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Assessment of pathogen removal efficiency of vertical flow constructed wetland treating Septage

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Abstract

A laboratory-scale Constructed Wetland (CW) system consisting of a sludge drying bed and a vertical flow wetland bed was utilized for the treatment of septage, focusing on the reduction of microbial contamination (specifically total coliforms) and helminth eggs. The system had a total area of 0.99 m^2. The sludge drying bed and vertical flow bed were filled with a combination of gravel with varying sizes (ranging from 5 to 40 mm) and washed sand. *Canna indica* plants were cultivated on both beds to facilitate phytoremediation processes.

The average inlet loads of Biochemical Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD), and Total Suspended Solids (TSS) were measured as 150 ± 65.7 g m^{-2} d^{-1}, 713 ± 443.9 g m^{-2} d^{-1}, and 309 ± 66.3 g m^{-2} d^{-1}, respectively. After treatment, the final effluent had an average load of 6 g m^{-2} d^{-1} for BOD, 15 g m^{-2} d^{-1} for COD, and 51 g m^{-2} d^{-1} for TSS, indicating that the CW system achieved an average removal efficiency of 88% for BOD, 87% for COD, and 65% for TSS in the septage wastewater.

The average influent load of total coliforms and helminth eggs was observed to be 4 x 10^8 Colony-Forming Units (CFU) m^{-2} d^{-1} and 3 x 10^7 eggs m^{-2} d^{-1}, respectively. However, the CW system demonstrated significant effectiveness in reducing microbial contamination, with an average removal efficiency of 99% for both total coliforms and helminth eggs. As a result, the total coliform and helminth egg loads in the effluent were reduced to 9 x 10^5 CFU m^{-2} d^{-1} and 1.6 x 10^4 eggs m^{-2} d^{-1}, respectively.

Introduction

Gradual increase in the Population is resulting in more septage discharge which is not being treated properly due to lack of proper management and high operational costs of the conventional methods. Removal of pathogens and other toxics from septage is a challenge in developing countries like India where proper management of septage is not practiced and standards for septage treatment are not well defined. Untreated septage is released into rivers and streams in India, causing them to deteriorate and become unfit for use. Septage contains numerous pathogens such as various coliforms[1,2] and helminths eggs[3,4] which have detrimental effects on human health once they reach into the body. Rise in temperature and run-off after precipitation increase the quantity of pathogens in the water body where septage is discharged. These pathogens are difficult to remove by conventional wastewater treatment methods. Constructed wetlands are an emerging approach that is being employed globally for septage treatment due to their ease of operation, adaptability, effectiveness, and low maintenance cost. Constructed wetlands (CW) are advantageous for septage treatment in developing and populated countries where it is difficult to set up large scale infrastructure due to high cost and requirement of larger area for wastewater treatment plant. Constructed Wetlands, whether vertical or horizontal, have proven to be effective at removing pathogens and other pollutants. The treated water collected after treatment by CW system can be used for various purpose such as irrigation and thus can be helpful in water scarce scenarios. Constructed wetlands (CW) remove microbial and physico- chemical pollutants from water

with the help of natural processes involving wetland vegetation and soils[5]. This study focuses on the removal of total coliform (TC) and helminths eggs (HE) collectively by the use of constructed wetland (vertical sub- surface flow).

There are different kinds of CW system used such as vertical sub- surface flow[6,7,8] horizontal sub-surface flow[9,10] and Up- flow sludge blanket[11,12] and hybrid constructed wetlands. new technologies like biological sand filters and biofilters are now being used for wastewater treatment[13] but CW are favorable because of their ease of operation, low cost establishment and better ability to remove microbial and organic pollutants. Vertical flow CW planted with Canna indica facilitated the treatment of dairy effluent by purifying BOD, COD, (NH4–N), and (PO4–P)[6]. Up- flow sludge blanket (UASB) is an effective anaerobic technique used for wastewater treatment which is cost effective and environment friendly, but their performance is limited due to presence of high residual pathogens and organic matter[12]. Vertical sub-surface flow (VSSF) constructed wetland systems (CWS) are widely accepted approach because of their better removal efficiency of pathogens and other pollutants[7,8,14]. Vertical subsurface flow CWs offer favourable oxygen transfer conditions and encourage nitrification[15]. Previous studies support the results by showing effective removal efficiency of CW systems in case of pathogens like coliform[13,16,17] and helminths eggs[4,18,19] along with reduction in BOD[13], COD[8], TDS[20] and TS[8]. Hybrid Imhoff tank and CW systems effectively removed total coliform and parasitic eggs by 99% from the wastewater, making the treated effluent fit for irrigation purpose[5]. Hybrid CW comprising horizontal and vertical subsurface flow CWs provided 99% removal of total coliform, 92% of organic matter (BOD, COD and TOC) and 88% of TSS[15]. A multistage CW system showed more than 5 log units reduction in total coliform and 100% removal of helminths eggs (HE) from wastewater[14]. Hybrid CW consisting of vertical flow CW planted with Arundodonax L. and horizontal flow CW planted with *Hibiscus esculentus L*., and *Solanummelongena L*., was used to treat dairy effluent; which showed average removal of TSS, BOD_3, total N, and P as 92.2 ± 6.1, 95 ± 3.8, 83.6 ± 9.0and 86.1 ± 10.0% respectively[21].

