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1 Evaluating the performance of horizontal sub-surface flow

2 constructed wetlands: A case study from southern India

- 3 Priyanka Jamwal^{1*}, Anjali V Raj¹, Lakshmi Raveendran¹, Shahana Shirin¹, Stephanie Connelly²,
- 4 Jagadeesh Yeluripati^{3a}, Samia Richards^{3b}, Lakshminarayana Rao⁴, Rachel Helliwell^{3b} and Matteo
- 5 Tamburini^{3c}
- 6 ¹ Centre for Environment and Development, Ashoka Trust for Research in Ecology and the
- 7 Environment (ATREE), Bangalore, India
- 8 ² Division of Infrastructure and Environment, James Watt School of Engineering, University of
- 9 Glasgow, Glasgow G12 8LT, UK
- ^{3a} Information and Computational Sciences, The James Hutton Institute, Craigiebuckler,
- 11 Aberdeen, AB15 8QH, Scotland, UK.
- ^{3b} Environmental and Biochemical Sciences, The James Hutton Institute, Craigiebuckler,
- 13 Aberdeen, AB15 8QH, Scotland, UK.
- ^{3c} Constructed wetland consultant, The James Hutton Institute, Craigiebuckler, Aberdeen, AB15
- 15 8QH, Scotland, UK.
- 16 ⁴ Centre for Sustainable Technologies, Indian Institute of Science (IISc), Bangalore, India
- 17 Abstract
- 18 Constructed wetlands are a nature-based engineering solution enabling polishing of septic tank
- 19 effluents at low-cost. However to date, the influence of planting on treatment efficiency
- 20 remains little understood. Here we report a case study evaluating the performance of two near-
- 21 identical Horizontal Sub-Surface Flow Constructed Wetlands (HSSF-CW) deployed at a school in
- southern India. The HSSF-CWs were of similar size and construction with the exception that one
- 23 system was planted (*Canna indica*) whilst the other was operated without plants. Both systems
- 24 were operated at similar hydraulic loading rate (HLR) and hydraulic retention time (HRT) of 84
- 25 mm day⁻¹ and 3.7 days, respectively to treat the effluent from septic tanks. The systems were
- 26 monitored fortnightly for one year and the performance kinetics, nutrient and organics removal
 27 efficiencies were evaluated. Significant reduction in biochemical oxygen demand (BOD₅) and
- chemical oxygen demand (COD) (p < 0.05) were observed in both systems with BOD₅ removal
- efficiency of 67 % and 61 % in the planted and unplanted systems, respectively. Whilst the
- 30 effluent from both systems met the environmental discharge standards set by Central Pollution
- 31 Control Board (CPCB), India, the total phosphorus (TP) and total suspended solids (TSS) removal
- 32 in the unplanted system were significantly greater than in the planted system. The first-order
- decay rate constants (K) obtained for TN (K_{TN}) and BOD₅ (K_{BOD_5}) in the planted system however
- 34 (0.16 day⁻¹ and 0.30 day⁻¹) were higher than in the unplanted system (0.09 day⁻¹ and 0.27 day⁻¹).

^{*} Corresponding author: Dr. Priyanka Jamwal, Fellow, Centre for Environment and Development, Ashoka Trust for Research in Ecology and the Environment (ATREE), Bangalore, India email: priyanka.jamwal@atree.org

- 35 Greater R² values obtained for the planted system (~ 0.90) suggests applicability of a first-order
- 36 decay model to assess contaminant degradation. Plants contributed to 7 % (0.3 gm/m²/day)
- BOD₅, 18 % (1.9 gm/m²/day) COD and 5 % (0.09 gm/m²/day) TN load removal. Our data
- 38 demonstrates that planting is effective in improving treatment efficiency in constructed
- 39 wetlands, and whilst the improvement is marginal here, it is noted that a rust infection could
- 40 have limited effectiveness of the plants in this case.

