

Performance of Vertical Subsurface Flow Constructed Wetlands for Mixed Wastewater Treatment: Physicochemical and Microbiological Assessment

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DOI: <https://doi.org/10.51244/IJRSI.2026.1308000315>

Received: 07 September 2026; Accepted: 12 September 2026; Published: 23 September 2026

ABSTRACT

The growing generation of mixed domestic wastewater necessitates effective decentralized treatment approaches. This study evaluated the treatment performance of vertical subsurface-flow constructed wetlands (VSSFCWs) receiving real mixed black and greywater from an institutional campus. Three comparable pilot-scale systems were operated under identical hydraulic and substrate conditions, comprising wetlands planted with *Phragmites australis*, *Arundo donax*, and an unplanted control. Treatment performance was assessed using physicochemical, nutrient, and microbiological indicators, with statistical and multivariate analyses to evaluate treatment consistency and pollutant interrelationships. All systems concurrently attenuated organic matter, solids, nutrients, and microbial indicators, confirming the multifunctional treatment capacity of the VSSFCW configuration. The *P. australis*-planted system exhibited the strongest treatment response, with mean removal efficiencies of 81.18% for chemical oxygen demand (COD), 75.29% for biochemical oxygen demand (BOD), 67.68% for total suspended solids (TSS), 65.50% for nitrate, and 67.25% for phosphate. The *A. donax* system showed intermediate performance, whereas the unplanted control exhibited comparatively lower removal. The treatment hierarchy remained statistically consistent across sampling events, and strong positive associations among water-quality parameters indicated coordinated pollutant attenuation. Principal component analysis further differentiated influent and treated wastewater, with the first principal component explaining 98.53% of the total variance. Overall, the findings demonstrate that vegetation can enhance the treatment functionality of VSSFCWs through the combined influence of the substrate, rhizosphere, and associated microbial processes. The study highlights the potential of vegetated VSSFCWs as a decentralized treatment strategy for complex institutional wastewater and emphasizes the importance of species selection under site-specific operating conditions.

Keywords: *Arundo donax*; Constructed wetlands; Microbial attenuation; Nutrient removal; *Phragmites australis*; Pollutant removal; Vertical flow

INTRODUCTION

Rapid urbanization, population growth, and increasing water consumption continue to intensify wastewater generation and place growing pressure on existing sanitation infrastructure. This challenge is particularly important in developing regions, where centralized wastewater treatment may be constrained by high infrastructure requirements, limited sewer connectivity, and operational limitations. Decentralized wastewater treatment systems (DEWTS) provide an alternative approach by treating wastewater closer to its source and reducing dependence on extensive collection and conveyance infrastructure. Recent studies have highlighted constructed wetlands (CWs) as important decentralized treatment technologies because of their ability to integrate physical, chemical, and biological treatment processes within relatively simple treatment configurations (Werkneh, 2024; Wu et al., 2023). Their potential for application at small communities, institutional facilities, and other decentralized locations makes them particularly relevant where conventional centralized treatment is difficult to implement.

Domestic and institutional wastewater contains a complex mixture of organic matter, suspended solids, dissolved constituents, nutrients, and microorganisms. Blackwater represents a particularly concentrated wastewater fraction because it contains human excreta and is a major source of organic matter and nutrients in domestic wastewater. It also contains a substantial proportion of pathogenic microorganisms, making inadequate treatment a potential environmental and public-health concern (Zhang et al., 2023). When blackwater is combined with greywater, the resulting wastewater contains pollutants from both toilet-derived and non-toilet sources. This creates a more complex treatment matrix in which organic loading, suspended solids, dissolved salts, nutrients, and microbial indicators can vary considerably with water use, occupancy, and daily activities. Such variability is especially relevant for institutional wastewater because wastewater generation is closely associated with population density, building use, and temporal patterns of water consumption. Consequently, evaluating treatment systems using real mixed wastewater provides a more representative assessment of their practical performance than studies based exclusively on synthetic wastewater.

Constructed wetlands have emerged as nature-based treatment systems that harness the combined activity of filter media, microorganisms, vegetation, and hydraulic processes to remove pollutants. Their treatment performance is governed by interactions among filtration, sedimentation, adsorption, biodegradation, plant uptake, and biogeochemical transformations within the wetland matrix (Wu et al., 2023). Among the major configurations, vertical subsurface-flow constructed wetlands (VSSFCWs) are particularly relevant for wastewater with appreciable organic and suspended-solid loads. In a VSSFCW, wastewater is intermittently distributed over the upper surface and moves vertically through a porous filter bed. This configuration promotes repeated contact among wastewater, substrate, microbial biofilms, and plant roots while facilitating air exchange within the pore spaces. The resulting oxygen availability in the upper and intermediate portions of the bed can support aerobic microbial processes, while spatially variable redox conditions can permit complementary transformations in deeper regions. Consequently, VSSFCWs can simultaneously address multiple pollutant groups through interconnected physical, chemical, and biological pathways (Wu et al., 2023; Werkneh, 2024). The filter substrate is a fundamental component of VSSFCW performance because it determines both the hydraulic characteristics of the bed and the extent of contact between wastewater and the treatment matrix. In addition to providing structural support, the substrate acts as a filtration medium and provides surfaces for microbial attachment and biofilm development. Its physical properties, including particle size, porosity, specific surface area, and hydraulic conductivity, influence wastewater distribution, oxygen transfer, and microbial colonization. The substrate's chemical characteristics can further determine its capacity for adsorption, precipitation, and other contaminant-retention processes (Wang et al., 2024). Thus, substrate selection requires a balance between adequate hydraulic conductivity and sufficient reactive surface area. Inappropriate media characteristics can impair flow distribution or limit pollutant–substrate interactions, whereas an appropriately selected substrate can support stable hydraulic movement while providing a favourable environment for microbial and physicochemical treatment processes. Organic matter and suspended solids are among the principal pollutant groups targeted by VSSFCWs. Suspended solids can be retained through filtration and sedimentation as wastewater passes through the porous medium. Organic matter, commonly represented by biochemical oxygen demand (BOD) and chemical oxygen demand (COD), can subsequently undergo biodegradation, adsorption, and physical retention. The relatively oxygenated conditions associated with vertical-flow systems can favour aerobic microbial degradation of biodegradable organic compounds. Field-scale and pilot-scale studies have demonstrated substantial organic-matter and solids removal in vertical-flow and other constructed-wetland configurations, although performance varies with hydraulic loading, influent characteristics, substrate properties, temperature, and system configuration (Wu et al., 2023; Werkneh, 2024). Nutrient removal is more complex because nitrogen and phosphorus are governed by different transformation and retention pathways. Nitrogen removal may involve nitrification, denitrification, microbial assimilation, plant uptake, and other microbial nitrogen transformations. The oxygen-rich conditions promoted by vertical flow can favour nitrification, whereas denitrification requires relatively oxygen-limited microsites and suitable electron-donor conditions. Therefore, nitrogen removal in VSSFCWs depends strongly on the spatial development of contrasting redox conditions within the filter bed. Nutrient-removal performance can also be modified by substrate characteristics, hydraulic operation, carbon availability, and vegetation (Kamilya et al., 2022; Huang et al., 2024). Phosphorus follows a different removal trajectory and is commonly associated with adsorption onto substrate surfaces, precipitation, microbial assimilation, and plant uptake. Unlike biological nitrogen

