Pollutant Removal from Sewage in Tropical Climate by Constructed Wetland System: An Asset for Irrigation

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Abstract. In the global outlook, letting of untreated sewage in existing river bodies deteriorates the water quality. The seepage likely depreciates the quality of ground water too. The quality of groundwater with special reference to India has tremendously gone down in the past twenty years leading to sour taste. On the other hand, agriculture sector is deprived of water in many places of India. A solution can be arrived concurrently by treating sewage and consuming the effluent in agricultural sector. First order kinetics was applied in constructed wetland system at different flow rates and optimised. At optimised HLR, effluent met the standards of discharge that can be utilized for agricultural/ irrigational purpose. The emanating major pollutants can be effectively treated using constructed wetland system under tropical climate. A few clippings at the onsite treatment illustrated the diversity of species thus adjoining sustainable biodiversity and treatment. Thus in tropical countries like India, constructed wetland system might pave solution not only for the treatment of sewage but in deploying the effluent in agricultural sector. A clean ecosystem can be achieved with sustainability.

1.   Introduction

Wastewater discharge from diverse sector releases a wide range of contaminants seeking profound attention of the environmentalists worldwide. Organic pollution of the rivers have a serious negative impact on human health and ecosystem. In the Indian scenario, about 62,000 MLD of domestic wastewater (sewage) is generated while the treatment capacity is only 37% of it. Remaining 63% of untreated sewage is let into rivers across the country including the Ganga River basin which supports almost 45% of living population (Times of India 2015). Agriculture being the backbone of the country strives due to water depletion. Henceforth, providing an effective solution correlating with environmental concern is focussed.

The sources that contribute to contamination of water bodies are bounteous and majorly anthropogenic. The River Yamuna in Delhi, India is highly polluted by domestic wastewater with elevated levels of ammonium concentration making it unfit for human consumption (Groeschke *et al*. 2017). Water quality of the upstream and downstream of the River Mandzoro was studied and reported by Baloyi *et al*. (2014) that there was deterioration in the water quality at the downstream due to the discharge of poor quality effluent from the sewage treatment plant. Indirect source of contamination may be due to urbanization, seepage of storm water, agricultural run-off and precipitation of atmospheric contaminants released owing to industrial evolution (Naderizadeh *et al*. 2016).

1.2. List of pollutants in domestic wastewater

The major pollutants in domestic wastewater that deteriorates the water quality when released in water bodies are cited.

1.2.1　　　Organic pollutants

The organic pollutants emanate majorly from domestic sewage, storm water, industrial effluent and agricultural run-off. Decomposition of organic pollutants results in the depletion of oxygen thus deeming it unfit for the survival of biotic life (Sharma & Gupta 2014). The organic pollutants from wastewater discharges had seriously affected the macro invertebrates in aquatic system. Globally the number of people affected by organic pollution of (Biological Oxygen Demand) BOD > 5 mg/L due to contamination of rivers was projected to be 2.5 billion in 2015 (Wen *et al.* 2017).

1.2.2　　　Nutrients

The major nutrients such as phosphorus and nitrogen are released in disagreeable amounts in domestic wastewater. The bounteous supply of nutrient leads to eutrophication. Excess nitrogen results in toxic algal blooms that gains entry via food chain and poses threat to aquatic life and humans leading to economic loss (Naden *et al.* 2016).

1.2.3　　　Heavy metals

Domestic wastewater contains heavy metals zinc, iron, cadmium, copper, aluminium, lead and manganese. A study conducted in Japan revealed that an average of 0.2–0.3 Cd, 1.6–1.9 Ni, 3.5-6.8 Pb, 0.8-1.4 Cr, 8.2-19.3 Mn, 9.4- 55.8 Cu, 44.3 – 62.7 Zn and 111- 293 Fe mg/ day/ person is released in domestic wastewater. Intrusion of sewage contamination has led to heavy metal concentration of lead, chromium, cadmium and nickel in underground water, surface water, soil and crop plants (Chino *et al.* 1991). The sources of heavy metal contamination in sewage are rainfall and soil erosion. These heavy metal containing aerosols usually accumulate on leaf surfaces in the form of fine particulates and can enter the leaves via stomata. Some of the human sources of heavy metals in wastewater effluents are metal finishing and electroplating, mining and extraction operations, textiles activities and nuclear power. Metal finishing and electroplating involve the deposition of thin protective layers into prepared surfaces of metal using electrochemical processes (Oghenerobor et al 2014)

1.2.4　　　Microbial contamination

Most of the rivers are polluted with fecal indicators such as total coliform, fecal coliform, *Escherichia coli* and fecal *Streptococci* due to contamination of excreta by humans and warm blooded animals. A study conducted in the rural sectors of Odisha, India by Schriewer *et al.* (2015) revealed human fecal markers in community water resources such as ponds (8%), tube wells (2%) and stored water (20%).

Bacterial analysis of River Ganges revealed that it is highly contaminated with coliforms, *Enterococcus faecalis*, *Actinomyces* sp., *Aerobacter aerogenes Staphylococcus aureus*, *Shigella* sp., *Bacillus* sp., *Salmonella* sp. and *Clostridium perfringens*. Hence consumption of Ganga water may lead to serious health risks (Bilgrami & Kumar 1998). Coliform infection, especially *E. coli* causes bloody diarrhea, nausea, vomiting, dehydration, fever and loss of appetite.

1.2.5　　　Emerging pollutants

Major emerging pollutants like carbamazepine, galaxolide and tonalide arise from pharmaceutical and cosmetic usage. Antibiotics sulfamethoxazole in wastewater is persistent and monitoring of wastewater for emerging pollutants is essential (Lamastra *et al.* 2016).

1.3　　　Essence of CWS in India

Water deficit is prevalent due to depletion of groundwater table. In India, agricultural sector consumes 82% of the total water supply. Hence, domestic wastewater can be treated and reused for agricultural purpose instead of letting it into water bodies thereby preserving its aesthetic value. Though many technologies are available, constructed wetland system (CWS) is the best ever green technology with less maintenance and operation cost to treat wastewaters for decades (USGS 2002).

2. Materials and Methods

2.1   Raw sewage- Source and analysis

Raw sewage was collected from the sewage treatment plant (STP) in Anna University, CEG campus, Chennai. Samples were collected on weekly basis for ten weeks and subjected to physico-chemical and biological analysis. All the analysis were carried out according to the standard procedures (APHA 2017).

2.2   Plants chosen for the study

Based on the literature study, the sedge *Cyperus alternifolius* was chosen for the study with the idea of converting the harvested biomass into some useful product. *Cyperus alternifolius* commonly known as umbrella sedge belongs to the family Cyperaceae. It is a perennial plant capable of growing to a height of 4- 6 feet with higher percentage of fine fibrous root biomass. Healthy plantlets were purchased from Sri Venkateshwara farm, Injambakkam, Chennai. The culms can be used in making mats, hats and thatching. The species is perennial and locally available.

2.3   Experimental setup for vertical flow constructed wetland system

The site for the experimental set up was chosen near the sample source, explicitly in the sewage treatment plant, Anna University, CEG campus, Chennai. The constructed wetland was built with 5mm acrylic sheets with the dimension of 1.2x0.6x 0.7 m provided with a slope of 0.5 and an outlet for effluent collection.

The reed bed consisted of three layers: pebbles (10 cm), blue metal chips (5 cm), coarse sand (15 cm) and fine sand (30 cm) from bottom to top. Blue metal chips was sandwiched between the sand and pebbles to avoid the percolation of sand into the gaps created in the pebble layer. The porosity of the wetland was calculated from the formulae

$$\text{Porosity \%} = \frac{\text{Void volume}}{\text{Total volume}} \times 100 \qquad \text{(Cresswell and Hamilton 2002)}$$

The wetland tank was covered with black cloth to prevent growth and interference of algae in the efficiency of the system. The experimental set up is shown in Figure 2.1.

