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ORIGINAL PAPER

Treatment of tannery wastewater in a pilot scale hybrid constructed wetland system in Arequipa, Peru

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Abstract

Tannery wastewater is an industrial effluent characterized by high and variable concentrations of complex pollutants. These contaminants pose a high risk to the environment if discharged into a body of water without undergoing proper treatment. This study evaluated the performance of a pilot-scale hybrid constructed wetland system (horizontal subsurface flow + free water surface flow) for tannery wastewater treatment. The pollutant removal efficiency of the hybrid constructed wetland was determined, and the chromium bioaccumulation and growth and survival parameters of the macrophytes *Isolepis cernua* and *Nasturtium aquaticum* were evaluated. The 5-day biological oxygen demand, the chemical oxygen demand, total suspended solids, total dissolved solids and chromium reached maximum levels (98%, 97%, 97%, 33% and 98%, respectively) after treatment in the pilot-scale hybrid constructed wetland. The average concentrations of the 5-day biological oxygen demand, chemical oxygen demand, total suspended solids and chromium were within the discharge limits established by national and international organizations for surface water bodies. The macrophytes had low levels of chromium bioconcentration and translocation, with the growth and survival, especially of *Isolepis cernua*, revealing a high capacity to adapt to the variability and possible toxic effects of tannery wastewater. In general, the pilot-scale hybrid constructed wetland proved to be a feasible alternative for the treatment of tannery wastewater in an important industrial zone in Peru.

Keywords Constructed wetlands · Hybrid system · *Isolepis cernua* · *Nasturtium aquaticum* · Phytoremediation · Tannery wastewater

Introduction

Wastewater treatment presents a great challenge, which implies dealing with metal ions and industrial compounds Tannery wastewater is an industrial effluent characterized by high and variable concentrations of complex pollutants, such as organic matter, nitrogen, suspended solids and chromium (Hashem et al. 2019; Da Silva et al. 2020). Several physicochemical methods have been developed to remove heavy metals from wastewaters. The processes most commonly used for the treatment of wastewater from tanneries include chemical coagulation, electrocoagulation, activated sludge and chemical precipitation (Batool and Saleh 2020). However, some of these physicochemical processes may be very expensive and these methods usually produce large quantities of toxic chemical sludge, whose disposal presents a major problem (Sultana et al. 2015). In Peru, tannery activities are carried out mainly in the cities of Trujillo, Arequipa and Lima, with Arequipa (Río Seco Industrial Park) representing 53% of the total companies of the leather industry in the country (Assessment and Environmental Control Agency 2017). In Arequipa, tannery effluents are subjected to a conventional physicochemical pre-treatment before being discharged into a receiving water body. However, in addition to having high operating costs, this procedure is insufficient and entails an inherent risk of environmental pollution (Assessment and Environmental Control Agency 2017).

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Constructed wetlands (CWs) are wastewater treatment systems that take advantage of the processes that occur in natural wetlands to improve treatment capacity, and are a cost-effective alternative for conventional wastewater treatment plants. The CWs are characterized by various economic and environmental benefits, such as low energy requirements, non-use of chemicals, easy maintenance and operation, and low economic cost (Stefanakis 2018). This approach is usually based on phytostabilization through the use of aquatic plants, especially emerging macrophytes that degrade and/or store contaminants in tissues and/or immobilize them in substrates (de Cabo et al. 2015). The removal of organic matter and pollutants has been shown to be efficient and consistent across many types of treatment wetlands, and the combination of the CWs in hybrid systems provides greater advantages in the removal of various pollutants (Zhang et al. 2015; Saeed and Khan 2019; Vymazal 2019).

The treatment of tannery wastewater involves numerous technical and operational challenges, in particular due to the intermittent and highly variable nature of chemical inputs, as well as to the high levels of biochemical oxygen demand (BOD) and chemical oxygen demand (COD). Other difficulties can include the high concentrations of total suspended solids (TSS) and chromium, and the intense odor and color of the water (Ramírez et al. 2019). Horizontal subsurface flow wetlands have shown the potential to significantly decrease these key parameters, since these systems improve the overall biological performance (Kassaye et al. 2020). In addition, the flow loses contact with atmospheric air, thereby reducing odors and the proliferation of vectors. In free water surface wetlands, rooted macrophytes grow and develop in the form of a mat with floating leaves on the water surface, which in turn generate new roots suspended in the water column. These new roots provide surface sorption and fixation of periphyton, as well as microbial propagation (Ashraf et al. 2018). In this way, they increase the plant's capacity to accumulate pollutants. These findings have generated interest in the evaluation of this type of wetlands in combination with other types of CWs to improve the treatment of industrial effluents (Saeed and Khan 2019).

Macrophytes contribute significantly to the primary production in CWs, and are a fundamental element of the trophic structure and in the recirculation of nutrients. During these processes, aquatic plants, sediment surfaces with adsorption capacity, and the metabolism of various microorganisms, can capture large amounts of pollutants and metals from these systems (Zhang et al. 2020). Moreover, wetland plants can rapidly adapt to several existing operational conditions, and are able survive the toxic effects and variability of wastewater (Papaevangelou et al. 2017). In Arequipa, southern Peru, the climate is dry and arid with intense solar radiation; hence, it is important to select aquatic plants adapted to the climatic conditions typical of the study area to be used in CWs. The emerging macrophyte species *Isolepis cernua*, commonly found in waterlogged soils with a high salinity in southern Peru, grows in the Yarabamba River, and *Nasturtium aquaticum* grows in the Tiabaya River. Both of these have high levels of organic matter and oxygen reactive substances (Montesinos-Tubée et al. 2019). Thus, these species were considered to be optimal for exploring their use in CWs as an option for the treatment of industrial tannery effluents. Although a few studies on the use of these species in CWs for wastewater treatment have already been published (Yin et al. 2014; Rahman et al. 2016), none has focused on the treatment of tannery wastewaters.