transformations, phosphorus retention is strongly dependent on the available sorption and precipitation capacity of the substrate and may decrease as reactive sites become progressively occupied (Wang et al., 2024).

Microbiological quality represents another important dimension of wastewater treatment because conventional physicochemical indicators alone do not adequately describe sanitary risk. Constructed wetlands can reduce microbial indicators through a combination of filtration, sedimentation, adsorption, predation, natural die-off, and interactions with microbial communities within the substrate and rhizosphere. The extent of microbial removal is influenced by hydraulic retention, filter-media characteristics, temperature, vegetation, and wastewater composition. Consequently, simultaneous evaluation of physicochemical and microbiological parameters provides a more comprehensive assessment of wetland treatment performance than monitoring organic matter and nutrients alone.

Vegetation is an important functional component of many VSSFCWs because plants modify the physical and biological environment within the filter bed. Root systems provide surfaces for microbial colonization, influence rhizosphere conditions, contribute to nutrient uptake, and can modify oxygen and carbon availability around the root zone. The influence of vegetation is therefore not limited to direct nutrient assimilation; plant-mediated changes in microbial habitat and redox conditions can also affect pollutant transformation. Experimental studies have demonstrated that plant presence can increase bacterial abundance and improve the removal of organic matter and nutrients compared with unplanted beds (Zahui et al., 2021). More recent research has further demonstrated that plant species can influence nitrogen transformation and microbial functional groups in vertical-flow systems, emphasizing that vegetation should be considered a functional design variable rather than merely a structural component (Huang et al., 2024). The treatment contribution of vegetation, however, is species-dependent. Differences in root architecture, biomass production, growth rate, rhizosphere development, nutrient requirements, and capacity to modify local physicochemical conditions can result in different treatment responses among macrophytes. This species effect is particularly relevant when selecting plants for site-specific VSSFCW applications. Comparative studies have demonstrated that plant species can substantially influence pollutant removal and microbial characteristics, although the magnitude and direction of the effect depend on wetland configuration and operating conditions (Zahui et al., 2021; Huang et al., 2024). Importantly, *Phragmites australis* and *Arundo donax* are both established macrophytes used in constructed-wetland applications. Their use in wastewater treatment has been documented under different climatic and hydraulic conditions, including systems treating university or institutional wastewater (Gholipour & Stefanakis, 2021). However, differences in plant performance cannot be generalized across all wastewater types and wetland configurations. Direct comparison within the same experimental configuration is therefore necessary to distinguish species-specific effects from differences caused by substrate, hydraulic conditions, or wastewater characteristics. An unplanted control provides an additional basis for resolving the contribution of vegetation. Because the filter medium itself can provide substantial filtration, adsorption, and microbial attachment surfaces, the performance of a planted wetland cannot be attributed solely to vegetation without an appropriate control. Comparison of planted and unplanted beds allows the additional contribution associated with plant establishment and the rhizosphere to be assessed while maintaining the same basic substrate and hydraulic configuration. Previous experimental studies have shown that planted vertical-flow wetlands can outperform unplanted systems, although the magnitude of improvement varies among plant species and treatment conditions (Zahui et al., 2021). Despite extensive research on constructed wetlands, important gaps remain in the evaluation of simple VSSFCWs treating mixed wastewater under actual institutional conditions. Much of the available literature focuses on domestic wastewater, specific wastewater streams, hybrid configurations, or individual pollutant groups. Field-scale evidence also indicates that treatment performance can vary substantially with wastewater characteristics, system configuration, substrate properties, and operational conditions (Werkneh, 2024). Moreover, comparative assessment of two macrophytes alongside an unplanted control within otherwise comparable VSSFCW units can provide stronger evidence regarding the contribution and relative performance of vegetation. Recent reviews have consequently emphasized the need for further investigation of suitable plant species, substrate configurations, long-term operation, and site-specific decentralized applications. Accordingly, the present study evaluated the treatment performance of VSSFCWs receiving mixed blackwater and greywater generated from an institutional setting. Three comparable VSSFCW units were assessed: VSSFCW1 planted with *Phragmites australis*, VSSFCW2 planted with *Arundo donax*, and VSSFCW3 maintained as an unplanted control. Treatment performance was evaluated using electrical conductivity (EC), total suspended solids (TSS), total dissolved

solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrate, phosphate, total coliform (TC), and total faecal coliform (TFC). The study further compared the relative performance of the planted and unplanted systems and examined the consistency and interrelationships of pollutant-removal responses using statistical and multivariate approaches. By integrating physicochemical, nutrient, and microbiological indicators with comparative vegetation assessment, this study provides a more comprehensive evaluation of single-stage VSSFCWs for mixed institutional wastewater treatment and identifies the relative treatment performance of the two tested macrophytes under a common system configuration.

