1 mg g^{-1} for root and tiller, respectively) than at 0 mmol l^{-1} Cr (0.4 mg g^{-1} for root and tiller).

On the other hand, root tissue K, P, Cu, Mn and B concentrations decreased with external Cr concentration (K: $r = -0.88$, $P < 0.05$; P: $r = -0.92$, $P < 0.01$; Cu: $r = -0.89$, $P < 0.05$; Mn: $r = -0.93$, $P < 0.01$; B: $r = -0.87$, $P < 0.05$; Fig. 2B–F). Finally, tissue Cu concentration in tillers diminished with increasing Cr concentration ($r = -0.89$, $P < 0.05$; Fig. 2D).

3.3. Chlorophyll fluorescence

Values of F_v/F_m declined, both at midday and dawn, with increasing Cr concentration, and this parameter was the most significantly correlative to Cr concentration (midday: 2 days, $r = -0.99$, $P < 0.0001$; 9 days, $r = -0.98$, $P < 0.0001$; and 15 days, $r = -0.99$, $P < 0.01$; dawn: 2 days, $r = -0.97$, $P < 0.01$; 9 days, $r = -0.98$, $P < 0.0001$; Fig. 3). The lowest F_v/F_m values were recorded at 10 and 20 mmol l^{-1} Cr at both dawn and midday after 9 days of treatment (ANOVA, $P < 0.0001$; Fig. 3B and E), and at 5 mmol l^{-1} Cr at midday after 15 days of treatment (ANOVA, $P < 0.01$; Fig. 3C). Furthermore, F_v/F_m was always lower at midday (*t*-test, $P < 0.05$; Fig. 3) and the reductions resulted mainly from the fact that values of Fm were lower at midday than at dawn (data not presented).

Plants grown at 10 and 20 mmol l^{-1} Cr showed fluorescence parameters outside the detection range of fluorimeter at both dawn and midday after 15 days of treatment, and therefore they have not been represented in Figs. 3 and 4.

The quantum efficiency of PSII (Φ_{PSII}) at midday showed a significant relationship with Cr concentration after 2 and 9 days of treatment (2 days, $r = -0.98$, $P < 0.01$; 9 days, $r = -0.96$, $P < 0.01$; Fig. 4A and B). By 2 days Φ_{PSII} values were significantly