Twelve plantlets were planted in the reed bed of each unit at equal intervals and saturated with diluted sewage for a period of 4 weeks for acclimatization and establishment of roots. The saturation aids in establishment of compact bed and association of microbial growth in reed bed and rhizome (Sehar et al. 2013).
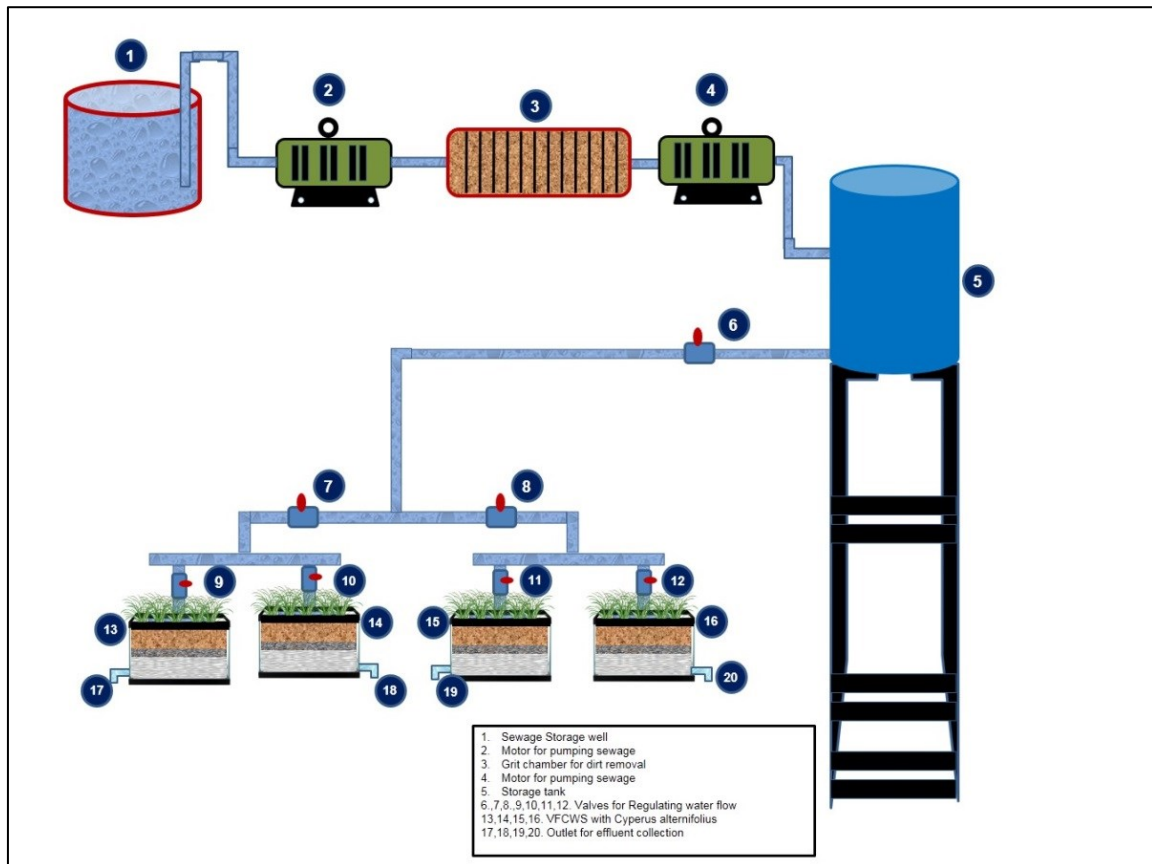
1. Sewage Storage well
2. Motor for pumping sewage
3. Grit chamber for dirt removal
4. Motor for pumping sewage
5. Storage tank
6.,7,8.,9,10,11,12. Valves for Regulating water flow
13,14,15,16. VFCWS with Cyperus alternifolius
17,18,19,20. Outlet for effluent collection

Figure 2.1 Experimental set up

Sewage was passed through grit chambers to segregate the debris and pumped into storage tank with a capacity of 210 liters. From the storage tank the wastewater was supplied into constructed wetland units. The influent was distributed evenly at the top through perforated pipe lines. Valves were set in the PVC pipes for regulation of water flow. Preliminary studies were conducted to monitor the plant growth of both species and compared with respective control.

2.4   Optimisation of HLR

The effect of HLR on removal efficiency was examined over a period of 16 weeks at four different hydraulic loading rates: 28 mm/d (20 L/d), 56 mm/d (40 L/d), 84 mm/d (60 L/d) and 112 mm/d (80 L/d). Hydraulic retention time (HRT) of respective HLR was calculated theoretically from the formulae (Lee et al 2015).

$$HRT = \frac{\text{Volume of wetland x porosity}}{\text{Flow rate}}$$

Influent and effluent were collected at sampling interval of 7 days. All samples were analysed for BOD, COD, TKN, TSS, TDS, phosphate, and heavy metals. However, microbiological analysis for total coliforms, faecal coliforms and *E. coli* were performed on fortnight basis for the same period of study. All the analysis were carried out in triplicates and averaged. The optimal performance of the wetland was evaluated based on mass removal rate, removal efficiency of the pollutants, areal removal rate constant and volumetric removal rate constant.

2.4.1   Calculations

The formulas used for the calculation are presented (Lee et al 2015).

$$\text{Removal efficiency \%} = \frac{C \text{ (influent)} - C \text{ (effluent)}}{C \text{ (influent)}} \times 100$$

Where C represents concentration in mg/L.
Mass removal rate was calculated using the formula

$$r = q \, (C \text{ (influent)} - C \text{ (effluent)})$$

Where q is the hydraulic loading rate in $m \, d^{-1}$. Mass removal rate is expressed in $g \, m^{-2} \, d^{-1}$. $q = Q/A$. where Q is the flow rate through the wetland and A is the area of the wetland. The geo coordinate of sample collection was Latitude: 13.0127 Longitude: 80.2364.

2.4.2   First order kinetics

First order degradation approach was used to predict the removal efficiency of the system. Areal removal rate constant and volumetric removal rate constant were calculated for the pollutants BOD, COD, TSS, TKN and TP. Kadlec & Wallace (2009) proposed the following equation for areal removal rate constant and the same equation was applied.

$$\ln \left[(C \text{ (effluent)} / C \text{ (influent)}) \right] = - K_A / q.$$

Where K_A is the areal removal rate constant in $m \, d^{-1}$ and q is the hydraulic loading rate in $m \, d^{-1}$.
The following equation for volumetric removal rate constant proposed by Reed et al. (1995) was applied.

$$\ln \left[(C \text{ (effluent)} / C \text{ (influent)}) \right] = - K_v \, t.$$

Where K_v is the volumetric removal rate constant in d^{-1} and t is the hydraulic retention time in the wetland.

3. Result and Discussion

3.1 Characteristics of sewage

The results of characterisation of the sewage with an average and standard deviation of sample size 10 are provided in Table 3.1. The pH range of domestic sewage from various cities of India ranges from 7 to 7.5 (CPCB 2005). The range of pH for domestic wastewater is 5.5 - 8 (Metcalf & Eddy 2003). But present study reveals that sewage is slightly alkaline in nature. Similar alkaline pH range is reported by Sonune et al. (2015). Fresh sewage is alkaline in nature. However, near neutral pH were also reported. If biological treatment is preferred the pH should be in the range of 6-8 for efficient action of microbes.

Electrical conductivity (EC) is a measure of the suitability of water for irrigation. Higher EC values indicates salinity. Irrigation with sewage contaminated water increases the electrical conductivity of the soil (Shresta et al 2017). The range of electrical conductivity in the present study revealed sewage as medium strength.

Table 3.1 Characteristics of sewage

Parameters	Mean ± S.D
Ph	7.8 ± 0.5
Electrical conductivity (μscm^{-1})	550 ± 100
BOD (mg/L)	250 ± 22
COD (mg/L)	360 ± 25
TKN (mg/L)	24 ± 5
Nitrate nitrogen (mg/L)	1.5 ± 0.5
Ammoniacal nitrogen (mg/L)	14 ± 2
Total phosphate (mg/L)	4.3 ± 0.9
Sulphate (mg/L)	48 ± 8
Chlorides (mg/L)	56 ± 5
TSS (mg/L)	312 ± 30
TDS (mg/L)	425 ± 35
Cadmium (mg/L)	BDL[*]
Chromium (mg/L)	0.23 ± 0.02
Copper (mg/L)	0.58 ± 0.03
Zinc (mg/L)	2.6 ± 0.23
Aluminium (mg/L)	2.3 ± 0.21
Nickel (mg/L)	0.12 ± 0.02
Iron (mg/L)	5.2 ± 0.5
Lead (mg/L)	0.22 ± 0.02
Total coliform (MPN/100 mL)	3.2×10^6 to 1.2×10^8
Fecal coliform (MPN/100 mL)	68.4×10^5 to 32×10^7
E. coli (MPN/100 mL)	12.3×10^4 to 3.3×10^6

BDL[*] - Below Detectable Limit (0.01 ppm)

Increase in soil salinity is attributed by usage of sewage in irrigation or leakage of sewers. The value of BOD for raw sewage is generally in the range of 100 – 400 mg/L in Indian cities. An average of 106 samples in Indian cities revealed a mean of 185.5 mg/L (CPCB 2005). An average BOD of 205 and 228.5 mg/L were reported in STPs located in Delhi and Madurai respectively. The typical range of BOD in domestic wastewater is 100-300 mg/L (Metcalf & Eddy 2003). The COD value ranges between 200-700 mg/L in 83% of observed cities in India with an average of 481 mg/L (CPCB 2005).

Biodegradability is a good index for organic degradation and calculated from BOD/COD ratio. Generally it ranges from 0.4–0.8 in raw sewage and differs for different types of wastewater. In our study, the BOD/COD ratio is 0.7 indicating that it can be best treated by biological means rather than chemical process.

The TKN (Total Kjeldahl Nitrogen) is the combination of ammoniacal nitrogen and organic nitrogen with a range of 20-85 mg/ L. In municipal wastewater, it ranges from 35 to 60 mg/L. TP (Total Phosphate) in domestic wastewater ranges from 5 to 10 mg/L. Sulphate concentration in sewage is 20-50 mg/L. Typical untreated municipal wastewater had a TSS (Total Suspended Solids) of 100-360 mg/L (Metcalf & Eddy 2003). Correlation between electrical conductivity and TDS was evaluated by Uwidia & Ukulu (2013) and our results well agree with it. Prerequisite changes in characterisation of wastewater is likely to occur from one location to another. Even in specific location, the composition varies from time to time.

The allowable limits of heavy metals by CPCB, India are presented in Table 3.2. Results revealed that heavy metals iron and lead exceeded the permissible limit. Hence, these 2 heavy metals were taken into consideration for further studies and other heavy metals zinc, aluminium, copper, cadmium, chromium and nickel were neglected as they are well below the allowable limits. However, the metals Cu, Zn, Ni are considered as micronutrients required for plant growth. Soil irrigated with sewage is contaminated with various heavy metals and long term application of sewage in irrigation may lead to piling up of heavy metal concentration in cultivable land and pass on to the ecosystem via food, posing threat to all living beings (Usharani & Vasudevan 2014).

