Salinity alleviates zinc toxicity in the saltmarsh zinc-accumulator *Juncus acutus*


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The potential importance of *Juncus acutus* for remediation of Zn-contaminated lands has been recognized, because of its Zn tolerance and capacity to accumulate Zn. Since it is also a halophyte, the extent to which salinity influences its Zn tolerance requires investigation. A factorial greenhouse experiment was designed to assess the effect of NaCl supply (0 and 85 mM NaCl) on the growth, photosynthetic physiology and tissue ions concentrations of plants exposed to 0, 30 and 100 mM Zn. Our results indicated that NaCl supplementation alleviated the effects of Zn toxicity on growth, as Zn at 100 mM reduced relative growth rate (RGR) by 60% in the absence of NaCl but by only 34% in plants treated also with NaCl. This effect was linked to a reduction in Zn tissue concentrations, as well as to overall protective effects on various stages in the photosynthetic pathway. Thus, at 85 mM NaCl plants were able to maintain higher net photosynthesis ($A_N$) than in the absence of added NaCl, although there were no differences in stomatal conductance ($g_s$). This contributed to preserving the trade-off between CO$_2$ acquisition and water loss, as indicated by higher intrinsic water use efficiency ($iWUE$). Hence, $A_N$ differences were ascribed to limitation in the RuBisCO carboxylation, manifested as higher intercellular CO$_2$ concentration ($C_i$), together with dysfunction of PSII photochemistry (in term of light harvest and energy excess dissipation), as indicated by higher chronic photoinhibition percentages and variations in the photosynthetic pigment profiles in presence of Zn under non-saline conditions.

**Keywords:** Chlorophyll fluorescence; Gas exchange; Halophyte; Photoinhibition; Salinity; Zn-stress.
1. Introduction

*Juncus acutus* L., is a caespitose, halophytic rush, with a sub-cosmopolitan distribution, that inhabits coastal marshes and dune slacks encompassing a wide range of salinity (Fernández-Carvajal, 1982). Together with various other *Juncus* species, it has been proposed as a bio-tool for wetland restoration projects around the world (Sparks et al., 2013; Marques et al., 2011). In particular, it has potential for the remediation of metal pollution, since it shows great tolerance to excess metals and the capacity to accumulate large amounts of them in its tissues without serious symptoms of toxicity (Mateos-Naranjo et al., 2014; Santos et al., 2014; Christofilopoulos et al., 2016). Medas et al. (2017) have recently suggested that *J. acutus* is able to optimize its response to metal pollution by tuning different biomineralization mechanisms with the minerals and geochemical conditions of the site. Previous studies of metal accumulation and its effects on the performance *J. acutus* have focused on zinc (Mateos-Naranjo et al., 2014; Santos et al., 2014; Christofilopoulos et al., 2016; Medas et al., 2017), although recently interactions of Zn with Cr, Ni and Cd have also been assessed (Christofilopoulos et al., 2016).

Zinc is an essential element for plant metabolism (Kabata-Pendias and Pendias, 2001). However, its excess can lead to various phytotoxicity effects on plant metabolism (Chaney, 1993), and specifically on halophytic species (Liu et al., 2016). The photosynthetic apparatus (i.e. Calvin cycle and photosystem functionality) is especially sensitive to this ion excess (Van Assche and Clijsters, 1986). Despite such potentially deleterious effects, *J. acutus* is regarded as Zn-hypertolerant, a feature attributable to a series of physiological and biochemical adaptations. In particular, Mateos-Naranjo et al. (2014) showed that carbon assimilation and the efficiency of PSII were not affected by high concentrations of Zn in the culture solution. Furthermore,
Santos et al. (2014) found that maintenance of the functionality of its photosynthetic apparatus was linked with its ability to overcome oxidative damage produced by excess Zn uptake, through the modulation of its antioxidant enzymatic machinery and efficient dissipation of the cellular redox potential consequent on Zn incorporation into chlorophyll molecules. These studies however, did not take account of the potential interaction of Zn with other important factors characteristic of marshes ecosystems, particularly salinity. It has been demonstrated that the accumulation of sodium in another halophyte, Spartina densiflora, can mitigate its responses to Zn-induced stress (Redondo-Gómez et al., 2011). Hence knowledge of the extent to which salinity might modulate the physiological responses of J. acutus to excess Zn is necessary for a realistic assessment of its metal toxicity thresholds and its potential for the remediation of zinc-polluted saltmarshes.

