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Enhanced remediation of tannery effluent in constructed wetlands augmented with endophytic bacteria

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ABSTRACT

The effluent generated during the manufacturing of leather has a complex composition that may lead to severe environmental damage. Constructed wetland (CW) has recently become one of the most economically feasible and easily applicable tools for the removal of pollutants from wastewater. The aim of this investigation is to evaluate the effect of augmentation with endophytic bacteria in CWs on the remediation of tannery effluent. Southern cattail (*Typha domingensis*) was vegetated in vertical flow CWs and inoculated with endophytes. Bacterial augmentation enhanced the removal of organic and inorganic pollutants from the wastewater and maximum reduction of 80% in chemical oxygen demand, 95% in biochemical oxygen demand in 5 d, and 95% in Cr was observed. Nutrients, lipids, ion content, sulphates, and chlorides showed similar reduction by the combined action of endophytes and southern cattail. Results reveal that CWs augmented with endophytic bacteria provided an efficient approach for clean-up of tannery wastewater.

Keywords: Tannery effluent; Constructed wetlands; Endophytic bacteria; Wastewater remediation; Plant–endophyte partnerships; *Typha domingensis*

1. Introduction

The growth of industry has helped the human beings to create better communities that are technologically and economically advanced. But, it has incurred undeniable consequences on natural environmental processes and health of the ecosystem as well. Worldwide, tanning industry is one of the main culprits of environmental degradation. In the Kasur district of Pakistan, over 300 tanneries work and release untreated effluent into natural waterways of the region, particularly the Sutlej and Ravi rivers [1]. The tannery effluent is loaded with dissolved and suspended solids, high chemical oxygen demand (COD) and biochemical oxygen demand in 5 d (BOD_5), ion content (Na^+ and K^+), lipids, and heavy metals, particularly Cr [2]. Therefore, tannery wastewater should be treated before its discharge into the environment.

Constructed wetlands (CWs) are basically an imitation of natural wetlands with some solid support holding suitable vegetation in contact with treatable effluent [3–5]. The use of bacteria in conjunction with plants is an established means of soil and water remediation [6–8]. Recently, the plant-bacterial synergistic strategy was successfully explored for the remediation of wastewater [9–11]. Plant associated microbes increase the pollutants removal capabilities of vegetation by degrading contaminants and promoting health and growth of plant [12–14]. The plants vegetated in CWs are selected on the basis of their capability to establish themselves in saturated soil environment [15–17].

Southern cattail is scientifically known as *T. domingensis* Pers. and belongs to the family Typhaceae. It is one of the most commonly found and dominant plants that can grow in

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aquatic and nutrient-rich environments [18–20]. This species is also reported to grow in industrial wastewater, which shows its ability to resist high levels of pollution [21,22]. It grows in the form of extensive monospecific stands that reproduce by vegetative propagation from rhizomes and also through dispersal of seeds [23]. Most importantly, it has a large biomass in the form of long leaves, dense shoots, and long dense roots – all in a very compact form [24] that makes it an ideal choice for use in CWs.

Regardless of the usefulness of augmentation of bacteria in CWs for industrial wastewater treatment [4,10,25], the combined use of endophytes and *T. domingensis* has been rarely tested in CWs for the remediation of tannery effluent. The aim of this study was to develop plant–endophytes synergism in CWs for the efficient remediation of tannery effluent. In this study, CWs vegetated with southern cattail was augmented with three endophytic bacteria, *Microbacterium arborescens* HU33, *Pantoea stewartii* ASI11, and *Enterbacter* sp. HU38, for the remediation of tannery effluent. Southern cattail was chosen due to its abundant growth in the tropical climate of Pakistan as well as its excellent hyperaccumulation capability in wetland systems [26,27].

2. Materials and methods

2.1. Sample collection and analysis

Effluent samples were collected from the main drain containing the wastewater from tanneries of Kasur. Collected samples were subjected to chemical analysis to estimate major environmental quality parameters according to standard methods as described in Table 1.

2.2. Bacterial strains

Three endophytic bacterial strains (*M. arborescens* HU33, *P. stewartii* ASI11, and *Enterobacter* sp. HU38) were chosen on the basis of their capability to degrade organic pollutants as well as to promote plant growth as reported by Khan et al. [28]. Moreover, these strains showed persistence in Cr-contaminated soil and water.

Pure cultures of these strains were separately cultivated in LB broth at 37°C for 24 h. Cells were harvested by centrifuging (10,000×*g*) and resuspended in 0.9% (w/v) NaCl solution. Optical density of suspension of each strain was adjusted at 600 nm and mixed together to get equal number of bacteria in the consortium. 100 mL of this consortium was used for inoculation in each CW where required.

2.3. Experimental setup

The CWs were prepared on small scale (length 36 cm × width 30 cm × height 15 cm) to treat wastewater. Twelve such systems were constructed by placing 1 inch layers of coconut shavings (bottom-most layer), gravel, sand, and soil in the same sequence in a netted basket. Nine plants of cattail were planted in each basket (Fig. 1). The basket was then placed on plastic tank (39 cm × 31 cm × 23 cm) carrying 20 L tap water. A pump was installed in each tank to ensure the continuous vertical circulation of water through the layers of gravel, sand, and soil. The plants were allowed to establish in tap water for 30 d.

When the plants got established after 30 d of vegetation, tap water was replaced with tannery effluent. The reactors were now divided into four treatments with three replicates of each. These treatments were:

Treatment 1 (T-1): Vegetation in tap water.
Treatment 2 (T-2): Vegetation in tannery effluent.
Treatment 3 (T-3): Vegetation in tannery effluent with bacterial inoculation.
Treatment 4 (T-4): Tannery effluent without vegetation or inoculation.

