Constructed wetland systems vegetated with different plants applied to the treatment of tannery wastewater

*Cristina S.C. Calheiros, António O.S.S. Rangel, Paula M.L. Castro**

Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

Keywords:
Constructed wetlands
Phytoremediation
Tannery wastewater
Horizontal subsurface flow
Phragmites australis
Typha latifolia

ABSTRACT

Wastewaters from leather processing are very complex and lead to water pollution if discharged untreated, especially due to its high organic loading. In this study the survival of different plant species in subsurface horizontal flow constructed wetlands receiving tannery wastewater was investigated. Five pilot units were vegetated with *Canna indica*, *Typha latifolia*, *Phragmites australis*, *Stenotaphrum secundatum* and *Iris pseudacorus*, and a sixth unit was left as an unvegetated control. The treatment performance of the systems under two different hydraulic loading rates, 3 and 6 $\mathrm{cm\,d^{-1}}$, was assessed. COD was reduced by 41–73% for an inlet organic loading varying between 332 and 1602 $\mathrm{kg\,ha^{-1}\,d^{-1}}$ and $\mathrm{BOD_5}$ was reduced by 41–58% for an inlet organic loading varying between 218 and 780 $\mathrm{kg\,ha^{-1}\,d^{-1}}$. Nutrient removal occurred to lower extents. *Phragmites australis* and *Typha latifolia* were the only plants that were able to establish successfully. Despite the high removal of organic content from the influent wastewater, during 17 months of operation, no significant differences in performance were observed between units.

Introduction

Constructed wetlands (CWs) can be used for primary, secondary and tertiary treatment of municipal or domestic wastewaters, stormwater, agricultural and industrial wastewaters such as landfill leachate, petrochemicals, food wastes, pulp and paper and mining, usually combined with an adequate pre-treatment (Kadlec et al., 2000). Although they are widely used for municipal wastewater, the application to industrial wastewater has to be carefully analyzed since its composition is frequently highly variable and the treatment needs are not the same. However, the use of CWs for the treatment of industrial wastewaters has increased over the past ten years (Korkusuz, 2005).

The discharge of tannery wastewaters can cause severe environmental problems due to its high chemical oxygen demand (COD) level and, sometimes, high chromium concentration and deep color content (Song et al., 2000). The effluent treatment systems for the tannery industry experience frequent problems due to the fact that they are often working over the capacity due to poor design, or to increases in production. In these situations, a CW can be potentially used to enhance the overall biological performance (Daniels, 2001b), or can be an alternative to the conventional biological treatment (Daniels, 2001a; Kucuk et al., 2003). However, no detailed studies on the treatment of tannery wastewater using CWs are known in the literature.

The choice of plants is an important issue in CWs, as they must survive the potential toxic effects of the wastewater and its variability. The most widely used CWs design in Europe is the horizontal subsurface flow system vegetated with the common reed (*Phragmites australis*) (Vymazal, 2005), although other plant species, such as cattails (*Typha* spp.) bulrushes (*Scipus* spp.) and reed canarygrass (*Phalaris arundinacea*) have

---

**Corresponding author.* Tel.: +351 22 5580059; fax: +351 22 5090351.
E-mail addresses: mccalheiros@mail.esb.ucp.pt (C.S.C. Calheiros), aorangel@esb.ucp.pt (A.O.S.S. Rangel), plcastro@esb.ucp.pt (P.M.L. Castro)

been used for both domestic and industrial wastewater treatment (Mbuligwe, 2005; Vymazal, 2005; Vymazal and Kröpfelová, 2005; Shepherd et al., 2001). In Portugal, the main macrophyte species used in CWs are *P. australis*, *Iris pseudacorus* (yellow iris) and *Cyperus* spp. In some systems, *Juncus effuses* (soft rush), other *Juncus* spp., and *Scirpus* spp. (bulrushes) are also found to establish spontaneously (Korkusuz, 2005).

The present study aimed at assessing the use of different plant species in CWs receiving wastewater from a tannery production plant. Six pilot units, with horizontal subsurface flow, were evaluated for a period of 17 months in terms of treatment performance and plant survival when subject to two different hydraulic loadings rates (HLRs).

Methods

Wetland site

The experimental pilot units were located at a leather company in the north of Portugal, and placed after a primary treatment of the effluent. This company has only the post-tanning and finishing operations, although in a complete productive cycle other operations are included, such as hide and skin storage, beamhouse, deliming, bating, pickling and tanning.

Wastewater sampling and analysis

The tannery wastewater to be applied to the pilot units was characterized over a one-year period. The following parameters were determined, based on standard methods (APHA, 1998): pH (Potentiometric Method), chemical oxygen demand (COD; Closed Reflux, Colorimetric 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 (NO_3^-; Nitrate Electrode Method), ammonia (NH_3; 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 analyses were done immediately after sample collection, otherwise were properly stored.

Constructed wetland pilot units

Five parallel pilot units were established with five different plant species—*Canna indica*, *T. latifolia*, *P. australis*, *Stenotaphrum secundatum* and *I. pseudacorus*—and filled with a substrate composed of light expanded clay (Filtralite® MR 3-8, maxit Group, Portugal). Another pilot unit was filled with the same substrate but was kept without plants. The characteristic size, d_c, of the substrate was between 3 and 8 mm, according to the technical description from the supplier company. The pH of the substrate was determined based on Houba et al. (1995).

The structure of the units was made of propylene with the following design characteristics: surface area of the bed, $A = 1.2\,m^2$, effective depth of the substrate, $h = 0.60$ m and average depth of liquid in the bed, $h_0 = 0.55$ m. Near the wastewater inflow and outflow of the beds a layer of coarse rock was put in place in order to facilitate the distribution of the effluent. Feeding of wastewater to the six pilot units was made through a perforated polyvinylchloride (PVC) rigid pipe with flow control.