This study employed a factorial experiment which aimed to: (1) investigate the influence of NaCl on the growth responses of J. acutus plants exposed to different Zn concentrations; (2) determine the extent to which this influence could be accounted for by impacts on its photosynthetic apparatus, both in terms of carbon assimilation and efficiency of light-energy use, and (3) assess the nutrient and Zn accumulation patterns consequent on the joint effects of treatment with elevated NaCl and Zn.

2. Material and Methods

2.1. Plant material

Seeds of Juncus acutus were collected in December 2013 from different individuals (n = 20) randomly selected from a well-established population in Doñana National Park (Huelva, SW Spain). The seeds were transported to the laboratory for
germination in a germination chamber (ASL Aparatos Científicos M-92004, Madrid, Spain) under the following conditions: photoperiod, 16/8 h light/darkness; temperature, 24/15°C; photon flux rate (400–700 nm), 35 µmol m⁻² s⁻¹. Germinated seedlings were immediately transferred to individual plastic pots (12 cm in depth, 0.5 L total volume) filled with perlite and placed in a glasshouse (University of Seville, Greenhouse Service) at controlled temperature of 25±3 °C, and a relative humidity of 40-60%, with natural day light (maximum quantum flux rate of 1000 µmol m⁻² s⁻¹). Pots were irrigated with nutrient solution (Hoagland and Arnon, 1938) before the onset of the experimental treatments.

2.2. Zn and NaCl experimental stress treatments

In June 2014, pots containing the *J. acutus* plants were randomly assigned to three Zn treatments (concentrations of 0, 30 and 100 mM) in factorial combination with two NaCl concentrations (0 and 85 mM) for 40 days. Zn and NaCl concentrations were established by combining Hoagland’s solution with appropriate amounts of ZnSO₄·7H₂O and NaCl, respectively. Thus, at the beginning of the experiment, the pots were placed in plastic trays containing appropriate solutions to a depth of 1 cm (10 replicate pots per stress treatment combination). In order to avoid changes of Zn and NaCl concentration caused by water evaporation from the nutrient solution, levels in the trays were monitored continuously throughout the experimental and topped up to the marked level with Hoagland’s solution (without additional ZnSO₄·7H₂O or NaCl). Furthermore, pH of the solution was monitored and adjusted to 6.5 - 7.0. The entire solution (including ZnSO₄·7H₂O and NaCl) in the trays was renewed weekly and their
positions were changed randomly every 2 days to avoid effects of environmental
heterogeneity inside the glasshouse.

After 40 days of exposure to the stress-inducing treatments, measurements of
growth, gas exchange, chlorophyll fluorescence, photosynthetic pigment concentrations
and tissue ion concentrations were made.

2.3. Growth measurements

Four plants from each treatment were harvested at the beginning of the
experiment and a further ten at the end. Plants were divided in roots and shoots and
these biomass fractions were oven dried (60°C for 48 h) and then weighed. In addition,
the number of dead tillers was recorded at the end of the experiment.

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

\[ RGR = \left( \ln B_f - \ln B_i \right) \cdot D^{-1} \, (g \, g^{-1} \, day^{-1}) \]

where \( B_f \) = final dry mass, \( B_i \) = initial dry mass (the mean of the four plants from
each treatment sampled at the beginning of the experiment) and \( D \) = duration of
experiment (days).

