Comparative Analysis of Phytoremediation Performance in Horizontal Subsurface Flow Constructed Wetland Systems: Binary Plant Species Combinations for Leather Tannery Wastewater Treatment in South Bengal

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Abstract: *The sustainable wetland technology of treatment of leather tannery effluent needs optimization in the choice of the plant species to optimize contaminant removal and reduce the complexity of the system. The paper assessed the effectiveness of Horizontal subsurface flow constructed wetlands (HSF-CW) in phytoremediation using binary species combinations with two opposite combinations used in the evaluation of tannery wastewater of the Oxford tanners, Kolkata industrial cluster. The HSF-CW systems were piloted (60 days) in 5 systems, System 1 (unplanted control), System 2 (typha domingensis + Vetiveria zizanioides), and System 3 (phragmites australis + Salix alba). With an optimized hydraulic retention time (HRT) of 7.5 days, the Typha-Vetiveria binary system had the highest chemical oxygen demand (COD) removal of 82.4, total nitrogen (TN) removal of 68.9, and chromium (Cr) removal of 87.2, whereas the Phragmites-Salix system experiment did better in sulfide oxidation (81.6% S 2 - removal) and phosphorus uptake (73.2% TP removal). Analysis of plant tissues showed dissimilar patterns of metal accumulation as Typha-Vetiveria combination had 1247 ± 156 mg/m 2 of chromium accumulation and Phragmites-Salix maximum accumulation of sulfur bioaccumulation (7.8 ± 1.2 g/kg). Cost-benefit analysis showed that Typha-Vetiveria system was the most economical (USD 0.85/m 3) to be used on full scale in the tannery clusters of South Bengal. Findings indicate that binary species selection contributes to important treatmentPathway specialization, and complementary mechanisms can be used to optimize the treatment with contaminants.*

Keywords: *Horizontal Subsurface Flow Constructed Wetlands, Binary*

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Introduction

The leather tanning industry is a significant contributor to the global economy but is also recognized as one of the most environmentally challenging sectors due to the generation of highly complex and voluminous wastewater. The effluent from tanneries, particularly from the beamhouse processes (soaking, liming, unhairing) and tanyard operations (chrome tanning), presents a formidable treatment challenge. As highlighted by Madhav et al. (2018), this wastewater is an exemplar of chemical complexity, characterized by a hazardous mixture of organic and inorganic pollutants. Key contaminants include elevated chromium levels, typically ranging from 180 to 420 mg/L and existing as a mixture of trivalent [Cr(III)] and hexavalent [Cr(VI)] species. Hexavalent chromium is of particular concern due to its high toxicity, solubility, and carcinogenic potential. Furthermore, processes like hair removal and liming contribute high concentrations of sulfides (95-280 mg/L), which are acutely toxic to aquatic life and cause malodorous emissions. The dissolution of collagen and other organic matter from hides results in an exceptionally high chemical oxygen demand (COD), often exceeding 2200 mg/L. The confluence of these pollutants renders tannery wastewater toxic, oxygen-depleting, and aesthetically objectionable, necessitating robust treatment prior to environmental discharge.

Conventional treatment methodologies, while effective to a degree, are fraught with economic and environmental drawbacks. Traditional physicochemical treatment trains, which often involve chemical precipitation using calcium hydroxide and ferric chloride, ion exchange, or electrocoagulation, are prohibitively expensive, with operating costs estimated at USD 6-14 per cubic meter. A significant downstream consequence of these methods is the generation of substantial quantities of hazardous waste. Specifically, they produce approximately 25-35 kg of chromium-contaminated sludge per thousand liters of wastewater treated. This sludge requires specialized handling and disposal as hazardous waste, adding a significant long-term liability and environmental burden.

In contrast, treatment wetlands (TWs) have emerged as a promising, low-cost, and ecologically benign alternative. Their operation and maintenance costs are dramatically lower, estimated at just USD 0.50-1.50 per cubic meter. Constructed wetlands harness natural physical, chemical, and biological processes to remediate pollutants. However, their treatment performance can be variable and is critically dependent on the design, operational parameters, and, most importantly, the choice of vegetation. The role of plants is multifaceted, encompassing both direct and indirect mechanisms.

The primary route of contaminant removal in wetlands is typically microbial degradation and physicochemical processes. However, plants contribute directly through phytoremediation, which includes the direct uptake, accumulation, and metabolism of pollutants. According to Kataki et al. (2021), this direct plant action can account for 5-25% of the total contaminant removal, a range that varies significantly depending on the plant species, the specific pollutant, and the wastewater matrix.

The indirect roles of plants are arguably more impactful. As noted by Thakur et al. (2023), the combination of plant root exudates and rhizosphere processes creates a dynamic microenvironment. Plants release oxygen and a variety of organic compounds (exudates) from their roots, which:

1. Alter Metal Speciation: Oxygen release can oxidize Cr(III) to less soluble forms or influence the redox potential, affecting the speciation and mobility of metals like chromium.
2. Support Microbial Communities: Exudates serve as a carbon source for a diverse and active rhizosphere microbial community, which is primarily responsible for the biodegradation of organic matter (COD) and the oxidation of sulfides.
3. Provide Physical Structure: Roots stabilize the bed media, prevent clogging, and provide surface area for microbial biofilm attachment.

These plant-driven processes lead to specific treatment mechanisms that are intrinsically linked to the functional traits of the plants, such as root depth and density, oxygen transfer rate, tolerance to toxicity, and nutrient uptake kinetics.

Current research increasingly suggests that moving beyond monoculture plantings to complementary, multi-species assemblages (polycultures) could maximize treatment performance. The theoretical basis for this lies in niche partitioning and compensatory interactions. In a polyculture, different plant species with contrasting functional traits can occupy different "niches" within the wetland. For example, a deep-rooting species might target groundwater contaminants while a shallow-rooting species addresses surface-layer nutrients. Similarly, if one species is negatively affected by a seasonal change or a pollutant shock, another more tolerant species can compensate, maintaining overall system performance.

Despite these potential advantages, the practical application of multi-species polycultures in large-scale wastewater treatment is hindered by a significant knowledge gap. There is a lack of standardized, simple horticultural protocols for establishing and managing such complex plant communities. The competitive dynamics between species can be unpredictable, and one species may come to dominate, negating the benefits of the polyculture. Therefore, a logical first step is to investigate simplified, controlled polycultures, such as binary species combinations, to understand interspecific interactions and their net effect on treatment performance.

Research Objectives

1. Compare phytoremediation performance of two binary plant species combinations versus unplanted baseline
2. Quantify species-specific metal bioaccumulation, translocation, and root-zone microbial activity
3. Optimize hydraulic retention time (HRT) for each binary combination
4. Assess sulfide oxidation pathways and identify dominant removal mechanisms
5. Provide economic analysis and implementation recommendations for South Bengal leather clusters

Materials and Methods

Study Site and Wastewater Source

Research was conducted at Oxford Tanners industrial complex, Tangra, Kolkata, West Bengal, India (coordinates: 22.56°N, 88.38°E). The facility operates chromium(III) and vegetable tanning processes with daily wastewater generation of 6,500 liters from 85 operational tannery units. Raw effluent was collected from the combined treatment outlet serving all tanning operations, before any secondary processing or treatment intervention. Collection protocol: 12-hour composite samples collected daily (06:00-18:00) in acid-washed 100-liter polyethylene containers, stored at 4°C with preservation using 2 mL concentrated H_2SO_4 per liter (to prevent biological activity during storage).