The presence of these pathogens has a correlation with the wastewater's physicochemical parameters such as BOD, COD, TDS, and TS. Septage contains a high BOD, COD, TDS, and TS in direct proportion to the amount of TC and HE. The media material and plant species have the greatest impact on the performance efficiency of CW system[22]. Filtration beds made up of materials like limestone, sawdust and coal, coupled with biological species like Glyceria, Acorus, Typha and Phragmites prove to be efficient in pollutant removal from wastewater[23]. Parallel wetland cells with several plant species, including Phragmites ,Typha and Cyperus removed 100% of the helminths eggs and 99.8% of protozoa[18]. Plantation and hydraulic retention time (HRT) are directly related to removal efficiency of constructed wetlands[4]. silex, granite and river gravel were used as filtration bed media with plant species Phragmites and Typha in CW system to treat municipal raw wastewater and the results showed significant decrease in organic and microbial pollutants. Sand and gravels (10 mm and 20 mm) were used as filtration media and Canna indica was planted in three labscale wetlands to treat dairy effluent at different HRT and the results showed removal of pollutants as follows: 64.2–74.5% for TSS, 45.3–63.1% for BOD, ammonium

nitrogen (NH4–N): 29.6–56.5% and phosphate phosphorous (PO4–P): 20.5–57.8%[6]. In a study of six vertical flow mesocosms with different media and plantations for six months, the sand media modified with 50% pumice and lemongrass plantation attained the best removal effectiveness, whereas the lowest removal was recorded in the sand media with no plantation[22]. Previous study confirms that hydraulic retention time has a significant effect on the removal efficiency of CW where maximum removal of pollutants was achieved at 48h HRT and minimum removal was reported at 12h HRT[6]. Optimum removal efficiency for total coliform was achieved at hydraulic load of 1250 L and HRT of 24hours[20]. In this study, Filtration media was made up of sand and gravels of different sizes, arranged in decreasing order of size from bottom to top. The plant species used was Canna Indica L., and the HRT for SDB and VSSF was 24 hours each.

Although CW system has been proved efficient for removal of pathogens but they might not be effective in every scenario in which case we need other aids to remove them. Constructed wetland systems along with electrochemical system show efficient removal of pathogens and micropllutants[24]. Coliforms were not entirely eliminated by CW system therefore an electrochemical cell with a cation exchange membrane was used to treat the wetland effluent after it had initially passed through the anodic chamber which resulted into an average 5-log unit reduction in coliform[25]. Biochar can also being used in CW systems for effective treatment of septage. Use of biochar makes the CW system more efficient by reducing the time taken for filtration, providing better removal of pollutants and upgrading the quality of treated water making it fit for reuse. Use of reed plant and its biochar in CW system showed significant reduction in coliform, BOD , COD and other pollutants in mere 72 hours[26].

This study sought to eliminate pathogens and organic contaminants with the implementation of a system composed of a sludge drying bed (SDB) and vertical subsurface flow constructed wetland. Studies focusing on wastewater treatment with the help of such a technology are scarce. Some studies focused on VSSF or SDB separately but none of them used both of these systems altogether for removal of helminthes eggs and total coliform along with TDS, TSS BOD, and COD. Due to improper management and discharge of sewage as well as the greater expense of wastewater treatment facilities, CW systems are of vital importance in India. This study helps to bridge the knowledge gap regarding removal of Helminthes eggs and total coliform by implementing a CW system comprising SDB and VSSF.

Methodology

Layout and construction of Laboratory-scale Vertical Subsurface Flow (VSSF) CW system

At Graphic Era University's research field station, a lab-scale Vertical sub-surface flow (VSSF) constructed wetland (CW) system was implemented, comprising a sludge drying bed, a vertical flow CW bed, and a filter (Fig. 1). These beds were interconnected in series, sloping at a 1% gradient from the inlet to the

discharge point. Each day, approximately 30 liters of septage underwent treatment in the CW system using an intermittent dosage regimen.

The sludge drying bed (SDB) served as the initial component of the CW system, responsible for dewatering and stabilizing the septage sludge. A cylindrical plastic container with a diameter of 41.5 cm was utilized as the SDB, which had a total area of 0.41 m² and a depth of 50 cm. To facilitate the dewatering process, the SDB's hydraulic retention time (HRT) was set to 24 hours. The effluent from the SDB was collected through a drainage pipe attached to its bottom and directed to the subsequent component, a vertical subsurface flow (VSSF) bed, connected in series.