41 Keywords:

42 HSSF-CW, Wastewater, Canna indica, removal kinetics, load reduction, plant uptake

43 1. Introduction

44 The release of untreated domestic wastewater, agricultural runoff and industrial effluents 45 results in the deterioration of surface water quality, loss of biodiversity and can cause serious 46 risk to public health (Mayo and Mutamba, 2004). In 2014, the Government of India (GOI) 47 launched the Swachh Bharat Mission (SBM) to eradicate open defecation and promote safe 48 sanitation in all households across India. The SBM mission led to construction of 102 million 49 new toilets across the country (Ministry of Urban Development, Government of India, 2017), 50 and, by the end of July 2019, the GOI declared rural India as open defecation free. Since the 51 mission was launched, numerous studies have been conducted to assess the impact of SBM on 52 the aquatic environment (Bhallamudi et al., 2019; NIUA, 2017; Rohilla et al., 2017). A number of 53 studies reported groundwater contamination of local aquifers as a result of black water disposal 54 via soak pits (Bhallamudi et al., 2019; Kumpel et al., 2017; Olonga et al., 2015), a low-cost 55 solution that allows leachate to percolate into the groundwater. To tackle this, septic tanks 56 were suggested as a preferred treatment system for deployment at household and community 57 level. However, given that septic tanks remove only 60 to 65 % of BOD₅, additional treatment 58 units are required to meet effluent discharge standards set by the Central Pollution Control 59 Board (CPCB) (DBT and CPCB, 2019). Low-cost decentralised treatment systems such as 60 constructed wetland have the potential to polish the effluent from septic tanks to remove 61 organics and nutrients, making effluent safe to discharge into the environment (Kivaisi, 2001; 62 Mairi et al., 2012).

63 Various studies have been conducted on the potential of constructed wetland technologies for 64 secondary/ tertiary treatment of wastewater in India (Juwarkar et al., 1995; Ramprasad and 65 Philip, 2018; Srivastava et al., 2020; Suganya and Sebastian, 2017). The type of constructed 66 wetlands used in India includes the PHYTORID systems (works on the principle of sub-surface 67 flow technology), horizontal/vertical sub-surface flow systems and hybrid reed bed systems, 68 with different types of plantation (DBT and CPCB, 2019; Haritash et al., 2015; Sharma et al., 69 2014; Suganya and Sebastian, 2017). Constructed wetlands, by design, often support a dense 70 growth of plants which are thought to be an important component of wetland systems with 71 emergent macrophytes most commonly used in sub-surface constructed wetlands (Schierano et

72 al., 2020; Wu et al., 2019).

In constructed wetlands, the treatment of wastewater occurs through a combination of 73 74 physical, chemical and biological processes (Sehar and Naz, 2016). Plants in constructed 75 wetlands have been shown to stabilize hydraulic conductivity, prevent erosion, promote 76 filtration, provide surface area for biofilms, uptake nutrients, release root exudates that are 77 utilized by biofilms and introduce additional fungi species and symbiotic bacteria to the wetland 78 ecosystem (Brix, 1997; Jason, 2007; Kadlec and Wallace, 2008; Stottmeister et al., 2003). 79 Planted constructed wetlands are reported to remove 70-95 % of organic matter and nutrients 80 from the wastewater (Albalawneh et al., 2016; Haritash et al., 2015; Ramprasad and Philip, 81 2018). Canna indica is widely used wetland plant species that is very effective in removing 82 nutrients from wastewater (Liang et al., 2017). Canna indica exhibits rapid growth rate and high 83 biomass production which provides surface area for the growth of biofilms, and, the fibrous 84 root system and root oxygen release enhances microbial activity (Lekshmi et al., 2020; Li et al., 85 2013). Canna indica also acts as a good phyto-accumulator for reducing contaminant load 86 (Suganya and Sebastian, 2017). Climatic conditions in India favors the growth of Canna indica 87 making it suitable for wetland vegetation in rural communities (Haritash et al., 2015). 88 Harvesting plants at regular intervals is important to sustain the contaminant removal efficiency 89 of the constructed wetlands (Wallace, 2015). Regular harvesting however adds to the extra 90 operational cost of constructed wetlands (Dotro et al., 2017), which is one of the key barriers 91 towards adoption and scaling up of planted constructed wetlands (Kivaisi, 2001). A growing 92 number of studies however report that microbial degradation, as compared to vegetation 93 uptake processes, acts as the major contributor to contaminant removal in constructed wetlands (da Costa et al., 2015, 2013; Ebrahimi et al., 2013; Iasur-Kruh et al., 2010). In 94 95 unplanted constructed wetlands systems, various processes contribute to treatment including 96 physical filtration and sedimentation remove suspended matter; chemical formation of 97 complexes can remove phosphorus; chemical precipitation can promote removal of iron and 98 heavy metals; and microbiological degradation in the biofilm promotes removal of dissolved 99 organic and inorganic matter (de Matos et al., 2018). 100 For effective deployment of constructed wetlands (with plants or without plants), confidence in