2.4. Photosynthetic physiology
Gas exchange and chlorophyll fluorescence parameters were measured on the same sections of randomly selected, fully developed photosynthetic tillers (n = 10) using an infrared gas analyzer (LI-6400-XT, Li-COR Inc., NE., USA) and a modulated fluorimeter (FMS-2; Hansatech Instruments Ltd., UK), respectively. The following gas exchange parameters were recorded at a light flux density of 1500 µmol photons m\(^{-2}\) s\(^{-1}\), ambient CO\(_2\) concentration (C\(_a\)) 400 µmol mol\(^{-1}\) air, leaf temperature of 25 °C and 50 ± 5 % relative humidity: net photosynthetic rate (A\(_N\)), stomatal conductance (g\(_s\)), intercellular CO\(_2\) concentration (C\(_i\)), and intrinsic water use efficiency (iWUE). The saturation pulse method was used to determine the energy yields of the Photosystem II (PSII) reaction centers: maximum quantum efficiency of PSII photochemistry (F\(_v\)/F\(_m\)), quantum efficiency of PSII (Φ\(_{PSII}\); Genty et al., 1989) and non-photochemical quenching (NPQ). As described by Schreiber et al. (1986), a 0.8 s saturating actinic light pulse of 15000 µmol m\(^{-2}\) s\(^{-1}\) was given, at dawn (stable, 50 µmol m\(^{-2}\) s\(^{-1}\) ambient light) and midday (1700 µmol photons m\(^{-2}\) s\(^{-1}\)), to photosynthetic tillers previously dark-adapted or exposed to light for 30 min.

Finally, the total chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid (C\(_x+c\)) contents of extracts obtained from randomly selected fully developed photosynthetic tillers (n = 5), were determined with a Hitachi U-2001 spectrophotometer (Hitachi Ltd., Japan), using three wavelengths (663.2, 646.8 and 470.0 nm). For more details, see Mateos-Naranjo et al. (2008). Concentrations of pigments (µg g\(^{-1}\)fw) were calculated according to Lichtenthaler (1987).

2.5. Tissue ion concentrations

Tiller and root samples taken from ten plants per treatment were dried at 80ºC for 48 h and ground, according to the protocols of Mateos-Naranjo et al. (2011). Then,
triplicate 0.5 g samples from each specific tissue were digested in 6 ml HNO₃, 0.5 ml HF and 1 ml H₂O₂. Ca, Mg, K, P, Na and Zn concentrations in the digests were measured by inductively coupled plasma (ICP) spectroscopy (ARL-Fison 3410, USA).

2.6. Statistical analysis

Statistical tests were performed in the software package Statistica v. 6.0 (Statsoft Inc.). Generalized linear models (GLM) were used to analyze the interactive effects of Zn and NaCl concentrations (as categorical factors) on the growth and physiological parameters (as dependent variables) of *J. acutus* plants. Multiple comparisons were analyzed by a LSD (post hoc) test. Before statistical analysis Kolmogorov-Smirnov and Brown-Forsythe tests were used to verify the assumptions of normality and homogeneity of variances, respectively. Differences between tiller and root ion concentrations were compared by the Student test (t-test).

3. Results

3.1. Effects of Zn and NaCl on growth

There were significant effects of both zinc and salinity on the RGR of *Juncus acutus* but no significant interactions (Table 1, GLM: salinity, p < 0.05; Zn, p < 0.01). Thus, in non-saline conditions RGR decreased 25% and 60% in plants grown at 30 and 100 mM Zn, respectively, compared to control; however, growth was much less affected by Zn in plants exposed to 85 mM NaCl (i.e. 11% and 34% for 30 and 100 mM Zn, respectively; Fig. 1A). Similarly, the percentage of dead tillers increased sharply with Zn concentration (GLM: Zn, p < 0.01), but this increase was less acute in plants grown in saline conditions (GLM: salinity, p = 0.07; Fig. 1B).
3.2. Effects of Zn and NaCl on photosynthetic physiology

