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Performance of Vertical Flow Constructed Wetlands Planted with Indigenous Species for Decentralized Wastewater Treatment and Biomass Production in Kerala, India

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ABSTRACT

This study evaluates the performance of tropical subsurface vertical flow constructed wetlands (VFCW) having indigenous plants as decentralized ecological treatment systems for municipal wastewater treatment combined with biomass production. The VFCW mesocosms were planted with lignocellulosic grass species suitable to climatic conditions of Kerala such as Cumbu Napier Hybrid grass (*Pennisetum purpureum)*, Gamba grass (*Andropogon gayanus*) and Palisade grass (*Urochloa brizantha*). The VFCWs were operated at a hydraulic loading rate (HLR) of $0.1md^{-1}$ and hydraulic retention time (HRT) of 1 day. During the study period, the planted VFCWs attained significant pollutant removal efficiency than the control system with an unplanted filter bed. The VFCW planted with Cumbu Napier Hybrid grass obtained average removal efficiencies of TSS (89.80%), BOD (89.90%), COD (78.10%), Nitrates (69.07%), TN (44.33%), and Phosphates (51.20%). In the VFCW system planted with Palisade grass, the average removal efficiencies observed were Turbidity (98.70%), TSS (89.50%), BOD (87.90%), COD (72.70%), Nitrates (62.07%), TN (43%), and Phosphates (47%). The treated effluent concentration from both the units conformed to the USEPA guidelines for non-potable water reuse standards. The average biomass yield of Cumbu Napier Hybrid grass during the study period was found to be significantly higher when compared to Gamba grass and Palisade grass.

INTRODUCTION

Inadequate access to drinking water and sanitation is one of the most ubiquitous challenges faced by humanity in developing countries. Constructed wetlands (CWs) are decentralized small-scale systems for wastewater treatment having low energy and minimal operational requirements (Brix 1994b, Gross et al. 2009, Hoffmann et al. 2011, Vasudevan et al. 2011). CWs are the artificial replica of natural wetlands, designed and developed to optimize the functions of plants, soil, and the rhizosphere microorganisms that occur in the natural wetlands for pollutant removal (Vymazal 2010). As it is an eco-friendly treatment process with low energy and maintenance requirements and evades the use of chemicals, CWs are largely recognized in many countries (Lee et al. 2009, Vymazal 2011, Avila et al. 2019). Subsurface vertical flow CWs are gaining significance as an eco-technological wastewater treatment technology and can play a vital role in realizing the concepts of ecological sanitation (Langergraber & Muellegger 2005, UN-HABITAT 2008, Masi 2009, Hoffmann et al. 2011, Pillai &Vijayan 2013). They can also be used as onsite flexible treatment systems that can be applied at an individual household level or on a community basis (Hoffmann et al. 2011).

The choice of plants is a significant aspect in determining the pollutant removal efficiency and performance of subsurface flow CWs. The plants influence the level of oxygen in the wetland bed, enable physical filtration, prevent VFCW systems from getting clogged and offer a large surface area for microbial colonization (Brix 1994a, 1994b, 1997). The most frequently used macrophyte in subsurface flow constructed wetlands is *Phragmites australis* most commonly used in Europe, Canada, Australia and parts of Asia and Africa. The second most commonly used plant for subsurface flow CWs is *Typha* (e.g. *latifolia, domingensis, orientalis* and *glauca*) spp. and they are used in North America, Australia, Africa, and East Asia. Yet another plant species is *Scirpus* (e.g. *lacustris, validus, californicus* and *acutus*) spp. largely used in North America, Australia, and New Zealand. *Juncus effusus* and *Eleocharis* sp. are mostly used in Asia, Europe and North America (Vymazal 2011). Moreover, some ornamental species such as *Iris pseudacorus* and *Canna* have been experimented in CWs in the tropical and subtropical countries (Ling et al. 2009, Abou-Elela & Hellal 2012). The efficiency of *Cyperus papyrus* for the treatment of municipal wastewater in subsurface flow CWs has been researched by many authors (Perbangkhem & Polprasert 2010, Abou-Elela

& Hellal 2012, Avila et al. 2019). The use of Napier grass (*Pennisetum purpureum*) in VFCWs has been reported for the treatment of greywater in India (Pillai &Vijayan 2013) as well as for swine wastewater treatment in Thailand (Klomjek 2016).

The CWs provide an efficient mechanism for the removal of nutrients while facilitating a suitable environment for the cultivation of grasses, a potential feedstock for ethanol production. An integrated approach for combining wastewater treatment with biomass productivity in subsurface flow CWs can realize environmental pollution control as well as biofuel production (Yi Chung et al. 2011). The biomass produced by plants provides supplementary values as cattle fodder, biofuel, medicines, pulp and paper, soil conditioner and compost. In addition, CWs offer environmental benefits such as green space, sequestration of carbon dioxide, creation of habitats for wildlife and preservation of biodiversity (Kadlec & Wallace 2009, Vymazal 2010, 2011, Perbangkhem & Polprasert 2010, Hoffmann et al. 2011). Thus, it becomes essential to identify local, resilient, and valuable perennial grasses with high biomass yield and potential for contaminant removal (Pillai & Vijayan 2013).

