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RESEARCH ARTICLE

Phragmites australis and *Schoenoplectus californicus* in constructed wetlands: Development and nutrient uptake

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Abstract

The goal of this work was to evaluate the development and nutrient uptake by *Phragmites australis* (Phr) and *Schoenoplectus californicus* (Sch) in horizontal subsurface flow constructed wetlands (HSSF) designed for wastewater treatment. Four HSSF systems with a surface area of 4.5 m^2 were planted with Phr and Sch. Wastewater was fed for 3 years at 1.6 to 4.8 g N m^{-2} d^{-1} and 0.2 to 0.6 g P m^{-2} d^{-1}. Nutrients (total nitrogen–TN and total phosphorus–TP), organic matter (chemical oxygen demand–COD) and solids (total suspended solids-TSS) were evaluated.

Nitrogen and phosphorus uptake were 7.52 g N m^{-2} and 0.83 g P m^{-2} for HSSF-Sch and 11.39 g N m^{-2} and 0.23 g P m^{-2} for HSSF-Phr. Showing a development of biomass of HSSF-Sch and HSSF-Phr were 1782 g DW m^{-2} and 385 g DW m^{-2}, respectively. Under these conditions, the removal efficiencies were 55-63% of COD and 88-92% of TSS for HSSF-Phr and 46-66% of COD and 83-91% of TSS for HSSF-Sch. TN removal was 23-24% for HSSF-Phr and 18-23% for HSSF-Sch. At the same time, removal for TP was -1 to 4% for HSSF-Phr and for HSSF-Sch was 9-13%.

Keywords: Constructed wetland, nutrients, *Phragmites australis, Schoenoplectus californicus*, wastewater

1. Introduction

The populations of rural areas from Chile are dispersed, and now the country has around two million of people in this situation, where only 15% of these populations have wastewater collection and treatment systems (Araya *et al*., 2014). Wastewater is characterized by high organic content (biochemical oxygen demand - BOD_5: 200-470 mg L^{-1} and chemical oxygen demand - COD: 200-740 mg L^{-1}), total nitrogen (35-100 mg TN L^{-1}), ammonium (6-66 mg NH_4^+-N L^{-1}), total phosphorus (6-30 mg TP L^{-1}), phosphates (6- 30 mg PO_4^{-3}-P L^{-1}) and total suspended solids -TSS (65-500 mg TSS L^{-1}) (Vera *et al.*, 2013; Araya *et al*., 2014). Constructed wetland (CW) is a technological alternative for removing organic matter and nutrients from wastewater (Vera *et al*., 2011; Abou-Elela *et al*., 2013). These engineering systems have been designed to utilize natural vegetation and soil processes and their associated microbial assemblages to assist in treating wastewater (Vymazal, 2011). Specifically, the CW with a horizontal subsurface flow (HSSF) removes between 67 to 84% of organic matter in terms of COD (loads of 7.4 to 76 g COD m^{-2} d^{-1}) (Vymazal and Kröpfelová, 2011), 85-91% of solids, with loads of 2.8 to 10 g TSS m^{-2} d^{-1}, 30-75% of total nitrogen, with a load of 2.6 a 10 g TN m^{-2} d^{-1}, and 15-65% of total phosphorous, with a load of 0.4 g TP m^{-2} d^{-1} (Rai *et al*., 2015). In this sense, an important part of the treatment in CW is attributable to the presence and activity of macrophytes and microorganisms (Brezinova and Vymazal, 2015; Rodríguez and Brisson, 2015). The particularly, research has shown that improvements in plant selection play an important role in nutrient removal from wastewater (Malecki-Brown *et al.*, 2010; Vymazal, 2011). The most commonly used genus of plants are the common reed (*Phragmites* spp.) and bulrush (*Schoenoplectus* spp.), due to their tolerance to substantial changes in pH (4-10), salinity (20–45 mg Cl^- L^{-1}), temperature (10–32 ºC) and nutrient assimilation (7300-20075 g N m^{-2} d^{-1}, 1095-5475150 g P m^{-2} d^{-1}) (Wallace and Knight, 2006; Vymazal, 2011; Neubauer *et al*., 2012).

Specifically, *Phragmites australis* (Phr) is a cosmopolitan macrophyte species, widely used in CW in Europe. Březinová and Vymazal (2015) reported that aboveground standing stock of Phr is in the range of 34 to 74 g N m^{-2}. Zhao *et al*. (2013) demonstrated that belowground nutrient storage increased during the growing season, with values of 13.2-36.1 g N m^{-2} and 3.0- 6.7 g P m^{-2}. Rodríguez and Brisson (2015) reported standing stock in aboveground biomass of *Phragmites* growing in natural stands in the range of 0.1–2.9 g TN m^{-2} d^{-1} removed to loads of 1.1-2.5 g TN m^{-2} d^{-1} and 0.05–0.83 g TP m^{-2} d^{-1} removed.