The units were filled with water and after three weeks the wastewater was applied. The systems then operated for 17 months under different hydraulic conditions. During the first two months, no monitoring of the efficiency of the system was carried out, apart from measuring the pH of the inflow and outflow. After that, for 11 months (corresponding to days 60–391), the systems were monitored under a HLR of $3\,cm\,d^{-1}$, with a nominal wetland detention time of 6.8 days. By the 8th month (between days 214 and 242), the systems were not fed continuously due to the shutdown of the tannery production plant, although during that period the level of the liquid in the units was kept at approximately 5 cm below the surface by adding of stored tannery wastewater as required. A second period of operation occurred subsequently during 4 months (corresponding to days 392–511) under a HLR of $6\,cm\,d^{-1}$, with a nominal wetland detention time of 3.4 days. Samples were periodically taken at the inlet and outlet of the CWs.

Plant material

The plant material used in the pilot units was chosen based on the plant species established in the surroundings of a wastewater discharge tank of the leather company: cattail (*T. latifolia*), yellow flag (*I. pseudacorus*), canna (*C. indica*) and St. Augustine grass (*S. secundatum*). These plants were transplanted from this site to the pilot units. The common reed (*P. australis*) was also used because it is very abundant in Portugal and it is frequently applied in CWs. It was transplanted from an industrial polluted site in Estarreja, Portugal (Oliveira et al., 2001). All the plants used for transplantation to the pilot units were apparently well established in their place of origin. The vegetation was planted by hand with a range of plants of $10\,m^{-2}$. The plant material was placed in the substrate at a depth equal to the operational water level of the units. The number of plants was monitored throughout operation.

Maintenance

The systems were inspected on, at least, a weekly basis concerning the overall functioning. Major attention was given to the inlet flow, which was checked twice a week, as obstruction of the pipes due to suspended solids in the effluent could occur. A general cleaning of the pipes was usually undertaken twice a month.

Data analysis

Statistical analysis was performed using the SPSS program (SPSS Inc., Chicago, IL, USA; Version 12.0). The data was analyzed through one-way analysis of variance (ANOVA) to compare the performance of each bed concerning the removal of BOD_5, COD, TSS, TKN, NH_3, NO_3^- and Total P. To detect the statistical significance of differences ($p<0.05$) between means of treatments, the Tukey test was performed.

Results

Tannery wastewater characterization

The characteristics of the tannery wastewater are presented in Table 1, demonstrating its variability during the year. The variations also occur through each working day. The variation of COD and pH, determined hourly, was analyzed during two different days—the average COD and pH were 2010 mg O_2 L^{-1} (±516) and 6.98 (±0.05), respectively, in one day, and 2068 mg O_2 L^{-1} (±446) and 7.93 (±0.08), respectively, in another day.

Table 1 – Mean composition of the tannery wastewater for 1 year. Average, minimum and maximum values (±SD) are shown

	Average ± SD	Minimum	Maximum
pH[a]	6.14 ± 1.10	4.62	8.13
COD[a] (mg O_2 L^{-1})	2250 ± 565	1100	3000
BOD$_5$[a] (mg L^{-1})	1000 ± 88	900	1200
TSS[b] (mg total solids L^{-1})	92 ± 36	58	200
TKN[b] (mg TKN-N L^{-1})	188 ± 17	150	220
NH$_3$[b] (mg NH$_3$-N L^{-1})	100 ± 14	75	135
NO$_3^-$[b] (mg NO$_3^-$-N L^{-1})	44 ± 9	36	67
Total P[b] (mg P L^{-1})	1.0 ± 0.7	0.1	2.0
Total Cr[b] (mg Cr L^{-1})	0.027 ± 0.075	<0.001	0.360
Cr VI[b] (mg Cr L^{-1})	0.004 ± 0.006	<0.001	0.020

[a] $n = 44$.
[b] $n = 24$.

Development of plant material in the pilot units

The growth of the plants was monitored during the operation of the pilot units. A limited natural colonization by some plants occurred in all units, except on the control unit, namely by *Aster squamatus*, *Picris echioides*, *Polypogon viridis*, *Dactylis glomerata*, *Paspalum paspalodes*, *Vicia sativa*, *Sonchus oleraceus* and *Rumex crispus*. No intervention was made to control their growth. The development of the plants in terms of total number of shoots is shown in Fig. 1. By the end of the first period (corresponding to day 391) of operation at an HLR of 3 cm d^{-1}, the counting of the existing number of plants was 7 for *Canna indica*, 10 for *T. latifolia*, 200 for *P. australis*, 5 for *I. pseudacorus*, while *S. secundatum* did not show any development. After the feed interruption period (between days 214 and 242), there was a 14% decrease for *C. indica*, a 17% decrease for *I. pseudacorus*, while there was an increase of 4% for *T. latifolia* and of 35% for *P. australis*. After the second period of operation, corresponding to four months (corresponding to days 392–511) at a higher HLR of 6 cm d^{-1}, the counting of the existing number of plants was 3 for *C. indica*, 25 for *T. latifolia*, 400 for *P. australis* and 9 for *I. pseudacorus*. *S. secundatum* almost disappeared.

Removal eficiency of the pilot units

The efficiency of the pilot units subject to the different hydraulic conditions was monitored through their operation, and the characteristics of the wastewater collected from the inflow and outflow of each pilot unit are shown in Tables 2 and 3.

In the first 2 months of wastewater application, the outflow pH of the units was more alkaline than in the following periods of operation, varying between 8.69 and 10.39 (data not shown). A decrease of the pH at the outflow of all pilot units occurred with time, but the pH was higher at the outflow than that observed at the inflow at all times. When operating at an HLR of 3 cm d^{-1}, the average pH at the inflow was 7.45, while the pH at the outflow, for the different units, varied between 7.95 and 9.88. When operating at an HLR of 6 cm d^{-1} the