The VSSF bed was constructed using an HDPE sheet measuring 0.76 m × 0.76 m × 0.53 m, providing a surface area of 0.58 m². Similar to the SDB, the VSSF bed also had a hydraulic retention time of 24 hours. The entire CW system occupied a total area of 0.99 m².

For filtration purposes, the beds were filled with sand and gravels. The SDB comprised four layers, with the topmost layer being 20 cm thick consisting of sand. The subsequent layers were each 10 cm thick and composed of gravels with sizes of 5 mm, 10 mm, and 10–40 mm, respectively.

In the VSSF bed, the first layer had a thickness of 20 cm and was filled with 5 mm-sized gravel, followed by a 10 cm layer of sand. The third and fourth layers, each 10 cm thick, consisted of gravels sized 10 mm and 20–40 mm, respectively.

Sample collection and analysis

Consecutive samples were taken at the S1, S2, and S3 sampling sites. Septage was collected daily and subjected to drying on a sludge drying bed (SDB) using a collection tank. The septage remained in the bed for 24 hours before being transferred to the subsequent vertical flow bed. The hydraulic retention time (HRT) for the vertical flow bed was set at 24 hours. The physico-chemical tests (BOD_5, COD, TSS, TDS) and biological tests (total coliform and helminth eggs) were conducted on the samples at the Environmental Science Laboratory at Graphic Era (Deemed to be University) in Dehradun, India (30.3165° N, 78.0322° E). TDS was measured using a calibrated EC-TDS Meter (CON100, Patsio), TSS was determined using a colorimetric method, BOD_5 was assessed using a 5-day incubation method, COD was analyzed using a reactor digestion method, total coliform was measured employing an ABC method, and helminth eggs were detected using an ABC method.

To identify statistically significant differences among the various parameters, including BOD_5, COD, TSS, TS, TC, and HE, statistical analyses were performed on the acquired results. The analyses were conducted using OrginPro 8.5 SR1 (Origin Lab Corporation, Northampton, MA, USA) and Microsoft Excel-2019.

Pollutant removal efficiency of CW system

The pollutant removal effectiveness of the hybrid-CW was assessed by calculating the purification and removal rates for multiple selected parameters over a six-month period from January to December 2021. The purification rate (%) was determined using the formula:

Purification rate (%) = {(Ci - Co)/Ci} * 100.....(1)

where Ci and Co represent the inlet and outlet concentrations of the parameter in milligrams per liter (mg/L).

Similarly, the removal rate (%) was calculated using the formula:

Removal rate (%) = {(Li - Lo)/Li} * 100.....(2)

where Li represents the inlet load in grams per square meter per day ($g/m^2/day$), and Lo represents the outlet load in grams per square meter per day ($g/m^2/day$).

The load (L) was determined using the equation:

Load (L) = Q * C / A.....(3)

Here, Q represents the flow rate measured in cubic meters per day (m^3/day), C denotes the concentration of the parameter in mg/L, and A represents the area in square meters (m^2).

Results and Discussion

BOD_5 and COD Load and Removal rate

The combination of a vertical subsurface flow (VSSF) bed and a sludge drying bed (SDB) has shown remarkable improvement in the removal efficiency of the BOD_5 load. The entire system achieved an average reduction of 93.4 ± 4.1% for BOD_5. Despite fluctuations in the influent BOD_5 load, ranging from 43.9 to 480.5 g m^{-2} d^{-1}, both the SDB and VSSF beds effectively contributed to the removal of organic pollutants. The SDB exhibited an average BOD_5 removal efficiency of 61.1 ± 13.5% with an average effluent load of 54.1 ± 13.96 g m^{-2} d^{-1}, while the VSSF bed demonstrated a higher average BOD_5 removal efficiency of 88.1 ± 4.9% with an average effluent load of 6.1 ± 2.72 g m^{-2} d^{-1}.

The significant increase (approximately 27%) in the BOD_5 removal efficiency of the VSSF bed compared to the SDB can be attributed to two factors: the variation in inlet BOD dosing loads and the presence of more aerobic conditions in the VSSF bed. The varying inlet BOD dosing loads likely contributed to the higher removal efficiency observed in the VSSF bed. Additionally, the design of the VSSF bed promotes a favorable aerobic environment, facilitating greater degradation of organic matter and further enhancing its performance in BOD_5 removal.

The treatment procedure consistently resulted in a decrease in the initial BOD_5 load, as indicated by the average BOD_5 load of the effluent (3.6 ± 1.59 g m^{-2} d^{-1}) compared to the influent load (62.1 ± 27.19 g m^{-2} d^{-1}) for the entire system. This signifies a significant reduction in the amount of organic matter present. The microbial degradation of organic compounds in the wastewater as it passed through the beds contributed to this reduction. The efficient functioning of the treatment system in reducing the organic load of the influent wastewater was demonstrated by the BOD_5 removal.

