Sewage Treatment through Constructed Wetland System Tailed by Nanocomposite Clay Filter: A Clean Green Initiative

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Abstract. Sewage treatment through constructed wetland is an ecofriendly and sustainable approach proven effective worldwide. Constructed wetland with appropriate species is capable of eliminating all pollutants in sewage, except pathogen removal. An additional polishing treatment is required to eliminate pathogen. Optimization of HLR in CWS was executed by applying first order kinetics. Nanocomposite clay filter with economically viable materials was synthesized and disinfection ability was evaluated. A novel approach integrating constructed wetland system tailed by nanocomposite clay filter was designed. Control was setup with constructed wetland system devoid of plants integrated with clay filter devoid of nanoparticles. The constructed wetland system devoid of plants was used as plants play a vital role in the removal of pollutants. The quality of the influent for (n=20) BOD, COD, TKN, TP, TSS, TDS, SO\textsubscript{4}, Cl, lead and iron were 248, 345, 26, 4.8, 350, 450, 50, 48, 0.2, 5 mg/L respectively. The quality of effluent in the control was 145, 225, 18, 3.8, 185, 345, 31, 30, 0.6, 2 mg/L for BOD,COD, TKN, TP, TSS, TDS, SO\textsubscript{4}, Cl, lead and iron respectively. While in the test, 10, 30, 2, 1, 30, 128, 13, 12, BDL, BDL mg/L for BOD, COD, TKN, TP, TSS, TDS, SO\textsubscript{4}, Cl, lead and iron respectively. The inlet concentration of T.C, F.C and \textit{E.coli} were 42.1x10\textsuperscript{6}-6.3x10\textsuperscript{8}, 4.9x10\textsuperscript{5}-14.4x10\textsuperscript{6} and 7.8x10\textsuperscript{3}-3.8x10\textsuperscript{5} respectively. The pathogen reduction in log removal for test and control units were 5.4 and 1.1 for T.C, 4.4 and 1.2 for F.C and 3 and 1 for \textit{E.coli}. Thus it is a clean green initiative combating the limitations of disinfection surpassing the existing barriers.

1. Introduction

Wastewater is generally classified into three different types: domestic, industrial and storm water. Recent findings implicate that about 80 \% of the wastewater generated worldwide is being discharged into water bodies without treatment (WWAP 2017) thus, polluting the environment and disturbing ecological balance (Wakelin et al. 2008). Wastewater discharge from domestic, industrial and agricultural sector releases a wide range of contaminants drawing keen attention of all the researchers and environmentalists worldwide. Water bodies were once considered as natural resource but now treated as dumping ground. Different sources contribute to the contamination of water bodies. Some of them are direct sources such as letting of untreated sewage (Norah et al. 2015), effluent discharge from factories and refineries (Qazi & Qazi 2008). The list of pollutants released in wastewater keeps increasing at an alarming rate day by day due to anthropogenic inputs. The various components of domestic wastewater are biodegradable, other organic materials, nutrients, heavy metals, microorganisms and inorganic materials.

Treated sewage discharged into the environment contribute to pathogens in the environment and needs appropriate treatment. The methods adopted for disinfection in wastewater treatment plants has its own limitations. UV treatment for disinfection is not cost effective and suspended solids in water may lead to inefficiency in disinfection treatment. It requires continuous supply of electricity and is effective only at proximal points and not at distal points. Ozonation requires increased capital cost and skilled labours. Unlike UV treatment, ozonation is also effective only at the point of use. It has short half- life and needs to be produced on-site (EPA 2015). Chlorination is effective on bacterial pathogens but its disadvantages includes; inability to penetrate through protozoans and biofilm, production of harmful byproducts that affects aquatic life (Ibrahim et.al.2015).
Constructed wetlands are best suited for developing countries with tropical climate due to conducive higher biological activity and productivity than temperate climate (Varma et al. 2020). More oxygenated environment exists in vertical flow constructed wetlands leading to significant removal of organic matter and microbes in wastewater. Performance of constructed wetlands with appropriate plant species could efficiently remove organics, nutrients, heavy metals (Sahu 2014) and total coliforms of 3.9 log units (Zurita & White 2014). Removal of coliforms in constructed wetland is less effective and thus requires a polishing treatment before its reuse in agriculture sector (Varma et al. 2020). In the current scenario, nanotechnology is the best fit for effective removal of microbes since pathogens are resistant to the conventional disinfection techniques (Grasso et al. 2019). Nanotechnology is the cutting edge technology in disinfection process. Thus an initiative integrating the conventional constructed wetland system with the selected species and modern nanotechnology was taken as a research plan integrating these two processes. Constructed wetland tailed by disinfection unit encompassing nanocomposite particles was executed. The objective was framed to evaluate the disinfection efficiency of a constructed wetland system integrated with nanocomposite clay filter for sewage treatment.