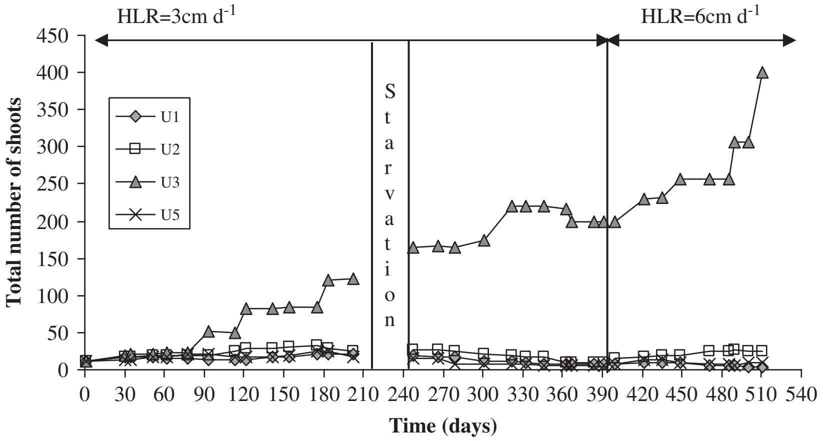

Fig. 1 – Variation in number of shoots in the pilot units during the systems operation. Unit 1: *Canna indica*, Unit 2: *Typha latifolia*, Unit 3: *Phragmites australis* and Unit 5: *Iris pseudacorus*. No loading occurred between days 214 and 242.

Table 2 – Mean composition of the inflow and outflow of the pilot units for a HLR of 3 cm d^{-1}. Minimum and maximum values are indicated in brackets

Parameters	Average results (min.–max.)						
	Inflow	U1[a]	U2[a]	U3[a]	U4[a]	U5[a]	U6[a]
pH[b]	7.45 (4.64–8.75)	8.64 (7.95–9.42)	8.61 (8.01–9.34)	8.69 (8.06–9.62)	9.03 (8.09–9.85)	9.15 (8.42–9.88)	8.77 (8.04–9.72)
COD[b] (mg O$_2$ L^{-1})	1966 (1108–3141)	882 (500–1400)	855 (550–1400)	833 (550–1290)	892 (554–1300)	884 (600–1325)	913 (600–1312)
BOD$_5$[c] (mg L^{-1})	875 (727–1080)	438 (350–525)	444 (350–550)	449 (400–530)	468 (380–570)	469 (400–560)	476 (400–600)
TSS[d] (mg total solids L^{-1})	75 (33–125)	19 (7–43)	18 (4–33)	19 (5–38)	21 (7–39)	20 (7–40)	20 (7–40)
TKN[c] (mg TKN-N L^{-1})	143 (90–230)	106 (62–170)	107 (65–180)	105 (62–176)	104 (57–173)	104 (56–165)	106 (67–168)
NH$_3$[c] (mg NH$_3$-N L^{-1})	74 (45–100)	59 (33–86)	59 (35–82)	61 (34–88)	61 (37–84)	60 (37–81)	59 (37–83)
NO$_3^-$[c] (mg NO$_3^-$-N L^{-1})	36 (20–60)	31 (18–50)	31 (18–51)	31 (18–52)	31 (17–48)	31 (18–52)	32 (18–52)
Total P[e] (mg P L^{-1})	0.30 (0.08–0.45)	0.42 (0.12–0.93)	0.37 (0.09–1.41)	0.44 (0.1–1.47)	0.46 (0.13–1.36)	0.56 (0.09–1.63)	0.40 (0.09–0.98)
Total Cr[d] (mg Cr L^{-1})	0.017 (<0.001–0.025)	<0.001	0.004 (<0.001–0.005)	0.012 (<0.001–0.025)	0.008 (<0.001–0.015)	0.005 (0.001–0.012)	0.006 (<0.001–0.012)
Cr VI[d] (mg Cr L^{-1})	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a] Units: U1: *Canna indica*, U2: *Typha latifolia*, U3: *Phragmites australis*, U4: *Stenotaphrum secundatum*, U5: *Iris pseudacorus*, U6: control.
[b] $n = 27$.
[c] $n = 10$.
[d] $n = 19$.
[e] $n = 13$.

Table 3 – Mean composition of the inflow and outflow of the pilot units for a HLR of 6 cm d^{-1}. Minimum and maximum values are indicated in brackets

Parameters	Average results (min.–max.)						
	Inflow	U1[a]	U2[a]	U3[a]	U4[a]	U5[a]	U6[a]
pH[b]	6.55 (5.84–7.13)	8.18 (8.03–8.54)	8.25 (8.13–8.48)	8.15 (8.06–8.37)	8.33 (8.15–8.64)	8.52 (8.33–8.78)	8.17 (8.01–8.38)
COD[b] (mg O$_2$ L^{-1})	2093 (1755–2669)	805 (684–900)	745 (600–870)	776 (672–880)	810 (900–700)	778 (672–870)	821 (740–915)
BOD$_5$[b] (mg L^{-1})	898 (740–1300)	436 (350–540)	436 (345–630)	449 (350–600)	449 (370–590)	465 (370–620)	453 (350–600)
TSS[b] (mg total solids L^{-1})	79 (66–100)	24 (20–33)	23 (19–31)	24 (19–30)	25 (18–32)	25 (19–33)	24 (19–32)
TKN[c] (mg TKN-N L^{-1})	126 (90–162)	94 (74–120)	96 (74–125)	97 (70–119)	96 (65–122)	94 (63–119)	95 (68–119)
NH$_3$[c] (mg NH$_3$-N L^{-1})	88 (75–100)	79 (70–87)	82 (73–94)	80 (72–90)	79 (70–88)	81 (73–88)	81 (72–93)
NO$_3^-$[c] (mg NO$_3^-$-N L^{-1})	30 (20–40)	28 (18–36)	27 (19–36)	28 (17–39)	27 (19–35)	27 (18–36)	28 (18–37)
Total P[b] (mg P L^{-1})	0.25 (0.20–0.38)	0.26 (0.20–0.39)	0.28 (0.21–0.38)	0.29 (0.21–0.38)	0.27 (0.21–0.39)	0.28 (0.21–0.4)	0.27 (0.21–0.39)
Total Cr[b] (mg Cr L^{-1})	0.010 (<0.001–0.027)	0.008 (<0.001–0.027)	0.022 (<0.001–0.080)	0.010 (<0.001–0.031)	0.012 (<0.001–0.026)	0.025 (0.001–0.049)	0.018 (<0.001–0.040)
Cr VI[b] (mg Cr L^{-1})	0.005 (<0.001–0.010)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a] Units: U1: *Canna indica*, U2: *Typha latifolia*, U3: *Phragmites australis*, U4: *Stenotaphrum secundatum*, U5: *Iris pseudacorus*, U6: control.
[b] $n = 12$.
[c] $n = 6$.

average pH of the inflow was 6.55, while the outflow values varied between 8.01 and 8.78. The performance of the units concerning organic matter and nutrients removal was measured thereafter.