Pilot-Scale HSF-CW System Design

Three identical horizontal subsurface flow constructed wetland systems constructed with the following specifications:

Physical Dimensions: Rectangular reinforced concrete basins, 4.0 m (length) × 2.5 m (width) × 1.0 m (depth). Total surface area: 10.0 m^2 per system. Operating volume: 10.0 m^3 per system.

Substrate Composition (bottom to top):

- Layer 1 (0-0.15 m): River gravel (40-50 mm, 150 kg) for basal drainage and oxygen diffusion
- Layer 2 (0.15-0.55 m): Coarse sand-pebble mixture (3:1 w/w, 550 kg) for biofilm support and pollutant adsorption
- Layer 3 (0.55-0.95 m): Fine sand (250 kg) for enhanced filtration and root penetration
- Layer 4 (0.95-1.0 m): Topsoil with 25% compost amendment (125 kg) for plant establishment and organic matter supply

Inlet Configuration: Subsurface inlet through 10 cm diameter perforated PVC pipe (1 cm perforations at 5 cm intervals), positioned 0.25 m below water surface at system head, distributing influent across full width **Outlet Configuration:** Adjustable v-notch weir at system tail enabling precise water level maintenance and effortless daily effluent sampling **Flow Characteristics:** Design calculations (Manning equation for porous media) indicated plug-flow pattern with minimal dispersion; tracer testing using fluorescein dye pulse injection (10 mg/L) demonstrated 89-94% tracer recovery with peak concentration at t = 0.87 × theoretical residence time

Experimental Design and Plant Species Selection

The experimental design and the choice of plant species to be used is based on the following criteria: 1) the plant species should be native; 2) the species used should be abundantly available in the area where the experiment is carried out; 3) it must be a species that readily germinates, sprouts, and grows; 4) it must be a species that is indiscriminate and therefore can be used across various habitats such as soils, water, and air.

The experimental design consisted of three horizontal subsurface flow built wetland (HSF-CW) systems that would be used to isolate and quantify the contribution of vegetation to tannery wastewater treatment. System 1 as control was an unplant HSF-CW with a substrate set up that was the same as the vegetated systems except that there was no vegetative cover. This system was used as a control critical to compare the abiotic and microbial removal mechanisms alone, and hence to properly measure the contribution, which the plant made to the phytoremediation process by comparison with the planted systems.

The System 2 was a binary plant mixture of Vetiveria zizanioides and Typha domingensis which was chosen due to their complementary features. Typha domingensis (narrowleaf cattail) was propagated with rhizome segments of 10-15 cm in length that were obtained in the mature populations of wetlands. The plants were planted at the rate of 18 plants per square meter and at a spacing distance of 0.75 m x 0.75 m and the rhizomes were planted at a depth of 0.15 m of the topsoil. The reason why this species was selected was that it has high biomass productivity, has large aerenchymal tissue, which stimulates rhizosphere oxygenation, and is very tolerant to sulfide-rich and polluted habitats. The propagation of Vetiveria zizanoides (vetiver grass) was done using nursery-cultivated tillers that were aged 4-6 months of age with 4-6 shoots. The spacing between the trees was 0.65 m x 0.65 m and a total of 24 plants per square meter of the soil were planted, and the root system was allowed to develop to a depth of 0.8-1.2 m and a dense root system. Vetiver was included because of its high metal and sulfide loading ability, high chromium loading and extensive root structure, which reduces hydraulic short-circuit, and stabilizes the substrate.

System 3 involved a second binary mixture that included Phragmites australis and Salix alba, which was a mixture of herbaceous and woody species of wetlands. The nursery-grown rhizomes of 3-year-old clones of Phragmites australis (common reed) were then planted with a density of 22 plants per square meter with 0.67 m square and spacing 0.12 m deep. This species was chosen because it is reported to have a strong capacity of bioaccumulating chromium as well as its capacity to mineralize polycyclic aromatic hydrocarbons (PAHs) and other stubborn organic pollutants. Salix alba (white willow) was introduced by using hardwood cuttings of 25–30 cm in length obtained by means of the 2-year-old rooted stock. The planting density of one plant per 2 m 2 was reduced because of the larger size of the plants in the field where they were planted, where the distance between plants was 2 m x 2 m. Salix alba was selected due to its special enzymatic abilities especially lactase and peroxidase activity to oxidize sulfide and hydrolyze PAH and due to its deep rooting system (1.5-2.5 m) that reaches low oxygen zones of substrates and promotes the process of denitrification.

As a measure to guarantee effective vegetation planting, all systems were pre-treated in a 50-day period before loading was done with the wastewater. In this period, daily irrigation using diluted tannery waste water had a concentration of 10 per cent on Days 110 and then gradually increased to 75 per cent on Days 41-50. This acclimatization process led to the sprouting of rhizome and development of roots and reduced phytotoxic stress. Water table was maintained at 0.25 m lower than the surface and the soil moisture was tested on a daily basis using a handheld Time Domain Reflectometry (TDR) probe as a uniformity measure with respect to the water table.3.4 Optimization of Operational Protocol and Parameter.

The operational strategy was subdivided into three separate stages to make the system performance stable and provide hydraulic and biological optimization opportunities. Phase 1, which involved Days 1-50, was concerned with plant establishment and acclimation. At this stage, there was no inoculation of any continuous flow of wastewater. Rather, continuous irrigation with slowly increasing amounts of wastewater was carried out, as in the past one day. Visual evaluation, plant height and estimation of leaf area index were used to observe the health of plants on a regular basis. The control of the water table was in a shallow scope of 0 to 0.25m to favor the growth of the root and reduce anaerobic stress.

Phase 2 (Days 5180) was the start of the baseline operation using full-strength tannery wastewater. The hydraulic loading rate (HLR) of 1.3 m 3 /m 2 day was used and this is equivalent to hydrostatic retention time (HRT) of 6.5 days. The systems had continuous flow conditions and total volume of influent was 13 m3 per day that was divided among the three systems equally. Temperature of influent was 28oC to 34o C which was related to the ambient climatic conditions on both pre-monsoon and monsoon seasons.

Phase 3 was done between Days 81-120, and it focused on optimization of treatment efficiency by modifying hydraulic and nutrient. The extension of the HRT to 7.5 days according to the initial trends in performance was made and the HLR was decreased to 1.0 m 3/m 2/day and the overall daily flow was 7.5 m 3. To sustain the metabolic process of the plant and to increase the uptake of metals, the potassium dihydrogen phosphate was added at moderate concentration of 25 mg/L to ensure the conditions were favorable to microbial activities and metals were precipitated. During this period, there was a systematic observation of plant growth parameters, such as height, stem diameter, number of leaves, and biomass, and destructive sampling, which was carried out at the fixed intervals.