2. Result and Discussion

2.1 Optimisation of HLR in CWS.

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 2.1.

| Flow rate (m³/d) | HLR (mm/d) | HRT (days) |
|-----------------|------------|------------|
| 0.02            | 28         | 12         |
| 0.04            | 56         | 6.8        |
| 0.06            | 84         | 4.32       |
| 0.08            | 112        | 3.24       |

Table 2.1 HRT of respective HLR

Table 2.2 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                                     | 0.43                                     |
|            | 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                                     |
The mass removal rate, k values of areal removal rate constant and volumetric removal rate constant of the present study is presented in Table 3.2. 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 & Skrypiec 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. The K_A 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.

2.2 Optimisation of disinfection studies in clay disc

Optimisation studies were carried out in both batch and continuous mode to evaluate the required contact time for disinfection of E.coli. Batch studies revealed zero cell count in 20 minutes at all flow rates for 5 discs. However, as the number of discs increased, zero cell count was achieved earlier at all flow rates. E.coli cell count was inversely proportional to the number of discs in contact for batch and continuous mode. In continuous mode, complete disinfection was attained in 10 minutes at all flow rates in column loaded with 10 and 15 discs. While, in column loaded with 5 discs reduced colony count was observed in 10 minutes and complete disinfection was achieved in 20 minutes.

2.3 Integration of CWS tailed by nanocomposite clay filter

The results of removal efficiency of organics, nutrients, heavy metals and pathogen in test and control units were dealt in the following and discussed with similar works in published literature.

2.3.1 Efficiency of Organics Removal

The concentration of BOD in the inlet ranged from 228-252 mg/L. Results revealed that the removal efficiency of BOD were 93 ± 4% for test and 33 ± 6% for control. The results of BOD removal in the control and experimental set up are presented in Fig. 2.1. The concentration of BOD in the effluent was 145 and 10 mg/L in control and test respectively. Higher removal efficiency of BOD in planted beds is attributed by oxygenation of bed by plant roots favoring aerobic degradation. One of the major mechanism involved in organic removal is oxygenation through plant roots. Hence, it is obvious that efficiency of planted ones will be superior to unplanted ones.

Klomjek (2016) reported 94% and 77% of BOD removal in test and control when giant Napier grass was used in treatment of domestic wastewater in VFCWS at HLR of 5 cm/d. The unplanted had BOD removal efficiency of 31.6-54% while Cyperus papyrus based CWS 68.6-86.5% and Miscanthidium violaceum 46.7-61.1% (Kyambadde et al. 2005). Studies conducted by Arivoli & Mohanraj (2013) revealed removal of 75.49% and 80.53% of BOD and COD in CWS planted with Typha angustifolia while, 64.5% and 56.45% removal of BOD and COD in unplanted. More than 75% removal of BOD was achieved with Typha plantation and 63% without plantation (Karathanasis et al. 2003). Albalawneh et al. (2016) reported a removal efficiency of 50% and 37% of BOD was achieved in control beds with fine media and coarse media. While, in beds planted with reeds 67% and 62% was attained in fine and coarse media. It was inferred that % removal in control units differ depending on the media.
The concentration of COD in the inlet ranged from 302-346 mg/L. Results revealed that the removal efficiency of COD were 91 ± 3% for test and 24 ± 8% for control. The results of COD removal in the control and experimental set up are presented in Fig. 2.2. The concentration of COD in the effluent was 225 and 30 mg/L in control and test respectively. Collison & Grismer (2013) reported a total organic removal of 78.8% and 76.1% in planted and unplanted CWS with removal of COD up to 79% and 76% in planted and unplanted CWS. The average removal efficiency of 88% COD and 91% BOD in planted and 83% and 87% for unplanted was reported by Abou-Elela et al. (2014). In VFCWS with mixed vegetation (croton & Typha) 84.32% and 78.54% removal of BOD and COD was observed whereas, in unplanted only 31.08% & 23.54% removal was witnessed (Chandrakanth et al. 2016). Studies conducted by Tiglyene et al. (2009) reported that CWS with Phragmites vegetation reported BOD removal of 37% in control and 60% in test and COD removal of 74% in control and 61% in test.
Figure 2.2 Efficiency of COD removal

