

Effect of HRT and seasons on the performance of pilot-scale horizontal subsurface flow constructed wetland to treat rural wastewater

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ABSTRACT

To find the effect of Hydraulic Retention Time (HRT) and seasons on the performance of horizontal subsurface flow constructed wetland (HSSF CW) in treating rural wastewater, a pilot scale unit 2.5 m × 0.4 m × 0.3 m size bed planted with *Typha latifolia* and *Phragmites australis* was operated for a 12-month duration. During the study 2, 4, 6, 8, and 10 days of HRT were maintained in winter, summer, and rainy seasons. The removal efficiency obtained ranged from 62.09 to 87.23% for Chemical Oxygen Demand, 69.58% to 93.32% for Biochemical Oxygen Demand₅ (BOD), 31.55% to 59.89% for Ammonia Nitrogen (NH₄-N), 15.18% to 52.90% for Total Kjeldahl Nitrogen (TKN), 21.02% to 50.21% for Phosphate Phosphorus (PO₄³⁻ P), 19.82% to 48.23% for Total phosphorus (TP), 74.93% to 93.10% for Faecal Coliform (FC) and 69.93% to 90.23% Total Coliform (TC). Overall, results showed that the performance of the unit was good. For statistical analysis two way ANOVA test followed by Tukey's test was used with a 95% level of significance. It was observed that the removal efficiency of the pollutants was increased with an increase in HRT. HRT of 6 days was found as adequate for significant removal of organic matter (COD and BOD). Seasonal removal efficiencies followed the order of summer > rainy > winter for all the parameters, but the difference was not statistically significant.

Key words: constructed wetlands, *Phragmites australis*, *Typha latifolia*, wastewater treatment

HIGHLIGHTS

- Sporadic study in the study area.
- Helps in the prevention of pollution.
- Low cost treatment solution for the rural areas.
- Helps to optimize the design of treatment unit.

1. INTRODUCTION

In most developing countries, wastewater treatment is not at an agreeable level due to economic and professional manpower constraints (Weerakoon *et al.* 2013). India constitutes about 70% of rural areas, where 90% of untreated wastewater is indiscriminately discharging directly without giving any treatment. Pit latrines and septic tanks are the most widely used onsite sanitation, improper maintenance of which is becoming a source of chemical pollutants and pathogenic bacteria into nearby groundwater sources, posing a threat to public health. This problem is due to the lack of expertise in the maintenance of systems and rural areas cannot afford expensive, conventional treatment systems (Shivendra & Ramaraju 2015). After the implementation of the Swachh Bharath Mission scheme by the Indian government to eliminate the open defecation system, the construction and usage of more toilets in the rural area have a stronger focus on maintenance of this discharge. Thus there is a serious need for effective and affordable wastewater management techniques. In this context, recently Horizontal Subsurface Flow Constructed Wetlands (HSSF CW) seem to have gained attention for successful treatment of organics, nutrients, and pathogens, and have also been accepted globally as cost-effective particularly for a small community (Jorsaraei *et al.* 2014). So far these treatment units are applied to treat a wide variety of wastewater that includes municipal wastewater, industrial effluents, agricultural and storm water runoff, greywater, and landfill leachate due to its many advantages including low energy consumption, ease of operation and maintenance, minimal sludge generation, and the creation of a visually appealing landscape (Puigagut *et al.* 2007; Sim *et al.* 2008; Ye & Li 2009; Merino-Solis *et al.*

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2015). In this system, the operational parameters such as Hydraulic Loading Rate (HLR), Organic Loading Rate (OLR) Hydraulic Retention Time (HRT), type of plants and filter media are expected to improve the water quality (Ewemoje *et al.* 2015). HRT is one of the significant parameters under consideration (Ghosh & Gopal 2010; Ewemoje *et al.* 2015; Sa *et al.* 2019). However, there are limited studies involving rural wastewater treatment using the HSSF CW unit in the southern part of India, especially regarding the design parameters. Additionally, no conclusive results are available on the influence of seasons in this region. In this regard, the present study aimed to find the effect of HRT on the removal efficiency of HSSF CW to treat rural wastewater over a period that covers different seasons. The key objectives were to evaluate the effect of operational parameters such as (a) hydraulic retention time and (b) season, on pollutant removal efficiency.