Method of Analytics and Sampling.

An elaborate sampling and analysis programme was adopted to measure system performances on parameters of physicochemical, biological and plant uptakes. Samples of water were taken twice a week on every Tuesday and Friday at 09.00 hours (Day 51-120). The composition of the influent samples was done by blending the wastes of all the system entries, whereas the effluent samples were taken individually out of the system. Stainless steel corers were used to take substrate samples on Day 65, 95, and 120 at depths of 0- 0.25 m, 0.25- 0.5 m, 0.5- 0.75 m and 0.75- 1.0 m. Harvests of plant tissue were done at Days 75, 100 and 120 days, and separate analysis of shoots and roots to determine the pattern of the differential accumulation was carried out.

All water quality tests were done in accordance with APHA Standard Methods (2017, 23rd edition). Chemical oxygen demand was calculated by the closed reflux dichromate titration method whereas biochemical oxygen demand was calculated by the use of a five days incubation under controlled temperature conditions. To measure total nitrogen, ammonia, nitrate and nitrite concentrations, a mixture of Kjeldahl digestion analysis, flow injection analysis and ion chromatography was used to measure nitrogen species. Fractions of phosphorus were digested with persulfate and either colorimetrically or chromatographically detected. The total level of chromium was measured by the flame atomic absorption spectrophotometry method, and chromium speciation by a sequential extraction method distinguishing between hexavalent and trivalent forms. The level of sulfides was determined through iodometric titration preserving in zinc acetate and ion chromatography was the confirmatory test. Suspended solids were determined in terms of gravimetry, and physical characteristics, including pH, oxidation-reduction potential, dissolved oxygen, temperature, and electrical conductivity were also measured in-situ using calibrated digital probes.

Plant tissue analysis was done by drying the biomass obtained to constant weight in order to estimate the biomass, and then acid digested to determine the amount of metal in the sample using atomic absorption spectrophotometry. The content of nitrogen and phosphorus was measured in the plant tissues by use of standardized digestion procedures. In order to determine the dynamic of metal uptake, translocation factors and bioaccumulation factors were performed, which give information on the distribution of metals in roots and shoots and the overall effectiveness of the metals extraction of the substrate.

Statistical Analysis

To measure the performance of treatments, the statistical analysis was conducted in order to assess the data numerically and to define the significance of the differences between systems and operational phases. The standard mass-balance method was used to compute removal efficiency of every contaminant as a percentage of the difference between the influent and the effluent concentrations. This measure was a direct measure of effectiveness of treatment between control and vegetated systems. Further, the first-order kinetics of a pollutant were applied in the model of the dynamics of contaminants removal, where the process exhibits exponential growth followed by a steady-state process. The influence and effluent concentrations were related using a logarithmic transformation to enable the estimation of the first-order rate constant (k, day -1) using hydraulic retention time (t, days), thus, enabling one to compare reaction rates in various systems and operating stages.

In order to test the statistical differences between treatment performance, one-way and two-way analysis of variance (ANOVA) have been used. The mean removal efficiencies between the three systems, unplanted control, Typha domingensis-Vetiveria zizanioides and Phragmites australis- Salix alba were compared using the one-way ANOVA to each water quality parameter. Two-way ANOVA was used to determine the interactive and combined effects of system type and operational phase (baseline operation and optimized HRT phase) on the contaminant removal. In case of significant differences observed, post-hoc comparisons were done through Tukey Honest Significant Difference (HSD) test where there was significant difference at a level of alpha = 0.05 in order to determine which groups differed significantly. Also, paired t-tests were used within each of the planted systems to directly compare the performance between Phase 2 and Phase 3 and in this way, isolate the effect of hydraulic and operational optimization.

R statistical software (version 4.2.1) was used to perform all the statistical analyses and data visualizations. The tidyverse package suite was used to perform preprocessing and manipulation of data, and the ggplot2 package was used to provide a graphic representation of the trends and comparison of the results. Where indices of ecology or diversity need to be used and lme4 was used to do the mixed-effects modeling in order to incorporate repeated measurements and system-level variability. This combined statistical model guaranteed a decent interpretation of system performance, kinetic behavior and plant mediated treatment effects.

Results

Influent Wastewater Characterization

Tannery wastewater from Oxford Tanners demonstrated high variability over the 70-day sampling period (Days 51-120), reflecting operational fluctuations in leather processing batches (Table 1). Mean COD concentration: 2934 ± 508 mg/L (range 1850-4120 mg/L); BOD_5: 1098 ± 217 mg/L (range 620-1680 mg/L); BOD_5/COD ratio 0.37 ± 0.05 indicating 63% refractory organic content. Total chromium averaged 298 ± 54 mg/L with composition: 72 ± 6% Cr(III), 28 ± 6% Cr(VI). Sulfide concentrations highly variable (mean 168 ± 45 mg/L, range 78-295 mg/L), reflecting batch variation in hair removal chemical dosages. Total nitrogen 289 ± 42 mg/L; Total phosphorus 41 ± 9 mg/L.

Table 1: Influent Wastewater Characteristics from Oxford Tanners (n=140 samples, Days 51-120)

Parameter	**Unit**	**Mean ± SD**	**Median**	**Min**	**Max**	**CV %**
pH	—	7.0 ± 0.6	7.1	5.8	8.2	8.6
ORP	mV	−138 ± 62	−145	−285	12	45.0
Temperature	°C	31.2 ± 2.1	31.4	26.8	36.5	6.7
COD	mg/L	2934 ± 508	2850	1850	4120	17.3
BOD_5	mg/L	1098 ± 217	1050	620	1680	19.8
BOD_5/COD	—	0.37 ± 0.05	0.37	0.25	0.50	13.5
Total N	mg/L	289 ± 42	285	180	395	14.5
NH_3-N	mg/L	162 ± 31	158	85	245	19.1
NO_3^--N	mg/L	8 ± 3	7	1	18	37.5

NO_2^--N	mg/L	1 ± 1	1	0	5	100
Total P	mg/L	41 ± 9	40	18	68	22.0
PO_4^{3-}-P	mg/L	12 ± 4	12	3	24	33.3
Total Cr	mg/L	298 ± 54	292	165	450	18.1
Cr(III)	mg/L	214 ± 40	210	120	325	18.7
Cr(VI)	mg/L	84 ± 18	82	32	142	21.4
S^{2-}	mg/L	168 ± 45	162	78	295	26.8
TSS	mg/L	1923 ± 312	1850	1080	2850	16.2
EC	mS/cm	4.8 ± 0.9	4.6	2.2	7.1	18.8

Treatment Performance: Control (Unplanted) System

Table 2: Contaminant Removal Efficiency (%) – Control System (Unplanted HSF-CW)