There were significant effects of salinity and Zn treatments on net photosynthetic rate (A$_N$) after 40 d of treatment (Table 1, GLM: salinity, p < 0.05; Zn, p < 0.01 and salinity x Zn, p < 0.01). Thus A$_N$ decreased progressively with increasing Zn concentration in plants grown at both NaCl concentrations. However, plants exposed to saline conditions maintained higher CO$_2$ assimilation rates at both increased concentrations of Zn than their non-saline counterparts (Fig. 2A). Very similar trends were recorded for stomatal conductance (g$_s$) but salinity did not significantly affect the responses to Zn (GLM: salinity x Zn, p = 0.06; Fig. 2B). In contrast, salinity significantly reduced the intercellular CO$_2$ concentration (C$_i$) (GLM: salinity, p < 0.05), whereas Zn concentration per se did not. However, C$_i$ values were reduced at the high salinity only in the presence of excess (30 or 100 mM) Zn (Fig. 2C). Salinity and Zn had synergistic effects on intrinsic water use efficiency (iWUE; GLM: salinity x Zn, p < 0.05). Thus, plants grown under saline conditions had consistently higher iWUE but the difference was only significant at 30 mM Zn (Fig. 2D).

Chlorophyll fluorescence parameters were also affected by the combination of Zn and salinity treatments. F$_{v}$/F$_{m}$ values, both at dawn and midday, tended to decrease with increasing Zn concentration in plants grown in non-saline conditions. However, in plants exposed to salinity, this effect was less marked and only evident at the highest Zn concentration treatment (Table 1, GLM$_{Md}$ and Pd: salinity x Zn, p < 0.05; Fig. 3A, B). Φ$_{PSII}$ values at dawn and at midday followed a similar pattern to those of F$_{v}$/F$_{m}$ (GLM$_{Md}$: salinity x Zn, p < 0.05; Fig. 3C,D), except that the differences in predawn values were minimal. NPQ values at midday increased markedly with Zn concentration, both in the absence and presence of salinity, but this effect was substantially stronger in the absence
of salinity (Table 1, GLM<sub>Md</sub>: salinity, p < 0.01 and Zn, p < 0.001; Fig. 3E). Predawn NPQ did not show any response to Zn or salinity, with values c. 0.15 in all cases (Fig. 3F).

The percentage of chronic photoinhibition increased progressively with increasing Zn concentration at both NaCl concentrations (Fig. 4A,B). However, this increment was more acute in plants grown under non-saline conditions. The percentage of dynamic photoinhibition did not vary with salinity or Zn treatments, except in plants grown at the highest Zn concentration and 85 mM NaCl, which showed a greater percentage inhibition than in the other treatments (Fig. 4A,B).

The concentration of chlorophyll a (Chl a) was decreased by excess Zn in the growth medium, although this reduction was entirely mitigated by salinity (Table 1, GLM: salinity x Zn, p < 0.01; Fig. 5A). Chlorophyll b (Chl b) and carotenoid (C<sub>x+c</sub>) concentrations did not show any response to excess Zn in plants grown in the absence of salinity, but they increased in those exposed to both Zn and salinity (GLM<sub>Chl b and C<sub>x+c</sub></sub>: salinity x Zn, p < 0.01; Fig. 5B,C).