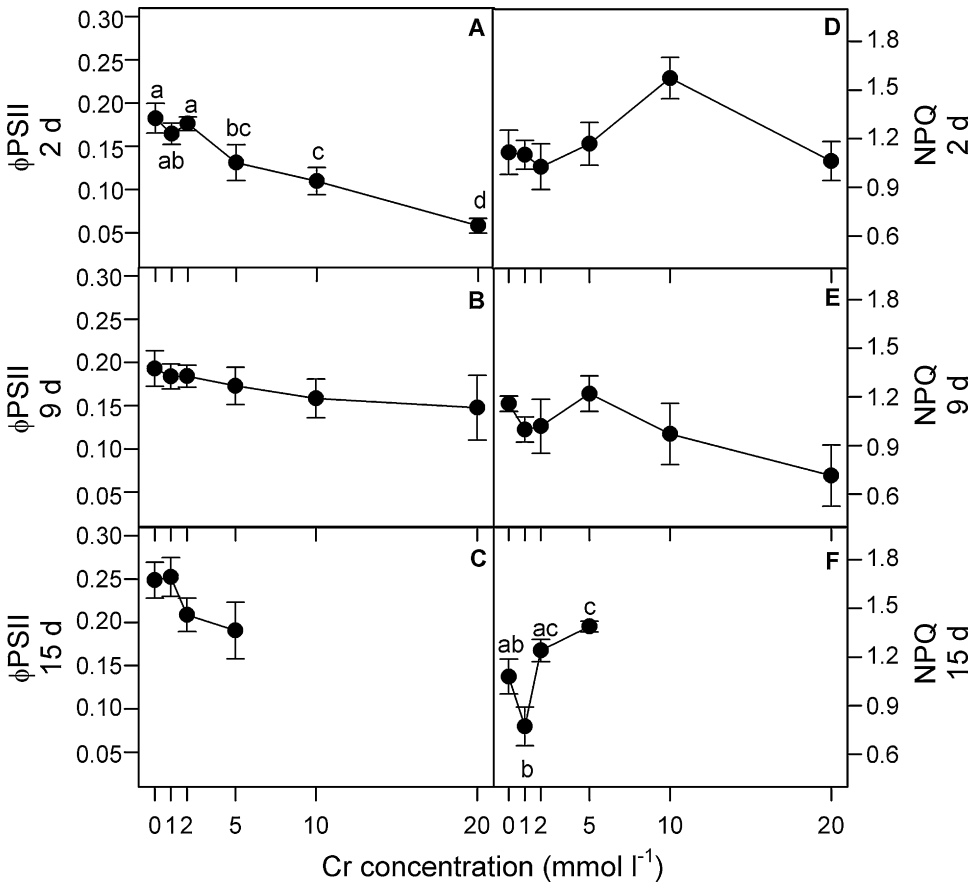

Fig. 4. (A–C) Quantum efficiency of PSII (Φ_{PSII}) and (D–F) non-photochemical quenching (NPQ) at midday of *Spartina argentinensis* in response to treatment with a range of Cr concentrations after 2 days (A and D), 9 days (B and E) and 15 days (C and F). Values represent mean ± SE, $n = 12$. Different letters indicate means that are significantly different from each other (LSD test, $P < 0.05$).

lower at 20 mmol l^{-1} Cr (ANOVA, $P < 0.01$; Fig. 4A). However, this effect disappeared after 9 days of treatment; there were no significant differences of Φ_{PSII} among Cr concentrations ($P > 0.05$; Fig. 4B and C). Finally, NPQ at midday did not show a significant relationship with Cr concentration at any time ($P > 0.05$; Fig. 4D–F).

3.4. Gas exchange

Net photosynthetic rate (A) declined with increasing Cr concentration during the course of the experiment (2 days: $r = -0.96$, $P < 0.01$; 9 days: $r = -0.93$, $P < 0.01$; 15 days: $r = -0.87$, $P < 0.05$); and the lowest A values were recorded at 10 and 20 mmol l^{-1} Cr (ANOVA, $P < 0.0001$; Fig. 5A–C). Furthermore, there was a significant relationship between RGR and A after 15 days ($r = 0.96$, $P < 0.01$).

Moreover, there were significant relationships between both stomatal conductance (Gs) and intercellular CO_2 concentration (Ci) and external Cr concentration at all sampling time (Fig. 5D–I); Gs decreased with increasing Cr concentration (2 days: $r = -0.91$, $P < 0.05$; 9 days: $r = -0.93$, $P < 0.01$; 15 days: $r = -0.97$, $P < 0.01$), while Ci increased (2 days: $r = 0.98$, $P < 0.0001$; 9 days: $r = 0.97$, $P < 0.01$; 15 days: $r = 0.98$, $P < 0.01$).

Water use efficiency (WUE) decreased significantly with Cr concentration during the course of the experiment (2 days: $r = -0.92$, $P < 0.01$; 9 and 15 days: $r = -0.90$, $P < 0.05$). Plants grown at 20 mmol l^{-1} Cr showed the lowest WUE at all sampling time (ANOVA, $P < 0.0001$; Fig. 6).

3.5. Photosynthetic pigment concentration

Pigment concentrations (Chl a, Chl b and C$x+c$, all in μg g fwt^{-1}) diminished with increasing external Cr concentration after 15 days of treatment (Chl a: $r = -0.95$, $P < 0.01$; Chl b: $r = -0.96$, $P < 0.01$; C$x+c$: $r = -0.97$, $P < 0.001$; Fig. 7). Plants treated with the two highest Cr concentrations showed the lowest pigments contents (ANOVA, $P < 0.0001$). Compared to the control, the reductions in Chl a, Chl b and C$x+c$ were ca. 68, 58 and 47%, respectively, for plants treated with 10 mmol l^{-1} Cr, and 90, 78 and 83% for 20 mmol l^{-1} Cr.

4. Discussion

Results showed that tillers of *Spartina argentinensis* are capable of accumulating substantial concentration of Cr, up to 15.1 mg g^{-1} DW from a 20 mmol l^{-1} Cr solution after 15 days of treatment. The maximum chromium concentration in the dry shoot matter of the hyperaccumulator *Prosopis laevigata* reached 5.5 mg g^{-1} [26], and ca. 6 mg g^{-1} in leaves of *Salvinia natans* [19]. 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 *S. argentinensis* exceeded the critical value (1.0) under all Cr treatments (Table 1), and the maximum BF value was recorded at 5 mmol l^{-1} Cr.