In addition to BOD_5, the measurement of Chemical Oxygen Demand (COD) was conducted. Chemical reactions and adsorption processes played a crucial role in reducing the non-biodegradable portion of COD. The influent COD load exhibited less variability compared to BOD_5, ranging from 241.9 to 413.4 g m^{-2} d^{-1}. The average COD load in the influent was 331.9 ± 41.5 g m^{-2} d^{-1}, approximately five times higher than the average BOD_5 load. The system achieved a significant COD load reduction with a removal efficiency of 95.7 ± 1.1%, while the SDB and VSSF beds individually achieved removal efficiencies of 73.2 ± 4.7% and 87.4 ± 3.3%, respectively. The average COD load in the SDB effluent was 215.4 ± 45.5 g m^{-2} d^{-1}, while in the VSSF effluent it was 24.4 ± 6.9 g m^{-2} d^{-1}. Notably, there were no significant differences observed in the COD and BOD_5 removal efficiencies of the VSSF bed.

By integrating SDB and VSSF beds, the strengths of each bed can be capitalized on to achieve synergistic effects. The SDB system can serve as an effective primary treatment stage, significantly reducing the initial COD load for the subsequent VSSF system.

Table 1
Average ± SD load of pollutants, total coliforms and total helminthes eggs in influent, and effluents of SDB and VSSF (Each data is average of 180 day's observation)

Parameter	Influent	SDB out	VSSF out
BOD_5 g m^{-2} d^{-1}	150 ± 65.7	54.1 ± 14.0	6.1 ± 2.7
COD g m^{-2} d^{-1}	713.4 ± 443.9	16.4 ± 96.5	15.0 ± 12.6
TSS g m^{-2} d^{-1}	309.9 ± 66.3	143.2 ± 20.8	50.9 ± 17.1
TS g m^{-2} d^{-1}	830.4 ± 130.3	413.5 ± 177.8	87.5 ± 37.7
*Total Coliforms(CFU m^{-2} d^{-1})	$4.3 \times 10^8 \pm 4.5 \times 10^8$	$3.9 \times 10^7 \pm 4.2 \times 10^7$	$9.0 \times 10^5 \pm 1.3 \times 10^6$
*Helminthes, (eggs m^{-2} d^{-1})	$3.7 \times 10^7 \pm 8.3 \times 10^6$	$3.3 \times 10^6 \pm 7.6 \times 10^5$	$1.6 \times 10^5 \pm 5.0 \times 10^4$

TSS and TS Load and Removal rate

The treatment process demonstrated effective removal of suspended solids, indicating the system's efficiency in physically filtering and settling solid particles. The system achieved removal efficiencies of

74.8 ± 4.5% for TSS and 82.0 ± 3.9% for TS. The dynamics of TSS and TS removal are illustrated in Fig. 3(c) and Fig. 3(d), respectively.

The influent TSS load ranged from 90.5 to 214.7 g m^{-2} d^{-1}, while the TS load ranged from 212.3 to 477.1 g m^{-2} d^{-1}. The average TSS and TS loads in the influent were recorded as 128.2 ± 27.4 g m^{-2} d^{-1} and 342.7 ± 54.7 g m^{-2} d^{-1}, respectively (Table 1). Interestingly, the average TS load in the influent was approximately three to four times higher than the TSS load, indicating a significant portion of pollutants in a dissolved state. The SDB played a crucial role in removing larger particulate matter, while the VSSF bed facilitated the settling and retention of finer suspended solids. The VSSF bed demonstrated slightly superior performance in TSS removal (70.7 ± 5.8%) compared to TS removal (65.3 ± 7.1%). The average TS load in the effluents of the SDB and VSSF beds was observed as 143.2 ± 20.8 g m^{-2} d^{-1} and 54.1 ± 11.1 g m^{-2} d^{-1}, respectively (Table 1).

Under the experimental conditions, the VSSF bed alone was capable of removing TSS and TS with average removal efficiencies of 70.7 ± 5.8% and 65.3 ± 7.1%, respectively, which were significantly higher than those achieved by the SDB (51.8 ± 12.1% for TSS and 45.6 ± 14.2% for TS), as shown in Fig. 3(c) and Fig. 3(d). These results align with previous studies [28,29] that highlight the role of the VSSF bed in TSS removal. The findings indicate that the combination of the SDB and VSSF effectively removes solid particles, improving water clarity and reducing the potential for sedimentation in receiving water bodies.

Total coliform and helminthes eggs Load and Removal rate

The assessment of a system's ability to provide microbiologically safe water is crucial, with a strong focus on the removal of total coliform bacteria and helminth eggs. These contaminants pose substantial risks to public health and environmental quality, especially in wastewater. The entire system demonstrated exceptional performance, achieving a removal efficiency of over 99% for both coliform bacteria and helminth eggs. The SDB alone exhibited an average removal of 91 ± 2% and 92 ± 3% (see Fig. 3(e) and (f)) of the initial influent counts of total coliforms (log_{10} 6 ± 5 CFU/100mL) and helminth eggs (log_{10} 6 ± 5 eggs/L), as shown in Fig. 2(e) and (f).