3.3. Effects of Zn and NaCl on tissue ion concentrations

Tissue ion concentrations were greater in roots than in tillers, except for K in all specific treatments and for P in plants grown at 100 mM Zn + 0 mM NaCl, 0 mM Zn + 85 mM NaCl and 30 mM Zn + 85 mM NaCl, (t-test, p < 0.05; Table 2). In addition, there were significant effects of salinity and Zn treatments on tissue ion concentrations except for K and Mn tiller concentrations (Table 1). Thus Zn concentrations increased markedly with the concentration of Zn in the growth medium in both roots and tillers, but this increment was more acute in the absence of NaCl addition (GLM: salinity x Zn, p < 0.01; Table 2). Furthermore, tissue Na concentrations were considerably greater
under saline conditions and tended to increase with the Zn concentration. Except for roots in presence of NaCl, where Na concentration showed a reduction with Zn augmentation (GLM: salinity x Zn, p < 0.01; Table 2). On the other hand, overall the concentrations of Mg, Ca, P and Mn in tillers and roots, and K in roots decreased with the increase of the concentration of Zn in the growth medium at both saline levels (Table 2). In general, the concentrations of these elements were significantly lower in plants grown with NaCl supplementation (Table 2).

4. Discussion

Understanding the effects of high metal concentrations on tolerant species and the thresholds for phytotoxicity is essential for the design and development of effective methodologies for environmental remediation. Similarly important is knowledge of possible interactions between metals, and between metals and other important environmental factors that may limit species distribution; in estuarine ecosystems interactions with salinity are relevant to the future use of halophytes that can cope with the growing problem of metal pollution of salinized lands (Kholodova et al., 2010).

This experiment confirmed previous work that had demonstrated hypertolerance to Zn stress in Juncus acutus (Mateos-Naranjo et al., 2014). Thus, the concentration of Zn required to kill 50% of its tillers after 40 days of exposure (LC50; Paschke et al., 2000) was greater than our most severe treatment of 100 mM. However, elevated concentrations of Zn in the culture solution progressively affected plant development, and this was particularly reflected in a clear reduction of RGR and an increase in the percentage of dead tillers. These deleterious effects are consistent with previously described general responses of vascular plants to excess Zn (Vaillant et al., 2005; Mateos-Naranjo et al., 2008; Santos et al., 2014). Nevertheless, we found that Zn
toxicity was partially counterbalanced by addition of NaCl to the growth medium, such that salinity-treated plants were able to maintain a higher RGR than their non-salinity treated counterparts. In addition, they reduced toxicity, as indicated by lower percentages of dead tillers at both 30 and 100 mM Zn. Therefore, the results suggest that salinity increases the tolerance of *J. acutus* to the toxic effects of high concentrations of Zn. This interaction is consistent with results for species not recognized as hypertolerant to Zn: Redondo-Gómez et al. (2011) demonstrated that the addition 170 mM NaCl to a growth medium with 1 mM Zn diminished the damage caused by metal excess in *Spartina densiflora*, and Han et al. (2013) reported similar amelioration of the effects of 100 µM Zn by the addition of 50 mM NaCl to the growth medium with in *Kosteletzkyia virginica*.

The mechanisms by which NaCl supplementation could enhance plant tolerance to elevated metal concentrations are not clear. Effects on metal uptake and translocation, and the resulting nutrient uptake balance have been described in certain estuarine species (Fitzgerald et al., 2003; Kadukova and Kalogerakis, 2007; Han et al., 2013). Redondo-Gómez et al. (2011) found that NaCl supplementation increased Zn accumulation in *S. densiflora* tissues compared with non-salinized plants, but this was accompanied by an overall improvement in nutrient uptake. Similar modifications in mineral content were recorded in *Kosteletzkyia virginica* tissues in response to salinity and Zn (Han et al. 2013), but in that case NaCl addition acted through a modification of Zn distribution rather than a decrease in plant Zn uptake capacity. In contrast, we found that although tissues Zn concentrations in *J. acutus* increased markedly with the external concentration in accordance with previous studies, this increase was progressively lower as tissue Na concentration increased in response to NaCl supplementation. Furthermore, salinity hindered the uptake of most nutrients in the
highest Zn concentration. These discrepancies may be ascribed to the severity of stress imposed, since a maximum concentration of 100 mM Zn was used in the present study whereas Redondo-Gómez et al. (2011) and Han et al. (2013) used only 1 mM and 100 µM, respectively. Reduced nutrient concentrations with the progressive accumulation of Na in roots and shoots have been found previously in other halophytes (Redondo-Gómez et al., 2007, 2010).

