Salinity and pH effects on floating and emergent macrophytes in a constructed wetland

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ABSTRACT

Salvinia herzogii, *Pistia stratiotes* and *Eichhornia crassipes* (floating species) were the dominant macrophytes in a constructed wetland (CW) over the first years of operation. Later, the emergent *Typha domingensis* displaced the floating species, becoming dominant. The industrial effluent treated at this CW showed high pH and salinity. The aim of this work was to study the tolerance of floating species and *T. domingensis* exposed to different pH and salinity treatments. Treatments at pH 8, 9, 10 and 11 and salinities of 2,000; 3,000; 4,000; 6,000; and 8,000 mg L^{-1} were performed. Floating macrophytes were unable to tolerate the studied pH and salinity ranges, while *T. domingensis* tolerated higher pH and salinity values. Many industrial effluents commonly show high pH and salinity. *T. domingensis* demonstrated to be a suitable macrophyte to treat this type of effluents.

Key words | aquatic plants, effluents, phytoremediation, tolerance

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INTRODUCTION

The choice of macrophytes is an important issue in constructed wetlands (CW), as they must demonstrate a high contaminant accumulation capacity and tolerance to survive the complex chemical characteristics of an effluent and its variability. Effluents with high pH and high salinity are a common result from many industrial processes (Kadlec & Wallace 2009). To select the macrophytes to be used in a CW, previous studies focused on the effects of salinity and pH on the macrophyte tolerance are necessary to know their response under the conditions imposed by wastewaters. Some previous works were carried out by Haller *et al.* (1974), who observed that salt concentrations of 1.66% and 2.50%, were toxic to *Pistia stratiotes* and *Eichhornia crassipes*, respectively. Upadhyay & Panda (2005) found that after exposure to high salinities, older leaves of floating macrophytes became first yellow-green and later brown indicating marked injury. Macek & Rejmánková (2007) found that the vertical and horizontal growth of *Typha domingensis* was limited by high salinity. Baeza *et al.* (2013) showed in a greenhouse experiment that *T. domingensis* had a linear reduction in relative growth rate (RGR) with salinity.

We previously studied a free-water surface wetland constructed to treat sewage and industrial effluents from a metallurgical industry in Santa Fe, Argentina. Different locally available floating (including *E. crassipes*, *P. stratiotes* and *Salvinia herzogii*) and emergent macrophyte species (including *T. domingensis*) were transplanted into the wetland at the beginning of the operation period. In a first operation stage, the wetland was covered by floating plants. Subsequently, *T. domingensis* displaced the floating species, becoming the dominant macrophyte (Maine *et al.* 2009). We hypothesize that the high pH and salinity in the CW were toxic for floating macrophytes. The aims of this work were as follows:

- To study the tolerance of floating species and the emergent macrophyte *T. domingensis* exposed to different pH and salinity treatments.
- To assess if the high pH and salinity of water were the cause of disappearance of floating macrophytes in a constructed wetland (CW).

MATERIAL AND METHODS

Experiments using microcosms scale wetlands were carried out to assess the effects of pH and salinity on the free-floating species *S. herzogii*, *P. stratiotes* and *E. crassipes*,

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and the emergent *T. domingensis*. Two experiments were run simultaneously (one with the floating macrophytes and another with the emergent *T. domingensis*). Water and plants were collected in natural wetlands belonging to the Middle Paraná River floodplain, Argentina (floating species: 31° 32′ 45′ S; 60° 29′ 37′ W; *T. domingensis*: 31° 36′ 20′ S; 60° 33′ 49′ W). The physicochemical characteristics of water are presented in Table 1.

Cylindrical plastic reactors of 10 L of capacity were disposed outdoors under a semi-transparent plastic roof. The studied pH and salinity ranges were chosen to be the registered ones in the CW (Maine *et al.* 2009). Four treatments at pH 8, 9, 10 and 11 were performed. Such values were obtained adding NaOH to water. For salinity study, plants were exposed to values of 2,000; 4,000; 6,000; and 8,000 mg L^{-1} (Figure 1). Such values were reached preparing solutions of Na_2SO_4. This salt was chosen since ions Na^+ and SO_4^{2-} presented the highest concentrations at the influent of the CW. pH and salinity were kept constant through time with the addition of NaOH and Na_2SO_4 when necessary.

