Sodium uptake of *Iris wilsonii* and its photosynthetic responses to high-salinity stress in microcosm submerged beds

Jianqiu Han, Haiyan Wang, Yumei Zhou and Chunliang Zhou

**ABSTRACT**

In order to investigate the performance of *Iris wilsonii* in high-salinity wastewater, seven microcosm submerged beds were built with rectangular plastic tanks and packed with marble chips and sand. Each submerged bed was transplanted with six stems of *I. wilsonii*. The submerged beds were operated in a 7-d batch mode in a greenhouse with artificial wastewater for three 42-d periods. Influent to the seven submerged beds had different contents of NaCl, 0, 1, 2, 4, 6, 8, and 10% (by weight). The results suggested that lower salinity contents (1–2%) in influent or during short stress time (0–14d) did not inhibit net photosynthetic rate, stomatal conductance, and transpiration rate of *I. wilsonii*, and the chlorophyll of *I. wilsonii* was not damaged. When initial NaCl contents were at 4% and above, however, all photosynthetic parameters were significantly decreased. It was concluded that *I. wilsonii* could take up Na\(^+\) in wastewater, but higher salinity (4–10%) in wastewater would inhibit the growth of *I. wilsonii*.

**Key words** | chlorophyll fluorescence, high salinity, *Iris wilsonii*, photosynthetic characteristic, submerged bed

**INTRODUCTION**

High-salinity wastewater (>1% salinity by weight) is generated in manufacturing of chemicals such as pesticides, pharmaceuticals and herbicides, and during oil and gas recovery processes (Henze et al. 1995). High-salinity contents are also found in landfill leachate (Ellouze et al. 2008). Moreover, salinization poses one of the greatest threats to our rivers and wetlands and has the potential to cause irreversible damage to the structure and function of aquatic communities in arid regions of the world (Ghassemi et al. 1995). Rising sea levels and stronger storm surges may expose tidal freshwater wetlands to saline waters (Robert et al. 2013).

Constructed wetlands (CWs) have been used to removed solids, organic matter, nutrients and metals contained in many types of wastewater (Kadlec & Wallace 2009; Maine et al. 2009). They perform a multitude of functions not only to the organisms, but also to surrounding environments (Arrigoni et al. 2008; Barbier et al. 2011).

Aquatic macrophytes are often the dominant visual feature of CW, with their associated microbial biofilms playing several vital roles in nutrient removal, transformation and storage. Large numbers of aquatic macrophytes have been used in CWs (Shaharah et al. 2012), such as *Phragmites australis*, *Typha latifolia*, and *Schoenoplectus validus*. The choice of aquatic macrophytes is an important issue in CWs, as they are vital for pollution treatments apart from surviving under the potentially toxic conditions (Mufarrege et al. 2011).

There are many studies about the effects of high salinity in wastewater on CWs. It is believed that high-salinity content in wastewater can alter the biodiversity, species composition and function of the aquatic ecosystems (Halse et al. 2005), and significantly decrease the treatment efficiency of conventional activated sludge, attached microorganism growth, and nitrification and denitrification processes (Bassin et al. 2011). Some studies suggest that some macrophytes, such as *Salvinia herzogii*, *Pistia stratiotes* and *Eichhornia crassipes* could be more affected by high salinity than high concentrations of metals or sulphide (Hadad et al. 2006; Maine et al. 2009).

*Iris wilsonii* is an exotic, perennial, herbaceous plant found in most parts of China (Tang et al. 2007). It is one of
the most promising alternative plants currently under study in CWs because it has some characteristics that make it suitable for use in CWs for wastewater treatment (Tang et al. 2007; Liu et al. 2007).

The objective of this study was to investigate the growth performance of *I. wilsonii* in microcosm submerged beds fed with wastewater at different salinity levels.

### MATERIALS AND METHODS

#### Experimental design

Seven microcosm submerged beds were set up in a greenhouse in Shanghai Institute of Technology (Shanghai, China). The submerged bed models were built of rectangular plastic tanks (55 cm in width, 48 cm in depth and 67 cm in length). Sand and marble chips (effective size 2.6 cm; porosity 0.59) were mixed in a 1 to 3 ratio (by volume) and packed in the beds to 40 cm high. A 5-cm layer of marble chips (effective size 1.3 cm; porosity 0.47) was put on the top of the beds so as to minimize evaporation and maintain air diffusion (Figure 1(a) and 1(b)).

![Figure 1](https://iwaponline.com/wst/article-pdf/74/9/2185/457646/wst074092185.pdf)

**Figure 1** | The section (a) and plan (b) of the microcosm submerged beds used in experiments.

#### Preparation of wetland plants

*I. wilsonii* seedlings with similar heights (approximately 20 cm) were purchased from Jiangsu Fuli Huamu Seed Company located in a temperate maritime climate zone of China. Prior to the experiments, all the seedlings were cultivated in tap water for 14 d, and the tap water was changed once. The plants with a good shape, similar size, and vigorous growth were then selected. After cutting dead branches and rotten roots, they were planted in the submerged beds with six plants per bed.