Notwithstanding the nutritional imbalance induced by Na accumulation, the lower concentrations of Zn in the tissues of plants grown in the presence of NaCl could help to explain their higher tolerance. Excess Zn accumulated in the tissues is likely to be toxic, affecting a variety of physiological and biochemical processes (Kabata-Pendias and Pendias, 2001). However, despite such reductions in tissue Zn concentration in J. acutus, it must be acknowledged that concentrations were still greater than the toxicity threshold for plants generally (Kabata-Pendias and Pendias, 2001). Consequently, other mechanisms must be involved in the ameliorative effect of NaCl on Zn toxicity in J. acutus.

Metal hypertolerance has been associated with various ecophysiological adaptations to metalliferous environments (Evangelou et al., 2004; Mateos-Naranjo et al., 2014; Santos et al., 2014). In particular, Mateos-Naranjo et al. (2014) indicated that Zn hypertolerance in J. acutus was linked with its capacity to maintain carbon assimilation and the efficiency of PSII even at Zn concentration of 100 mM. In contrast we found a clear deleterious effect of Zn at this concentration on the photosynthetic apparatus in the present experiment; this discrepancy may be attributable to different experimental and measurement conditions. Although AN (along with gs) decreased considerably with increasing Zn concentration, plants grown at 85 mM NaCl were able to maintain higher AN values than their non-saline counterparts. However, this positive
effect cannot be attributed to alleviation of stomatal limitation, since $g_s$ values did not vary between salinity levels in either Zn treatment. Therefore, differences in $A_N$ value between NaCl levels and each specific Zn concentration treatment could be explained by non-stomatal limitations (Flexas and Medrano, 2002). In this regard, Perez-Romero et al. (2016) found that photosynthesis activity was more limited by mesophyll conductance ($g_m$) than $g_s$ in *Salicornia ramossisima* in response to Cd. Moreover, $g_m$ has been widely implicated in photosynthetic responses patterns to salinity (Flexas et al., 2012). Hence, it is possible that $A_N$ differences between salinity levels in *J. acutus* plants at the same Zn concentration could be linked with $g_m$ variations; however this area requires further research. Another possibility relates to impairment of major carbon-assimilation enzyme activities, such as RuBisCO that may degrade the photosynthetic pathway under metal stress (Perfus-Barbeoch et al., 2002; Khan and Khan, 2014). A degree of metal tolerance has been demonstrated in the maintenance such enzyme functions (Ying et al., 2010; Pérez-Romero et al., 2016). Taking into account these issues, the higher $C_i$ in *J. acutus* plants grown without NaCl addition suggests that differences in carbon assimilation between salinity treatments could have been linked to limitation in RuBisCO carboxylation capacity (Mateos-Naranjo et al., 2008, 2014).

On the other hand, the greatest photosynthetic tolerance to Zn-induced stress under saline conditions was associated with the highest integrity and functionality of the photochemical apparatus of *J. acutus*. It is known that Zn is concentrated in chloroplasts and interacts with the PSII donor, inhibiting the photosynthetic fixation of CO$_2$ and the Hill reaction (Prasad and Strzalka, 1999). In addition, Monnet et al. (2001) indicated that the destruction of antenna pigments would affect the efficiency of PSII. Our results revealed that $F_v/F_m$ and $\Phi_{PSII}$ values were affected by elevated Zn and this effect was
more acute in plants grown in absence of NaCl, suggesting that NaCl alleviates Zn-induced, excess-light photoinhibition. Furthermore, under non-saline conditions and in presence of Zn, NPQ values were higher, which indicates that more of the absorbed energy would have been dissipated as heat and would not taken the photochemical pathway (Flexas et al., 2012). In line with our results, Padinha et al. (2000) and Mateos-Naranjo et al. (2008) also found that Zn stress affected the PSII photochemistry of the halophytes Spartina maritima and S. densiflora, respectively. Damage to photosynthetic components may lead to an increase of photoinhibition (Werner et al., 2002), a phenomenon that affects photosynthetic productivity and, consequently, plant growth (Melis, 1999). This fact could contribute to explaining our growth data, since chronic photoinhibition percentage increased in presence of Zn under non-saline conditions, whereas this increased photoinhibition was ameliorated under saline conditions, although less so in plants exposed to 100 mM Zn. However, these plants showed a greater dynamic photoinhibition percentage compared to other treatments, which would indicate an overcompensation effect of the excess of energy fixed, through thermal dissipation mechanisms, thereby protecting the leaf from light-induced damage (Maxwell and Johnson, 2000). In addition, the benefit of NaCl supplementation to photosynthetic-pigment concentration in the presence of Zn could contribute to explaining its positive effects on the photosynthetic apparatus efficiency of J. acutus.

