Zinc tolerance and accumulation in the halophytic species *Juncus acutus*

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ABSTRACT

The research on species with capacity to tolerate and accumulate zinc is of paramount importance for phytoremediation purposes. An experiment was designed to investigate the effect of Zn from 0 to 100 mmol l^{-1} on the growth, photosynthetic apparatus and nutrient uptake of the halophytic species *Juncus acutus*. Gas exchange, chlorophyll fluorescence and photosynthetic pigments concentration were measured. We also determined total zinc, magnesium, potassium, phosphorus and sodium concentrations, as well as C/N ratio. *J. acutus* showed high tolerance to Zn-induced stress, since all plants survived and none of them showed any toxicity symptoms, such as chlorosis, necrosis or growth reduction at concentrations up to 100 mmol l^{-1} Zn. The integrity and functionality of the photosynthetic apparatus were unaffected even at zinc concentrations greater than 500 mg Kg^{-1} on tillers. Likewise, nutrient absorption was relatively unaffected. Zn tolerance was associated with the capacity to accumulate Zn in roots (with values up to 2500 mg Kg^{-1}) and largely avoid its transport to tillers. These characteristics, along with its ability to establish in a wide variety of ecosystems, render this species a useful phytostabilizer for revegetation of Zn-contaminated lands.

Keywords: Growth response; metal toxicity; nutrient absorption; photosynthesis; Zn-stress; photoinhibition.

1. Introduction

Environmental pollution by heavy metals is a serious problem worldwide, increasing in parallel with the development of human technology. Government, the industry and the public now recognize the potential dangers that metals pose to human health (Duruibe et al., 2007) through the food chain and the health of terrestrial and aquatic communities and ecosystems (Kabata-Pendias and Pendias, 2001). The danger of toxic metals is aggravated by their immutable nature and indefinite persistence in the environment (Garbisu and Alkorta, 2001; Aycicek et al., 2008). Among heavy metals, Zn is considered the main industrial pollutant of both terrestrial and aquatic environments (Barak and Helmke, 1993) and has the greatest mobility and bioavailability of all elements (Morillo et al., 2004). Although Zn is an essential microelement with many roles in plant metabolism (Kabata-Pendias and Pendias, 2001), its excess can lead to toxic effects in plants (Chaney, 1993), with specific effects on the Calvin cycle and photosystem activity (Van Assche and Clijsters, 1986).

Many remediation strategies have been considered to counter the detrimental effects of Zn excess, including physical, chemical and biological methods that immobilize or remove metals from the environment (Marques et al., 2011). Phytoremediation has recently gained importance on account of its cost-effective, long-term applicability and because it is an ecofriendly, promising clean-up solution for a wide variety of contaminated sites (Weis and Weis, 2004). This methodology depends on the use of plants to act upon the contaminants, by extracting, degrading or immobilizing them (Marques et al., 2011). The research on species which can be useful in metal phytoremediation has become a major issue (Zhang et al., 2010) and these

71 species should be chosen on the basis of their capacity to tolerate and accumulate
72 particular contaminants (Marques et al., 2011).
73 There exists a wide variation in sensitivity to metal exposure. However, exists a
74 lack of knowledge about metal toxicity thresholds for native plant species (Ross and
75 Kaye, 1994) and for species used to restore sites contaminated by heavy metals, such as
76 salt marshes. Species of genus *Juncus* have been employed in wetland restoration
77 projects around the world (Sparks et al., 2013; Marques et al., 2011), but the
78 information on the tolerance and accumulation patterns of heavy metals in these species
79 is really scarce. The present study is focused on the species *Juncus acutus* L., a
80 halophytic densely caespitose plant with subcosmopolitan distribution that is common
81 in Spanish coastal marsh communities and can be found growing in sediments
82 containing 100–4800 ppm Zn in several estuaries of the Iberian Peninsula (Sáinz and
83 Ruiz, 2006). Moreover, this species has a wide ecological range, tolerating soils with
84 high levels of sulphates and chlorides (Fernández-Carvajal, 1982) and soils with a sandy
85 texture and hydric stress during the dry summer season. Our hypothesis is that all these
86 circumstances highlight the potential of *J. acutus* to be used for metal remediation in
87 polluted areas. However, no studies have analyzed its growth and physiological
88 responses to zinc excess.
89 The aim of this study was to evaluate the tolerance of *J. acutus* to elevated
90 concentration of zinc in relation of its survival, growth and photosynthetic response, and
91 quantify the capacity of this species for accumulating this element.
92
93 **2. Materials and Methods**
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95 *2.1. Plant material*

96

97 Seeds of *J. acutus* were collected in December 2011 from the natural marshes of
98 Doñana National Park (37º 15´ N - 6º 58´W; SW Spain) and stored at 4ºC (in darkness)
99 for three months. After that, seeds were placed into a germinator for a month (ASL
100 Aparatos Científicos M-92004, Madrid, Spain) and subjected to an alternating diurnal
101 regime of 16 h of light (photon flux rate, 400-700 nm, 35 μmol m^{-2} s^{-1}) at 25ºC and 8 h
102 of darkness at 12ºC. Seedlings were then planted in individual plastic pots (11 cm of
103 diameter) filled with perlite and placed in a glasshouse with controlled temperature of
104 21-25ºC, 40-60% relative humidity and natural daylight (minimum and maximum light
105 flux: 250 and 1000 μmol m^{-2} s^{-1} respectively). Pots were carefully irrigated with 20%
106 Hoagland's solution (Hoagland and Arnon, 1938) as necessary. All the pots received the
107 same irrigation.