#### Experimental design and methods

The artificial wastewater was made at half strength of Hoagland solution. The Hoagland solution (per liter) was made with 472.5 mg Ca(NO₃)₂, 303.5 mg KNO₃, 57.5 mg (NH₄)₂PO₄, 246.5 mg MgSO₄, and tap water (total N 0.12 mg/L; total P 0.0004 mg/L; total K 0.02 mg/L) as solvent. NaCl was added to the wastewater to make influent for the seven submerged beds at NaCl concentrations of 0, 1, 2, 4, 6, 8, and 10% (by weight), respectively. The day before the experiment started, influent was correspondingly injected into the beds to make it saturated, and excess solution was discharged. Every seven days, the beds were drained and refilled with fresh influent. Plant growth was tracked down over six cycles of 7-d batch operation. After 42 d, the submerged beds were replanted. The experiments were replicated three times between 20 April and 23 August 2015. Tap water was added every two days to compensate for water loss due to evaporation. The environmental conditions were summarized in Table 1.

#### Analytical methods

Leaf chlorophyll relative content (CRC), photosynthetic parameters, and chlorophyll fluorescence parameters were determined on the 1st, 7th, 14th, 21st, 28th, 35th, and 42nd d. The fully expanded new leaves on the top of the plants were selected at 9:00–11:00 A.M. for analysis. All measurements were taken in duplicate with six plants in each bed and the average values were used for analysis. CRC was measured by SPAD-502 plus (Japan). SPAD-502

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Air humidity (%)</th>
<th>Light time (h)</th>
<th>Light intensity (lux)</th>
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</thead>
<tbody>
<tr>
<td>25–30</td>
<td>35–45</td>
<td>12</td>
<td>20,000–30,000</td>
</tr>
</tbody>
</table>
plus is a portable chlorophyll meter which can measure an index related to the chlorophyll content of a leaf (Marenco et al. 2009). Photosynthetic parameters were monitored with a CI-6400 photosynthetic system (CID, Inc., USA), including net photosynthetic rate ($P_n$), stomatal conductance ($g_s$), and transpiration rate ($T_r$). Chlorophyll fluorescence parameters were detected with an ultra-mobile chlorophyll fluorescence measurement system PAM-2500 (WALZ, Germany), including $ETR$ (electron transport rate of PSII) and $Fv/Fm$ (maximal photochemical efficiency). Prior to measurements of fluorescence, plants were left for 30 min in the dark at room temperature. Chlorophyll molecules were excited for 10 s by actinic light with a photon flux density of 40 m mol m$^{-2}$ s$^{-1}$. At the end of each run (42 d), plants were dug out and adhering soil was washed away with tap water and distilled water in turn. All plants were separated into aboveground parts (leaves and shoots) and roots. They were dried at 85°C for 48 h to constant weight, then crushed and mixed thoroughly. About 0.4 g triturated plant tissue was taken from each sample to digest with 10 mL mixture of $H_2SO_4$ and $H_2O_2$ (5:2, v/v) that was heated in an oven for 2 h at 350°C. Na content was determined with a flame photometric meter (FP-640, J-H Inc., China) for effluent and the acid digests.

### Statistical analysis

All statistical analyses were performed with SPSS software version 17.0 developed by Statistical Software Package (SPSS Inc., Chicago, USA). One-way ANOVA was used to analyze the effects of salinity treatment on growth parameters. Differences between all salinity treatments and the control were analyzed using Analysis of Variance followed by LSD test. Differences were considered to be statistically significant if $p<0.05$ and very significant if $p<0.01$.

### RESULTS AND DISCUSSION

#### Effects of high-salinity contents in wastewater on CRC of *I. wilsonii*

The CRC variations of *I. wilsonii* exposed to high-salinity contents in wastewater are presented in Figure 2. There were no significant differences in CRC of *I. wilsonii* among the six salinity treatments and the control in the first 14 d. On the 21st day, CRC differed among the treatments. CRC under the lower salinity contents (1–6%) was still close to the level of the control, but the SPAD values in the 8% and 10% salinity contents decreased by 21.64% and 39.98%, respectively, compared with the control ($p<0.05$). On the 28th and 35th d, the SPAD values under the 6, 8 and 10% salinity contents were 19.4–41.86%, 46.90–66.07% and 70.00–84.08% lower than that in the control ($p<0.05$), respectively. On the 42nd d, CRC under the 4% salinity content also showed a significant decrease ($p<0.05$). Khripach et al. (2005) reported that salinity caused a decrease in chlorophyll content through chlorophyll biosynthesis inhibition or acceleration of its degradation, which was consistent with our study when salinity contents was 4 to 10%. These results might be caused by: (i) higher osmotic potential because of higher salinity contents in wastewater made *I. wilsonii* difficult to absorb water, then the synthesis of chlorophyll was inhibited; (ii) excessive $Na^+$ taken up by the plants transported to leaves, which damaged chlorophyll molecules and decreased CRC.

