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Unveiling the Potential of Novel Macrophytes for the Treatment of Tannery Effluent in Vertical Flow Pilot Constructed Wetlands

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Abstract: The phytoremediation potential of macrophytic species has made them an inevitable component of constructed wetlands (CWs) for the treatment of industrial effluents. The macrophytes must have tolerance for the harsh conditions imposed by effluents for an effective establishment of the CW system. In this context, the basic purpose of this work was to investigate the efficacy of five indigenous emergent macrophytes (*Brachiaria mutica*, *Canna indica*, *Cyperus laevigatus*, *Leptochloa fusca*, and *Typha domingensis*) for the remediation of tannery effluent in vertical subsurface flow CWs. The ability of each macrophytic species to tolerate pollution load and to remove pollutants from the effluent was assessed. The effect of tannery effluent on the survival and growth of macrophytes was also studied. The treated tannery effluent samples were analyzed for electrical conductivity (EC), pH, biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), chlorides (Cl^-), sulphates (SO_4^{2-}), oil and grease, and Cr levels. All of the studied macrophytes significantly decreased the pollution load of tannery effluent, and the higher nutrient content of effluent stimulated their growth without any signs of negative health effects. *Leptochloa fusca* and *T. domingensis* performed better in removing pollutants and showed higher growth rates and biomass than other tested macrophytes and can be considered preferred species for use in CWs treating tannery effluent. *Brachiaria mutica* showed morphologically better results than *C. indica* and *C. laevigatus*.

Keywords: tannery effluent; wastewater treatment; phytotechnology; wetland plants; tanning industry; constructed wetlands

1. Introduction

Tanning is a pollution intensive industry, discharging effluent of complex composition to the environment that is difficult to depurate by conventional methods [1]. Use of constructed wetlands (CWs) as biogeochemical engineered systems for sustainable remediation of tannery effluent is a promising solution that integrates several components such as macrophytes, filter media, and microorganisms to accelerate the removal of pollutants [2–4]. Low operation and maintenance costs and environmental and socio-economic sustainability have supported the wide use of CWs for the treatment of a variety of wastewaters in comparison to conventional wastewater treatment technologies [5–8]. In the current scenario of limited resources, CWs can also fulfill the increasing demand of water and energy sources for the agricultural and industrial sectors [9].

Macrophytes are vital for the structural and functional integrity of CWs, and they play a crucial role in pollution reduction. They perform many functions: provide stability, facilitate filtration, prevent clogging, promote microbial growth, transport oxygen to the root zone, take up nutrients, and accelerate removal of pollutants in CWs [10–14].

The type of macrophytes used in treatment wetlands may influence the microbial population present [15–17], but the composition of the water to be treated may influence both plant growth and microbial composition. Therefore, macrophytes are a crucial factor in CWs design, because the response of macrophytes to effluent with the same composition varies from species to species [7,18].

The general requirements for the macrophytes to be used in CWs for particular wastewater treatment include ecological acceptability, tolerance of pollutants, tolerance to local climatic conditions, rapid establishment, propagation, growth, and high pollutant removal efficiency [19–21]. Specific requirements vary depending on the role of macrophytes in CWs, such as design of wetland employed, mode of operation, flow pattern, loading rate, and nature of wastewater [22,23]. Furthermore, for efficient treatment of tannery effluents through CWs, selection of specific macrophytic species in relation to their removal mechanism is essential to achieve maximum pollutant removal in a minimum time period, under particular conditions [12,24,25].

Previously, a large number of macrophytes, such as *Brachiaria decumbens*, *C. indica*, *Iris pseudacorus*, *Penisetum purpureum* [26], *Juncus effusus* [27], *Scirpus americanus*, *Typha latifolia* [28], *Cyperus kylinga*, *Cyperus rotundus*, *Ludwigia parvifloria*, *Marselia quadrifolia* [29], *Cyperus esculentus*, *Typha angustifolia*, *Vetiveria nemoralis*, *Vetiveria zizanioides* [30], *Leersia hexandra* [31], *Borassus aethiopum*, *Cyperus alternifolius*, *Parawaldeckia karaka*, *T. domingensis* [32], *B. mutica*, *L. fusca*, and *T. domingensis* [33] have been used in CWs to treat tannery effluent.