Schoenoplectus californicus (Sch) is found from (37°0′0″ north, 120°0′0″ west) California to (54°0′0″ south, 70°0′0″ west) Tierra del Fuego. Sch is an annual or perennial plant with triangular stems up to 3 m tall and roots that penetrate down to 70-80 cm (Vymazal, 2011). The foliar biomass production of *Schoenoplectus* ranges between 350-2000 g DW m^{-2} (Malecki-Brown *et al*., 2010; Neubauer *et al*. 2012). Neubahuer *et al.* (2012) found that Sch foliar biomass contained 12.15 g TN m^{-2} and 1.06 g TP m^{-2}, while rhizomes contained 0.4 g TN m^{-2} and 0.06 g TP m^{-2}. The goal of this work was to evaluate the development and nutrient uptake by Phr and Sch in subsurface flow constructed wetlands designed for wastewater treatment.

2. Materials and Methods

2.1. Study area

The wetland system is located in Hualqui (36°59’26.93” south and 72°56’47.23” west), Biobío Region, Chile. The influent entering to the HSSF was wastewater

from a treatment plant that serves a rural community of 20,000 inhabitants. During the period 2011 to 2014, the average daily temperatures were 11.3 ºC in fall (March to June), 9.8 ºC in winter (June to September), 14.1 ºC in spring (September to December) and 15 ºC in summer (December to March). There were marked seasonal trends of rainfall, with maximum rainfall in the fall and winter (3.1 mm d^{-1}) and minimum rainfall of 0.6 mm d^{-1} in the spring and summer.

2.2. HSSF constructed wetlands

The HSSF system consisted of four horizontal subsurface flow wetland units. The wastewater used in the HSSF was extracted after pre-treatment (40 mm bars), and then turn to a primary treatment, consisting of sand trap-degreaser (630 L), septic tank (1200 L) and pumping tank (630 L). Then, the HSSF was then fed by gravity (López *et al.*, 2015). Each HSSF unit had an area of 4.5 m^2, a total volume of 1.28 m^3 and a water table of 0.4 m in depth. The support medium was gravel, with an average height of 0.57 m (Rojas *et al*., 2013., Navia *et al*., 2003). Figure 1a shows the HSSF system: two units were planted with the macrophyte species *Phragmites australis* and labeled (HSSF-Phr1) and (HSSF-Phr2), respectively; and two units were planted with the species *Schoenoplectus californicus* macrophyte and labeled (HSSF-Sch1) and (HSSF-Sch2), respectively (López *et al*., 2015). The operational and climatic parameters for the HSSF units were: hydraulic loading (HL) between 19.8-44.6 mm d^{-1}, and a hydraulic retention time (HRT) between 3 and 7 days. The evapotranspiration rate (ET) presented maximum variations between winter (1.1 mm d^{-1}) and summer (4.0 mm d^{-1}).

2.3. Sampling strategy

The system was implemented in July 2011. The HSSF were operated for 1163 days. Samplings were taken in the spring (S), summer (Sm), fall (F) and winter (W) over the entire operational period.

In each HSSF, the following measurements were monitored *in situ*: a) temperature, b) pH, c) oxidation reduction potential (ORP) and d) dissolved oxygen (DO). The parameters are presented as the average between fall and winter (F/W) and spring and summer (S/Sm) for each year. HSSF wastewater influent and effluent samples were characterized physicochemically every season. This analysis determined COD, total suspended solids (TSS), TN and TP. *In situ* and physicochemical characterizations were done every 15 days (López *et al*., 2015).

2.4. Macrophyte sampling

The sampled plants were evaluated with measurements: height (from the base to the apex) and relative abundance (stem m^{-2}), with a PVC quadrant of 1 m^2 (Neubauer *et al*., 2012). In addition, above and belowground biomass were determined and a proximate analysis was made.

All macrophyte analyses were done in spring (November 2012), winter (August 2013) and summer (January 2014). Each HSSF was separated into three zones: Zone A (initial zone); Zone B (middle zone); and Zone C (output zone). The surface area of each zone was 1.5 m^2 (Lopez *et al*., 2015).

2.5. Analytical methods

To characterize the influent and effluent of each HSSF, the samples were filtered through Whatman 0.45 µm pore size membrane filters. The physicochemical parameters, COD, TN, TP and TSS, were

measured according to the protocols described in Standard Methods (APHA, 1998). The *in situ* parameters, pH, ORP and temperature, were measured in each HSSF unit using a portable multiparameter OAKTON (PC650–480485). DO was measured using a portable oximeter (oxi 330i/set Hanna HI 9146-04) (Vera *et al*., 2014; López *et al.*, 2015).

Aboveground and belowground biomass were obtained by dewatering at 75 °C for 24 h, until reaching constant weight (Neubauer *et al.*, 2012). Proximate analysis was performed for below and aboveground biomass of Phr and Sch. TN was analyzed by digestion and distillation and TP by calcination and colorimetry (Neubauer *et al*., 2012).

