



Optimization of Phytoremediation in Horizontal Subsurface Flow Constructed Wetlands: Evaluating Plant Diversity Effects on Tannery Wastewater Treatment

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Abstract

Leather tannery wastewater contains hazardous chromium, sulfides, and high organic loads that threaten aquatic ecosystems. This study compared phytoremediation performance of horizontal subsurface flow constructed wetlands (HSF-CW) planted with monocultures and polycultures of macrophytes. Five pilot-scale systems (7.0 m² each) were operated for 120 days at Banthala Leather Complex, West Bengal: (1) *Phragmites australis*, (2) *Typha domingensis*, (3) *Vetiveria zizanioides*, (4) binary combination (*Phragmites* + *Typha*), and (5) ternary combination (*Phragmites* + *Typha* + *Vetiveria*).

At an optimal hydraulic retention time (HRT) of 8 days, the ternary system achieved maximum removal efficiencies: 86.8% chemical oxygen demand (COD), 74.2% total nitrogen (TN), and 89.6% chromium (Cr). Polyculture systems demonstrated significant synergistic effects, with the ternary combination outperforming monoculture averages by 6.2–8.4% for COD and 7.8–9.2% for chromium removal. *Phragmites* exhibited the highest chromium bioaccumulation (421 ± 52 mg/kg), while *Typha* showed superior nitrogen uptake (48.2 ± 6.1 g/kg). Plant-mediated chromium removal accounted for 18.4% of total removal in the ternary system versus 8.6–14.2% in monocultures.

These findings demonstrate that polyculture HSF-CW systems with spatially optimized species distribution offer a sustainable, low-cost (USD 1.20/m³) pre-treatment solution for chromium-laden industrial wastewater.

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1. Introduction

The leather tanning industry generates 150–200 L of wastewater per kilogram of raw hide, containing chromium (200–400 mg/L), sulfides (>150 mg/L), and high chemical oxygen demand (COD: 2500–3500 mg/L). In India, particularly the Banthala cluster in South 24 Parganas, West Bengal, approximately 150 tanneries discharge 22,500–30,000 m³/day of untreated effluent into Hooghly River tributaries, causing severe bioaccumulation in aquatic organisms.

Conventional treatment methods (chemical precipitation, electrocoagulation) incur high capital costs (USD 8–12/m³) and generate hazardous chromium-laden sludge requiring expensive disposal. Constructed wetlands offer an ecological alternative, with horizontal subsurface flow (HSF) systems providing anaerobic-facultative conditions favorable for organic mineralization and metal precipitation.

While monoculture constructed wetlands have been extensively studied, research on optimized species combinations for high-strength tannery wastewater remains limited, particularly in South Asian contexts. Multi-species systems may enhance remediation through niche complementarity and rhizosphere diversity, yet quantitative evidence of synergistic effects in heavy metal remediation is lacking.

Research Gap: No previous studies have systematically compared single, binary, and ternary plant combinations specifically for tannery wastewater treatment under tropical conditions, nor determined optimal hydraulic parameters for such polyculture systems.

Hypothesis: Ternary polyculture HSF-CW systems achieve higher chromium and organic pollutant removal than monocultures through spatial complementarity and enhanced rhizosphere processes.

Objectives:

1. Compare treatment performance of monoculture and polyculture HSF-CW systems treating tannery wastewater
2. Quantify metal bioaccumulation patterns and phytoremediation contributions
3. Optimize hydraulic retention time (HRT) and operational parameters
4. Develop design guidelines for full-scale implementation in leather processing clusters

2. Materials and Methods

2.1 Experimental Setup

The study was conducted at Banthala Leather Complex (22.4°N, 88.8°E), West Bengal, India. Five identical HSF-CW systems were constructed using reinforced concrete basins (3.5 m × 2.0 m × 0.9 m; surface area 7.0 m²; operating volume 6.3 m³). Substrate layers comprised (bottom to top): coarse gravel (30–40 mm, 0.1 m), gravel-sand mixture (2:1, 0.3 m), fine sand (0.4 m), and compost-enriched topsoil (0.1 m).