Moreover, we found that Cr levels were much higher in *S. argentinensis* aerial structures than in the subterraneous structures from 10 mmol l^{-1}Cr. Yadav et al. [28] reported that Cr accumulation in shoots of *Jatropha curcas* was relatively low due to transport barriers. Thus, these barriers could limit the ion transport into tillers

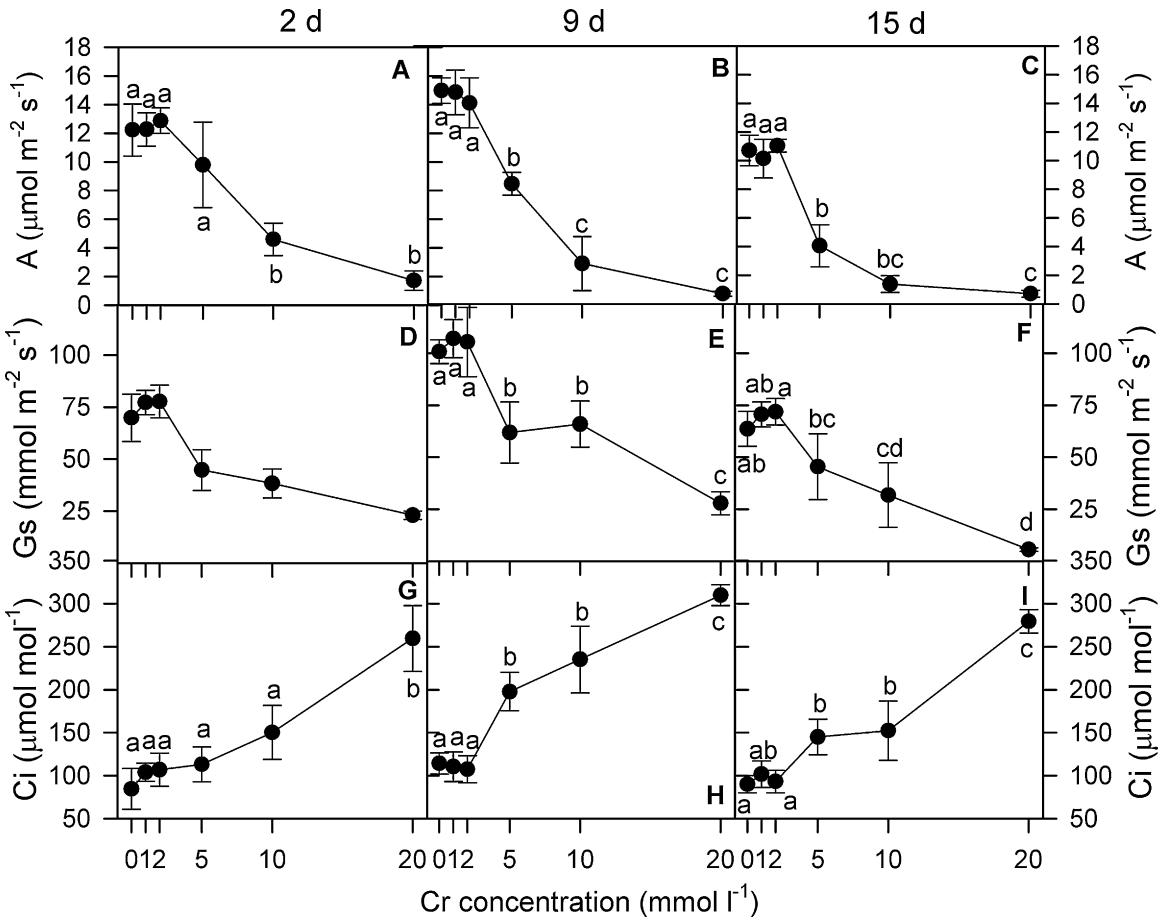

Fig. 5. (A–C) Net photosynthetic rate (*A*), (D–F) stomatal conductance (Gs), (G–I) intercellular CO_2 concentration (Ci) in *Spartina argentinensis* in response to treatment with a range of Cr concentrations after 2 days (A, D, G), 9 days (B, E, H) and 15 days (C, F, I). Values represent mean ± SE, $n = 6$. Different letters indicate means that are significantly different from each other (LSD test, $P < 0.05$).