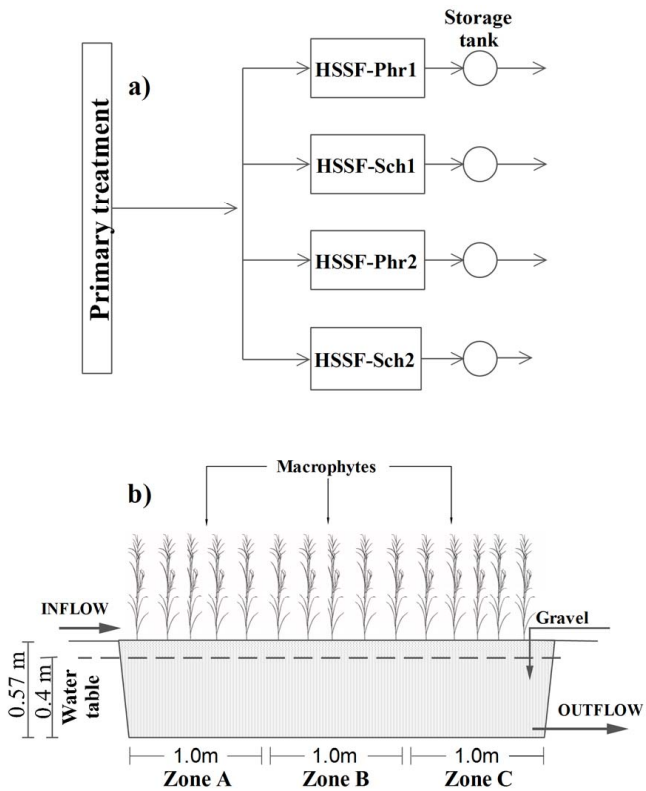

Figure 1. a) Configuring the pilot system of constructed wetlands. HSSF-Phr1 and HSSF-Phr2: units planted with *Phragmites australis*. HSSF-Sch1 and HSSF-Sch2: units planted with *Schoenoplectus californicus*; b) Characteristics and sampling points of the HSSF.

2.6. Statistical analyses

Statistical analyses were performed for each HSSF using the statistical program INFOSTAT (Di Rienzo *et al*., 2011). Previously, data were subjected to a normality test (the Shapiro–Wilk test) to determine the appropriate statistical tests for comparison. To compare the first HSSF to the second HSSF (HSSF-Phr1 vs. HSSF-Phr2; HSSF-Sch1 vs. HSSF-Sch2), (a) data with a normal distribution were analyzed using a paired t-test and (b) data without normal distribution were analyzed with a Wilcoxon test.

To compare the first HSSF and second HSSF between the treatment lines (HSSF-Phr vs. HSSF-Sch), (a) data with a normal distribution were analyzed with a paired t-test and (b) data without normal distribution were analyzed with a Wilcoxon test. Furthermore, to compare the influence of the seasons (spring, Summer, Fall and Winter), (a) data with a normal distribution were analyzed by ANOVA and (b) data without a normal distribution were analyzed by a Kruskal–Wallis test. For all statistical tests, the significance level was $\alpha = 0.05$ (López *et al*, 2015).

Table 1. Physicochemical characterization of the influent

Year	Seasons	Parameter (mg L^{-1})			
		COD	TSS	TN	TP
2011	Spring	296 ± 71	275 ± 128	93 ± 11	17 ± 6
	Summer	210 ± 66	342 ± 194	89 ± 1	14 ± 2
2012	Fall	260 ± 38	457 ± 77*	92 ± 24	15 ± 1
	Winter	420 ± 72*	425 ± 154	88 ± 65	14 ± 3
2013	Fall	194 ± 78	147 ± 115	68 ± 4*	13 ± 1
	Winter	172 ± 32*	137 ± 44*	120 ± 16	14 ± 2
	Spring	318 ± 112	256 ± 44	107 ± 18	15 ± 3
	Summer	289 ± 9	124 ± 33	91 ± 15	13 ± 2

n=42 for COD; n=38 for TSS; n=31 for TN and TP. * Significant differences between seasons ($p \leq 0.05$).

3. Results

3.1. Characterization of the influent

Table 1 shows the results of the physicochemical characterization of wastewater influent for 1163 days. During the monitoring period, the influent concentrations variations of COD were 17 mg L^{-1} (less than 10%) higher in the warm seasons (S/Sm) than in the cold seasons (F/W). Significant differences for COD during winter 2012 and 2013 compared to the others seasons are observed. On the other hand, TSS concentrations presented a maximum increase of 228 mg L^{-1} in the cold seasons (fall and winter in 2012). TSS significant differences during fall (2012) and winter (2013) with respect to the others seasons are observed. The nutrients contained in the influent varied less than 10 mg L^{-1}, between 1 to 9 mg L^{-1} for TN and between 0.5-2 mg L^{-1} for TP. NT significant differences are observed during Fall 2013 and other seasons ($p \leq 0.05$). No significant differences between seasons were observed for TP ($p \geq 0.05$).

3.2. In situ parameters

Table 2 shows the in situ parameters measured for HSSF-Phr and HSSF-Sch. The pH levels did not change significantly between F/W and S/Sm, with variations between 6.8 and 7.1 for both species ($p \geq 0.05$). Temperatures during S/Sm were on average 20.3 ºC, with a maximum of 25.4 ºC. During F/W, the average temperature was 12.05 ºC, with a minimum of 6.4 ºC. The oxidation reduction potential for HSSF-Sch was on average -258 mV and -256 mV for HSSF-Phr in both periods. DO was less than 0.46 mg L^{-1} for both species throughout the monitored period. No significant differences between species of macrophytes for *In situ* parameters were observed ($p \geq 0.05$).

Table 2. Seasonal in situ parameters in the HSSF. n= 42 for pH, temperature (ºC), ORP (mV) and DO (mg/L).