- the translation of design principles to the field is essential. Presently, constructed wetlands
- design and deployment for use in sanitation systems lacks design principles, and, understanding
- 103 of the removal kinetics of contaminants in field conditions remains limited (Davis, 1995; Mairi
- 104 et al., 2012; Rohilla et al., 2017). Here we present a case study based on field data describing
- the operation and performance of two near identical wetland systems deployed in rural India.
- 106 The objective of the study is to a) assess and compare the performance of planted and
- 107 unplanted horizontal subsurface flow constructed wetland (HSSF-CW) systems b) estimate the
- 108 contaminant removal kinetics in the planted and unplanted HSSF-CW systems and c) assess the
- 109 contribution of vegetation to contaminant load removal.

110 2. Methodology

111 2.1 Description of constructed wetlands

112 The HSSF-CW systems studied are one of a number of technologies deployed to provide a 113 complete, closed-loop decentralised wastewater treatment and recycling system at Berambadi Government Primary School (11°45′44″ N; 76°34′03″ E) located in Berambadi village, 114 115 Chamarajanagar district, Karnataka (Subramanian et al., 2020). The area receives an annual 116 rainfall of 915 mm and has an average annual temperature of 22.9 °C (Sekhar, 2016). 117 Berambadi Primary School serves around 180 students and 5 teachers. The HSSF-CW systems 118 reported in this study were deployed to provide secondary treatment to the effluent from two 119 anaerobic systems (septic tanks) that serve as primary treatment for the black water and 120 greywater (hand wash facility provided in the toilet block) generated at the school's toilet 121 block. The planted HSSF-CW is connected to a plug-flow septic tank and the unplanted HSSF-CW 122 to a conventional septic tank, both of which generate effluents of similar quality. The gravel bed 123 of each HSSF-CW has dimension 4.4 m×1.0 m× 0.9 m (length×width×height) with sampling ports 124 (locations of sampling pipes numbered 1-10) distributed along the length (Fig 1). The base of 125 each wetland slopes towards the outlet with a 1% gradient. The outflow from each HSSF-CW is collected through an outflow pipe located at the bottom of the CWs. A riser pipe is used to 126 maintain the water depth (0.8 m) in each of the HSSF-CW systems. For discharge monitoring, 127 128 outflow from each system was collected separately in two graduated circular tanks. The 129 overflow from the tanks was finally discharged into an open storm water drain.