The main objective of this study was to evaluate the performance of a pilot-scale hybrid constructed wetland (HCW) system (horizontal subsurface flow + free water surface flow) in the treatment of tannery wastewater. The specific objectives were to determine the pollutant removal efficiency, and to evaluate the growth and survival parameters of aquatic plants, as well as the Cr bioaccumulation in the HCW system.

Materials and methods

Pre-treated tannery wastewater

Tannery wastewater, previously subjected to a conventional primary physicochemical treatment, was collected from a local tannery industry in Arequipa, Peru, and stored in tanks. Then, this was added as an influent to the HCWs. The physicochemical characteristics of the influent for each pilot-scale wetland unit are described in Table 1.

Emerging plants and growth conditions

The native plants *Isolepis cernua* (Vahl) Roem. & Schult. and *Nasturtium aquaticum* Wahlenb were collected from the Yarabamba River (16°29′9"S; 71°31′41"W) and the Tiabaya

Table 1 Physicochemical characteristics of the influent for each pilot-scale hybrid constructed wetland unit

Parameter	Concentration (means ± SD)
Temperature (°C)	17.6 ± 0.5
pH	8.7 ± 0.2
EC (μS·cm^{-1})	4705 ± 165
BOD_5 (mg·L^{-1})	649.3 ± 39.3
COD (mg·L^{-1})	2412 ± 1345
TSS (mg·L^{-1})	272.5 ± 117.5
TDS (mg·L^{-1})	2355 ± 85
Cr (mg·L^{-1})	8.11 ± 4.86

River (16°27′30"S; 71°33′47"W), respectively, in the south of Arequipa city. Both these areas have pseudo-pristine characteristics. The collected plants were washed in situ, and then in the laboratory they were thoroughly washed under running water to remove any remaining material adhered to the surface. For experimental studies, both plants species, of similar biomass, were maintained in a previously filtered nutrient solution composed of cattle manure and distilled water at 5% p/v. The water conditions of the wetlands were: temperate between 18 and 22 °C, pH 7–8 and with sunlight exposure. Plants were maintained under these conditions for the 45 days for acclimatization and also during the experimental time of exposure to pre-treated tannery wastewater.

Experimental design

Design of constructed wetlands

The HCW system consisted of two treatment stages: a first stage of horizontal subsurface flow (HSF) with *I. cernua* plants, and a second stage of free water surface flow (FWSF) with *N. aquaticum* plants (Fig. 1). The interior of each treatment unit was lined with a 0.75 mm impermeable geomembrane to avoid leaching. Each unit was 1.25 m long, 0.80 m wide and 0.15 m deep. In order to avoid clogging, gravel (30 to 70 mm in diameter, 8.5 uniformity coefficient, and 10.8% porosity) was used as the filter material in the inlet and outlet of both types of wetlands, and sand (2 to 6 mm in diameter, 3.2 uniformity coefficient, and 35.0% porosity) was used in the center of the wetland. The gravel and fine sand substrates were collected from the plant collection sites. The flow of each system was regulated with individual valves and PVC water distribution pipes with holes on their surface. This ensured the continuous, regular and constant flow of the tannery wastewater through the filtration bed. A vertical piping system was available in the central area to control the wetland water height and to maintain adequate oxygenation of the system. The influent entered the unit through a PVC pipe (D_{10}: 18 mm) and was distributed perpendicular to the inlet via aligned holes to produce a laminar flow in both types of wetlands.