In this regard, the most commonly used macrophyte was *Phragmites australis* for tannery wastewater treatment [34–38]. Calheiros et al. [39,40] have extensively studied various aspects of tannery effluent treatment through CWs by using *Arundo donax*, *C. indica*, *P. australis*, *Sarcocornia fruticosa*, and *Stenotaphrum secundatum*.

Klomjek and Nitisoravut [18] have tested eight emergent plant species (*Echinodorus cordifolius*, *Digitaria bicornis*, *Cyperus corymbosus*, *V. zizanioides*, *Spartina patens*, *B. mutica*, *L. fusca*, and *T. angustifolia*) in CW systems for their tolerance to salinity stress by spiking municipal wastewater with common salt. They found that *E. cordifolius* and *V. zizanioides* were not tolerant to the imposed saline conditions (14–16 mS/cm), and *B. mutica* died after the completion of the experiment. Therefore, use of appropriate macrophytes can accelerate removal of pollutants in CW systems over time [41].

In the present study, five indigenous macrophytes, viz., *B. mutica*, *C. indica*, *C. laevigatus*, *L. fusca* and *T. domingensis*, well acclimatized to the local climatic conditions, were investigated for their ability to remove pollutants, particularly Cr, from tannery effluent in CWs. Here, use of *B. mutica*, *C. laevigatus*, and *L. fusca* are new additions.

The lack of detailed research and information concerning the tolerance, growth characteristics, and treatment performance of macrophytes in CWs facing complex industrial effluents, such as hypersaline effluents from tanneries, is an important issue that begs for more investigation; the present study was conducted to deepen this knowledge. Its aims were (1) to evaluate the efficacy of the indigenous macrophytes to treat tannery effluent, and (2) to assess the effects of tannery effluent on the survival and growth of these macrophytes vegetated in CW systems.

2. Materials and Methods

2.1. Design Configuration of CWs

A total of 15 similar vertical flow CW treatment systems were prepared on a small scale (51 cm × 28 cm × 30 cm) by planting five plant species in triplicate with a total water storage capacity of each system of 20 L. Approximately 2.5 cm layers each of coconut shavings, gravel, sand, and soil (from bottom to top in the same order) in a netted basket (51 cm × 28 cm × 13 cm) were placed over a plastic container fed with tap water (Figure 1a). *Leptochloa fusca* and *B. mutica* were grown vegetatively by inserting stem cuttings in phytoreactors, while to grow *C. indica*, *C. laevigatus*, and *T. domingensis*, their rhizomes were planted and allowed to establish for 45 days in tap water.