Table 3.2   Comparison of heavy metal concentration in sewage with permissible limit

Heavy metal	Concentration in sewage	Permissible limit
Cadmium (mg/L)	BDL[*]	2
Chromium (mg/L)	0.23 ± 0.02	2
Copper (mg/L)	0.58 ± 0.03	3
Zinc (mg/L)	2.6 ± 0.23	5
Aluminium (mg/L)	2.3 ± 0.21	5
Nickel (mg/L)	0.12 ± 0.01	3
Iron (mg/L)	5.2 ± 0.5	3
Lead (mg/L)	0.22 ± 0.02	0.1

BDL[*] - Below Detectable Limit (0.01 ppm)

3.2   Optimisation of HLR – First order kinetics

Published literature on optimisation studies revealed that application of first order kinetics fitted well for removal efficiency of BOD, COD, TSS, TKN and TP. The theoretical HRT for the respective HLR with flow rate is presented in Table 3.3.

Table 3.3 HRT of respective HLR

Flow rate	HLR	HRT (days)
0.02 m^3/d	28 mm /d	12
0.04 m^3/d	56 mm /d	6.8
0.06 m^3/d	84 mm /d	4.32
0.08 m^3/d	112 mm /d	3.24

The mass removal rate, k values of areal removal rate constant and volumetric removal rate constant of the present study is presented in Table 3.4. In the present study, first order kinetics fitted well for removal of organics and nutrients till 84 mm /d. The removal efficiency at the highest HLR of 112 mm /d is slightly less. It may be due to little over loading and spillage of influent. Similar condition was explained by Trang *et al.* (2010), at HLR 146 mm /d. In HFCWS, applying Kickuth equation of first order kinetics revealed that hydraulic and pollutant loading strongly influenced wetland performance in removal of organics. Monad model of first order kinetics predicted the removal of nitrogen in constructed wetland system (Gajewska & Skrzypiec 2018). Trang *et al.* (2010), conducted experiments under tropical climatic conditions at 4 HLRs: 31 mm /d, 62 mm /d, 104 mm /d and 146 mm /d and concluded that applying first order kinetics fitted well for all parameters up to 104 mm/ d. In the present study, K_v and K_A values were similar for BOD, COD, TKN and TSS while, the values of TP was little lesser confirming the significant removal range of the pollutants.

Higher values were obtained at HLR 84 mm /d for organics (BOD and COD) and nutrients (TKN and TP) removal confirming the optimal load of existing system. The quality of influent corresponds to typical municipal wastewater and the quality of effluent remained consistent after a period of 9 weeks at all hydraulic loading rate. Stabilization of the system was attained after maximal growth of plants to a height of 5-6 feet in 9 weeks. The removal efficiency remains constant in a fully matured VFCWS (Stefanakis & Tsihrintzis 2012).

Table 3.4 Rate constant values and mass removal rate at different HLRs

Parameters	HLR mm/d	Mass removal rate (g m^{-2} d^{-1})	Areal removal rate constant K_A (m d^{-1})	Volumetric removal rate constant K_V (d^{-1})
BOD	28	4.06	0.02	0.07
	56	9.07	0.06	0.17
	84	18.06	0.18	0.52
	112	20.38	0.15	0.43
COD	28	6.16	0.02	0.08
	56	13.16	0.06	0.17
	84	26.04	0.18	0.5
	112	28.56	0.14	0.40
TSS	28	6.9	0.04	0.12
	56	14.4	0.09	0.25
	84	23.9	0.18	0.51
	112	28.5	0.17	0.49
TKN	28	0.57	0.04	0.12
	56	1.21	0.10	0.27
	84	1.97	0.19	0.54
	112	2.35	0.18	0.50
TP	28	0.07	0.02	0.08
	56	0.18	0.07	0.20
	84	0.30	0.13	0.37
	112	0.33	0.12	0.33

3.2.1　　　Removal of organics in VFCWS

The concentration of BOD in the influent ranged from 216-252 mg/L. As the HLR increases, the removal rate of organics increased till 84 mm /d with HRT of 4.32 days. Maximum removal efficiency of organics was achieved at 84 mm /d. The BOD concentration in the influent and effluent with removal efficiency of VFCWS is depicted in Figure 3.1. In the present study, a maximum of 80 - 88% removal of BOD was achieved at 84 mm /d. BOD removal of 50-56%, 54-63% and 64-73% was achieved for 28 mm/d, 56 mm/d and 112 mm/d respectively. The respective concentration of BOD in the effluent at maximal removal % was 95, 78, 25 and 58 mg/L for 28 mm/d, 56 mm/d, 84 mm /d and 112 mm/d respectively. The removal of BOD increased steadily till 9 weeks. However, the removal % tends to remain stable and constant after 9 weeks. The reason might be due to time period to attain the stabilisation of the system. Moreover, the plants reached a maximum height of 5 feet within that time duration indicating a fully matured VFCWS. In a fully matured VFCWS as the HLR increases, the rate of organic removal increases (Stefanakis & Tsihrintzis 2012).

According to Klomjek (2016) when HLR was increased from 2 cm/d to 5 cm/d the removal efficiency of BOD increased from 86 ± 4% to 94 ±1% in CWS planted with giant Napier grass. Transformation of pollutants vary with depth and is a crucial factor in determining contaminant removal by affecting the redox status and dissolved oxygen level in CWS. The microbial community is highly active near the root zone favouring organic removal (Prajapati *et al*. 2017).

Yang *et al*. (2017) reported that as the OLR increases, the removal rate of BOD and COD in aerobic bio filters increased. The efficiency of wetland in organic removal is higher in summer than other seasons (Ramakrishna Rao *et al*. 2013). The removal efficiency of VFCWS for organic removal is reported to be above 90% in several studies (Luederitz *et al*. 2001). Removal of 95.3–99% of BOD was achieved in hybrid CWS (Lee *et al*. 2015).
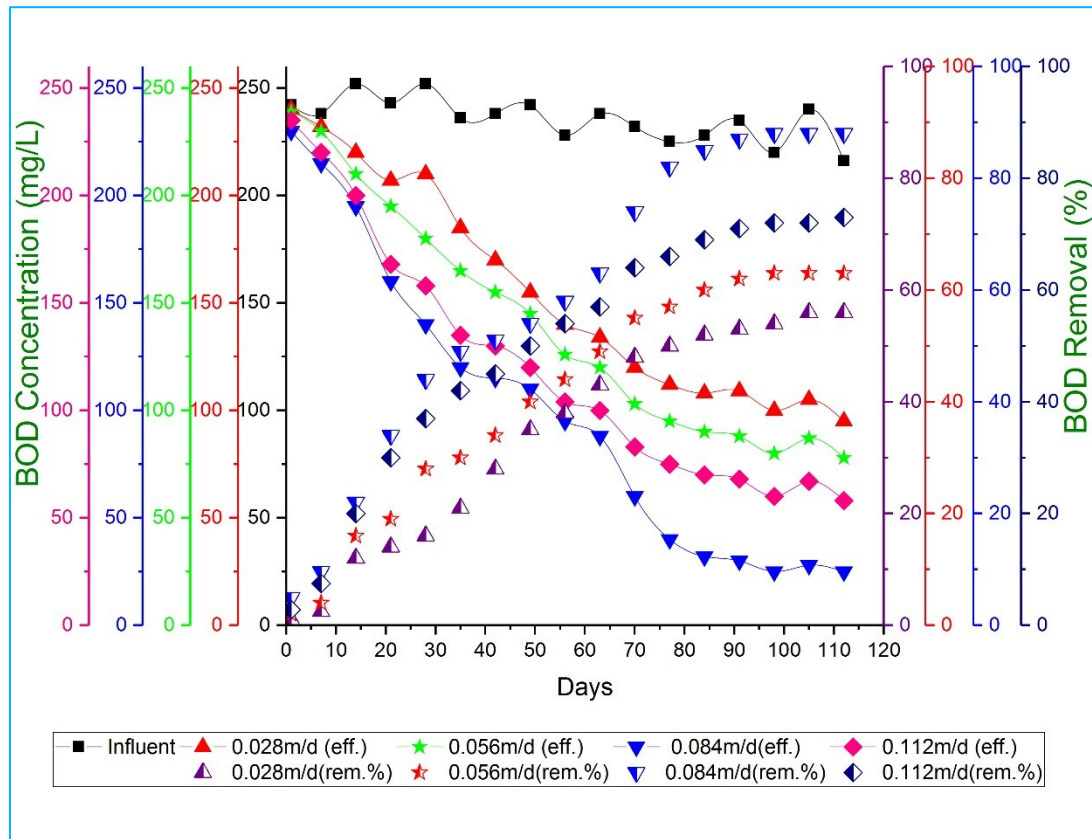
Figure 3.1 BOD removal in VFCWS