Finally the greater tolerance to Zn in plants treated with NaCl was linked with a better water balance, an idea supported by the overall higher iWUE values. Thus, these plants would be able better to preserve the trade-off between CO₂ acquisition for growth and water loss, as indicated the higher A_N and the invariable g_s values compared with their counterparts not treated with NaCl. Han et al. (2013) also found a positive effect of NaCl supplementation on water relations, in Kosteletzkyia virginica, under Zn excess.
This beneficial effect could be linked with the key role of Na accumulation in plant osmotic adjustment (Shabala et al., 2009). Hence, it is possible that the higher Na concentration in tissues of J. acutus under saline conditions and the reduction in $g_s$ in the presence of Zn might help to alleviate any water stress ascribed to Zn toxicity.

5. Conclusions

We may conclude that the presence of NaCl in the growth medium, at concentrations representative of estuarine environments, considerably reduces the deleterious effects of elevated Zn concentrations on the growth and development of J. acutus. This beneficial effect was largely mediated by the reduction of Zn levels in J. acutus tissues, together with an overall protective effect on its photosynthetic apparatus, manifested as improved carbon harvesting, functionality of the photochemical apparatus (PSII) and photosynthetic pigment concentrations. Furthermore, amelioration by NaCl was linked with the maintenance of a more advantageous water balance. These ecophysiological characteristics would enhance the fitness and competitive ability of J. acutus in zinc-polluted estuaries and saltmarshes, providing a tolerant bio-tool for the management and restoration metal pollution in salinized lands.
Acknowledgements

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**Table 1.** Generalized linear model (GLM) results for the growth, physiological and tissues ions concentration of *J. acutus* plants in response to Zn and NaCl concentration (as categorical variables) and its interaction. * Significance level 95% and ** Significance level 99%. Md (midday), Pd (predawn), T (tiller and R (root)).

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<td>[P]_T</td>
<td>0.02*</td>
<td>0.00**</td>
<td>0.04*</td>
</tr>
<tr>
<td>[P]_R</td>
<td>0.00**</td>
<td>0.00**</td>
<td>0.01**</td>
</tr>
<tr>
<td>[Mn]_T</td>
<td>0.78</td>
<td>0.00**</td>
<td>0.88</td>
</tr>
<tr>
<td>[Mn]_R</td>
<td>0.00**</td>
<td>0.00**</td>
<td>0.00**</td>
</tr>
</tbody>
</table>
Table 2. Ion concentration in tiller and roots of *Juncus acutus* treated with a range of Zn concentration in combination with 0 mM and 85 mM NaCl, after 40 days. Values represent mean ± SE, n = 5.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Tiller concentration</th>
<th>Root concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zn (mM)</strong></td>
<td><strong>NaCl (mM)</strong></td>
<td><strong>Zn (mg Kg⁻¹)</strong></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>32.3 ± 0.5ᵃ</td>
</tr>
<tr>
<td>0</td>
<td>85</td>
<td>304.6 ± 1.4ᵇ</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>611.7 ± 0.8ᶜ</td>
</tr>
<tr>
<td>0</td>
<td>85</td>
<td>87.3 ± 0.7ᵃ</td>
</tr>
<tr>
<td>0</td>
<td>85</td>
<td>2479.0 ± 0.3ᶜ</td>
</tr>
<tr>
<td>100</td>
<td>85</td>
<td>1969.2 ± 1.1ᶠ</td>
</tr>
</tbody>
</table>