MATERIALS AND METHODS

Study area

The study was conducted at the campus of Central University of South Bihar (CUSB), Gaya, Bihar, India. The experimental site is located at 24°52'58.206" N and 84°53'44.0592" E, at an elevation of approximately 125 m above mean sea level. The region has a subtropical climate, with hot summers, cold winters, and a monsoon season mainly extending from June to September. The area receives an average annual rainfall of approximately 944 mm, with an average relative humidity of about 67%. Relative humidity generally ranges from approximately 43% in March to 89% in August. The region experiences high summer temperatures, with a mean maximum temperature of approximately 45.2 °C, while winter temperatures can decrease to approximately 4.0 °C.

Experimental design

A field-scale experimental setup comprising three identical pilot-scale vertical subsurface-flow constructed wetlands (VSSFCWs) was established at the CUSB campus (Fig. 1). The three units were operated independently under the same hydraulic and substrate conditions to evaluate the effect of vegetation on wastewater treatment performance. Each VSSFCW unit measured 1.5 m in length × 0.6 m in width × 0.5 m in depth, with a 1% slope towards the outlet. The units were designed to maintain subsurface vertical flow, with wastewater introduced at the upper surface of the filter bed and collected at the outlet after passing through the substrate. The three units were designated as VSSFCW1, VSSFCW2, and VSSFCW3. VSSFCW1 was planted with *Phragmites australis*, while VSSFCW2 was planted with *Arundo donax*. VSSFCW3 was maintained as an unplanted control. The two plant species were selected from locally available riparian vegetation. Plant material was collected from nearby riverside areas and multiplied under nursery conditions before transplantation. Plants of comparable size and growth stage were used for the two vegetated systems to minimize differences in initial plant establishment. All three VSSFCW units were filled with locally available river gravel with a particle size of 6-15 mm. The gravel served as the filter medium and provided support for plant growth and surfaces for microbial attachment. The same substrate material and bed dimensions were maintained in all three units.

Wastewater collection and wetland operation

Wastewater was collected from the university sewer network receiving wastewater from student hostels, the canteen, laboratories, and academic buildings. The wastewater therefore represented a mixed stream containing contributions from blackwater and greywater generated within the institutional campus. The collected wastewater was transferred to a sedimentation cum storage tank before application to the VSSFCWs. Preliminary settling was allowed in the tank to remove readily settleable solids and coarse material. The pretreated wastewater was supplied separately to each VSSFCW in batch mode. Approximately 108 L of wastewater was applied to each unit per batch. The same loading volume was used for all three systems. A hydraulic retention time (HRT) of 24 h was maintained throughout the experimental period. Wastewater was allowed to pass vertically through the gravel bed under subsurface-flow conditions and was collected from the outlet after the specified retention period. The three units were operated simultaneously under comparable environmental and hydraulic conditions. No additional aeration or chemical treatment was applied to any of the systems. The experimental operation was conducted from April 2025 to September 2025.

Sampling and wastewater quality analysis

Influent and effluent samples were collected from the inlet and outlet of each VSSFCW at regular sampling intervals during the experimental period. For each sampling event, approximately 1 L of grab sample was collected in clean polypropylene (PP) sampling bottles. Samples were transported to the laboratory and analysed on the same day to minimize changes in their physicochemical and microbiological characteristics. The samples were analysed for pH, electrical conductivity (EC), total suspended solids (TSS), total dissolved solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrate, phosphate, total coliform (TC), and total fecal coliform (TFC). The physicochemical analyses were performed following standard procedures described in Standard Methods for the Examination of Water and Wastewater (APHA, 2005).

The pH and EC of the wastewater were measured using calibrated digital meters. TSS was determined using the standard filtration and gravimetric method, while TDS was determined using the appropriate standard procedure. BOD was measured using the standard incubation method, and COD was determined using the dichromate digestion method. Nitrate and phosphate concentrations were determined using standard analytical procedures described by APHA (2005).

Microbiological analysis

Microbiological quality was assessed by determining total coliform (TC) and total fecal coliform (TFC) concentrations in the influent and effluent samples. The Most Probable Number (MPN) method was used for the initial detection and estimation of coliform bacteria. For the analysis, appropriate sample dilutions were inoculated into multiple tubes containing MacConkey lactose broth with inverted Durham tubes. The inoculated tubes were incubated at 37 °C for 24 h. Tubes showing positive reactions were selected for further confirmation by subculturing onto nutrient agar and incubating for an additional 24 h at 37 °C. The bacterial load was also determined using the serial dilution and colony-forming unit (CFU) method. Appropriate serial dilutions were prepared from the wastewater samples and inoculated onto suitable culture media. Following incubation, the developed colonies were counted, and the microbial concentration was expressed as CFU/L. The same procedure was applied to influent and effluent samples from all three VSSFCW units.

Pollutant removal efficiency

The removal efficiency of each pollutant was calculated from the measured influent and effluent concentrations using Eq. (1):

$$RE (\%) = (C_{in} - C_{out}) / C_{in} \times 100 \quad (1)$$

Where RE is the pollutant removal efficiency (%), C_{in} is the influent concentration, and C_{out} is the effluent concentration. The same equation was applied to the microbial indicators, with concentrations expressed as CFU/L.

Statistical analysis

The treatment performance of VSSFCW1, VSSFCW2, and VSSFCW3 was evaluated using the measured concentrations and calculated removal efficiencies across the sampling events. As the three VSSFCW units were evaluated during the same sampling events, the observations were considered matched. Differences among the treatment conditions were assessed using the Friedman test. Pairwise differences were subsequently evaluated using the Wilcoxon signed-rank test with Holm adjustment for multiple comparisons. Statistical significance was considered at $p < 0.05$. Pearson correlation analysis was performed to determine the relationships among the removal efficiencies of the investigated pollutants. Principal component analysis (PCA) was further applied to standardized water-quality data to identify major patterns of variation among the influent and the three VSSFCW treatment conditions. These statistical analyses were used to provide an integrated assessment of pollutant-removal patterns and differences among the vegetated and unplanted systems.