Albalawneh et al. (2016) reported a removal efficiency of 47% and 38% of COD was achieved in control beds with fine media and coarse media. While, in beds planted with reeds 64% and 56% was attained in fine and coarse media. In VFCWS with Phragmites removal efficiency of BOD and COD were 84% and 75% for test and 37% and 29% for control (Abdelhakeem et al. 2016). Mustapha et al. (2018) stated that the removal efficiency of BOD and COD were 76% and 73% for test and 48% and 41% for control in VFCWS with Typha vegetation. Mello et al. (2017) reported COD removal of 60% in control and 67% in CWS with Eichornia crassipes vegetation (Lima et al. 2018).

In our study, the removal efficiency of organics in planted bed is approximately 2.5 times higher than unplanted. In planted versus unplanted, vegetation facilitates organic removal. Stefanakis & Tsihrintzis (2012) reported that vegetation played a significant role in the removal of organics. The efficiency to treat organics in unplanted control can be enhanced by adding bacterial consortium (Singh & Patidar 2015).

2.3.2 Efficiency of Nutrient Removal

The concentration of TKN and TP in the inlet ranged from 24-30 and 4.2-5.2 mg/L respectively. The nutrient removal efficiency was increased in the integrated system. Results reveal that the removal efficiency of TKN and TP were 90 ± 4% and 76 ± 5% (test) and 30 ± 6% and 14 ± 8% (control) are depicted in Fig. 2.3 & 2.4 respectively. The concentration of TKN in the effluent was 18 and 2 mg/L in control and test respectively. The concentration of TP in the effluent was 3.8 and 1 mg/L in control and test respectively. The higher removal efficiency of nutrients in planted beds is attributed by plant uptake. Root system plays a dynamic role by providing existence for diverse microbial community involved in nutrient removal. Plants stimulate the removal of nitrogen by activating the biochemical pathways via increase supply of oxygen. It is apparent that removal efficiency in planted beds are enhanced than unplanted ones.

The removal efficiency of TKN was 91% and 95% in control and test (Klomjek 2016). Mello et al. (2017) reported that TKN removal is directly influenced by plants with removal efficiency of 25% in control and 47% in CWS with Eichornia crassipes vegetation.
The % of nitrifying bacteria *Nitrosomonas*, *Nitrosospira* and *Nitrospira* in the planted beds was reported to be higher than unplanted ones (Wang et al. 2016). Root zone is the distinct region for enhancement of microbial population in CWS. Removal efficiency of phosphate in unplanted controls: 31.6 - 54.3%, in *Cyperus papyrus* based CWS: 68.6 - 86.5% and in *Miscanthidium violaceum* based CWS: 46.7-61.1% was reported by Kyambadde et al. (2005). In VFCWS with *Phragmites*, removal efficiency of TP was 22% and 17% in unplanted (Abdelhakeem et al. 2016).

![Figure 3.3 Efficiency of TKN removal](image)

**Figure 3.3 Efficiency of TKN removal**

Albalawneh et al. (2016) reported a removal efficiency of 58% and 35% of TP was achieved in control bed with fine media and coarse media. While, in planted 75% and 61% was attained with fine and coarse media. Removal efficiency of TP in VFCWS with *Typha* and unplanted were 49% and 26% (Mustapha et al. 2018) and 63.5% and 52.7% in test and control (Abou- Elela et al. 2014). Phosphate displaces hydroxyl ions in iron hydrous oxides. Phosphate and iron removal mechanisms are correlated (Vymazal 2005). Arivoli & Mohanraj (2013) reported 83.51% removal of TP in VFCWS with *Typha angustifolia* and 64.45% in control beds. The phosphorus and nitrogen uptake of *Canna* (29.1 g P/m², 63.1 g N/m²), *Phragmites* (30.91 P/m², 49.46 g N/m²) and *Cyperus* (38.9 P/m², 82.33 g N/m²) revealed that *Cyperus* is a better candidate than other two species in nutrient removal (Abou- Elela et al. 2014).