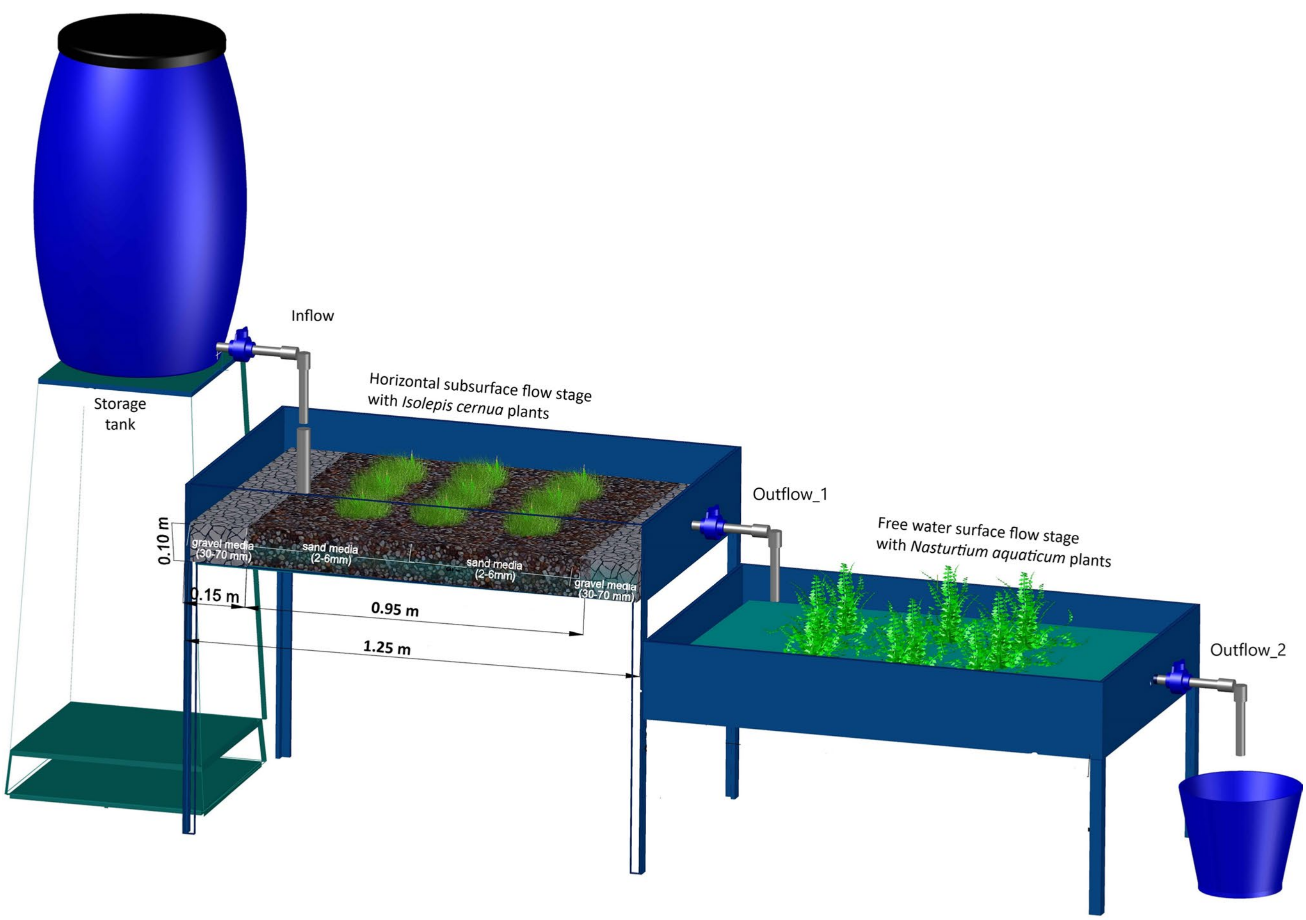

Fig. 1 Diagram of the pilot-scale hybrid constructed wetland (HCW) system

Operating conditions of wetlands

All CW units were irrigated simultaneously with a constant flow of pre-treated tannery wastewater (Table 1). Taking into account the average water temperature of the Yarabamba and Tiabaya rivers in the coldest month (16 °C), and considering the optimal BOD_5 levels (30 mg O_2 L^{-1}) for body discharge of surface water, according to Peruvian legislation—Supreme Decree No. 003-2002-Ministry of Production—(Ministry of Environment, Peru 2002), an average constant flow rate of 0.016 m^3 day^{-1} was determined. Therefore, for the first stage of horizontal subsurface flow, the hydraulic loading rate (HLR) was 14 mm d^{-1} and the hydraulic retention time (HRT) was 4 days, while for the second stage of free water flow, the HLR was 18 mm d^{-1} and the HRT was 2 days. To evaluate the efficiency of the pilot-scale HCW system in the remediation of tannery wastewater, the following three treatments with increasing concentrations of tannery wastewater were used: *T*-50% (50% of pre-treated tannery wastewater + 50% tap water); *T*-75% (75% pre-treated tannery wastewater + 25% tap water); and *T*-100% (only pretreated tannery wastewater). The *T*-50%, *T*-75% and *T*-100% treatments with aquatic plants were compared with a control treatment that consisted of undiluted concentrations of the pre-treated tannery wastewater and with no plants involved in the removal processes (*T*-Control). Each treatment was performed in duplicate. The experiment was conducted for 14 days, and samples were collected from each of the treatments at the beginning and end of the experimental period.

Sample collection and laboratory analysis

Water monitoring and analysis

Water samples were collected from the influent (Inflow) of the HCW system, and from the effluents of the HSF stage (Outflow_1) and the FWSF stage (Outflow_2). The parameters temperature, pH and electrical conductivity (EC) were analyzed in situ using a field probe, with the aim of observing the continuous behavior of the HCW system. Total chromium levels (Cr), the 5-day biological oxygen demand test (BOD_5), chemical oxygen demand (COD), total suspended solids (TSS) and total dissolved solids (TDS) were analyzed immediately after sampling, according to standard methods (APHA et al. 2012). For Cr, immediately after collection, the water samples were acidified with HNO_3 (63%) to pH ≤ 2 and filtered with 0.45 µm filter paper. Finally, the Cr content was analyzed using an Agilent 7500cx Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Agilent Technologies, California). A blank was prepared using the same protocol without wastewater (only reagents). A spiked sample was also prepared to verify the Cr recovery percentages by spiking a 5-mL water sample with variable amounts of individual standard solutions. The recovery of the spiked sample was 90–110% (data not shown).

Plant monitoring and analysis

The *I. cernua* and *N. aquaticum* plants exposed in the experimental units were removed after 14 days of treatment to measure the accumulation of total Cr in the tissues (Fig. 2). The harvested plants were thoroughly washed with tap water to remove adhered sediments, rinsed with deionized water and dried with absorbent paper. The shoot (stem and leaf) and root parts were separated manually. Individual plant height, maximum root length and leaf length of each plant were recorded over the treatment period to assess species growth, according to Arreghini et al. (2017). The corresponding fractions were oven-dried at 105° C for 1 h. Samples of the dried plant material (5 g) were carbonized in an oven at 450 °C for 4 h, and the ashes were digested using a mixture of concentrated HNO_3 (63%) and HCl (20%) (3:1) (v/v) (Bertrand et al. 2016). For metal determination, solid residues were removed by centrifugation, and the samples were diluted with ultrapure water to a final volume of 25 mL. Finally, the Cr content was analyzed using a FAAS (air-acetylene flame atomic absorption spectrometer, Perkin Elmer 3110, USA). Digestion blanks were prepared and analyzed following the same procedure. The accumulation of Cr in tissues was expressed as mg·kg^{-1} dry weight (DW).