The Sludge Drying Bed (SDB) played a crucial role in effectively eliminating both bacteria and helminth eggs, reducing their load from the septage. The high removal efficiency of the SDB can be attributed to the gradual filtration of septage through sand and multiple layers of differently sized gravels. The effluent from the SDB exhibited average counts of 4 for total coliforms and 5 for helminth eggs (on a log10 scale).

The configuration of the VSSF bed, including the filter depth, significantly influenced the removal of pollutants and pathogens. It demonstrated the ability to remove 95 ± 3% and 93 ± 1% (refer to Fig. 3(e) and (f)) of total coliforms and helminth eggs, respectively. It is noteworthy that despite fluctuations in the counts of total coliforms and helminth eggs, both beds performed equally well in reducing the microbial counts.

Correlation study

The correlation matrix (Fig. 4) of the entire system illustrates the interrelationships among key water quality parameters, such as COD, BOD_5, TSS, TS, total coliform (TCOL), and helminth eggs (HE), spanning from influent to effluent. The correlation coefficients offer valuable insights into the strength and direction of these relationships, providing a comprehensive understanding of the interconnectedness of these parameters.

In the correlation matrix encompassing the entire system, notable positive correlations were identified between the influent and effluent values for the same parameters. These correlations include COD inf vs. COD eff (r = 0.99), TCOL eff vs. TCOL inf (r = 0.996), HE eff vs. HE inf (r = 0.85), and TS inf vs. TS eff (r = 0.66). These results indicate that the composition of the influent significantly influences the quality of the effluent, emphasizing the effectiveness of the SDB and VSSF combination in accurately reflecting the influent characteristics in the treated water.

Strong positive correlations were also observed between distinct parameters, namely COD inf vs. BOD eff (r = 0.78) and COD eff vs. BOD inf (r = 0.74), suggesting that an increase in organic pollutants measured by COD corresponds to higher levels of BOD.

Moderate positive correlations were found between TSS eff vs. TS inf (r = 0.66) and TSS eff vs. TS eff (r = 0.60), indicating that the presence of suspended solids is associated with higher concentrations of total solids in the water samples. Additionally, weaker positive correlations were identified between COD inf and TSS inf (r = 0.48), COD eff and TSS inf (r = 0.34), TS eff and COD inf (r = 0.43), and TS eff and COD eff (r = 0.37). These findings suggest that elevated levels of organic pollutants coincide with increased amounts of both suspended and total solids in the water.

In terms of microbiological parameters, a weak positive correlation was observed between TS eff and HE inf (r = 0.25), indicating a potential association between organic pollution and the presence of helminth eggs. However, no significant correlation was found between TCOL and HE, despite similar removal efficiencies of the SDB and VSSF. This lack of correlation may be attributed to fluctuations in the counts of both parameters from influent to effluent.

Overall, these correlation findings highlight the interconnected nature of water quality parameters and provide insights into their relationships within the treatment system.

Conclusions

By optimizing the design and operational parameters of each system and considering the specific requirements of the treatment site, the combined approach of Sludge Drying Bed-VSSF (Vertical Flow Constructed Wetland) holds great potential for achieving high levels of pollutant and pathogen removal. The Vertical Flow Constructed Wetland system, equipped with pretreatment by sludge drying bed, has proven to be efficient in achieving impressive removal rates. In septage wastewater, it demonstrated

removal rates of 88% for BOD, 87% for COD, and 65% for TSS. Additionally, the CW (Constructed Wetland) system exhibited significant effectiveness in reducing microbial contamination, consistently achieving an average removal efficiency of 99% for both total coliforms and helminth eggs. These results not only showcase the efficiency of the combined SDB-VSSF approach but also highlight its contribution to sustainable and environmentally friendly practices.

Declarations

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Author contributions

P.K.S. developed and executed the study. SS executed the study and wrote the manuscript; S.U. contributed to writing; A.R, J.M.R, B.R, P.N., P.K. and P.B. contributed to discussions throughout the study. All authors helped in refining and revising the manuscript.

Competing interests

The authors declare no competing interests.

Data availability

All experimental results have been included in the manuscript. The data can be made available upon reasonable request to the corresponding author.

Statement of experimental research and field studies on plant

- Experimental research and field studies involving plants, including the collection of plant material, strictly adhere to the relevant guidelines and legislation at the institutional, national, and international levels.
- Permission was obtained from the University administration to collect and utilize *Canna indica* from the University's agricultural experimental station for experimental work.
- The identification of the *Canna indica* plant sample was performed by Dr. Gajendra Singh, a Scientist at USAC, India.