RESULT AND DISCUSSION

Overall treatment performance of VSSFCWs

The three vertical subsurface-flow constructed wetlands (VSSFCWs) demonstrated consistent reductions in organic matter, suspended and dissolved solids, nutrients, and microbial indicators. The influent wastewater was characterized by relatively high organic and solids loads, with mean BOD and COD concentrations of 272.7 and 681.6 mg/L, respectively. TSS, TDS, and EC were 114.7 mg/L, 630.6 mg/L, and 898.9 μ S/cm, respectively (Table 1). Following treatment, concentrations of all measured pollutants decreased, with VSSFCW1 generally producing the lowest effluent concentrations, followed by VSSFCW2 and VSSFCW3 (Table 1; Fig. 1). Organic matter showed the most pronounced treatment response. BOD declined to 66.1 mg/L in VSSFCW1, while COD decreased to 124.3 mg/L. The corresponding mean removal efficiencies were 75.29% and 81.18%, respectively (Table 2). VSSFCW2 and VSSFCW3 also achieved substantial organic-matter removal, although their performance was progressively lower. The temporal profiles further showed that this treatment pattern was maintained across the sampling period rather than being associated with a single sampling event (Fig. 2).

The strong reduction in BOD and COD reflects the capacity of VSSFCWs to provide multiple complementary removal pathways. Vertical-flow systems promote contact between wastewater, filter media, attached microbial communities, and plant roots. Organic matter can consequently be removed through physical retention and adsorption followed by microbial biodegradation. The vertical configuration also favours oxygen transfer into the bed, which can support aerobic degradation of readily biodegradable organic matter. Constructed wetlands are recognized as multifunctional treatment systems in which substrate, microbial biofilms, vegetation, and hydraulic conditions jointly determine pollutant removal. TSS was reduced from 114.7 mg/L in the influent to 37.0 mg/L in VSSFCW1, corresponding to 67.68% mean removal (Tables 1 and 2). TDS and EC were also substantially reduced, with the highest mean reductions occurring in VSSFCW1. The simultaneous decrease in TSS, TDS, and EC indicates that the treatment response extended beyond particulate organic matter to include a substantial fraction of dissolved constituents. However, EC and TDS are bulk indicators and do not identify the specific dissolved species responsible for the observed reduction. Substrate-mediated retention, adsorption, precipitation, plant uptake, and microbial interactions may collectively contribute to changes in dissolved constituents, although these individual pathways were not directly quantified in the present study. Nutrient concentrations also decreased consistently across the three systems. Nitrate declined from 6.55 mg/L in the influent to 2.26 mg/L in VSSFCW1, while phosphate declined from 5.60 to 1.83 mg/L (Table 1). The corresponding removal efficiencies in VSSFCW1 were 65.50% for nitrate and 67.25% for phosphate (Table 2). These results are consistent with the multifunctional nature of constructed wetlands, where nitrogen removal can involve microbial transformation and plant-associated processes, while phosphorus may be retained through adsorption, precipitation, microbial assimilation, and plant uptake.

Microbial indicators followed the same overall treatment pattern. Total coliform and total fecal coliform concentrations decreased substantially after treatment, with the highest reductions occurring in VSSFCW1 (Tables 1 and 2). Such reductions can result from the combined effects of filtration, sedimentation, adsorption, biological antagonism, and natural die-off within the wetland matrix. However, the persistence of coliform indicators in the treated effluents indicates that the VSSFCWs function primarily as treatment systems rather than complete disinfection barriers.

Overall, the results demonstrate that the VSSFCWs were capable of simultaneous treatment of multiple wastewater-quality constituents. The consistent decline observed across the concentration profiles and sampling events (Fig. 1 & 2) indicates that treatment was not restricted to a particular pollutant class or sampling period. This broad treatment response is an important characteristic of constructed wetlands because their performance results from the integrated action of physical, chemical, and biological processes rather than from a single removal mechanism.

Planted versus unplanted systems and species-specific performance

A clear performance distinction was observed between the planted and unplanted systems. VSSFCW1 and VSSFCW2 consistently outperformed the unplanted VSSFCW3 for the measured pollutants, demonstrating the

contribution of vegetation to overall treatment performance. VSSFCW1 achieved the highest removal for every parameter evaluated, followed by VSSFCW2 and VSSFCW3 (Table 2). The same hierarchy was evident in the concentration distributions and temporal reduction profiles (Figs. 1 and 2). The superior performance of the planted beds can be attributed to the additional functions introduced by the plant–substrate–microbial interface. Plant roots increase the reactive surface area within the bed and provide niches for microbial colonization. Rhizosphere development can also modify local redox conditions and stimulate microbial processes involved in organic-matter and nutrient transformation. Importantly, recent evidence indicates that the contribution of macrophytes to wetland treatment may be predominantly indirect, through modification of rhizospheric microbial communities and the physicochemical environment, rather than through direct plant uptake alone.

Between the two planted systems, VSSFCW1 planted with *Phragmites australis* was consistently superior to VSSFCW2 planted with *Arundo donax*. The advantage of VSSFCW1 extended across all measured pollutant groups, including organic matter, solids, nutrients, and microbial indicators. For example, COD and BOD removal in VSSFCW1 reached 81.18% and 75.29%, respectively, compared with 74.44% and 67.42% in VSSFCW2. Similarly, nitrate and phosphate removal were higher in VSSFCW1 than in VSSFCW2 (Table 2). The statistical comparison further supports this species-level difference. The pairwise analysis showed significant differences between VSSFCW1 and VSSFCW2 for the pollutant parameters after adjustment for multiple comparisons. Thus, the superior performance of VSSFCW1 was not simply an apparent difference in mean removal but represented a consistent treatment advantage across the sampling events.

The observed superiority of *P. australis* is scientifically plausible because this species is widely established in constructed-wetland applications and is capable of developing an extensive rhizosphere that supports microbial activity and interactions with the treatment substrate. Reviews of constructed-wetland vegetation have also identified *P. australis* among the commonly effective macrophytes used for wastewater treatment. However, plant performance is strongly dependent on wastewater characteristics, hydraulic conditions, substrate properties, climate, and plant development. Therefore, the present result should be interpreted as species superiority under the specific hydraulic and substrate conditions of this study, rather than as evidence that *P. australis* is universally superior to *A. donax*. Comparative studies have shown that the relative performance of wetland plants can vary with system configuration and target pollutants.