The relative growth rate (RGR) was calculated as: RGR = $(\mathrm{Ln}M_2 - \mathrm{Ln}M_1) / (t_2 - t_1)$, where M_1 and M_2 are dry weight mass at t_1 (initial time) and t_2 (final time), respectively, and expressed as mg kg^{-1} d^{-1}. The survival percentage (% SP) was calculated as: % SP = $I_s/I_t \times 100$, where I_s and I_t are the number of surviving individuals and the number of total individuals exposed to different treatments with pre-treated tannery wastewater, respectively. The removal efficiency percentage was determined as: Removal efficiency (%) = $(1 - C_o/C_i) \times 100$, where C_i and C_o are the concentrations in the inflow and outflow in mg L^{-1}, respectively.

Statistical analysis

Data were statistically analyzed using the software InfoStat version 1.1. Treatments were compared using the analysis of variance (ANOVA) to determine significant differences (at a significance level of $p < 0.01$) and with a posteriori test of DGC ($p < 0.05$). The assumptions of ANOVA (normality, independence and homoscedasticity) were previously verified analytically and graphically for each of the measured variables. The variables that were not normally distributed were fourth-root transformed before performing statistical analysis.

Fig. 2 Plants of *I. cernua* and *N. aquaticum* extracted from experimental units after 14 days of exposure to tannery wastewater. **a** *I. cernua* after exposure to the *T*-75% treatment. **b** *I. cernua* after exposure to the *T*-100% treatment. **c** *N. aquaticum* after exposure to the *T*-75% treatment. **d** *N. aquaticum* after exposure to the *T*-100% treatment

Results and discussion

HCW system removal efficiency

Table 2 presents the results of the physicochemical characteristics of the influent and the effluents of the HCW in each phase of the tannery wastewater treatment, as well as the respective removal efficiency (%). The average values of temperature, pH and EC measured in water in the HCW were 20.8 ± 0.5 °C, 8.0 ± 0.1 and 3529 ± 204 $\mu S{\cdot}cm^{-1}$, respectively. The variation in pH (from 7.2 to 8.9) was within the range established for discharges into sewers and surface water bodies by Peruvian legislation. The EC varied between 1270 $\mu S{\cdot}cm^{-1}$ and 5401 $\mu S{\cdot}cm^{-1}$ over the study period, and these values were below those of the typical levels of production processes in the tannery industry (Hashem et al. 2019; Da Silva et al. 2020; Zapana-Huarache et al. 2020). Electrical conductivity decreases mainly by absorption of the substrate and roots, and by ion uptake of plants (Bakhshoodeh et al. 2017). In this study, after treatment, the HCW units with the presence of macrophytes (*T*-50%, *T*-75% and *T*-100%) showed lower EC values than the control unit without plants (*T*-Control) (Table 2), which could have been due to ion absorption by the plants.

The concentrations of BOD_5 and COD in the HCW system varied from 2.8 to 688.6 $mg{\cdot}O_2{\cdot}L^{-1}$, and from 22.2 to

Table 2 Physical and chemical characteristics of inflow and outflow from a hybrid constructed wetland system in each treatment phase and its respective removal efficiency (%)

Treatment	Sampling point	Temperature (°C)	pH	EC (µS·cm^{-1})	BOD_5 (mg·O_2·L^{-1})	COD (mg·O_2·L^{-1})	TSS (mg·L^{-1})	TDS (mg·L^{-1})	Cr (mg·L^{-1})
T-50%	Inflow	17.6 ± 0.5	8.7 ± 0.2	2443 ± 8	321.6 ± 22.7	1281.2 ± 747.9	134.3 ± 56.8	1165 ± 30	4.30 ± 2.71
	Outflow_1	16.6 ± 0.3	7.6 ± 0.1	1905 ± 295	9.5 ± 4.6	32.7 ± 10.5	4.3 ± 0.6	955 ± 145	0.01 ± 0.00
	Removal efficiency %				97.04	97.45	96.78	18.05	99.74
	Outflow_2	22.4 ± 1.1	8.2 ± 0.2	1570 ± 300	6.2 ± 2.1	38.7 ± 4.5	4.6 ± 0.2	780 ± 150	0.09 ± 0.00
	Removal efficiency %				98.06	96.98	96.60	33.06	98.01
T-75%	Inflow	17.6 ± 0.5	8.7 ± 0.2	3483 ± 169	480.1 ± 22.6	1776.8 ± 966.2	204.4 ± 88.1	1756 ± 99	5.97 ± 3.50
	Outflow_1	19.7 ± 1.6	7.7 ± 0.1	3386 ± 163	38.4 ± 8.8	89.9 ± 19.6	13.5 ± 5.3	1694 ± 84	0.02 ± 0.01
	Removal efficiency %				92.01	94.94	93.38	3.52	99.61
	Outflow_2	22.1 ± 0.4	8.4 ± 0.0	2798 ± 462	6.6 ± 1.1	51.0 ± 6.5	5.0 ± 1.1	1532 ± 282	0.06 ± 0.01
	Removal efficiency %				98.63	97.13	97.55	12.74	98.93
T-100%	Inflow	17.6 ± 0.5	8.7 ± 0.2	4705 ± 165	649.3 ± 39.3	2412.1 ± 1345.5	272.5 ± 117.5	2355 ± 85	8.11 ± 4.86
	Outflow_1	19.3 ± 1.5	7.6 ± 0.1	4382 ± 373	174.5 ± 46.4	258.5 ± 55.8	26.6 ± 10.4	2186 ± 187	0.03 ± 0.01
	Removal efficiency %				73.12	89.28	90.22	7.18	99.57
	Outflow_2	23.0 ± 0.6	8.3 ± 0.2	3368 ± 376	11.7 ± 3.5	67.5 ± 6.6	6.2 ± 1.7	1700 ± 184	0.05 ± 0.01
	Removal efficiency %				98.20	97.20	97.72	27.81	99.36
T-Control	Inflow	17.6 ± 0.5	8.7 ± 0.2	4705 ± 165	649.3 ± 39.3	2412.1 ± 1345.5	272.5 ± 117.5	2355 ± 85	8.11 ± 4.86
	Outflow_1	20.4 ± 2.3	7.7 ± 0.2	4383 ± 521	120.1 ± 30.6	208.7 ± 42.0	7.3 ± 3.2	2200 ± 256	0.04 ± 0.01
	Removal efficiency %				81.50	91.35	97.31	6.58	99.57
	Outflow_2	22.9 ± 0.4	8.1 ± 0.0	4108 ± 724	8.9 ± 3.1	55.9 ± 11.9	7.0 ± 1.4	2083 ± 381	0.06 ± 0.01
	Removal efficiency %				98.62	97.68	97.45	11.57	99.22
Peruvian National Standard (MA, 2002) for discharge to:	Sewer network		6.0–9.0		500	1500	500		2
	Surface water bodies		5.0–8.5		30	50	30		0.5