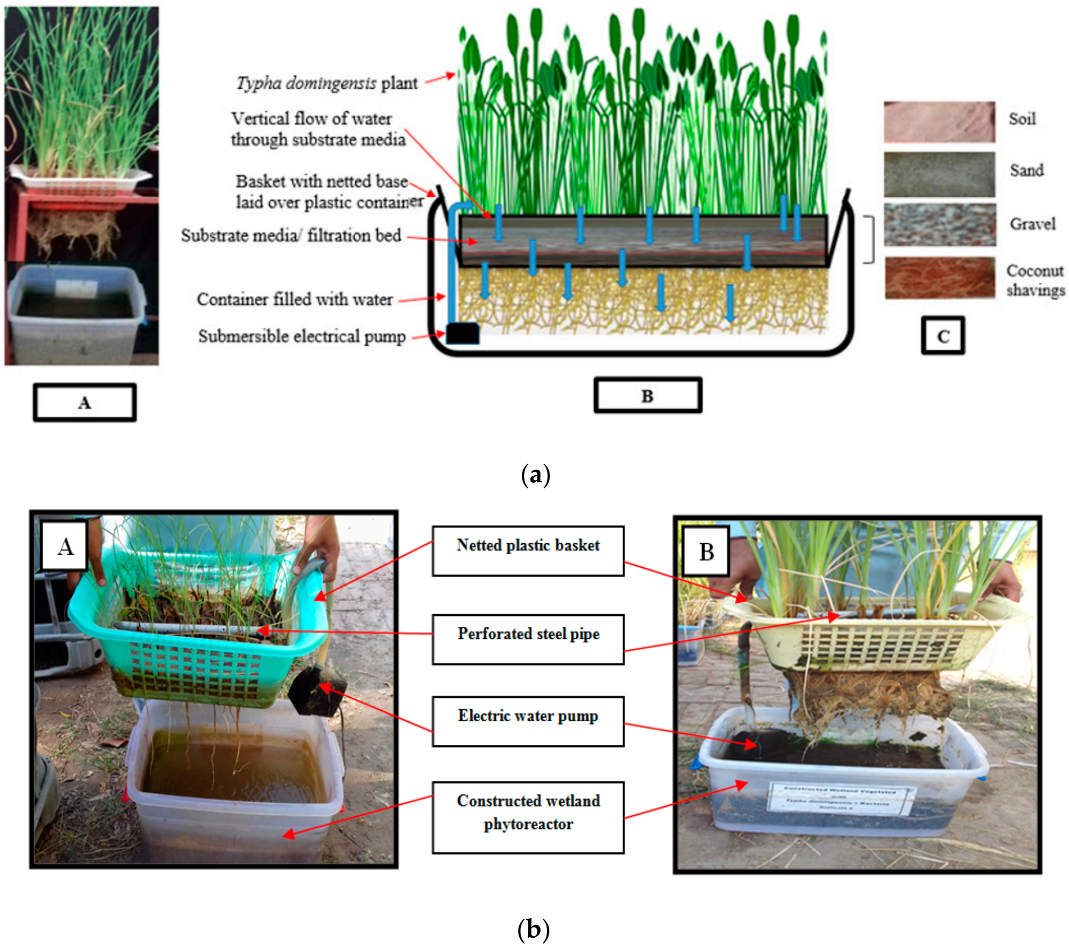

Figure 1. (**a**) Schematic diagram of a vertical flow constructed wetland phytoreactor. Photographic image of constructed wetland phytoreactor planted with *Typha domingensis* (A), configuration of constructed wetland showing vertical flow of water through filtration bed by electrical pump (B), and filtration media/substrate comprising soil, sand, gravel, and coconut shavings (C); (**b**) Vertical flow constructed wetland phytoreactors vegetated with *Leptochloa fusca* (A) and *Typha domingensis* (B) at different growth stages showing installation of an electric water pump for circulation of water by a perforated steel pipe through the wetland surface.

Continuous circulation of tap water, and later on of effluent through wetland surface, was ensured using a perforated steel pipe attached to electrical pumps (Figure 1b). Average hydraulic loading rate

(HLR) was set to 300 mL/min. After the plants were fully established (45 days), these phytoreactors were filled with 25% tannery effluent, followed by 50%, 75%, and 100% depending on their survival at each concentration at a seven-day interval. Average hydraulic retention time (HRT) of each phytoreactor at different concentration levels was approximately seven days. Tannery effluent samples were collected from each phytoreactor every week till there was no further reduction in pollutant level, and finally the pollutant removal capacity of each plant species was evaluated.

2.2. Collection and Characterization of Tannery Effluent

Tannery effluent samples were collected at the inlet of the common effluent treatment plant (CETP) receiving wastewater from tanneries in the industrial area of Kasur, Pakistan. The collected sample was subjected to various physical–chemical analyses to estimate major wastewater quality parameters including chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total dissolved solids (TDS), total suspended solids (TSS), sulphates, chlorides, oil and grease, and Cr using standard methods [42] as shown in Table 1.

Table 1. Physical–chemical characteristics of tannery effluent.

Parameters	Values (Mean ± SD)	NEQS	Parameters	Values (Mean ± SD)	NEQS
Color appearance	Black	Grey	TS (mg/L)	13,710 ± 608	NG
Color intensity (m^{-1})	61.2 ± 1.2	NG	TSS (mg/L)	2854 ± 230	150
Odor	Foul smell	NG	TSeS (mg/L)	8 ± 0.1	NG
Temperature (°C)	31 ± 0.3	40	SO_4^{2-} (mg/L)	1789 ± 113	NG
pH	8.0 ± 0.4	6–10	Cl^- (mg/L)	3315 ± 249	1000
EC (mS/cm)	16.5 ± 1.4	NG	Total Cr (mg/L)	134 ± 5.8	1.0
TDS (mg/L)	10,560 ± 978	3500	Cr^{6+} (mg/L)	0.48 ± 0.15	0.25
COD (mg/L)	5634 ± 245	150	Cr^{3+} (mg/L)	133 ± 1.4	0.75
BOD_5 (mg/L)	2910 ± 341	80	Oil and grease (mg/L)	145 ± 5.6	10

BOD_5: Biochemical oxygen demand; COD: Chemical oxygen demand; EC: Electrical conductivity; NEQS: National Environmental Quality Standards of Pakistan for industrial effluent discharge; NG: Not given; TDS: Total dissolved solids; TS: Total solids; TSS: Total suspended solids; TSeS: Total settleable solids. All measurements were performed in triplicates.