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Figures

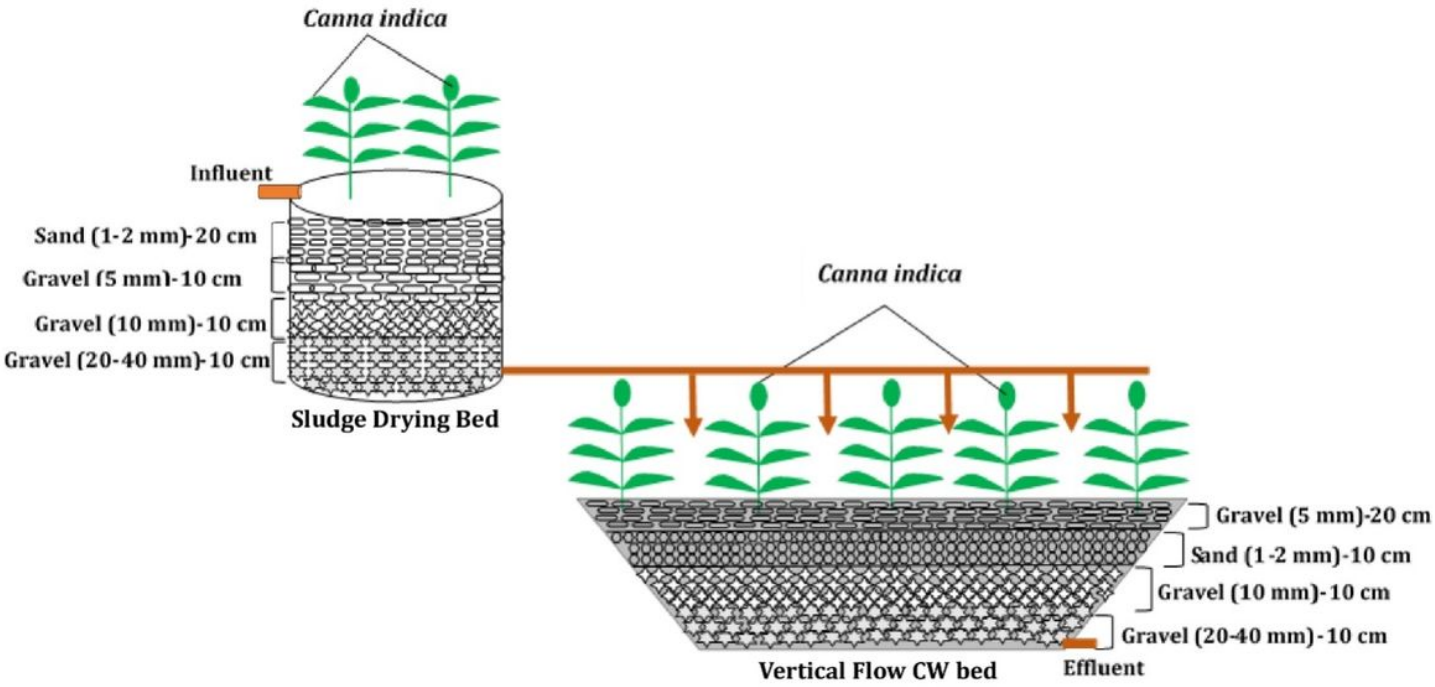


Figure 1

Schematic diagram of Constructed wetland system for treatment of Septage