3757.6 mg·O_2 L^{-1}, respectively, over the study period. The treated effluents generated wastewater with a concentration of $BOD_5 < 30$ mg·O_2·L^{-1}, which is the maximum value allowed for discharges to surface water bodies by Peruvian legislation. Regarding COD, the treated effluents generated wastewater with a concentration of COD < 1500 mg·O_2·L^{-1}, which is the maximum value allowed for sewer network discharges by Peruvian legislation. In surface water bodies (COD < 50 mg·O_2·L^{-1}), lower values were only observed in the *T*-50% and *T*-75% treatments. These results are comparable to those recorded by Zapana-Huarache et al. (2020), who reported COD values within the maximum limits permitted by Peruvian legislation for discharges to surface water bodies, but not within those for a sewer network. In addition, the aquatic plants of this study revealed a tolerance to average COD levels of 2412.1 mg·O_2·L^{-1}. Other authors have reported macrophytes that were tolerant to effluents, with COD concentrations similar to or higher than the ones recorded in this study (Saeed et al. 2012; Alemu and Leta 2015).

The highest COD and BOD_5 removal percentages in the outflow_1 of the HFS wetland were recorded at *T*-50%, and the lowest values were observed at *T*-100% and *T*-Control (Table 2). The BOD_5 removal percentage in outflow_2 of the HCW was greater than 98% in all treatments (Table 2). This result was higher than the previously reported values of BOD_5 removal of 77% by Alemu and Leta (2015) and of 87.1% by Bakhshoodeh et al. (2017), which were both recorded for the treatment of tannery wastewater through horizontal subsurface flow CWs. In addition, the COD removal percentage in outflow_2 of the HCW exceeded 97% in all treatments. This result was higher than the recorded average levels of COD removal of 62.2% by Ramírez et al. (2019) in a hybrid wetland and of 74.5% by Bakhshoodeh et al. (2017) in a horizontal subsurface flow CW.

Organic mass removal rates were 5.04, 7.57, 10.20 and 10.24 g $m^{-2}d^{-1}$ for BOD_5 and 19.88, 27.61, 37.51 and 37.70 g $m^{-2}d^{-1}$ for COD for T-50%, T-75%, T-100% and T-Control, respectively. These results are comparable to the average value of BOD_5 (13.4 g $m^{-2}d^{-1}$) recorded by Dotro et al. (2011) in a horizontal subsuperficial flow CW including the *Typha latifolia* species. However, they are lower than the average values of BOD_5 (36.3 g $m^{-2}d^{-1}$) and COD (61.5 g $m^{-2}d^{-1}$) reported by Calheiros et al. (2012) in the treatment of tannery wastewater through horizontal subsurface flow CWs. In addition, they and also lower than the mean BOD_5 (249 g $m^{-2}d^{-1}$) and COD (678 g $m^{-2}d^{-1}$) recorded by Saeed et al. (2012) in tannery wastewater in hybrid wetlands with the *P. autralis* species.

The biodegradability of tannery wastewater, which is limited due to the presence of recalcitrant compounds (Carballeira et al. 2016), can be estimated by the BOD_5/COD ratio (Siwiec et al. 2018) of effluent quality parameters. In the present study, the BOD_5/COD ratio was found to be within the range 0.25 to 0.27 in the inflow of HCW units, implying a low biological decomposition of the organic matter in the system (Saeed et al. 2012; Madera-Parra et al. 2015). However, the BOD_5/COD ratio (0.30 to 0.68) in outflow_1 revealed an increase in biodegradability at the HSF stage, at a level similar to values reported for municipal and domestic wastewater (Siwiec et al. 2018).

As well as absorbing inorganic compounds and organic pollutants in CWs, plants also release oxygen and carbon compounds into the rhizosphere, which provides a habitat for many decomposing microbes, and increases the microbial diversity and activity (Liu et al. 2015). The fact that the BOD_5 and COD levels in outflow_1 were lower than the inflow values, and that the levels in the outflow_2 were approximately those of the minimum discharge standards (Table 2), could have been due to the presence of a variety of microorganisms. The different micro-sites, such as the anaerobic areas of the interstices of the substrates and the aerobic areas around plant roots and rhizomes, generate favorable environments for the degradation of organic compounds (Gomes et al. 2018).