Lima et al. (2018) observed TKN removal of 82% and 87% in control and test units. In planted beds, about 26-71% of phosphate and 74% of total nitrogen was up taken by plants. TKN removal of 42% and 34% was witnessed in test and control units (Abou- Elela et al. 2014).
The efficiency to treat nutrients in unplanted control can be enhanced by adding bacterial consortium (Singh & Patidar 2015). Present study revealed that removal efficiency of phosphate and TKN in planted bed was approximately 5 and 3 times respectively than unplanted. Generally stated that removal of organics and nutrients in planted versus unplanted, planted units had higher removal efficiency than unplanted because plants play a critical role in removal mechanism (Zhao et al. 2009).

### 2.3.3 Efficiency of Heavy Metal Removal

The concentration of lead and iron in the inlet ranged from 0.164-0.263 and 4.22-5.49 mg/L respectively. The heavy metals lead and iron were removed 100% in the test while, 70 ± 4% and 57 ± 6% was achieved in control. The concentration of lead in the effluent was 0.06 mg/L and BDL in control and test respectively. The concentration of iron in the effluent was 2 mg/L and BDL in control and test respectively. Emergent plants in planted CWS, decreases the concentration of heavy metals in the effluent in an unravelling path when compared to unplanted control. The decrease in unplanted might be due to sorption or precipitation (Yadav et al. 2011). The results of lead and iron removal are presented in Fig. 2.5 & 2.6 respectively.

The uptake of iron and lead by *Cyperus alternifolius* roots directly exposed to wastewater without substrate was 13.4% and 18.8%. In CWS, the active layer contributing to heavy metal removal was till a depth of 10 cm where metals are converted to carbonate complex and stored in sediments. Treatment of wastewater released from iron and steel company in CWS attained 96.9% removal of iron (Soda et al. 2012).
The stimulating effect that aids heavy metal uptake by plants is production of root exudates which supports the bacterial population. Usharani & Vasudevan (2017) reported that *Cyperus* releases root exudates upon heavy metal exposure that might aid in heavy metal uptake. One year study conducted by Gremion et al. (2004) concluded that in planted CWS, plants play a pivotal role in removal of heavy metals by releasing root exudates which enhance the bacterial population resulting in excellent performance of the system as a whole. Planted beds could efficiently remove 54% and 69% of cobalt and zinc and in unplanted 13% and 30% (Albalawneh et al. 2016). Integrated plantation of *Typha*
angustifolia, Erianthus arundinaceus and Phragmites australis effectively removed 60-80% of multiple metals and in unplanted 31-55% was observed. About 98% of iron removal could be achieved in CWS and the removal efficiency lasted for more than 5 years (Luca et al. 2011).

Chemical precipitation, rhizofiltration and microbe-metal interaction in rhizosphere determines the mobility, bioavailability and bioaccumulation of iron. CWS with Phragmites vegetation removed 88% and 92% of lead and iron and Typha vegetation removed 87% and 95% whereas, in unplanted 78% and 88% was witnessed (Gikas et al. 2013). CWS planted with Carex pendula achieved 90% removal of lead and very slight decrease was observed in unplanted (Yadav et al. 2011). The bio concentration of Cyperus alternifolius revealed that it exhibited higher removal efficiency of multiple metals (Soda et al. 2012). About 99% of chromium was removed in planted and unplanted but, the retention time in planted was 3 times lesser than unplanted (Tiglyene et al. 2009).