2.3. Operation and Maintenance of CWs

The CW phytoreactors were checked for maintenance on a daily basis to ensure proper functioning. The main concern of these inspections was to attend to the pump that was responsible for making the effluent flow through the filtration bed of CWs because its obstruction could occur due to the suspended solids in the effluent. Reduction in water volume by evapotranspiration in all the containers was replaced by tap water on a daily basis.

2.4. Analysis of Effluent Quality

During the experimental period, effluent samples from each phytoreactor were collected on a weekly basis. The effect of different macrophytes on the pollutant removal ability of CWs was observed by subjecting each treated sample to physical and chemical analyses including color, EC, pH, TDS, COD, BOD_5, TSS, SO_4^{2-}, Cl^-, oil and grease, and Cr according to standard methods [42].

2.5. Macrophytes Growth and Biomass Yield

To observe the effect of pollutants present in tannery effluent on the growth of all macrophytes (Figure 2), morphological parameters were measured immediately after harvesting the plants at the end of the experiment. The shoot length of plants was measured with a scale taking into account the distance of planting from topsoil, and root length was also measured in the same way, from the depth of planting to the bottom below the netted basket. Shoots of all macrophytes were harvested by cutting at soil level in the wetland reactors, while roots were harvested by removing from the filtration bed and cutting at the base of the netted basket of wetland phytoreactors to measure the fresh weight. Dry weight of root and shoot was measured after drying in an oven at 60 °C for a week.

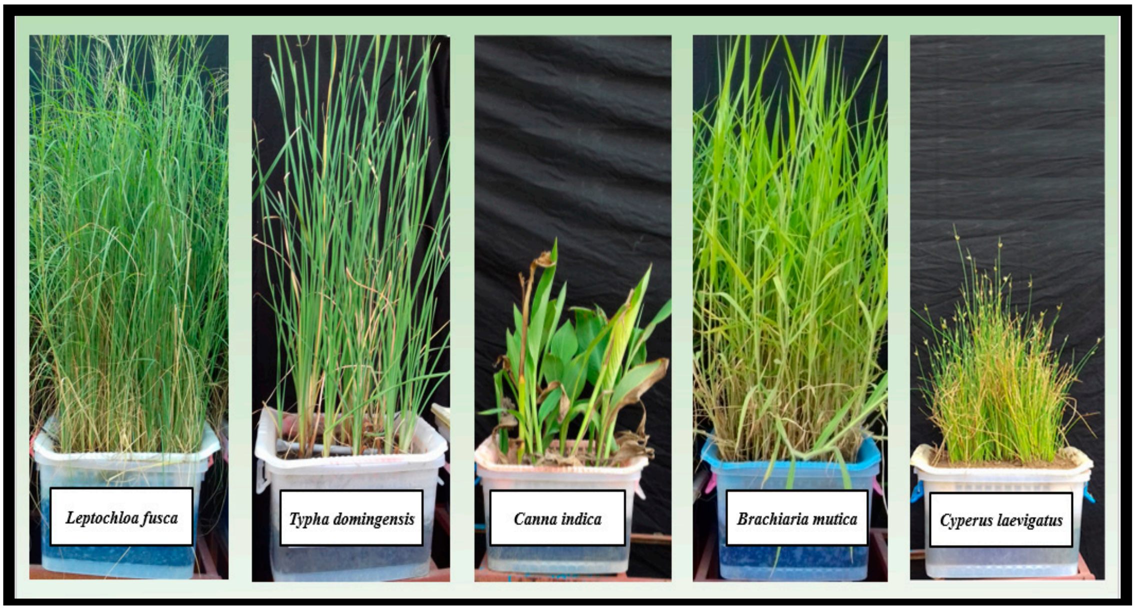

Figure 2. Constructed wetland phytoreactors vegetated with different macrophytic plants for the treatment of tannery effluent.

2.6. Data Analysis

The data gathered from experimentation was analyzed using statistics software version 8.1 (Statistix, Tallahassee, FL, USA). Significant statistical differences ($p < 0.05$) among macrophytes for pollution reduction were determined by one-way analysis of variance (ANOVA), and variation in pollutant removal efficacy among tested macrophytes was determined by least significant difference (LSD) test.