The concentration of COD in the influent ranged from 320-360 mg/L. The COD concentration and removal efficiency of VFCWS is shown in Figure 3.2. Maximum of 78-88% removal of COD was attained at 84 mm /d while, removal of 52-60%, 59-66% and 66- 72% was achieved for 28, 56 and 112 mm/d respectively. The respective concentration of COD in the effluent at maximal removal % was 135, 115, 40 and 95 mg/L for 28 mm/d, 56 mm/d, 84 mm /d and 112 mm/d respectively. As in the case of BOD, a steady increase in the removal of COD was observed till a period of 9 weeks and stabilization of the system was achieved after 9 weeks. Most of the studies in CWS reveal 76-99% and 78.80-98.46% removal of BOD and COD respectively (Qomariyah *et al*. 2017). Our results correlate with the findings of Ebrahimi *et al*. (2013) where, an average of 83% of COD removal from municipal wastewater was attained by *Cyperus alternifolius*. Studies conducted by Shahi *et al*. (2013) revealed that *Cyperus alternifolius* was a better candidate than *Phragmites australis* for removal of organics, nutrients and heavy metals from the system except microbiological parameter. Comparative results of removal efficacy of two different species revealed that *Phragmites australis* was effective in removal of organics than *Typha latifolia* (Andreo- Martinez *et al.* 2016) with a removal efficiency of 96.4% BOD and 84.6% COD.
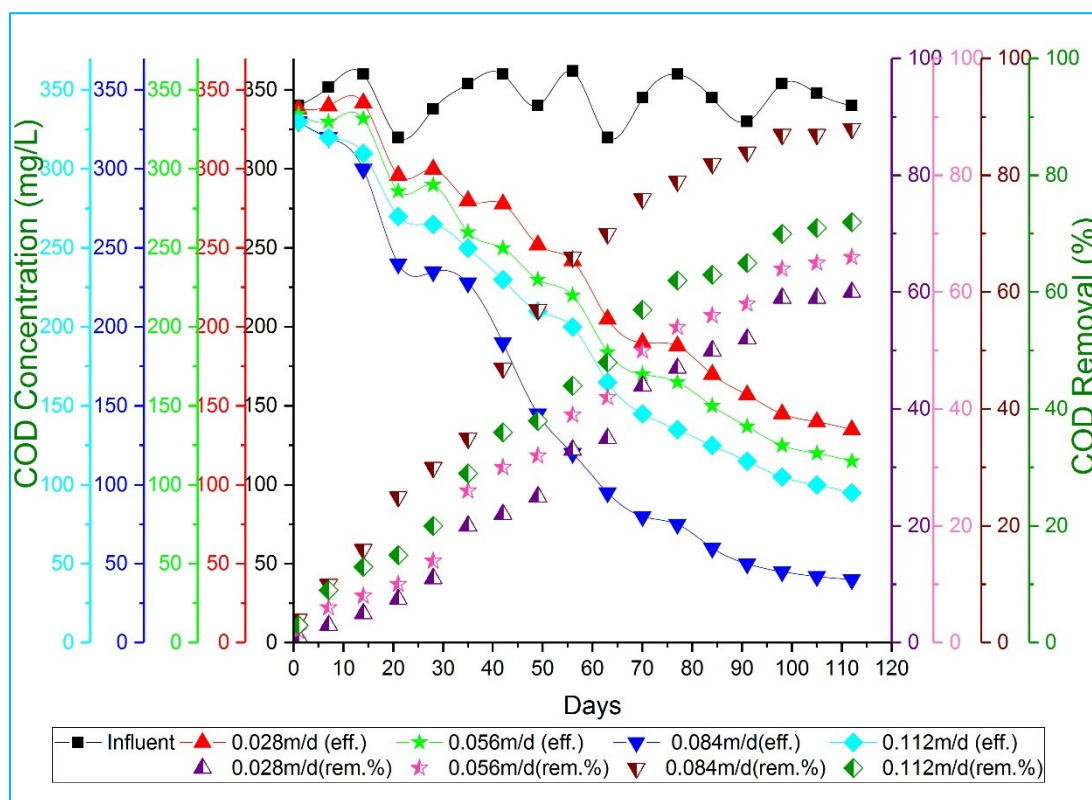
Figure 3.2 COD removal in VFCWS

Generally, CWS achieves an outlet of less than 25 mg/L of BOD in summer with maximum removal of 94.9% BOD and 96.7% COD (Tole *et al*. 2014). Above 15ºC, 90.5% of BOD and 90.8% COD removal was achieved and lesser removal efficiency is reported at temperature lesser than 15ºC (Akratos & Tsihrintzis 2007). India being a country tropical climate, maximal removal of organics can be well accomplished.

CWS planted with *Typha angustifolia* and *Phragmites australis* had > 75% BOD removal in domestic wastewater (Karathanasis *et al.* 2003). About 96.1% BOD and 94.5% COD was attained in CWS planted with *Typha latifolia*, *Cyperus papyrus* and *Phragmites australis* (Nzengya & Wishitemi 2001). CWS treating sewage planted with *Eichornia crassipes* is capable of removing 95.89% and 97% of BOD and COD   (Yadav *et al.* 2011).

In VFCWS, BOD and COD removal efficiency of CWS planted with *Phragmites australis* were 92.3% and 91.7% (Gikas *et al.* 2007), 77.99% BOD removal and 76.16% COD removal (Sudarsan *et al.* 2015), 84% and 75% of BOD and COD removal (Abdelhakeem *et al.* 2016). From this it can be inferred that even for the same species the removal rate differs depending upon diverse factors: geographical condition, design of the wetland, substrate used and the quality of the influent. The removal of organics is much higher in vertical flow than horizontal flow constructed wetlands (Lee *et al*. 2015). *Cyperus papyrus* and *Phragmites mauritianus* efficiently removed 81.22% COD and 78.37% BOD while, *Cyperus* alone contributed to 73.76% COD and 75.78% BOD removal (Nzabuheraheza *et al*. 2012). CWS treating domestic wastewater with integrated plantation of *Typha* and *Phragmites* showed a removal efficiency of 92% BOD and 86% COD (Mirunalini *et al*. 2014). In hybrid CWS, treating domestic wastewater planted with *Paspalidium flavidum* 97.55% of COD and 97.5% of BOD removal was achieved (Sehar *et al*. 2013).

3.2.2 Removal of nutrients in VFCWS

The concentration of TKN in the influent ranged from 24-30 mg/L. As the HLR increases, the removal of nutrients increased till 84 mm /d with HRT of 4.32 days. The influent and effluent concentration and TKN removal efficiency of VFCWS is shown in Figure 3.3. From the results it can be inferred that maximum removal of 82-84% of TKN was accomplished at 84 mm /d and removal efficiency of 63-70%, 60-76% and 65-72% was attained for 28 mm/d, 56 mm/d and 112 mm/d

respectively. The respective concentration of TKN in the effluent at maximal removal % was 7.5, 6, 4 and 7.2 mg/L for 28 mm/d, 56 mm/d, 84 mm /d and 112 mm/d respectively. At the maximum HLR 112 mm /d, a slight decrease in removal efficiency might be due to over loading. The stability of the system in nutrient removal is attributed by an increase in the number of lateral roots. Accomplishment of lateral root production might be achieved when the plant attains maximal growth.

Nitrogen is up taken by plants, stored in sediments and apart from that microbial nitrification and denitrification process takes place. Biological process of nitrification involves 2 steps: The conversion of ammonia into nitrite and conversion of nitrite into nitrate. *E. coli* is reported to reduce nitrate to ammonia (Gonzalez *et al.* 2006). Denitrification was retarded at higher salinity of 15 ppm (Wu *et al.* 2008). The wastewater characteristics of the present study confine salinity of medium strength which does not have much interference with denitrification process.

In a fully matured VFCWS as the HLR increases, the rate of nitrogen removal increases (Stefanakis & Tsihrintzis 2012). Lower HRT in CWS is associated with incomplete denitrification of wastewater because N removal requires longer HRT than organic removal (Lee *et al.* 2015). As OLR increases, the removal rate of ammoniacal nitrogen in aerobic biofilters increased (Yang *et al.* 2017). Nitrogen removal is higher in vertical flow because it provides the suitable conditions for nitrification process and removal of 19-53.3% of TKN was achieved in hybrid constructed wetland system (Lee *et al*. 2015).
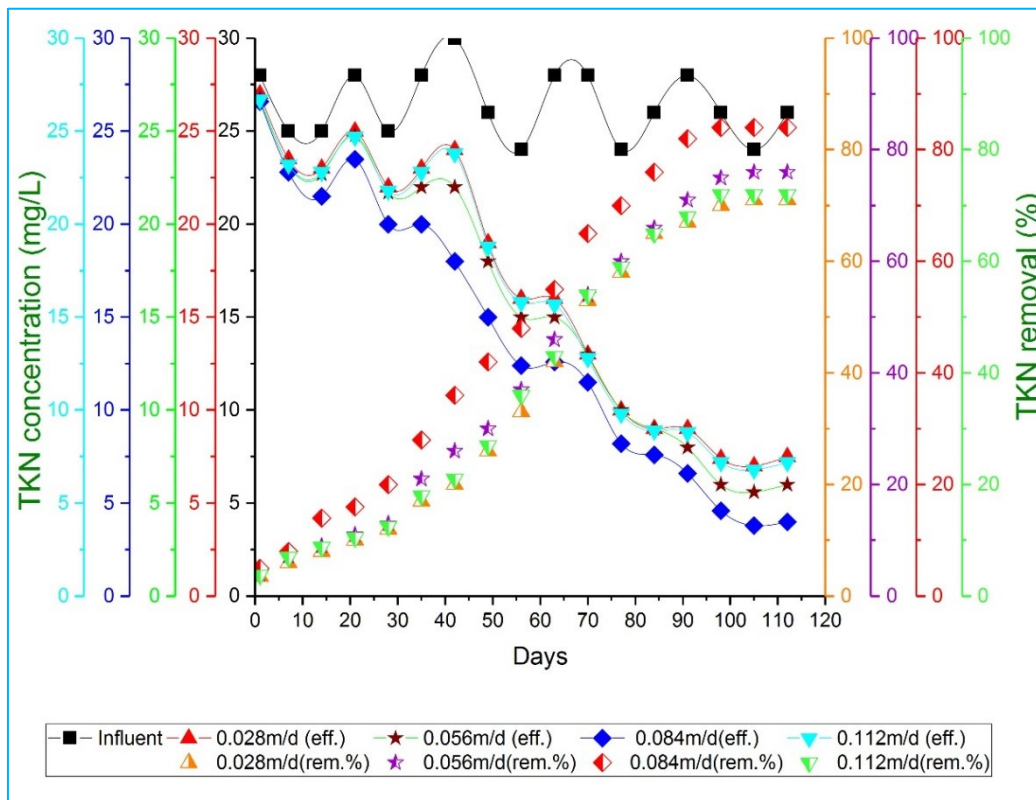