2. MATERIALS AND METHODOLOGY

2.1. Pilot-scale setup description

The pilot-scale HSSF CW unit was set up in the open-air laboratory, PET Research Center (12.5161°N, 76.8790°E), Mandya, Karnataka, India. The rectangular-shaped unit made of stainless steel with a dimension of 2.5 m × 0.4 m × 0.3 m is prefabricated and installed. The design of the reactor has done by using the Kickuth method (Kadlec & Wallace 2008; UN-Habitat 2008; Patel & Dharaiya 2013; Dotro *et al.* 2017). The arrangement had been done in the reactor to expel excess rainfall to avoid surface flow conditions. The stabilized porosity was 0.45 at the time of reaching the steady-state condition. As polyculture being the least susceptible to seasonal variation, and provides consistent treatment, two locally available plants were used, namely *Phragmites australis* and *Typha latifolia*, which exhibit the highest oxygen release rate of 1.4 mg/h/plant and 1.0 mg/h/plant respectively (Meng *et al.* 2014). The plant density was of 16 no/m² bed area during plantation and later reached 34 no/m² at the time of operation.

2.2. Synthetic wastewater preparation

Rural wastewater consists of grey water and black water was collected at the household level from various villages in the study area and analyzed to know the characteristics of pre-settled wastewater. The synthetic wastewater was prepared to simulate the best characteristics of rural wastewater (Zhao *et al.* 2010) but the modification was done to adjust COD/N to optimize the performance (Camacho *et al.* 2007; Cheng *et al.* 2011). Compounds used for simulation were Glucose (C₆H₁₂O₆), which is the main source of organic loading, the source of Nitrogen is Ammonium chloride (NH₄Cl), and the source of phosphorus is Hydrogen Potassium Phosphate (K₂HPO₄). The septage sludge collected from the wastewater treatment plant was added to get the biological characteristics (Weerakoon *et al.* 2013). Prepared wastewater is fed to the feeding tank and channeled through a constant head arrangement to maintain a predetermined flow rate.

2.3. Operation of the unit

The pilot plant setup initially fed with gradually increasing wastewater/tap water ratios and eventually completely replaced by wastewater. The reactor reached steady-state condition after 6 months. The first experimental run was in October 2017. During the operation, the hydraulic loading rate was adjusted to get the desired HRTs of 2, 4, 6, 8, and 10 days in all three seasons. The sampling campaigns were carried during winter (October 2017 to January 2018), summer (February to May 2018), and rainy (June to September 2018). Water samples were analyzed for Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Ammonia Nitrogen (NH₄-N), Total Kjeldahl Nitrogen (TKN), Phosphate Phosphorus (PO₄³⁻ P), Total Phosphorus (TP), Faecal Coliform (FC) and Total Coliform (TC). All the parameters were analyzed as per standard procedure (APHA 2012).

2.4. Statistics

Experiment results were statistically evaluated using SPSS[®] 24 and Microsoft Excel 2013 software. Data normality and homoscedasticity were checked in the Shapiro Wilk W test and Levene test. Comparison of removal efficiency between seasons and HRT were performed using two-way ANOVA and post hoc analysis was performed by Tukey's test with 95% significance level of difference ($p < 0.05$).

3. RESULT AND DISCUSSION

The average concentration of synthetic wastewater and rural wastewater is shown in Table 1. The details of the operational parameters are tabulated in Table 2. During the study period pH, dissolved oxygen, and the temperature of wastewater were taken into consideration and recorded for the operation of the pilot plant setup. The

Table 1 | Average concentration of synthetic wastewater loaded into the HSSF CW throughout the study period

Parameters	Average	SD
COD (mg/L)	253.19 (263.15)	36.39 (20.35)
BOD (mg/L)	210.34 (200.32)	24.55 (15.32)
NH ₄ -N (mg/L)	40.86 (7.83)	5.30 (2.23)
TKN (mg/L)	52.23 (9.23)	8.14 (1.56)
PO ₄ -P (mg/L)	3.61 (2.32)	0.44 (0.52)
TP (mg/L)	5.97 (6.88)	0.34 (2.98)
FC *10 ⁸ cfu/100 mL	6.52 (7.02)	0.89 (1.79)
TC *10 ⁸ cfu/100 mL	8.35 (9.19)	1.04 (1.35)

Values in parentheses represent average concentration of presettled rural wastewater.