3. Results

3.1. Characterization of Tannery Effluent

The effluent was characterized in terms of physical–chemical parameters using APHA (American Public Health Association) [42] standard methods as shown in Table 1. The effluent was characterized by high salt contents, as shown by the high electrical conductivity levels (16.5 mS/cm) and high contents of dissolved solids (10,560 mg/L). In addition, BOD_5 (2910 mg/L), COD (5634 mg/L), and Cr (134 mg/L) concentrations were far beyond the permissible limits set by National Environmental Quality Standards (NEQS) of Pakistan for industrial effluent discharge.

3.2. Treatment Performance of Macrophytes

The performance of studied macrophytes to remove pollutants from tannery effluent was evaluated for six weeks after their establishment. *Leptochloa fusca* achieved the highest mean removal percentage (64% BOD_5; 51% COD; 56% TDS; 67% TSS; 42% SO_4^{2-}; 38% Cl^-; 47% oil and grease; and 55% Cr). For *T. domingensis* the values were 59% BOD_5; 47% COD; 43% TDS; 69% TSS; 49% SO_4^{2-}; 46% Cl^-; 43% oil and grease; and 48% Cr, while for *B. mutica*, mean removal percentage was 39% BOD_5; 31% COD; 38% TDS; 53% TSS; 25% SO_4^{2-}; 30% Cl^-; 26% oil and grease; and 35% Cr. The treatment with *C. indica* and *C. laevigatus* showed the lowest removal of all pollutants. The efficacy of both *L. fusca* and *T. domingensis* treatments in reducing pollutant concentrations were relatively higher than other tested macrophytes. In the present study, the lowest values of pollution variables were observed for effluents treated with *C. indica* and *C. laevigatus* (Figure 3). The initial and final characteristics of tannery effluent after treatment with different plant species for the studied pollution parameters and their comparison with NEQS are shown in Table 2.

Table 2. Pollutant reduction in tannery effluent by CW phytotechnology and its comparison with NEQS.

Pollution Parameters (mg/L)	Before Treatment Mean ± SD	Characteristics of Tannery Effluent after Treatment by CWs Planted with Different Plant Species Mean ± SD					NEQS
		L. fusca	*T. domingensis*	*C. indica*	*B. mutica*	*C. laevigatus*	
BOD_5	2910 ± 341	1047 ± 131	1193 ± 56	1717 ± 165	1775 ± 48	2008 ± 129	80
COD	5634 ± 245	2760 ± 201	2986 ± 237	3775 ± 179	3887 ± 160	4281 ± 242	150
TDS	10,560 ± 978	5913 ± 563	6019 ± 732	7180 ± 945	6547 ± 504	7708 ± 660	3500
TSS	2854 ± 230	942 ± 76	884 ± 146	1541 ± 98	1341 ± 43	1455 ± 94	150
Sulphates	1789 ± 113	1038 ± 104	912 ± 74	1413 ± 140	1342 ± 49	1288 ± 125	NG
Chlorides	3315 ± 249	2055 ± 130	1790 ± 167	2354 ± 248	2320 ± 173	2586 ± 204	1000
Oil and grease	145 ± 5.6	77 ± 2.3	82 ± 12	116 ± 5.1	107 ± 6.6	110 ± 9.3	10
Cr	134 ± 5.8	60 ± 2.4	69 ± 10.7	84 ± 4	87 ± 3.1	103 ± 8.5	1

Each value is a mean of three replicates, ±standard deviation; NEQS: National Environmental Quality Standards of Pakistan for industrial effluent discharge; NG: Not given.