Subsurface flow CWs are often significant for developing countries in tropical regions with warm and humid weather throughout the year (Kivaisi 2001, Chelliapan et al. 2011, Caselles-Osorio et al. 2011, Almuktar et al. 2018). The possibility of applying CWs as decentralized ecological sanitation systems is substantial in India, but the rate of adoption and replication of the technology has been extremely slow (Pillai & Nair 2015). This study aims to assess the performance of tropical subsurface vertical flow constructed wetland (VFCW) mesocosms using native grass species for the treatment and reuse of wastewater. The VFCW units using different grass species appropriate to the tropical conditions of Kerala such as Cumbu Napier Hybrid grass (*Pennisetum purpureum*), Gamba grass (*Andropogon gayanus*) and Palisade grass (*Urochloa brizantha*) were evaluated and compared for their overall performance and effectiveness in the treatment and utilization of municipal wastewater. The biomass yield from the different planted VFCWs was also studied and compared.

MATERIALS AND METHODS

Description of the Study Site

This research was conducted in Thiruvananthapuram, the capital city of the state of Kerala, which is located on the southwestern tropical Malabar coast of India. Though the city has a separate sewerage and drainage system, the coverage is only about 37% and 50% respectively. In the uncovered areas of the city, sewage from households is disposed to septic tanks, borehole latrines and community toilets. The remaining untreated wastewater gets directly discharged into open drains, canals, streams, rivers and other surface water bodies. Even though the city has a centralized sewage treatment plant (STP) of capacity 107 MLD constructed at Muttathara to treat the sewage load of the entire corporation area, the inflow to the STP is only 44 MLD. The existing gap in the sewerage network of the city is about 63% (TMC & KWA 2016).

Characterization of Influent Wastewater

Municipal wastewater used as an influent to the VFCWs was obtained from the outlet of the grit chamber of the STP located at Muttathara in the district of Thiruvananthapuram. The grab samples were collected manually and analysed for the following parameters: pH, Temperature, Turbidity, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), nitrates, Total Nitrogen (TN), phosphates and heavy metals. The physico-chemical analysis of the influent and treated samples was carried out as per the standard methods. (APHA 2005). The influent BOD concentration ranged from 175 $mg.L^{-1}$ to 192 $mg.L^{-1}$, while COD values varied between 390 $mg.L^{-1}$ and 430 $mg.L^{-1}$. The concentration of suspended solids ranged from 259 $mg.L^{-1}$ to 302 $mg.L^{-1}$. The average concentration of nitrates, TN and phosphates were 3.37 $mg.L^{-1}$, 52.6 $mg.L^{-1}$ and 13.58 $mg.L^{-1}$ respectively. The presence of heavy metals such as copper, lead, chromium, mercury and cadmium were found to be less than 0.01 $mg.L^{-1}$. The detailed characterization of the influent municipal wastewater is presented in Table 1.

The influent organic loading rate (OLR) used in this study varied between 17.5 and 19.2 g BOD_5 $m^{-2}d^{-1}$ at a constant hydraulic loading rate (HLR) of 0.1 md^{-1}. The

Table 1: Characteristics of influent municipal wastewater.

Parameters	Unit	Average ± SD
pH	-	6.73 ± 0.21
Temperature	°	28 ± 0.79
Turbidity	NTU	130 ± 4.03
TSS	$mg.L^{-1}$	279 ± 15.25
BOD	$mg.L^{-1}$	180 ± 5.06
COD	$mg.L^{-1}$	412 ± 13.63
Nitrates	$mg.L^{-1}$	3.37 ± 0.39
Total Nitrogen	$mg.L^{-1}$	52.6 ± 4.54
Phosphates	$mg.L^{-1}$	13.58 ± 0.79
Heavy metals (Cu, Cd, Cr, Hg, Pb)	$mg.L^{-1}$	< 0.01

OLR and HLR recommended by Brix and Arias (2005) for VFCWs in Denmark were 10-40 g BOD_5 $m^{-2}d^{-1}$ and 0.05-0.06 md^{-1}. Prochaska et al. (2007) experimented with OLR of 20-40 g BOD_5 $m^{-2}d^{-1}$ and HLR 0.08-0.17 md^{-1} in N. Greece. In warm climates, an OLR of 30-35 g BOD_5 $m^{-2}d^{-1}$ and HLR of 0.2 md^{-1} has been successfully experimented (Hoffmann et al. 2011). Stefanakis & Tsihrintzis (2012) used three high OLR of 89.9, 105.7 and 180.9 g BOD_5 $m^{-2}d^{-1}$ at HLR of 0.19, 0.26 and 0.44 md^{-1} to treat synthetic wastewater for long term in VFCWs.

VFCW System Configuration

The VFCW systems were constructed in the campus of an educational institution in the district of Thiruvananthapuram in Kerala. The design criteria of the subsurface VFCWs were taken from the constructed wetlands manual (USEPA 2000, Kadlec et al. 2000, Brix & Arias 2005, UN-HABITAT 2008). The experimental VFCW mesocosms (labelled VFCW1-VFCW3) were made of rectangular plastic containers each of length 0.65 m, width 0.45 m and depth 0.45 m. To study the effect of macrophytes in the removal of contaminants, a control VFCW without plants was used. The characteristics and design parameters of the VFCW units are presented in Table 2.