	Year	Period	Parameter			
			pH	T (ºC)	ORP (mV)	DO (mg L^{-1})
Phragmites australis	2011	Spring/Summer	7.0 ± 0.0	21.0 ± 0.2	-233.9 ± 5.0	0.45 ± 0.0
	2012	Fall/Winter	6.8 ± 0.1	12.8 ± 0.1	-246.5 ± 8.3	0.68 ± 0.1
	2013	Fall/Winter	7.0 ± 0.0	11.6 ± 0.0	-272.3 ± 4.1	0.23 ± 0.0
		Spring/Summer	6.9 ± 0.0	19.6 ± 0.2	-271.4 ±1.8	0.37 ± 0.1
Schoenoplectus californicus	2011	Spring/Summer	7.1 ± 0.0	21.6 ± 0.1	-242.8 ± 4.3	0.44 ± 0.0
	2012	Fall/Winter	6.9 ± 0.0	12.4 ± 0.3	-228.8 ± 10.1	0.67 ± 0.1
	2013	Fall/Winter	6.9 ± 0.0	11.4 ± 0.1	-287.3 ± 2.3	0.21 ± 0.0
		Spring/Summer	6.9 ± 0.0	19.0 ± 0.1	-273.6 ± 4.1	0.48 ± 0.1

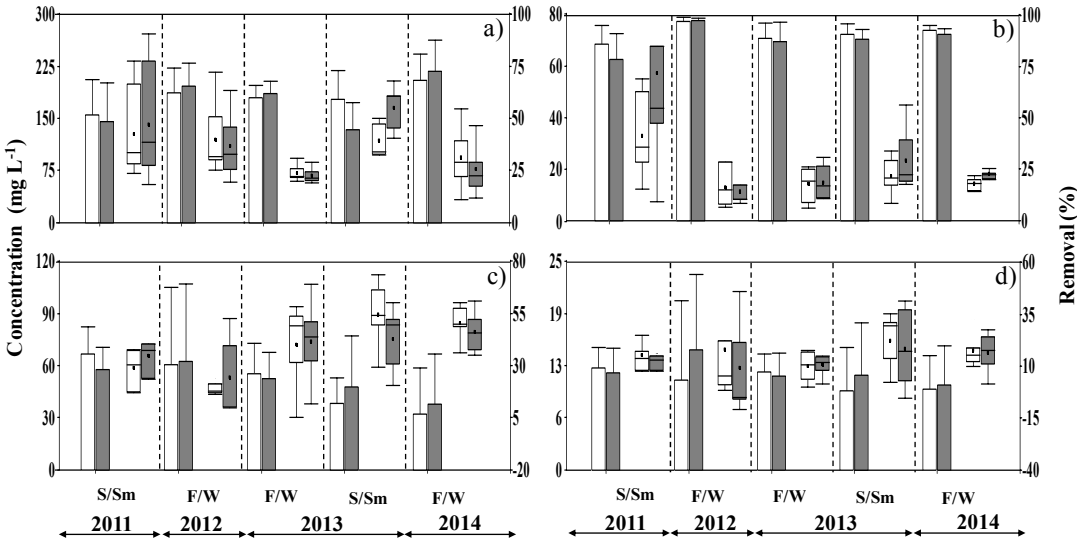

Figure 2. Effluent concentration (box plot) and mean removal efficiency (bar chart) for HSSF-Phr (□) and HSSF-Sch (■). (a) COD; (b) TSS; (c) TN; (d) TP.

3.3. Effluent concentrations and removal efficiencies

Figure 2 shows the average effluent concentrations (box-plot) and removal efficiency for of COD, TSS, TN and TP (bar chart) by HSSF-Phr (white) and HSSF-Sch (gray) during the monitored seasons. Average COD removal by HSSF-Phr was 55 and 63% for S/Sm and F/W, with effluent outlet concentrations of 122 and 110 mg L^{-1}, respectively. HSSF-Sch eliminated 46 and 66% in S/Sm and F/W, with effluent concentrations of 110 and 84 mg L^{-1}. Removal increased from the first to the third year by 17% and 24% for HSSF-Phr and HSSF-Sch, respectively. No significant differences between species were observed ($p \geq 0.05$). Removal of solids presented less variation (10 %) between species and seasons, with removal efficiencies of 88 and 92 % for HSSF-Phr and 83 and 91 % for HSSF-Sch in S/Sm and F/W, respectively.

The average outlet concentrations were 20 mg TSS L^{-1} for HSSF-Phr and 28 mg TSS L^{-1} for HSSF-Sch. No significant differences between species and seasons were observed ($p \geq 0.05$).

TN removal decreases varied seasonally by 15 to 28 %. There were minor variations between species and seasons of less than 10 %, with an average of 23 and 24% removal in F/W and S/Sm for HSSF-Phr, respectively, and 23 and 18 % in F/W and S/Sm for HSSF-Sch, respectively. The same tendency occurred with TP, it is noted a removal decreasing over the three years, with 10% removal for HSSF-Phr and HSSF-Sch in S/Sm (first year) falling to 1% in F/W of the third year of operation. Removal for HSSF-Phr was between -1 to 4% and for HSSF-Sch was 9-13% with concentrations of effluent for both species of 12 to 14 mg L^{-1}. No significant differences between species or seasons were observed for NT and PT ($p \geq 0.05$.