Figure 3.3 TKN removal in VFCWS

The concentration of phosphate in the influent ranged from 4.2-5.2 mg/L. The influent and effluent concentration and TP removal efficiency of VFCWS is shown in Figure 3.4. Results reveal maximum removal of 66-68% of TP at 84 mm /d and removal efficiency of 30-36%, 52-56% and 54-60% for 28 mm/d, 56 mm/d and 112 mm/d respectively. The respective concentration of TP in the effluent at maximal removal % was 4.6, 2.9, 2 and 1.4 mg/L for 28 mm/d, 56 mm/d, 84 mm /d and 112 mm/d respectively. Phosphate removal in CWS is majorly accomplished by adsorption and precipitation in sand. In our study about three fourth of the media is constituted by sand that might have played a major role. River sand has excellent phosphate removal property (Trang *et al.* 2010). The mechanism for phosphate removal might be due to adsorption and/or precipitation in sand filter, plant uptake and microbial action.
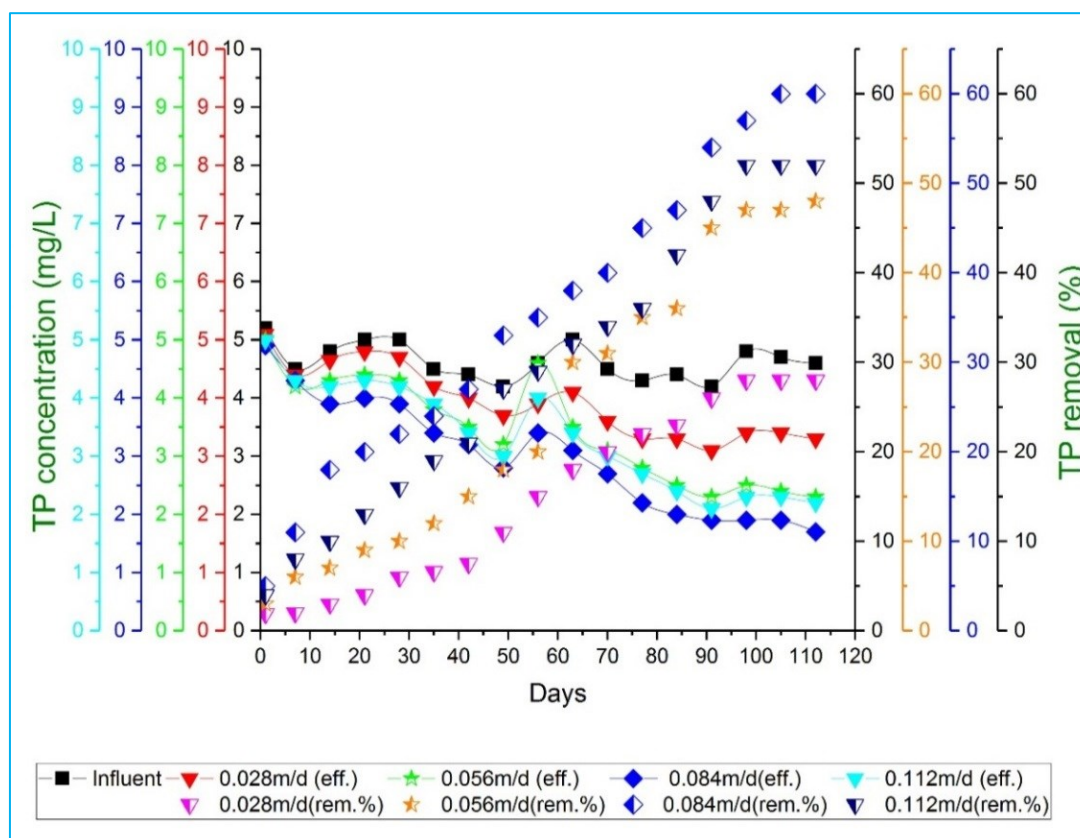
Figure 3.4 TP removal in VFCWS

Emblematic removal of phosphorus in the wetlands ranges from 40- 60%. However, *Cyperus* species is capable of up taking phosphate equivalent to *Phragmites* and our results correlate with Gikas *et al*. (2007). Latrach *et al.* (2015) reported a removal of 82 and 80% of TKN and TP from domestic wastewater when multi layered substrate was used. Gikas *et al.* (2007) reported removal of 80.3% TKN and 67.3% TP in wetlands planted with *Phragmites australis*. Above 90% of nutrient removal can be achieved in wetlands depending on the performance of species and system as a whole (Luederitz *et al.* 2001).

In the previous studies a removal of 58.03% and 27.5% of TP was achieved by *Phragmites australis* in VFCWS and HFCWS respectively indicating that vertical flow achieves higher removal % than horizontal for the same species (Sudarsan *et al.* 2015, Mesquita *et al.* 2017). Maximum of 63.2% of TP was reported in hybrid constructed wetland (Lee *et al.* 2015). The microbial enzyme phosphatase is responsible for phosphate removal in CWS. The microbes in wastewater and soil contributes to 7% and 6% and adsorption in soil contributes to 71%. According to Kumar *et al.* (2011) a maximum of 64-75% of TP is removed by adsorption process in the system, 9-19% by plant uptake and 7-12% by microbial metabolism. *Cyperus papyrus* has the ability to uptake 28.5% of nitrogen and 11.2% of phosphate from the system (Kyambadde *et al.* 2005).

The efficiency of wetland in nutrient removal is higher in summer than other seasons (Ramakrishna Rao *et al*. 2013, Mesquita *et al.* 2017). CWS with *Eichornia crassipes* treating sewage revealed removal of 43.07% and 49.03% for nitrogen and TP (Yadav *et al*. 2011). Temperature majorly influences the removal of ammoniacal nitrogen, total nitrogen and total phosphate (Akratos & Tsihrintzis 2007). In HFCWS, *Phragmites* is efficient in removing 79.5% of N and 83.7% of TP (Andreo- Martinez *et al.* 2016). CWS with integrated plantation of *Typha* and *Phragmites* could achieve 84% of TN and 75% of TP removal (Mirunalini *et al.* 2014). In hybrid CWS, planted with *Paspalidium flavidum* 89.35% of TP was removed from domestic wastewater influent (Sehar *et al*. 2013).

3.2.3   Removal of coliform in VFCWS

The pathogen removal in CWS of the present study reveals 99.99 % removal of total coliforms (TC), fecal coliforms (FC) and *E.coli* corresponding to 2-3 log removal. The range of TC in the inlet ranges from 4.3×10^6-37.8×10^7 MPN/100mL respectively. Consistent removal of TC, FC and *E.coli* was attained after 8 weeks period of time. Log removal of TC increased from 0.93-2.3, 0.8-1.7, 0.6-1.6 and 0.14-0.5 for 28 mm/d, 56mm/d, 84mm/d and 112 mm /d respectively during the first seven weeks.
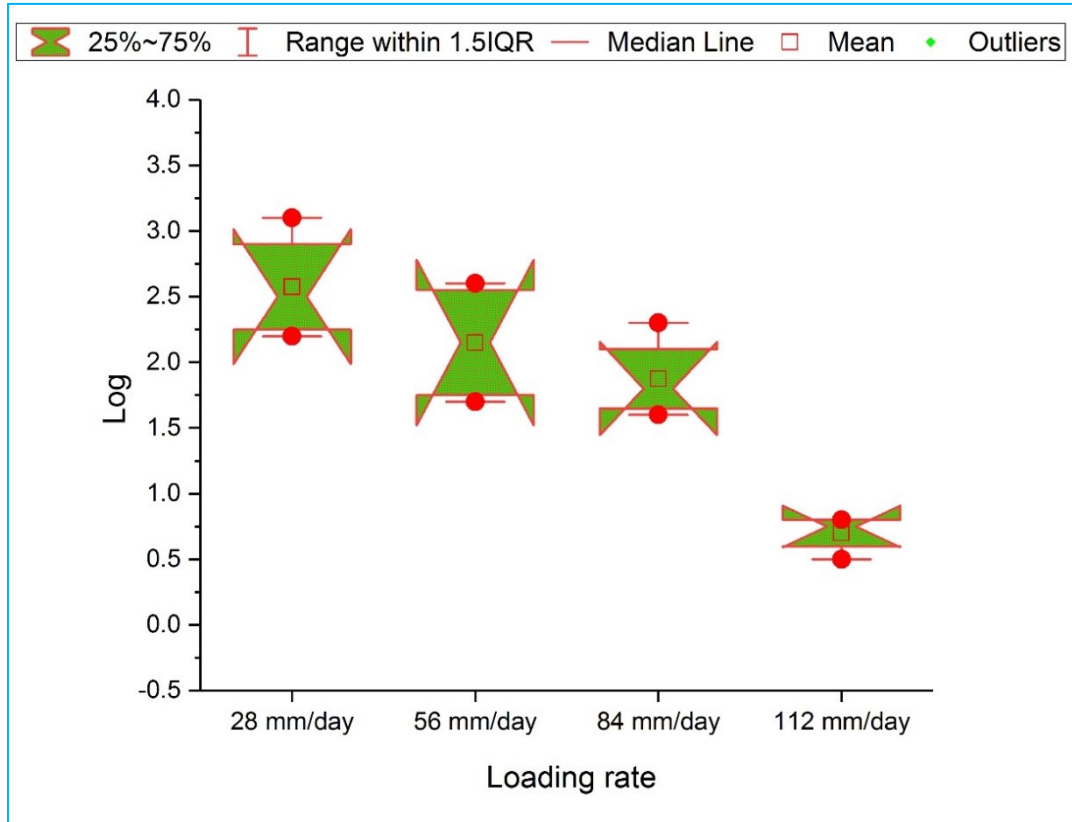
Figure 3.5 Reduction of TC at different HLRs

After 8th week, a maximum of 3.1 log reduction was achieved at 28 mm/d whereas, 2.6 log removal, 2.3 log removal and 0.9 log removal was achieved for 56mm/d, 84mm/d and 112 mm /d respectively. The log reduction of total coliforms in CWS at different HLR is presented in Figure 3.5. The lowest HLR had the retention time of 12 days. Increased retention time might have favoured maximum removal. About 99.99% of pathogen reduction is reported by several authors in different constructed wetlands with different species equivalent to 3-4 log reduction (Tole *et al*. 2014).