The wastewater was treated for 27 d. Treated wastewater samples were collected after every 3 d from each tank till no further degradation was observed. These wastewater samples were subjected to different physicochemical tests. The concentration of heavy metals was measured in the collected wastewater samples and in plant tissues as described earlier [2].

2.4. Bacterial persistence

The survival/persistence of the endophytes in different components of CWs was determined as demonstrated by Afzal et al. [29]. Briefly, effluent was plated on LB agar supplemented with 100 mg/L Cr and incubated at 37°C for 24 h. On the other hand, roots and shoots were first surface sterilized and then homogenized in 0.85% NaCl solution. The homogenate was then plated on LB agar plates supplemented with 100 mg/L Cr. Colony forming units (CFUs) were counted and colonies that were morphologically identical to those of inoculants were subjected to restriction fragment length polymorphism (RFLP) to identify inoculated bacterial strains from the total bacterial population present in plant biomass and effluent as described earlier [30].

Table 1
Analyzed parameters and responsive methods

Parameter	Method	Reference
Chemical oxygen demand (COD)	Closed reflux, colorimetric method	[41]
Biochemical oxygen demand (BOD)	5-d BOD test	[41]
Ions (Na^+ and K^+)	Flame photometric method	[2]
Nutrients (N and P)	Colorimetric methods	[41]
Solids	–	[41]
pH and EC	Electrometric method	[41]
Heavy metals (Cr, Cu, Fe, Mn, Ni, Ba, Cd, and Pb)	Atomic absorption spectrophotometer	[2]

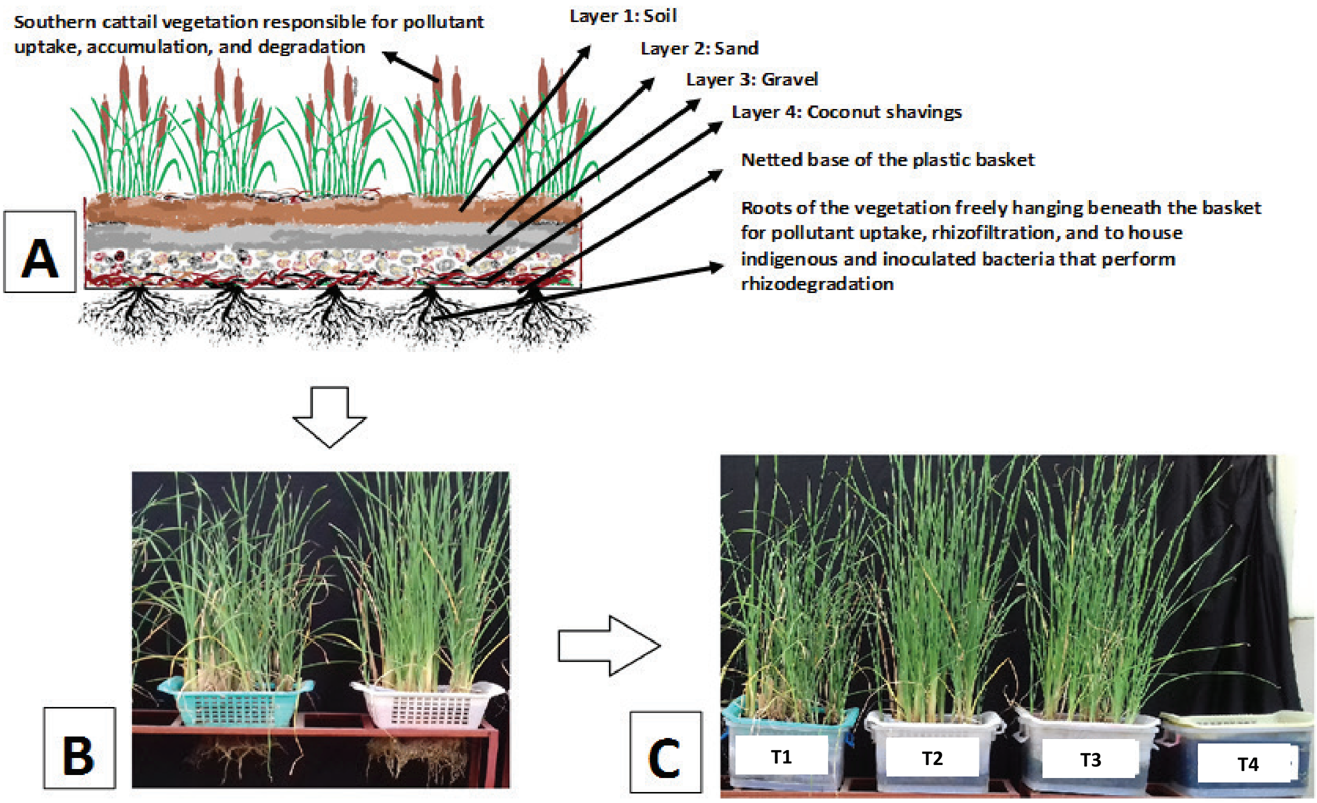

Fig. 1. Schematic diagram of the experiment. Netted baskets were prepared with 1-inch layers of soil, sand, gravel, and coconut shavings (A), *Typha domingensis* growth in the netted baskets (B), and one representative of three replicates of four different treatments with netted baskets placed in plastic tanks (C). Vegetation of *T. domingensis* in constructed wetlands having tap water (T-1), tannery effluent (T-2), tannery effluent and augmentation of endophytes (*Microbacterium arborescens* HU33, *Pantoea stewartii* ASI11, and *Enterobacter* sp. HU38) (T-3), and tannery effluent without vegetation and bacterial inoculation (T-4).