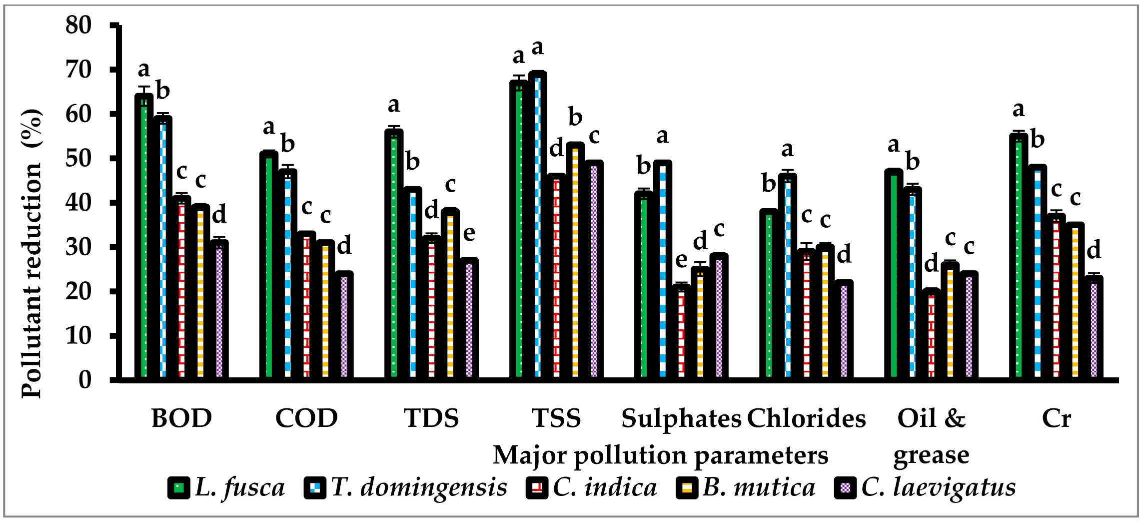

Figure 3. Pollutant reduction in tannery effluent by different macrophytes in constructed wetlands. Error bars indicate standard deviation among three replicates. Labels (a)–(e) indicate statistically significant differences ($p < 0.05$) among plant species at a 5% level of significance.

3.3. Growth and Biomass Yield of Macrophytes

All macrophytes exhibited good survival at all concentrations of tannery effluent. *Typha domingensis*, *L. fusca*, and *B. mutica* showed relatively rapid growth in the tannery effluent compared to *C. indica* and *C. laevigatus*. By the end of the experiment, length and weight (both fresh and dry) of the root and shoot of all macrophytes were determined. Among all the macrophytes, *T. domingensis* exhibited the highest length, followed by *L. fusca*, then *B. mutica*, *C. indica*, and *C. laevigatus*. *Typha domingensis* reached heights up to 147 cm and *L. fusca* heights of 132 cm, while heights were 121 cm for *B. mutica*, 51 cm for *C. indica*, and 43 cm for *C. laevigatus*, as shown in Figure 4. The measurement of fresh and dry biomass yield at the end of the experiment revealed that the weight (both fresh and dry) of *B. mutica* shoots was higher than the remaining macrophytes, while maximum fresh biomass of root was observed by *L. fusca* and dry biomass by roots of *T. domingensis* (Figure 5).

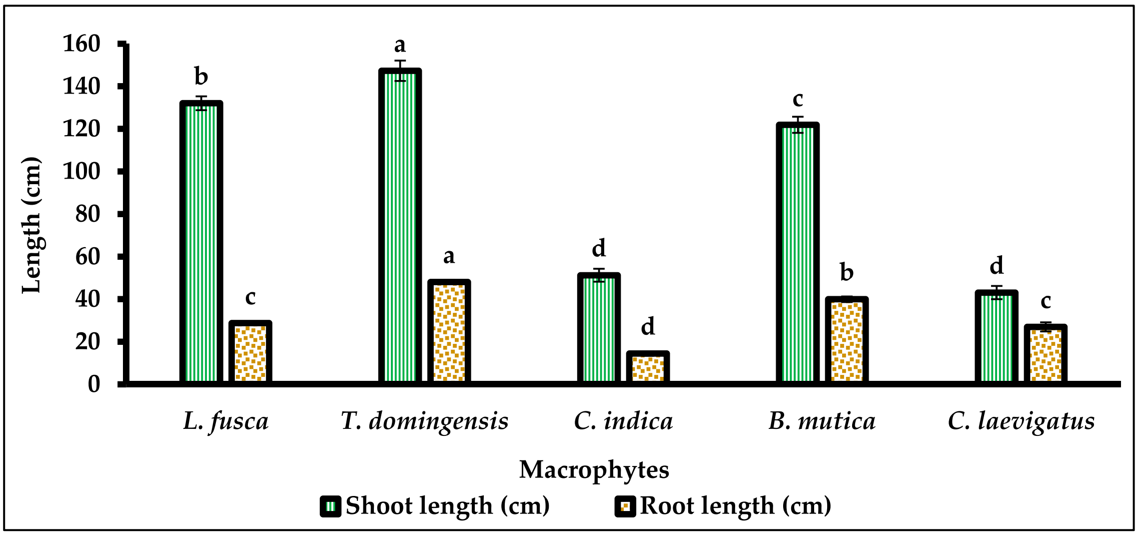

Figure 4. Relative growth pattern of different macrophytes in constructed wetlands fed with tannery effluent. Error bars indicate standard deviation among three replicates. (**a**–**d**) indicate statistically significant differences ($p < 0.05$) within plant tissues across species at a 5% level of significance.