The depth of filter bed in a subsurface flow CW is normally limited to almost the rooting depth of plants so that the plants are in constant contact with the influent wastewater and can contribute to the treatment process (UN-HABITAT 2008). In this study, the substrate materials used as filter media consisted of gravel, coarse sand, and coco-peat (coir fibre pith). The lowest layer of the filter bed was filled with a 10 cm thick layer of gravel (porosity= 0.42) of size varying from 10 to 20 mm. Above that, a 20 cm thick layer of coarse river sand (d_{10} = 0.3 mm, uniformity coefficient =4, porosity =0.39) was laid followed by an 8 cm thick layer of coco-peat. The coco-peat used in the study had a pH of 6.2, bulk density 0.09 g cm^{-3}, electrical conductivity 0.16 $mScm^{-1}$ and porosity 0.65. In order to prevent any accumulation of water, a 2 cm thick gravel layer was placed on the top surface of the wetland bed. The VFCW systems were provided with an inlet and outlet arrangement. The slope of the bottom bed was oriented 1% towards the outlet. The schematic representation of the filter bed is illustrated in Fig. 1.

Table 2: Design Parameters of experimental VFCW units

Parameters	Unit	Value
Length	m	0.65
Width	m	0.45
Depth	m	0.45
Design flow	m^3d^{-1}	0.03
Hydraulic Loading Rate	m d^{-1}	0.1
Hydraulic Retention Time	day	1
Slope	%	1

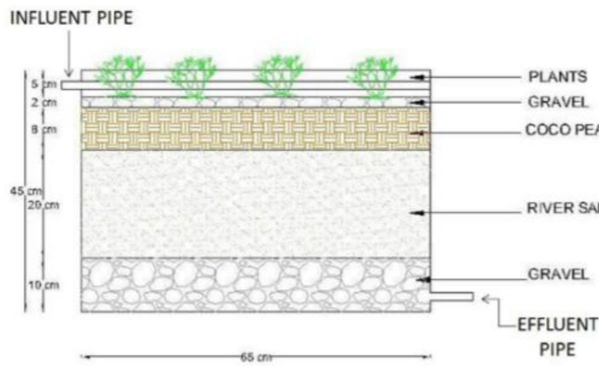

Fig. 1: Schematic representation of the filter bed profile in the VFCW mesocosms.

Plant Species

The fodder grass species used in this study were collected from the forage farm of Kerala Agricultural University (KAU). The grass species planted in the first (VFCW1) unit was Cumbu Napier Hybrid grass (*Pennisetum purpureum*) which is an interspecific hybrid between fodder Cumbu (*Pennisetum glaucum*) and *Napier grass* (*P. purpureum* Schumach). This hybrid variety termed "CO5" was developed by KAU and is regarded as a valuable fodder grass with high biomass productivity. It can be grown perennially in tropical regions and is adapted to the climate of Kerala. The grass is reported to have profuse tillering capacity, high yield potential, quick regeneration, high leaf to stem ratio, high dry matter and crude protein content and is also recognized as a potential biofuel crop. The second experimental (VFCW2) system was planted with Gamba Grass (*Andropogon gayanus*) which is a common perennial forage grass of the tropical regions with short rhizomes. The third experimental (VFCW3) system was planted with Palisade grass (*Urochloa brizantha*) which is a rhizomatous perennial grass and often used as a forage for livestock. The grass is best adapted to humid and sub-humid tropics and survives drought better than many other tropical species of grasses (KAU 2011).

Construction and Operation of VFCW Systems

The stem cuttings of Cumbu Napier hybrid grass and the slips of Gamba and Palisade grass were planted in the wetland bed of VFCW1, VFCW2, and VFCW3 respectively. The plant density provided was 14 plant stems/m^2. Initially, for the establishment of the grasses, they were daily watered with freshwater for 30 days. Thereafter, an acclimatization period of 30 days was provided by feeding the VFCW systems with municipal wastewater. The treatment of wastewater in the control and planted VFCW systems was further continued for ten months from March 2019 to December 2019. The influent municipal wastewater was initially collected in a feeding tank, which was then fed intermittently to the VFCWs through the inlet. During the entire treatment period, wastewater was fed into the VFCW systems at a HLR of 0.1 m/day. A distribution pipe with perforations at the bottom was used to uniformly distribute the wastewater onto the surface of the wetland bed. The wastewater fed on to the surface of the bed percolated vertically down through the different layers of the filter media and the treated effluent was collected from the outlet. Hydraulic retention time (HRT) of 1 day was provided in the experimental and control VFCW systems. Between successive feeds, a dosing interval of 2 days was given.

Influent and treated wastewater were sampled monthly from the inlet and outlet of the VFCW units and analysed for various physical and chemical parameters. During the entire operational period, the biomass from the planted VFCW systems was harvested four times. The initial harvesting was done after 4 months of planting and the subsequent harvests at an interval of 2 months. The grasses were cut approximately 5 cm above the bed surface. The above-ground biomass yield of the grasses from the planted VFCW systems was estimated and assessed for their nitrogen and phosphorus constituents.