2.5. Effect of wastewater and endophytes on plant growth

To observe the consequences of the effluent on plant health and the potential of endophytic inoculation to reverse this effect, plant material was harvested and morphological assessments were carried out. Length of root and shoot as well as the fresh and dry weight of root and shoot was recorded. Dry weight of root and shoot was measured by drying plant material at 60°C for a week in an oven as explained earlier [31].

2.6. Maintenance of CWs

Along with the collection of samples from each tank, the CWs reactors were also inspected for maintenance on daily basis in order to ensure proper functioning. The main concern of these inspections was to look after the pumps for their proper functioning and flow of wastewater through the layers of gravel, sand, and soil.

2.7. Statistical data analysis

The data gathered from experimentation were statistically analyzed using Minitab software (version 15). Significant statistical differences ($p < 0.05$) among the four treatments were determined by one-way analysis of variance. This helped to compare the average values obtained for all environmental parameters measured in this investigation.

3. Results

3.1. Tannery effluent characteristics

The physicochemical characteristics of the tannery effluent are given in Table 2. The effluent was found to be highly polluted with both organic and inorganic chemicals, and having pollution levels higher than that allowed by the National Environmental Quality Standards (NEQS) of EPA-Pakistan (Environmental Protection Agency, Pakistan) [32]. The dissolved and suspended solids, COD, BOD, Na^+ and K^+ ion content, heavy metals, and lipid content were found higher than the allowed limits for industrial effluent.

3.2. Effect of endophytes inoculation on efficiency of CWs

The CWs vegetated with southern cattail and inoculated with endophytes showed the highest pollutants removal efficiency while the gravel-bed without vegetation showed lowest efficiency. This pollutants removal pattern was observed for not only the major environmental quality parameters, such as dissolved solid content, BOD_5, COD, lipids, and Cr (Fig. 2), but for minor parameters such as colour intensity, ion content, suspended solids, coliforms, and pathogenic bacteria as well (Table 3).

Electrical conductivity (EC) of the effluent decreased with a simultaneous decrease in Na^+ and K^+ ion content.

Table 2
Physicochemical characteristics of the tannery effluent

Parameter	Value	NEQS	Parameter	Value	NEQS
Colour appearance	Black	Grey	HCO_3 (mg/L)	2,912 ± 920	NG
Colour intensity (m^{-1})	76.2 ± 0.2	NG	Na (mg/L)	388 ± 26	250
Odour	Foul smell	NG	K (mg/L)	119 ± 11	NG
Temperature (°C)	30 ± 0.6	40	Ca (mg/L)	163 ± 1.5	NG
pH	7.9 ± 0.4	6–10	Mg (mg/L)	64 ± 0.5	NG
EC (mS/cm)	20.2 ± 1.3	NG	Total Cr (mg/L)	247 ± 5.8	1.0
TDS (mg/L)	12,928 ± 1,549	3,500	Cr^{6+} (mg/L)	0.78 ± 0.05	0.25
COD (mg/L)	6,066 ± 1,335	150	Cr^{3+} (mg/L)	246 ± 17.2	0.75
BOD (mg/L)	3,860 ± 612	80	Cu (mg/L)	1.12 ± 0.001	1.0
TS (mg/L)	18,715 ± 1,208	NG	Fe (mg/L)	17.38 ± 2.4	2.0
TSS (mg/L)	4,800 ± 230	150	Mn (mg/L)	4.33 ± 0.010	1.5
TSeS (mL/L)	11 ± 0.3	NG	Ni (mg/L)	1.22 ± 0.001	1.0
TOC (mg/L)	1,851 ± 46	NG	Pb (mg/L)	0.24 ± 0.021	0.5
TN (mg/L)	752 ± 29	NG	Ba (mg/L)	1.15 ± 0.062	1.0
NO_3 (mg/L)	590 ± 22	NG	Cd (mg/L)	2.66 ± 0.45	0.1
PO_4 (mg/L)	19.8 ± 0.3	NG	Co (mg/L)	1.06 ± 0.04	NG
SO_4 (mg/L)	2,693 ± 203	NG	Oil and grease (mg/L)	362 ± 22.5	10
Cl (mg/L)	5,600 ± 1,876	1,000	Coliform ($mL^{-1} \times 10^3$)	2.64 ± 0.20	NG
CO_3 (mg/L)	1.8 ± 0.42	NG	CFU ($mL^{-1} \times 10^6$)	73 ± 12	NG

Each value is a mean of three replicates; ±, indicates standard deviation; NG, not given; NEQS, National Environmental Quality Standards; TS, total solids; TSS, total suspended solids; SS, settleable solids; and TN, total nitrogen.