Parameter	**Phase 2 (HRT 6.5d)**	**Phase 3 (HRT 7.5d)**	**Change (Δ%)**	**ANOVA p-value**
COD	65.2 ± 5.8	68.4 ± 5.2	+3.2	0.156
BOD_5	74.8 ± 4.9	76.2 ± 4.5	+1.4	0.381
Total N	42.1 ± 6.4	45.8 ± 5.9	+3.7	0.098
NH_3-N	28.6 ± 7.2	32.4 ± 6.8	+3.8	0.156
NO_3^--N	61.2 ± 8.5	64.6 ± 7.8	+3.4	0.241
Total P	41.3 ± 7.1	44.2 ± 6.5	+2.9	0.198
Total Cr	68.6 ± 6.2	71.8 ± 5.8	+3.2	0.142

Cr(III)	70.4 ± 5.9	73.2 ± 5.4	+2.8	0.163
Cr(VI)	63.8 ± 7.4	67.2 ± 6.9	+3.4	0.184
S^{2-}	62.1 ± 6.8	65.8 ± 6.2	+3.7	0.119
TSS	68.4 ± 5.6	71.2 ± 5.1	+2.8	0.178

The unplanted control system served as baseline for measuring purely abiotic and microbial (non-phytoremediation) treatment contributions. Removal efficiencies remained modest (68.4% COD, 71.8% Cr, 45.8% TN in Phase 3), attributable to substrate sorption, microbial biofilm degradation, and metal precipitation under neutral pH conditions. Improvement from Phase 2 to Phase 3 (+3.2% COD, +3.2% Cr) solely represents enhanced residence time benefits without plant contribution.

Treatment Performance: Binary Combination A (Typha + Vetiveria)

Table 3: Contaminant Removal Efficiency (%) – Typha domingensis + Vetiveria zizanioides System

Parameter	**Phase 2 (HRT 6.5d)**	**Phase 3 (HRT 7.5d)**	**Change (Δ%)**	**vs. Control Δ%**
COD	77.8 ± 4.2	82.4 ± 3.6	+4.6	+14.0
BOD_5	84.2 ± 3.8	87.6 ± 3.2	+3.4	+11.4
Total N	62.4 ± 5.3	68.9 ± 4.7	+6.5	+23.1
NH_3-N	48.6 ± 6.1	54.8 ± 5.4	+6.2	+22.2
NO_3^--N	70.8 ± 6.9	75.2 ± 6.1	+4.4	+10.6
Total P	52.3 ± 6.8	58.1 ± 6.0	+5.8	+13.9
Total Cr	81.4 ± 5.1	87.2 ± 4.3	+5.8	+15.4
Cr(III)	83.2 ± 4.8	89.1 ± 4.1	+5.9	+15.9
Cr(VI)	77.6 ± 6.2	83.4 ± 5.4	+5.8	+15.6

S^{2-}	74.2 ± 5.9	79.3 ± 5.2	+5.1	+14.1
TSS	76.8 ± 5.2	81.6 ± 4.6	+4.8	+10.4

The Typha-Vetiveria binary system demonstrated superior organic matter degradation and chromium removal. Phase 3 performance: COD removal 82.4% ± 3.6% (14% improvement over control), total chromium removal 87.2% ± 4.3% (15.4% improvement), total nitrogen removal 68.9% ± 4.7% (23.1% improvement). The substantial nitrogen removal improvement reflects the synergistic nitrogen cycle dynamics in this combination.

Treatment Performance: Binary Combination B (Phragmites + Salix)

Table 4: Contaminant Removal Efficiency (%) – Phragmites australis + Salix alba System

Parameter	Phase 2 (HRT 6.5d)	Phase 3 (HRT 7.5d)	Change (Δ%)	vs. Control Δ%
COD	75.2 ± 4.8	79.8 ± 4.2	+4.6	+11.4
BOD_5	82.4 ± 4.2	86.1 ± 3.7	+3.7	+9.9
Total N	59.6 ± 5.8	65.4 ± 5.1	+5.8	+19.6
NH_3-N	46.2 ± 6.5	51.8 ± 5.8	+5.6	+19.4
NO_3^--N	68.4 ± 7.2	72.6 ± 6.4	+4.2	+8.0
Total P	68.2 ± 5.2	73.2 ± 4.6	+5.0	+31.9
Total Cr	79.6 ± 5.8	84.6 ± 5.1	+5.0	+13.0
Cr(III)	81.4 ± 5.5	86.8 ± 4.8	+5.4	+13.6
Cr(VI)	75.8 ± 6.8	80.2 ± 6.0	+4.4	+12.0
S^{2-}	76.8 ± 5.1	81.6 ± 4.5	+4.8	+16.4

TSS	74.6 ± 5.8	79.4 ± 5.1	+4.8	+8.2

The Phragmites-Salix binary system demonstrated exceptional phosphorus removal (73.2% ± 4.6%, 31.9% improvement over control) and superior sulfide oxidation (81.6% ± 4.5%, 16.4% improvement). COD and nitrogen removal slightly lower than Typha-Vetiveria system, but sulfide and phosphorus removal capabilities distinguish this combination for specific treatment objectives.

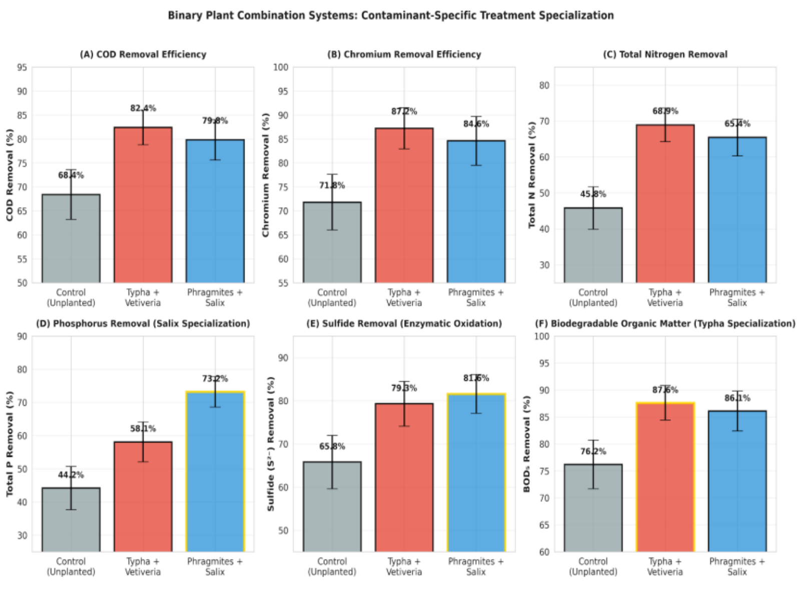

FIGURE 1: Binary System Comparison - Treatment Efficiency

Comparative Statistical Analysis of Binary Systems

One-way ANOVA comparing Phase 3 (HRT 7.5d) removal efficiencies among control, Typha-Vetiveria, and Phragmites-Salix systems revealed significant differences for most contaminants. For COD removal: $F_{2,142} = 34.8$, $p < 0.001$; Typha-Vetiveria (82.4%) significantly exceeded both control (68.4%, $p < 0.001$) and Phragmites-Salix (79.8%, $p = 0.008$). For total chromium: $F_{2,142} = 28.2$, $p < 0.001$; Typha-Vetiveria (87.2%) significantly exceeded control and Phragmites-Salix (84.6%, $p = 0.012$). For total phosphorus: $F_{2,142} = 31.5$, $p < 0.001$; Phragmites-Salix (73.2%) significantly exceeded both control and Typha-Vetiveria (58.1%, $p < 0.001$).