Table 2 | Main operational parameter of HSSF CW system

Parameter	Unit	1 HRT	2 HRT	3 HRT	4 HRT	5 HRT
Hydraulic retention time	days	2.0	4.0	6.0	8.0	10.0
Hydraulic loading rate	l/m ² /d	56.3	28.1	18.8	14.1	11.3
Organic loading rate						
Chemical Oxygen Demand (COD)	g/m ² /d	14.23	7.12	4.74	3.56	2.85
Biochemical Oxygen Demand (BOD)	g/m ² /d	11.81	5.91	3.94	2.95	2.36
Ammonia Nitrogen (NH ₄ -N)	g/m ² /d	0.41	0.21	0.14	0.10	0.08
Total Kjeldahl Nitrogen (TKN),	g/m ² /d	0.53	0.26	0.18	0.13	0.11
Phosphate Phosphorus (PO ₄ ³⁻ P)	g/m ² /d	0.20	0.10	0.07	0.05	0.04
Total Phosphorus (TP)	g/m ² /d	0.28	0.14	0.09	0.07	0.06
Faecal Coliform (FC) × 10 ¹²	cfu/m ² /d	0.37	0.18	0.12	0.09	0.07
Total Coliform (TC) × 10 ¹²	cfu/m ² /d	0.47	0.23	0.16	0.12	0.09

monthly air temperature observed during the study period summarized in Table 3. Data showed that the highest temperature recorded in April was 36 °C, while the lowest was 18 °C in December.

Though there was moderate variation in the temperature, the mean temperature variation was within the optimum range (20–35 °C) (Kadlec & Reddy 2001). The analysis of temperature data reveals that the climatic conditions of the study area are favorable for the growth and survival of plants grown in constructed wetlands throughout the year (Zamora-Castro *et al.* 2019). The removal efficiency of COD, BOD, NH₄-N, TKN, TP, PO₄³⁻ P, FC, and TC obtained during the study period is shown in Table 4. Figure 1 represented the box plot of mean removal efficiency under different HRT and seasons.

3.1. Overall performance evaluation of pilot plant setup

3.1.1. Organic matter removal

The average inflow concentration of COD and BOD were 253.19 mg/L and 210.34 mg/L respectively. The removal efficiency achieved ranges from 62.09 to 87.23% for COD and 69.58% to 93.32% for BOD (Figure 1(a))

Table 3 | Variation of air temperature in study area during operation period of HSSF CW system

Yr 2017-18	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep
T max °C	28	28	28	30	32	34	36	33	29	29	28	30
T avg °C	26	25	24	26	28	31	32	30	27	26	26	27
T min °C	21	19	18	19	20	23	25	24	22	22	21	21

Table 4 | Mean value of removal efficiency for different HRT and seasons in HSSF CW treatment system

HRT days Parameters	2 Efficiency (%)	4 Efficiency (%)	6 Efficiency (%)	8 Efficiency (%)	10 Efficiency (%)
Winter season					
COD	62.09	70.74	80.07	84.05	86.41
BOD	69.58	76.95	86.14	89.25	92.66
NH ₄ -N	31.55	38.74	49.23	56.25	59.39
TKN	15.18	24.53	37.20	47.51	52.69
PO ₄ -P	21.02	30.58	40.34	45.35	48.50
TP	19.82	27.23	37.51	44.34	48.70
FC	74.93	80.88	82.43	89.61	91.73
TC	69.93	77.15	79.30	86.1	89.6
Summer season					
COD	65.03	74.43	82.79	86.11	88.34
BOD	73.78	80.34	88.08	92.22	94.93
NH ₄ -N	35.77	43.32	53.93	62.87	64.23
TKN	18.48	27.69	42.96	51.45	55.44
PO ₄ -P	23.89	34.53	43.32	50.20	52.40
TP	23.55	30.32	40.80	47.06	50.65
FC	75.92	81.43	84.93	93.48	95.32
TC	73.34	79.95	83.38	90.94	93.09
Rainy season					
COD	63.27	71.43	80.96	85.78	87.23
BOD	71.66	78.07	87.29	90.91	93.32
NH ₄ -N	33.60	40.37	50.90	57.42	59.89
TKN	18.62	26.43	41.09	47.22	52.90
PO ₄ -P	22.86	32.08	42.12	48.33	50.21
TP	20.08	28.45	35.67	45.78	48.23
FC	74.80	82.45	84.93	91.41	93.10
TC	72.20	78.37	81.32	88.30	90.23

and 1(b)). The leguminous microorganism that is attached to the plant's roots and sediment surface carry out the biodegradation process (Saeed & Sun 2012). Depending on the availability of dissolved oxygen, organic matter is removed primarily by aerobic microbiological or anaerobic biodegradation. The overall efficiency of the unit was good for organic matter removal.