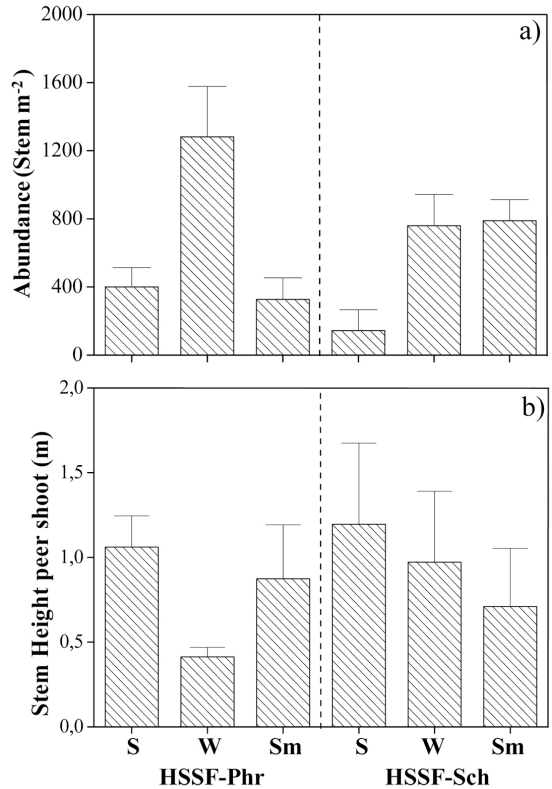

Figure 3. a) Seasonal abundance for *Phragmites australis* (HSSF-Phr) and *Schoenoplectus californicus* (HSSF-Sch). b) Seasonal stem height per shoot. a) *Phragmites australis*; b) *Schoenoplectus californicus*

3.4. Macrophyte development and nitrogen uptake

Figure 3 shows the plant abundance and height for HSSF-Phr and HSSF-Sch. Figure 3a shows the number of individuals and/or stems for HSSF-Phr (400 ± 112 stems), which was significantly 64% higher than HSSF-Sch (143 ± 122 stems) during spring ($p \leq 0.05$). For the first year (winter 2012), the number of stems increased 68% and 81% for HSSF-Phr and HSSF-Sch, respectively. In turn, in the summer the number of individual plants in HSSF-Phr decreased by 75% due to the effect of a pest (Family: Aphididae). However, plants in HSSF-Sch were not affected by the pest and maintained their abundance, increasing the number of stems by 3.6% and reaching coverage of 85%. Stem height varied seasonally by 18 to 61% for both species. On the other hand, stem size in HSSF-Phr decreased significantly ($p \leq 0.05$) by 61%, from 1.06 m (spring-466 d) to 0.41 m during the period of senescence (winter-780 d), but increased size (0.87 m) during summer (986 d). Stem size in HSSF-Sch decreased by 40% over time.

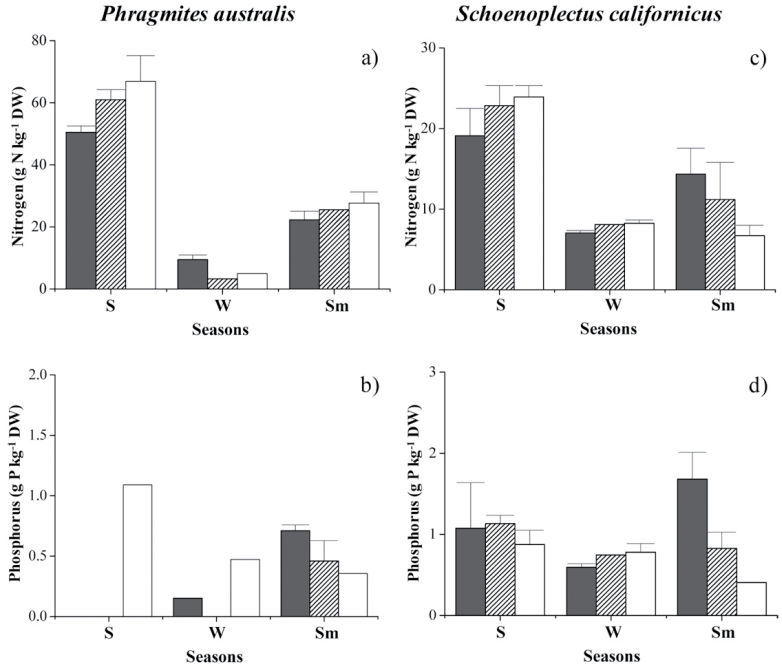

Figure 4. Nitrogen and phosphorus content in plant tissue. (a) and (b) units planted with *Phragmites australis*; (c) and (d) units planted with *Schoenoplectus californicus*. Zone A (■); Zone B ( ) and Zone C (□).