Total solids in wastewater comprise both TSS and TDS, which encompass particles suspended and dissolved in water, such as silt and clay, decaying organic matter, industrial waste, and different organic and inorganic ions. The concentrations of TSS and TDS in the HCW ranged from 2.5 to 390.1 mg·L^{-1} and from 630 to 2780 mg·L^{-1}, respectively. The treated effluents generated wastewater with a concentration below the standard established by Peruvian legislation of a maximum of TSS < 30 mg·L^{-1} for discharges to surface water bodies. For the HSF stage, the highest removal percentages of TSS in outflow_1 were found for T-50% and T-Control, with the lowest values recorded at *T*-75% and *T*-100% (Table 2). On the other hand, during exposure in the HCW systems after the FWSF stage, TSS removal in outflow_2 was above 97% in all treatments. This value was similar to that reported by Ashraf et al. (2018) for the treatment of tannery effluents in vegetated CWs, with and without endophytes. Bakhshoodeh et al. (2017) observed that suspended solid removal occurs mainly through a filtration mechanism by the roots of the plants and the substrate in CWs. However, in our study, TSS removal was similar in both the vegetated and non-vegetated treatments. For TDS, HCW revealed a low removal efficiency of 11 to 33%. Similarly, Calheiros et al. (2012) reported values of 2 to 30% of TDS removal in CW treatment units. However, the non-removed TDS fraction might not have had any negative effects on the performance of the treatment systems of this study or on the growth of the macrophytes. Stefanakis (2018) observed optimal levels of operation of their wastewater treatment systems, with TDS levels above 10,000 mg·L^{-1} and without implications for plant growth.

In the present investigation, the Cr concentration in treated wastewater in the HCW systems ranged from 0.01 to 12.98 mg·L^{-1} In each unit, this reached values below the limits allowed for discharges to surface water bodies (Cr < 0.5 mg·L^{-1}) by Peruvian legislation, and moreover, below those imposed by more stringent international standards (Cr < 0.042 mg L^{-1}) (US EPA 2003). The decrease in Cr in the CWs may have been a consequence of its chemical reduction. This can occur under anoxic conditions and in the presence of an electron donor, such as bivalent iron and/or sulfides, as well as a few other reducing compounds of tannery wastewater (Ton et al. 2015; Alemu et al. 2019; Wu et al. 2019). This metal can be stored in the substrate, mainly through precipitation and/or complexation reactions with organic matter (Madera-Parra et al. 2015). Here, the percentages of Cr removal in the effluents outflow_1 and outflow_2 of the treatment units were 98% (Table 2). These removal rates were similar to those observed by Alemu and Leta (2015) and Ramírez et al. (2019) in tannery wastewater treated in pilot-scale horizontal subsurface flow CWs. However, they were greater than the removal rates observed by Zapana-Huarache et al. (2020) in tannery wastewater treated in bioreactors. The Cr removal rates of our study were 67.7, 94.6, 129.0, and 128.8 mg $m^{-2}d^{-1}$ in *T*-50%, *T*-75%, *T*-100% and *T*-Control, respectively. These were comparable to the mean Cr values of 135 mg $m^{-2}d^{-1}$ recorded by Dotro et al. (2011) in a horizontal subsurface flow CW with *Typha latifolia*. A similar Cr removal rate was observed in undiluted tannery wastewater in the vegetated and un-vegetated treatments of this study. Therefore, the substrate (i.e., porous media and biofilm) could be an important removal mechanism of Cr (Papaevangelou et al. 2017).

Measurements in *I. cernua* and *N. aquaticum*

The choice of plants plays an important role in CWs, since they must survive the possible toxic effects of tannery wastewater and tolerate effluent variability. Table 3 shows the relative growth rate (RGR) measured in the macrophytes *I. cernua* and *N. aquaticum* used in the pilot-scale HCW systems at *T*-50%, *T*-75% and *T*-100% over 14 days, compared with that of these species at the collection sites of the Yarabamba and Tiabaya rivers. The RGR values for *I. cernua* at *T*-50% and *T*-75% were similar to those observed in this species at the Yarabamba river collection site, but significantly higher than those recorded at *T*-100%. For *N. aquaticum*, the RGR values at T-50% were similar to those observed in this plant at the Tiabaya River sampling site, but significantly higher than those recorded at *T*-75% and *T*-100%. The slight increase in RGR at *T*-100% of *I. cernua* and at *T*-75% and *T*-100% of *N. aquaticum* could have been due to a delay in the growth of both species in the culture media with Cr concentrations in the tannery effluents. Concerning this, in phytoremediation processes of tannery wastewater, Cr has been shown to induce plant growth changes (Alemu et al. 2019) and a delay of the growth in *N. officinale* (Klimek-Szczykutowicz et al. 2019) and in *Typha domingensis* (Mufarrege et al. 2018).

The accumulation of heavy metals in aquatic plants causes significant physiological and biochemical responses in the growth of different plant organs (Sultana et al. 2015). For example, the accumulation of Cr in macrophyte tissues can affect the photosynthetic system and nutrient uptake, causing alterations in the morphological parameters, which in turn affect plant growth (Arán et al. 2017). Ton et al. (2015) reported signs of metal toxicity, such as leaf chlorosis, shoot wilting and short root with tip necrosis, in plants exposed to Cr. In our study, signs of toxicity in both plant species were more severe in the undiluted tannery wastewater than in diluted treatments (Fig. 2).