of *S. argentinensis* up to 5 mmol l^{-1} Cr. Accordingly, the transfer factor (TF), which is defined as the ratio of the metal concentration in shoots to that in roots [27], was lower than 1.0 for 1–5 mmol l^{-1} Cr treatments. In contrast, TF values were higher than 1.0 at 10 and 20 mmol l^{-1} Cr (Table 1). Nonetheless, phytorremediation efficiency depends on plant biomass and the ability of metal to be translocated to the shoots [29]. Thus, transport factor (TF′) from chromium accumulation could be a better parameter in indicating metal transport efficiency than TF [30]. The TF′ values of *S. argentinensis* were higher than 1.0 from 5 mmol l^{-1} Cr, which indicates that this species displays a higher ability to transfer Cr from roots to tillers at higher Cr concentrations. These results show that *S. argentinensis* has the basic characteristics of a Cr-hyperaccumulator.

S. argentinensis plants responded to the Cr^{6+} supply in nutrient solution by developing visible symptoms of stress at the two highest Cr concentrations, such as tiller chlorosis. This visible symptom of Cr toxicity was also verified by Paiva et al. [20] in *Eichhornia crassipes* at 10 mmol l^{-1} Cr. Relative growth rate of plants of *S. argentinensis* exposed between 1 and 5 mmol l^{-1} Cr had no significant differences compared with the control. With the increase of Cr concentration in the medium, growth decreased gradually, compared to the control, the reductions in RGR for 10 and 20 mmol l^{-1} Cr were both 53%. Reduced growth at high Cr concentrations may be partly ascribed to lower net photosynthetic rate (*A*) recorded at 10 and 20 mmol l^{-1} Cr; but not exclusively, since A values were similar at 5 and 10 mmol l^{-1} Cr after 15 days of treatment, and there were no significant differences in growth rate between 0 and 5 mmol l^{-1} Cr. In this way, it has been argued that the reduced growth observed at higher Cr treatments might be also due to increased tissue permeability [31]. It might also result from inhibition of cell division or from accumulation of excess Cr in different plant parts [4]. Thus, reduction in growth can be linked to the high Cr accumulation, as in this case plants have to spend extra energy to cope with the high Cr concentrations in the tissues [32]. This could explain the disparity recorded between A and Φ_{PSII} in the later stage of our experiment; A values were marked as lower at high Cr concentrations for all sampling time, while Φ_{PSII} did not show significant differences among treatments from 9 days. Furthermore, this physiological process could be a relevant mechanism to protect *S. argentinensis* against excess of radiation under high Cr, since the relatively stable NPQ across the chromium range could indicate that Cr does not produce an increase in thermal dissipation in the PSII antennae.

There were very clear effects of chromium on A and stomatal conductance (Gs) in our experiment from 5 mmol l^{-1} Cr; and the

Table 1
Bioaccumulation coefficient (BC), transport factor from concentration (TF), and from accumulation (TF′) in *Spartina argentinensis* in response to treatment with a range of Cr concentrations after 15 days.

Cr concentration (mmol l^{-1})	BC	TF	TF′
1	4.3	0.0	0.1
2	4.0	0.0	0.1
5	6.7	0.9	4.4
10	4.2	1.9	8.7
20	3.0	5.1	19.7