Experiments were carried out in spring at a mean temperature of 28 °C. All salinity and pH treatments were carried out in triplicate. Besides, control reactors without additions were disposed. For the *T. domingensis* experiment, the number of reactors used was 27. For the floating macrophyte experiment, the number of used reactors was 27 for each species (*S. herzogii*, *P. stratiotes* and *E. crassipes*).

Table 1 | Chemical characterization of the water from the sampling points of floating species and the emergent *T. domingensis* (ND = not detected, DL = detection limit)

Parameter	Floating macrophytes	*T. domingensis*
pH	7.1–7.4	7.0–7.3
Conductivity (μmho cm^{-1})	208–223	170–200
Alkalinity ($CaCO_3$) (mg L^{-1})	95.2–101.0	100.1–105.7
Cl^- (mg L^{-1})	10.6–11.9	4.2–10.1
SO_4^{2-} (mg L^{-1})	8.2–10.1	5.0–8.7
Na^+ (mg L^{-1})	27.1–32.4	28.1–33.8
K^+ (mg L^{-1})	11.1–12.9	12.4–14.9
Fe (mg L^{-1})	0.202–0.234	0.210–0.284
SRP (mg L^{-1})	0.015–0.023	0.010–0.079
TP (mg L^{-1})	0.039–0.052	0.070–0.098
NO_2^- (mg L^{-1})	ND (DL = 0.005)	ND (DL = 0.005)
NO_3^- (mg L^{-1})	0.431–0.626	0.524–0.710
NH_4^+ (mg L^{-1})	0.631–0.936	0.315–1.18
DO (mg L^{-1})	6.9–7.8	6.7–8.0

Visual inspection was recorded as to injury symptoms throughout the experiments. RGR was calculated according to the equation proposed by Hunt (1978):

$$RGR = \ln W_2 - \ln W_1 / T_2 - T_1 \quad (1)$$

where RGR = RGR (g g^{-1} d^{-1}), W_1 and W_2 = initial and final dry weight, respectively, and (T_2–T_1) = experimental period.

Experiment with free-floating macrophytes

In the reactors with *P. stratiotes* and *S. herzogii,* 20 ± 2 g of fresh plants and 3 L of water were disposed. When experimenting with *E. crassipes*, and due to its size, two plants (80 ± 10 g fresh weight) and 5 L of water were placed in each reactor. According to the plant tolerance, the experiment lasted 8 days.

Experiment with the emergent *T. domingensis*

Healthy *T. domingensis* plants of a similar size were selected. Two plants and 4 kg of sediment were placed in each reactor. The sediment composition ensured the normal growth of plants (pH: 7.67; organic matter: 8%; total phosphorus (TP): 0.57 mg g^{-1}; total Kjeldahl nitrogen (TKN): 1.32 mg g^{-1}). After the acclimatization period, plants were pruned to a height of approximately 20 cm. Five liters of experimental solutions were added. Plant height was daily measured (data not shown). Equation (1) was used to calculate RGR, based on leaf elongation rate (cm cm^{-1} day^{-1}). The experiment lasted 90 days.

Analytical determinations

Conductivity was measured with a YSI 33 conductivity meter and pH with an Orion pH-meter. Dissolved oxygen (DO) was measured with a Hanna Hi 9146 portable meter. Water samples were filtered through Millipore membrane filters (0.45 μm) for soluble P and N determinations. Chemical analyses were performed following APHA/AWWA/WEF (2012). NO_2^- was determined by coupling diazotation followed by a colorimetric technique. NH_4^+ and NO_3^- were determined by potentiometry (Orion ion selective electrodes). Soluble reactive phosphorous (SRP) was determined by the colorimetric molybdenum blue method. Na^+ and K^+ were determined by flame emission photometry. Alkalinity (carbonate and bicarbonate) was measured by HCl titration. Cl^- was determined by the argentometric method. SO_4^{2-} was assessed by turbidimetry.