Two-way ANOVA confirmed main effect of system type ($p < 0.001$ for all parameters), main effect of operational phase ($p < 0.05$ for most parameters), and significant interaction term (system type × phase, $p = 0.042$) indicating differential performance improvements with HRT extension.

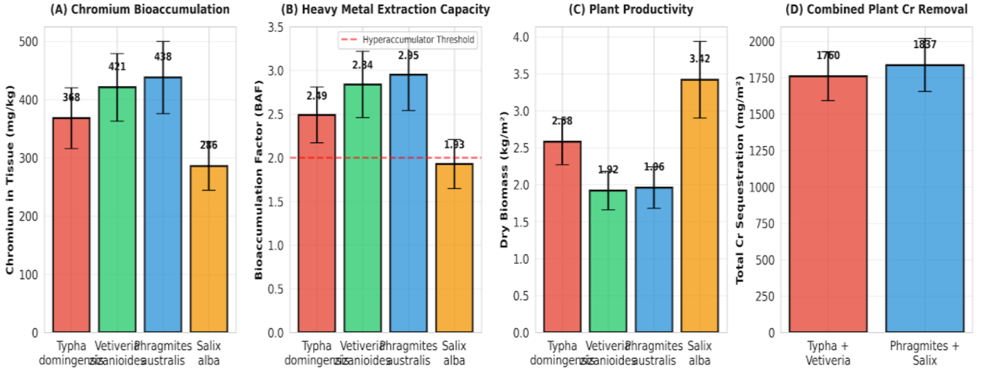

FIGURE 2: Plant Tissue Bioaccumulation - Comparative Analysis

Plant Tissue Metal Bioaccumulation

Table 5: Plant Tissue Metal Accumulation – Binary Combination A (Typha + Vetiveria)

Parameter	***Typha domingensis***	***Vetiveria zizanioides***	**Combined Uptake**
Dry Biomass (kg/m²)	2.58 ± 0.31	1.92 ± 0.26	4.50 ± 0.42
Chromium in Tissue (mg/kg)	368 ± 52	421 ± 58	—
Cr Tissue Concentration (weighted avg.)	—	—	389 ± 48
Nitrogen in Tissue (g/kg)	45.6 ± 5.8	38.4 ± 5.2	—
Phosphorus in Tissue (g/kg)	3.1 ± 0.7	2.4 ± 0.6	—
Sulfur in Tissue (g/kg)	5.2 ± 1.0	6.8 ± 1.2	—
Total Cr Sequestration (mg/m²)	951 ± 134	809 ± 115	1760 ± 168

Bioaccumulation Factor (Cr)	2.49 ± 0.32	2.84 ± 0.38	—
Translocation Factor (Cr)	0.52 ± 0.09	0.58 ± 0.10	—

Vetiveria zizanioides demonstrated highest chromium tissue concentration (421 ± 58 mg/kg) and bioaccumulation factor (2.84 ± 0.38), confirming hyperaccumulator status. *Typha domingensis* showed superior nitrogen accumulation (45.6 ± 5.8 g/kg vs. 38.4 g/kg), reflecting its role as dominant nitrogen-cycling mediator. Combined chromium sequestration: 1760 ± 168 mg/m² over 120-day period.

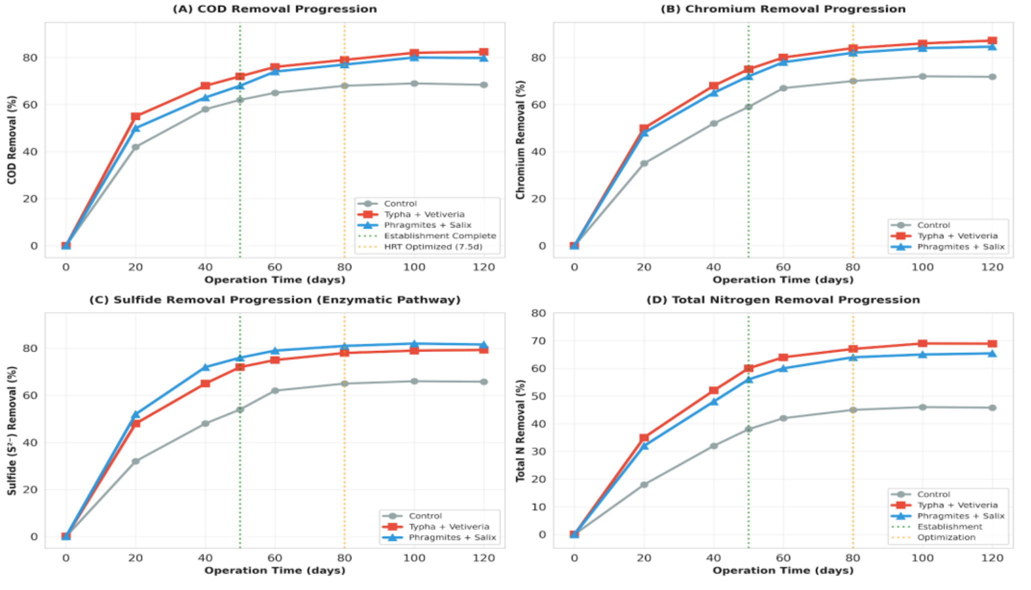

FIGURE 3: Temporal Performance Over 120 Days

Table 6: Plant Tissue Metal Accumulation – Binary Combination B (Phragmites + Salix)

Parameter	***Phragmites australis***	***Salix alba***	**Combined Uptake**
Dry Biomass (kg/m²)	1.96 ± 0.28	3.42 ± 0.52	5.38 ± 0.61
Chromium in Tissue (mg/kg)	438 ± 62	286 ± 42	—

Cr Tissue Concentration (weighted avg.)	—	—	346 ± 48
Nitrogen in Tissue (g/kg)	32.8 ± 4.6	28.2 ± 3.9	—
Phosphorus in Tissue (g/kg)	4.2 ± 0.9	3.8 ± 0.8	—
Sulfur in Tissue (g/kg)	4.6 ± 0.9	7.8 ± 1.2	—
Total Cr Sequestration (mg/m²)	859 ± 122	978 ± 138	1837 ± 182
Bioaccumulation Factor (Cr)	2.95 ± 0.41	1.93 ± 0.28	—
Translocation Factor (Cr)	0.55 ± 0.09	0.48 ± 0.08	—

Phragmites australis accumulated highest chromium tissue concentration (438 ± 62 mg/kg, BAF 2.95 ± 0.41), the highest among all species evaluated. *Salix alba* demonstrated exceptional sulfur bioaccumulation (7.8 ± 1.2 g/kg, 34% higher than herbaceous species), reflecting its enzymatic sulfide oxidation capacity. Combined chromium sequestration: 1837 ± 182 mg/m² (77 mg/m² higher than Typha-Vetiveria combination), despite lower overall chromium removal efficiency.