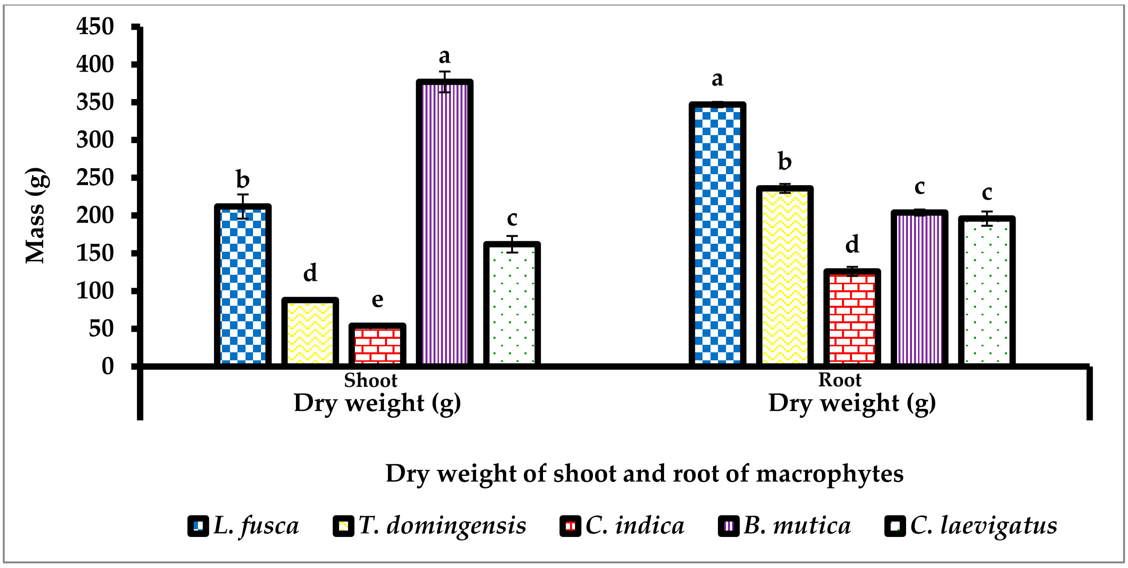

Figure 5. Biomass production of aquatic macrophytes in constructed wetlands fed with tannery effluent. Error bars indicate standard deviation among three replicates. (**a**–**e**) indicate statistically significant differences ($p < 0.05$) among plant species for shoot and root dry weight at a 5% level of significance.

4. Discussion

It is essential to assess the feasibility of aquatic macrophytes to be used in CWs for treating high salinity effluent, because the performance of CWs may be compromised if macrophytes fail to establish [33,43,44]. In the present study, the efficacy of macrophytes to remove pollutants from saline tannery effluent having high organics and Cr loadings and biomass yield was assessed during a time period of three months. All the macrophytes developed and proliferated varyingly in the tannery effluent, without showing any signs of toxicity.

Morphological characteristics and growth behavior of macrophytes revealed great differences among all the experimental species that were exposed to tannery effluent. This difference in growth among macrophytes over time is due to their intrinsic nature [40,45–47].

In the present work, use of halophytes (*L. fusca* and *T. domingensis*) in CW systems to treat tannery effluent proved better in terms of rapid growth and pollutant removal than the other tested macrophytes, due to their salinity tolerance and metal uptake capacity [33,48]. Removal of BOD_5 and COD, the major pollution indicators of industrial effluents, was comparatively higher in treatment wetlands vegetated with halophytes than other macrophytes. The ability of halophytes to accumulate salts and metals made them preferred candidates for salt phytoremediation of saline effluents [48–50]. However, bioaccumulation of salts and metals including Cr within macrophytes has not been assessed in the present work that may affect final removal, disposal, and/or reuse of harvested macrophytes and overall operation and maintenance costs of CW systems.