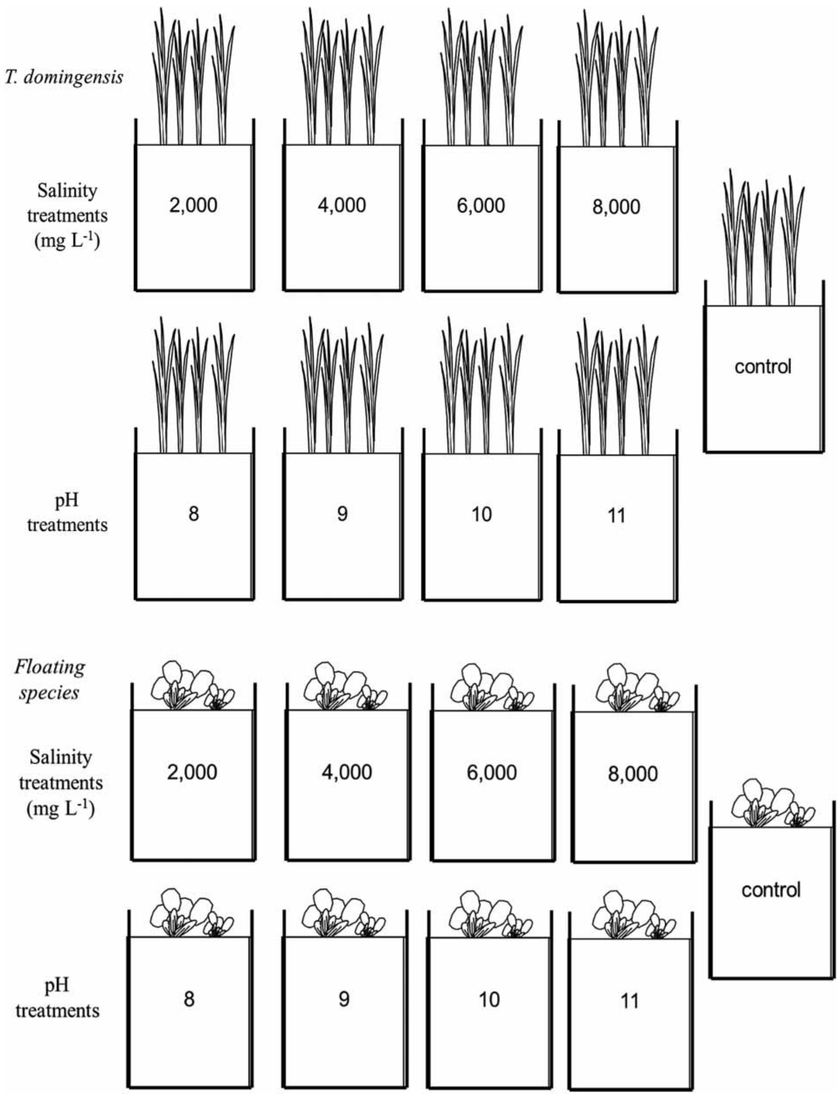

Figure 1 | Schematic of the salinity and pH treatments studied in *T. domingensis* and the floating macrophytes (*P. stratiotes*, *S. herzogii* and *E. crassipes*), and control reactors in each case.

Chlorophyll concentration

This parameter was measured initially and at the end of the experiments. The increase in chlorophyll concentration was expressed as a percentage. Chlorophyll was extracted with acetone for 48 h in cold darkness (3–5 °C). Transmittances of the extracts at wavelengths of 645 and 665 nm were recorded with a spectrophotometer UV-Vis (Westlake 1974).