RESULTS AND DISCUSSION

Performance and Treatment Efficiency of VFCW Systems

The efficiency of subsurface constructed wetlands can be expressed in terms of percent concentration reduction and percent mass removal of the pollutants (Kadlec & Wallace 2009, Stefanakis &Tsihrintzis 2012). In this study, the overall performance and treatment efficiency of the different VFCW units were analysed on the basis of average influent and effluent concentrations and percent removal of the pollutants. Fig. 2 presents the removal of pollutants (percent removal efficiency) in the control and planted VFCWs during the entire operational period. The concentrations of the treated effluent from the various systems are given in Table 3.

Turbidity: Turbidity was significantly reduced in the effluent obtained from the control as well as the planted VFCW mesocosms. In all the experimental VFCWs planted with Cumbu Napier grass, Gamba grass and Palisade grass, the mean turbidity removal efficiency observed was greater than 97%. The control system without plants obtained a mean turbidity removal of 87.84%. The removal of turbidity in the various VFCWs during the study period is shown in Fig. 2a.

Suspended solids: The TSS removal efficiency in the various VFCW mesocosms with time is shown in Fig.2b. The VFCW1 system planted with Cumbu Napier grass had a mean TSS removal of 89.80%, whereas for planted systems with Gamba grass (VFCW2) and Palisade grass (VFCW3) it was observed as 78.45% and 89.50% respectively. In the control system, the removal efficiency observed was 65.39%. The mean values of concentrations of TSS in the final effluent treated using control and planted systems are shown in Table 3.

Removal of TSS in CWs can be attributed to sedimentation, filtration, interception, adsorption and root zone treatment. The voids and media grain structure have a significant influence on the trapping of the suspended solids during the flow path (Abdelhakeem et al. 2016, Tsihrintzis 2017, Avila et al. 2019). The substrate materials such as sand, coco-peat,

Table 3: Statistical data of concentration of various physico-chemical parameters in the effluent obtained after treatment from the VFCW systems (mean value ± standard deviation)

Parameters	Unit	Treated effluent from the control system	Treated effluent from VFCWs planted with			Treated effluent quality standards (MoEFCC, India)	
			Cumbu Napier Hybrid grass (VFCW1)	Gamba grass (VFCW2)	Palisade grass (VFCW3)	Into inland surface water	On land for irrigation
pH	---	6.9 ± 0.02	7.1 ± 0.32	7 ± 0.12	7.1 ± 0.32	6-9	6-9
Turbidity	NTU	15.80 ± 1.02	1.55 ± 0.73	3.03 ±1.54	1.70 ± 1.40	---	---
TSS	$mg.L^{-1}$	96.67 ± 11.70	28.37±12.15	60.16 ± 13.85	29.18 ±13.10	100	100
BOD	$mg.L^{-1}$	112.67 ± 5.86	18.20 ±13.94	42.56 ± 27.31	21.83 ± 12.19	30	100
COD	$mg.L^{-1}$	267.02± 12.52	90.23 ± 24.19	119.25 ± 45.07	112.54 ± 39.46	250	250
Nitrates	$mg.L^{-1}$	3.17 ± 0.37	1.05 ± 0.66	1.44 ± 0.67	1.29 ± 0.62	10	Not specified
TN	$mg.L^{-1}$	45.42 ± 3.32	29.06 ± 5.05	30.90 ± 5.98	29.75 ± 6.21	---	---
Phosphates	$mg.L^{-1}$	12.80 ± 0.73	6.61 ± 1.53	7.01 ± 1.63	7.19 ± 1.46	5	Not specified

