Treatment of industrial wastewater with two-stage constructed wetlands planted with *Typha latifolia* and *Phragmites australis*

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ABSTRACT

Industrial wastewater treatment comprises several processes to fulfill the discharge permits or to enable the reuse of wastewater. For tannery wastewater, constructed wetlands (CWs) may be an interesting treatment option. Two-stage series of horizontal subsurface flow CWs with *Phragmites australis* (UP series) and *Typha latifolia* (UT series) provided high removal of organics from tannery wastewater, up to 88% of biochemical oxygen demand (BOD_5) (from an inlet of 420 to 1000 mg L^{-1}) and 92% of chemical oxygen demand (COD) (from an inlet of 808 to 2449 mg L^{-1}), and of other contaminants, such as nitrogen, operating at hydraulic retention times of 2, 5 and 7 days. No significant ($P < 0.05$) differences in performance were found between both the series. Overall mass removals of up to 1294 kg COD ha^{-1} d^{-1} and 529 kg BOD_5 ha^{-1} d^{-1} were achieved for a loading ranging from 242 to 1925 kg COD ha^{-1} d^{-1} and from 126 to 900 kg BOD_5 ha^{-1} d^{-1}. Plants were resilient to the conditions imposed, however *P. australis* exceeded *T. latifolia* in terms of propagation.

Introduction

Tannery wastewater may cause severe environmental impacts due to the pollutant levels that are commonly present; the main problems, depending on the productive cycle, are the presence of solids, nitrogen, chromium and the high organic loading. This issue has been identified by the European Commission and is described in detail in the reference document on best available techniques for the tanning of hides and skins (EC, 2003). The need for environmental and economically feasible systems is a real demand worldwide. Emphasis is given here to tannery wastewater since the search for best available technologies to accomplish the legal discharge targets may contribute, in a certain way, to the preservation of this industry, which in Portugal is considered to be of great importance due to the historical and economic value that it represents (INETI, 2000).

Constructed wetlands (CWs) have proven, to different extents, to be an interesting option for several types of industrial wastewaters that are to be treated by biological means (Vymazal, 2008; Kadlec et al., 2000), including those that have originated from the tannery industry (Calheiros et al., 2008b, 2007; Kucuk et al., 2003; Daniels, 2001). In Portugal, CWs are among the treatment processes that have no specific regulations. Most of the CWs are applied for domestic sewage and municipal wastewater treatment and only few are used for industrial purposes (Dias et al., 2006). The diversity of CW configurations makes them versatile for implementation. The target pollutant and removal efficiencies are set so the dimensioning and the scaling of the system are adequate; caution must be paid to issues such as whether the system will operate as the sole treatment or will it be integrated in an existing plant. Mechanisms underlying the functioning of these systems may be found in the literature (Kadlec et al., 2000); the choice of substrate, plant species, basin compartmentation, liners, flow structure, and other CWs components, influence their capital cost (Kadlec et al., 2000) and may be crucial for granting of the project implementation. For tannery industry wastewater treatment, detailed research data on efficiency of CWs, performance and adequate set-up are still lacking. However, efforts have already been taken for, selecting plant species tolerant to this peculiar wastewater (Calheiros et al., 2007), selecting suitable supporting media or substrate (Calheiros et al., 2008b) and for approaching the bacterial dynamics (Aguilar et al., 2008; Calheiros et al., 2009).

In order to add knowledge to the operational conditions of CWs, the performance of two constructed wetland units (CWUs) arranged in a two-stage series, one planted with *Phragmites australis* and another planted with *Typha latifolia*, was evaluated for different hydraulic loading rates (HLRs) of tannery wastewater. At the same time, the development and propagation of these plants and their enzymatic and physiological responses were followed.

Methods

Constructed wetlands

The monitoring of two series (named as UT for the series planted with *Typha* and UP for the series planted with *Phragmites*)

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of horizontal subsurface flow CWs was undertaken in a site located at the wastewater treatment plant of a leather company in the North of Portugal (Fig. 1). The surface area of each bed was 1.2 m^2 (length: 1.2 m and width: 1 m), the effective depth of the substrate was 0.60 m and the average depth of the liquid in the bed was 0.55 m. The substrate used in the series was Filtralite®MR 3–8 (FMR), with particle size ranging from 3 to 8 mm (from maxit to Argilas Expandidas, SA – Portugal).

The first unit of both *T. latifolia* series (UT1) and *P. australis* series (UP1) had been in operation for 17 months receiving tannery wastewater (Calheiros et al., 2007). The plants used in UT2 and UP2, *T. latifolia* and *P. australis*, respectively, were transplanted from an industrial polluted site in Estarreja, Portugal (Oliveira et al., 2001) in a range of 10 plants m^{-2}. The systems were filled with water for three weeks prior to the application of the wastewater, and were then aligned to work in series and operated for 31 months under different hydraulic conditions and interruptions in feed. Briefly, for 2 months the first unit of each system was subjected to a HLR of 18 cm d^{-1}. By the third month the systems were not fed for 24 days due to the shutdown of the production plant and a second period of operation occurred subsequently during 23 months under a HLR of 6 cm d^{-1}. During this time the wastewater supply was stopped twice. By day 479 plants were clipped, leaving around 10 cm of aboveground plant material. A third period of operation occurred during six months under a HLR of 8 cm d^{-1}, and the systems were not fed due to the shutdown of the production plant by the second month within that period. The overall hydraulic retention time in the two-stage system series, for each HLR was 7, 2 and 5 days (in the order of application). Maintenance of the systems was done according to Calheiros et al. (2007). The systems were inspected on, at least, a weekly basis concerning the overall functioning, and a general cleaning of the pipes was usually undertaken twice a month. Major attention was paid to the inlet and outlet flows – measurements were made at the sampling times, and no relevant differences were observed.

Plant parameters

Macrophytes number and height (starting from the substrate level) were registered at least monthly. For that, each CWU was divided into four zones (Fig. 1) – A and C corresponded to the inlet and outlet zones, respectively, and B and D corresponded to the left and right sides of the CWU (inlet reference point) – and at the beginning three plants were marked in each zone. Generally, plants were visually inspected on a weekly basis for toxicity signs, such as chlorosis, necrosis and malformation.

Chlorophyll content. For chlorophyll analysis, fresh circular discs from mature leaves of plants collected at the inlet and outlet of the CWUs were cut with a 10.5 mm corer, and were extracted in *N,N'*-dimethylformamide. Chlorophyll *a* and *b* content was determined according to Wellburn (1994).