108

109 *2.2. Stress treatments*

110

111 In October 2012, after five months of seedling culture, the pots (with between 5
112 and 6 tillers) were randomly allocated still inside the glasshouse to five Zn treatments
113 (six pots per tray, one tray per Zn treatment): 0, 10, 30, 60 and 100 mmol l^{-1} Zn. The
114 treatment with 0 mmol l^{-1} Zn was considered the control treatment. Zinc treatments
115 were established by combining 20% Hoagland´s solution and $ZnSO_4 \cdot 7H_2O$ of the
116 appropriate concentration. The control, 0 mmol l^{-1} Zn treatment, had exactly 0.002
117 mmol l^{-1} Zn, as Hoagland´s solution contains a small amount of Zn as an essential trace
118 nutrient. Zn concentrations were chosen to cover variations recorded by Sáinz and Ruiz,
119 (2006) in the salt marshes of the joint estuary of the Tinto and Odiel Rivers.

At the beginning of the experiment, 1 l of appropriate solution was placed in each tray (Hoagland al 20% + $ZnSO_4.7H_2O$) to a depth of 1cm. During the experiment, the levels of trays were monitored and topped up to the marked level with 20% Hoagland´s solution (without additional $ZnSO_4.7H_2O$) to limit the change of Zn concentration caused by water evaporation from the nutrient solution. Also, the entire solution (including $ZnSO_4.7H_2O$) was changed every three days.

2.3. Growth analysis

At the beginning and the end of the experiment (after 50 days of treatment) three and five entire plants, respectively, from each treatment were dried at 80ºC for 48 h and weighed. Also, before and after the Zn treatment, the number and height of all fully developed tillers were measured.

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

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

where B_f = final dry mass, B_i = initial dry mass (an average of the three plants from each treatment dried at the beginning of the experiment) and D = duration of experiment (days).

2.4. Gas exchange

Measurements were taken on random, fully developed photosynthetic tillers (n =

10, two measurements per plant) using an infrared gas analyser in an open system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) after 50 days of treatment. Maximum net photosynthetic rate (A), intercellular CO_2 concentration (C_i) and stomatal conductance to CO_2 (G_s) were determined at CO_2 concentration of 400 µmol CO_2 mol^{-1} air, temperature of 25-30ºC, 42.4 ± 0.4% relative humidity and a photon flux density of 1000 µmol m^{-2} s^{-1} once a steady-state was reached. A, C_i and G_s were calculated using standard formulas of Von Caemmerer and Farquhar (1981). Photosynthetic area was approximated as the area of a cylinder. Intrinsic water use efficiency (WUE_i) was calculated as the ratio between A and G_s.

2.5. Tiller water content

Tiller water content (TWC) was calculated after 50 days of treatment as (n = 5, one measurement per plant):

$$TWC = (FW - DW)/FW \times 100$$

where FW is the fresh mass of the tillers and DW is the dry mass after oven-drying at 80ºC for 48 h.

2.6. Photosynthetic pigments

At the end of the experimental period, photosynthetic pigments in fully developed, photosynthetic tillers (n=5) from each treatment were extracted using 0.05 g of fresh material in 10 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 $gfwt^{-1}$) were obtained through calculation following Lichtenthaler (1987).

2.7. Measurement of chlorophyll fluorescence

Chlorophyll fluorescence was measured using a portable modulated fluorimeter (Mini-PAM, Heinz Walz, Germany) after 50 days of treatment, in tillers similar to those used previously. Measurements were made on each plant in the five zinc treatments (n = 10, two measurements per plant). Light and dark-adapted fluorescence parameters were measured at dawn (stable 75 µmol m^{-2} s^{-1} ambient light) and at midday (1500 µmol m^{-2} s^{-1}) to investigate whether zinc concentration affected the sensitivity of plants to photoinhibition (Qiu et al., 2003).

Plants were dark-adapted for 30 minutes using leaf–clips 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) too small to induce significant physiological changes in the plant (Schreiber et al., 1986). The data stored were an average taken over a 1.6 seconds period. Maximal fluorescence level in this state (F_m) was measured after applying a saturating actinic light pulse of 10000 µmol m^{-2} s^{-1} for 0.8 s (Bolhàr-Nordenkampf and Öquist, 1993). The value of F_m was recorded as the highest average of two consecutive points. Values of the variable fluorescence ($F_v = F_m - F_0$) and maximum quantum efficiency of PSII photochemistry (F_v/F_m) were

194 calculated from F_0 and F_m. This ratio of variable to maximal fluorescence can be used
195 to quantify photoinhibition (Maxwell and Johnson, 2000).
196 The same tiller section of each plant was used to measure light-adapted
197 parameters. Steady state fluorescence yield (F_s) was recorded under ambient light
198 conditions. A saturating actinic light pulse of 10000 µmol m^{-2} s^{-1} for 0.8 s was then used
199 to produce the maximum fluorescence yield (F_m') by temporarily inhibiting PSII
200 photochemistry.
201 Using fluorescence parameters determined in both light- and dark-adapted states,
202 the following were calculated: quantum efficiency of PSII ($\Phi_{PSII} = (F_m' - F_s)/ F_m'$)
203 (Genty et al., 1989); photochemical quenching (qP = $(F_m' - F_s) / (F_m' - F_0')$, where F_0'
204 corresponds to open reaction center traps in the light-acclimated state), and non-
205 photochemical quenching (NPQ = $(F_m - F_m') / F_m'$; Schreiber et al., 1986).
206 Photochemical quenching gives an indication of the proportion of PSII reaction centres
207 that are open (Maxwell and Johnson, 2000).
208
209 *2.8. Chemical analyses of plant tissue samples*
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211 In accordance with protocols of Mateos-Naranjo et al. (2008), at the end of the
212 experiment, tiller and root samples were dried at 80ºC for 48 h and ground. Tillers and
213 roots were carefully washed with distilled water before any further analysis. Then, 0.5 g
214 samples from tillers and roots (taken from five plants per treatment) were digested in
215 triplicate with 6 ml HNO_3, 0.5 ml HF and 1 ml H_2O_2. Ca, Mg, K, P, Na and Zn
216 concentrations in tillers and roots were measured by inductively coupled plasma (ICP)
217 spectroscopy (ARL-Fison 3410, USA). Total N and C concentrations were determined
218 for undigested dry samples with an elemental analyzer (Leco CHNS-932, Spain).