The lower performance of the unplanted VSSFCW3 nevertheless confirms that the gravel matrix alone retained a substantial treatment capacity. This is important because it indicates that vegetation enhanced, rather than solely determined, pollutant removal. The resulting performance hierarchy-*P. australis* > *A. donax* > unplanted control-therefore reflects the combined effects of the common gravel substrate and the additional biological functions associated with vegetation. Recent reviews similarly emphasize that substrate, vegetation, microbial communities, and hydraulic conditions operate as an interconnected treatment system.

Statistical consistency and correlation among wastewater-quality parameters

The statistical analysis confirmed the consistency of the observed treatment-stage differences. Kendall's coefficient of concordance (W) was 1.00 for EC, TSS, TDS, BOD, COD, nitrate, phosphate, total coliform, and total fecal coliform, while pH showed $W = 0.77$ (Fig. 3). All parameters showed statistically significant concordance ($p < 0.001$). The high W values indicate that the ranking of treatment stages remained highly consistent across sampling events. In practical terms, the systems followed a stable treatment hierarchy rather than exhibiting random changes in relative performance.

The lower concordance for pH is consistent with its comparatively narrow variation across the systems. Unlike the pollutant parameters, pH remained close to neutrality throughout treatment, indicating that the VSSFCWs did not induce major shifts in the overall acid–base condition of the wastewater. This stability is relevant because extreme pH changes could alter microbial activity and nutrient transformation pathways.

The Pearson correlation analysis revealed predominantly strong positive relationships among the wastewater-quality parameters (Fig. 4). Correlation coefficients ranged from 0.61 to 0.98, with particularly strong associations among TSS, TDS, BOD, COD, nitrate, phosphate, and microbial indicators. The strongest relationships included TDS-BOD ($r = 0.98$), BOD-TFC ($r = 0.97$), COD-TFC ($r = 0.97$), and TDS-TFC ($r =$

0.96). EC showed comparatively weaker, although still positive, relationships with COD and TFC. The strong positive associations indicate that changes in different pollutant groups occurred in a coordinated manner across the treatment systems. This supports the interpretation that the observed treatment response was multidimensional, involving simultaneous changes in organic matter, solids, nutrients, dissolved constituents, and microbial indicators. Such coordinated behaviour is expected in constructed wetlands because several pollutant-removal pathways operate concurrently within the same substrate and rhizosphere environment.

Nevertheless, correlation should not be interpreted as evidence of direct causality. Strong associations may arise because several parameters respond simultaneously to the same treatment processes or to changes in overall wastewater quality. Therefore, the correlation analysis primarily demonstrates the coherence of the treatment response, rather than establishing specific mechanistic relationships among individual pollutants.

Multivariate differentiation of treatment stages

Principal component analysis further demonstrated the strong differentiation of wastewater quality across the treatment stages. PC1 explained 98.53% of the total variance, whereas PC2 and PC3 accounted for only 0.72% and 0.34%, respectively (Fig. 7). Thus, the first three principal components represented approximately 99.6% of the total variability in the dataset. The PCA score plot showed clear separation of influent and treated samples along PC1 (Fig. 5). Influent samples occupied the positive region of PC1, whereas samples from the VSSFCWs were positioned on the negative side. The three treatment systems also showed distinct clustering, with VSSFCW1, VSSFCW2, and VSSFCW3 occupying progressively differentiated positions along the principal-component space. This separation is consistent with the concentration and removal patterns presented in Figs. 1 and 2 and Tables 1 and 2.

The loading analysis showed that all measured wastewater-quality parameters contributed strongly to PC1 (Fig. 6). The similar direction and magnitude of the PC1 loadings indicate that this component represents an integrated wastewater-quality treatment gradient, rather than a pollutant-specific axis. The dominance of PC1 therefore reflects the simultaneous reduction of multiple pollutant classes through the treatment sequence.

The PCA results provide an important multivariate confirmation of the univariate findings. The clear separation between influent and treated samples indicates that the VSSFCWs produced a systematic transformation of the overall wastewater-quality profile. The further separation among VSSFCW1, VSSFCW2, and VSSFCW3 demonstrates that the treatment systems did not produce identical effluent signatures. Instead, their performance differed in a consistent direction, with the *P. australis*-planted system producing the strongest overall shift in wastewater quality.

The agreement between PCA, Kendall's W, and Pearson correlation analysis strengthens the interpretation of the experimental results. Kendall's W demonstrated consistency among treatment stages, Pearson correlation revealed coordinated changes among parameters, and PCA integrated these changes into a single multivariate treatment gradient. Such complementary statistical evidence reduces the likelihood that the observed performance hierarchy resulted from isolated behaviour of individual parameters.

CONCLUSION

The VSSFCWs demonstrated a strong capacity for simultaneous improvement of physicochemical and microbiological wastewater quality. Treatment was not restricted to a particular pollutant group; instead, organic matter, suspended and dissolved constituents, nutrients, and microbial indicators were reduced concurrently. Vegetation was an important factor differentiating treatment performance. Both planted systems consistently outperformed the unplanted control, confirming that the plant-associated rhizosphere provided additional treatment functionality beyond that of the gravel matrix alone. Among the two tested macrophytes, *Phragmites australis* produced the most effective overall treatment response under the conditions of this study, indicating its suitability for the investigated VSSFCW configuration. The statistical analyses demonstrated that this performance hierarchy was consistent across the monitoring period. The strong correlations among wastewater-quality parameters further indicated a coordinated treatment response, while PCA clearly separated the influent and treatment stages along a dominant overall water-quality gradient.

Collectively, the findings demonstrate that VSSFCWs can function as integrated treatment systems in which substrate filtration, microbial activity, and vegetation-associated processes act concurrently. The results also highlight the importance of plant selection when designing vegetated VSSFCWs. However, the residual microbial indicators and the study-specific nature of plant performance indicate that additional polishing may be required where stringent reuse or microbiological standards apply. Longer-term studies incorporating hydraulic loading, seasonal operation, nutrient transformation pathways, and rhizosphere microbial dynamics would further strengthen the basis for scaling this treatment approach.

ACKNOWLEDGEMENTS

The authors express their gratitude to the University Grant Commission for financial assistance in terms of a fellowship to one of the authors. Also, the authors are thankful to the Department of Environmental Science, Central University of South Bihar, Gaya, for providing essential laboratory facilities and logistical support throughout the study.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest

All the authors declare that there are no financial or non-financial conflicts of interest.