Peroxidase activity (POD) determination. POD was determined in root and leaf samples of *T. latifolia* and in root, leaf and stem samples of *P. australis*, which were collected at the inlet and outlet of the CWUs and immediately frozen in liquid nitrogen. The experimental procedure described in USEPA (1994) for plant peroxidase activity determination was followed (three replicates). One gram of fresh plant tissue was ground with a calcium chloride solution. The crude extract was added to an assay mixture and absorbance readings were taken in a spectrophotometer (Novaspec II, Visible spectrophotometer) at 510 nm.

Physico-chemical analysis

Wastewater samples were taken, in general twice each month, at the inlet and outlet of the CWUs and the following parameters were determined based on Standard Methods (APHA, 1998): pH, colour (Spectrophotometric Method), chemical oxygen demand (COD; Closed Reflux, Titrimetric Method), biochemical oxygen demand (BOD_5; 5-Day BOD Test), total suspended solids (TSS; Total Solids Dried at 103–105 °C Method), Kjeldahl nitrogen (TKN; Kjeldahl Method), nitrate nitrogen (NO_3^-—N; Nitrate Electrode Meth-

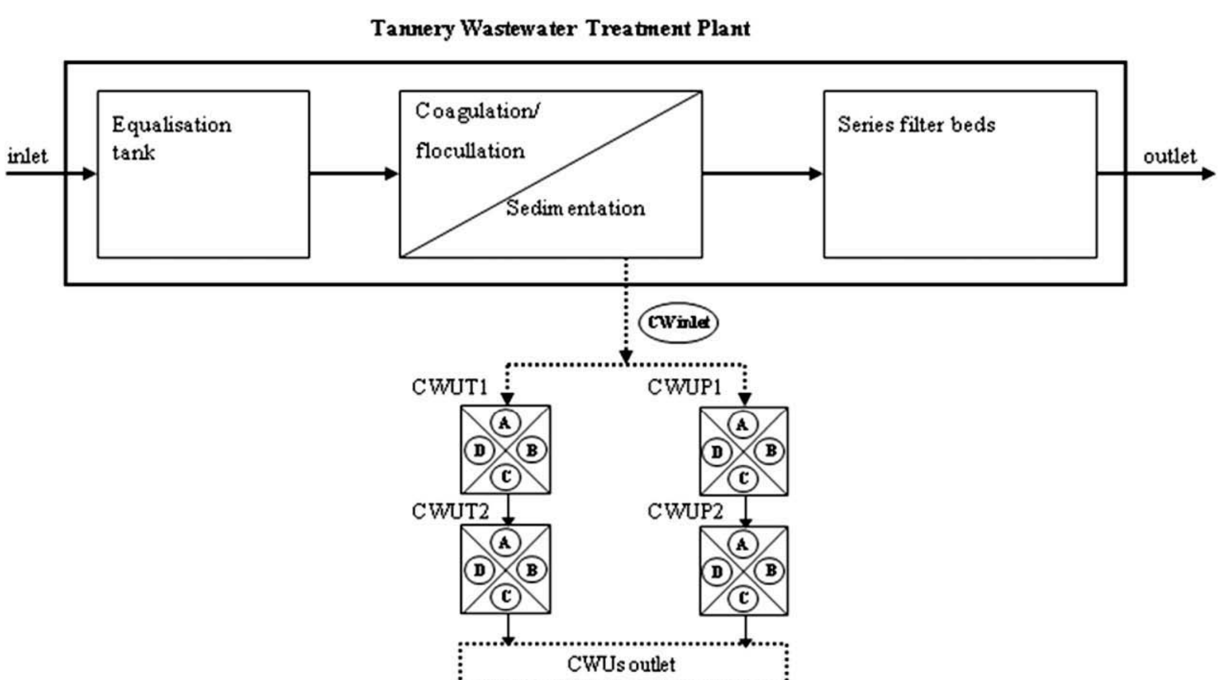

Fig. 1. Schematic representation of the constructed wetland units (CWUs) (UT1 and UT2: units with Filtralite®MR 3-8 planted with *Typha latifolia*, UP1 and UP2: units with Filtralite®MR 3-8 planted with *Phragmites australis*).

od), ammonia nitrogen (NH_3–N; Phenate Method), total phosphorus (Total P; Manual Digestion and Flow Injection Analysis for Total Phosphorus), total chromium (Total Cr; Nitric Acid Digestion followed by the Colorimetric Method) and hexavalent chromium (Cr(VI); Colorimetric Method). The sulphates determination (SO_4^{2-}; Turbidimetric Method) was done based on the Association of Official Analytical Chemists (AOAC, 1995). Wastewater temperature was measured at the inlet and outlet of the CWUs and also 15 cm below the substrate surface in the middle of the units, using a thermometer. The dissolved oxygen (DO), conductivity and salinity (Sal.) were registered with a WTW handheld multi-parameter instrument 340i at the inlet and outlet of the units.

Data analysis

Statistical analysis was performed using the software SPSS (SPSS Inc., Chicago, IL, USA; Version 12.0). Data were analyzed through Student's *t*-test at a significance level of $P < 0.05$ to compare the performance between units for the removal of the contaminants and those from the chlorophyll and POD analyses were also analyzed. When applicable, values were presented as the mean ± standard error. Correlations were performed with different variables and Spearman's correlation coefficients were determined.

Results

Plant development and analysis

The plant growth and number of shoots were monitored during the CWUs operation and results are presented in Fig. 2. When the first feeding period started (HLR of 18 cm d^{-1}) the number of shoots was 28 for UT1, 13 for UT2 and 13 for UP2. The number of shoots in the first unit with *Phragmites* (UP1) was not monitored since this plant was well established with around 400 shoots at the beginning of the operation in series, with signs of continuing proliferation. At the beginning of the second feeding period (by day 92) the number of shoots was 27 for UT1, 15 for UT2 and 21 for UP2. By day 765, at the beginning of the third feeding period, the number of shoots was 52 for UT1, 62 for UT2 and 201 for UP1. At the end of the experiment, by day 928, the total number of shoots was 56 for UT1, 65 for UT2, 305 for UP2, while UP1 remained proliferous.

Concerning the shoot countings in each zone (A, B, C, and D), by the end of the experiment, counts were lower at the entrance for all the units (point A) when compared to the other zones (B, C and D). For example, UP2 started with three shoots in each zone and at the end of the experiment the shoot countings were 43 in A, 82 in B, 87 in C and 93 in D.

In relation to the height of the plants, small differences were registered along the systems operation. In general, plants developed and proliferated without showing signs of toxicity.

The presence of aphids and other plant insects was prevalent in *T. latifolia* in the warmer periods (spring/summer), and the presence of the snail *Helix aspersa* was also observed. In the *P. australis* unit millipedes of the family Julidae (Diplopoda, Julida) were found. Occasionally, garden spiders (*Araneus diadematus*) and leafhoppers (Hemiptera: Cicadellidae) were seen in all the units.