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220 *2.9. Statistical analysis*

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222 Statistical analysis was carried out using Statistica v. 6.0 (Statsoft Inc.). Pearson
223 coefficients (r) were calculated to assess correlation between different variables. Data
224 were analysed by means of a one-way analysis of variance (*F*-test). Data were first
225 tested for normality with the Kolmogorov-Smirnov test and for homogeneity of
226 variance with the Brown-Forsythe test. Significant test results were followed by Tukey
227 tests for identification of important contrasts. Differences between measurements of
228 fluorescence at dawn and midday were compared by the Student test (*t*-test).

229

230 **3. Results**

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232 *3.1. Growth*

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234 Total dry mass of plants under 100 mmol l^{-1} Zn was lower than for all the other
235 treatments except that of 60 mmol l^{-1} Zn (Anova, $P < 0.05$; Fig. 1A). Compared to the
236 control, the reduction in total dry mass for 100 mmol l^{-1} Zn was 38% after 50 days of
237 treatment (Fig. 1A).

238 A similar trend was reported for RGR (Anova, $P < 0.05$; Fig. 1B) and this was
239 correlated with the reduction in the number of tillers ($r = 0.97$, $P < 0.01$; Fig. 1C) but
240 not with mean height of tillers. However, mean height of tillers was also smaller in 100
241 mmol l^{-1} Zn treatment than in control (Anova, $P < 0.05$; Fig. 1D). Compared to the
242 control, the reduction in RGR for 100 mmol l^{-1} Zn treatment was 21% (Fig. 1B).

243

3.2. Gas exchange

Net photosynthetic rate (A), stomatal conductance (G_s) and intercellular CO_2 concentration (C_i) did not vary with zinc treatments, with values around 12 µmol CO_2 m^{-2} s^{-1}, 0.62 mol H_2O m^{-2} s^{-1} and 350 µmol CO_2 mol^{-1} air respectively (Table 1).

Similarly, Zn increment did not affect intrinsic water use efficiency (WUE_i) and tiller water content (TWC) of *J. acutus* after 50 days of treatment, with around 20 µmol CO_2 mol^{-1} H_2O and 75% respectively (Table 1).

3.3. Photosynthetic pigments

Pigment concentrations (Chl *a*, Chl *b* and C*x*+*c*) decreased significantly with Zn concentration (Chl *a*: r = -0.6, P < 0.05; Chl *b*: r = -0.68, P < 0.05; C*x*+*c*: r = -0.74, P < 0.5; Fig. 2A-C). However, only in plants grown at 100 mmol l^{-1} Zn there was a significant reduction compared to the control treatment (Anova, P < 0.05), with a mean reduction ca. of 30% for all photosynthetic pigments.

3.4. Chlorophyll fluorescence

F_v/F_m values at dawn were uniformly high at all external Zn concentrations, with values around 0.84 (Fig. 3A). F_v/F_m at midday were always lower than at dawn (t-test, P < 0.05), probably because of the lower F_m at midday than at dawn (data not presented). F_v/F_m at midday did not show differences among treatments (Anova, P > 0.05).

Φ_{PSII} at dawn and at midday did not vary with Zn concentration, but values at midday were lower than at dawn in all treatments (t-test, $P < 0.05$; Fig. 3B).

Finally, NPQ showed no relationship with Zn concentration at dawn, whereas at midday it increased compared to the control when the plants were exposed to external concentrations of 30 mmol l^{-1} Zn or higher (Anova, $P < 0.05$; Fig. 3C).

3.5. Chemical analyses of plant tissue samples

At the end of the experiment, tissue Zn concentration was greater in roots than in tillers (t-test, $P < 0.05$). Tillers Zn concentration increased gradually with external Zn concentration ($r = 0.91$, $P < 0.01$). The trajectory of Zn concentration in roots was different, showing an increment which was marked from 0 to 10 mmol l^{-1} Zn but gentler from 10 to 60 mmol l^{-1} Zn (Anova, $P < 0.05$; Fig. 4A). In contrast, roots and tillers Ca, K, Mg and P concentrations showed no significant overall response to Zn concentration, although roots and tillers Ca and Mg concentrations were lower at the highest level of zinc than in control (Anova, $P < 0.01$; Fig. 4B, F). Contrary, root K and P concentrations were lower in absence of zinc than in the rest of treatments (Anova, $P < 0.01$; Fig. 4D, E). On the other hand, tissue Na concentration in roots was higher than in tillers, increasing with external Zn concentration in both tissues ($r = 0.98$, $P < 0.01$; $r = 0.96$, $r = 0.01$, for roots and tillers respectively; Fig. 4C).