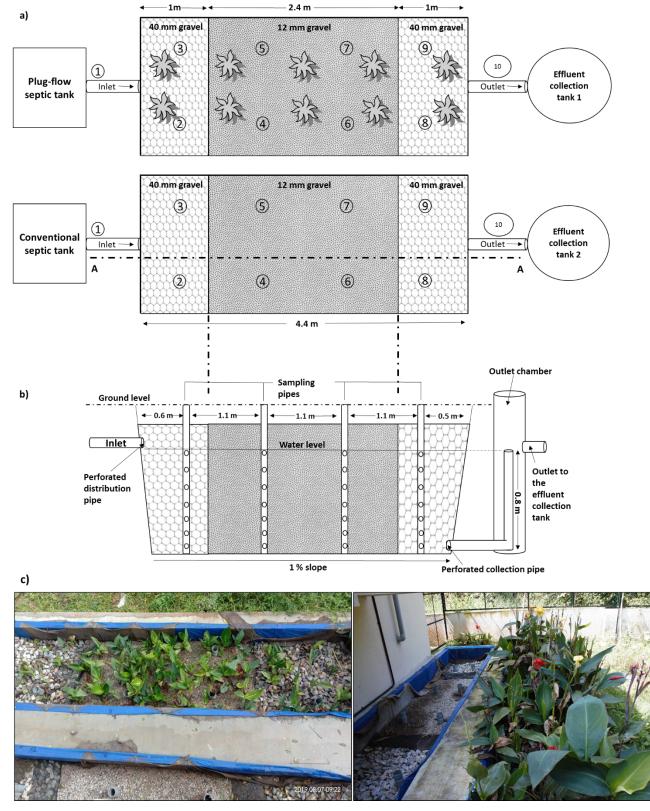


Fig 1: a) Flow diagram of the plan view, b) elevation view (section 'AA') and c) field image of the
 HSSF-CW systems.

134 2.2 Flow measurements and operating conditions

- 135 The volumetric flow rate to each of the HSSF-CWs was estimated using data collected on water 136 consumption in the toilets. The water consumption was monitored via a float gauge connected
- 137 to overhead tanks supplying water to the toilets. Samples for water quality analysis and water
- 138 consumption data was collected simultaneously during the working hours. The average water
- 139 consumption in the toilets was 740 L day⁻¹ and the average outflow from each HSSF-CW was
- 140 350 L day⁻¹. The calculated hydraulic loading rate (HLR) and the hydraulic retention time (HRT)
- 141 were 84 mm day⁻¹ and 3.7 days respectively. The porosity of both HSSF-CW systems were
- 142 calculated using equation 1 and was approximately 32 % (Conley et al., 1991).

143
$$Porosity(\%) = \frac{V_V}{V_T} \times 100$$

144 Where, V_V is the void volume determined at the beginning of the study by pouring a known 145 amount of water into the wetland bed filled with aggregate material and V_T is the total volume 146 of the bed.

(1)

- 147 Both systems were operational from January 2019 and the water quality monitoring was
- carried out fortnightly from January to December 2019. The systems were operating without
- plants until July 2019. To meet the objectives of the study, in July 2019 young Canna indica
- 150 plants (with similar shoot length and dry biomass around 17.7 g/plant) propagated from
- 151 rhizome cuttings were transplanted into one of the HSSF-CW systems at a planting density of 20
- 152 plants/m². The plants were allowed to acclimatize for one month and samples were collected
- 153 for water quality analysis and efficiency evaluation/comparison of two systems(Mantovi et al.,154 2003).

155 2.3 Water Sampling and analytical methods

156 Water samples were collected from ten locations (perforated pipes) provided across each 157 system (Fig 1, sample points 1 to 10). The inflow (outflow from the septic tanks) samples were 158 collected from the sampling ports provided during the time of construction. Water samples 159 were collected in sterile borosilicate glass bottles (100 ml) and plastic bottles (500 ml) (LDPE, 160 medical-grade USP Class VI autoclavable) and were tested for physical, chemical and biological 161 parameters. Samples were preserved at a pH < 2 for some parameters (*) using sulfuric acid to 162 minimize precipitation and microbial degradation. Samples were stored at temperature < 4° C 163 during transportation to the Water and Soil Laboratory at the Ashoka Trust for Research in 164 Ecology and Environment (ATREE). All water quality parameters were tested according to 165 methods described in APHA Standard Methods for the Examination of Water and Wastewater 166 (Federation and Association, 2005). Temperature, pH, electrical conductivity (EC) and dissolved 167 oxygen (DO) were measured onsite using a YSI Pro 1030 and YSI Pro ODO sensors (YSI, Yellow 168 springs, USA), and nitrate-N was measured onsite using a Hach Nitrate Pocket Colorimeter (Hach Company, Loveland, USA). Ammonium-N*, total nitrogen (TN)*, orthophosphate-P 169 170 (ortho-P) and total phosphorus (TP)* were analyzed photometrically (Merck KGaA, Darmstadt, 171 Germany). Biochemical oxygen demand (BOD₅) and chemical oxygen demand (COD)* were