Research Involving Human Participants and/or Animals

This research did not involve human participants or animals.

Informed Consent

Not applicable.

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Table 1. Physicochemical and microbiological parameters in influent and VSSFCW effluents (Mean $\pm$ standard deviation)

Parameter	Influent	VSSFCW1	VSSFCW2	VSSFCW3
pH	6.71 $\pm$ 0.16	6.91 $\pm$ 0.18	6.76 $\pm$ 0.15	6.67 $\pm$ 0.09
EC (μ S/cm)	898.9 $\pm$ 13.6	369.8 $\pm$ 21.1	503.5 $\pm$ 26.4	567.6 $\pm$ 22.5
TSS (mg/L)	114.7 $\pm$ 1.8	37.0 $\pm$ 6.0	46.3 $\pm$ 4.6	56.1 $\pm$ 6.7
TDS (mg/L)	630.6 $\pm$ 9.5	310.2 $\pm$ 24.8	330.4 $\pm$ 41.0	375.0 $\pm$ 48.1
BOD (mg/L)	272.7 $\pm$ 24.3	66.1 $\pm$ 11.7	87.0 $\pm$ 17.0	115.5 $\pm$ 23.4
COD (mg/L)	681.6 $\pm$ 60.7	124.3 $\pm$ 43.2	168.9 $\pm$ 55.5	207.7 $\pm$ 67.7
Nitrate (mg/L)	6.55 $\pm$ 0.20	2.26 $\pm$ 0.26	2.76 $\pm$ 0.61	3.33 $\pm$ 0.60
Phosphate (mg/L)	5.60 $\pm$ 0.14	1.83 $\pm$ 0.14	2.38 $\pm$ 0.17	2.84 $\pm$ 0.47
Total coliform (CFU/L)	391,188 $\pm$ 16,744	124,004 $\pm$ 11,521	137,278 $\pm$ 16,740	165,108 $\pm$ 19,736
Total fecal coliform (CFU/L)	350,625 $\pm$ 5,693	108,144 $\pm$ 16,445	125,109 $\pm$ 19,445	137,620 $\pm$ 21,389

Table 2. Mean removal efficiency of physicochemical and microbiological parameters in the three VSSFCW systems.

Parameter	VSSFCW1	VSSFCW2	VSSFCW3
EC (μ S/cm)	58.88	44.0	36.88
TSS (mg/L)	67.68	59.62	51.05
TDS (mg/L)	50.82	47.64	40.58
BOD (mg/L)	75.29	67.42	56.69
COD (mg/L)	81.18	74.44	68.58
Nitrate (mg/L)	65.50	57.96	49.33
Phosphate (mg/L)	67.25	57.47	49.11

Total coliform (CFU/L)	68.25	64.79	57.65
Total fecal coliform (CFU/L)	69.12	64.28	60.71

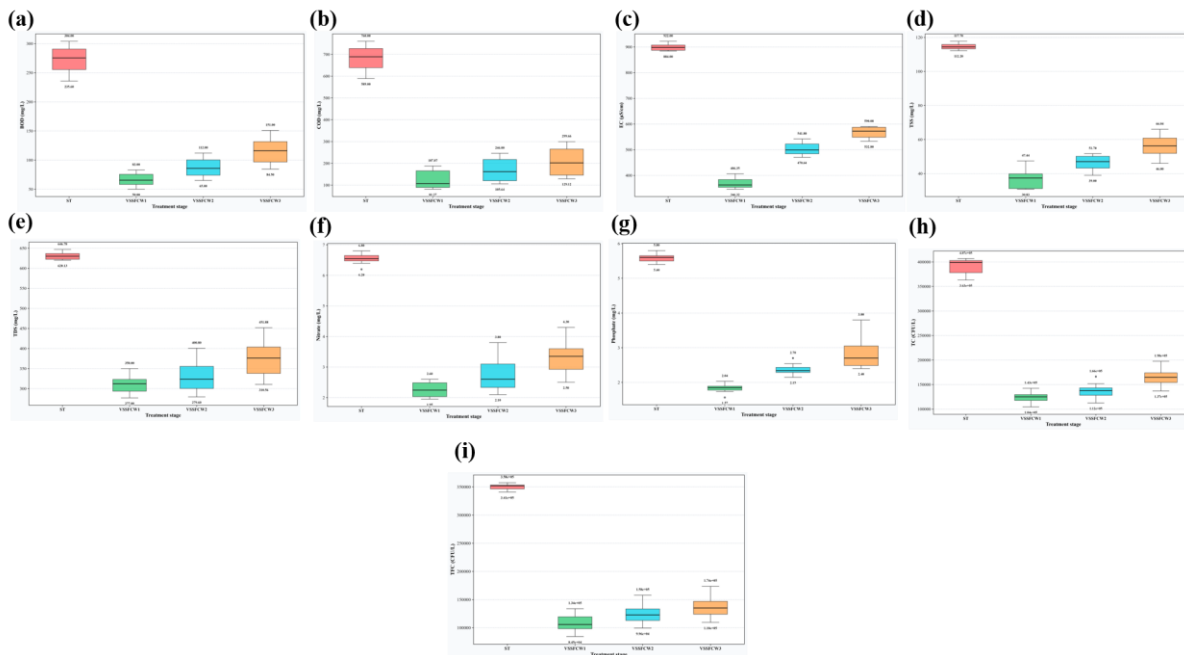


Fig. 1. Concentration profiles of wastewater quality parameters across the influent (ST) and VSSFCW1, VSSFCW2, and VSSFCW3.