The concentration of FC in the inlet ranged from 4.9×10^5 to 11.5×10^6 MPN/100 mL. The log reduction of fecal coliforms in CWS at different HLR is presented in Figure 3.6.
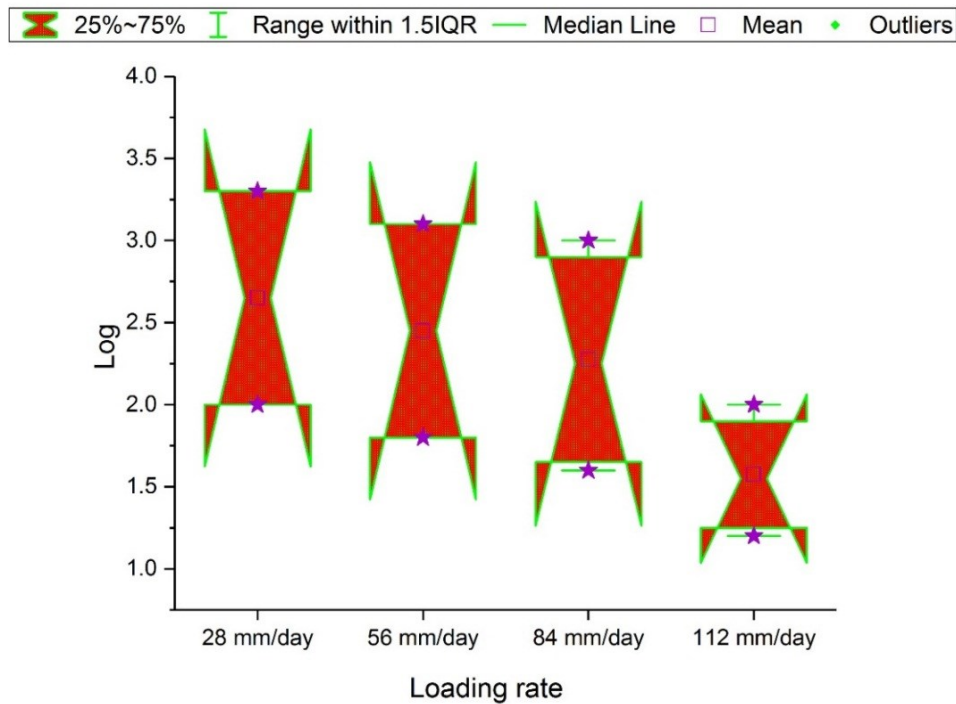
Figure 3.6 Reduction of FC at different HLRs

Log removal of FC increased from 0.08-2, 0.06-1.8, 0.04-1.6 and 0.02-1.2 for 28 mm/d, 56 mm/d, 84 mm/d and 112 mm/d respectively during the first seven weeks. After 8th week, a maximum of 3.3 log reduction was achieved at 28 mm/d while, 3.1 log reduction, 2.9 log reduction and 2 log reduction was achieved for 56mm/d, 84mm/d and 112 mm /d respectively.

Among the four wetland species: *Cyperus papyrus*, *Cyperus alternifolius, Typha latifolia* and *Phragmites mauritianus*; *Cyperus alternifolius* and *Typha latifolia* were effective in significant removal of *Salmonella* and *E.coli* above 98% followed by *Cyperus papyrus*. The pathogen removal % of *Phragmites mauritianus* was least (Kipasika *et al*. 2016). The highest removal of 96% and 89% total coliforms and *E.coli* was reported in subsurface flow wetlands (Reinso *et al.* 2008). Domestic wastewater treatment with *Cyperus papyrus* attained 99.99% removal of fecal coliforms equivalent to 2 log units (Mburu *et al*. 2008). Reduction of 1.28, 1.21 and 1.01 log units of total coliform, fecal coliform and *E.coli* was attained in multilayered substrates of CWS without plantation (Latrach *et al*. 2015).
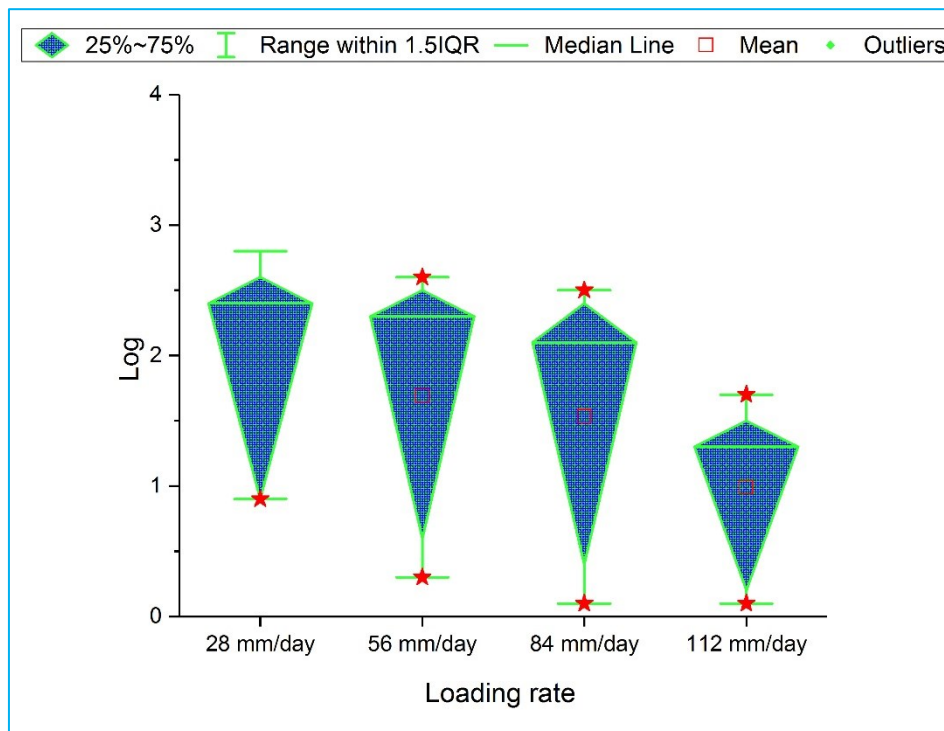
Figure 3.7 Reduction of *E.coli* at different HLRs

After 99.9% removal of *E.coli,* total coliforms and fecal coliforms, pathogens still persists in the effluent than the permissible limit of STP except membrane bioreactor and further research initiative is needed to achieve complete disinfection (Hendricks & John pool 2012).

The range of *E.coli* in the inlet ranges from 5.9×10^4 to 37.8×10^5 MPN/100mL. The log reduction of *E.coli* in CWS at different HLR is presented in Figure 3.7. Log removal of *E.coli* increased from 0.8-1.9, 0.3-1.4, 0.1-1.6 and 0.1-1.3 for 28 mm/d, 56mm/d, 84mm/d and 112 mm /d respectively during the first seven weeks. After 8th week a maximum of 2.8 log reduction was achieved at 28 mm/d while, 2.6.1 log reduction, 2.4 log reduction and 1.7 log reduction was achieved for 56 mm/d, 84 mm/d and 112 mm /d respectively. The effectiveness of *E.coli* removal in CWS majorly depends on the filtering mechanism. Reduction of 4.7 log of *E.coli* was reported in sand filtration (Seeger *et al.* 2016). Sand beds are capable of removing 1.2-2.7 log unit of total coliforms and 1.5-3.5 log unit of *E.coli* (Bohorquez *et al.* 2016). Fine media had an increased log reduction than coarse media (Albalawneh *et al*. 2016).

Typha latifolia in CWS potentially removed 96.8-99.7% of fecal coliforms throughout a period of 17 months (Smith *et al*. 2005). Karathanasis *et al*. (2003) reported more than 93% removal of fecal coliforms in CWS planted with *Typha latifolia*. In hybrid CWS with *Paspalidium flavidum* 98.6% removal of fecal coliform in domestic wastewater was reported by Sehar *et al*. (2013). The pathogen removal in CWS is not adequate and requires an additional treatment for disinfection. Maximum of 6 log reduction is recommended by WHO (2006) for wastewater reuse in agriculture.

3.2.4   Removal of TSS and TDS in VFCWS

The fate of suspended organic matter under anaerobic condition in CWS is presented in Figure 3.8. The microbes involved in degradation of organic matter are discussed under the section: micro flora in CWS.