The level of chlorophyll pigments *a* and *b*, for plants in each unit, is shown in Table 1. When the inlet and outlet zones of each unit in the summer of 2005 were compared no significant ($P < 0.05$) differences were found, except for UP2, where the levels at the outlet were significantly ($P < 0.05$) higher than those at the inlet. The second analysis was undertaken in the summer of 2006 although most of the plant leaves were dry and for that reason, at the inlet of UT1, this determination was not done. No significant ($P < 0.05$) differences were found between the inlet and outlet of UT2, UP1 and UP2 units.

The results of POD in the plants are presented in Table 2. This analysis was not undertaken for UT1 due to the fact that most of the plant leaves were dry at the time of collection. No significant ($P < 0.05$) differences were found between inlet and outlet of each unit, concerning the different plant parts (leaf, stem and root).

3.2. CWUs performance

In Tables 3–5 the results of the physico-chemical analysis corresponding to the inlet and outlet of *Typha* and *Phragmites* series,

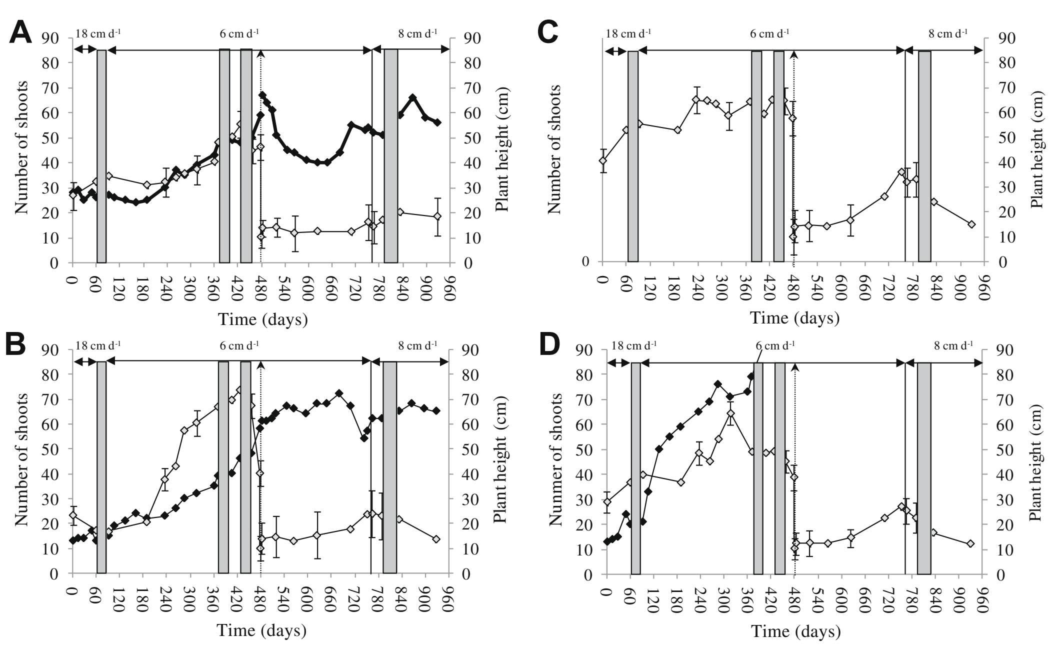

Fig. 2. Variation in the number of shoots and the height of the plants in the pilot units. (A) UT1, (B) UT2, (C) UP1 and (D) UP2. The values of plant height are means ±1 SEM. ▭ No loading occurred, ...Clipping of the plants at the day 479, ♦ Number of shoots, ◇ Plant height.

Table 1
Chlorophyllous pigments of the plants in the constructed wetlands units in series ($n = 5$).

Units	Date CW site	June 2005 Chlorophyll (mg g^{-1} FW)			June 2006 Chlorophyll (mg g^{-1} FW)		
		a	*b*	*a/b*	*a*	*b*	*a/b*
UT1[a]	Inlet	0.7 ± 0.1	0.32 ± 0.03	2.1	nd	nd	
	Outlet	0.7 ± 0.1	0.31 ± 0.02	2.2	0.5 ± 0.1	0.3 ± 0.1	1.9
UT2[a]	Inlet	0.5 ± 0.2	0.3 ± 0.2	1.6	0.49 ± 0.03	0.22 ± 0.02 0.	2.25
	Outlet	0.9 ± 0.1	0.33 ± 0.02	2.8	0.7 ± 0.2	18 ± 0.03	3.7
UP1[a]	Inlet	0.10 ± 0.03	0.09 ± 0.01	1.18	1.80 ± 0.05	0.64 ± 0.01	2.82
	Outlet	0.14 ± 0.01	0.067 ± 0.005	2.10	1.7 ± 0.1	0.5 ± 0.1	3.2
UP2[a]	Inlet	0.18 ± 0.02	0.09 ± 0.01	1.99	1.6 ± 0.2	0.31 ± 0.05	5.0
	Outlet	1.4 ± 0.1	0.8 ± 0.1	1.8	1.1 ± 0.2	0.33 ± 0.05	3.4

[a] Units: UT1 and UT2: *T. latifolia* + Filtralite®MR3-8, UP1 and UP2: *P. australis* + Filtralite®MR3-8 nd: not determined; values presented are means ± 1 SEM.

under different HLRs, are presented. In Table 6 the overall removal efficiency of each series (UT and UP) is shown. The wastewater composition at the inlet of the CWUs varied along with time according to the tannery production process. The removal of organic matter (COD and BOD_5), nutrients (TKN and NH_3), colour and SO_4^{2-} did not vary significantly ($P < 0.05$) between series UT1–UP1 and UT2–UP2.

The average wastewater inlet pH ranged between 6.36 and 7.82 and for the outlet of CWUs it ranged between 7.82 and 8.27. Concerning the conductivity and salinity, inlet averaged between 7.29 and 8.01 mS cm^{-1} and between 0.39% and 0.41%, respectively. For the outlet of the CWUs the variation was between 5.38 and 7.89 mS cm^{-1} and between 0.28% and 0.44%, respectively. The DO was low at the CWUs inlet, varying between 0.1 and 1.9 mg L^{-1} as was also for the outlet, varying between 0.1 and 1.8 mg L^{-1}. A trial was undertaken to measure the DO in the CWUs, 15 cm below the substrate surface, with a probe. Along two days this probe was settled in the units, at the inlet and outlet zones, and measurements were taken every two hours in a 10-h period each day. For the inlet zone of *Typha* series, the concentration varied between 0.17 and 0.42 mg L^{-1} for UT1 and 0.14 and 1.42 mg L^{-1} for UT2. For the outlet zone, the concentration varied between 0.10 and 0.22 mg L^{-1} for UT1 and 0.11 and 1.23 mg L^{-1} for UT2. Concerning the inlet zone of *Phragmites* series the concentration varied between 0.15 and 0.51 mg L^{-1} for UP1 and 0.15 and 1.68 mg L^{-1} for UP2. For the outlet zone the concentration varied between 0.11 and 0.21 mg L^{-1} for UP1 and 0.09 and 1.65 mg L^{-1} for UP2.