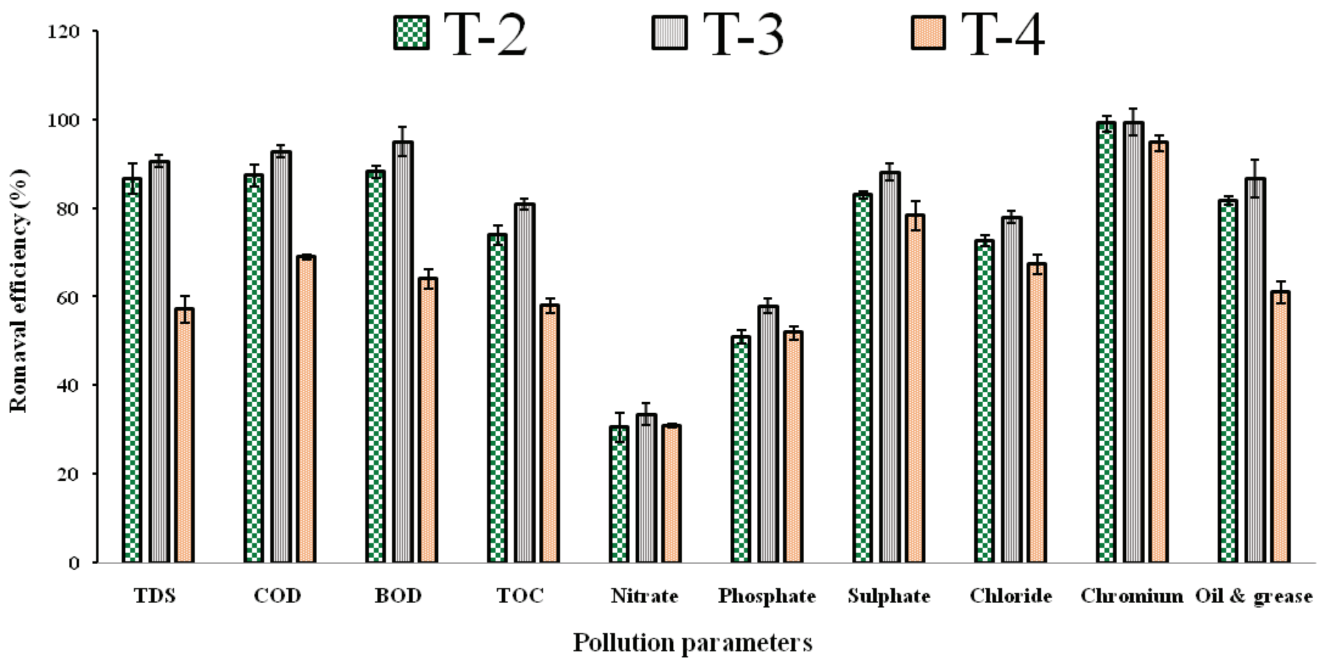

Fig. 2. Removal of total dissolved solids (TDS), COD, BOD, total organic carbon (TOC), nitrate, phosphate, sulphate, chloride, chromium, and oil and grease from tannery effluent in constructed wetlands having *Typha domingensis* only (T-2), *T. domingensis* and endophytes *Microbacterium arborescens* HU33, *Pantoea stewartii* ASI11, and *Enterobacter* sp. HU38 (T-3), and without vegetation (T-4).

The combined application of vegetation and endophytes resulted in a decrease in EC from 20.2 to 2.07 mS/cm as compared with a decrease in EC to 8.66 mS/cm in gravel-bed without vegetation. Similarly maximum COD and BOD_5 reduction of 92% and 95%, respectively, were observed in inoculated CWs in comparison with 87% and 88% in CWs without inoculation (Fig. 2). Sulphates, Cl^-, suspended and dissolved solids, lipids, and total hardness (Mg^+ and Ca^{2+})

Table 3
Effect of *Typha domingensis* vegetation and endophytic inoculation on remediation of tannery effluent in constructed wetland

Parameter	*Typha domingensis*				*Typha domingensis* and bacterial consortium[a]				Without vegetation			
	Time (d)				Time (d)				Time (d)			
	0	9	18	27	0	9	18	27	0	9	18	27
pH	7.9 ± 0.4	8.0 ± 0.3	7.3 ± 0.1	7.8 ± 0.3	7.9 ± 0.4	7.5 ± 0.2	6.8 ± 0.1	7.4 ± 0.2	7.9 ± 0.4	8.2 ± 0.3	7.8 ± 0.4	8.5 ± 0.2
EC (mS/cm)	20.2 ± 1.3	8.3 ± 0.6	4.5 ± 0.3	1.5 ± 0.4	20.2 ± 1.3	7.1 ± 1.1	3.6 ± 0.2	2.1 ± 0.1	20.2 ± 1.3	16.2 ± 1.0	12.9 ± 0.7	8.66 ± 0.2
TS (mg/L)	18,715 ± 998	9,314 ± 107	838 ± 35	987 ± 21	18,715 ± 998	8,720 ± 48	639 ± 15	233 ± 18	18,715 ± 998	14,300 ± 119	11,203 ± 42	7,991 ± 16.5
TSS (mg/L)	4,800 ± 230	126 ± 22	43 ± 5	11 ± 0.5	4,800 ± 230	113 ± 19	36 ± 7	10 ± 1.4	4,800 ± 230	566 ± 51	143 ± 32	13 ± 4
TSeS (mL/L)	11 ± 0.3	3.3 ± 0.3	1.8 ± 0.1	0.4 ± 0.1	11 ± 0.3	2.4 ± 0.1	1.3 ± 0.2	0.4 ± 0.1	11 ± 0.3	7.1 ± 0.6	2.8 ± 0.2	0.5 ± 0.1
Na (mg/L)	388 ± 26	130 ± 12	76 ± 3.3	42 ± 0.5	388 ± 26	105 ± 4	52 ± 1.3	38 ± 1.8	388 ± 26	269 ± 12	142 ± 3.5	84 ± 5.8
K (mg/L)	119 ± 11	38 ± 17	23 ± 4.2	6.7 ± 0.4	119 ± 11	32 ± 13	20 ± 2.9	5.5 ± 1.6	119 ± 11	97 ± 22	53 ± 6.5	17 ± 1.7
Ca (mg/L)	163 ± 1.5	121 ± 2.8	84 ± 3	54 ± 12	163 ± 1.5	106 ± 1.4	70 ± 3	42 ± 13	163 ± 1.5	138 ± 6.5	95 ± 1.1	67 ± 2.5
Mg (mg/L)	64 ± 0.5	41 ± 2.8	24 ± 1.4	16.7 ± 1.8	64 ± 0.5	36 ± 1.5	19 ± 0.6	8.9 ± 0.3	64 ± 0.5	57 ± 1.5	44 ± 2.3	35 ± 11.9
Coliform ($mL^{-1} \times 10^3$)	2.6 ± 0.2	1.8 ± 0.3	1.1 ± 0.7	0.9 ± 0.3	2.6 ± 0.2	1.5 ± 0.5	1.1 ± 0.1	0.7 ± 0.1	2.6 ± 0.2	1.9 ± 0.2	1.60 ± 0.2	1.3 ± 0.1
CFU ($mL^{-1} \times 10^6$)	73 ± 12	36 ± 4.5	21 ± 1.9	13 ± 0.5	73 ± 12	31 ± 4	17 ± 2.2	9 ± 0.2	73 ± 12	42 ± 1.9	29 ± 0.5	16 ± 0.7