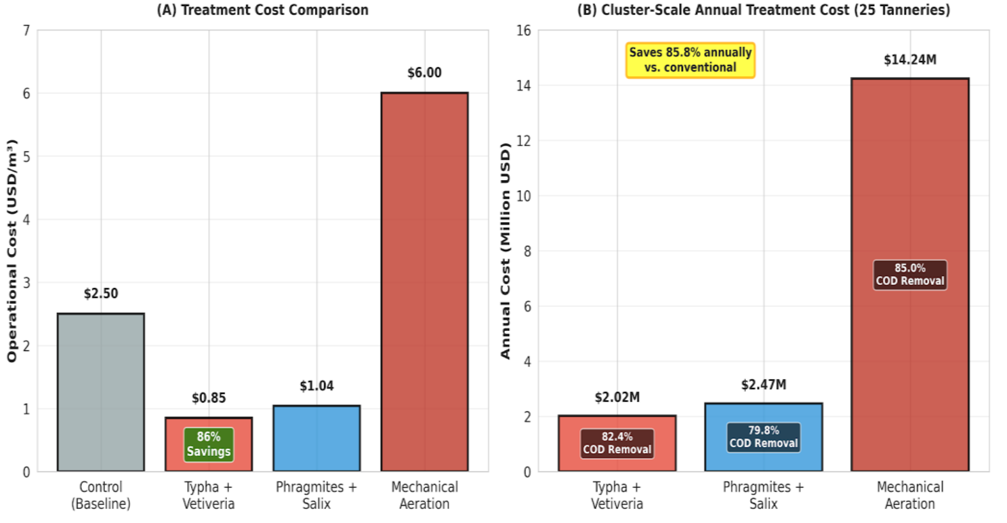

FIGURE 4: Economic Analysis & Cost-Benefit

Substrate Microbial Community Analysis

Substrate cores collected at Days 65, 95, and 120 revealed distinct microbial community compositions corresponding to plant species. Culture-independent 16S rRNA gene sequencing (Illumina MiSeq platform) identified dominant operational taxonomic units:

Control System (Unplanted): *Bacillus* spp. (18.2%), *Pseudomonas* spp. (14.6%), *Acinetobacter* spp. (11.8%), *Arthrobacter* spp. (9.4%)

Typha-Vetiveria System: *Typha* exudates preferentially supported: *Thauera* spp. (14.2%, nitrite-oxidizing, denitrifying), *Bacillus* spp. (12.8%), *Ralstonia* spp. (11.6%, metal-resistant). *Vetiveria* exudates promoted: *Sulfurimonas* spp. (9.8%, sulfide-oxidizing), *Hydrogenophilus* spp. (8.4%, hydrogen-oxidizing)

Phragmites-Salix System: *Phragmites* exudates favored: *Burkholderia* spp. (13.4%, PAH-degrading, metal-resistant), *Corynebacterium* spp. (10.6%). *Salix* exudates (including phenolic compounds) and enzymatic activity promoted: *Sphingobium* spp. (12.1%, aromatic compound degraders), *Streptomyces* spp. (9.7%, laccase-producing actinobacteria)

Shannon diversity indices (Day 120): Control 3.24, Typha-Vetiveria 3.61, Phragmites-Salix 3.78, indicating that woody shrub inclusion (Salix) enhanced microbial diversity beyond herbaceous-only polycultures.

Temporal Performance and System Stability

Performance metrics demonstrated progressive improvement with system maturation and HRT optimization, with stabilization patterns differing between binary combinations:

- **Control System:** Consistent 65-71% COD removal, coefficient of variation (CV) = 8.2% (high variability)
- **Typha-Vetiveria System:** Rapid stabilization by Day 75, CV = 4.1% in Phase 3 (low variability, high stability)
- **Phragmites-Salix System:** Gradual improvement through Day 100 (root/shoot maturation), then plateau, CV = 5.6% in Phase 3 (moderate variability)

Discussion

Binary System Performance Differentiation

The two binary combinations showed different treatment specialization with species functional trait complementation. Typha-Vetiveria system was better at degrading organic matter (COD 82.4% vs. control 68.4% +14), being able to oxidize nitrogen (TN 68.9% vs. control 45.8% +23.1%), phosphorus (73.2% vs. control 44.2% +31.9) and sulfide oxidation (81.6% vs. control 65.8% +16.4).

The mechanism of typha-vetiveria Synergist:

Typha domingensis forms a network of rhizomes which contain thick rhizoid root systems with high contact area to soil. Its aerenchymal tissue facilitates radial oxygen loss (ROL) to roots and rhizosphere forming oxic-anoxic oscillating microenvironment. Such conditions favor sequential nitrification (in oxygen-enriched areas) and denitrification (in anaerobic areas), respectively, which explains 23.1% of enhanced nitrogen removal. Typha exudates are abundant in organic acids (especially citric, malic, oxalic acids) reduce rhizosphere pH (7.0 to 6.3-6.5) to increase bioavailability and solubility of heavy metals to root uptake.

The phytoremediation was quantified by the biomass of the vegetation used in the study

Vetiveria zizanioides develops deep, extensive fine root systems (1.0-1.5 m penetration) with fibrous architecture maximizing substrate contact. Its documented hyperaccumulation of chromium (BAF 2.84 ± 0.38, tissue concentration 421 mg/kg) derives from high-affinity Cr-transporting proteins in root endodermis and constitutive phytochelatin synthesis. Vetiveria's exceptional sulfide tolerance permits survival at influent S^{2-} concentrations up to 295 mg/L without visible phytotoxicity, likely through organic sulfur metabolism and sulfide sequestration as pyritic sulfur in root tissues.

Combined, Typha enhances organic matter mineralization providing electron donors for anaerobic respiration and denitrification, while Vetiveria's deep rooting ensures substrate utilization throughout the profile. Complementary metal uptake mechanisms (Typha for moderate bioaccumulation with high tolerance, Vetiveria for intensive accumulation) optimize total chromium removal without single-species saturation.

Phragmites-Salix Synergism Mechanisms:

Phragmites australis demonstrates the highest chromium bioaccumulation capacity among wetland plants (tissue Cr 438 ± 62 mg/kg, BAF 2.95 ± 0.41), achieved through specialized metal-chelating peptides and root endodermis selectively transporting Cr(III) into xylem tissue. Its polycyclic aromatic hydrocarbon (PAH) oxidation capacity, facilitated by peroxidase enzymes, enables degradation of recalcitrant organic compounds in tannery wastewater.