The potential removal of pollutants by macrophytes is influenced both by growth rate and biomass yield through uptake of nutrients from concentrated effluents. Species of aquatic macrophytes that have rapid growth and capacity to produce high biomass are more effective for the treatment of tannery effluents [51]. In the present study, *C. indica* and *C. laevigatus* were less effective in removing pollutants than *B. mutica*, *L. fusca*, and *T. domingensis*, probably because of their slow growth pattern and low biomass yield.

The effectiveness of macrophytes to remediate effluent is enhanced by harvesting biomass to maintain high rates of vegetative growth in the macrophytes population, and consequently to create a positive effect on the removal rate of pollutants as evidenced by Yang et al. [52]. Rozema et al. [53] also reported enhanced pollutant removal efficiencies by frequent harvesting of *T. latifolia* and *J. torreyi* in CWs. However, in the present study, it was not necessarily due to the relatively short experiment period.

Plant biomass produced after phytoremediation can be used in an environmentally friendly way as soil conditioner or green fertilizer to reduce secondary pollution [54,55]. For example, it can be used profitably in subsequent effluent treatment experiments to meet the initial nutrients requirement for the establishment of plants in CWs. Pat-Espadas et al. [56] suggested the use of residual waste as construction material to avoid undesirable environmental and health effects.

In the present study, reduction of pollutants from tannery effluent in CWs by *B. mutica, L. fusca*, and *T. domingensis* was attributed to their extensive root growth, which served as an active zone for degradation of organic and sequestration of inorganic pollutants by microbes. Macrophytes capable of developing an extensive root system in tannery effluent are more effective in removing its pollutant concentration as evidenced by earlier studies [44,57–60]. According to Jamshidi et al. [61], wetlands vegetated with *Phragmites* sp. exhibited high rates of pollutant reduction due to the development of an extensive root system that increased oxygen transfer efficiency, thus enhancing contribution of microbial biomass towards treatment.

Among the tested macrophytes, *B. mutica*, *L. fusca*, and *T. domingensis* turned out to be quite resilient and effective for the purpose of treating highly polluted tannery effluent, particularly high concentrations of salts, Cr, and organic compounds.

Moreover, the large-scale adoption of CWs technology for the treatment of highly complex tannery effluent still requires much fundamental and applied research for effective remediation, to achieve multipurpose wastewater treatment, biomass reuse/recycle, coping water scarcity, and environmental protection. In this regard, some progressive and novel approaches can be applied to CW systems like bioaugmentation, biostimulation, genetically engineered plants or microbes, and salt phytoremediation to improve their performance and efficiency.

5. Conclusions

The selection of appropriate macrophytes in CWs is crucial for effective treatment of highly polluted wastewaters generated by tanneries. This investigation demonstrated that treatment wetlands vegetated with *L. fusca* and *T. domingensis* can survive and flourish and facilitate removal of pollutants in hypersaline tannery effluent. While *B. mutica* grows rapidly in toxic tannery effluent, it requires frequent harvesting as plants die after reaching full maturity. The results showed that *L. fusca* and *T. domingensis* were clearly superior for metal uptake and solids, as well as organics removal over other macrophytes under local climatic conditions. The originality of this research work is the use of halophytic species (*L. fusca*) in CWs to combat salinity stress posed during the treatment of hyper saline tannery effluents.

Therefore, this study suggests the use of native halophytes for the phytoremediation of tannery effluents in CW bioengineered systems built economically in an environmentally friendly way.

Author Contributions: Conceptualization: M.N., Z.A.Z., M.A. (Muhammad Afzal), and M.F.S.; Data curation: S.A. (Sobia Ashraf), S.A. (Sana Ashraf), and A.M.; Formal analysis and software: M.F.S., N.A.A.-S., Y.R. and S.A. (Sana Ashraf); Investigation: N.A.A.-S., Y.R., and B.A.A.; Methodology: M.N., A.M., and M.A. (Majed Alotaibi); Supervision: M.N., M.A. (Muhammad Afzal), and Z.A.Z.; Resources: M.N., Z.A.Z., M.F.S. N.A.A.-S. B.A.A. and M.A. (Majed Alotaibi); Funding acquisition: M.F.S., B.A.A., N.A.A.-S., and A.M.; Writing—Original draft: S.A. (Sobia Ashraf) and S.A. (Sana Ashraf); Writing—Review and editing: M.A., B.A.A., K.A.A., M.F.S., N.A.A.-S., Y.R., and A.M. All authors have read and agreed to the published version of the manuscript.

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