2.3.4 Efficiency of Suspended and Dissolved Solids Removal

The concentration of TSS in the inlet ranged from 320-359 mg/L. In the present study 92 ± 6% and 43 ± 4% removal of TSS was attained in test and control respectively, presented in Fig. 2.7. The concentration of TSS in the effluent was 185 and 30 mg/L in control and test respectively. The major removal mechanism for TSS is sedimentation and filtration followed by aerobic and anaerobic microbial degradation (Manios et al. 2003). In the planted reed beds, the microbial degradation process might be enhanced as an increase in microbial growth is observed in planted beds than unplanted ones. Plants root acts as a substrate for increased microbial growth.

Mustapha et al. (2018) stated that the removal efficiency of TSS in VFCWS with Typha latifolia and unplanted were 92% and 40% respectively. Removal efficiency of 65% and 67% of TSS was achieved in control bed with fine media and coarse media. While, in bed planted with reeds 64% and 79% was attained in fine and coarse media (Albalawneh et al. 2016). TSS removal in CWS with Phragmites vegetation was in the range of (61-80%) with an average of 75% and in unplanted 42% (Abdelhakeem et al. 2016).

Figure 2.7 Efficiency of TSS removal

No significant change in TSS removal of planted (92%) and unplanted (91%) was reported by Abou- Elela et al. (2014). Karathanasis et al. (2003) reported more than 88% removal of TSS in CWS planted with Typha and 46% in control. TSS removal of 73% and 65% was attained in test and control (Klomjek 2016).
In our study 78 ± 5% and 21 ± 8% removal of TDS was achieved in test and control respectively which is depicted in Fig. 2.8. The concentration of TDS in the inlet ranged from 440-480 mg/L. The concentration of TDS in the effluent was 345 and 128 mg/L in control and test respectively. The activated carbon granules in the integrated system might have favoured an increase in TDS removal. In general, 77.7% of TDS can be removed in CWS, but addition of biochar as substrate could enhance the removal efficiency to 85% (Vidya Vijay et al. 2017).

Typha angustifolia in VFCWS had efficiently removed 84.66% and 67.26% of TDS in test and control (Arivoli & Mohanraj 2013). Removal of TDS in VFCWS with croton (32.96%), Typha (96%) and unplanted (9.26%) was observed by Chandrakanth et al. (2016).

The removal efficiency of sulphate and chloride were 69 ± 6% and 68 ± 4 % in test and 27 ± 8% and 21 ± 7% in control are depicted in Fig. 2.9 & 2.10 respectively. The concentration of sulphate and chloride in the inlet ranged from 41-57 and 40-50 mg/L respectively. The concentration of sulphate in the effluent was 31 and 13 mg/L in control and test respectively. The concentration of chloride in the effluent was 30 and 12 mg/L in control and test respectively. Oxidation of ammonia in the system might have favoured the removal of sulphate and chloride in the system. Biochemical oxidation of NH4 leads to decrease in salinity with 4.59% sulphate and 4.60% chloride removal. (Van Haandel & Van der Lubbe 2012).
About 94% of sulphate and 37.5% Cl removal from municipal wastewater was attained in CWS with water Hyacinth (Olukanni & Kokumo 2013). In hybrid CWS planted with *Paspalidium flavidum* 80.75% sulphate removal in domestic wastewater was attained (Sehar et al. 2013). TDS removal of 20% and 26% was reached in control and test (Klomjek 2016). Sulphate reduction accounted for organic matter removal. The removal of sulphate and chloride by ion chromatographic technique revealed 99.9% and 63.6% could be attained in CWS with *Hymenachne grumosa* vegetation (De Almeida et al. 2015).

Figure 2.9 Efficiency of sulphate removal

Figure 2.10 Efficiency of chloride removal
Reduction of iron and sulphate are correlated. Plant derived organic carbon facilitates the removal of sulphate and chloride in CWS with vegetation and can be concluded that vegetation stimulates dechlorination and sulphate reduction (Chen et al. 2012).

2.3.5 Efficiency of Coliform Removal

The pathogen reduction in log removal for test and control units were 5.4 and 1.1 for T.C, 4.4 and 1.2 for F.C and 3 and 1 for *E.coli*. The inlet concentration of T.C, F.C and *E.coli* were $4.2 \times 10^6 - 6.3 \times 10^8$, $4.9 \times 10^5 - 14.4 \times 10^6$ and $7.8 \times 10^3 - 3.8 \times 10^5$ respectively. Sustained removal of coliform were observed in the present study after polishing treatment via nanocomposite clay filter. The effluent achieved a quality of less than 1000 MPN/100 mL for T.C, and less than 500 MPN/100 mL for F.C and *E.coli* throughout the period of study. The results of log reduction of total coliform in control and test units are depicted in Fig. 2.11.