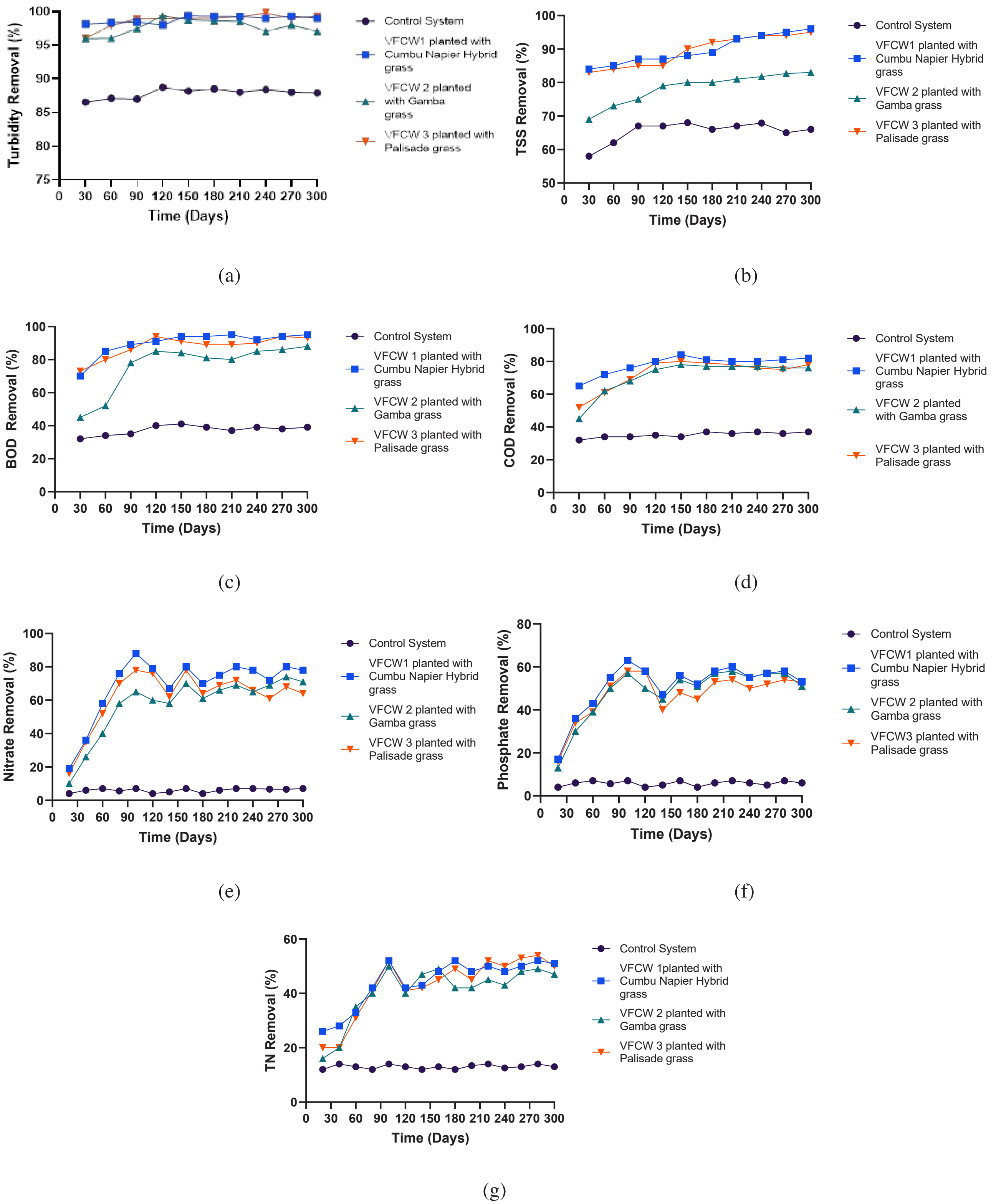

Fig. 2: Removal of various pollutants in the control and planted VFCW systems during the entire operational period (a) Turbidity; (b) TSS; (c) BOD; (d) COD; (e) Nitrates; (f) Phosphates; (g) TN.

and gravel, as well as the roots of the plants, acted as the filters to trap the suspended particles (Brix 1994a, USEPA 2000). Vertical flow systems are highly efficient in removing suspended solids, provided the bed clogging problems are managed through a load and rest operation regime (Kadlec & Wallace 2009). The removal efficiency obtained from the control and planted systems indicated the positive influence of plants in the removal of suspended solids. This signifies the role of root zone treatment and filtration by impaction of suspended particles in the roots and stems of the plants in subsurface flow systems.

Organic matter: In the control unit, the mean BOD removal efficiency observed was only 37.4%. For planted VFCWs, the mean removal efficiency of BOD observed was 89.9%, 76.4%, and 87.9% for systems vegetated with Cumbu Napier Hybrid grass, Gamba grass, and Palisade grass respectively (Fig. 2c). In the VFCW1 using Cumbu Napier hybrid grass, the mean COD removal efficiency was observed as 78.1% while the removal efficiency of other systems planted with Gamba grass and Palisade grass was 71.1% and 72.7%. The control unit attained a mean COD removal of 35.2% (Fig. 2d). In the planted VFCWs, it was observed that the performance improvement in the removal of organic matter occurred after about 90 days of treatment. This trend continued with the growth of the plants and stabilized thereafter, attaining an almost steady removal rate during the study period.

Attached and suspended bacterial growth is mainly responsible for the removal of soluble organic compounds which are degraded aerobically and anaerobically. The removal of BOD in the planted VFCW systems occurs due to the biodegradation of organic matter that takes place in the biofilm together with the roots of plants and stems and the surface of the substrate (UN -HABITAT 2008, Avila et al. 2019). The intermittent flow regime in the VFCWs enables the formation of a vadose zone allowing for diffusion of atmospheric oxygen into the CW media (Kadlec & Wallace 2009).

Nutrient removal: The concentration of nitrates, total nitrogen, and phosphates in the influent and treated effluent were analysed to determine nutrient removal in the planted and control VFCWs. The results showed that the nutrient removals in the planted VFCWs were significantly higher than that in the control system. This indicated the importance of the presence of plants and the uptake of nutrients by them. The VFCW1 using Cumbu Napier Hybrid grass attained high removal of nitrate, phosphate, and TN in comparison to other planted systems. The mean removal efficiencies obtained for the VFCW1 system planted with Cumbu Napier hybrid grass were: Nitrates (69.07%), Phosphates (51.2%) and TN (44.33%). In VFCW2 planted with Gamba grass, the removal efficiencies observed were as follows: Nitrates (57.47%), Phosphates (48.27%), Total Nitrogen (40.87%). For the VFCW3 system planted with Palisade grass, the mean removal efficiencies observed were: Nitrates (62.07%), Phosphates (47%) and TN (43 %). Removal of nitrates, phosphates and TN in the different VFCWs during the treatment period is shown in Figs. 2e, 2f and 2g, respectively.