The concentration of TSS in the influent ranges from 300-360 mg/L. The results of TSS removal in CWS at different HLR and the concentration in inlet and outlet is presented in Figure 3.9. Results revealed maximum removal of 80-88% TSS with the discharge quality of < 50 mg/L at 84 mm/d whereas, 70-75%, 75-80% and 78-83% removal was achieved for 56mm/d, 84mm/d and 112 mm /d respectively. The respective concentration of TSS in the effluent at maximal removal % was 45, 40, 35 and 38 mg/L for 28 mm/d, 56 mm/d, 84mm/d and 112 mm/d respectively.
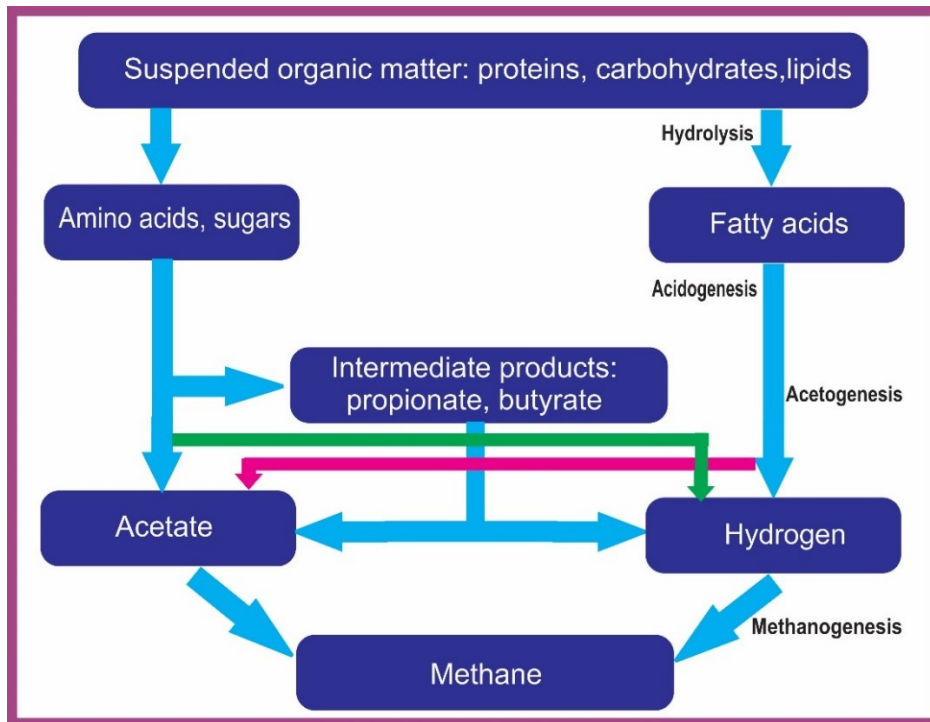
Figure 3.8 Fate of suspended organic matter under anaerobic condition in CWS

Removal of TSS in the system is majorly attributed by filtration and sedimentation. The organics in the sediments are further degraded by the microbes in the system. The lateral roots of *Cyperus* adds an additional impact in the mechanism of filtration. The root zone of plants contribute as filter media for highest removal of TSS (Chandrakanth *et al.* 2016).

VFCWS treating domestic wastewater with *Phragmites australis* is reported to remove 93.2% (Gikas *et al.* 2007) and *Typha latifolia* > 88% of TSS (Karathanasis *et al.* 2003). HFCWS with *Phragmites australis* effectively removed TSS of 84.3% (Li *et al.* 2017) and kenaf 79% (Albalawneh *et al.* 2016).
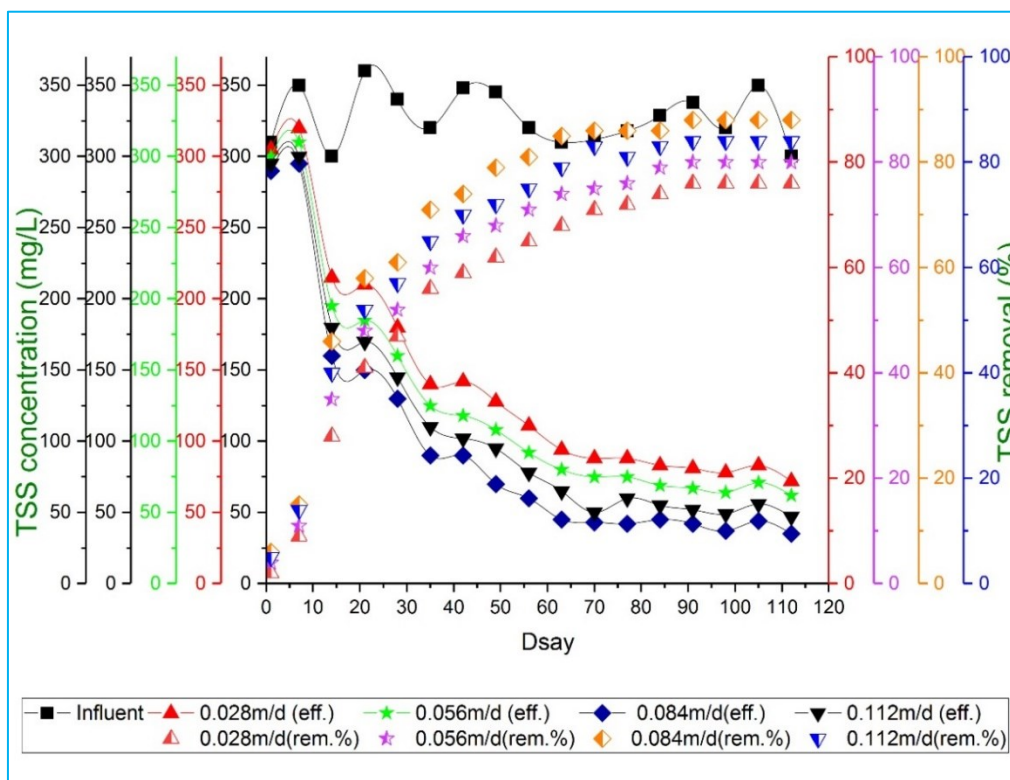

Figure 3.9 TSS Removal in VFCWS

Manios *et al.* (2003) reported that among different substrates, gravel reed bed planted with *Typha* had the best removal of TSS more than 95% with effluent concentration of less than 10 mg/ L. About 95-97% of TSS removal throughout the year was achieved irrespective of season (Smith *et al.* 2006). Synergistic effect of *Typha, Cyperus* and *Phragmites* in CWS could remove 97.6% of TSS (Nzengya & Wishitemi 2001) while, *Typha* and *Phragmites* efficiently removed 96% (Mirunalini *et al.* 2014). *Eichornia crassipes* efficiently removed 82% of TSS in sewage (Yadav *et al.* 2011).Integration of *Cyperus papyrus* and *Phragmites mauritianus* potentially removed 80.01% of TSS while *Cyperus* species alone could achieve only 79% removal (Nzabuheraheza *et al.* 2012). Generally, TSS removal of 85% was achieved in CWS irrespective of the plant species.

The concentration of TDS in the influent ranges from 430-480 mg/L. The results of TDS removal in CWS at different HLR is presented in Figure 3.10.
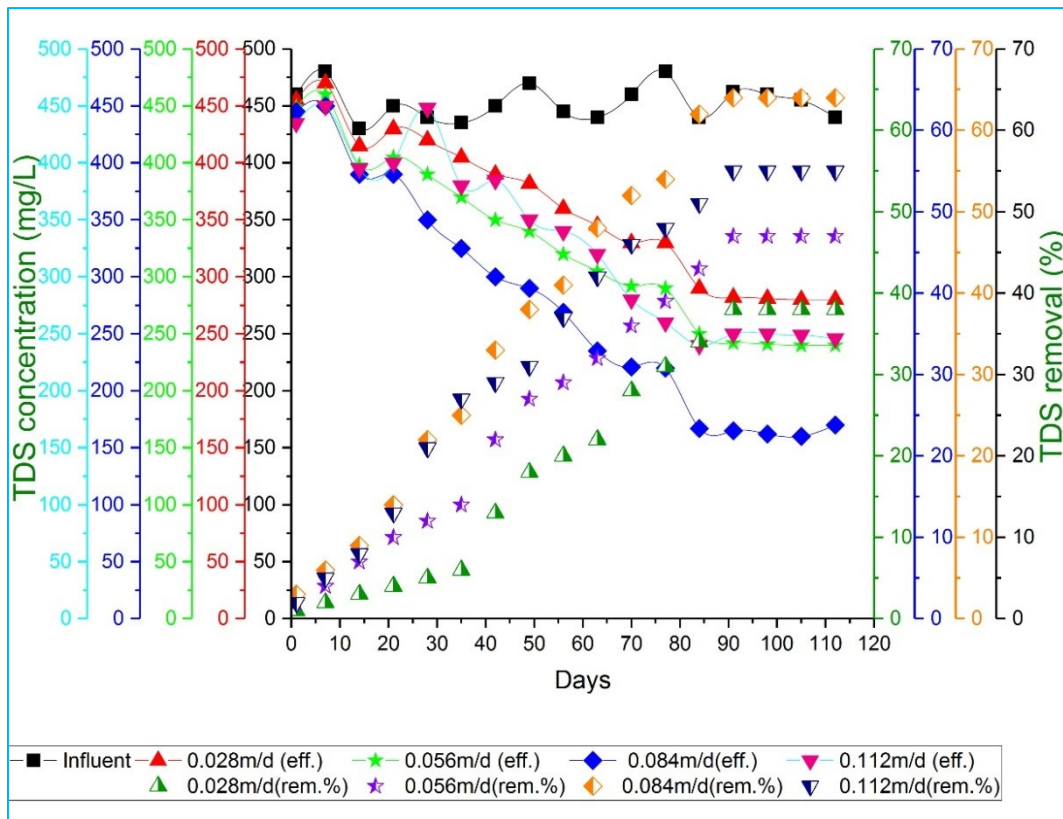

Figure 3.10 TDS Removal in VFCWS

Maximum removal of 60-64% was attained at 84 mm/d and removal of 34-38%, 43-47% and 51-55% was achieved for 28 mm/d, 56 mm/d and 112 mm/d respectively. The respective concentration of TDS in the effluent at maximal removal % was 280, 240, 170 and 246 mg/L for 28 mm/d, 56 mm/d, 84 mm/d and 112 mm/d respectively. The prime mechanism of dissolved solids is via microbial degradation.

Integration of *Cyperus papyrus* and *Phragmites mauritianus* potentially removed 72.07% of TDS while *Cyperus* species alone could achieve only 71% (Nzabuheraheza *et al.* 2012). In HFCWS 84% of TDS removal was reported when *Phragmites* was used (Andreo- Martinez *et al.* 2016). VFCWS treating domestic wastewater with *Phragmites australis* is reported to remove 57.34% of TDS (Sudarsan *et al.* 2015). *Eichornia crassipes* efficiently removed 71% of TDS in sewage (Yadav *et al.* 2011).