[a]Mixture of equal numbers of cells of *Microbacterium arborescens* HU33, *Pantoea stewartii* ASI11, and *Enterobacter* sp. HU38.

were reduced in the tannery effluent by treating with CWs; however, the removal efficiency for all these pollutants was enhanced with the introduction of endophytic bacteria.

3.3. Translocation for heavy metals in plant tissues

Chromium, the most concerning heavy metal in tannery effluent, was removed more than 95% by the combined application of vegetation and endophytes in CWs (Fig. 2). All other metals including Cu, Ni, Pb, Cd, Co, Fe, Mn, and Ba also showed better uptake by the vegetation in the presence of endophytes (Table 4). The metals were mainly accumulated in roots rather than their little translocation in the shoot. For most metals, the plant showed greater tendency to translocation of metals into the shoot in the presence of endophytes (Table 5). This effect was particularly distinct for Cu, Pb, Cd, Co, and Ba which showed more accumulation in the shoot with bacterial inoculation.

3.4. Effect of endophytes inoculation on plant growth

Fresh and dry biomass and length data of the plant showed that the plant survived better in the tannery effluent (T-2 and T-3) as compared with tap water (T-1; Table 6). Plant growth and health were further improved by the introduction of endophytes in CWs (T-3).

3.5. Bacterial persistence

The inoculation of endophytes in CWs increased bacterial population in different compartments of the plant. RFLP analysis revealed that 56% of bacteria belonged to the inoculated endophytes while others were indigenous to the plant or the effluent (Table 7). Furthermore, within the biomass, persistence of inoculated endophytes was greater in the root as compared with the rhizosphere, bulk soil, and shoot.

4. Discussion

In this investigation, tannery effluent was treated in vertical flow CWs vegetated with southern cattail in the presence and absence of augmentation of endophytic bacteria. The most important component of the CWs here was the southern cattail, which was selected here for its hyperaccumulating nature. Being a hyperaccumulator plant, it has natural metabolic and hydraulic processes which enable it to remove diverse pollutants, particularly heavy metals, from the substrate in which it grows [27]. It is reported to survive effectively in polluted water while maintaining continuous nutrient and contaminant uptake [24] that renders it effective for restoration of the quality of industrial wastewater [10,27]. In this research, an interesting observation was the better growth of southern cattail in the tannery effluent (T-2 and T-3) as compared with that in tap water (T-1; Table 6). The improvement in the growth of southern cattail in the effluent can be due to the complex composition of tannery effluent that largely comprises of inorganic chemicals that may act as nutrients for the plant without being toxic due to its hyperaccumulating and tolerant nature [24,26,33]. On the other hand, tap water contains little or no nutrients to support the fastidious growth of the plant (T-1). Addition of endophytes

Table 4
Removal of heavy metals by *Typha domingensis* planted in vertical flow constructed wetland

Heavy metal	Plant part					
	Root (mg/kg)			Shoot (mg/kg)		
	T-2	T-3	*F* ratio	T-2	T-3	*F* ratio
Cr	95 ± 5.5	110 ± 4.5	13.4*	33 ± 1.2	40 ± 2.3	21.84**
Cu	16.5 ± 0.9	20.5 ± 0.5	45.28**	10 ± 0.8	12 ± 0.3	16.44*
Ni	106 ± 5	134 ± 7.5	3.87^{NS}	6 ± 0.4	10 ± 1	41.38**
Pb	2.5 ± 0.15	3.5 ± 0.1	92.31**	5 ± 0.1	5 ± 0.1	0.00^{NS}
Cd	2.5 ± 0.2	3.5 ± 0.3	23.08**	5 ± 0.3	5.5 ± 0.5	2.21^{NS}
Co	6 ± 0.2	7 ± 0.19	39.42**	5 ± 0.12	5 ± 0.05	0.00^{NS}
Fe	9,025 ± 213	9,675 ± 339	7.91*	2,270 ± 165	2,540 ± 188	3.50^{NS}
Mn	375 ± 12	415 ± 22	7.64^{NS}	510 ± 25	660 ± 15	79.41**
Ba	5.5 ± 0.2	9.5 ± 0.5	165.52**	14 ± 0.7	13 ± 0.3	5.17^{NS}

All values are mean (n = 3) ± standard deviation.
**Highly significant results $p < 0.01$.
*Significant $p < 0.05$ and NS = non-significant results $p > 0.05$ at 5% significance level by one-way analysis of variance. T-2 = *T. domingensis* without bacterial inoculation; and T-3 = *T. domingensis* with bacterial inoculation.