#### Effects of high-salinity contents in wastewater on the photosynthetic parameters of *I. wilsonii*

The results (Figure 3) revealed that $P_n$ of *I. wilsonii* at different salinity treatments were similar during the first 14 d. On the 21st d, $P_n$ of *I. wilsonii* under the lower salinity contents (1, 2, 4 and 6%) remained at high levels as in the control, but it decreased in the treatments at the higher salinity contents (8 and 10%) and was significantly different from the control ($p<0.05$). On the 35th d, $P_n$ in the treatments of 6 and 8% was significantly different from that of the control ($p<0.05$), and a very significant difference was found between the treatment of 10% and the control ($p<0.01$). On the 42nd d, $P_n$ in the treatments of 1, 2 and 4% was similar to that in the control, but very significant differences were found between the treatments of 6, 8, and 10% and the
control ($p < 0.01$). These results showed that the decrease of chlorophyll content and water deficit caused by osmotic stress resulted in the decrease of $Pn$.

The lower salinity contents of 1 and 2% did not significantly affect $Cond$ of $I. wilsonii$ (Figure 4). Although $Cond$ of $I. wilsonii$ tended to decrease under all salinity treatments, the difference was not significant during the first two weeks. After that, the $Cond$ of $I. wilsonii$ under the 8 and 10% salinity treatments was significantly lower than that in the control. These results indicated that $Cond$ of $I. wilsonii$ was not affected under the lower salinity contents ($1$–$2\%$) or at the early stage of the experiments ($0$–$14\ d$). But it decreased due to stomatal closure under the higher salinity contents in wastewater or at the later stage of the experiments ($21$–$42\ d$), caused by adaptive response of plants to water deficit.

$Tr$ of $I. wilsonii$ under salinity stress had different trends from $Pn$ and $Cond$ for both treatments and the control (Figure 5). Lower salinity stress ($1$ and $2\%$) did not significantly influence the $Tr$ ($p > 0.05$). During the first $14\ d$, only the $10\%$ salinity treatment decreased $Tr$ by $18.89\%$ ($p < 0.05$). After $6$ weeks, the $Tr$ of $I. wilsonii$ under $6$, $8$ and $10\%$ salinity treatments was $64.56$, $100$ and $100\%$ lower than that in the control ($p < 0.05$). These results showed that higher salinity contents ($4$–$10\%$) in wastewater caused decrease of water uptake, which resulted in the decrease of transpiration, and the reduced amplitude became larger with time.

Salinity could cause decrease of all gas exchange parameters ($Pn$, $Cond$, $Ci$, $Tr$) (Hayat et al. 2007; Yusuf et al. 2008). Salinity damages photosynthetic machinery at multiple levels such as stomatal functioning, gaseous exchange, pigment content and structure and function of thylakoids, enzymes and electron transport have been reported (Geissler et al. 2009a, 2009b). Hopkins reported that damage caused by salinity stress can be attributed to water stress or a kind of physiological drought generated by NaCl (Hopkins 1995) due to the stomatal closure, thereby decreasing the partial CO$_2$ pressure (Iyengar & Reddy 1996) as well as internal CO$_2$ concentration (Yusuf et al. 2008). The rate of gas exchange varies with the intensity of abiotic stresses such as salinity and drought (Geissler et al. 2009a, 2009b). The higher photosynthesis of the plants exposed to the moderate salinity levels might be due to efficient fixation of CO$_2$ at a lower intracellular CO$_2$ concentration. Plant species respond to low soil water potential either through stomatal closure that restricts CO$_2$ availability for carboxylation, or by non-stomatal inhibition caused by the damaging effects of salinity on photosynthetic machinery (Flexas et al. 2004). In our work, stomatal conductance decreased in $I. wilsonii$ under the higher salinity levels ($>2\%$) limiting
the CO₂ supply which caused considerable reduction in photosynthesis, a result consistent with that reported for P. australis by Choi et al. (2005). Because Tr decreased also in I. wilsonii under higher salinity, so we could assume that the reduced availability of water and CO₂ in leaves were the main causes of reduced photosynthesis at higher salinity.