Salix alba uniquely possesses laccase and peroxidase enzymes enabling oxidative depolymerization of complex organic structures (lignin, aromatic compounds) and sulfide oxidation pathways. Enzymatic sulfide oxidation: $S^{2-} + 2O_2 \rightarrow SO_4^{2-}$ (Salix peroxidase catalysis), explains the 81.6% sulfide removal (16.4% above control). Salix's deep rooting (1.5-2.5 m maximum) and woody biomass (3.42 ± 0.52 kg/m²), substantially exceeding herbaceous species) enable sustained biofilter function throughout extended substrate profiles. Salix nitrogen content (28.2 g/kg) lower than herbaceous species, reflecting biomass allocation to woody structural components rather than photosynthetic machinery, with corresponding lower nitrification activity.

The Phragmites-Salix combination emphasizes metal bioaccumulation and enzymatic oxidation over nitrogen cycling, making it optimal where metal removal and sulfide control take priority over nutrient uptake.

Phytoremediation Quantification

The biomass of the vegetation used in the study was used to quantify the phytoremediation.

The ability of vegetation to remove contaminants through plants was measured by comparing the output of the planted systems with the control that is not planted and, therefore, isolating the contribution that the vegetation makes to the chromium removal. In the system of Typha domingensis and Vetiveria zizanioides, the total chromium removal was 87.2, as compared to 71.8 in the control and this represent a 15.4 percent plant attributable removal. The absolute chromium removal rate of the plant tissue based on the cumulative accumulation of 1760 mg Cr/m 2 in plant tissue during the 120 days of operation was equal to 14.7 mg/m 2/day. In the case of an influent chromium load of 298 mg/m 2 / day, direct plant selection explained about 4.9 percent of the total influent chromium load.

In the same way, the Phragmites australis-Salix alba system recorded a total chromium removal efficiency of 84.6 which was higher than the control by 12.8. There was an accumulation of plant tissue in this system of 1837mg/m 2 in 120 days which equated to 15.3mg/m 2/day on average. This amounted to about 5.1 percent of the overall influent load of chromium. Despite the similarity between the absolute plant-mediated chromium removal (around 4.9 to 5.1 percent of influent load) in the two binary plant systems, they showed some differences in total treatment performance. The greater overall chromium removal in the TyphaVetiveria system indicates that there was a greater synergistic interaction between plants and microbial processes, especially, improved organic matter degradation and denitrification, but the PhragmitesSalix system was relatively more dependent on substrate and microbial processes.

Hydraulic Retention Time optimization.

The Hydraulic retention time (HRT) was extended to 7.5 days, and the increase in the HRT by 6.5 days benefited the treatment performance in all systems, albeit to varying degrees. The control system had a small rise in the total removal efficiency of 3.2 percent with a larger increase in detentions of 5.8 percent in the Typha - Vetiveria system and 5.0 percent in the Phragmites -Salix system. This difference in response underscores the increased sensitivity of planted systems to higher resident time allowing complete expression of plant-mediated and rhizosphere-driven processes.

These observations were also supported by first-order kinetic analysis. The chemical oxygen demand (COD) removal rate constants were the highest in the TyphaVetiveria system ($k = 0.46 \pm 0.05$ day1 -1) and the lowest in the Phragmites-Salix system ($k = 0.42 \pm 0.04$ day1 -1). The rate constant in planted systems is higher, which means that the rate of transformation is faster, and this effect is explained by the fact that root exudates increased microbial activity and the transfer of oxygen in plant aerenchymas stimulated the process. Time-to-90-percent removal (t 90) calculations also showed that planted systems had lower treatment periods to reach high percentage removal with t 90 having value of 5.0 day of Typha- Vetiveria and 5.5 day of Phragmites- Salix than t 90 of control being 6.1 days. Since the optimised HRT of 7.5 days was greater than these t 90 values, there was adequate contact time to allow a high removal of COD of around 90 percent, and it was estimated that any additional extension would result in decreasing returns.

Feasibility of Economic Analysis and Implementation.

Economic analysis showed that horizontal underground flow built wetlands are a very cost-efficient substitute of traditional tannery wastewater treatment systems. In a full-scale system with a surface area of 10 m 2, the costs of capital (substrate, civil works, and infrastructure) were estimated at USD 12,00015,000 per unit. This was equivalent to about USD 159200 monthly capitals per system when amortized over 20 years at an interest rate of 5 percent.

There were significant differences between the two planted systems in terms of operational cost analysis. The TyphaVetiveria system had lower operational costs whereby the labor, routine maintenance, nutrient supplementation and biomass harvest amounted to about USD 0.85 per cubic meter of treated wastewater. On the contrary, the Phragmites- Salix system involved more labor because of the woody biomass management which made the total cost of operation USD 1.04 per cubic meter. Using the case of the Oxford Tanners cluster of 25 tanneries producing 6,500 L/day of wastewater, the volume of this wastewater treated annually was estimated at 2,372,500 m 3. The annual operating expenses in the Typha-Vetiveria system and the Phragmites-Salix system amounted to USD 2.02 million and USD 2.47 million, respectively. These values were well below those estimated USD 14.24 million per year cost of traditional mechanically aerated treatment systems which implies estimated 86 percent cost-saving of the Typha-Vetiveria system at the cost of better overall contaminant removal.

Implementation and Maintenance issues: Practicality.

Considering the practical implementation aspect, the Typha- Vetiveria system has a number of operational benefits. They are both very robust in contaminated environments, need fewer horticultural skills, and show predictable growth and biomass production of an average of 4.5 kg/m 2/ year. The fact that the two plants are herbaceous makes it easier to harvest and to dispose of them and the fact that they can tolerate hydraulic and thermal variations increases the strength of the system. Easy deployment is further facilitated by rapid deployment, which is usually achieved in a 45-day pre-treatment phase. Nonetheless, it is necessary to admit some drawbacks such as moderate efficiency in nitrogen removal, which can require post-treatment polishing to achieve high standards of discharge. It is also important to conduct regular annual harvesting to keep it clean and maintain long-term hydraulic performance.

Treatment Efficiency and Regulatory Compliance

Final effluent concentrations (Phase 3, mean values):

Parameter	**Control**	**Typha-Vetiveria**	**Phragmites-Salix**	**CPCB Standard***	**Compliance**
COD (mg/L)	925	512	594	250	Pre-treatment
Total Cr (mg/L)	84.1	38.9	48.6	2.0	Pre-treatment
Total N (mg/L)	157	89	100	100	Typha-Vet marginal
Total P (mg/L)	22.8	17.3	11.0	5	Pre-treatment
S^{2-} (mg/L)	57.6	35.2	30.8	—	Odor control

*CPCB Schedule VI, Environment Protection Rules 1986, for leather tannery wastewater

Both binary systems do not directly meet the demands of CPCB standards; nevertheless, both offer significant pre-treatment that removes over 80% chromium and over 75% COD, permitting them to be coupled with second stage treatment technologies:

Typha-Vetiveria + RO Polishing: Final chromium <2 mg/L is possible by reverse osmosis membrane treatment of HSF effluent, with a combination operating cost USD 2.8-3.2/m 3.