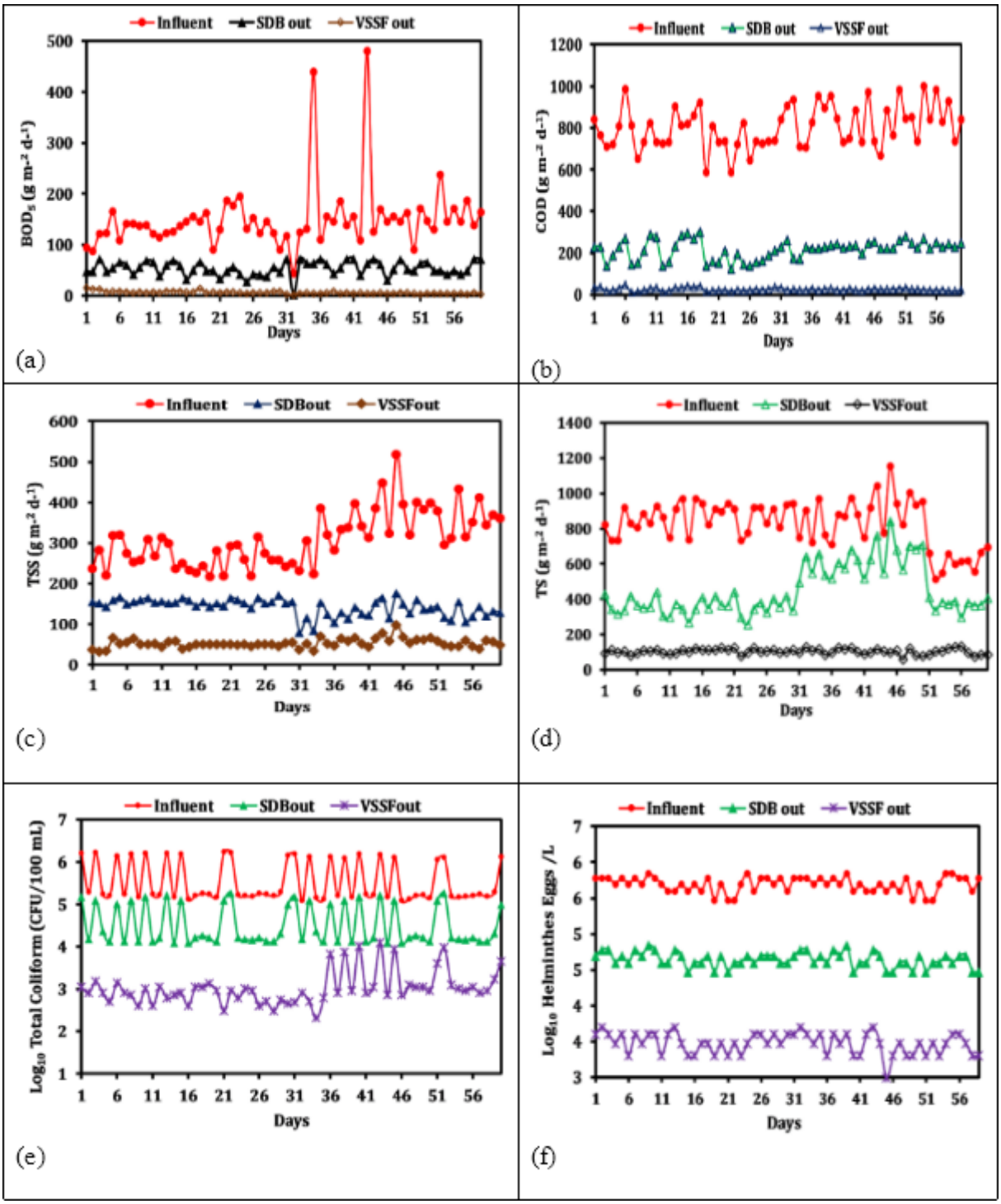


Figure 2

Dynamics of (a)-BOD, (b)COD, (c)-TSS, (d)-TS, (e)-total coliforms, and (f)-total helmminthes in influent, and effluents of SDB, and VSSF, with respect to HRT (days)