Series UT and UP presented an overall COD and BOD_5 removal efficiency of 79 ± 2% (for an inlet varying between 808 and 2449 mg L^{-1}) and 71 ± 2% (for an inlet varying between 420 and 1000 mg L^{-1}), respectively, reaching in some stages removal levels of up to 92% for COD and 88% for BOD_5. Fig. 3 shows, as an example, the patterns of COD for the first (UP1) and second (UP2) units of *Phragmites* series, when subjected to different HLRs and interruptions in feed along time, after which the removal efficiency always resumed. The lowest COD concentrations were detected at a HLR of 6 cm d^{-1}. For this loading rate, the COD removal efficiency for the Spring/Summer (April–September) and Autumn/Winter (October–March) periods showed no significant ($P < 0.05$) differences for both series. Air temperature varied between 19 and 27 °C during the HLR of 18 cm d^{-1}, between 6.1 and 29.5 °C for 6 cm d^{-1} and 15.5 and 27.3 °C for 8 cm d^{-1}. The inlet and outlet wastewater temperatures varied between 6.5 and 32.0 °C and 5.0 and 28.9 °C. The temperatures were also registered 15 cm below the surface of each unit, each time that a sample collection took place, and the values varied between 3.5 °C and 29.0 °C. Spearman's correlation analyses of the COD removal efficiency for each unit (UT1, UT2, UP1 and UP2) versus the air temperature and temperature below the substrate surface were performed – no significant ($P < 0.05$) correlations were found between these factors.

A linear correlation between mass loading and mass removal is shown in Fig. 4. High mass loadings were applied to the first unit of each series, where mass removals were higher; the different HLRs that were applied combined with fluctuations in the wastewater composition, typical of the tannery sector, allowed for the wide range of mass loadings to reach the systems. Concerning the organic mass loadings for the first unit of *Typha* (UT1) and *Phragmites* (UP1) series, they varied between 485 and 3849 kg ha^{-1} d^{-1} for COD and between 252 and 1800 kg ha^{-1} d^{-1} for BOD_5, with maximum mass removals of 1869 kg COD ha^{-1} d^{-1} and 675 kg BOD_5 ha^{-1} d^{-1}. For the second unit, the inlet organic loading ranged between 162 and 2160 kg ha^{-1} d^{-1} for COD and between 114 and 1188 kg ha^{-1} d^{-1} for BOD_5, with maximum mass removals of 877 kg COD ha^{-1} d^{-1} and 383 kg BOD_5 ha^{-1} d^{-1}. No significant ($P < 0.05$) differences were found between UT1 and UP1 and between UT2 and UP2 for COD and BOD_5 mass removal under long-term operation. Considering the series overall mass removals, maximum values of 1294 kg COD ha^{-1} d^{-1} and 529 kg BOD_5 ha^{-1} d^{-1} were achieved for an inlet varying between 242 and 1925 kg COD ha^{-1} d^{-1} and 126 and 900 kg BOD_5 ha^{-1} d^{-1}, respectively.

Each series presented a TSS removal efficiency of 89 ± 1% (for an inlet varying between 32 and 324 mg L^{-1}), reaching levels of up to 97% in some occasions, with no clogging tendency occurring. TKN removal efficiencies were lower, 55 ± 1% for UT and 57 ± 1% for UP, considering an inlet varying between 87 and 160 mg L^{-1}, reaching in some stages removal levels of up to 67%. The same was found for NH_3, for which average removals of 50 ± 1% for UT and 55 ± 1% for UP were achieved considering an inlet varying between 60 and 98 mg L^{-1}, reaching in some stages higher removal levels, up to 69%. Colour removal efficiencies attained for an inlet

Table 2
POD activity of the plants in the constructed wetlands units in series ($n = 3$).

Units	CW site	POD activity (ΔA min^{-1} g^{-1} FW)		
		Leaf	Stem	Root
UT1[a]	Inlet	n.d.	nd	0.4 ± 0.2
	Outlet	n.d.	n.d.	0.4 ± 0.1
UT2[a]	Inlet	1.8 ± 0.3	nd	2.3 ± 0.8
	Outlet	1.2 ± 0.2	nd	2.1 ± 1.0
UP1[a]	Inlet	3.6 ± 1.6	9.6 ± 1.9	7.2 ± 0.7
	Outlet	1.8 ± 0.8	5.7 ± 0.8	17.7 ± 4.1
UP2[a]	Inlet	2.3 ± 0.4	26.4 ± 15.8	15.8 ± 0.6
	Outlet	4.4 ± 1.3	65.8 ± 12.0	18.2 ± 7.3

[a] Units: UT1 and UT2: *T. latifolia* + Filtralite®MR3-8, UP1 and UP2: *P. australis* + Filtralite®MR3-8 nd: not determined; values presented are means ±1 SEM.

Table 3
Mean composition of the water at the inlet and outlet of the pilot units for a HLR of 18 cm d^{-1} (2 days HRT per series). Minimum and maximum values are indicated in brackets ($n = 7$).

Parameters	Average results (min.–max.) for each unit outlet				
	Inlet	UT1[a]	UT2[a]	UP1[a]	UP2[a]
pH	7.82 (7.66–8.10)	8.27 (8.09–8.65)	8.21 (8.10–8.55)	8.18 (8.06–8.54)	8.26 (8.07–8.61)
Conductivity (mS cm^{-1})	7.39 (6.64–8.31)	6.15 (5.04–7.64)	6.77 (5.74–7.91)	6.58 (6.03–7.35)	6.18 (5.20–7.16)
Salinity (%)	0.41 (0.36–0.46)	0.37(0.31–0.44)	0.33 (0.27–0.42)	0.36 (0.33–0.40)	0.33 (0.28–0.39)
DO (mg O_2 L^{-1})	nd	nd	nd	nd	nd
COD (mg O_2 L^{-1})	1629 (1354–2138)	883 (710–1100)	610 (512–701)	906 (720–1200)	614 (500–713)
BOD_5 (mg L^{-1})	826 (720–1000)	554 (487–660)	410 (365–458)	564 (500–650)	392 (353–441)
TSS (mg L^{-1})	168 (98–324)	30 (21–43)	10 (8–12)	28 (22–34)	10 (7–13)
Total P (mg L^{-1})	0.31 (0.21–0.43)	0.24 (0.17–0.31)	0.22 (0.16–0.30)	0.25 (0.18–0.33)	0.22 (0.13–0.32)
Color (Pt/Co)	nd	nd	nd	nd	nd
TKN (mg TKN–N L^{-1})	136 (110–150)	97 (81–110)	59 (49–72)	93 (53–104)	57 (52–68)
NH_3 (mg NH_3–N L^{-1})	76 (63–87)	46 (38–53)	35 (30–42)	46 (39–53)	36 (30–41)
NO_3^- (mg NO_3^-—N L^{-1})	47 (37–54)	31 (23–35)	23 (17–28)	31 (26–38)	24 (15–30)
SO_4^{2-} (mg SO_4^{2-} L^{-1})	1531 (934–2206)	1087 (745–1479)	923 (599–1180)	1091 (686–1435)	941 (521–1199)
Total Cr (mg L^{-1})	0.370 (0.021–0.885)	0.110 (0.006–0.322)	0.081 (0.001–0.276)	0.122 (0.010–0.368)	0.083 (0.009–0.263)
Cr(VI) (mg L^{-1})	<0.001	<0.001	<0.001	<0.001	<0.001