Figure 4 shows seasonal nitrogen and phosphorus content for HSSF-Phr and HSSF-Sch. Nitrogen content for both species was between 57-90% for HSSF-Phr and 50-64% for HSSF-Sch, being higher in spring than in the other seasons, significant values ($p \leq 0.05$). In spring, plant nitrogen content increased along the wetland a 20% for HSSF-Sch and 24% for HSSF-Phr. Nitrogen content in spring was 50 and 19 g N kg^{-1} DW in the intake zone (Zone A) of the wetland and 66 and 23 g N kg^{-1} DW in the output zone (Zone C) for HSSF-Phr and HSSF-Sch, respectively. No significant differences between macrophytes ($p \geq 0.05$), but they were significant between zone A and zone C of the wetland ($p \leq 0.05$). Plant nitrogen content was significantly lowest in winter (6 to 8 g N kg^{-1} DW), with variations of 25% for HSSF-Phr and HSSF-Sch ($p \leq 0.05$). In summer nitrogen content in Phr increased significantly by 19 g N kg^{-1} DW ($p \leq 0.05$), while it increased by only 2.6 g N kg^{-1} DW in Sch. On average during the three monitored seasons plant phosphorus content was significantly ($p \leq 0.05$) higher (40%) for HSSF-Sch, with 0.86 g P kg^{-1} DW, than for HSSF-Phr, with 0.51 g P kg^{-1} DW. P content was more stable for HSSF-Sch (maximum variation of 33%) over the three seasons, while P content for HSSF-Phr varied by up to 72%, significantly different values ($p \leq 0.05$). In summer phosphorus content was significantly ($p \leq 0.05$) higher (57-75%) in the Zone A (0.7 to 1.6 g P kg^{-1} DW) than Zone C (0.3 and 0.4 g P kg^{-1} DW) for HSSF-Phr and HSSF-Sch, respectively.

Table 3. Biomass production and foliar nitrogen and phosphorous content in HSSF-Phr and HSSF-Sch.

Parameter	Unit	Seasons					
		Spring		Winter		Summer	
		HSSF-Phr	HSSF-Sch	HSSF-Phr	HSSF-Sch	HSSF-Phr	HSSF-Sch
Biomass Production	g DW m^{-2}	654 ± 48	444 ± 40	1405 ± 281	1392 ± 220	385 ± 145	1782 ± 177
Content plant tissue	g N m^{-2}	11.39 ± 0.63	3.25 ± 0.57	1.28 ± 0	1.24 ± 0.06	1.73 ± 0.5	7.52 ± 0.76
	g P m^{-2}	0.23 ± 0.00	0.45 ± 0.02	0.08 ± 0.00	0.12 ± 0.00	0.19 ± 0.02	0.83 ± 0.03

HSSF biomass production and nutrient content for each species is presented in Table 3. Stem propagation was 32% higher for HSSF-Phr (654 g DW m^{-2}) than for HSSF-Sch (444 g DW m^{-2}) in spring (466 d), while stem propagation in winter (780 d) presented only minor variations of 1% (13 stems). However, in summer (986 d) the number of stems in HSSF-Phr decreased significantly ($p \leq 0.05$) by 73% due to pest problems, while the number of plants in HSSF-Sch increased in the same period by 21% (1782 g DW m^{-2})

Table 3 shows the nutrient content in the HSSF species. The maximum seasonal variations in nitrogen content were 10 g N m^{-2} for Phr and 6.28 g N m^{-2} for Sch (Statistically significant values with $p \leq 0.05$). Major differences (73%) were observed between species in spring and summer (Statistically significant differences with $p \leq 0.05$), while there was little variation in nitrogen content (3%) for either species in winter (0.04 g N m^{-2}). Significant differences between seasons and macrophyte species are observed in the content of NT ($p \leq 0.05$).

On average the phosphorus content for HSSF-Sch was 0.46 g P m^{-2}, which was 65% higher than that of HSSF-Phr (0.16 g P m^{-2}). In turn, the phosphorus content was higher in spring (0.22 g P m^{-2} for Phr and 0.64 g P m^{-2} for Sch) and summer (0.19 g P m^{-2} for Phr and 0.83 g P m^{-2} for Sch) than in winter (0.08 g P m^{-2} for Phr and 0.12 g P m^{-2} for Sch). Significant differences between seasons and macrophyte species are observed in the content of PT ($p \leq 0.05$).

4. Discussion

The average concentrations of the physicochemical influent used in this study were 270 mg COD L^{-1}, 94 mg TN L^{-1} and 14 mg TP L^{-1}, which are in agreement with those determined by Henze *et al*. (2002) who propose a classification for wastewater, determining that the wastewater used in this study correspond to concentrated. The influent solids (270 mg TSS L^{-1}) were 50% higher than those found by García *et al.* (2004), due to the rural origin of the influent, presenting increased solid entrainment.

In situ measurement in the HSSF found no differences in pH values (6.8-7.1) between species or seasons. These values are consistent with the findings of Vymazal and Kröpfelová, (2011), and García *et al*. (2004), who worked with wastewater with pH levels ranging from 6.5 to 7.7. Temperature in the HSSF was directly conditioned by seasonal characteristics associated with a Mediterranean area, with maximum seasonal variations of 10 °C. Furthermore, considering ORP ranges (-233 to -287 mV) and DO concentration (less than the 0.7 mg L^{-1}), an anaerobic condition was

determined for the HSSF. This agrees with the findings of García *et al.* (2004) and Vyzamal (2011), who determined values between -351 to -390 mV and less than 0.1 mg DO L^{-1} for HSSF.