A study claims the maximum COD removal rate of 91.5% and Ammonia nitrogen reduction of 99.9% for municipal wastewater (Langergraber 2005). A study on optimization of the performance of wetlands carried out by Valsero and the team observed better efficiency of *Typha angustifolia* than *Phragmites australis* in the removal of organic matter (Hijosa-Valsero *et al.* 2010). Removal of particulate organic matter is attributed to filtration through the bed of wetland and retained organic matter by aerobic respiration in which oxygen is the terminal electron acceptor and in anoxic conditions by aerobic organisms in which nitrates are the terminal electron acceptor (Garcia *et al.* 2010).

3.1.2. Nitrogen removal

The nitrogen compounds inflow concentrations were 40.86 mg/L and 52.23 mg/L for NH₄⁻N and TKN. The removal efficiency was ranged between 31.55% to 59.89% and 15.18% to 52.90% (Table 3 and Figure 1(c) and 1(d)). In the present research work, nitrogen removal was moderate, which is in line with previous findings (Tyroller *et al.* 2010; Butterworth *et al.* 2013; Nivala *et al.* 2013; Butterworth *et al.* 2016). The lesser removal efficiency in HSSW CW may be due to lack of oxygen availability, there was no sufficient nitrification, resulting in

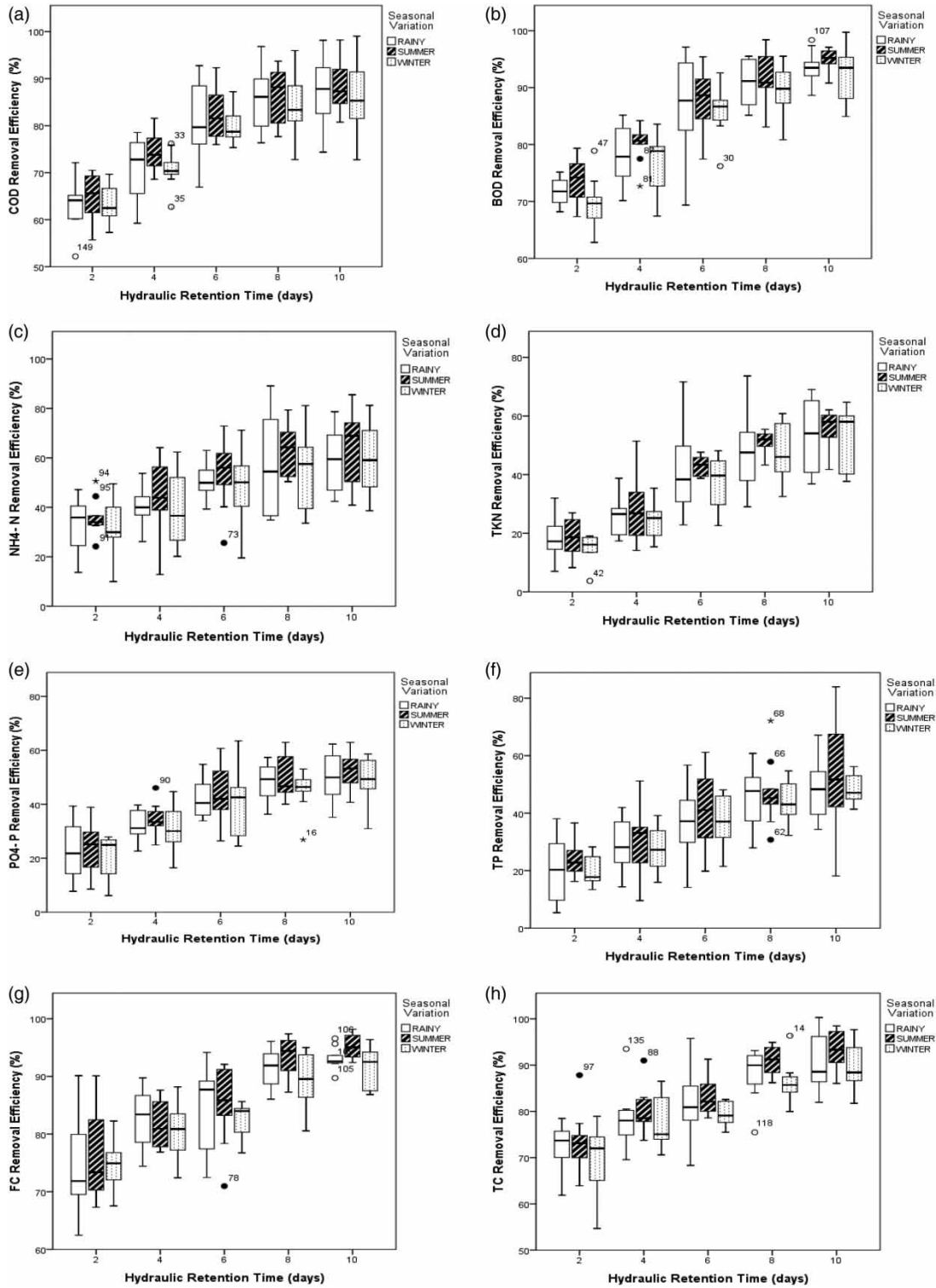


Figure 1 | Box plot representing the mean percentage removal efficiency of (a) COD, (b) BOD, (c) TKN, (d) NH₄-N, (e) PO₄³⁻ P, (f) TP, (g) FC and (h) TC under different HRT and seasons during the study period.

higher effluent concentration. According to Vymazal, with a pH of 6.5 to 8.5, the nitrification process will be optimum at the temperature range of 30 to 40 °C and ammonification will be 40 to 60 °C (Vymazal 2007). Higher temperature may improve the microbial activity and thereby nitrogen removal efficiency. Recent studies conducted by Lai and co-workers claim the efficiency of nitrogen removal decreases with the increase in COD/N ratio. This might be the reason for the halt of nitrogen removal by 67% (Lai et al. 2020). Labella and the team

evaluated the influence of forced aeration in HF in which $\text{NH}_4\text{-N}$ removal performance was increase by 69% (Labella *et al.* 2015). Similar attempts can be made in the present study to improve efficiency.

3.1.3. Phosphorus removal