Table 4 shows the survival percentages of the macrophytes *I. cernua* and *N. aquaticum* exposed in the pilot-scale HCW system, at *T*-50%, *T*-75% and *T*-100% for 14 days, compared with those of these species at the collection sites of the Yarabamba and Tiabaya rivers. The survival percentage values for *I. cernua* at *T*-50% and *T*-75% were 100% for plants of this species at the Yarabamba river collection site. For the *T*-100% treatment, however, a decrease in the survival of the species (93.0%) was observed. The survival of *N. aquaticum* individuals decreased with increasing concentration of tannery wastewater. Moreover, the lowest survival was recorded for *T*-100% (*T*-50% > *T*-75% > *T*-100%), with survival found to be lower for all treatments than in its natural habitat for 14 days. The macrophyte *I. cernua* showed an ability to grow and survive, similar to that reported for other species, such as *Phragmites sp.* (Ramírez et al. 2019) and *Cyperus papyrus* and *Typha domingensis* (Alemu and Leta 2015), under exposure to complex effluents such as tannery wastewaters in CWs systems.

Table 4 Survival percentage (%) of aquatic plants ($n=8$) in the different wetland units and sampling sites

Aquatic plant	Sampling site	*T*-50%	*T*-75%	*T*-100%
I. cernua	100.0 (Yarabamba River-16°29′9"S; 71°31′41"W)	100.0	100.0	93.0
N. aquaticum	100.0 (Tiabaya River-16°27′30"S; 71°33′47"W)	87.5	60.0	52.5

Table 3 Relative growth rate (RGR) (mg·$kg^{-1}d^{-1}$) of aquatic plants ($n=8$) in the different wetland units and sampling sites

Aquatic plant	Sampling site	*T*-50%	*T*-75%	*T*-100%
I. cernua	0.056 (Yarabamba River-16°29′9"S; 71°31′41"W)	0.063 ± 0.015 a	0.052 ± 0.021 a	0.024 ± 0.008 b
N. aquaticum	0.038 (Tiabaya River-16°27′30"S; 71°33′47"W)	0.041 ± 0.012 a	0.018 ± 0.011 b	0.013 ± 0.007 b

Significant differences ($p<0.05$) according to the DCG test are shown in lowercase letters

To evaluate the capacity of accumulation and transfer of Cr in the tissues of *I. cernua* and *N. aquaticum*, the Cr accumulated by these species in their aquatic ecosystems was compared with that amassed in the treatments with greater concentrations of tannery wastewater (*T*-75% and *T*-100%). These experiments were performed in triplicate. The variations in Cr content in the root and shoots of the aquatic plants during the experimental period are shown in Fig. 3. For all treatments, the Cr concentration in the root of *I. cernua* was always higher than in the shoot, whereas in *N. aquaticum*, the Cr concentration in the root was similar to that recorded in the shoot. This result suggests a differential capacity of Cr uptake from the root of *I. cernua* in waterlogged substrates, such as those of the HSF stage, and also from leaves and floating roots of *N. aquaticum*, when they were in contact with water in the FWSF stage. These results found for Cr accumulation from the water column were in agreement with previous studies (Amin et al. 2018; Gomes et al. 2019). The differences in average Cr accumulation between *I. cernua* (24.13 ± 2.57 mg kg^{-1}) and *N. aquaticum* (20.34 ± 2.37 mg kg^{-1}) during exposure were not significant ($p = 0.5421$), reflecting a similar ability of both species to capture Cr. The efficiency of Cr phytoremediation depends on many factors, including the physicochemical properties of substrates and water, Cr bioavailability, and the capture and storage capacity of plants (Sinha et al. 2018). In the present investigation, the high levels of TDS recorded in the effluents after treatment in the CWs (Table 2) might have restricted the uptake and accumulation of Cr in *I. cernua* and *N. aquaticum*.

Chromium accumulation in the root and shoot of *I. cernua* and *N. aquaticum* in the *T*-75% and *T*-100% treatments revealed a significant increase, compared to the amount accumulated in both species in their natural environments of the Yarabamba and Tiabaya rivers, respectively. This feature has been previously reported for emergent plants employed in CWs, such as *Gynerium sagittatum*, *Colocasia esculenta* and *Heliconia psittacorum* (Madera-Parra et al. 2015) and *Leersia hexandra* (Liu et al. 2015).

The bioconcentration (BCF) and translocation (TF) factors of Cr in *I. cernua* and *N. aquaticum* from HCW systems are shown in Table 5. The BCF is the most common index used to estimate the potential of a plant for phytoremediation, and is calculated as the ratio between the total concentration of the metal in the plant and its concentration in the culture medium (Arán et al. 2017). Root BCF was higher than that observed in the shoot of both aquatic plant species (Table 5). In *I. cernu*a, the BCF was similar for the *T*-75% and *T*-100% treatments, whereas in *N. aquaticum*, the BCF was higher in *T*-75% than in *T*-100%. However, these aquatic plants were not able to bioconcentrate the Cr of tannery wastewater at the levels