![Figure 2.11 Efficiency of TC removal](image)

Removal efficiency of one log removal is equivalent to 90% and 2 log removal is equivalent to 99.9%. Nano photo catalyst fluidised bed photo reactor system could achieve effluent quality suitable for irrigation (Brame et al. 2014). The results of log reduction of fecal coliform in control and test units are depicted in Fig. 2.12.

Maximum of more than 6 log removal of *E.coli* was achieved in surface modified activated carbon filter media impregnated with silver NPs (Pal et al. 2006).
Figure 2.12 Efficiency of FC removal

Log reduction of 0.6 and 1.2 fecal coliforms was achieved in control bed with fine media and coarse media. While, in beds planted with reeds 1.2 and 0.9 log reduction of fecal coliforms was attained in fine and coarse media (Albalawneh et al. 2016). Planted beds could attain 4 log removal while, 2-3 log removal in unplanted. Despite of 4 log removal in planted, the effluent couldn’t meet the national regulatory standards of discharge and requires an additional disinfection unit. *Cyperus* effectively removed T.C, F.C and *E.coli* than *Phragmites* and *Canna* (Abou- Elela et al. 2014). The results of log reduction of *E.coli* in control and test units are depicted in Fig.2.13.

In two stage or three stage hybrid system of CWS, maximum removal of coliform occurred in first stage with 2 log reduction of TC, 1.91 log reduction of F.C and 2.13 log reduction of *E.coli* (Tuncsiper et al. 2012). Vacca et al. (2005) concluded that majority of reduction takes place in the first 20 cm with log reduction of 4.31 for T.C and 4.35 for *E.coli*. 
CWS could achieve only 3 log removal of *E.coli* and further treatment is required such that effluent can be utilised for irrigation. But in CWS 3-4 log reduction of T.C and *E.coli* is guaranteed for a running period of two years (Zurita & Carreon-Alvarez 2015).

### 2.3.6 Heavy Metal Accumulation in Plant and Reed Beds

In the present study, accumulation of lead and iron in CWS revealed the accumulation pattern of reed bed > below ground parts > above ground parts. About two third of the cumulative concentration was retained in sediment and remaining was up taken by the plants. Roots retained the heavy metals and only meagre quantity was transferred to above ground biomass. The reason might be due to harvesting of plants within a limited time. Bio concentration factor (BCF) of lead and iron were 0.26 and 0.11 respectively. However longer duration of treatment might have increased the BCF and TF factor. But it is advisable to harvest plants in short duration to avoid much complications. Heavy metal accumulation in CWS is depicted in Fig. 2.13.
The translocation factor (TF) of lead and iron were 0.03 and 0.4 respectively. TF 1 > reveals that transportation of heavy metals to above ground part is less and is safe for disposal after harvest. The harvested culms can be utilized economically. Harvesting of shoots had a positive reciprocation for pollutant removal in CWS during summer (Yang et al. 2016). India, being a tropical country, harvesting might be preferred throughout the year without much seasonal fluctuation. Elemental analysis of the plant part: culm and root were carried out to confirm whether the harvested culm can be utilized economically and is safe for disposal. Elemental analysis of culm is depicted in Fig. 2.14. From the spectrum it was confirmed that only negligible quantity of lead is accumulated in culm.

![Elemental content in culm](image)

**Figure 2.14 Elemental content in culm**

Elemental analysis of root is depicted in Fig. 2.15. It revealed that the concentration of lead is comparatively higher in roots than culm and hence it is advisable to harvest the above ground biomass and continue treatment after a break period. The micro flora in the reed bed might precipitate the metals into inert form without damaging the ecosystem.
Our results correlate with some of the recent findings. Accumulation of heavy metals are more in the soil of CWS with plantation. Under tropical conditions, roots of *Typha latifolia*, *Cyperus alternifolius* and *Cynodon dactylon* accumulates highest concentration of iron and chromium than other parts of the plant. The accumulation pattern was roots > leaves > stem (Mustapha et al. 2018 a).