Table 5
Translocation of metals in *Typha domingensis*

Translocation factor (TF$[Metal]_{Shoot}/[Metal]_{Root}$)									
Treatments	Heavy metals								
	Cr	Cu	Ni	Pb	Cd	Co	Fe	Mn	Ba
T-2	0.26	0.60	0.06	2.01	2.01	0.83	0.25	1.36	2.54
T-3	0.30	0.58	0.07	1.42	1.57	0.71	0.26	1.59	1.36

T-2 = *T. domingensis* without bacterial inoculation, and T-3 = *T. domingensis* with bacterial inoculation.

Table 6
Effect of tannery effluent and bacterial inoculation on length and fresh and dry weight of root and shoot of *Typha domingensis* plants vegetated in vertical flow constructed wetlands

Treatments	Length (cm)		Fresh weight (g)		Dry weight (g)	
	Root	Shoot	Root	Shoot	Root	Shoot
T-1	31 ± 2.04	119.38 ± 3	616 ± 32	315 ± 14	182 ± 3.4	66 ± 2.5
T-2	38 ± 1.53	124.46 ± 5.8	688 ± 17	345 ± 16	216 ± 9	82 ± 5
T-3	43 ± 2.9	129.54 ± 1.2	834 ± 26.8	417 ± 25	280 ± 8	88 ± 9.9
One-way analysis of variance (ANOVA) test at 95% confidence level						
F ratio	21.93**	5.27*	54.66**	22.96**	142.34**	9.01*

*$p < 0.05$ represents significant results.
**$p < 0.01$ represents highly significant results for length and weight of plant root and shoot, each value is mean ± standard deviation. Number of replicates was 3 for each treatment. T-1 (*T. domingensis* in tap water), T-2 (*T. domingensis* in effluent), and T-3 (*T. domingensis* in effluent with bacterial inoculation).

Table 7
Bacterial population in the rhizosphere, bulk soil, root, and shoot of *Typha domingensis* planted in vertical flow constructed wetlands

Treatments	Rhizosphere (CFU $g^{-1} \times 10^6$)	Bulk soil (CFU $g^{-1} \times 10^6$)	Root (CFU $g^{-1} \times 10^6$)	Shoot (CFU $g^{-1} \times 10^6$)
T-2	3.6 ± 0.12	2.18 ± 0.11	3.3 ± 0.10	1.7 ± 0.23
T-3	4.5 ± 0.09	3.19 ± 0.17	5.4 ± 0.33	2.9 ± 0.14

Each value is mean (three replicates) ± standard deviation. T-2 = *T. domingensis* only, and T-3 = *T. domingensis* and endophytic bacteria.

supported plant growth even further and resulted in more above and belowground biomass that is clearly attributable to the production of plant growth promoting chemicals, such as phytohormones and organic acids, by inoculated bacteria. Once loaded with pollutants, the plant parts, such as leaves and shoots can be harvested in order to help the plant perform further uptake. The harvested plant material can be incinerated or used for bioenergy production.

In this study, although CWs vegetated with southern cattail effectively remediated tannery effluent, a better efficiency of pollutants removal was obtained by augmentation of endophytes in CWs. Because of symbiotic relationship, the vegetation provides an optimum microenvironment within its endosphere and rhizosphere for the inoculated bacteria to thrive [34], while the bacteria in return release plant growth promoting chemicals (such as 1-aminocyclopropane-1-carboxylate-deaminase, indole-3-acetic acid, siderophore, chitinase, pectinase, cellulase, salicylic acid, and hydrogen cyanide) and degrade toxic contaminants to aid plant in surviving and decontaminating the effluent [13,16,35]. A significantly higher reduction in BOD_5, COD, and TOC was observed in inoculated CWs than uninoculated CWs. In inoculated CWs, BOD was removed up to 95% while both COD and TOC showed a removal of more than 80% (Fig. 2). While cattail brought significant reduction in these parameters on its own, the removal efficiency of CWs was further enhanced with the augmentation of pollutant-degrading endophytes. In an earlier study, Calheiros et al. [36] reported that CWs vegetated with *Phragmites australis* and *Typha latifolia*, effectively removed pollutants from tannery effluent in a period of 17 months, whereby a reduction of 41%–73% was observed in COD and 41%–58% in BOD_5. In this study, a better pollutant removal efficiency was achieved after 27 d than previous study; it can be deduced that enhanced performance of CWs is possible with an application of beneficial bacteria, which is in concordance with previously reported finding [8,10].

In addition, a much greater reduction in EC was observed in inoculated CWs as compared with uninoculated CWs. By the inoculation of endophytes in CWs, the EC reduced to 2.07 mS/cm that rendered the effluent completely safe for agricultural use [8,37]. A reduction in EC occurred as a result of the removal of Na^+ and K^+ ions from the effluent. Although, nutrient content was also removed by CWs and gravel bed, this removal was significantly less than that obtained in inoculated CWs (Fig. 2). In a previous research, southern cattail was reported to effectively remove phosphate and nitrate [8,10] which is contrary to what has been observed here. In this study, removal of less than 40% and 60% was obtained for NO_3 and PO_4^{2+}, respectively, the required amount was taken up by the plant and rest remained in the effluent (Fig. 2). This can be because of insufficient PO_4^{2+} solubilization by organic acids, being released by indigenous and inoculated bacteria. Furthermore, effective denitrification of NO_3 require anaerobic environment; however, in CWs used here the prevalent conditions were aerobic, as oxygen was provided beneath the netted basket to the root system both by aerenchyma cells of plant as well as through dissolution in effluent from atmosphere due to its continuous vertically downward circulation, thus enhancing the degradation of organics but reducing denitrification processes.