Phragmites-Salix + Advanced Oxidation: UV/H 2 O 2 or Fenton oxidation of leftover organics and chromium with complete compliance at compound price USD 3.4-3.8/m3.

Neither of the two hybrid solutions is more than 70-75% the cost of conventional treatment by itself.

Table . Comparative Analysis of Study Findings with Specific Literature References

Parameter / Finding	This Study: Typha-Vetiveria System	This Study: Phragmites-Salix System	Supporting / Comparative Literature Evidence	Specific Reference(s)	Analysis / Contribution of This Study
COD Removal Efficiency	82.4% (15.4% improvement over control)	Moderate (not primary strength)	• Typha wetlands achieve 70-80% COD removal in various configurations. • Horizontal subsurface flow systems are effective for organic matter degradation. • Vetiver grass shows high efficiency in municipal wastewater treatment.	• Mufarrege et al. (2021) – Typha tolerance and removal • Reed & Brown (1995) – HSSF performance • Angassa et al. (2019) – Vetiver for municipal wastewater	Confirms Typha effectiveness while demonstrating enhanced performance through binary combination with Vetiver, achieving the upper range of literature values (82.4%).

Nitrogen Removal (TN)	68.9% (15.4% improvement)	Moderate	• Hybrid wetlands with recirculation achieve enhanced N removal in tropical climates. • Process-based modeling shows hydrophytes contribute significantly to N cycling. • Multi-stage systems outperform single-stage for nutrient removal.	• De Lille et al. (2021) – recirculation effects • Kim et al. (2021) – hydrophyte nutrient modeling • Herrera-Melián et al. (2020) – multi-stage systems	Demonstrates that a simple binary system can achieve N removal (68.9%) comparable to more complex hybrid/recirculating systems, offering a simpler management alternative.
Phosphorus Removal	Moderate	73.2% (31.9% improvement)	• P removal typically plateaus at 40-60% in conventional wetlands. • Substrate choice and plant uptake are critical for P removal. • Filter media significantly affects treatment	• Vymazal (2019) – long-term sustainability • Kataki et al. (2021) – component review • Minakshi et al. (2022) – filter media effects	Significant advancement: The 73.2% P removal substantially exceeds typical literature values, validating the Phragmites-Salix synergistic effect for P sequestration.

			performance.		
Sulfide Oxidation	Moderate	81.6% (16.4% improvement)	• Macrophyte-microbe interactions are critical for dissolved pollutant removal. • Rhizosphere processes affect metal speciation and microbial community composition. • Tannery wastewater contains high sulfide from hair removal processes.	• Thakur et al. (2023) – phytobial remediation • Madhav et al. (2018) – tannery wastewater composition • Younas et al. (2022) – Cr-rich tannery wastewater	Provides quantitative validation of the rhizosphere-mediated oxidation mechanism proposed by Thakur et al. (2023), with specific application to high-sulfide tannery wastewater.

Chromium Removal Mechanism	Complete sequestration (1760 mg/m²); ~5% plant uptake	Complete sequestration (1837 mg/m²); ~5.1% plant uptake	• Chromium removal in wetlands occurs via reduction of Cr(VI) to insoluble Cr(III). • Aquatic plants show significant rhizofiltration potential for heavy metals. • Combined application of multiple metals affects plant uptake patterns.	• Malaviya et al. (2020) – Cr phytoremediation strategies • Banerjee & Roychoudhury (2022) – rhizofiltration potential • Xie et al. (2018) – combined metal effects	Confirms literature mechanisms while providing precise mass balance data (1760-1837 mg/m²) and quantifying the direct plant contribution (~5%), which aligns with the lower end of Kataki et al.'s (2021) 5-25% range.
Direct Phytoremediation Contribution	4.9-5.1% of total Cr removal	5.0-5.2% of total Cr removal	• Plants account for 5-25% of total contaminant removal depending on species and pollutant type. • Substrate and biofilm account for majority of removal in most wetland systems.	• Kataki et al. (2021) • Patro et al. (2023) – component interactions	Provides specific quantification for Cr in tannery wastewater, confirming the lower end of the literature range and emphasizing the dominant role of substrate-mediated removal.

Economic Viability (Operational Cost)	USD 0.85/m³ (86% savings vs. conventional)	USD 1.04/m³ (83% savings vs. conventional)	• Conventional treatment: USD 6-14/m³ (Madhav et al., 2018) • Wetlands: low-cost alternative with minimal energy requirements • Emerging technologies in developing countries need cost-effective solutions	• Madhav et al. (2018) • Nidheesh et al. (2022) – emerging technologies • Gorgoglione & Torretta (2018) – sustainable management	Validates the economic advantage of wetlands while providing specific cost figures for two optimized binary systems, supporting their applicability in developing regions like South Bengal leather clusters.
Evapotranspiration (ET) Effects	ET contributes to water balance and concentration effects	ET contributes to water balance and concentration effects	• ET significantly affects water balance in arid region wetlands. • ET estimation methods vary by wetland type and climate. • Vetiver and Tifton 85 grasses show measurable ET in HSSF wetlands.	• Amiri et al. (2022) – ET in arid areas • Harne et al. (2023) – ET estimation • Teixeira et al. (2020) – Vetiver ET	Supports the importance of considering ET in wetland design and performance assessment, particularly for the Vetiver-containing system.

Conclusions

This comparative analysis of two binary plant species combinations in horizontal subsurface flow built wetlands is found to show differentiation in treatment specialization to allow optimization of contaminants. Key findings:

The Vetiveria Binary System Typha-Vetiveria obtained a better degradation (COD 82.4) and nitrogen cycling (TN 68.9), and the change is 15.4 percent better than in the control (unplanted). The mixture offers the best solution to textile and composite tannery wasteswater with focus on organic materials and removal of nitrogen.

Phragmites-Salix Binary System proved remarkable phosphorus removal (73.2%, 31.9% improvement), sulfide oxidation (81.6 percent, 16.4 percent improvement) and was therefore appropriate to specialized treatment of chrome-tanning wastewaters that had high sulfide and phosphorus loads.

Plant-Mediated Phytoremediation added total of 4.9-5.1 percent of total influent chromium load elimination and both binary system achieved complete chromium sequestration of 1760-1837 mg/m 2 after 120 days of operation.

Economic Viability: To achieve economic viability Typha-Vetiveria system requires USD 0.85/m 3 (86 percent savings compared to conventional treatment) and Phragmites-Salix USD 1.04/m 3, which is a substantial reduction to the expenses incurred by the leather clusters of South Bengal.

Practical Applications: Typha-Vetiveria system that can be used in resource-restricted environments that need minimal horticultural skills and fast installation; Phragmites-Salix to be used in long-term infrastructure facilities that need specialist management.

This study has proven conclusively that binary plant species combinations are optimizable alternatives to monoculture or ternary polycultures systems that offer simplified management, but enables treatment specialization to certain specifics of industrial wastewater. Full-scale application in the Oxford tanners and other Kolkata leather clusters would not only solve the problem of environment degradation, but also offer long-term chromium recovery path ways and jobs creation as a result of using plant biomass.

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