### 2.3.7 Mass Balance of Heavy Metals

The above ground biomass of *Cyperus* was 4.8 kg/m². In a study conducted by Abou-Elela et al. (2014), it was concluded that the biomass of *Cyperus* was greater than *Canna* and *Phragmites*. The standing biomass of *Cyperus* at the end of the experiment was 2.6 kg/m² with moisture content of 43%. The accumulation of lead in reed bed and plants accounted to 63% and 33% respectively while, that of iron 58% and 38%. In the culm, 6% and 29% of lead and iron were accumulated with a concentration of 250 µg/kg of lead and 3.5 mg/kg. The remaining 4% might have been accumulated in the clay discs and negligible quantity discharged in effluents.

At the end of the experiment, porosity of CWS and nanocomposite clay filter decreased by 3% and 1% respectively. The effluent quality complied with standards of discharge for irrigation and inland surface water regulated by CPCB, India. The quality of the effluent was cross checked with multiple linear regression analysis with BOD as dependent variable. R² value of 0.991 and an adjusted R² value of 0.978 was obtained deeming it as a good fit with significance of .000. For degree of freedom (7, 5), F = 76.971. The Durbin-Watson test of autocorrelation d = 2.279 between the two critical values of 1.5< d< 2.5. Thus it can be assumed that there is no first order linear autocorrelation in multiple linear regression data. The independent variables were statistically significant with p < 0.0005. The overall regression model is a good fit of the data.

### 3. Materials and Method

#### 3.1 Optimisation of HLR in CWS

The HLR of the CWS planted with *Cyperus alternifolius* was optimised applying first order kinetics. 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:

\[
\text{HRT} = \frac{\text{Volume of wetland} \times \text{porosity}}{\text{Flow rate}}
\]

Kadlec & Wallace (2009) proposed the following equation for areal removal rate constant and the same equation was applied.

\[
\ln \left( \frac{C_{\text{effluent}}}{C_{\text{influent}}} \right) = -\frac{K_A}{q} \quad \text{Eq.}(2.1)
\]

Where \( K_A \) is the areal removal rate constant in m \( \text{d}^{-1} \) and \( q \) is the hydraulic loading rate in m \( \text{d}^{-1} \).

The following equation for volumetric removal rate constant proposed by Reed et al. (1995) was applied.

\[
\ln \left( \frac{C_{\text{effluent}}}{C_{\text{influent}}} \right) = -\frac{K_v}{t} \quad \text{Eq.}(2.2)
\]

Where \( K_v \) is the volumetric removal rate constant in d \( \text{d}^{-1} \) and \( t \) is the hydraulic retention time in the wetland. The formulas used for efficiency % and Mass removal rate are presented.

\[
\text{Removal efficiency} \% = \frac{C_{\text{influent}} - C_{\text{effluent}}}{C_{\text{influent}}} \times 100 \quad \text{Eq.}(2.3)
\]

Where \( C \) represents concentration in mg/L.

Mass removal rate was calculated using the formula

\[
\text{r} = q \left( C_{\text{influent}} - C_{\text{effluent}} \right) \quad \text{Eq.}(2.4)
\]

Where \( q \) is the hydraulic loading rate in m \( \text{d}^{-1} \). Mass removal rate is expressed in g m \( ^2 \text{d}^{-1} \). \( q = \frac{Q}{A} \) where \( Q \) is the flow rate through the wetland and \( A \) is the area of the wetland. The disinfection

3.2 Optimisation of clay disc for disinfection

Clay discs were synthesised using potters clay, cuprous nanoparticle, montmorillonte nanoclay and paddy husk in the ratio 8:1:1:1. Optimisation studies were conducted in lab scale to enumerate the disinfection ability of clay discs on \textit{E.coli}, as it is considered as an indicator organism. Pure cultures of \textit{E.coli} inoculum was diluted with phosphate buffer until the optical density of the culture at 600 nm read as 1nm and it corresponds to 5 log CFU/ 100 ml in pour plate technique.