The average inflow of PO_4^{3-} and TP were 3.61 mg/L and 5.97 mg/L respectively and removal efficiencies ranged from 21.02 to 50.21% and 19.82% to 48.23% respectively (Figure 1(e) and 1(f)). Major processes in concern with the removal of phosphorous in constructed wetlands are adsorption, precipitation, and plant uptake (Saeed & Sun 2017; Sa *et al.* 2019). These results are consistent with previous literature studies (Xu *et al.* 2006; Ewemoje *et al.* 2015). Tanner and his team conducted an experimental investigation on the removal of total phosphorus that claims increase in total phosphorus removal efficiency of up to 38% with planted wetlands (Tanner *et al.* 1995). According to Vymazal (2007), adsorption due to a gravel bed in the wetland is limited and the involvement of plants is considered to be less with a constructed wetland. In the present study, as no special media materials are used for the phosphorus, there is moderate removal efficiency.

3.1.4. Coliform removal

In the present study, the average inflow concentration of FC and TC were 6.52×10^8 cfu/100 mL, 8.35×10^8 cfu/100 mL respectively. The overall removal efficiency achieved was ranged from 74.93 to 93.10% for FC, 69.93% to 90.23% for TC (Table 4), (Figure 1(g) and 1(h)). Even though the reduction of total coliform doesn't serve as an indication for the pathogen removal, the fecal coliform count is prominently considered in this order and it is claimed that there is significant coliform removal with constructed wetlands (Alufasi *et al.* 2017). Cell death is the major factor in coliform removal; other factors such as filtration, adsorption, and sedimentation play a role in coliform removal. Oxidation, predation, inactivation of cells, temperature, and water chemistry also significantly influence the pathogen removal in constructed wetlands (Weber & Legge 2008; Vymazal 2011; Makvana & Sharma 2013; Karimi *et al.* 2014; Wu *et al.* 2016). The coliform group of bacteria was reduced in higher order and the effluent would still have a high potential for containing pathogens, and would need to be disinfected before discharge.

3.2. Effect of HRT

The relationship between applied mass loading rate and corresponding effluent concentration of COD, BOD, $\text{NH}_4\text{-N}$, TKN, TP, PO_4^{3-} P, FC, and TC in all three seasons is represented in Figure 2. From the graph, it is clear that in all the seasons, effluent concentration increases as hydraulic loading increases. Since higher loading implies smaller HRT, resulting in higher effluent concentration thereby lower removal efficiency. This data is useful to the designer to fix the required loading rate, thereby HRT to get the desired effluent concentration within the preset limit. Table 5 represents the statistical analysis results from two-way ANOVA and post-hoc Tukey's test during the study period.