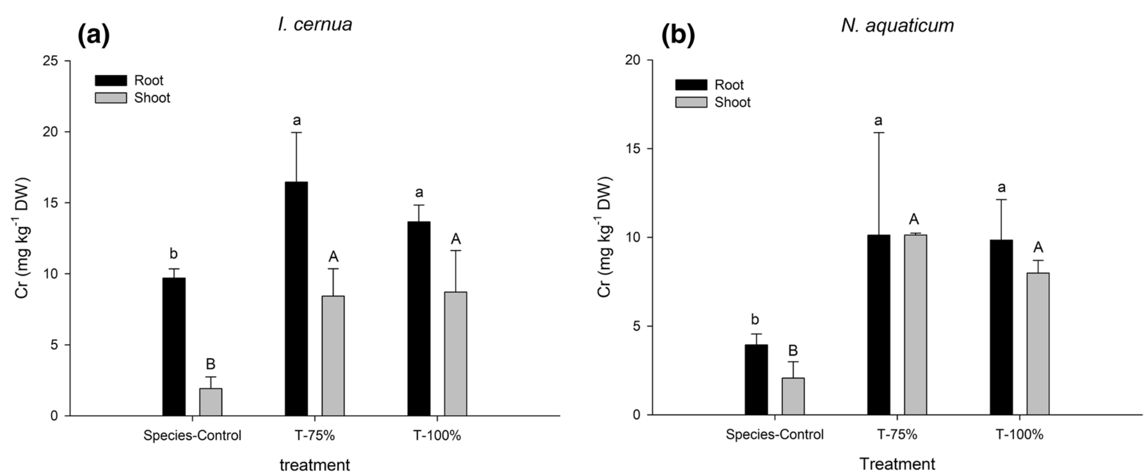

Fig. 3 Accumulation of Cr in root and shoot (stem + leaf) of *I. cernua* **a** and *N. aquaticum* (b) at different concentrations of tannery wastewater and in their aquatic ecosystem (Species-Control). Significant differences ($p < 0.05$) according to the DCG test are shown by lowercase and uppercase letters for root and shoot, respectively

Table 5 Bioconcentration factor (BCF) and translocation factor (TF) of Cr in *I. cernua* and *N. aquaticum* from a hybrid constructed wetland system

Treatment (Wetland units)	*I. cernua*			*N. aquaticum*		
	BCF_{root}	BCF_{shoot}	TF	BCF_{root}	BCF_{shoot}	TF
T-75%	2.34 ± 0.14	1.62 ± 0.36	0.69 ± 0.15	2.15 ± 0.55	1.40 ± 0.18	0.50 ± 0.14
T-100%	2.02 ± 0.44	1.01 ± 0.28	0.65 ± 0.09	0.83 ± 0.39	0.78 ± 0.36	0.94 ± 0.42

shoot (stem + leaf)

that have been usually reported for hyperaccumulators (Romero-Hernández et al. 2017). The TF is an indicator of the ability of the plant to transfer metal from below-ground to above-ground biomass, with levels greater than 1 indicating a high translocation capability (Papaevangelou et al. 2017). However, in *I. cernua* and *N. aquaticum*, the TF value was less than 1, indicating a low translocation of Cr from root to shoot. In fact, a low Cr translocation toward the shoots and Cr retention in the roots is common in macrophytes with an important radical system, for example, in *Limnobium laevigatum* (Arán et al. 2017).

Several factors contribute to a greater accumulation of Cr in roots, such as barriers or the lack of transport mechanisms suitable for Cr transport from roots to shoots (de Cabo et al. 2015), a high sorption in the cell walls of roots, thus preventing the translocation of the metal to the aerial parts (Mufarrege et al. 2018), accumulation in the root cell internal organelles, mainly inside the vacuoles and plastids (Sinha et al. 2018) and rhizosphere bacterial activity, which can facilitate metal accumulation in the roots (Amin et al. 2018). In our investigation, the fact that no toxicity symptoms were observed in *I. cernua* and *N. aquaticum* (Fig. 2a and b) was probably related to a low translocation from below-ground to above-ground plant parts, which minimized the stress imposed by the surrounding water and sediment (Papaevangelou et al. 2017).

As a management strategy, the phytoremediation of metal (loids) requires the harvest of plants to prevent the return of the pollutants to the environment by decomposition processes, which could cause the contamination of nearby water bodies. One of the main issues related to Cr removal by using constructed wetlands is the post-treatment management of the plant biomass harvest at the end of each vegetation and/or experimentation cycle (Sultana et al., 2015). After harvesting, management through advanced techniques, including composting and compaction, combustion and gasification, phytomining and pyrolysis, is essential (Mohanty, 2016; Vocciante et al. 2019). However, some of these techniques still present a challenge for their large-scale use, since they require effective and safe handling in order for the different processes to be feasible. In the present study, the contaminated plants were treated as hazardous waste using the recommended methods, as indicated by Peruvian legislation, of end disposal including approved secure landfills. Nevertheless, in the opinion of the authors of this study, for the implementation of full-scale constructed wetlands for wastewater treatment, harvested plants could be used for the production of bio-energy by burning the biomass under safe under safe controlled conditions. In this way, the volume could be reduced and the remaining ash could be used as bio-ore, utilizing an appropriate processing strategy for metal recovery.

Conclusion

The pilot-scale HCW system was efficient for treating high-strength tannery wastewater, with the treated effluent meeting the acceptable minimum national and international discharge standards. Overall, the contaminant removal obtained by the HCW system was higher than 98%, 97%, 97% and 98% for BOD_5, COD, TSS and Cr, respectively. In contrast, TDS was reduced by between 11 and 33%. There was a maximum organic mass removal rate of 10.24 g $m^{-2}d^{-1}$ for BOD_5 and 37.70 g $m^{-2}d^{-1}$ for COD, with similar maximum Cr removal rates observed in the vegetated and non-vegetated treatments of about 120 mg·Cr·$m^{-2}d^{-1}$.

The two aquatic macrophytes *I. cernua* and *N. aquaticum* were tested for concurrent removal of Cr. These macrophytes showed an uptake of this heavy metal in the root under different treatments for high concentrations of tannery wastewater (75 and 100%), but a low capacity of translocation to the leaf in both species. In addition, these plants removed the metal without any noticeable effects on survival and growth in the diluted treatment of tannery wastewater. The relative growth and survival of *I. cernua* in the 50 and 75% treatments were similar to the ones observed at the Yarabamba River collection site. For *N. aquaticum*, the relative growth for the 50% treatment was similar to that observed at the Tiabaya River sampling site.

Future studies will focus on long-term and full-scale research of different types of wetlands, as well as the importance of aquatic plants, with the aim of improving the current understanding of the CW operation in industrial areas of Peru.

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Compliance with ethical standards

Conflict of interest The author has declared no conflict of interest. The study has carried out by the author.

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