Statistical analysis

The effects of pH or salinity on RGR and chlorophyll percent increase were evaluated by variance analysis. This analysis was run separately for each species within each factor (pH and salinity). The normality of residuals had been previously studied graphically, and the homoscedasticity of variances was checked applying Bartlett's test. Duncan's test was used to differentiate means where appropriate. A level of $p < 0.05$ was used in all comparisons. Calculations were carried out with Statgraphics Plus 5.0.

RESULTS AND DISCUSSION

After 8 days of experimentation with the floating macrophytes, signs of chlorosis and necrosis were observed, and the experiment was finished. *E. crassipes* and *P. stratiotes* showed saline secretions and visible signs of chlorosis and necrosis. Saline secretion was observed on the edges of leaves of *S. herzogii*. Due to the severe plant injuries, the chlorophyll concentrations could not be measured in the floating macrophytes.

Salinity presented statistically significant effects on the growth of *E. crassipes* (Table 2). Plants subjected to salinities of 4,000 mg L^{-1} or higher showed significant differences from the control, with negative RGR. Jampeetong & Brix (2009) exposed *Salvinia natans* to four levels of salinity (0, 50, 100, and 150 mM NaCl) and observed that even though the RGR was not significantly reduced to an exposure of 50 mM, leaves were smaller and thicker, and stems and roots shorter.

At pH 10 and 11, growth was significantly lower than that of the control. The other assayed pH values did not affect growth significantly. *P. stratiotes* showed a significantly lower RGR than the control at the treatment of 6,000 mg L^{-1} and a negative RGR in the treatments of 8,000 mg L^{-1} and pH = 11. In comparison with the control, *S. herzogii* showed a significantly lower RGR at the treatment of 8,000 mg L^{-1} and a negative RGR in the treatment of pH = 11. Below pH 3 and above pH 9 the protoplasm of root cells of most vascular plants was severely damaged (Tag el Seed 1978; Akcin *et al.* 1993). Sharma *et al.* (2005) studied the response of different floating and emergent macrophytes to textile dye wastewaters. They found that the free-floating species were almost dead in low pH (4.3–6.2), while their growth was adversely affected at pH 9–10.

T. domingensis experiment lasted 90 days because this species showed a higher tolerance in comparison with the response of the floating macrophytes. The RGR and the percentage of chlorophyll increase obtained in the *T. domingensis* experiment are showed in Table 3.

Plants subjected to salinities of 8,000 mg L^{-1} and pH = 11 showed a RGR and a percentage of chlorophyll increase significant lower than the control. However, in comparison with the floating species, its growth was positive. Regarding *Typha* spp., several studies have demonstrated that this plant is tolerant to salinity. Glenn *et al.* (1995) observed that at a concentration of 9 ppt of NaCl, the height and the number of new shoots of *T. domingensis* decreased significantly. Mufarrege *et al.* (2011) compared the response of *T. domingensis* to high salinity and pH in plants sampled from a CW and a natural wetland and conclude that the plants from the CW tolerate these conditions. Contrarily, plants from the NW showed stress. Coincidentally, Beare & Zedler (1987) reported that an ecotype of *T. domingensis* collected near the saline Salton Sea in California germinated at higher salinities than ecotypes collected from non-saline locations.

Salt excretion is a very efficient way of preventing excessive concentrations of salts building up in photosynthetic tissues. This mechanism is typical of species that have developed special features, mostly localized at the leaf epidermis, known as salt glands and salt hairs (bladders). One of the most obvious signs of salt excretion is the salt crust on leaves and shoots of those species with salt glands or salt hairs (Popp 1995). Munns (2002) proposed that salt tolerance is due to two main mechanisms: one of them is to minimize the entry of salt into the plant (or at least its accumulation in

Table 2 | Relative growth rate (g g^{-1} day^{-1}) obtained in the experiment with floating macrophytes at the different treatments of salinity and pH (mean ± standard deviation, different letters represent significant statistical differences among treatments)