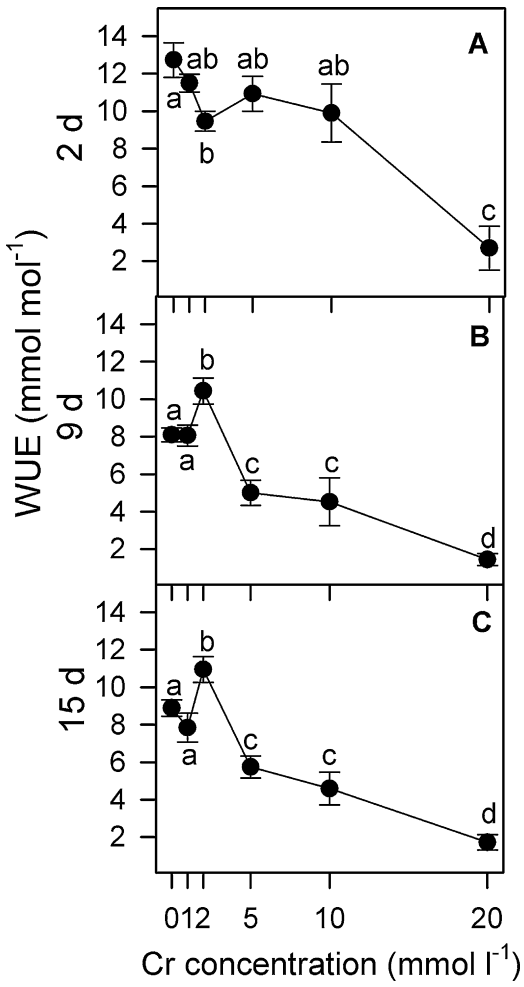

Fig. 6. (A–C) Water use efficiency (WUE) in *Spartina argentinensis* in response to treatment with a range of Cr concentrations after 2, 9 and 15 days (A, B and C, respectively). Values represent mean ± SE, $n = 6$. Different letters indicate means that are significantly different from each other (LSD test, $P < 0.05$).

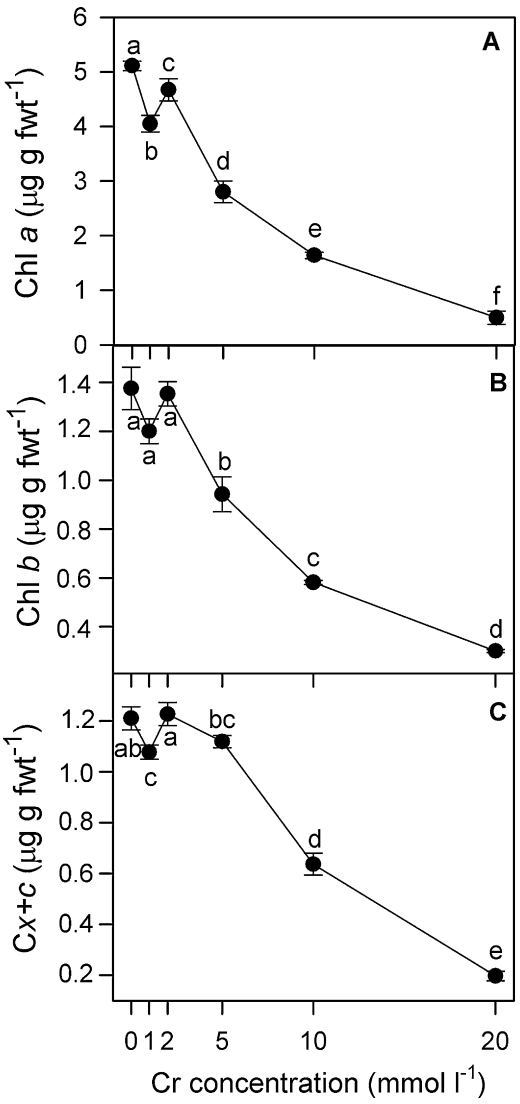

Fig. 7. (A) Chlorophyll *a* (Chl *a*), (B) chlorophyll *b* (Chl *b*) and (C) carotenoid (C*x* + *c*) concentrations in *Spartina argentinensis* in response to treatment with a range of Cr concentrations after 15 days. Values represent mean ± SE, $n = 5$. Different letters indicate means that are significantly different from each other (LSD test, $P < 0.05$).