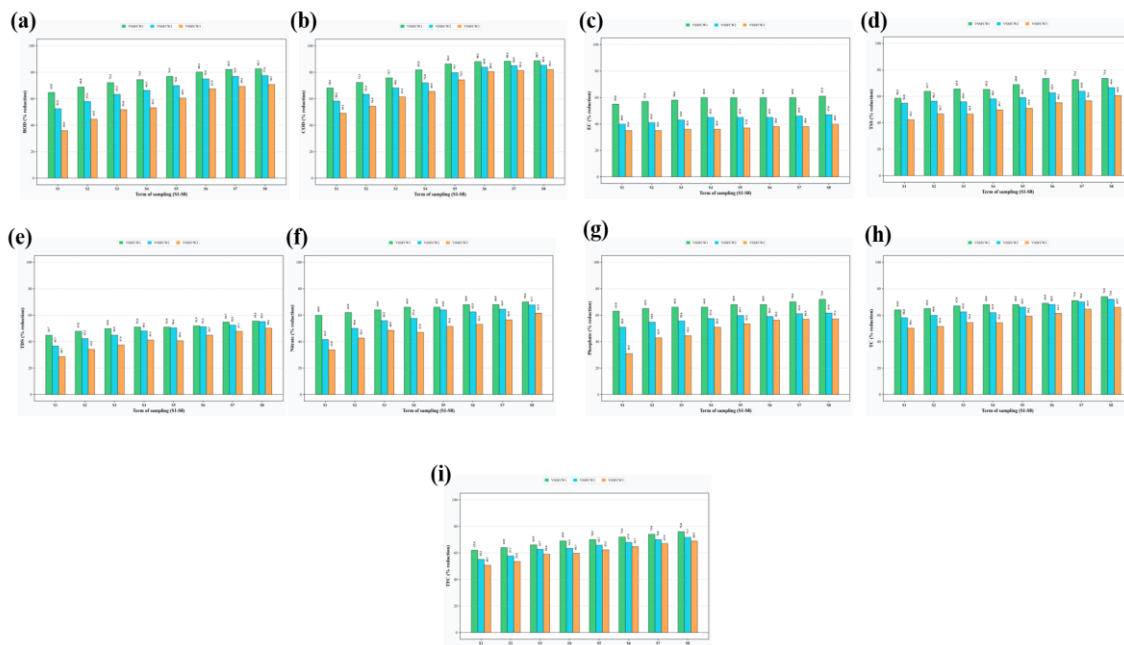


Fig. 2. Reduction (%) of wastewater quality parameters across VSSFCW1, VSSFCW2, and VSSFCW3.

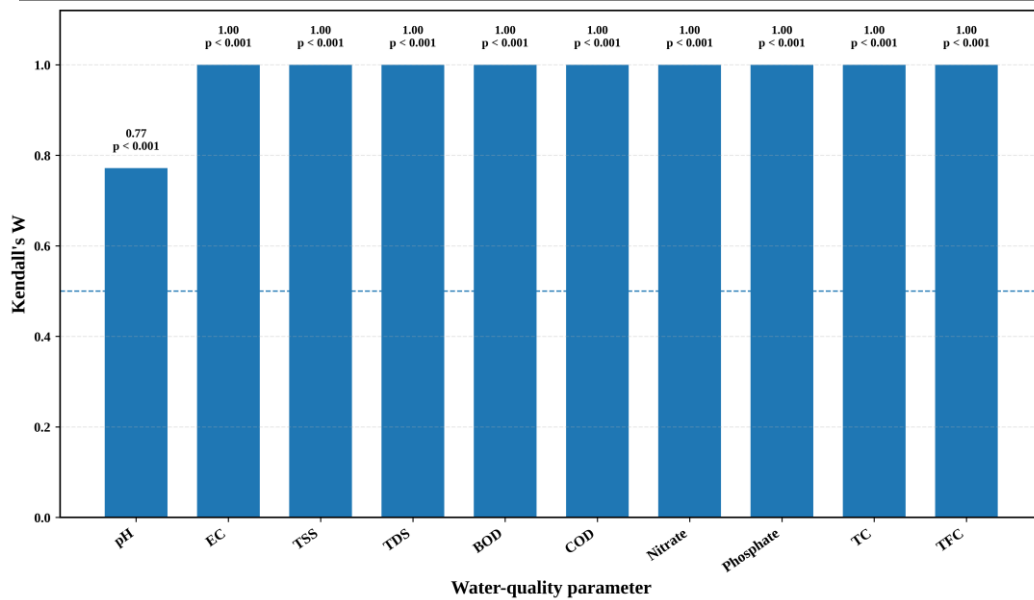


Fig. 3. Kendall's coefficient of concordance (W) for wastewater quality parameters across treatment stages.

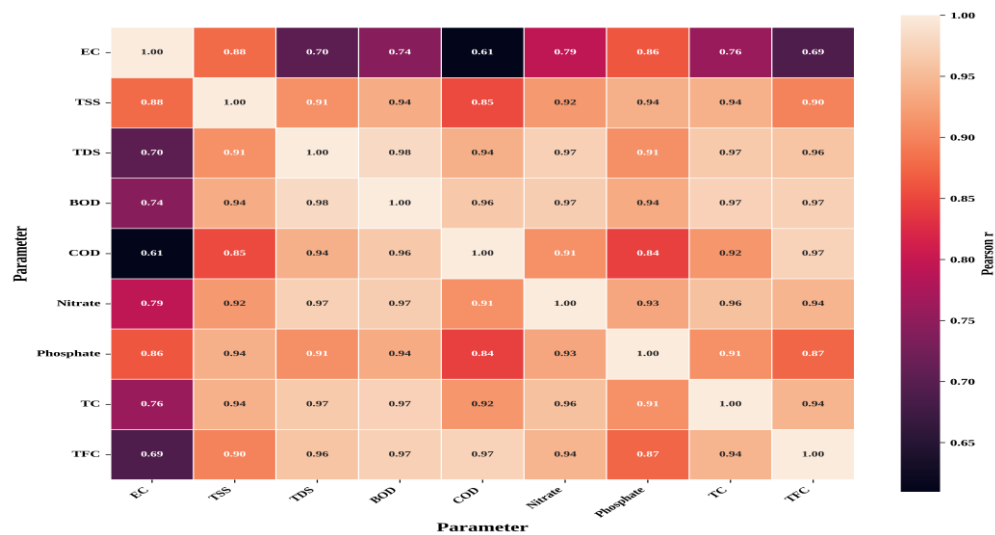


Fig. 4. Pearson correlation matrix of wastewater quality parameters across treatment stages.