Finally, C/N ratio was higher in roots than in tillers and this ratio decreased in presence of zinc in the nutrient solution for both tissues (Fig. 5), with little difference among Zn treatments.

4. Discussion

293

294 The research on biological resources which could be used as bio-tools for
295 managing heavy metal pollution is one of the fundamental guidelines for the design and
296 development of effective methodologies for environmental remediation. Hence, the
297 location of species with a high capacity for metal-accumulation and for growth under
298 metal-polluted conditions can be of paramount importance for the remediation of metal
299 pollution (Tripathi et al., 2007; Zhang et al., 2010).

300 Our experiment showed that *J. acutus* demonstrated hypertolerance to zinc
301 stress, since all plants were able to survive even with external Zn concentrations as high
302 as 100 mmol l^{-1}. Furthermore, *J. acutus* plants did not show any visible Zn toxicity
303 symptoms such as chlorosis, necrosis or a strong growth inhibition even at
304 concentrations of zinc in *J. acutus* above-ground tissues of 560 mg Kg^{-1}. This zinc
305 concentration is higher than the upper toxic levels of 100-500 mg Kg^{-1} dry mass
306 considered for high plants (Kabata-Pendias and Pendias, 2001). This tolerance is higher
307 than that reported for other species of genus *Juncus*, such *J. articulatus*, which showed
308 no live leaf biomass at Zn concentration of 0.3-1 mmol l^{-1} (Matthews et al., 2004).
309 Furthermore, hypertolerance of *J. acutus* was supported by the high value of the
310 effective concentration of Zn (EC50, substrate Zn concentration resulting in 50 percent
311 biomass reduction; Paschke et al., 2000), greater than 100 mmol l^{-1} Zn. At this
312 concentration, *J. acutus* showed a 38 percent of biomass reduction after 50 days of
313 treatment. *J. acutus* EC50 value is considerably higher than those reported by several
314 authors for many different species. For example, Paschke et al. (2000, 2006) found plant
315 EC50 values of 1.2-3.4 mmol l^{-1} Zn for five reclamation grass species and six
316 restoration forbs used for restoration of contaminated areas. In the current experiment,
317 RGR underwent only a 21% reduction in plants grown at 100 mmol l^{-1} Zn. This

318 decrease was attributed to a reduction in the number of tillers rather than to a reduction
319 in the mean height of tillers. This fact indicated that *J. acutus* would be able to maintain
320 the state of development of its tillers regardless of the external Zn concentration, as
321 indicated by the few differences between different treatments in total height of tillers.
322 Compared with our results, Matthews et al. (2004) found an important total biomass
323 reduction by zinc treatment at 0.7 mmol l^{-1} in *Juncus effusus*, a concentration
324 considerably lower than those used in our experiment. Moreover, Stefani et al. (1991)
325 found that initial growth of seedlings of *J. acutus* was strongly inhibited by Pb
326 concentrations from 0.00195 mmol l^{-1} upwards, and by Cu and Cd concentration from
327 0.00012 mmol l^{-1}, but these specific metal discrepancies could be attributed to different
328 tolerance mechanisms.
329 On the other hand, tolerant plants could be classified into plants that tolerate a
330 high uptake of metals in roots but avoid their transport to above-ground tissues, and
331 plants that accumulate metals and preferentially transport metals to aerial parts (Pollard
332 et al., 2002). In our glasshouse experiment, Zn levels were much higher in *J. acutus*
333 subterranean structures than in the aerial structures, reaching values ca. 2500 mg Kg^{-1} in
334 the roots. These results revealed that *J. acutus* could have the basic characteristics of a
335 tolerant plant with high capacity for the phytostabilization of metal in its belowground
336 structures. In accordance with this, Fizgerald et al. (2003) found that the roots of marsh
337 plants overall accumulate more metals than the above-ground biomass. The lower Zn
338 concentration in tillers of *J. acutus* compared to that in roots could be related to the
339 development of mechanisms such as compartmentation, which would control ion
340 transport into tillers, thereby improving plant tolerance to heavy metals. This species
341 may have accumulated most Zn in the roots to minimize Zn translocation to
342 aboveground tissues. The sequestering of metals into tissue or cellular compartments,

343 which are less sensitive to such metals, has been described as a tolerance mechanism
344 (Kabata-Pendias and Pendias, 2001; Weis and Weis, 2004) that entails restriction of
345 both upward movement into shoots (avoidance mechanism) and translocation of excess
346 metals into leaves (Verkleij and Schat, 1990). However, when Zn is present in an
347 extremely high concentration in the nutrient solution, it can be translocated from the
348 roots and accumulated within the shoots (Kabata-Pendias and Pendias, 2001), which
349 could explain the Zn increase in the tops of *J. acutus*.