decrease of A involved a parallel decline of water use efficiency at all stages of the experiment. Nonetheless, the decline of A may be attributed to stomatal and/or non-stomatal limitations [33]; thus, Cr stress can affect photosynthesis in terms of CO_2 fixation, electron transport, photophosphorylation and enzyme activities [34]. Therefore, if the limitation of A in *S. argentinensis* were due to Gs, there would be a reduction in intercellular CO_2 concentration (Ci). According to Ci, the photosynthesis does not show a correlation between both parameters. The increase of Ci may be explained by modifications of RuBisCO activities of *S. argentinensis*. The inhibition in enzyme activity in presence of heavy metals could be due to substitution of Mg^{2+} in the active site of RuBisCO subunits by metal ions [35]. In our case, we recorded a modification of Cr/Mg concentration ratio (due to increase of Cr and unchanged Mg), which could be linked with a decrease in RuBP carboxylase. Likewise, Mateos Naranjo et al. [36] concluded that, in *Spartina densiflora*, zinc entailed a simultaneous reduction in A and Gs without direct relation. The decrease of the stomatal conductance may be related to an alteration in the K/Ca ratio in the guard cells and/or to the alterations in the abscisic acid concentration, which controls the stomatal movement [37]. Accordingly, we recorded higher K concentrations for 5–20 mmol l^{-1} Cr, whereas Ca did not change with increasing Cr content.

Paiva et al. [20] explained that another reason for the decrease in A caused by Cr^{6+} was probably the damage suffered by the photosynthetic apparatus based on the decrease in the maximum quantum efficiency of PSII photochemistry (F_v/F_m). In our study, F_v/F_m did show a significant reduction at midday compared to dawn values. At midday, the reduction in F_v/F_m values indicated that *S. argentinensis* 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. Moreover, F_v/F_m was the most sensitive parameter to Cr concentration at all stages of the experiment, denoting that chromium excess enhances photoinhibition induced by light stress. Paiva et al. [20] similarly reported that photochemical activities of chloroplasts were significantly reduced at Cr concentrations of 10 mmol l^{-1} Cr in the nutrient medium. Photoinhibition is caused by damage to photosynthetic components, and this effect can be either short-term and reversible (dynamic photoinhibition) or long-term and irreversible (chronic photoinhibition) [38]. F_v/F_m values at dawn remained lower than control parameters for unstressed plants [39] after 15 days of treatment, this fact revealing a chronic photoinhibition.

On the other hand, Boonyapookana et al. [40] found that the decrease in photosynthesis, promoted by increased Cr concentra-

tion in nutrient solution, is associated with biochemical changes, causing inhibition of chlorophyll synthesis. Thus, the decrease in chlorophyll and total carotenoid contents of *S. argentinensis* 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 Cr-induced decrease in the chlorophyll level has been widely reported [20]. Dhir et al. [19] explained that this decline in chlorophylls levels could be due to (i) reduction of Fe content, (ii) reduced efficiency of enzymes involved in chlorophyll biosynthesis, and (iii) replacement of central Mg^{2+} molecule in chlorophylls by heavy metals. In the case of *S. argentinensis*, we recorded lower Fe concentrations in presence of Cr. In this way, Kabata-Pendias and Pendias [1] reported that Cr additions resulted in decreased concentrations of almost al major nutrients in tops and of K, P, Fe, and Mg in roots. In our experiment the presence of Cr reduced uptake of K, P, Fe, Cu, Mn and B. The antagonistic interaction between Cr and Mn, Cu and B has also been reported by Turner and Rust [41]. Finally, Kabata-Pendias and Pendias [1] claimed that Cr can alter the fine structure of chloroplasts and the chloroplasts membranes, and Paiva et al. [20] confirmed that the presence of Cr can provoke structural alterations in thylakoids.

In conclusion, *S. argentinensis* showed symptoms of toxicity (chlorosis) from 10 mmol l^{-1} Cr; although photosynthetic symptoms of stress were recorded from 5 mmol l^{-1} Cr after 15 days of treatment (reductions in leaf gas exchange, chlorophyll *a* fluorescence parameters and photosynthetic pigment concentrations). Moreover, results of concentration of Cr in roots and shoots, BF, TF and TF′ indicate that *S. argentinensis* has the basic characteristics of a Cr-hyperaccumulator and may potentially be useful for restoring Cr-contaminated sites. However, further studies are necessary because the behaviour of Cr may change when it occurs naturally in soils.