- analyzed by methods described in APHA standard methods, 2005. Faecal coliforms (FC) were
- 173 detected and enumerated using Colilert 18 (IDEXX Laboratories, Westbrook, USA).

174 2.4 Data analysis

- 175 Statistical analysis of data was conducted using IBM SPSS Statistics 23.0 (Armonk, NY: IBM
- 176 Corp.). Statistical difference between the influent and effluent quality of two systems were
- 177 determined using an independent sample t-test. Linear regression graphs were plotted using
- 178 Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA; USA).

179 2.5 Calculations

- **180** *Contribution of plants to contaminant removal*
- 181 The contribution of plants to contaminant removal was estimated as the difference
- 182 (percentage) in load reduction between two systems. Mass loading of contaminants at the inlet
- and outlet of each were estimated using equation 2. The percentage reduction in the
- 184 contaminant loads were estimated using equation 3.

185 Contaminant load (L)
$$(mg. day^{-1}) = C \times Q \times 1000$$
 (2)

- 186 Where C is the contaminant concentration (mg/L) and Q is the flow rate (L/day)
- 187 The contribution of plants to contaminant removal was estimated using equation 2

188 Contaminant load reduction (%) =
$$\frac{(L_i - L_o)}{L_i} X100$$
 (3)

- 189 Where,
- 190 L_i is the contaminant load at the inlet and L_o is the contaminant load at the outlet.

191 *Contaminant removal kinetics*

- 192 The first-order plug-flow model was used to describe the removal of TN, TP, BOD₅ and COD. The
- 193 removal rate constant K was calculated by linear regression using the integrated first-order
- 194 plug-flow equation (4) (Sooknah and Wilkie, 2004):

195
$$\ln\left(\frac{C_i}{C_o}\right) = K_T t$$
 (4)

- 196 Where C_i is the influent contaminant concentration in mg/L and C_o is the effluent contaminant 197 concentration.
- K_T is the temperature-dependent first-order volumetric rate constant (day⁻¹), and t is the
 Hydraulic Retention Time (day).
- 200 3. Results and discussion
- 201 3.1 Performance of planted vs. unplanted constructed wetlands
- 202 The effluent quality of both systems met the discharge standards set by CPCB (MOEF & CC,
- 203 2017) with BOD₅ and COD less than 30 mg/L and 250 mg/L respectively (Table 2). Whilst the

- water quality characteristics of influent and effluent of each of the HSSF-CWs was observed to
 be variable (Table 2), this is common of systems deployed in the field under real-world
- 206 operating conditions.

207 The DO levels at the inlet of both HSSF-CW systems were very low since the systems receive 208 primary treated effluent from anaerobic systems. Despite this, a significant reduction (p < 0.05) 209 in the effluent BOD₅ and COD was observed in each system and the planted HSSF-CW offered 210 slightly higher removal of both BOD₅ and COD. This can be attributed to release of oxygen 211 through the roots of *Canna indica* which facilitates the decomposition of organic matter by 212 aerobic microbes (Gaballah et al., 2020). In addition, the exchange of oxygen through 213 perforated stand pipes provided for sample collection might have contributed to the 214 degradation of organic matter in both systems. An average of 67 %, 61 % reduction in BOD₅ and 215 53 %, 38 % reduction in COD was observed in planted and unplanted HSSF-CW respectively 216 (Table 2). Removal efficiency here is lower than that reported by Haritash et al., 2015 who 217 observed 70-96 % and 64-99 % reduction in BOD₃ (BOD measured after 3 days of incubation at 218 27 °C) and COD respectively in HSSF-CW planted with Canna lily (HRT ~ 24 hours). The 219 contaminant removal efficiency depends on composition of contaminants/constituents present 220 in the inflows to the constructed wetland system (D. Wang et al., 2014). The system reported in 221 the study cited by Harish et al. 2015 was fed with synthetic wastewater containing readily 222 degradable constituents such as glucose, potassium dihydrogen phosphate (KH_2PO_4), 223 ammonium sulphate [$(NH_4)_2SO_4$], and potassium nitrate (KNO_3), whilst the systems reported 224 here were fed with the effluent from septic tanks. Thus, whilst removal efficiencies are lower, 225 both systems reported here may be qualitatively described as well functioning with respect