350 Metal hypertolerance in plants has been described as an ecophysiological
351 adaptation to metalliferous environments (Evangelou et al., 2004). In our experiment,
352 the analysis of physiological measurements of *J. acutus* corroborated this idea because
353 neither WUE_i, TWC, nor A were affected by Zn concentration, even when it was as
354 high as 100 mmol l^{-1}. Vaillant et al. (2005) reported that the photosynthetic activity of
355 four *Datura* species decreased at 2.5 mmol l^{-1} Zn in nutrient solution. Mateos-Naranjo
356 et al. (2008) and Cambrollé et al. (2013) reported that other marshes plants, such as
357 *Spartina densiflora* and *Limoniastrum monopetalum*, showed reductions in A at external
358 Zn concentrations of 10 and 60 mmol l^{-1}, respectively. Similarly, there were not any
359 effects whatsoever of Zn on G_s and C_i. Although net photosynthetic rates depend on
360 mesophyll conductance and carboxylation capacity of Rubisco apart from stomatal
361 conductance (Flexas et al., 2008; Perez-Martin et al., 2009), the invariable A across the
362 whole range of external Zn indicated that the photosynthetic apparatus of *J.acutus* could
363 be able to accommodate to prolonged exposure to high external zinc concentration, this
364 involving considerable physiologic plasticity. In fact, F_v/F_m at dawn and midday did not
365 change across the same range of external Zn, this ratio being commonly used to
366 quantify photoinhibition (Maxwell and Johnson, 2000), a phenomenon that affects
367 photosynthetic productivity and, consequently, plant growth (Melis, 1999). Therefore,

368 the long-term effects of the highest Zn concentration on the growth rate of *J. acutus*
369 could be due to the different development of the photosynthetic area rather than to
370 variations in net photosynthetic rate. Hence, similar rates of CO_2 assimilation could be
371 more than compensated for by a greater photosynthetic area in low Zn concentration.
372 This response might provide positive feedback, since larger photosynthetic areas would
373 induce higher growth rates which would in turn induce more photosynthetic area,
374 amplifying the difference between plants at different zinc concentrations over time.
375 Thus, in our experiment, the largest differences in RGR were related to variations in the
376 number of tillers, which might be related to differences in photosynthetic area and hence
377 to reduction in light interception. In line with our results, Delperee and Lutts (2008) also
378 found that growth inhibition was not correlated with CO_2 assimilation rate for *Solanum*
379 *lycopersicum* under cadmium stress conditions. This was explained by the presence of
380 several mechanism of tolerance related with oxidative stress control and the protection
381 of photosystems. It is possible that *J. acutus* used the same protection system. In this
382 respect, the hypertolerance of *J. acutus* to Zn stress was also reflected in the integrity
383 and functionality of its photochemical apparatus. Several studies have reported a direct
384 effect of zinc on the photosynthetic electron transport chain (Vaillant et al., 2005;
385 Mateos-Naranjo et al., 2008), which may be associated with a substantial stress
386 response. Our data showed that F_v/F_m values were always lower at midday than at
387 dawn, a fact that indicated that *J. acutus* experienced some degree of dynamic
388 photoinhibition at the higher light flux. Several authors have defined dynamic
389 photoinhibition as a reversible mechanism controlling the dissipation of excess
390 luminous energy by means of thermal dissipation (NPQ), which is in agreement with
391 the greater NPQ at midday than at dawn in our data. This was supported by the lower
392 Φ_{PSII} at dawn than at midday, a decrease due to the increase in NPQ, which indicates

393 that the plants dissipate light as heat, thereby protecting the leaf from light-induced
394 damage (Maxwell and Johnson, 2000). Dawn values of F_v/F_m were close to optimal
395 values for unstressed plants (approximately 0.84; Bjorkman and Demmig, 1987), this
396 fact revealing no presence of chronic and irreversible photoinhibition.
397 On the other hand, the reduction in the absorption of essential mineral elements
398 has been described as one of the effects of heavy metals on plants (Chaney, 1993;
399 Kabata-Pendias and Pendias, 2001). In this regard, our mineral nutrient analyses
400 indicated that the presence of zinc in nutrient solution did not generate large nutritional
401 imbalance in *J. acutus* plants, especially in tillers tissues, although root and tillers Ca
402 and Mg concentrations were lower at the highest zinc level. The interactions Zn-Ca and
403 Zn-Mg have been previously described by several authors (Kabata-Pendias and Pendias,
404 2001). Thus, the reduction in Mg concentration in tillers could be linked with a
405 decrease in chlorophyll content recorded in this experiment, since the most familiar role
406 of Mg in photosynthesis is as the central atom of the chlorophyll molecule (Shaul,
407 2002). Finally, Na concentration for tillers and roots increased with external Zn
408 concentration. Redondo-Gómez et al. (2011) determined that the accumulation of Na in
409 the tissues of *Spartina densiflora* favored recovery of the photosynthetic apparatus of
410 this species against zinc excess. This result is linked with the high integrity showed by
411 photosynthetic apparatus of *J. acutus* to Zn stress.
412
413 **5. Conclusions**
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415 *J. acutus* shows a high tolerance to zinc-induced stress, as proved the fact that all
416 plants were able to survive and did not show any visible Zn toxicity symptoms, such as
417 chlorosis, necrosis or a strong growth inhibition at concentrations up to 100 mmol l^{-1}

418 Zn. Likewise, unaffected photosynthesis and efficiency of PSII photochemistry
419 apparatus might indicate that *J. acutus* is not experiencing metal toxicity, despite the
420 fact that Zn concentrations recorded in its tillers tissues (> 500 mg Kg^{-1}) were greater
421 than toxicity thresholds recorded for plants. Furthermore, Zn excess did not affect water
422 relations of this species and overall absorption of essential mineral elements. All these
423 results suggest that *J. acutus* is a hypertolerant species to zinc. Moreover, the capacity
424 of this species to accumulate great amount of Zn in its roots (> 2500 mg Kg^{-1} Zn) could
425 be accounted for by the development of such mechanisms as compartmentation, which
426 could control the ion transport into tillers, thereby improving its tolerance to Zn.
427 Consequently, the hypertolerance to zinc proved by these results, together with its
428 ability to establish in a wide variety of ecosystems, reflect that this species is suitable as
429 a phytostabilizer for revegetation of Zn-contaminated lands.
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Figure captions

Fig. 1. Growth analysis of *Juncus acutus* in response to treatment with a range of Zn concentrations for 50 d. Total dry mass (A); relative growth rate, RGR (B); number of tillers (C) and mean height of tillers (D). Values represent mean ± SE, n = 5. Different letters indicate means that are significantly different from each other (Tukey test, $P < 0.05$).