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References

[1] A. Kabata-Pendias, H. Pendias, Trace Elements in Soils and Plants, CRC Press, Boca Ratón, FL, 2001.
[2] D. Subrahmanyam, Effects of chromium toxicity on leaf photosynthetic characteristics and oxidative changes in wheat (*Triticum aestivum* L.), Photosynthetica 46 (2008) 339–345.
[3] R. Eisler, Chromium Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review, Fish and Wildlife, Service, U.S. Department of the Interior, Washington, DC, 1986.
[4] H. Diwan, A. Ahmad, M. Iqbal, Chromium-induced modulation in the antioxidant defense system during phenological growth stages of Indian mustard, Int. J. Phytoremediat. 12 (2010) 142–158.
[5] R. Font, M. del Río, A. de Haro, Use of near infrared spectroscopy to evaluate heavy content in *Brassica juncea* cultivated on the polluted soils of Guadiamar river area, Fresen. Environ. Bull. 11 (2002) 777–781.
[6] P. Sampanpanish, P.W. Ongsapich, S. Khaodhiar, E. Khan, Chromium removal from soil by phytoremediation with weed plant species in Thailand, Water Air Soil Poll. 6 (2006) 191–206.
[7] S. Xu, P.R. Jaffe, Effects of plants on the removal of hexavalent chromium in wetland sediments, J. Environ. Qual. 35 (2006) 334–341.
[8] F. Bareen, S. Khilji, Bioaccumulation of metals from tannery sludge by *Typha angustifolia* L., Afr. J. Biotechnol. 7 (2008) 3314–3320.
[9] S. Mangkoedihardjo, R. Ratnawati, N. Alfianti, Phytoremediation of hexavalent chromium polluted soil using *Pterocarpus indicus* and *Jatropha curcas* L., World Appl. Sci. J. 4 (2008) 338–342.
[10] Q. Lin, I.A. Mendelssohn, The combined effects of phytoremediation and biostimulation in enhancing habitat restoration and oil degradation of petroleum contaminated wetlands, Ecol. Eng. 10 (1998) 263–274.
[11] Q. Lin, I.A. Mendelsoohn, M. Sudan, K. Lee, A. Venosa, The dose-response relationship between No. 2 fuel oil and the growth of the salt marsh grass, *Spartina alterniflora*, Mar. Pollut. Bull. 44 (2002) 897–902.
[12] L. Cambrollé, S. Redondo-Gómez, E. Mateos-Naranjo, M.E. Figueroa, Comparison of the role of two *Spartina* species in terms of phytostabilization and bioaccumulation of metals in the estuarine sediment, Mar. Pollut. Bull. 56 (2008) 2037–2042.
[13] A.L. Cabrera, A. Willink, Biogeografía de América Latina, Serie de Biología, Monografía N° 13, OEA, Washington, DC, 1973.
[14] S.R. Feldman, V. Bisaro, J.P. Lewis, Photosynthetic and growth responses to fire of the subtropical-temperate grass *Spartina argentinensis* Parodi, Flora 199 (2004) 491–499.
[15] S.R. Feldman, J.P. Lewis, Effect of fire on *Spartina argentinensis* Parodi demographic characteristics, Wetlands 27 (2007) 785–793.
[16] M.C. Petenello, S.R. Feldman, Efecto de hidrocarburos sobre la germinación de especies potenciales biorremediadoras de suelos contaminados, Anál Semillas 3 (2009) 85–87 (in Spanish).
[17] D. Hoagland, D.I. Arnon, The water culture method for growing plants without soil, Calif. Agric. Exp. Stn. Bull. 347 (1938) 1–39.
[18] C. Prado, L. Rodríguez-Montelongo, J.A. González, E.A. Pagano, M. Hilal, F.E. Prado, Uptake of chromium by *Salvinia minima*: effect on plant growth, leaf respiration and carbohydrate metabolism, J. Hazard. Mater. 177 (2010) 546–553.
[19] B. Dhir, P. Sharmila, P.P. Saradhi, S.A. Nasim, Physiological and antioxidant responses of *Salvinia natans* exposed to chromium-rich wastewater, Ecotox. Environ. Saf. 72 (2009) 1790–1797.
[20] L.B. Paiva, J.G. de Oliveira, R.A. Azevedo, D.R. Ribeiro, M.G. da Silva, A.P. Vitória, Ecophysiological responses of water hyacinth exposed to Cr^{3+} and Cr^{6+}, Environ. Exp. Bot. 65 (2009) 403–409.