In terms of organic matter removal (COD and BOD_5) no significant differences were found between the different pilot units. For an HLR of 3 and 6 cm d^{-1} the average COD inflow concentration was 1966 and 2093 mg O_2 L^{-1}, respectively. The removal efficiencies of the pilot units varied from 41% to 67% for an HLR of 3 cm d^{-1}, and from 54% to 73% for an HLR of 6 cm d^{-1}. The average BOD_5 inflow concentration was 875 and 898 mg L^{-1} for an HLR of 3 and 6 cm d^{-1}, respectively. At the outflow of the pilot units the removal efficiency varied between 41 and 55% for an HLR of 3 cm d^{-1}, and between 41% and 58% for an HLR of 6 cm d^{-1}.

The TSS inflow concentration ranged between 33 and 125 mg total solids L^{-1} and the removal efficiencies varied between 48% and 92% for 3 cm d^{-1} and between 62% and 77% for 6 cm d^{-1}. There were no significant differences between the units for the two HLRs.

Nutrients removal was in general low for the two hydraulic conditions. The TKN and NH_3 inflow concentrations ranged between 90–230 mg TKN-N L^{-1} and 45–100 mg NH_3-N L^{-1}. For the HLR of 3 cm d^{-1}, the removal efficiencies varied from 18% to 42% for TKN and 11–27% for NH_3. For an HLR of 6 cm d^{-1} the average removal efficiencies for TKN and NH_3 were in the range of 16–30% and 2–16%, respectively. There were no significant differences between the units for the two HLRs. The NO_3^- inflow concentration ranged between 20 and 60 mg NO_3^--N L^{-1}; at the outlet concentration varied between 17 and 52 mg NO_3^--N L^{-1}.

Phosphorus and chromium (total and hexavalent) were only detected at low concentrations at the inflow and outflow of the units. For phosphorous removal, there were no significant statistical differences between the pilot units. The chromium concentration at the outflow of the units was below the detection limit, being the average inflow concentration of 0.010 mg Cr L^{-1}. These low levels are due to the fact that the leather production plant does not use chromium in high amounts.

Fig. 2 shows, as an example, the pattern of COD removal during the system operation at the two HLRs for the *P. australis* unit. The outflow values of COD, for all units, gradually decreased at the beginning of operation but remained fairly constant thereafter. The efficiency of COD removal was not affected by the feed interruption period, corresponding to the tannery plant shutdown at day 214. When the feed was restarted, after one month, the efficiency was resumed. The COD removal efficiency was not significantly affected by the time of the year, varying between 41% and 67% in Spring/Summer (April–September) and between 41% and 65% in Autumn/Winter (October–March).

Relationship between removal efficiency and organic load

The removal of COD, BOD_5 and TSS was proportional to the influent load. The linear relationship between mass removal and mass loading is illustrated in Figs. 3–5. For an HLR of 3 cm d^{-1}, the organic loading (COD) inflow of the units varied between 332 and 942 kg ha^{-1} d^{-1}, being the mass removal between 152 and 582 kg ha^{-1} d^{-1}. For an HLR of 6 cm d^{-1}, the organic loading (COD) varied between 1053 and 1602 kg ha^{-1} d^{-1}, being the mass removal between 573 and 1092 kg ha^{-1} d^{-1}. In terms of BOD_5, the organic loading varied from 218 to 324 kg ha^{-1} d^{-1}, for a 3 cm d^{-1}, being the mass removal between 92 and 168 kg ha^{-1} d^{-1}, while for an HLR of 6 cm d^{-1} it varied between 444 and 780 kg ha^{-1} d^{-1}, and the mass removal was between 198 and 456 kg ha^{-1} d^{-1}. The maximum and minimum TSS loading was, respectively, 10 and 38 kg ha^{-1} d^{-1} for 3 cm d^{-1} and 40 and 60 kg ha^{-1} d^{-1} for 6 cm d^{-1}, being the correspondent mass removals variations of 6–33 and 25–45 kg ha^{-1} d^{-1}.

Discussion

In this study, six constructed wetland pilot unit systems receiving tannery wastewater operated for 17 months using horizontal subsurface flow under two different HLRs, 3 and

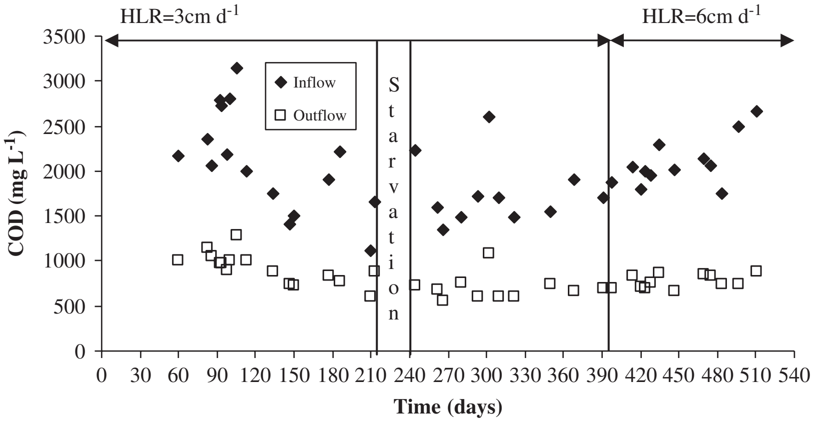

Fig. 2 – COD at the inflow and outflow of the *Phragmites australis* pilot unit during the time of operation, for the two HLRs applied. No loading occurred between days 214 and 242.