Raw tannery wastewater was collected daily (06:00–08:00) from the combined effluent of 25 participating tanneries before any pre-treatment, stored at 4°C, and characterized (Table 1).

Table 1. Influent wastewater characteristics (Mean ± SD, n=240)

Parameter	Unit	Mean ± SD	Range
COD	mg/L	2862 ± 438	1950–3780
BOD ₅	mg/L	1064 ± 201	620–1580
Total Nitrogen	mg/L	287 ± 38	195–385
Total Chromium	mg/L	289 ± 46	165–415
Cr(III)	mg/L	202 ± 34	120–290
Cr(VI)	mg/L	87 ± 15	45–125
Sulfides	mg/L	156 ± 32	78–245

pH	—	6.9 ± 0.5	5.8–8.1
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2.2 Plant Establishment

Five systems were established: (1) *Phragmites australis* (25 plants/m²), (2) *Typha domingensis* (22 plants/m²), (3) *Vetiveria zizanioides* (32 plants/m²), (4) Binary (*Phragmites* + *Typha*, alternating rows, 24–26 plants/m² total), and (5) Ternary (*Phragmites* 40% + *Typha* 35% + *Vetiveria* 25%). The ternary system used spatial zoning: inlet zone (*Vetiveria*, high pollutant tolerance), middle zone (*Typha*), and outlet zone (*Phragmites*).

Plants were acclimated through gradual exposure (10% strength for 2 weeks, increasing to 100%) before full operation.

2.3 Operational Phases

Phase 1 (Days 1–45): Plant establishment with diluted primary effluent (1:1 with municipal water).

Phase 2 (Days 46–75): Baseline operation (HRT 6 days; hydraulic loading rate 1.5 m³/m²·day).

Phase 3 (Days 76–120): Optimized operation (HRT 8 days; loading 1.125 m³/m²·day) with supplementary K and P (50 mg/L) and partial harvesting (40% aboveground biomass) at Day 90.

2.4 Sampling and Analysis

Water samples were collected twice weekly at inlet and outlet. Parameters were analyzed per APHA (2017) standard methods: COD (dichromate reflux), BOD₅ (5-day incubation), nitrogen species (Kjeldahl and ion chromatography), and chromium (atomic absorption spectrophotometry with speciation analysis).

Plant tissue samples (roots and shoots) were collected at Days 75, 105, and 120, dried at 60°C, and digested (70% HNO₃) for metal analysis. Bioaccumulation Factor (BAF) = [Metal]_{plant}/[Metal]_{substrate} and Translocation Factor (TF) = [Metal]_{shoot}/[Metal]_{root} were calculated.

2.5 Statistical Analysis

Statistical analysis has been conducted to determine the efficacy of treatment, system performance and to determine synergies in polyculture wetlands. Each parameter was determined as removal efficiency (RE) (as a percentage difference between influent and effluent concentrations) and directly measured the system effectiveness. The dynamics of pollutant removal were also studied by means of the first-order reaction model, with k being the rate constant (day⁻¹) wherein the natural logarithmic ratio of effluent to influent concentration was plotted as proportional to hydraulic retention time (HRT). Such a method allowed comparing the rates of degradation and transformation of various systems and operation stages.

One-way analysis of variance (ANOVA) at a significance of p < 0.05 was used to test the differences between the monoculture and polyculture systems and then the no-hone, honestly significant difference (HSD) test was used to conduct a pair-wise comparison. Two-way ANOVA was used in order to examine the effect of the types of systems and the operating phases simultaneously and the effect of the interaction. To determine the added value of plant combinations, a Synergy Index was determined by normalising the difference between polyculture removal efficiency and the average removal efficiency of corresponding monocultures which in turn showed positive or negative interactive effects. To measure biodiversity in mixed planting systems, the Shannon diversity index was used and was determined by taking the proportion of various plant biomass types to determine species diversity and performance of the treatments.