The processes for nitrogen removal in CWs are varied including volatilization of ammonia, nitrification, denitrification, plant and microbial uptake, ammonification, nitrate reduction to ammonium, anaerobic ammonia oxidation, sorption, desorption, burial, and leaching (UN-HABITAT 2008). But only very few processes eventually remove TN from wastewater while most processes just convert nitrogen into its various other forms. The pH values of the effluent from the planted systems were just above 7, which indicated that conditions were suitable for nitrification within the wetland bed. Ammonia gets oxidized to nitrate with the help of nitrifying bacteria in the aerobic zones of the VFCWs. The oxygen essential for nitrification is supplied by atmospheric transmission and leakage from the roots of the plants. In vertical flow constructed wetlands, very high nitrification proceeds but, due to the absence of entirely anaerobic conditions in the wetland bed, denitrification is very limited in these systems (Vymazal 2007, 2010).

The processes for the removal of phosphorus in constructed wetlands include adsorption, complexation and precipitation, storage, plant uptake (with subsequent harvest) and biotic assimilation (UN-HABITAT 2008, Vymazal 2010). Removal of phosphorus is generally reported to be low in subsurface constructed wetlands unless special media with high sorption capacity are used (Vymazal 2007). The results obtained show that the removal of phosphates is effective in the planted VFCWs when compared to the control system (Fig. 2f).

The grasses were harvested four times during the treatment period and it was observed that during each cutting cycle, the removal of nutrients increased with the growth of the plant and then slightly declined as the plants reached a maturing stage. In order to study the direct contribution of plants in nutrient removal, the harvested above-ground biomass was analysed for nitrogen and phosphorus. The average nitrogen uptake for Cumbu Napier, Gamba and Palisade grass was obtained as 37 $g.m^{-2}$, 11.12 $g.m^{-2}$ and 20.5 $g.m^{-2}$ whereas, the average phosphorus uptake was 2.7 $g.m^{-2}$, 0.81 $g.m^{-2}$ and 1.2 $g.m^{-2}$ respectively. This is supported by the values reported in the literature for above-ground nitrogen ranging from 2-64 $g\ Nm^{-2}$ and for phosphorus in the range 0.01-19 $g\ P\ m^{-2}$ (Vymazal 2007). According to Langergraber

(2005), for a subsurface CW treating municipal water, potential nutrient uptake of about 1.9% of the influent nitrogen and phosphorus loading can be expected.

Comparison of Treatment Efficiency

For all the physical and chemical parameters analysed, the planted VFCW mesocosms obtained high removal efficiency than the control system without plants. The results indicated the significance of plants, their rooting systems and associated microorganisms.

In the case of planted vertical flow wetlands, high pollutant removal efficiency was observed in VFCW1 using Cumbu Napier Hybrid grass when compared to the systems planted with Gamba grass and Palisade grass. The mean pollutant removal efficiency of the VFCWs during the entire study period is shown in Fig. 3.

The phytoremediation potential of the different grasses can be attributed to their root morphology, tillering rate, adaptation to the polluted environment, regeneration capacity, nutrient uptake and biomass productivity. The best removal efficiencies were observed in the system planted with Cumbu Napier hybrid grass with high nutrient uptake and biomass yield. Both Cumbu Napier Hybrid grass and Palisade grass has tolerated very well the treatment conditions showing high vegetative growth and biomass productivity when compared to Gamba grass. Cumbu Napier and Palisade grass have a profuse root system, penetrating deep into the soil and an abundance of fibrous roots spreading into the topsoil horizons. This deep, dense and fibrous rooting system can enhance the root zone treatment by facilitating more microbial fixation sites, sufficient residence time of wastewater, entrapment of suspended particles, large surface area for adsorption of contaminants, assimilation in plant tissues and oxygen for the oxidation of organic and inorganic matter in the rhizosphere. Whereas, Gamba grass has a shallow root system with fibrous roots close to the surface and short rhizomes which can be one of the reasons for its comparatively low removal of organic matter and suspended solids. The biomass yield of Gamba grass was found to be much less when compared to Cumbu Napier and Palisade grass (Fig. 5).

Statistical Analysis

Tukey's multiple comparison test was used for statistical analysis in order to determine significant statistical differences in the performance and efficiency of wastewater treatment between the different groups of treatments (control system, VFCW1, VFCW2, and VFCW3). All statistical analyses were performed at 0.05 significant levels. The statistical analysis was carried out using the software package Graph Pad Prism 8.2.1.