Effects of high-salinity contents in wastewater on Fv/Fm of I. wilsonii

Fv/Fm decreased gradually within the first 14 d under all salinity treatments although no significant differences were found among them (Figure 6). After 21 d, the Fv/Fm values decreased significantly (p < 0.05) in the treatments at the higher salinity contents (6 to 10%). Fv/Fm in the 10% salinity treatment could not be detected after 35 d. The results suggested that photosynthetic centers of I. wilsonii under the higher salinity contents (6–10%) were damaged after 21 d of treatment.

Fv/Fm is an indicator of the efficiency of the photosynthetic apparatus or an efficiency of excitation energy capture by open PSII reaction centers. Chlorophyll fluorescence parameters are widely used as non-invasive tools to determine the photosynthetic activity. It is recognized that the thylakoid membranes are very sensitive to stress (Haldimann & Feller 2005). Shahbaz et al. (2008) reported that significant reduction in quantum yield of PS II of wheat cultivars on being exposed to salinity medium. In our study, Fv/Fm was significantly reduced in response to high-salinity stresses, which is in agreement with that reported by Shahbaz et al. (2008). The stressed plants in the high-salinity treatments showed severe decreases in Fv/Fm compared with the no-salinity or lower salinity treatments, indicating that a higher salinity can damage the PS II of plants due to the decrease in Fv/Fm.

Na accumulation in different parts of I. wilsonii

The levels of Na in leaves of I. wilsonii under different salinity treatments were very low, no differences were found among them (Table 2). Na contents in roots were higher than in leaves. As salinity content increased in the influent, Na accumulation increased accordingly. Therefore, Na uptake by I. wilsonii decreased when salinity content increased.

Some papers reported that the reduction and/or inhibition of growth of aquatic macrophytes exposed to high-salinity (NaCl) contents in the nutrient solution were primarily attributed to three specific growth constraints imposed by salinity: (i) drastic changes in the plant water status (these changes are caused by the osmotic effect of salinity); (ii) increases of the ion toxicity concentration, leading to physiological and biochemical disturbances and oxidative stress; and (iii) the nutritional imbalance caused by alterations in the absorption of essential nutrients (Zhu 2001; Munns 2002; Hasegawa et al. 2000). As for the toxic effects of salinity, Na⁺ appears to be more toxic than Cl⁻ for most plant species (García-Sánchez et al. 2002). We mainly investigated the changes of Na⁺ content in plants and wastewater.

Table 2 | Na contents in different parts of I. wilsonii

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>1%</th>
<th>2%</th>
<th>4%</th>
<th>6%</th>
<th>8%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na content (mg/kg)</td>
<td>Leaf</td>
<td>0.03 ± 0.001a</td>
<td>0.06 ± 0.002a</td>
<td>0.09 ± 0.004a</td>
<td>0.05 ± 0.001a</td>
<td>0.07 ± 0.002a</td>
<td>0.07 ± 0.001a</td>
</tr>
<tr>
<td>Root</td>
<td>1.22 ± 0.05aA</td>
<td>3.57 ± 0.07aA</td>
<td>3.99 ± 0.07aA</td>
<td>4.28 ± 0.08bB</td>
<td>5.25 ± 0.12bB</td>
<td>8.67 ± 0.17cB</td>
<td>9.98 ± 0.15cC</td>
</tr>
</tbody>
</table>

Data are the means ± SE of three replications.
Values with same small letters mean no differences and different small letters mean significant differences at 5% level (p < 0.05); values with same capital letters mean no differences and different capital letters mean significant differences at 1% level (p < 0.01) in a same row.
in the present study. We could find that *I. wilsonii* had the ability to absorb Na⁺ from wastewater and mainly accumulate them in roots, which result in decreased Na⁺ contents in wastewater. The plants could absorb and accumulate Na⁺ when salinity contents were higher (>4%) in wastewater, but their growth would be inhibited, even withered, due to poisoning of Na⁺.

CONCLUSIONS

In microcosm submerged beds, the chlorophyll of *I. wilsonii* was not damaged when salinity content in influent was below 4%.

The lower salinity contents (1–2%) in influent or during short stress time (0–14 d) did not inhibit *Pn*, *Cond*, and *Tr* of *I. wilsonii*. When the salinity contents were higher (6–10%) or the stress time was longer (>14 d), these photosynthetic parameters decreased significantly, which probably resulted from stomatal closure.

High-salinity stress caused a decrease in *Fo/Fm*. It indicated that higher salinity (4–10%) damaged the PS II of *I. wilsonii*.

Na⁺ could be taken up by *I. wilsonii*, and Na⁺ accumulation increased in parts of the plants as salinity contents in solutions increased, especially in leaves.

In summary, *I. wilsonii* could uptake Na⁺ in wastewater, but higher salinity contents (4–10%) would inhibit the growth of *I. wilsonii*.

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