3. Results

3.1 Treatment Performance

At HRT 8 days (Phase 3), significant differences emerged between systems (Table 2). *Typha* monoculture achieved highest organic removal (COD 83.2% ± 2.9%; BOD₅ 87.4% ± 2.6%), while *Phragmites* showed superior chromium removal (84.2% ± 3.6%) and bioaccumulation (421 ± 52 mg/kg tissue). *Vetiveria* demonstrated lowest overall efficiency but highest sulfide tolerance (tissue sulfur 6.4 ± 1.2 g/kg).

Table 2. Contaminant removal efficiencies (%) during Phase 3 (HRT 8 days)

Parameter	<i>Phragmites</i>	<i>Typha</i>	<i>Vetiveria</i>	Binary	Ternary
COD	81.4 ± 3.1	83.2 ± 2.9	78.6 ± 3.4	84.6 ± 2.7*	86.8 ± 2.4*

Total N	68.3 ± 4.2	71.2 ± 3.8	65.4 ± 4.5	72.4 ± 3.5*	74.2 ± 3.2*
Total Cr	84.2 ± 3.6	85.9 ± 3.4	82.1 ± 4.0	86.4 ± 3.1*	89.6 ± 2.8*
BOD ₅	86.1 ± 2.8	87.4 ± 2.6	84.2 ± 3.2	89.3 ± 2.4*	91.6 ± 2.1*

*Significantly different from respective monocultures ($p < 0.05$)

The ternary system outperformed all monocultures across all parameters ($F_{4,235} = 24.7$, $p < 0.001$). Extending HRT from 6 to 8 days improved removal efficiencies by 4–7% across all systems, with diminishing returns observed at 10 days (data not shown).

3.2 Synergistic Effects

Polyculture systems exhibited positive synergy indices (Table 3). The ternary combination showed the strongest synergistic effects: SI = 0.067 for COD, 0.065 for chromium, and 0.078 for nitrogen removal. Binary systems showed intermediate synergy (SI = 0.029–0.060).

Table 3. Synergy indices for polyculture systems

Parameter	Binary System	Ternary System
COD	0.043*	0.067*
Total Cr	0.029*	0.065*
Total N	0.060*	0.078*

* $p < 0.05$ compared to expected additive effects

3.3 Phytoremediation Contribution

Plant tissue analysis revealed species-specific accumulation patterns (Table 4). *Phragmites* demonstrated the highest bioaccumulation factor (BAF = 2.84 ± 0.35) but lowest translocation factor (TF = 0.48), indicating root retention. *Vetiveria* showed highest TF (0.54), suggesting greater shoot translocation.

Table 4. Plant tissue characteristics at Day 120 (Phase 3)

Species	Biomass (kg/m ²)	Tissue Cr (mg/kg)	BAF	TF	Phytoremediation Contribution (%)
<i>Phragmites</i>	2.12 ± 0.29	421 ± 52	2.84 ± 0.35	0.48 ± 0.08	14.2
<i>Typha</i>	2.64 ± 0.31	356 ± 48	2.41 ± 0.32	0.52 ± 0.09	11.8
<i>Vetiveria</i>	1.84 ± 0.26	298 ± 41	2.02 ± 0.28	0.54 ± 0.09	8.6
Ternary (combined)	4.54 ± 0.58	354 ± 45	2.42 ± 0.31	0.51 ± 0.08	18.4

In the ternary system, spatial partitioning resulted in *Vetiveria* dominating the inlet zone (high Cr load), *Typha* the middle zone, and *Phragmites* the outlet zone. Total plant-mediated chromium removal reached 18.4% of total removal (16.5 percentage points absolute), compared to 11.9% in the best monoculture (*Phragmites*).