The statistical data of effluent concentration obtained after treatment from the control and planted VFCW systems is given in Table 3. The box-whisker plots for the effluent concentrations of each parameter during the study period are shown in Fig. 4 (a,b,c,d,e,f). The final effluent values were compared with the Indian standards of treated effluent quality for disposal into inland surface water as well as onto land for irrigation (MoEFCC India 2016). Results proved that the final effluent concentration from the control and all the planted VFCWs reached the Indian standards required for disposal onto land for irrigation. The effluent concentration values were also compared with the USEPA guidelines for water reuse (USEPA 2004). The effluent concentration from VFCW1 and VFCW3 conformed to the standards required for non-potable reuse of water according to the guidelines given by USEPA.

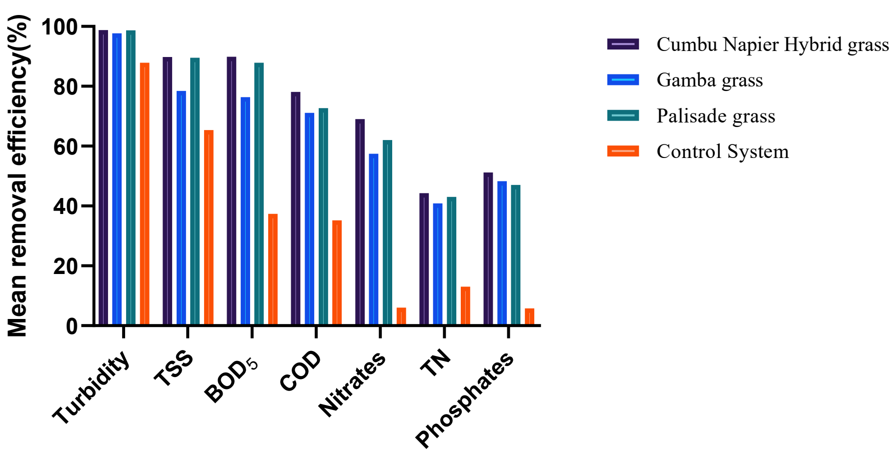

Fig. 3: Mean removal efficiency of pollutants in the different VFCW systems.

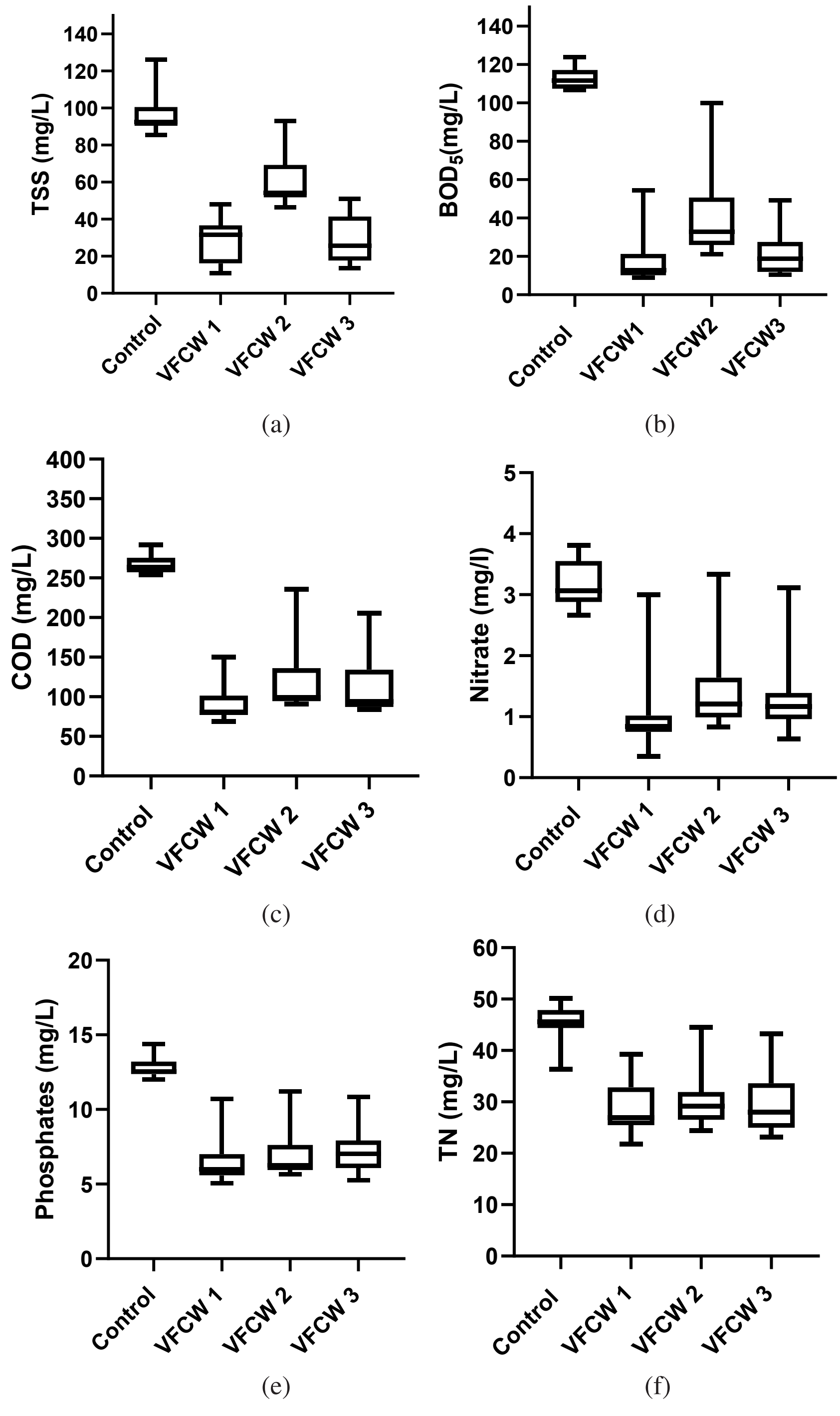

Fig. 4: Box-Whisker graphs for effluent concentrations during the study period for (a) TSS; (b) BOD; (c) COD; (d) Nitrates; (e) Phosphates; (f) TN.