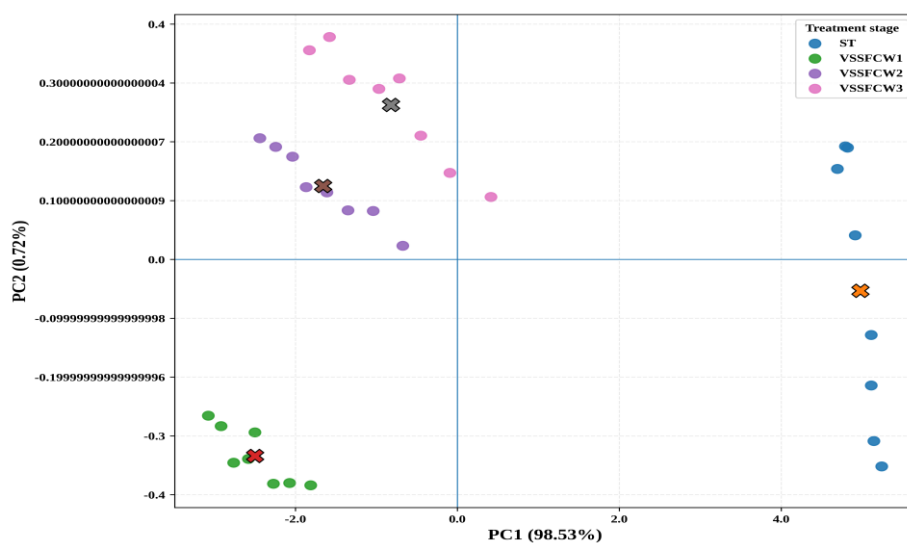


Fig. 5. Principal component analysis (PCA) score plot showing the distribution of wastewater samples across treatment stages.

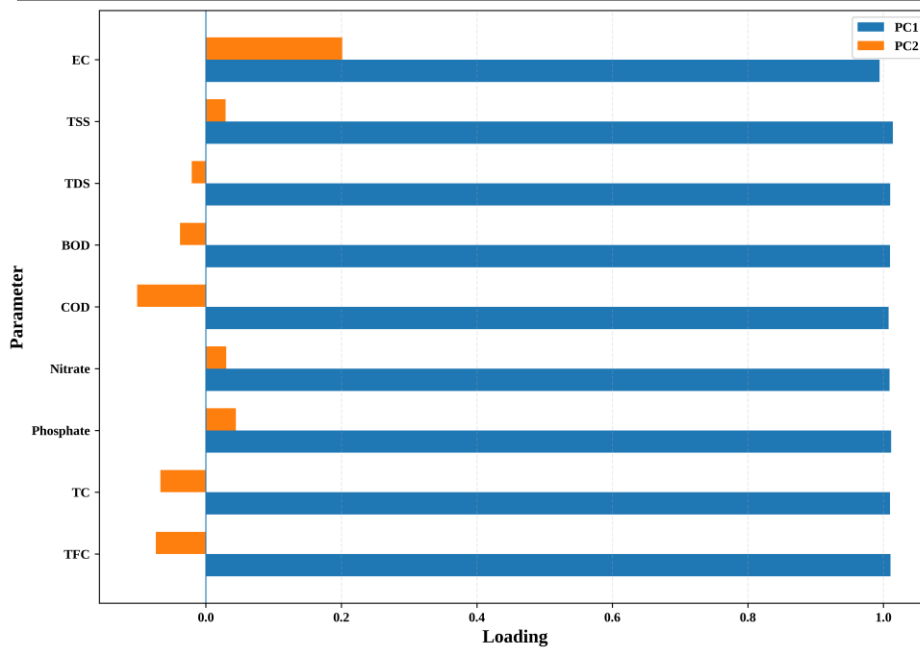


Fig. 6. Principal component loadings of wastewater quality parameters on PC1 and PC2.

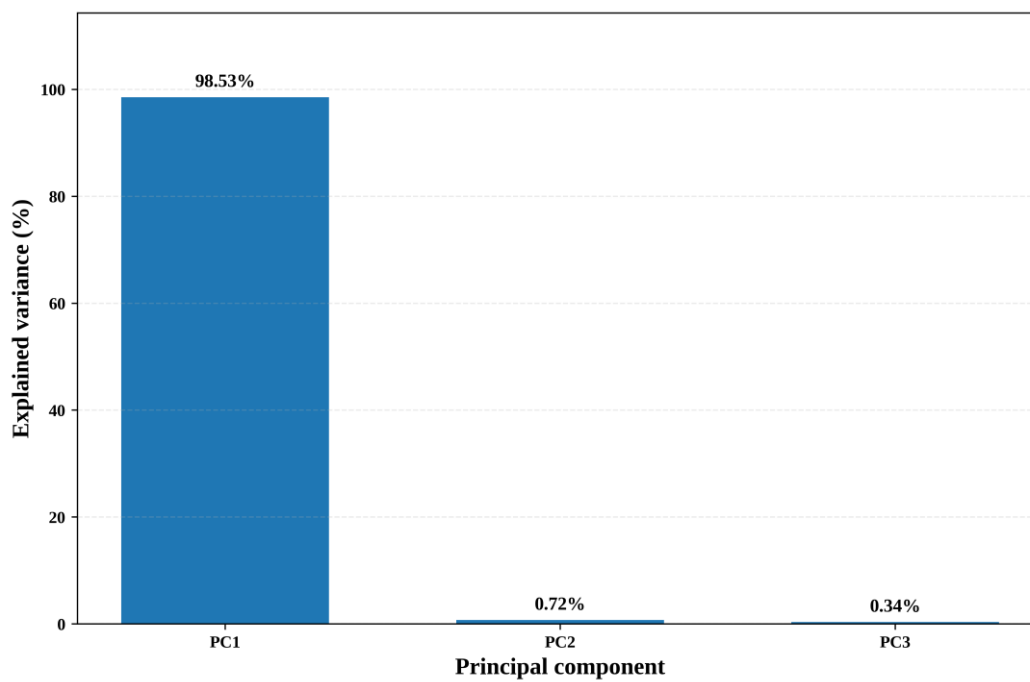


Fig. 7. Explained variance of the principal components (PC1-PC3) in the PCA.