Fig. 2. Chlorophyll a, Chl *a* (A); Chlorophyll b, Chl *b* (B) and carotenoids, C*x*+*c* (C) concentrations in randomly selected, fully developed photosynthetic tiller of *Juncus acutus* in response to treatment with a range of Zn concentrations for 50 d.Values represent mean ± SE, n = 5. Different letters indicate means that are significantly different from each other (Tukey test, $P < 0.05$).

Fig. 3. Maximum quantum efficiency of PSII photochemistry, F_v/F_m (A); quantum efficiency of PSII, Φ_{PSII} (B) and non-photochemical quenching, NPQ (C) at dawn (○) and at midday (●) in randomly selected, fully developed photosynthetic tillers of *Juncus acutus* in response to treatment with a range of Zn concentrations for 50 d. Values represent mean ± SE, n = 10. Different letters indicate means that are significantly different from each other (Tukey test, $P < 0.05$).

Fig. 4. Concentration of Zn (A); calcium, Ca (B); sodium, Na (C); potassium, K (D); phosphorus, P (E) and magnesium, Mg (F) in tillers (○) and roots (●) of *Juncus acutus* in response to treatment with a range of Zn concentrations for 50 d. Values represent mean, n = 5. Different letters indicate means that are significantly different from each other (Tukey test, $P < 0.05$).

Fig. 5. C/N ratio for tillers (○) and roots (●) of *Juncus acutus* in response to treatment with a range of Zn concentrations for 50 d. Values represent mean, n = 5. Different

583 letters indicate means that are significantly different from each other (Tukey test, $P <$

584 0.05).

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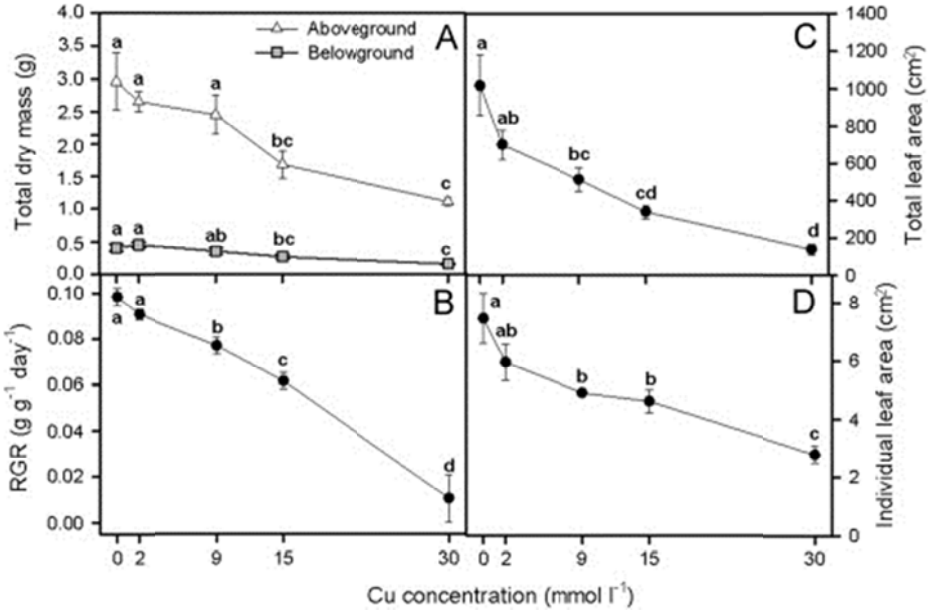

Fig. 1.

Growth analysis of *Atriplex halimus* in response to treatment with a range of Cu concentrations over 20 d. Total dry mass (above- and belowground biomass) (A), relative growth rate, RGR (B), total leaf area (C) and individual leaf area. Values represent mean±SE, n=6. Different letters indicate means that are significantly different from each other (Tukey test, p<0.05).11111