For both COD and BOD, there was a significant increase in the removal efficiencies with every increase in HRT up to 6 days; that is, from 2 to 4 days and from 4 to 6 days ($p > 0.05$). Beyond this, further increases in HRT from 6 to 8 days and 10 days did not bring significant changes ($p < 0.05$). This implies that 6 days HRT was found as adequate for the removal of organic matter in higher order. The removal efficiency achieved at this HRT was up to 82.79% and 88.08%. In the study of Akrotos and Tsihrintzi, HRT of 8 days was adequate (Akrotos & Tsihrintzi 2007) wherein the result is comparable. HRT of 3 days was adequate to treat the secondary effluent with a concentration of 108.66 mg/L in the previous study conducted by Ghosh (Ghosh & Gopal 2010), whereas in the present study treatment of primary effluent with a higher concentration of 250.19 mg/L might have demanded higher HRT in the present study.

With $\text{NH}_4\text{-N}$ and TKN removal, HRT plays a significant role. For $\text{NH}_4\text{-N}$, the result showed significance in only 4 to 6 days. The TKN result showed a significant increase in removal efficiencies for every increase in HRT from 2 to 4 days, 4 to 6 days and then 6 to 8 days; that is, for 4, 6 and 8 days ($p < 0.05$). But between 8 and 10 days the increase in value is insignificant ($p > 0.05$). But the result showed that beyond 6 days HRT, there is still a significant increase in the removal of efficiency for 8 days. The results showed that nitrogenous organic matter requires longer HRT than carbonaceous matter.

Similar to organic compounds, for P-PO_4^{3-} up to 8 days, there is a significant increase in each shift of HRT; that is, ($p < 0.05$). Thereafter there is no significant increase. But TP showed a significant difference only when HRT increased from 4 to 6 days ($p < 0.05$), not for any other HRTs. The result indicates that for PO_4^{3-} P, though 8 days is required, for TP up to 6 days there is a significant increase. The corresponding highest efficiencies obtained for