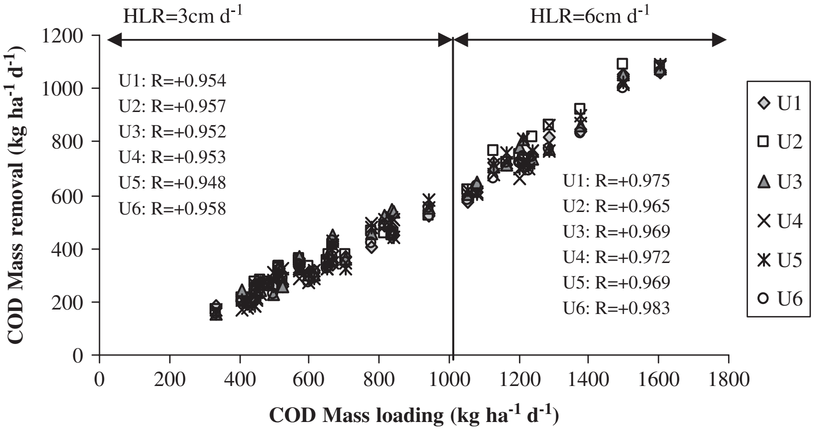

Fig. 3 – COD loading vs. COD removal. Unit 1: *Canna indica*, Unit 2: *Typha latifolia*, Unit 3: *Phragmites australis*, Unit 4: *Stenotaphrum secundatum*, Unit 5: *Iris pseudacorus* and Unit 6: control. The coefficients of the linear correlation are represented all with $p < 0.05$.

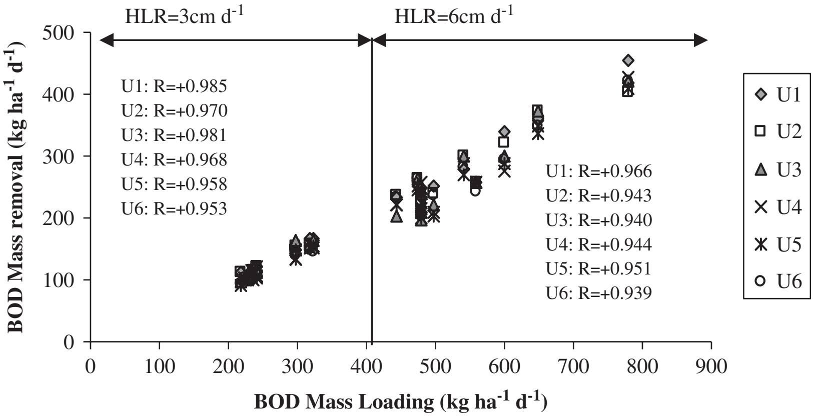

Fig. 4 – BOD_5 loading vs. BOD_5 removal. Unit 1: *Canna indica*, Unit 2: *Typha latifolia*, Unit 3: *Phragmites australis*, Unit 4: *Stenotaphrum secundatum*, Unit 5: *Iris pseudacorus* and Unit 6: control. The coefficients of the linear correlation are represented all with $p < 0.05$.

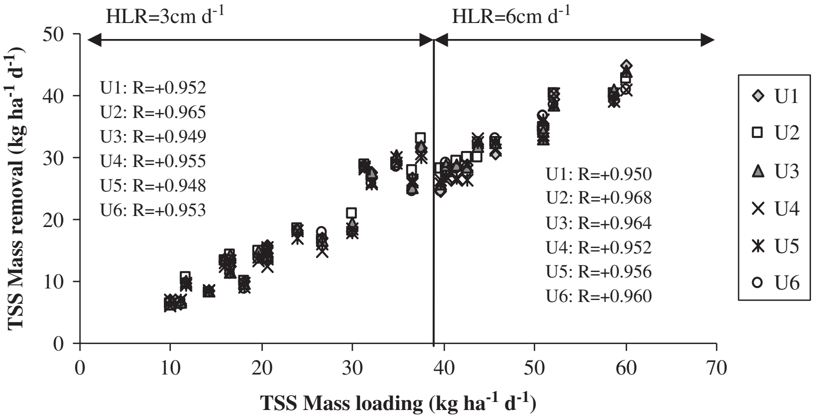

Fig. 5 – TSS loading vs. TSS removal. Unit 1: *Canna indica*, Unit 2: *Typha latifolia*, Unit 3: *Phragmites australis*, Unit 4: *Stenotaphrum secundatum*, Unit 5: *Iris pseudacorus* and Unit 6: control. The coefficients of the linear correlation are represented all with $p < 0.05$.

$6\,\mathrm{cm\,d^{-1}}$. In the leather manufacturing, different operations and processes result in effluents with different compositions (Song et al., 2000), and wastewater treatment systems should withstand such variability. The type of wastewater may influence crucially the survival of plants in a CW. In the present study, the patterns of the tannery wastewater produced were characterized. Prior to the operation of the pilot units, the one year assessment of the chemical characteristic of the wastewater has revealed that the composition was variable, with the COD ranging from 1100 to $3000\,\mathrm{mg\,O_2\,L^{-1}}$ and the BOD_5 from 900 to $1200\,\mathrm{mg\,L^{-1}}$. The inflow to the pilot units varied thereafter in a similar manner.