[a] Units: UT1and UT2: *T. latifolia* + Filtralite®MR3-8, UP1 and UP2: *P. australis* + Filtralite®MR3-8; nd: not determined.

Table 4
Mean composition of the water at the inlet and outlet of the pilot units for a HLR of 8 cm d^{-1} (5 days HRT per series). Minimum and maximum values are indicated in brackets ($n = 6$).

Parameters	Average results (min.–max.) for each unit outlet				
	Inlet	UT1[a]	UT2[a]	UP1[a]	UP2[a]
pH	6.36 (5.84–7.37)	7.82 (7.16–8.19)	8.07 (7.84–8.19)	8.03 (7.78–8.18)	8.19 (7.99–−8.32)
Conductivity (mS cm^{-1})[b]	8.01 (6.95–9.54)	7.89 (5.21–9.27)	7.65 (6.92–8.34)	7.42 (6.21–9.29)	7.26 (5.79–9.22)
Salinity (%)[b]	0.45 (0.38–0.54)	0.44 (0.28–0.52)	0.42 (0.38–0.46)	0.41 (0.34–0.52)	0.40 (0.31–−0.52)
DO (mg O_2 L^{-1})[b]	0.5 (0.2–1.0)	0.4 (0.1–0.7)	0.3 (0.1–0.6)	0.3 (0.2–0.7)	0.3 (0.6–0.2)
COD (mg O_2 L^{-1})	1908 (1751–2100)	921 (819–984)	412 (312–474)	952 (785–1168)	382 (333–−421)
BOD_5 (mg L^{-1})	728 (620–860)	406 (330–450)	236 (210–293)	388 (330–420)	217 (180–260)
TSS (mg L^{-1})[b]	107 (98–121)	33 (30–36)	12 (11–15)	34 (31–38)	13 (10–17)
Total P (mg L^{-1})	0.21 (0.13–0.31)	0.17 (0.11–0.27)	0.11 (0.07–0.21)	0.15 (0.10–0.26)	0.13 (0.08–0.24)
Color (Pt/Co)	242 (221–280)	141 (112–163)	112 (87–131)	135 (108–161)	106 (78–121)
TKN (mg TKN–N L^{-1})	128 (121–134)	79 (73–87)	51 (48–55)	83 (76–95)	51 (46–56)
NH_3 (mg NH_3–N L^{-1})	83 (76–90)	48 (43–53)	37 (30–42)	47 (43–52)	34 (28–37)
NO_3^- (mg NO_3^-—N L^{-1})	46 (43–55)	35 (33–38)	27 (25–30)	34 (31–40)	26 (23–29)
SO_4^{2-} (mg SO_4^{2-} L^{-1})	215 (177–295)	137 (103–184)	107 (68–156)	137 (103–184)	107 (70–150)
Total Cr (mg L^{-1})	0.043 (0.010–0.120)	<0.001	<0.001	<0.001	<0.001
Cr(VI) (mg L^{-1})	<0.001	<0.001	<0.001	<0.001	<0.001

[a] Units: UT1and UT2: *T. latifolia* + Filtralite®MR3-8, UP1 and UP2: *P. australis* + Filtralite®MR3-8.
[b] $n = 7$.

Table 5
Mean composition of the water at the inlet and outlet of the pilot units for a HLR of 6 cm d^{-1} (7 days HRT per series). Minimum and maximum values are indicated in brackets ($n = 27$).

Parameters	Average results (min.–max.) for each unit outlet				
	Inlet	UT1[a]	UT2[a]	UP1[a]	UP2[a]
pH[b]	6.61 (4.43–8.46)	8.07 (7.42–8.93)	8.07 (7.46–8.52)	7.99 (5.84–8.99)	8.04 (6.39–8.88)
Conductivity (mS cm^{-1})[c]	7.29 (4.74–10.05)	6.47 (4.31–10.12)	5.38 (2.34–8.02)	6.43 (5.05–9.67)	5.84 (3.99–9.12)
Salinity (%)[c]	0.39 (0.25–0.56)	0.35 (0.23–0.57)	0.28 (0.10–0.43)	0.35 (0.26–0.54)	0.31 (0.20–0.51)
DO (mg O_2 L^{-1})[d]	0.7 (0.1–1.9)	0.4 (0.1–1.1)	0.5 (0.2–1.8)	0.5 (0.1–1.3)	0.4 (0.1–1.3)
COD (mg O_2 L^{-1})	1598 (808–2449)	550 (270–883)	242 (120–356)	559 (289–1022)	262 (105–428)
BOD_5 (mg L^{-1})	706 (420–860)	314 (190–455)	156 (100–230)	323 (198–460)	161 (75–250)
TSS (mg L^{-1})	80 (32–134)	24 (10–40)	9 (4–14)	25 (7–42)	9 (5–14)
Total P (mg L^{-1})	0.41 (0.10–0.95)	0.32 (0.07–0.80)	0.27 (0.04–0.69)	0.31 (0.07–0.75)	0.26 (0.04–0.70)
Color (Pt/Co)[e]	304 (132–610)	122 (78–231)	92 (55–132)	127 (91–200)	93 (62–128)
TKN (mg TKN–N L^{-1})	122 (87–160)	84 (56–100)	56 (36–65)	83 (53–104)	54 (34–64)
NH_3 (mg NH_3–N L^{-1})	81 (60–98)	54 (35–64)	42 (26–53)	54 (31–69)	41 (21–53)
NO_3^- (mg NO_3^-—N L^{-1})	43 (17–59)	30 (14–45)	24 (12–34)	28 (13–42)	21 (10–30)
SO_4^{2-} (mg SO_4^{2-} L^{-1})[b]	297 (78–1231)	189 (37–775)	138 (23–612)	190 (34–821)	137 (20–553)
Total Cr (mg L^{-1})[b]	0.366 (0.010–2.500)	0.157 (<0.001–0.635)	0.108 (<0.001–0.525)	0.152 (<0.001–0.646)	0.094 (<0.001–0.486)
Cr(VI) (mg L^{-1})[b]	<0.001	<0.001	<0.001	<0.001	<0.001

[a] Units: UT1and UT2: *T. latifolia* + Filtralite®MR3-8, UP1 and UP2: *P. australis* + Filtralite®MR3-8.
[b] $n = 28$.
[c] $n = 24$.
[d] $n = 16$.
[e] $n = 10$.