Removal efficiencies by HSSF for COD, TSS, TN and TP did not show seasonal differences in Fall/Winter (12.05 °C) and Spring/Summer (20.3°C). However, there were differences over time during the HSSF operation, which concurs with what Vymazal (2011) found in analyzing HSSFs several countries (Sweden, Norway, the USA and the Czech Republic). Vymazal determined that bacterial activity is not changed with low temperatures. Rather, supplies of carbon and nutrients are the main factors affecting HSSF efficiency. This is why COD removal efficiency improves over time, with a maximum removal of 72% (F/W in the third year.). However, this is lower than what was found by Vymazal and Kröpfelová (2011), who obtained removal efficiencies of 80 to 94%.

Nitrogen removal was 30% (0.98 g N m^{-2} d^{-1} removed) in the first year and less efficient (12%) by the spring of the third year (0.7 g N m^{-2} d^{-1} removed). Chen *et al*. (2014) determined that the efficiency of CW decreased from 1.05 to 1.10 g N m^{-2} d^{-1} in the initial stage to 0.23 to 0.31 g N m^{-2} d^{-1} in the final stage. This could be explained by the fact that TN loading increased from 2.6 to 3.1 g N m^{-2} d^{-1}. Vymazal (2007) found that at removal efficiencies of 42% TN loading was 1.7 g N m^{-2} d^{-1}. Therefore, the low removal rates in the HSSF could be due to limited ammonium nitrification, which in turn is due to lack of dissolved oxygen under permanent saturation (Vymazal and Kröpfelová, 2011).

The efficiency of P removal by HSSF decreased steadily over the first three years, which concurred with Vohla *et al.* (2005), who found a decrease from 74.5 to 24.7 g P m^{-2} yr^{-1}, which could be caused by washing Fe content out of the substrate. Mander *et al*. (2003) observed a decrease of 20.7 kg P yr^{-1} to 5.1 kg P yr^{-1} in an HSSF, which was attributed to the less efficient saturation of the support material. In other research, Vera *et al.* (2014) determined that the use of gravel as a support medium contributed to 20-50% of HSSF P removal.

Biomass in HSSF-Phr developed more rapidly during spring, with 400 stems and biomass production of 654 g DW m^{-2} (over 75% coverage), than HSSF-Sch, which presented less coverage at 50% (143 and 444 g DW m^{-2}). This can be explained according to determined by Wallace and Knight, (2006) who indicated that Phr has a propagation rate of 10 m yr^{-1} while Sch has one of 15-30 cm yr^{-1}. The propagation rate of Phr is classified as very rapid and invasive (Wallace and Knight, 2006).Tanner (1996) found a density of 758 stems m^{-2} in only 90 days from the start of cultivation. Along the same line, biomass production in HSSF was 23% higher than that found by Vymazal and Köpfelová (2005) and Zheng *et al*. (2016) in a CW with Phr production between 500-600 g DW m^{-2} (first year). Subsequently, stem production in winter (1281 stems m^{-2}) matched that determined by Rodríguez and Brisson (2015) and Vymazal and Köpfelová (2005), who observed the development of 1366 stems m^{-2} in winter (first year) and biomass production between 1000 and 1600 g DW m^{-2}. Stem and biomass production for Sch during spring (143 stems and 444 g DW m^{-2}) and winter (760 stems and 1392 g DW m^{-2}) coincided with Neubauer *et al*. (2012), who found that *Schoenoplectus californicus* increased biomass from 522 g m^{-2} in 2009 to 729 g m^{-2} in 2010. In the summer 2013 HSSF-Phr was affected by aphids (family Aphididae), resulting in decreases of 75% in the number of stems (325 stems) and 72% in biomass (385 g DW m^{-2}) in the HSSF-Phr. Meanwhile, the HSSF-Sch was not affected by the aphid attack and biomass increased 21% (1782 g DW m^{-2}). This is because aphids prefer species like *Phragmites* spp., since *Phragmites* is a secondary host of aphid in late spring and early summer, almost without exception plant that

supports high aphid populations (Medina *et al*., 1986; Tscharntke, 1992; Mook and Wiegers, 1999). There is also evidence that reeds growing at wet sites are more heavily infested than at dry sites. When *Phragmites* is colonized, the aphids feed on the nutrients in the phloem that extract from the leaves of *Phragmites*. This result in a decrease of the growth, the leaves are rolled and if the attack is severe can dry the plant, generating a reduction in the production of final biomass (Mook and Wiegers, 1999). Furthermore, photosynthesis is reduced, due to physical effects (fallen leaf) and biological (fungi) generated by excretions of sugars by aphids. Moreover, aphids can transmit toxic substances and/or phytopathogenic virus to *Phragmites* (Medina *et al.*, 1986). Also, aphids impact on the nutritional chemistry of plants, related negatively to the P content in leaves and roots (Larsen *et al.*, 2015). Because of this, it is very important to consider the susceptibility to diseases when evaluating the selection of macrophytes in constructed wetlands. Despite the above, improved biomass production is expected in this study. Specifically, because it has been determined that maximum biomass development for Phr is between the third and fourth growing season. Therefore, we expect improved biomass development and consequently improved HSSF efficiency (Vymazal and Köpfelová, 2005).