The choice of different plant species should take into account some factors such as the rooting depth, plant productivity and tolerance to high loads of wastewater (Brix, 1994). The main emergent macrophyte species used in CWs in the Mediterranean countries are *Canna* spp., *Iris* spp., *Cyperus* spp., *Typha* spp., *Phragmites* spp., *Juncus* spp., *Poaceae* spp. and *Paspalum* spp. (Korkusuz, 2005). In this study, plant distribution and propagation varied according to the species planted in the pilot units. *P. australis*, which is considered as highly invasive, and *T. latifolia* were the species that were better established in the units, while the propagation and development of *Iris* spp. and *Canna* spp. was reduced. The former plants supported different hydraulic conditions. The potential of using *P. australis* and *T. latifolia* in CWs is found in literature in several studies dealing with domestic and industrial—tannery, dye-rich wastewater, food processing and high-strength winery–wastewater treatment (Vymazal, 2005; Vymazal and Krőpfelová, 2005; Mbuligwe, 2005; Kucuk et al., 2003; Shepherd et al., 2001; Vrhovšek et al., 1996). Vymazal (2005) also reported the successful use of *Iris* spp. (*pseudacorus* and *sibrica*) in CWs for urban wastewater treatment. In a similar manner to *Iris* spp. and *Canna* spp., the *S. secundatum* used in the present study was also clearly not suitable for tannery wastewater treatment as it was not able to survive. Less frequently used plants have also been tested in CWs for wastewater treatment. Klomjek and Nitisoravut (2005) evaluated the feasibility of using CWs to remove pollutants from saline wastewater using eight emergent plants. They reported that among *Typha angustifolia*, *Digitaria bicornis*, *Cyperus corymbosus*, *Brachiaria mutica*, *Vetiveria zizaniodes*, *Spartina patents*, *Leptochloa fusca* and *Echinodorus cordifolius*, the first two, *T. angustifolia* and *D. bicornis*, were clearly superior for nitrogen uptake and BOD_5 removal. Maine et al. (2006), in a free water surface CW, tested several locally available macrophytes (*Eichhornia crassipes*, *Typha domingensis* and *Pontederia cordata*) in order to treat wastewater from a metallurgic industry, aiming at nutrient and metal removal. The *E. crassipes* became dominant and covered about 80% of the water surface, and *T. domingensis* and *Panicum elephantipes* developed as accompanying species, with 14% and 4% cover, respectively. The tannery wastewater applied to the CWs, in the present study, may have a negative effect upon the growth of wetland species due to the complexity of its composition. There are several possible causes for the inhibition of plant growth, such as the presence of toxic levels of contaminants and deficiency of nutrients for plant development. The pH of the substrate may have also influenced plant growth. In the first weeks of application of the wastewater to the plant units, the inflow was slightly alkaline and the outflow wastewater was strongly alkaline, as the pH of the substrate was strongly alkaline (9.75). With time, the outflow pH stabilized to moderately alkaline. The optimum pH for plant development is considered to be between 3.0 and 8.5 for *T. latifolia*, 3.7 to 8.0 for *P. australis*, 6.0 to 7.5 for *I. pseudacorus* (USEPA, 2000a), 5.5 to 7.5 for *C. indica* (Jett, 2005) and 6.0 to 8.5 for *S. secundatum* (Smith and Valenzuela, 2002). It is possible that the plants could have had some difficulty in developing and propagating considering that the pH of the matrix, particularly at the beginning of operation, was out of their optimum range.

CWs with subsurface horizontal flow usually provide high removal of organic matter (BOD_5 and COD) and suspended solids but lower nutrient removal (Vymazal, 2005; Kadlec et al., 2000). In this study, for the HLR of $3\,\mathrm{cm\,d^{-1}}$, the maximum removal efficiency in terms of COD was 67% for an average inflow of $1966\,\mathrm{mg\,O_2\,L^{-1}}$ ($590\,\mathrm{kg\,ha^{-1}\,d^{-1}}$), 55% for BOD_5 for an average inflow of $875\,\mathrm{mg\,L^{-1}}$ ($263\,\mathrm{kg\,ha^{-1}\,d^{-1}}$) and 92% for TSS for an average inflow of $75\,\mathrm{mg\,L^{-1}}$ ($23\,\mathrm{kg\,ha^{-1}\,d^{-1}}$). Concerning the HLR of $6\,\mathrm{cm\,d^{-1}}$, the maximum removal efficiency in terms of COD was 73% for an average inflow of $2093\,\mathrm{mg\,O_2\,L^{-1}}$ ($1256\,\mathrm{kg\,ha^{-1}\,d^{-1}}$), for BOD_5 was 58% for an average inflow of $898\,\mathrm{mg\,L^{-1}}$ ($539\,\mathrm{kg\,ha^{-1}\,d^{-1}}$) and for TSS was 77% for an average inflow of $79\,\mathrm{mg\,L^{-1}}$($47\,\mathrm{kg\,ha^{-1}\,d^{-1}}$). The COD at the outlet of the CWs was in average 850 (± 191) $\mathrm{mg\,O_2\,L^{-1}}$ despite the different HLRs applied, which may indicate that a part of the COD from the tannery wastewater is not readily biodegradable in the system under the different hydraulic retention times tested. Daniels (2001a) reported that in the first year of operation of a five-day retention time pilot root-zone treatment with reeds, 80–85% of the COD of a tannery effluent was removed, for an inlet COD level ranging from 1000 to $2000\,\mathrm{mg\,L^{-1}}$. In another study reported by the same author, carried out with effluent of the manufacture of finished shoe upper leather from multisource wet blue with a typical COD inflow of $1160\,\mathrm{mg\,L^{-1}}$, reductions of 85%, 82% and 70% COD were obtained, for CWs planted with two subspecies of *Glyceria maxima* and *Phragmites*, respectively, in a five day root–zone system. Both species proved to be extremely robust and survived shock dosing, long periods of drying out, total immersion and cold (Daniels, 1998). However, no detailed information is provided on the hydraulic loadings applied to the systems. In contrast with these high removal efficiencies, Kucuk et al. (2003) reported a maximum COD removal of 30% for a tannery wastewater with an inlet concentration of $300\,\mathrm{mg\,L^{-1}}$, using a hydraulic retention time of 8 days. It is thus clear that the specificity of the different tannery wastewaters affect their biodegradability in a CW.