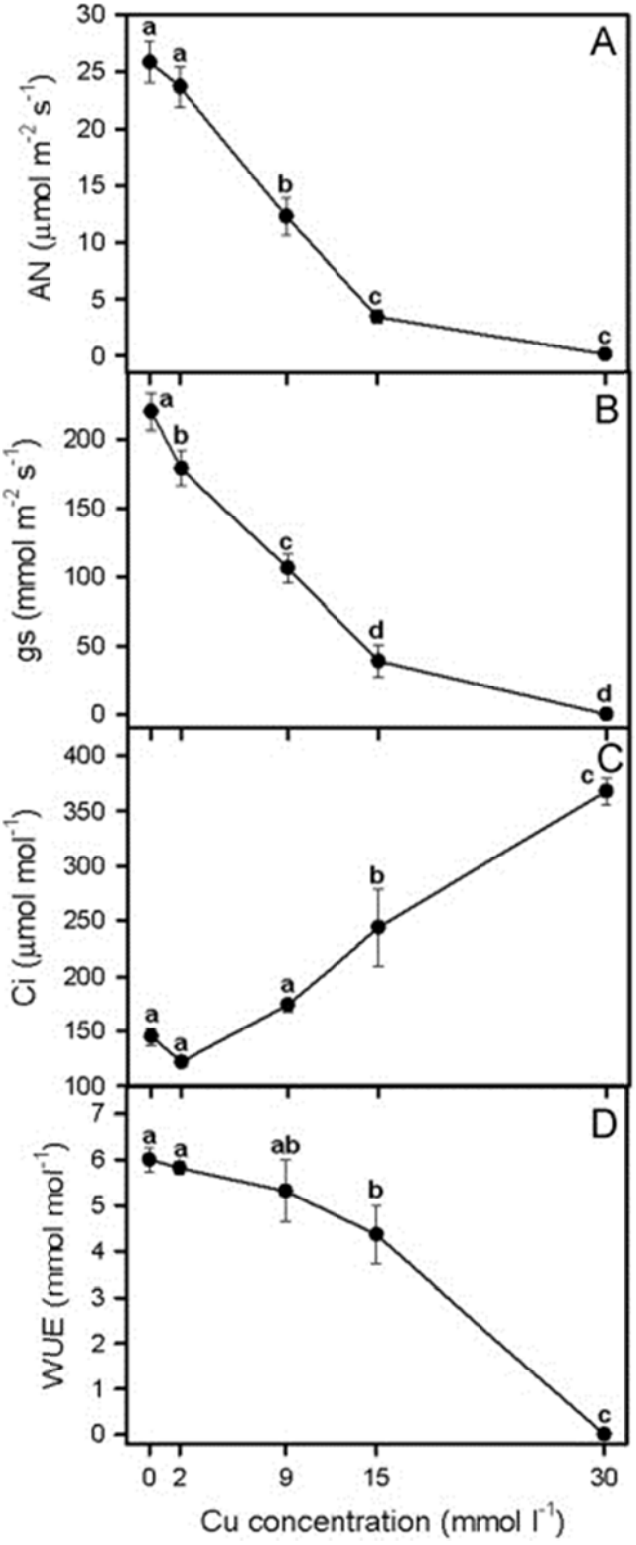

Fig. 2.

Net photosynthetic rate, AN (A), stomatal conductance, gs (B), intercellular CO2 concentration, Ci (C) and water use efficiency, WUE (D) in randomly selected, fully expanded leaves of Atriplex halimus in response to treatment with a range of Cu concentrations over 20 days. Values represent mean±SE, n=12. Different letters indicate means that are significantly different from each other (Tukey test, $p<0.05$).

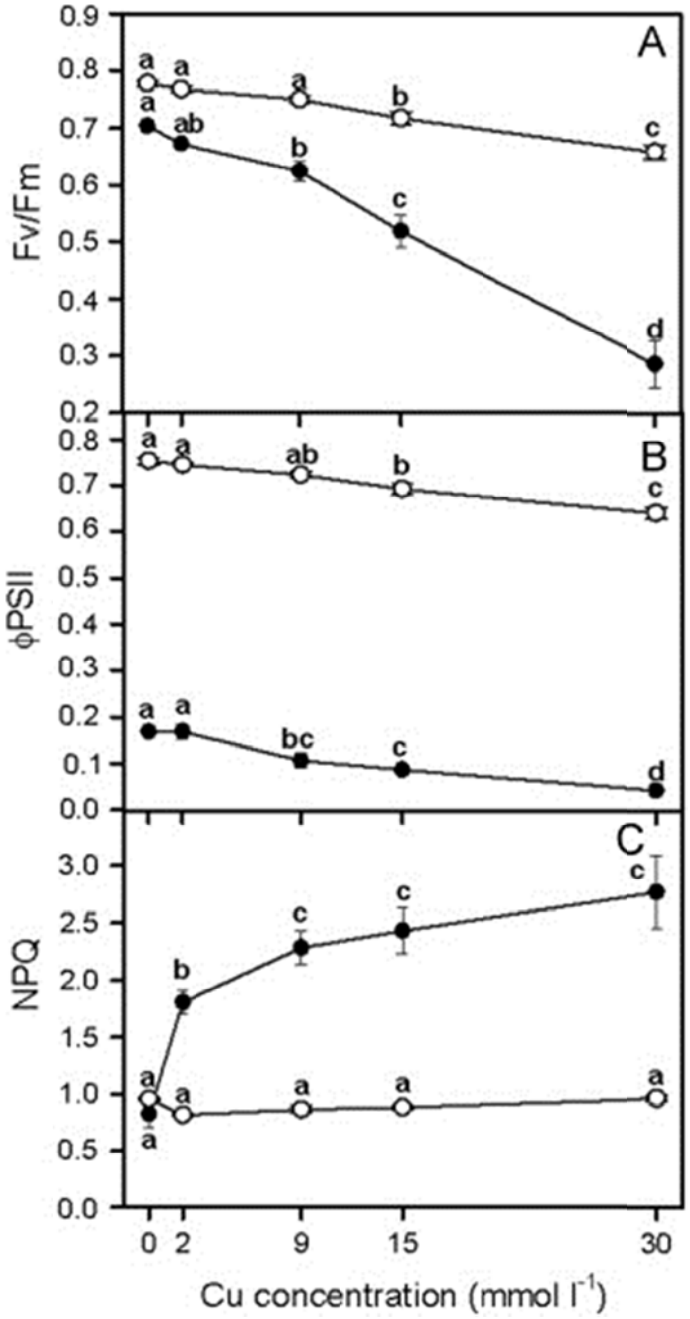

Fig. 3.