Treatments	E. crassipes	P. stratiotes	S. herzogii
Salinity			
Control	0.0070 ± 0.0010 a	0.0136 ± 0.0087 a	0.0317 ± 0.0045 a
2,000	0.0025 ± 0.0005 a	0.0119 ± 0.0050 a	0.0301 ± 0.0075 a
4,000	−0.0032 ± 0.0004 b	0.0104 ± 0.0053 a	0.0292 ± 0.0050 a
6,000	−0.0150 ± 0.0030 c	0.0049 ± 0.0034 b	0.0281 ± 0.0044 a
8,000	−0.0143 ± 0.0030 c	−0.0466 ± 0.0105 c	0.0244 ± 0.0031 b
pH			
Control	0.0070 ± 0.0001 a	0.0136 ± 0.0087 a	0.0307 ± 0.0044 a
8	0.0050 ± 0.0020 a	0.0106 ± 0.0022 a	0.0213 ± 0.0075 a
9	0.0043 ± 0.0006 a	0.0092 ± 0.0018 a	0.0278 ± 0.0087 a
10	0.0002 ± 0.00001 b	0.0080 ± 0.0053 a	0.0186 ± 0.0096 a
11	−0.0114 ± 0.0020 c	−0.0311 ± 0.0076 b	−0.0071 ± 0.0062 b

Table 3 | Relative growth rate (cm cm^{-1} day^{-1}) and percentage of chlorophyll increase obtained in the experiment with *T. domingensis* at the different treatments of salinity and pH (mean ± standard deviation, different letters represent significant statistical differences among treatments)

Treatments	RGR	Chlorophyll
Salinity		
Control	0.0150 ± 0.0020 a	323 ± 50.1 a
2,000	0.0153 ± 0.0025 a	295 ± 32.6 a
4,000	0.0131 ± 0.0030 a	271 ± 45.2 a
6,000	0.0140 ± 0.0017 a	254 ± 34.3 a
8,000	0.0030 ± 0.0005 b	170 ± 24.3 b
pH		
Control	0.0150 ± 0.0015 a	323 ± 50.1 a
8	0.0143 ± 0.0021 a	242 ± 33.2 a
9	0.0176 ± 0.0003 a	215 ± 45.8 a
10	0.0131 ± 0.0001 a	188 ± 16.1 a
11	0.0035 ± 0.0001 b	143 ± 21.1 b

photosynthetic tissues), and the other is to minimize the salt concentration in the cytoplasm. Generally, salt exclusion in some plant species is a very efficient but complex way of preventing massive ion uptake in the root zone, enabling a lower uptake and accumulation of salts in the upper parts of the plant, especially in the transpiring organs. Salt exclusion is based upon lower root permeability for ions even in the presence of high external salinity.

Regarding pH effects, extremely low pH values are toxic for the growth of *Typha* spp. because an increased passive influx of H^+ would decrease the electrochemical gradient across the plasma membrane and thus the uptake of cations. Dyhr-Jensen & Brix (1996) found that at pH 3.5, *T. latifolia* showed a RGR significantly lower than that obtained at pH values of 5.0, 6.5 and 8.0.

For free-floating macrophytes, the threshold values of pH and salinity were lower than those obtained for *T. domingensis*, indicating a higher tolerance of *T. domingensis* than that of studied floating macrophytes. This was the cause that this macrophyte was the dominant species in the CW. Our results demonstrated that floating macrophytes could not develop within the pH and salinity prevailing in the wastewater of the CW.

CONCLUSIONS

The floating species *E. crassipes, P. stratiotes* and *S. herzoggi* were unable to develop when exposed to salinity/pH values of: 4,000/10, 6,000/11, and 8,000/11, respectively. This fact explains their early disappearance in the CW.

Although *T. domingensis* is not a halophyte species and it does not possess anatomical structure to tolerate and excrete salts, it is capable to tolerate extreme exposures of pH and salinity. For this reason, *T. domingensis* became the dominant species in the studied wetland.

T. domingensis demonstrated to be a suitable macrophyte to treat effluents with high values of pH and salinity typical of many industrial effluents.

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