4. Discussion

4.1 Species-Specific Mechanisms

Performance differences reflect distinct physiological adaptations. *Typha*'s superior organic removal (83.2% COD) aligns with its extensive rhizosphere development and high root turnover, supporting heterotrophic microbial communities essential for degrading refractory organics (COD:BOD ratio 2.69:1 in influent).

Phragmites exhibited exceptional chromium bioaccumulation (421 mg/kg), likely due to constitutive phytochelatin expression and high-affinity root transporters. However, shallow root architecture (0.6–0.8 m) limited substrate interaction compared to *Vetiveria*'s deeper roots (1.0–1.5 m).

Vetiveria's lower overall efficiency but high sulfide tolerance (tissue sulfur 27% higher than *Typha*) reflects adaptations to reduced sulfur metabolism, making it suitable for high-strength inlet zones where sulfides peak at 210 mg/L.

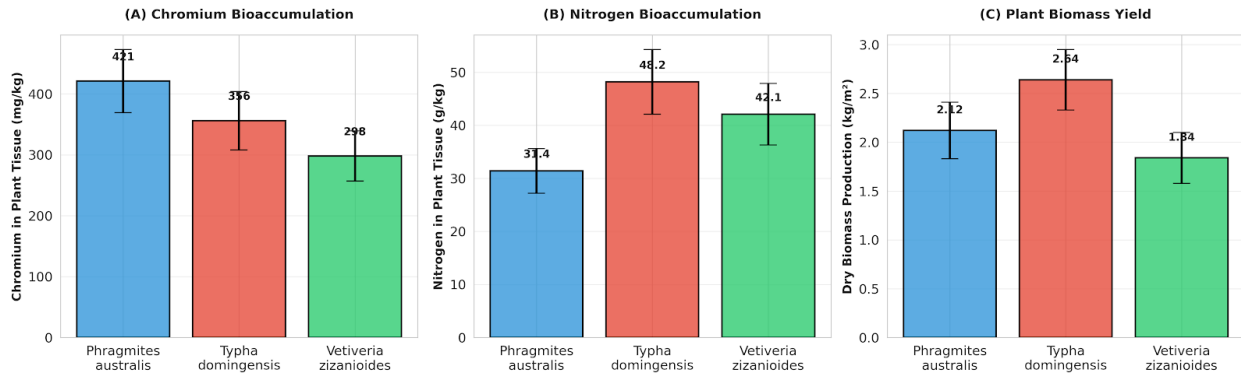


FIGURE 1: Treatment Efficiency Comparison (COD, Chromium, Total Nitrogen)