Table 6
Mean removal efficiency of each series (UT and UP) concerning different hydraulic retention times (2, 5 and 7 days). Minimum and maximum values are indicated in brackets.

HRT[b]	7d[A]			5d[B]			2d[C]		
Parameters		% Removal			% Removal			% Removal	
	Inlet	UT[a]	UP[a]	Inlet	UT[a]	UP[a]	Inlet	UT[a]	UP[a]
COD (mg L^{-1})	1598 (808–2449)	84 (61–92)	83 (56–92)	1908 (1751–2100)	78 (75–82)	80 (77–83)	1629 (1354–2138)	62 (60–67)	62 (57–67)
BOD_5 (mg L^{-1})	706 (420–860)	77 (57–85)	77 (56–88)	728 (620–860)	67 (56–76)	70 (61–77)	826 (720–1000)	50 (44–56)	52 (48–59)
TSS (mg L^{-1})	80 (32–134)	88 (75–96)	90 (83–95)	107 (98–121)	88 (85–90)	85 (81–89)	168 (98–324)	93 (89–97)	90 (86–97)
Color (Pt/Co)	304 (132–610)	60 (38–90)	60 (39–88)	242 (221–280)	54 (51–61)	56 (46–65)	nd	nd	nd
TKN–N (mg L^{-1})	122 (87–160)	53 (39–65)	55 (44–67)	128 (121–134)	60 (56–63)	60 (54–66)	136 (110–150)	57 (51–61)	58 (53–62)
NH_3–N (mg L^{-1})	81 (60–98)	48 (36–61)	49 (34–67)	83 (76–90)	56 (51–67)	58 (51–69)	76 (63–87)	54 (52–57)	53 (49–57)
SO_4^{2-} (mg L^{-1})	297 (78–1231)	56 (19–81)	56 (23–80)	215 (177–295)	51 (38–65)	50 (36–63)	1531 (934–2206)	37 (22–53)	37 (19–52)

[a] Series: UT: *T. latifolia* + Filtralite®MR3-8, UP: *P. australis* + Filtralite®MR3-8.
[b] HRT: Hydraulic Retention Time; nd: not determined.
[A] HLR per unit 6 cm d^{-1}.
[B] HLR per unit 8 cm d^{-1}.
[C] HLR per unit 18 cm d^{-1}.

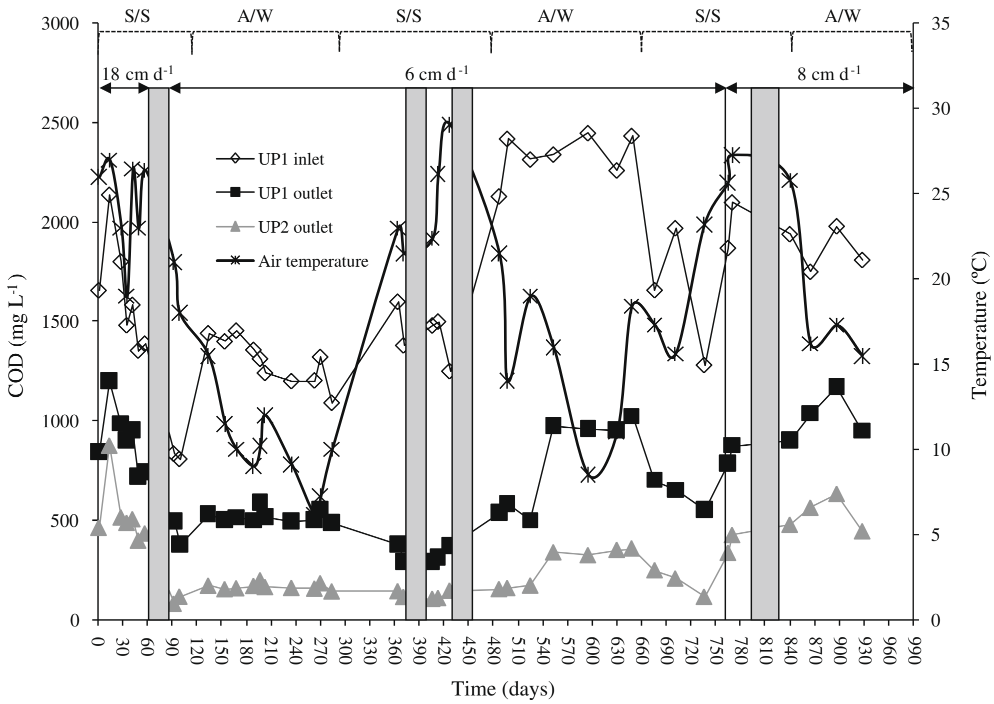

Fig. 3. COD at the inlet and outlet of the UP1 and UP2 units, during the time of operation, for the three HLRs applied. ▒ No loading occurred. Air temperature at the time of sampling collection is presented. Periods of Spring/Summer and Autumn/Winter are represented as S/S and A/W, respectively.

varying between 132 and 610 Pt/Co were 58 ± 4% for UT and 59 ± 4% for UP, reaching in some stages high removal levels – up to 90%. Each series presented a SO_4^{2-} removal efficiency of 52 ± 2% (for an inlet varying between 78 and 2206 mg L^{-1}), reaching removal levels of up to 81%. Phosphorus and chromium (total and hexavalent) were only detected at low concentrations at the inlet and outlet of the CWUs.

In Table 7 a brief summary of the performance of the systems in terms of organic matter, TSS, nitrogen and phosphorous removal is presented.

4. Discussion

In this study the performance, in terms of contaminant removal with special focus on organic loading, and plant development, of two series of CWs planted with *T. latifolia* and *P. australis* was evaluated.

4.1. CWs plant development and analysis

P. australis was the plant that established most successfully in the CWUs, although *T. latifolia* also showed a good resistance to the alterations in the loadings applied to the systems. By the end of the run, after 928 days, at the inlet zone (A) of the CWUs, the number of shoots for *T. latifolia* units (UT1 and UT2) and *P. australis* (UP1 and UP2) was lower when compared to the that at other zones (B, C, D). This behavior has been reported already (Calheiros et al., 2008b; Shepherd et al., 2001) and was attributed to the fact that at the inlet the organic loading was higher.