Nitrogen content for Phr varied between the growing season (spring) and senescence (winter) from 11.39 to 1.28 g N m^{-2}. This coincides with Tanner (2001), who found N accumulations in fall of 26 to 47 g N m^{-2} and in summer (3rd growing season), of 69 g N m^{-2}. Moreover, it has been found that from 0.1 to 0.25 g N m^{-2} d^{-1} is released from living plant tissue during senescence. In particular, average N content (4.8 g N m^{-2}) for HSSF-Phr was less than 86% according to Brezinová and Vymazal (2015) and Zhang *et al*. (2016), who determined an N content between 30-34.8 g N m^{-2} (mean concentration in stems and leaves) for Phr, while, N content in Sch (1.24 to 7.52 g N m^{-2}) was similar to what was determined by Weller *et al*. (2015) and Malecki-Brown *et al.* (2010), who found N content for *Schoenoplectus* spp., *S. californicus* and *S. americanus* was 5.3 g N m^{-2}, 0.7-2.7 g N m^{-2}, and 2.9 g N m^{-2}, respectively.

In turn, the P content accumulated in the plants in this study (0.08 to 0.19 g P m^{-2}) was less than determined by Zheng *et al*. (2016), who found accumulations between 1.5-3.0 g P m^{-2}.

The variability in N and P accumulation between the macrophyte species used in this study reflects the different ways some plant species respond to nutrient-favorable conditions (concentrated wastewater). Some species have greater apparent use efficiency, increasing growth to maximize production, while others tend toward a more conservative strategy of accumulating nutrients, as is the case with *Phragmites australis* (Tanner 1996). Chen *et al*. (2014) determined that plant N consumption increases over time, being 0.9 to 1.7 times greater in the final stages of monitoring, due to root development and plant maturity. This coincides with the results obtained with Sch in our study, which with time increased biomass cover by about 85%, as well as increasing the amount of nutrients in tissues.

On average HSSF-Sch had more (40%) phosphorus in tissues with 0.86 g P kg^{-1} DW and 0.51 g P kg^{-1} DW than did HSSF-Phr over the three monitored seasons. In turn, P content was higher (0.42 g P m^{-2}) in spring and summer than in winter (0.1 g P m^{-2}). It has been reported that the P accumulation is in the range of 0.2 to 10.5 g P m^{-2} in various types of CW and for HSSF it is 0.7 to 5.5 g P m^{-2} (Vymazal and Kröpfelová, 2011). Malecki-Brown *et al*. (2010) determined that *Schoenoplectus californicus* has a close to addition 2.5 g P kg^{-1}DW and 0.55 g P m^{-2}.

Nitrogen assimilation by Phr and Sch were 2 to 12% and 2 to 28%, respectively. Less than 6% of the N

load that entered the HSSF was assimilated by either plant species. In an investigation of 41 wetland plants, McJannet *et al*. (1995) found that N concentrations in plant tissues ranged from 0.25 to 2.14% and P concentrations from 0.13 to 1.07% (Stottmeister *et al*., 2003). This is corroborated by Vymazal and Kröpfelová (2011), who found that P and N levels incorporated by *Phragmites australis* in HSSF were 2.3% and 3.7%, respectively. Vohla *et al*. (2005) determined that the P uptake by plants was 6.1% and microorganisms to 4.4% of total removal. P assimilation in this study was less than 5% for Phr and between 4 and 9% for Sch. Coinciding, as determined by Vymazal and Kröpfelová (2011); Vohla *et al*. (2005) and Zheng *et al.* (2016), who delivered values assimilation by macrophytes in HSSF 2.3 -6.1% P and 3.7-6.2% of N coming into the CW.

In short, a low incidence of plants in removing N and P (less than 6%) contained in the wastewater is evident. However, the role that plants in constructed wetlands has been defined beyond assimilation of nutrients. Thus, it has been determined that constructed wetlands influence directly on regulation of temperature, microbial biomass development, contribute organic matter, aeration rhizosphere, transport of gases, water flow regulation, release of root exudates, esthetic contribution, among others (Chen *et al*., 2014; Button *et al*., 2015; Carballeira *et al*., 2016).

On the other hand, it has been determined that the temporal variability of nutrients and development of biomass accumulate mainly in shoot tissues in spring-summer, but are transferred to below-soil storage organs during senescence. Indeed, it has been suggested that the removal efficiency in HSSFs can be maximized by make crop before fall to prevent translocation due to senescence.

5. Conclusions

There were no differences in removal efficiencies by HSSF for COD, TSS, TN and TP between the F/W (12.05 °C) and S/Sm periods (20.3 °C). However, there were differences over time.

By the end of the three-year study, biomass (1782 g DW m^{-2}) and coverage (85%) of HSSF-Sch were higher than those of HSSF-Phr (385 g DW m^{-2} and 64%). However, the low level of development of HSSF-Phr at the end of the monitoring period was mainly due to an attack by aphids.

The nutrient content in tissues of Sch and Phr presented markedly seasonal trends, with the highest concentrations of nitrogen (7.52 for Sch and 11.39 g N m^{-2} for Phr) and phosphorus (0.23 for Phr and 0.83 g P m^{-2} for Sch) during the growing seasons (spring and summer). Therefore, Phr and Sch are capable of removing a maximum of 6% of the N and P loads applied to the HSSF.

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