Maximum quantum efficiency of PSII photochemistry, F_v/F_m (A), quantum efficiency of PSII, Φ_{PSII} (B) and non-photochemical quenching (C) at midday (●) and at dawn (○) in randomly selected, fully expanded leaves of *Atriplex halimus* in response to treatment with a range of Cu concentrations over 20 days. Values represent mean±SE, n=10. Different letters indicate means that are significantly different from each other (Tukey test, p<0.05).

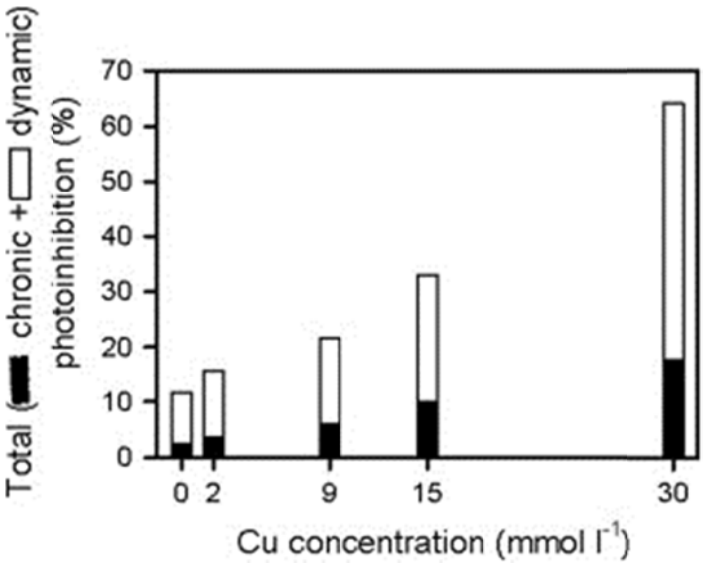

Fig. 4.

Total, chronic and dynamic photoinhibition in randomly selected, fully expanded leaves of *Atriplex halimus* in response to treatment with a range of Cu concentration over 20 days. Values represent mean±SE, *n*=10.

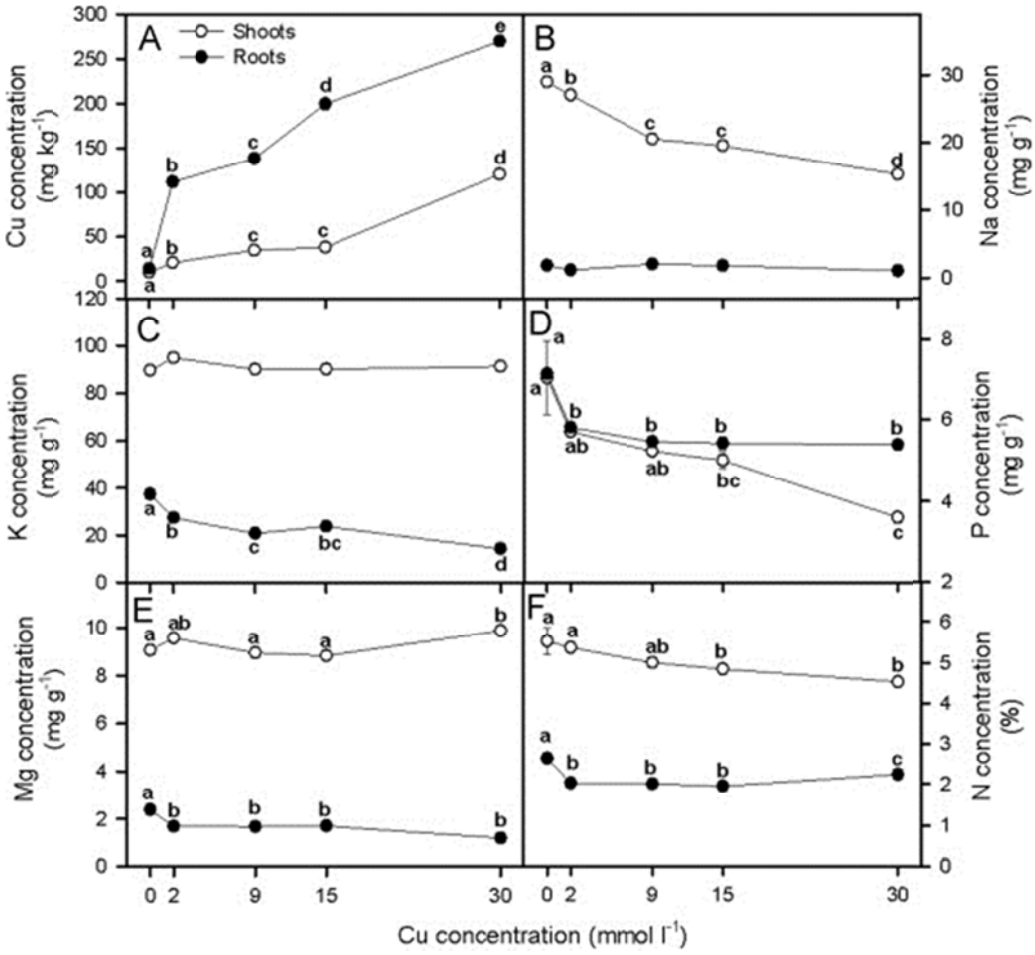

Fig. 5.

Total copper, Cu (A), total sodium, Na (B), total potassium, K (C), total phosphorous, P (D), total magnesium, (E) and total nitrogen (F) concentrations on a dry weight basis for above- (○), and belowground biomass (●) of *Atriplex halimus* in response to treatment with a range of Cu concentrations over 20 days. Values represent mean, *n*=6. Different letters indicate means that are significantly different from each other (Tukey test, $p<0.05$).