Different letters indicate means that are significantly different from each other.
Figure legends

**Fig. 1.** Relative growth rate, RGR (A) and percentage of dead tillers (B) in *Juncus acutus* plants in response to a treatment with a range of Zn concentration with (●) and without (○) NaCl addition, after 40 days. Values represent mean ± SE, n = 10. Different letters indicate means that are significantly different from each other (LSD test, P < 0.05).

**Fig. 2.** Net photosynthetic rate, $A_N$ (A), stomatal conductance, $g_s$ (B), intercellular CO$_2$ concentration, $C_i$ (C), and intrinsic water use efficiency, $i$WUE (D) in randomly selected, fully developed photosynthetic tiller of *Juncus acutus* treated with a range of Zn concentration with (●) and without (○) NaCl addition, after 40 days. Values represent mean ± SE, n = 10. Different letters indicate means that are significantly different from each other (LSD test, P < 0.05).

**Fig. 3.** Maximum quantum efficiency of PSII photochemistry, $F_v/F_m$ (A,B), quantum efficiency of PSII, $Φ_{PSII}$ (B,C), and non-photochemical quenching, NPQ (D,E), at midday and predawn in randomly selected, fully developed photosynthetic tiller of *Juncus acutus* treated with a range of Zn concentration with (●) and without (○) NaCl addition, after 40 days. Values represent mean ± SE, n = 10. Different letters indicate means that are significantly different from each other (LSD test, P < 0.05).

**Fig. 4.** Total chronic and (●) and dynamic (○) photoinhibition percentage in randomly selected, fully developed photosynthetic tiller of *Juncus acutus* treated with a range of Zn concentration at 0 mM (A) and 85 mM (B) NaCl concentration, after 40 days. Values represent absolute percentage per each specific treatment.
Fig. 5. Chlorophyll a, Chl a (A), chlorophyll b, Chl b (B) and carotenoids, Cx+c (C) concentrations in randomly selected, fully developed photosynthetic tiller of *Juncus acutus* treated with a range of Zn concentration with (●) and without (○) NaCl addition, after 40 days. Values represent mean ± SE, n = 5. Different letters indicate means that are significantly different from each other (LSD test, P < 0.05).
Fig. 1

A

RGR (g g⁻¹ d⁻¹)

0 mM NaCl
85 mM NaCl

B

Dead tiller (%)

0 30 100

Zn concentration (mM)

0 3 6 9 12 15 18 21 24 27

0.000 0.003 0.006 0.009 0.012 0.015 0.018 0.021 0.024 0.027 0.030

a a bc ab b c d c bc
Fig. 2
Fig. 3

Midday

Predawn

\[ 0 \text{ mM NaCl} \]
\[ 50 \text{ mM NaCl} \]

F<sub>i</sub>/F<sub>M</sub>

\[ \psi_{\text{cell}} \]

NPO

Zn concentration (mM)
Fig. 4

![Bar chart showing total photoinhibition (%) at 0 mM NaCl and 85 mM NaCl with different Zn concentrations (0, 30, 100 mM) for Chronic and Dynamic treatments.](image)

- **0 mM NaCl**
  - Bar A: Zn concentration (mM) - 0, 30, 100
  - Bar B: Zn concentration (mM) - 0, 30, 100

- **85 mM NaCl**
  - Bar A: Zn concentration (mM) - 0, 30, 100
  - Bar B: Zn concentration (mM) - 0, 30, 100

Legend:
- **Chronic**
- **Dynamic**