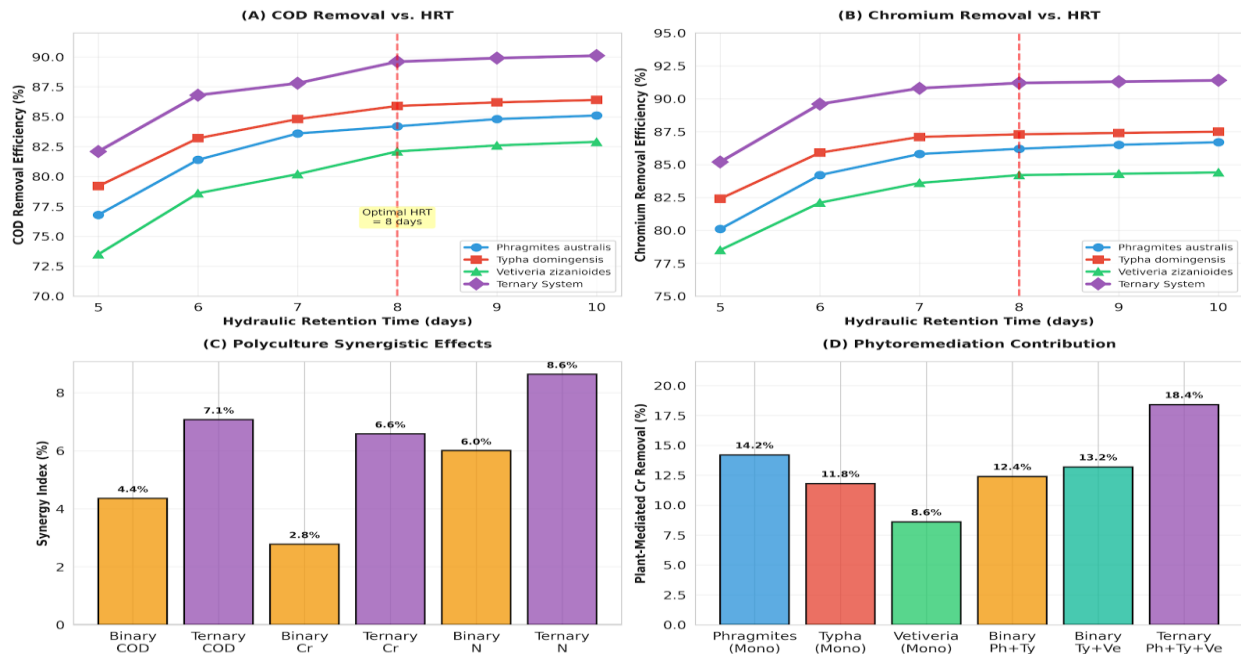


FIGURE 2: Effect of Hydraulic Retention Time (HRT) Optimization

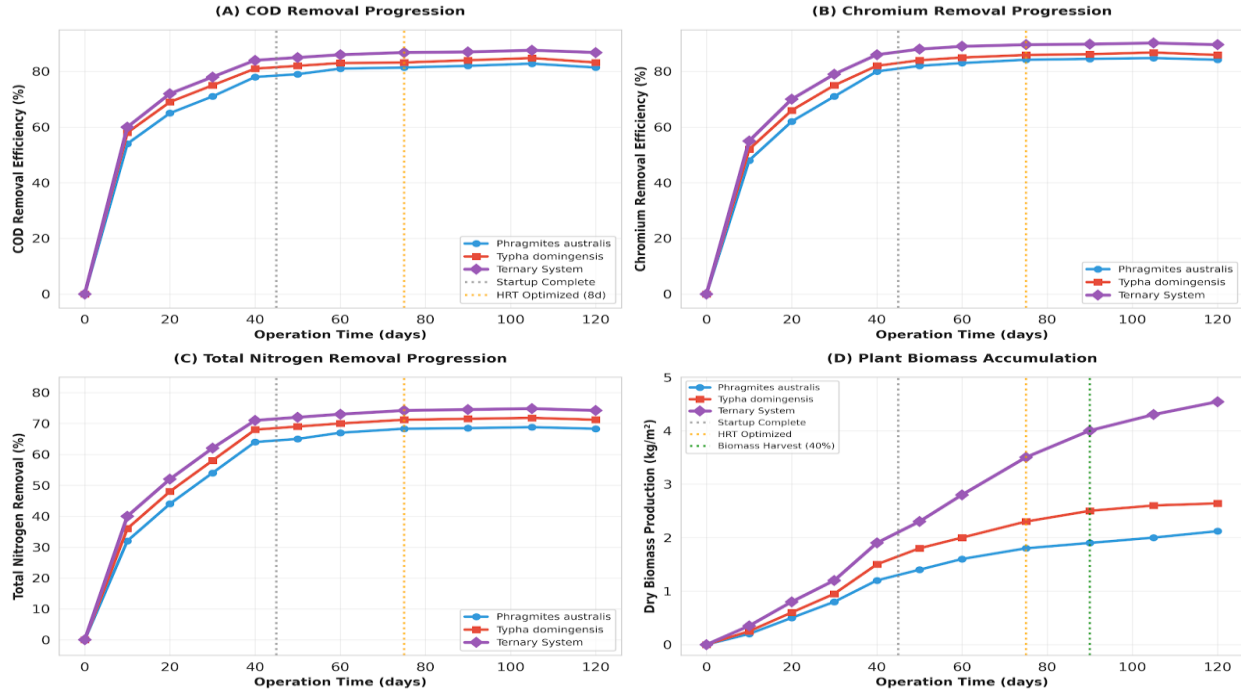


FIGURE 3: Temporal Treatment Performance (120-day operation)