[21] S. Redondo-Gómez, E. Mateos-Naranjo, A.J. Davy, F. Fernández-Muñoz, E.M. Castellanos, T. Luque, M.E. Figueroa, Growth and photosynthetic responses to salinity of the salt-marsh shrub *Atriplex portulacoides*, Ann. Bot. 100 (2007) 555–563.
[22] K. Maxwell, G.N. Johnson, Chorophyll fluorescence—a practical guide, J. Exp. Bot. 51 (2000) 659–668.
[23] S. Redondo-Gómez, C. Wharmby, J.M. Castillo, E. Mateos-Naranjo, C.J. Luque, A. de Cires, T. Luque, A.J. Davy, M.E. Figueroa, Growth and photosynthetic responses to salinity in an extreme halophyte, *Sarcocornia fruticosa*, Physiol. Plantarum 128 (2006) 116–124.
[24] S. Von Caemmerer, G.D. Farquhar, Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves, Planta 153 (1981) 377–387.
[25] H.K. Lichtenthaler, Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, Methods Enzymol. 148 (1987) 350–382.
[26] L. Buendía-González, J. Orozco-Villafuerte, F. Cruz-Sosa, C.E. Barrera-Díaz, E.J. Vernon-Carter, *Prosopis laevigata* a potential chromium (VI) and cadmium (II) hyperaccumulator desert plant, Bioresour. Technol. 101 (2010) 5862–5867.
[27] Y.B. Sun, Q.X. Zhou, L. Wang, W. Liu, Cadmium tolerance and accumulation characteristics of *Bidens pilosa* L. as a potential Cd-hyperaccumulator, J. Hazard. Mater. 161 (2009) 808–814.
[28] S.K. Yadav, M. Dhote, P. Kumar, J. Sharma, T. Chakrabarti, A.A. Juwarkar, Differential antioxidative enzyme responses of *Jatropha curcas* L. to chromium stress, J. Hazard. Mater. 180 (2010) 609–615.
[29] G. Shi, Q. Cai, Cadmium tolerance and accumulation in eight potential energy crops, Biotechnol. Adv. 27 (2009) 555–561.
[30] F. Wu, W. Yang, J. Zhang, L. Zhou, Cadmium accumulation and growth responses of a poplar (*Populus deltoids* x *Populus nigra*) in cadmium contaminated purple soil and alluvial soil, J. Hazard. Mater. 177 (2010) 268–273.
[31] A.K. Sen, N.G. Mondal, Salvinia natans as the scavenger of Hg (II), Water Air Soil Pollut. 34 (1987) 439–446.
[32] M. Israr, S.V. Sahi, J. Jain, Cadmium accumulation and antioxidative responses in the Sesbania drumondii Callus, Arch. Environ. Contam. Toxicol. 50 (2006) 121–127.
[33] J. Flexas, H. Medrano, Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revised, Ann. Bot. 89 (2002) 183–189.
[34] A.K. Shanker, C. Cervantes, H.L. Tavera, S. Avudainayagam, Chromium toxicity in plants, Environ. Intern. 31 (2005) 739–753.
[35] A. Siedlecka, Z. Krupa, Rubisco activity maintenance in environmental stress conditions-how many strategies, Cell. Mol. Biol. Lett. 9 (2004) 56–57.
[36] E. Mateos Naranjo, S. Redondo-Gómez, J. Cambrollé, T. Luque, M.E. Figueroa, Growth and photosynthetic responses to zinc stress of an invasive cordgrass, *Spartina densiflora*, Plant Biol. 10 (2008) 754–762.
[37] H. Marschner, Mineral Nutrition in Higher Plants, Academic Press, Harcourt B, Company Publishers, London, 1999.
[38] C. Werner, O. Correia, W. Beyschlag, Characteristic patterns of chronic and dynamic photoinhibition of different functional groups in a Mediterranean ecosystem, Funct. Plant Biol. 29 (2002) 999–1011.
[39] O. Björkman, B. Demmig, Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins, Planta 170 (1987) 489–504.
[40] B. Boonyapookana, E.S. Upatham, M. Kruatrachue, P. Pokethitiyook, S. Singhakaew, Phytoaccumulation and phytotoxicity of Cd and Cr in duckweed *Wolffia globosa*, Int. J. Phytoremediat. 4 (2002) 87–100.
[41] M.A. Turner, R.H. Rust, Effect of chromium on growth and mineral nutrition of soybeans, Soil Sci. Soc. Am. Proc. 35 (1971) 755–758.