According to statistical analysis for the removal of suspended solids, organic matter and nutrients, there was a significant difference ($p<0.05$) in the treatment between the control system and all the experimental planted VFCW systems (VFCW1, VFCW2, and VFCW3). Among the planted VFCWs, in the removal of TSS, Tukey's multiple comparison results proved that there was a significant difference ($p<0.05$) between VFCW1 and VFCW2 as well as between VFCW2 and VFCW3. But there was no significant difference ($p= 0.999$) between VFCW1 and VFCW3 in the removal of TSS.

Similar results were obtained for the removal of BOD among the planted systems. The VFCW1 and VFCW3 removed BOD efficiently, but there was no significant difference between the two. Whereas significant difference ($p<0.05$) was observed in the treatment between VFCW1 and VFCW2 as well as between VFCW2 and VFCW3.

There was no significant difference amongst the VFCWs planted with different species for the removal of COD, nitrates, TN, and phosphates.

Biomass Yield

During the study period of ten months, the grasses planted in the different experimental VFCW systems were harvested four times. The first cutting was done after 120 days of planting and the subsequent cuttings at an interval of 60 days. The average green biomass yield of Cumbu Napier hybrid grass from the four harvests was 4.5 $kg.m^{-2}$, whereas the average green yield of Gamba grass and Palisade grass were 1.36 $kg.m^{-2}$ and 1.91 $kg.m^{-2}$ respectively. The average dry biomass yield of Cumbu Napier hybrid grass, Gamba grass, and Palisade grass were obtained as 1.5 $kg.m^{-2}$, 0.45 $kg.m^{-2}$, and 0.64 $kg.m^{-2}$ respectively. The biomass yield of Cumbu Napier Hybrid grass was found to be significantly ($p<0.05$) higher than that of Gamba grass and Palisade grass. The average biomass yield of the different grasses obtained from four harvests is presented in Fig. 5.

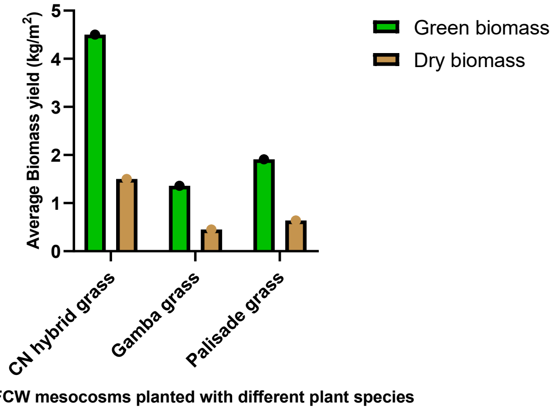

Fig. 5: Average above ground biomass yield from the planted VFCWs.

From the study, it was also observed that the biomass yield of all the grasses declined after each cutting cycle. This can be due to the restrictions in the space and availability of nutrients in the VFCW mesocosms as the plant grows. In this study, the grasses were grown in the VFCW systems without applying any external fertilizer, but the plants extracted the required nutrients and water from the influent wastewater. This is supported by Klomjek (2016) who reported on the feasibility of using Giant Napier grass (*Pennisetum purpureum* cv. King grass) and Dwarf Napier grass (*Pennisetum purpureum* cv. Mott) in vertical flow wetlands for the treatment of swine wastewater in Thailand. The potential of Napier Bajra Hybrid grass (*Pennisetum purpureum X Pennisetum typhoides)* for greywater treatment and its high biomass yield in VFCWs has also been reported by Pillai & Vijayan (2013).

CONCLUSIONS

The study indicated the significance of plant presence and the role of root zone treatment in removing the pollutants in VFCWs. The planted systems using Cumbu Napier Hybrid grass and Palisade grass attained high pollutant removal efficiency, though there was no significant statistical difference between the two. The final effluent from the control and planted systems complied with the Indian standards of treated effluent quality required for irrigation. The green and dry biomass yield of Cumbu Napier Hybrid grass were found to be significantly higher when compared to Gamba grass and Palisade grass.

The VFCWs planted with Cumbu Napier hybrid grass and Palisade grass indicated their suitability to be used as ecological sanitation systems for the decentralized treatment of municipal wastewater and its reuse with regards to subsequent valuable biomass production. In addition to improvement in water quality, the plant harvest provides value-added materials, which can considerably reduce the expenses of treatment. Further investigations are required to investigate the bioethanol production potential of these lignocellulosic grass species.

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