The high contents of heavy metals were another factor of concern in the tannery effluent being discharged in environment. In this study, significant amount of all heavy metals was removed by treating tannery effluent in CWs. Southern cattail absorbed and stored large amounts of heavy metals in its root tissues (Table 4) with small amounts being translocated into the shoot (Table 5). Tadesse and Seyoum [38] similarly reported Cr-removal up to 97%–99% from tannery effluent by CWs vegetated with *T. domingensis*, *Parawaldeckia karaka*, and *Borassus aethiopum*, most of the metals were accumulated in the roots. In another study, Cr removal efficiency of 90%–99% was reported by Dotro et al. [39] using CWs. In the present research, southern cattail was observed to be an effective accumulator of heavy metals, particularly Cr with removal efficiency higher than 95%. Nevertheless, an enhancement in the removal efficiency of heavy metals was observed by introduction of endophytic bacteria in CWs which can be due to increased bioavailability of the metals to the plants [40].

5. Conclusions

- The CWs, vegetated with southern cattail, were found to be a promising technology to remediate tannery effluent.
- The augmentation of endophytes in CWs enhanced the growth of southern cattail and the remediation of tannery effluent.
- Endophytes showed persistence and colonization in the root and shoot of the plants and also enhanced the population of bacteria in different compartments of the plant. Endophytes showed more persistence in the root as compared with shoot.
- Further studies are needed to explore the abundance and activity (gene expression) of the inoculated endophytes in the rhizosphere and endosphere of the plant vegetated in CWs by using culture-independent approaches.

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References

[1] S.R. Tariq, M.H. Shah, N. Shaheen, A. Khalique, S. Manzoor, M. Jaffar, Multivariate analysis of selected metals in tannery effluents and related soil, J. Hazard. Mater., 122 (2005) 17–22.
[2] M. Afzal, G. Shabir, S. Iqbal, T. Mustafa, Q.M. Khan, Z.M. Khalid, Assessment of heavy metal contamination in soil and groundwater at leather industrial area of Kasur, Pakistan, Clean Soil Air Water, 42 (2014) 1133–1139.
[3] J. Vymazal, Constructed wetlands with horizontal subsurface flow in the Czech Republic: two long-term case studies, Desal. Wat. Treat., 4 (2009) 40–44.
[4] K. Gunes, B. Oktar, S. Ayaz, A.S. Gunay, G. Ozden, K. Disbudak, C. Senduran, T. Dikerler, E. Metin, F. Masi, Large scale constructed wetland implementation projects in Turkey in salt lake special environmental protection area, Desal. Wat. Treat., 51 (2013) 4758–4767.
[5] H. Wu, J. Zhang, H.H. Ngo, W. Guo, Z. Hu, S. Liang, J. Fan, H. Liu, A review on the sustainability of constructed wetlands for wastewater treatment: design and operation, Bioresour. Technol., 175 (2015) 594–601.