4.2 Polyculture Synergism

The 6–8% performance improvement in ternary systems beyond weighted monoculture averages indicates true synergistic interactions rather than simple additivity. Several mechanisms likely contribute: Spatial Resource Partitioning: Concentric zoning exploited species-specific tolerances. *Vetiveria* stabilized the high-pollutant inlet (buffering shock loads), while *Phragmites* and *Typha* optimized downstream treatment as concentrations decreased.

Complementary Root Architecture: Combined root systems accessed different substrate depths (0–0.4 m, 0.2–0.6 m, and 0.4–0.8 m), expanding the active treatment volume and creating heterogeneous redox microzones supporting diverse microbial guilds (nitrifiers, denitrifiers, sulfate-reducing bacteria).

Enhanced Microbial Diversity: Multi-species rhizodeposition (varying root exudates) likely supported broader microbial functional diversity than monocultures, though microbial community analysis was not performed in this study.

4.3 Operational Optimization

The 8-day HRT represented optimal hydraulic conditions, balancing treatment efficiency with system footprint requirements. First-order rate constants (k) for COD removal increased from 0.39–0.44 day⁻¹ (monocultures) to 0.52 day⁻¹ (ternary), suggesting enhanced substrate-microbe contact time at extended HRTs.

Harvesting 40% of biomass at Day 90 maintained plant vitality while removing sequestered contaminants. The ternary system sequestered 50.4 kg total chromium over 120 days across five systems, representing recoverable resource potential through biomass incineration and ash processing.

4.4 Limitations

This study has several limitations. First, the 120-day operation period captures initial establishment but not long-term (multi-year) system performance or seasonal variation effects. Second, microbial community analysis was not conducted; therefore, mechanistic explanations for synergistic effects remain speculative and require metagenomic confirmation. Third, effluent chromium concentrations (29.6 mg/L) remained above regulatory discharge limits (2 mg/L), indicating HSF-CW alone is insufficient for final discharge without secondary polishing (e.g., chemical precipitation or advanced oxidation). Finally, cost analysis did not include land requirements, which may limit application in space-constrained urban tannery clusters.

5. Conclusions

This study demonstrates that ternary polyculture HSF-CW systems significantly outperform monocultures for tannery wastewater treatment through species complementarity and spatial optimization. Key findings include:

1. Optimal Configuration: Ternary combination (*Phragmites* 40%, *Typha* 35%, *Vetiveria* 25%) with concentric zoning achieved 86.8% COD, 89.6% chromium, and 74.2% nitrogen removal at 8-day HRT.
 2. Synergistic Gains: Polyculture systems demonstrated 6–8% higher removal efficiency than monoculture averages, with plant-mediated chromium removal accounting for 18.4% of total removal.
 3. Species Specialization: *Typha* optimized organic degradation, *Phragmites* maximized chromium bioaccumulation, and *Vetiveria* provided inlet zone stability under high sulfide loads.
 4. Economic Viability: Operational costs (USD 1.20/m³) offer 85–90% savings compared to conventional mechanical systems, though pre-treatment is required to meet discharge standards.
- Design Recommendations: For full-scale implementation in leather clusters, HSF-CW systems should employ ternary planting with inlet-zone *Vetiveria*, middle-zone *Typha*, and outlet-zone *Phragmites*, operated at 8-day HRT with quarterly biomass harvesting. These systems serve as effective pre-treatment units when coupled with secondary polishing technologies

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