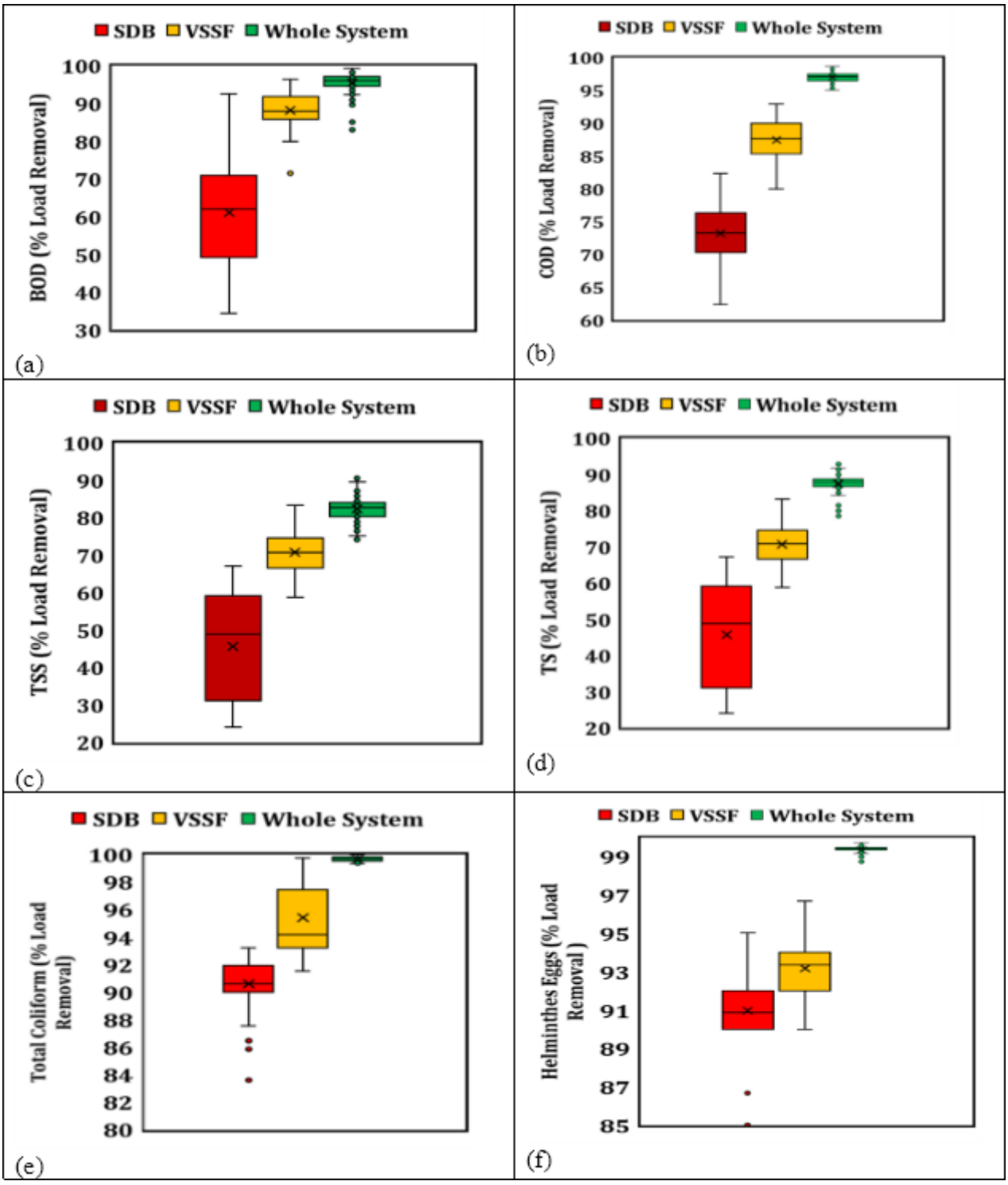


Figure 3

Performace of SDB, VSSF, and whole system in percentage load removal of (a)-BOD, (b)COD, (c)-TSS, (d)-TS, (e)-total coliforms, and (f)-total helmminthes

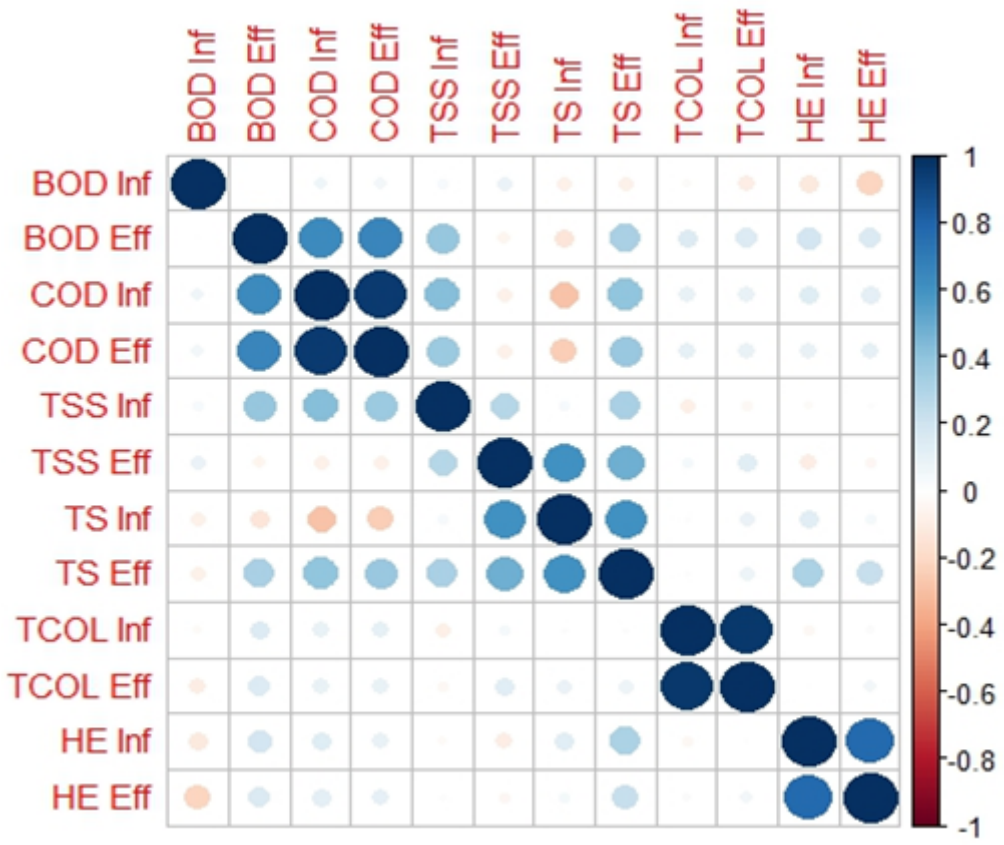


Figure 4

Matrix-correaltion studies (influent to effluent) of BOD_5, COD, TSS, TS, total coliform, and total helminthes