[6] N. Weyens, D. van der Lelie, S. Taghavi, L. Newman, J. Vangronsveld, Exploiting plant-microbe partnerships to improve biomass production and remediation, Trends Biotechnol., 27 (2009) 591–598.
[7] M. Afzal, Q.M. Khan, A. Sessitsch, Endophytic bacteria: prospects and applications for the phytoremediation of organic pollutants, Chemosphere, 117 (2014) 232–242.
[8] A. Ijaz, G. Shabir, Q.M. Khan, M. Afzal, Enhanced remediation of sewage effluent by endophyte-assisted floating treatment wetlands, Ecol. Eng., 84 (2015) 58–66.
[9] T.R. Saeed, R. Afrin, A. Al Muyeed, G. Sun, Treatment of tannery wastewater in a pilot-scale hybrid constructed wetland system in Bangladesh, Chemosphere, 88 (2012) 1065–1073.
[10] M. Shehzadi, M. Afzal, M.U. Khan, E. Islam, A. Mobin, S. Anwar, Q.M. Khan, Enhanced degradation of textile effluent in constructed wetland system using *Typha domingensis* and textile effluent-degrading endophytic bacteria, Water Res., 58 (2014) 152–159.
[11] M.Y. Sultana, A.K.M.M.B. Chowdhury, M.K. Michailides, C.S. Akratos, A.G. Tekerlekopoulou, D.V. Vayenas, Integrated Cr(VI) removal using constructed wetlands and composting, J. Hazard. Mater., 281 (2015) 106–113.
[12] A. Arslan, A. Imran, Q.M. Khan, M. Afzal, Plant-bacteria partnerships for the remediation of persistent organic pollutants, Environ. Sci. Pollut. Res., 24 (2017) 4322–4336.
[13] K. Fatima, A. Imran, I. Amin, Q.M. Khan, M. Afzal, Plant species affect colonization patterns and metabolic activity of associated endophytes during phytoremediation of crude oil-contaminated soil, Environ. Sci. Pollut. Res., 23 (2016) 6188–6196.
[14] M. Shehzadi, K. Fatima, A. Imran, M.S. Mirza, Q.M. Khan, M. Afzal, Ecology of bacterial endophytes associated with wetland plants growing in textile effluent for pollutant-degradation and plant growth-promotion potentials, Plant Biosyst., 150 (2016) 1261–1270.
[15] H. Brix, Do macrophytes play a role in constructed treatment wetlands? Water Sci. Technol., 35 (1997) 11–17.
[16] C. Calheiros, A. Teixeira, C. Pires, A. Franco, A. Duque, L. Crispim, Bacterial community dynamics in horizontal flow constructed wetlands with different plants for high salinity industrial wastewater polishing, Water Res., 44 (2010) 5032–5038.
[17] A. Ijaz, A. Imran, M. Anwar-ul-Haq, Q.M. Khan, M. Afzal, Phytoremediation: recent advances in plant-endophytic synergistic interactions, Plant Soil, 405 (2016) 179–195.
[18] B. Lorenzen, H. Brix, K. McKee, I. Mendelssohn, S. Miao, Seed germination of two Everglades species, *Cladium jamaicense* and *Typha domingensis*, Aquat. Bot., 66 (2000) 169–180.
[19] N. Abdel-Ghani, A. Hegazy, G. El-Chaghaby, *Typha domingensis* leaf powder for decontamination of aluminium, iron, zinc and lead: biosorption kinetics and equilibrium modeling, Int. J. Environ. Sci. *Technol*., 6 (2009) 243–248.
[20] D. Mani, C. Kumar, Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation, Int. J. Environ. Sci. Technol., 11 (2013) 843–872.
[21] M. Maine, N. Sune, H. Hadad, G. Sánchez, C. Bonetto, Nutrient and metal removal in a constructed wetland for wastewater treatment from a metallurgic industry, Ecol. Eng., 26 (2006) 341–347.
[22] G. Di Luca, M. Maine, M. Mufarrege, H. Hadad, G. Sánchez, C. Bonetto, Metal retention and distribution in the sediment of a constructed wetland for industrial wastewater treatment, Ecol. Eng., 37 (2011) 1267–1275.
[23] H. Stewart, S. Miao, M. Colbert, C. Carraher, Seed germination of two cattail (*Typha*) species as a function of Everglandes nutrient levels, Wetlands, 17 (1997) 116–122.
[24] J. Grace, The effects of nutrient additions on mixtures of *Typha latifolia* L. and *Typha domingensis* pers. along a water-depth gradient, Aquat. Bot., 31 (1988) 83–92.
[25] A. Ijaz, Z. Iqbal, M. Afzal, Remediation of sewage and industrial effluent using bacterially assisted floating treatment wetlands vegetated with *Typha domingensis*, Water Sci. Technol., 74 (2016) 2192–2201.
[26] A. Hegazy, N. Abdel-Ghani, G. El-Chaghaby, Phytoremediation of industrial wastewater potentiality by *Typha domingensis*, Int. J. Environ. Sci. Technol., 8 (2011) 639–648.
[27] A. Mojiri, H. Aziz, M. Zahed, S. Aziz, M. Selamat, Phytoremediation of heavy metals from urban waste leachate by southern cattail (*Typha domingensis*), Int. J. Sci. Res. Environ. Sci., 1 (2013) 63–70.
[28] M.U. Khan, A. Sessitsch, M. Harris, K. Fatima, A. Imran, M. Arslan, M. Afzal, Cr-resistant rhizo- and endophytic bacteria associated with *Prosopis juliflora* and their potential as phytoremediation enhancing agents in metal-degraded soils, Front. Plant Sci., 5 (2015) 755.
[29] M. Afzal, S. Yousaf, T.G. Reichenauer, A. Sessitsch, The inoculation method affects colonization and performance of bacterial inoculant strains in the phytoremediation of soil contaminated with diesel oil, Int. J. Phytorem., 14 (2012) 35–47.
[30] M. Afzal, S. Yousaf, T.G. Reichenauer, M. Kuffner, A. Sessitsch, Soil type affects plant colonization, activity and catabolic gene expression of inoculated bacterial strains during phytoremediation of diesel, J. Hazard. Mater., 186 (2011) 1568–1575.
[31] N. Tara, M. Afzal, T.M. Ansari, R. Tahseen, S. Iqbal, Q.M. Khan, Combined use of alkane-degrading and plant growth-promoting bacteria enhanced phytoremediation of diesel contaminated soil, Int. J. Phytorem., 16 (2014) 1268–1277.
[32] National Environmental Quality Standards (NEQS), Environmental Protection Agency (EPA), Islamabad, Pakistan, 1999.
[33] F. Gao, Z.-H. Yang, C. Li, W.-H. Jin, Saline domestic sewage treatment in constructed wetlands: study of plant selection and treatment characteristics, Desal. Wat. Treat., 53 (2015) 593–602.
[34] J. Gaiero, C. McCall, K. Thompson, N. Day, A. Best, K. Dunfield, Inside the root microbiome: bacterial root endophytes and plant growth promotion, Am. J. Bot., 100 (2013) 1738–1750.
[35] S. Khan, M. Afzal, S. Iqbal, Q.M. Khan, Plant-bacteria partnerships for the remediation of hydrocarbon contaminated soils, Chemosphere, 90 (2013) 1317–1332.
[36] C.S. Calheiros, A.O. Rangel, P.M. Castro, Constructed wetland systems vegetated with different plants applied to the treatment of tannery wastewater, Water Res., 41 (2007) 1790–1798.
[37] Natural Resources Conservation Service, National Engineering Handbook; Part 651: Agricultural Waste Management Field Handbook, Washington, D.C., USA, 1999.
[38] A. Tadesse, L. Seyoum, Evaluation of selected wetland plants for removal of chromium from tannery wastewater in constructed wetlands, Ethiopia, Afr. J. Environ. Sci. Technol., 9 (2015) 420–427.
[39] G. Dotro, S. Castro, O. Tujchneider, N. Piovano, M. Paris, A. Faggi, M. Fitch, Performance of pilot-scale constructed wetlands for secondary treatment of chromium-bearing tannery wastewaters, J. Hazard. Mater., 239 (2012) 142–151.
[40] M. Maine, H. Hadad, G. Sánchez, G. Di Luca, M. Mufarrege, S. Caffaratti, M. Pedro, Long-term performance of two free-water surface wetlands for metallurgical effluent treatment, Ecol. Eng., 98 (2017) 372–377.
[41] A. Greenberg, L. Clesceri, A. Eaton, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, D.C., USA, 1995.