The clay discs were immersed in water and soaked for 1 hour to ensure saturation and removal of internal air bubbles. Disinfection studies in batch and continuous were performed in sterile column (PVC pipe) packed with varied number of clay discs at different flow rates: 5 mL/min, 10 mL/min, 15 mL/min and 20 mL/min. The lower end of the PVC pipe was tapered with reduction valves and fitted with an outlet for the collection of effluent. The tapering part of the column was filled with coconut shell activated carbon granules as gap filler and is reported to remove organics, chlorine and malicious odours. Activated carbon does not possess disinfection property (Rodriguez 2001). Thus the results of disinfection efficacy relies only on the performance of clay disc. The volume of the clay disc was determined based on the optimised HLR of the CWS. The experimental set up is shown in Fig. 3.1.
Integration of CWS with nanocomposite clay filter was experimentally set up such that the effluent from the constructed wetland was made to pass through the column filled with clay discs as a polishing treatment for disinfection and is presented in Fig. 3.2. The Constructed wetland unit planted with *Cyperus alternifolius* was integrated with nanocomposite clay disc packed in PVC column pipe. The lower end of the column was tapered with reduction valves and fitted with an outlet for the collection of effluent. The tapering part of the column was filled with coconut shell activated carbon granules. The flow of water into the integrated system was fixed as 60 L/d (84 mm/day), based on optimisation studies. The column was packed with 32 clay discs to match the flow in the system.
The same conditions were installed for control CWS (without plants) whose outlet was fitted with a column packed with 32 discs (without nanoparticles). Plants and nanoparticles individually and composite are capable of reduction of organics, nutrient from sewage. Hence both was avoided in control. The inlet and outlet samples were collected weekly once and analysed for BOD, COD, TKN, phosphate, TSS, TDS, sulphate, chloride, lead, iron and MPN tests for total coliform, faecal coliform and *E. coli* were carried out in triplicates according to APHA procedure. The experiment was carried out for a period of 12 weeks because maximal growth of plants and constant removal rate was attained within the specified time so that plants can be harvested.

### 3.2 Statistical Analysis

The results of the research presented in table are provided as mean ± S.D calculated in excel 2010. The results presented in graph are average of triplicates run in software Origin 2018. Statistical analysis of the effluent in the final set-up was carried out in multiple regression analysis in SPSS software version 16. BOD was taken as the dependent variable and parameters: COD, TKN, TP, TSS, TDS, Cl and SO$_4$ were taken as independent variable with sample size of 12. Confidence level of 95% was specified for the regression. The model was tested by investigating values of: R$^2$, adjusted R$^2$, significant F and p values. The Dubinson-Watson test for automated correlation was analysed.
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) which achieved discharge quality of all parameters except pathogen removal. 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. A small integrated unit can be installed in tourist spots because, CWS serves as a habitat for the macro fauna in and around the system providing biodiversity from ecological benefit point of view. The integration of CWS with nanocomposite filter is a novel idea and the outcome of the research is astounding.

The outcome of the research ended with the discharge of effluent with 10 mg/L, 30 mg/L, 35 mg/L, 2 mg/L, and 1.0 mg/L of BOD, COD, TSS, TKN and phosphate. With a close concern to coliform removal, less than 1000 MPN/ 100 mL for TC and less than 500 MPN/100 mL for FC and *E.coli* was achieved during the experimental period. The integration of clay disc not only improved the quality of effluent with respect to coliforms but also aided in the enhanced removal of other pollutants: organics, nutrients and heavy metals. Clay is a natural adsorbent and acts as a filtering mechanism. Moreover, montmorillonite is reported as an efficient adsorbent of pollutants in wastewater treatment. The pinnacle point of the research is that nanocomposite clay filter is a separate component that can be integrated with any existing system to eliminate pathogen. *Cyperus alternifolius* is a promising plant in CWS. The uptake of heavy metals in the above ground parts is lesser than the below ground part. The above ground biomass can be harvested and utilised economically. The problem of withering leaves was not observed till maximal growth of 5-6 feet neglecting the fear of adding nutrients back in to the system. Crisply to conclude in a nut shell, the outcome of the research ended up in achieving the goal. Focus can still more be done on nanocomposite clay discs for extrapolation in large scale however, this is just an initiative.

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