Phragmites is known to be the most frequently used plant worldwide in subsurface flow constructed wetlands (Kadlec et al., 2000). Calheiros et al. (2008a) carried out toxicity tests to assess the effect of tannery wastewater on *Trifolium pratense*, *T. latifolia* and *P. australis*, and higher germination levels were achieved for *P. australis*. Furthermore, this plant has a high poten-

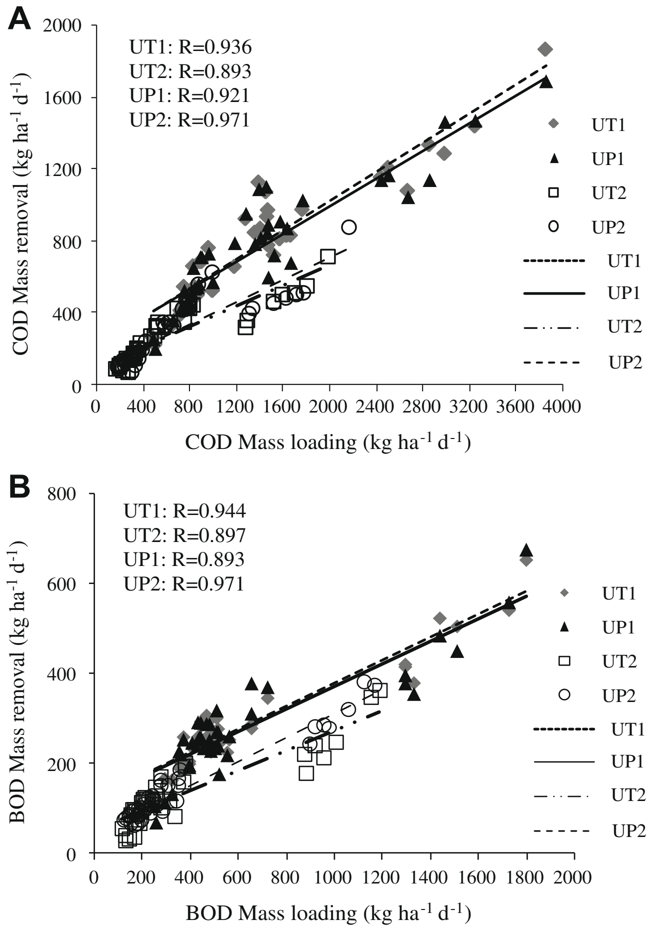

Fig. 4. Removal of organics during system operation. (A) COD Mass loading vs. COD Mass removal, (B) BOD_5 Mass loading vs. BOD_5 Mass removal. The coefficients of the linear correlation are represented with $p < 0.05$.

tial to extract and accumulate chromium, characteristic that may be interesting to the tannery industry since this heavy metal poses a major environmental concern. Calheiros et al. (2007) had previously reported on a successful establishment of *P. australis* and *T. latifolia* in CWs for tannery wastewater when compared to other plants, such as *Iris pseudacorus*, *Canna indica* and *Stenotaphrum secundatum*. Kucuk et al. (2003) has also reported on the use of *P. australis* in a CW with subsurface flow for tannery wastewater treatment.

Plants did not visually show signs of phytotoxicity, however their height was lower than those of plants occurring in the environment of origin. This fact may be attributed to the complex composition and toxicological load of tannery wastewater, which has been shown to affect the development of different plant species (Calheiros et al., 2008a). Nevertheless, the plants have survived different HLRs, and thus different organic loadings, and interruptions in the system feed for periods up to one month.

The chlorophyll content of *P. australis* in the second determination was in general higher than in the first one, which may explain the apparently higher resistance of this plant to the application of the wastewater. For *T. latifolia* the differences between the two sampling times were not so evident. Chlorophyll loss may be induced by several causes such as hostile environmental pressures of natural or anthropogenic origin (Hendry et al., 1987). Also, peroxidase activity may be related to water stress in plants (Klar et al., 2006). However, more studies would be required to assess the detoxification pathway of these plants and the wastewater toxicity on plant photosynthesis in order to infer about the stress responses.

4.2. Wastewater treatment performance in CWUs

Calheiros et al. (2007) reported on the efficiency of CWs treating tannery wastewater subjected to HLRs of 3 and 6 cm d^{-1}; in the present study a wider range of hydraulic conditions were used – HLRs of 6, 8, 18 cm d^{-1} to evaluate the tolerance capacity of these systems and, furthermore, units were arranged in series.

The pH, conductivity and salinity of the wastewater are in the range of what was reported in other studies (Calheiros et al., 2008b, 2007; Kucuk et al., 2003). The DO measured at the inlet was low since the wastewater had a high organic content. These low levels were also measured in the substrate and at the wastewater outlet, with no considerable oscillation along the operation. This may occur because the systems are operating in subsurface flow mode and those conditions are generally considered as anoxic or anaerobic. The amount of DO in the inlet wastewater is considered to be only a negligible portion of the DO used in the bed and the outflow concentration provides little information about the processes occurring there (Vymazal and Kröpfelová, 2008).

The organic pollutant load, measured as COD and BOD_5, of the wastewater treated in the present study, is very high. The production process is based on the conversion of wet blue into finish leather and according to Daniels (2001) the resultant wastewater for this type of process is recognised as particularly difficult to be treated biologically. Kucuk et al. (2003) reported that the highest COD removal (30%), in a subsurface flow CW, was obtained at a hydraulic retention time of 8 days for an inlet concentration of 210 mg L^{-1}; in the present study the highest percentage removal was 92% for inlet levels varying between 808 and 2449 mg L^{-1}. To a higher extent Aguilar et al. (2008), with a three-stage CW (405 m^2) operating with a flow of 20 L min^{-1}, managed to achieve removal efficiencies between 96 and 98% for COD (of a inlet 12,340–17,520 mg L^{-1}) and 93 and 95% for BOD (of a inlet 675–1320 mg L^{-1}). Also, Prabu and Udayasoorian (2007) investigated the pollutant removal in CWs treating pulp and paper mill effluent, in terms of BOD_5 (68–138 mg L^{-1}), COD (988–1387 mg L^{-1}) and total soluble solids (160–310 mg L^{-1}), and high percentage removals were achieved for units planted with *P. australis* (77%, 62% and 77%, respectively), *T. latifolia* (74%, 55% and 72%, respectively) and *C.*

Table 7
Summary of treatment performance for different HRTs.