3.2.5   Removal of Heavy Metals in VFCWS

The concentration of lead in the influent ranged from 0.156 - 0.263 mg/L. With respect to the removal of heavy metals Fe and Pb in our study, the effluent collected from outlets at different HLR confined the limits of discharge. However, the removal % varied at different HLR involved in the study. A maximum of 90-94% removal of lead was accomplished at 84 mm /d confronting effluent

standards as per CPCB (2005). Lead removal of 65-66%, 74-77% and 78-80% was achieved for 28, 56 and 112 mm/d respectively. The respective concentration of lead in the effluent at maximal removal % was 0.062, 0.042, 0.011 and 0.036 mg/L for 28 mm/d, 56 mm/d, 84 mm/d and 112 mm/d respectively. The influent and effluent concentration with lead removal efficiency at different HLR is presented in Figure 3.11. Efficient removal of heavy metals by biological treatment is achieved best at pH 8.8 (Rajasulochana & Preethty 2016). The alkaline pH of the sewage might be one of the reason for efficient removal. The lateral roots of *Cyperus* play a major part in heavy metal uptake.

Metals may be retained in the sediments either in oxidized/ reduced soil conditions (Sinicrope *et al*. 1992). A study conducted on the comparison of three species of *Cyperus*: *Cyperus alternifolius*, *Cyperus prolifer* and *Cyperus textilis* for uptake and tolerance of heavy metals aluminium and iron revealed that, *Cyperus alternifolius* was the best fit for phytoremediation. However few reports are available about the genus *Cyperus* in heavy metal removal (Ayeni 2016).
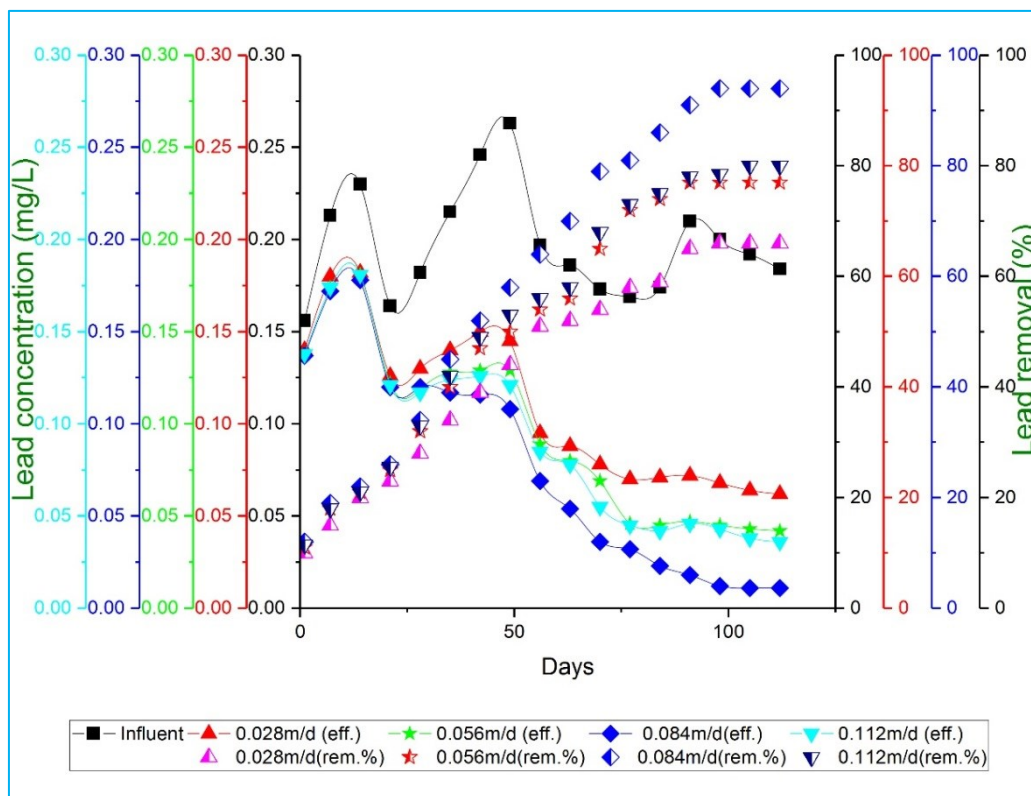

Figure 3.11 Lead Removal in VFCWS

Phragmites australis could efficiently remove 88% lead and 92% iron. *Typha* could proficiently remove 87% lead and 95% iron (Gikas *et al*. 2013). The removal of lead by substrates is influenced by the concentration of iron in the system (Ren *et al*. 2016). An average of 60. 6% removal of lead was observed in HFCWS planted with *Phragmites australis* (Li *et al*. 2017).

The concentration of iron in the influent ranged from 3.28 to 5.82 mg/L. A maximum of 82-85% removal was attained at 84 mm/d confronting discharge quality. Iron removal of 70- 72%, 77-78% and 76-79% was achieved for 28 mm/d, 56 mm/d and 112 mm/d respectively. The respective concentration of lead in the effluent at maximal removal % was 1.5, 1.2, 0.8 and 1.1 mg/L for 28 mm/d, 56 mm/d, 84 mm/d and 112 mm/d respectively. Heavy metals are removed as their bicarbonate due to bacterial production of bicarbonate alkalinity and as insoluble sulphide. The reduction of metals to non-mobile forms is achieved by microbial activity in wetlands and the reducing conditions are afforded by sulphate reducing bacteria. Iron and lead are precipitated into insoluble sulphides in CWS (Sheoran & Sheoran 2006).The influent and effluent concentration with iron removal efficiency at different HLR is presented in Figure 3.12.

Iron uptake by plants might be favoured as it is one of the critical component involved in many physiological process of plant: DNA synthesis, respiration, photosynthesis, mitochondrial and chloroplast metabolism. When iron enters the xylem, it complexes with citrate (Rout & Sahoo 2015).
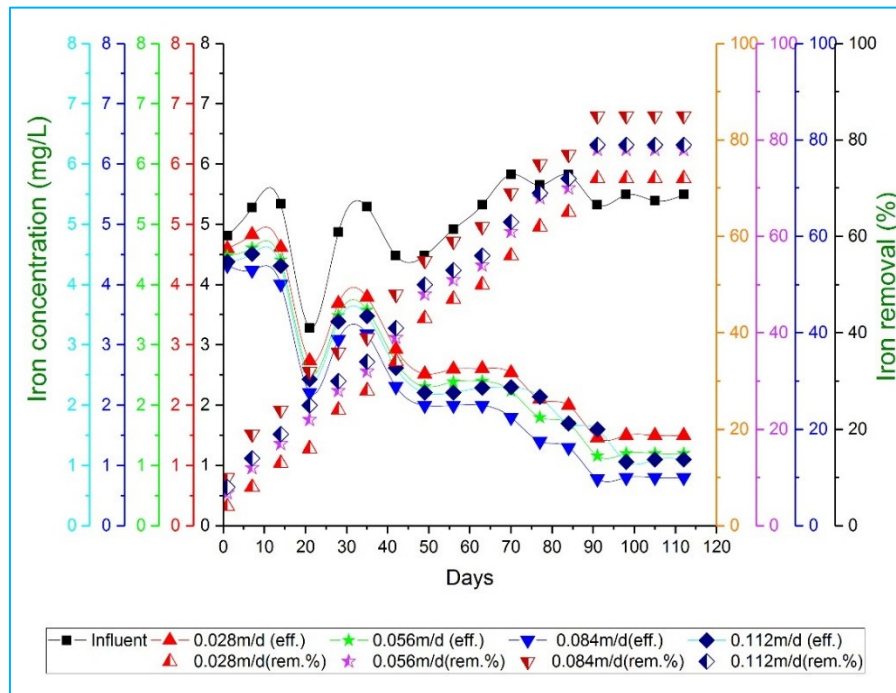

Figure 3.12 Iron Removal in VFCWS

Plant cell wall has cationic exchange sites named phytochelatins that detoxify and balance homeostasis of heavy metal uptake. Metal reduction can be tolerated in the body mass of plants without showing negative effects on its growth (Sheoran & Sheoran 2006). Heavy metals present in wastewater are effectively removed by the wetland mesocosms at different hydroperiods (Sinicrope *et al*. 1992).

4. Conclusion

Among the diverse existing wastewater treatments schemes, CWS secures a peculiar place for its aesthetic value in addition to low cost maintenance. The optimal HLR for VFCWS planted with *Cyperus alternifolius* was 84 mm/d (60L/d) with discharge quality which met the standards of discharge for agriculture and inland water. Hence the effluent can be utilized either for irrigation or used for ground water recharge. Vegetation is the major factor contributing the removal of organics and nutrients. The dynamic role of microbes in removal of organics, nutrients and heavy metal removal is inevitable and secures a noteworthy place in CWS. CWS not only aids in treatment but also biodiversity thus favouring a successful ecological balance apart from sustainable treatment of sewage. A few clippings of visitors indicating biodiversity in CWS clicked at the time of sample collection is presented in Figure. 4.1. The different species such as caterpillar, grasshopper, snail etc were observed in the constructed wetland system indicating it as a clean green way in sewage treatment without affecting the ecosystem and biodiversity.

Figure 4.1 Visitors at CWS indicating biodiversity

In a nutshell it can be concluded that for tropical countries CWS is not only the evergreen technology that provides solution for wastewater treatment but the best for deterrence of exploitation of diversity. The harvested plants can be left decayed because the oxidation of the heavy metals were changed such that it remains less toxic. The findings related to this are continued in the further studies.

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