Nutrients removal occurred to lower extents, but was found to be within the range reported by other authors for subsurface horizontal flow CWs (Mantovi et al., 2003; García et al., 2005), although for different types of wastewater. Wetland plants are known to take up nutrients but this amount may be insignificant compared to the wastewater inflow loading (Brix, 1994, 1997). According to Vymazal (2005) the removal of nutrients (nitrogen and phosphorus) is usually low in CWs and does not exceed 50% when dealing with municipal sewage. Also, according to Tanner (2001), on an annual basis, the net accumulation of nutrients in plant tissues in mature wetlands is reduced. However, factors such as climate, nutrient regime and type of plant can play a significant role in this mechanism (Vymazal, 2005; Tanner, 2001). The phosphorus uptake capacity of macrophytes is reported to be lower than the nitrogen uptake capacity (Brix, 1994). In the present study, due to the low inflow levels of phosphorus, we cannot infer about the capacity of the different plants to remove it from the wastewater. The removal of chromium was also negligible in the present study, but it is possible that differences in chromium removal would have been achieved between the units if the concentration of the metal in the effluent was higher, as different plants have different capacities to remove heavy metals (Maine et al., 2006; Kadlec et al., 2000).

In the present study the ability of treating wastewater at high organic loading—typical of tannery wastewater—was evaluated. The organic loading removal achieved in the pilot units was high, up to 1092 kg COD ha^{-1} d^{-1}, 456 kg BOD_5 ha^{-1} d^{-1} and 45 kg TSS ha^{-1} d^{-1}, increasing with the influent load in a linear correlation. This behavior corresponds to a first-order kinetics, showing that the removal rate was proportional to the inflow amount. In Tanner (2001) monotonic relationships between BOD and COD mass loading and removal rates are also presented, with little difference between planted and unplanted beds. Shepherd et al. (2001) used a CW for winery wastewater treatment with similar average organic loadings, varying between 345 and 1640 kg COD ha^{-1} d^{-1}, and reported removal efficiencies of ca. 99%, while in the present study a maximum removal efficiency of 73% was achieved. For horizontal subsurface beds, some authors do not recommend the application of organic loadings higher than 67 kg BOD_5 ha^{-1} d^{-1} (Metcalf and Eddy Inc., 1991) or out of the range of 67–157 kg BOD ha^{-1} d^{-1}, and for TSS 45–168 kg TSS ha^{-1} d^{-1} (USEPA, 2000b). García et al. (2004) have reported that in order to obtain a BOD_5 removal of 90%, for urban wastewater, an organic surface loading of 200 kg ha^{-1} d^{-1} should not be exceeded (based on the first year of operation system). For the treatment of tannery wastewater, there is a need for appropriate design parameters.

No significant differences were found between the planted and unplanted systems during the 17 months of operation of the systems. Plants can contribute to wastewater treatment processes in a number of ways, such as settlement of suspended solids, providing surface area for microorganisms, increasing uptake of nutrients and trace elements and providing oxygen release (Kadlec et al., 2000; USEPA, 2000a; Brix, 1994, 1997). The beneficial role of plants in CWs is not always evident, and that seems to depend on several parameters, such as the time length of operation, type of vegetation and characteristics of the wastewater. Huang et al. (2000) found no difference in the removal of ammonium and TKN from domestic wastewater as a result of the presence of plants in a CW. Baptista et al. (2003) also reported similar performances in a CW planted with *Phragmites* and in unplanted units, when treating filtered beer with an average BOD_5 concentration of 104 mg L^{-1} (91% and 92% removal, respectively). The similar performance of the planted and unplanted beds obtained in the present study may be explained by the fact that the units had not reached maturity. A complete root–rhizome development for a newly CW may require 3–5 years (Kadlec et al., 2000). For *Phragmites*, three to four growing seasons are usually needed to reach maximum standing crop but in some systems it may take even longer (Vymazal and Kröpfelová, 2005). Vrhovšek et al. (1996) studied the application of CWs with *Carex gracillis* and *P. australis* to the secondary treatment of wastewaters from a food processing plant, showing that depuration improved with bed maturity, and after several years a continuous efficiency of more than 90% was obtained regarding the removal of BOD_5, COD and orthophosphate. Other studies show improvements in performance in planted beds. Mbuligwe (2005) reported for the treatment of a dye-rich wastewater, with an organic loading of 106 kg COD ha^{-1} d^{-1}, cattail and coco yam units outperformed an unplanted unit in terms of organic removal efficiency by 17.5–21.7%, being the organic removals of 86.8, 92.1 and 64.4 kg COD ha^{-1} d^{-1}, respectively. However, there are authors who suggest that the primary benefits of vegetation in these systems may be mainly insulation and esthetics (Mæhlum and Stålnacke, 1999).

The findings from this study show that the application of CWs systems to the treatment of tannery effluents may be an attractive approach for integrated secondary treatment, but longer term operation of such units is important to ascertain for the role and benefits of the plants in such systems.

Conclusions

The aim of this study was to investigate the application of different plant species in CWs receiving wastewater from a tannery production plant. The conclusions are as follows:

1. The plants *T. latifolia* and *P. australis* were the plant species better adapted to tannery wastewater in terms of survival and propagation.
2. CWs with horizontal subsurface flow seem to be a viable alternative for reducing the organic matter content from tannery wastewater, being able to tolerate inflow fluctuations, including interruptions in the feed.
3. Nutrient removal is low when compared with the removal efficiencies achieved for COD and BOD_5.
4. The fact that the plant beds may have not reached maturity may contribute to the similar performance obtained for vegetated and unvegetated units.

Acknowledgments

The authors thank Dias Ruivo, Curtumes e Produtos Industriais, Lda for making possible the establishment of this project. Cristina S.C. Calheiros wishes to thank a research grant from Fundação para a Ciência e Tecnologia (FCT), Portugal (SFRH/BDE/15507/2004). The work was supported by the Project POCI/AMB/60126/2004 financed by Medida V.4-Acção V.4.1 of the Programa Operacional Ciência e Inovação 2010 (Fundação para a Ciência e Tecnologia). The authors thank maxit—Argilas Expandidas, SA that kindly offered Filtralite®. We thank Dr. Paulo Alves from Departamento de Botânica, Faculdade de Ciências, Universidade do Porto, for help in identification of plant species.

References

APHA, 1998. Standard Methods for the Examination of Water and Wastewater, twentieth ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

Baptista, J.D.C., Donnelley, T., Rayne, D., Davenport, R.J., 2003. Microbial mechanisms of carbon removal in subsurface flow wetlands. Water Sci. Technol. 48 (5), 127–134.