Parameter	HRT (d)	Concentration (mg L^{-1})		Removal (%)	Loading (kg ha^{-1} d^{-1})	
		In	Out		In	Mass removal
COD	7[a]	1598	252	83	479	404
	5[b]	1908	397	79	802	651
	2[c]	1629	612	62	1466	916
BOD_5	7[a]	706	159	77	212	164
	5[b]	728	226	68	306	221
	2[c]	826	401	51	743	383
TSS	7[a]	80	9	88	24	21
	5[b]	107	13	88	45	40
	2[c]	168	10	93	151	142
TKN–N	7[a]	122	55	54	37	20
	5[b]	128	51	60	54	34
	2[c]	136	58	57	123	71
NH_3–N	7[a]	81	42	48	24	13
	5[b]	83	35	57	35	21
	2[c]	76	36	53	69	37
TP	7[a]	0.41	0.27	38	0.09	0.04
	5[b]	0.21	0.12	43	0.13	0.05
	2[c]	0.31	0.22	27	0.28	0.08

[a] HLR per unit 6 cm d−1.
[b] HLR per unit 8 cm d^{-1}.
[c] HLR per unit 18 cm d^{-1}.

pangorei (64%, 49% and 67%, respectively). Mantovi et al. (2003) reported removal efficiencies above 90% for COD (859–2312 mg L^{-1}) and BOD_5 (242–887 mg L^{-1}), although the wastewater to be treated by the CWs had originated from dairy parlor effluent and domestic sewage.

The organics removal in the CWUs was quite stable along seasons. Spearman's correlation analyses performed between the COD removal efficiency for each unit versus the air temperature and temperature below the substrate surface corroborated such a conclusion. However, climatic conditions may influence performance to different extents (Kadlec et al., 2000). According to the Köppen classification (Kottek et al., 2006), the Portuguese climate in Minho region is classified as Csb, i.e., temperate climate with rainy winters and dry summers with mild temperatures.

The maximum pollutant removal rates for CWs are dependent on several design parameters, such as type of flow, substrate, hydraulic and organic loadings, and the toxicity of the wastewater. The organic loadings tested in this study are much higher than those suggested in the literature for other types of wastewaters (since no specific guidelines are detailed for tannery wastewater) – to attain a final BOD_5 of 10–30 mg L^{-1} a loading rate of 67–157 kg ha^{-1} d^{-1} is recommended in the EPA manuals (*e.g.* USEPA, 2000). Vymazal (2001) presented a linear relationship between inflow and removed loadings (COD and BOD_5), as was also verified in the present study for higher mass loadings, indicating a good predictability of the amount that could be removed in horizontal subsurface flow CWs. Shepherd et al. (2001) applied high-strength (up to 1640 kg COD ha^{-1} d^{-1}) winery wastewater to a subsurface flow CW combined with an upflowing sand prefilter and removal rates of 98% were reported.

Typically, in a wetland treatment system, the removal efficiency concerning TSS is in the range of 80–90% (Kadlec et al., 2000). The high suspended solid removal achieved in the present study can be attributed to the physical processes occurring in the wetland which involve sedimentation, filtration and adsorption (USEPA, 2000). However, in general, for specific solids removal purposes, it is mandatory that the wastewater treatment comprises a proper primary system before CWs so that their functional integrity is assured and no clogging occurs.

Although CWs were capable of removing nitrogen and phosphorus, this happened to a lower extent than for organics (represented by COD and BOD_5). These findings were in the range found by other authors (Calheiros et al., 2008b, 2007; Mantovi et al., 2003) and to some extent are expected for this type of systems (Kadlec et al., 2000).

The operation of CWs in two stages is intended to improve the wastewater treatment performance. Langergraber et al. (2008) reported that the elimination of BOD_5, COD and NH_4–N by a two-stage vertical flow CW (2 m^2 per person equivalent) was twice compared to that by a single stage (4 m^2 per person equivalent) when the former operated with the double organic load. Daniels (2001) also applied a multi-stage polishing plant for domestic effluent. Mantovi et al. (2003) applied a series of two horizontal subsurface flow CWs with *P. australis* for dairy parlor wastewater treatment and the high pollutant removal allowed the discharge of effluents onto surface waters. Other combinations, such as an hybrid system with a three-stage horizontal flow (with *I. pseudacorus*) – vertical flow (with *P. australis*) - horizontal flow (with *I. pseudacorus*) CWs configuration, was found to be very effective for agricultural wastewater treatment, where COD (50.3 ± 7.2 mg L^{-1}), total N (35.1 ± 6.5 mg L^{-1}) and total phosphorus (3.0 ± 0.8 mg L^{-1}) had removal rates of 95.1%, 68.4% and 94.3%, respectively (Seo et al., 2008).

Based on our studies at lower hydraulic retention times, such as 2 days, the efficiency of, the CWs was not sufficient to attain high levels of organic matter removal. In the case of 7 day retention time, the performance of the systems allowed, in some cases, the wastewater discharge to be in compliance to the Portuguese law for tanneries (*Portaria no. 512/92 de 22 de Junho*), concerning the COD, BOD_5, TSS, total chromium and pH parameters. We can assume that for high-strength wastewater derived from a complex sector, such as tannery, an organic loading of 212 kg BOD_5 ha^{-1} d^{-1} should not be exceeded if the BOD discharge limits for tannery industry are set as a goal (Table 7). Arrangement in two-stage units allows operational flexibility and cost savings. In case of unexpected events, such as wetland contamination due to pretreatment failure, the first unit can be isolated and the effluent may be redirected to the second unit, without stopping the wastewater treatment. Taking advantage of the gravity flow no pumps are required and, as seen in the present case, there is no need for using recirculation in the system. The application of a CW as a reliable wastewater treatment technology, in terms of economic and efficient sustainability, may be a viable choice in order to fulfill the discharge requirements. The data presented here support this remediation technology as suitable for complex wastewaters, contributing to the compliance of an integrated water management system.

5. Conclusions

Two series of two-stage horizontal subsurface flow CWs, operating under different organic loadings, were evaluated in terms of performance, establishment and adequacy to be applied to tannery wastewater treatment. The main conclusions of this investigation are summarized as follows.

- CWs can be subjected to higher organic and hydraulic loadings than those suggested in the literature, allowing for wastewater treatment under different scenarios of operation such as shock loadings and interruptions in feed, which is advantageous for industrial applications.
- The systems were able to deal with situations commonly found in industrial scenarios, such as fluctuations in the organic loadings and interruptions in the feed, without showing clogging symptoms.
- Units in two-stage alignment provided gains in terms of a higher extent of industrial wastewater treatment under long-term operation and in terms of the robustness of the system, with no requirements concerning wastewater recirculation.
- The two series were similar in performance and both plants were able to propagate and establish. However, for building systems at a greater scale, *P. australis* is considered very interesting since it showed high propagation through the CWs, avoiding jeopardizing the treatment success concerning plant establishment.

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