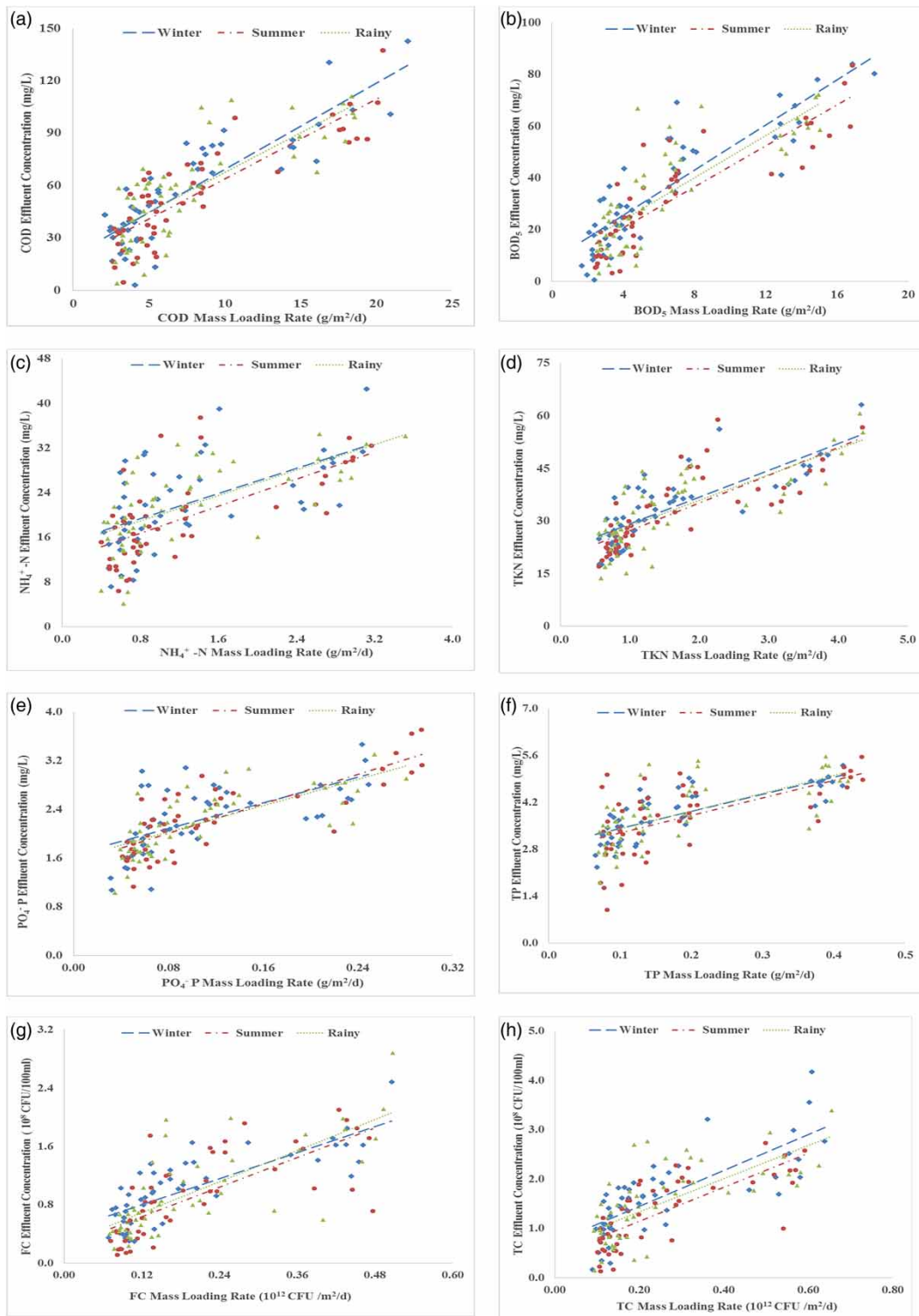


Figure 2 | Relationship between the mass loading rate and effluent concentration during study period for: (a) BOD, (b) COD, (c) TKN, (d) NH₄-N, (e) PO₄³⁻-P (f) TP (g) FC and (h) TC.

8 days were 50.20% and 47.06%. Sa and team conducted a study and got the highest removal of 73% for TP in 6 days HRT for an inlet concentration of 41.5 ± 10.4 mg/L for HSSF CW planted with *Cyperus alternifolius* (Sa *et al.* 2019). The type of plant species, and media might have contributed to higher efficiencies.

The coliform groups of bacteria also showed significant dependency on HRT. Findings from previous studies submitted that coliform removal is mainly subjective to hydraulic characteristics and the presence of vegetation

Table 5 | Two way ANOVA and post-hoc Tukey's test *p*-Value for COD, BOD, NH₄⁻N, TKN, TP, PO₄³⁻ P, FC and TC under HRT and seasonal variation and combined effect during the study period

Factor	COD removal (%)	BOD removal (%)	NH ₄ N removal (%)	TKN removal (%)	PO ₄ P removal (%)	TP removal (%)	FC removal (%)	TC removal (%)
Two-way Analysis Of Variance								
HRT	0.080	0.067	0.151	0.115	0.090	0.347	0.095	0.085
Season	8.61E-36*	5.71E-43*	5.28E-15*	5.06E-36*	5.77E-28*	1.02E-17*	1.23E-30*	1.19E-24*
HRT × Season	1.000	0.999	1.000	0.996	1.000	1.000	0.925	0.419
Tukey's Test – Seasonal Variation								
Winter – Summer	0.067	0.065	0.143	0.094	0.073	0.362	0.003	0.061
Summer – Rainy	0.368	0.186	0.363	0.527	0.552	0.392	0.151	0.659
Rainy – Winter	0.644	0.318	0.855	0.569	0.474	0.998	0.285	0.337
Tukey's Test – HRT Variation								
2–4 days	0.000*	0.000*	0.221	0.002*	0.000*	0.061	0.000*	0.000*
4–6 days	0.000*	0.000*	0.019*	0.000*	0.000*	0.009*	0.269	0.306
6–8 days	0.069	0.023	0.184	0.004*	0.047*	0.050	0.000*	0.000*
8–10 days	0.682	0.123	0.960	0.215	0.799	0.727	0.388	0.597

**P*- Value <0.05.

(Kipasika *et al.* 2016; Wu *et al.* 2016; Soler *et al.* 2019) For FC, an increase of HRT from 2 to 4 days observed a significant difference ($p < 0.05$) but not from 4 to 6 days. Again for 8 days the increase was significant ($p > 0.05$). In resemblance with FC, TC also followed the same shape of significance. These findings are in line with the previous finding by Soler *et al.* (2019). The author found that HRT influence is highly significant on the removal of coliform and may yield up to 96% removal at 4 days HRT in surface wetlands. Present research work results indicate that, even with a lower HRT, the system is capable of removing fecal coliform at a higher level, irrespective of the seasons in the trophic region. The effect of HRT is simple to explain: the longer the HRT, the longer the bacteria are exposed to unfavorable conditions (Vymazal & Kropfelova 2015; Kipasika *et al.* 2016).