226 BOD₅ and COD removal.

227 No significant difference (p>0.05) was observed in ammonium-N removal efficiencies between 228 the two systems. Nitrate-N concentration was observed to increase significantly in the effluent 229 of both the planted and unplanted HSSF-CW systems. This can be accounted to the low C/N 230 ratio of the influent (2.0) which facilitates the colonization of nitrifying community and 231 nitrification (Hu et al., 2009; Zhu et al., 2014). The net increase in nitrate-N concentration 232 observed in the planted HSSF-CW was significantly lower indicating uptake/assimilation of 233 nitrate-N by plants (Table 1). Some studies have reported 280 -500 % increase in nitrate-N 234 concentration in both planted and unplanted constructed wetland, suggesting conversion of 235 ammonium-N to nitrate through the nitrification process (Dornelas et al., 2009; lasur-Kruh et 236 al., 2010). Aeration through perforated pipes present in the systems of this study might have 237 further facilitated the metabolism of nitrifying microbial community. No significant difference 238 was observed in the average TN concentrations suggesting the major role of biofilms in 239 nitrogen assimilation in both systems. Relative to the unplanted system, an increase of 5 % in 240 ortho-P and 7 % in TP concentration were observed at the outlet of planted HSSF-CW. Many 241 studies have reported the presence of more efficient and high concentration of inorganic P-242 solubilizing bacteria and organic P mineralizing bacteria in the rhizosphere soil than in the non-243 rhizosphere soil (Cao et al., 2018; Qian et al., 2010; Rodríguez and Fraga, 1999; Teng et al., 244 2019). Hence the increase in ortho-P concentration at the outlet of planted HSSF-CW could be

- accounted to increased solubilization of insoluble P in the system and the incompetency of
- 246 Canna plants to assimilate ortho-P due to rust infection. Again, the relative increase of TP in the
- 247 planted HSSF-CW could have arisen due to rust infections observed in *Canna indica* and
- subsequent release of phosphorus assimilated into the plant biomass back to the effluent due
- to necrosis (lasur-Kruh et al., 2010; Garcia et al., 2010; Dotro et al., 2017; Wallace, 2015).

Lower TSS removal was observed in planted HSSF-CW as compared to the unplanted HSSF-CW. This

- 251 was unexpected as vegetation is thought to promote TSS removal via settling, interception and
- filtration by vegetation. Again, this may have been influenced by the onset of rust infections in the
- 253 Canna plants. The appearance of yellow pustules in the foliage of Canna plants were observed after a
- few days of planting and resultant immature leaf drying and dropping as a result of the infections in *Canna indica* can contribute to lower TSS removal in planted HSSF-CWs (Kadlec and Wallace, 2008).
- *Canna indica* can contribute to lower TSS removal in planted HSSF-CWs (Kadlec and Wallace, 2008).
 One log order reduction in the faecal coliform (FC) levels was observed across each of the two systems.
- 257 Similar FC removal rate were observed for CW operated for 8 years in Rabat (Morocco) (Boano et al.,
- 257 Similar PC removal rate were observed for CW operated for 8 years in Rabat (Morocco) (Board et al., 258 2020). Various studies reported the persistence of FC in organic sediments and low die-off rates in
- wastewater (Anderson et al., 2005). The data suggests that accumulation of suspended solids within
- the filtration media have contributed to low die-off of FC (Reed, 1993). Poor TP and TSS removal in the
- 261 planted HSSF-CW as compared to unplanted HSSF-CW could be attributed to the development of
- 262 infection in *Canna indica*.