Brix, H., 1994. Functions of macrophytes in constructed wetlands. Water Sci. Technol. 29 (4), 71–78.

Brix, H., 1997. Do macrophytes play a role in constructed treatment wetlands? Water Sci. Technol. 35 (5), 11–17.

Daniels, R., 1998. You're now entering the root zone—investigation: the potential of reed beds for treating waste waters from leather manufacture. World Leather 11 (7), 48–50.

Daniels, R., 2001a. Enter the root-zone: green technology for the leather manufacturer, part 1. World Leather 14 (4), 63–67.

Daniels, R., 2001b. Enter the root-zone: green technology for the leather manufacturer, part 3. World Leather 14 (6), 85–88.

García, J., Aguirre, P., Barragán, J., Mujeriego, R., Matamoros, V., Bayona, J.M., 2005. Effect of key design parameters on the efficiency of horizontal subsurface flow constructed wetlands. Ecol. Eng. 25, 405–418.

García, J., Aguirre, P., Mujeriego, R., Huang, Y., Ortiz, L., Bayona, J.M., 2004. Initial contaminant removal performance factors in horizontal flow reed beds used for treating urban wastewater. Water Res. 38, 1669–1678.

Houba, V.J.G., Van Der Lee, J.J., Novozamsky, I., 1995. Soil Analysis Procedures—Other Procedures (Soil and Plant Analysis, part 5B). Department of Soil Science and Plant Nutrition, sixth ed. Wageningen Agricultural University Syllabus, Wageningen, The Netherlands.

Huang, J., Reneau, J.R.R.B., Hagedorn, C., 2000. Nitrogen removal in constructed wetlands employed to treat domestic wastewater. Water Res. 34 (9), 2582–2588.

Jett, J.W., 2005. Plant pH preferences. West Virginia University Extension Service, USA.

Kadlec, R.H., Knight, R.L., Vymazal, J., Brix, H., Cooper, P., Haberl, R., 2000. Constructed wetlands for pollution control—processes, performance, design and operation. IWA Scientific and Technical Report No. 8. IWA Publishing, London, UK.

Klomjek, P., Nitisoravut, S., 2005. Constructed treatment wetland: a study of eight plant species under saline conditions. Chemosphere 58, 585–593.

Korkusuz, E.A., 2005. Manual of Practice on Constructed Wetlands for Wastewater Treatment and Reuse in Mediterranean Countries. Report, MED-REUNET II Support Programme (EC Project No: INCO-CT-2003–502453), AGBAR FOUNDATION, Spain. 〈http://med-reunet.org//〉.

Kucuk, O.S., Sengul, F., Kapdan, I.K., 2003. Removal of ammonia from tannery effluents in a reed bed constructed wetland. Water Sci. Technol. 48 (11–12), 179–186.

Mæhlum, T., Stålnacke, P., 1999. Removal efficiency of three cold-climate constructed wetlands treating domestic wastewater: effects of temperature, seasons, loading rates and input concentrations. Water Sci. Technol. 40 (3), 273–281.

Maine, M.A., Suñe, N., Hadad, H., Sánchez, G., Bonetto, C., 2006. Nutrient and metal removal in a constructed wetland for wastewater treatment from a metallurgic industry. Ecol. Eng. 26, 341–347.

Mantovi, P., Marmiroli, M., Maestri, E., Tagliavini, S., Piccinini, S., Marmiroli, N., 2003. Application of a horizontal subsurface flow constructed wetland on treatment of dairy parlor wastewater. Bioresour. Technol. 88, 85–94.

Mbuligwe, S.E., 2005. Comparative treatment of dye-rich wastewater in engineered wetland systems (EWSs) vegetated with different plants. Water Res. 39, 271–280.

Metcalf and Eddy,Inc., 1991. In: Tchobanoglous, G., Burton, F.L. (revisors), Wastewater Engineering: Treatment, Disposal and Reuse, third edition. McGraw-Hill Publishing, NY, USA.

Oliveira, R.S., Dodd, J.C., Castro, P.M.L., 2001. The mycorrhizal status of *Phragmites australis* in several polluted soils and sediments of an industrialised region of Northern Portugal. Mycorrhiza 10, 241–247.

Shepherd, H.L., Grismer, M.E., Tchobanoglous, G., 2001. Treatment of high-strength winery wastewater using a subsurface-flow constructed wetland. Water Environ. Res. 73 (4), 394–403.

Smith, J., Valenzuela, H., 2002. Sustainable Agriculture Cover Crops: St. Augustinegrass Fact Sheet; College of Tropical Agriculture and Human Resources, University of Hawai'i at Manoa; Hawaii, USA; CTAHR-Aug., SA-CC-4.

Song, Z., Williams, C.J., Edyvean, R.G.J., 2000. Sedimentation of tannery wastewater. Water Res. 34 (7), 2171–2176.

Tanner, C.C., 2001. Plants as ecosystem engineers in subsurface-flow treatment wetlands. Water Sci. Technol. 44 (11–12), 9–17.

USEPA (2000a). A Handbook of Constructed Wetlands, A Guide to Creating Wetlands for: Agricultural Wastewater, Domestic Wastewater, Coal Mine Drainage, Stormwater in the Mid-Atlantic Region.—Volume 1: General Considerations. United Sates Environmental Protection Agency, Washington DC, New York, USA.

USEPA (2000b). Wastewater Technology Fact Sheet—Wetlands: Subsurface Flow. EPA 832-F-00-023. United Sates Environmental Protection Agency, Washington DC, New York, USA.

Vrhovšek, D., Kukanja, V., Bulc, T., 1996. Constructed wetland (CW) for industrial waste water treatment. Water Res. 30 (10), 2287–2292.

Vymazal, J., 2005. Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. Ecol. Eng. 25, 478–490.

Vymazal, J., Krőpfelová, L., 2005. Growth of *Phragmites australis* and *Phalaris arundinacea* in constructed wetlands for wastewater treatment in the Czech Republic. Ecol. Eng. 25, 606–621.