3.3. Effect of seasonal variation

Seasonal variation in the removal of nutrients in wastewater has often occurred. Figure 2 indicates a seasonal comparison of effluent concentration in which the smaller the slope, the more efficient the unit is. The removal efficiency of COD and BOD in summer was up to 88.34% and 94.93% respectively whereas in winter it was up to 86.41% and 92.66% with rainy season at 87.23% and 93.32%. Statistical test result (Table 4) showed no significant variation ($p > 0.05$) (Figure 2(a) and 2(b)). According to the previous literature, aerobic and anaerobic bacterial activities play a significant role in the reduction of carbonaceous organic matter and these can survive and act efficiently up to the temperature of 5 °C (Saeed & Sun 2012). From Table 3 it is clear that air temperature in the study area is higher, which is suitable for organic matter removal. Hence there is no statistically significant variation has occurred among the season.

For NH₄⁻N and TKN, the removal efficiency in winter was up to 59.39% and 52.69% whereas in summer it rose to 64.23% and 55.44% and the rainy season showed the performance in between summer and winter, 59.89% and 52.90% respectively (Figure 2(c) and 2(d)). The Akrotos and Tsihrintzi studies showed significant seasonal variation since in the subtropics region the temperature drops below 15 °C and at this temperature the bacteria responsible for removal of nitrogen and macrophytes of the treatment do not work properly (Akrotos & Tsihrintzi 2007; Chang *et al.* 2013; Giustinianovich *et al.* 2016). In this study, the statistical test showed significant no seasonal variation ($p > 0.05$) between the seasons which may be due to, as the study area falls under the tropical region even in the coldest season, the temperature ranges above 20 °C, which does not alter the performance of the unit in any season. The minor seasonal variation may be due to the summer period has been more efficient in removing the NH₄⁻N might in part be related to better oxic conditions due to higher oxygen released by plants roots, which promote the nitrification process (Liang *et al.* 2017).

The seasonal variation of phosphorus compound followed the same trend as organic matter (Figure 2(e) and 2(f)). Highest in summer up to 52.40% and 50.65%, followed by 50.21% and 48.23% in the rainy season and 48.50% and 48.70% in winter respectively. This insignificant seasonal variation ($p < 0.05$, Table 5) may be due to during winter dead microbial mass and litter being decomposed and releasing phosphorus from the precipitates, resulted in increased solubilization. Liang and team observed increased phosphorus removal results in winter due to the gradual development of the microbial community and the assimilation of P by the microbes (Liang *et al.* 2017).

For both FC and TC, the highest efficiency was obtained in summer; that is, 95.32% and 93.09%, followed by rainy season at 93.10% and 90.23%, and least during winter at 91.73% and 89.60%, respectively (Figure 2(g) and 2(h)). So summer months may positively affect the pilot unit and might have increased their ability to remove coliforms. The lower removal in the winter can be attributed to the lower microbial activity and alternatively, a substantial reduction in root biomass during the winter season may limit microbial attachment surface area and filtering capacity of the substrate. When statistically tested, the seasonal variation that affected the removal efficiency of both FC and TC was insignificant ($p < 0.05$) (Table 5).

The increased rainfall during the rainy season may have increased hydraulic loading, thereby decreasing HRT and resulting in lesser removal efficiency. However, provision is made in the reactor to avoid the effect of excess rainfall runoff. Overall during the study period, the result showed that the season did not affect the performance of the unit significantly for any parameter under consideration. Previous studies reported that the net effect of seasonal variation in wetlands is greatly influenced by plant-mediated oxygen transfer, which will be affected by cold temperatures and it occurs mainly in temperate zones (Stein *et al.* 2006; Zamora-Castro *et al.* 2019). As the study area lies in a tropical region no seasonal variation has been found.

4. CONCLUSION

Based on the obtained result, the following conclusion can be drawn.

- HSSF CW showed good removal efficiency for organic matter (88.34% for COD) and coliforms (93.09% for TC) but moderate removal for nitrogen (55.44% for TC) and phosphorus (50.65% for TP). In this study, the removal efficiency of all the parameters increased with the increase in HRT. The effect of HRT was statistically significant and HRT of 6 days was found to be adequate for removal of organic matter, and for all other parameters higher HRT is recommended. All parameters exhibited a seasonal trend of summer > rainy > winter in the pollutant removal efficiency but variation was not statistically significant. Further studies are required to assess the long-term monitoring of the treatment unit by the application of rural wastewater at effective HRT based on the experimental results by the development of scale-up techniques.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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