Parameters	Planted HSSF-CW			Unplanted HSSF-CW		
(n = 5)	Inlet	Outlet	Percent	Inlet	Outlet	Percent
	(Mean± SD)	(Mean± SD)	change	(Mean± SD)	(Mean± SD)	change
Temperature (°C)	24.4 ± 1.7	23.2 ± 1.0	-	25.0 ± 1.8	23.3 ± 1.1	-
рН	8.0 ± 0.2	7.7 ± 0.1	-	8.3 ± 0.0	7.9 ± 0.1	-
EC (µS/cm)	1842 ± 301	1812 ± 269	-	2425 ± 312	2264 ± 281	-
TSS (mg/L)	125.8 ± 67.5	87.1 ± 69.4	-5 %	203.0 ± 103.7	69.5 ± 37.8	61 %
Nitrate-N (mg/L)	1.9 ± 2.0	4.0 ± 4.0	-297 %	2.3 ± 0.8	7.5 ± 5.8	-387 %
Ammonium-N	87.1 ± 28.2	76.1 ± 24.0	7 %	114.3 ± 28.9	98.4 ± 25.7	11 %
(mg/L)						
TN (mg/L)	114.0 ± 31.5	95.9 ± 18.6	12 %	153.5 ± 16.7	140.8 ± 8.7	8 %
Ortho-P (mg/L)	10.1 ± 0.7	10.5 ± 1.4	-5 %**	17.0 ± 2.8	15.1 ± 1.8	10 %**
TP (mg/L)	10.7 ± 0.5	11.5 ± 1.5	-7 %**	18.2 ± 3.7	16.0 ± 2.8	11 %**
DO (mg/L)	0.43 ± 0.16	0.29 ± 0.02	26 %	0.40 ± 0.04	0.28 ± 0.03	29 %
BOD₅ (mg/L)	76.1 ± 36.8	24.1 ± 13.2	67 %	88.1 ± 52.5	30.6 ± 9.0	61 %
COD (mg/L)	233.8 ± 116.7	108.5 ± 52.0	53 %	321.3 ± 201.2	170.6 ± 63.8	38 %
FC (MPN/ 100 mL)	1.37×10 ⁵ ± 8.88×10 ⁴	1.89×10 ⁴ ± 1.67×10 ⁴	1 log	2.73×10 ⁵ ± 2.91×10 ⁵	1.27×10 ⁴ ± 1.10×10 ⁴	1 log

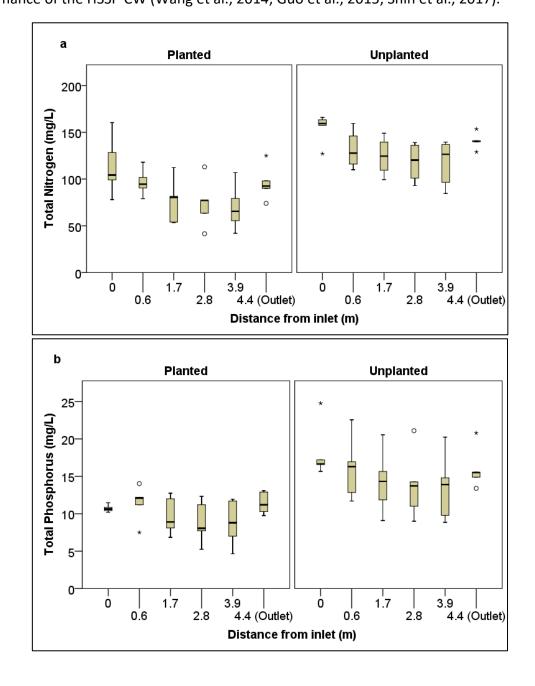
263 **Table 1:** Influent and effluent contaminant levels in planted and unplanted HSSF-CW

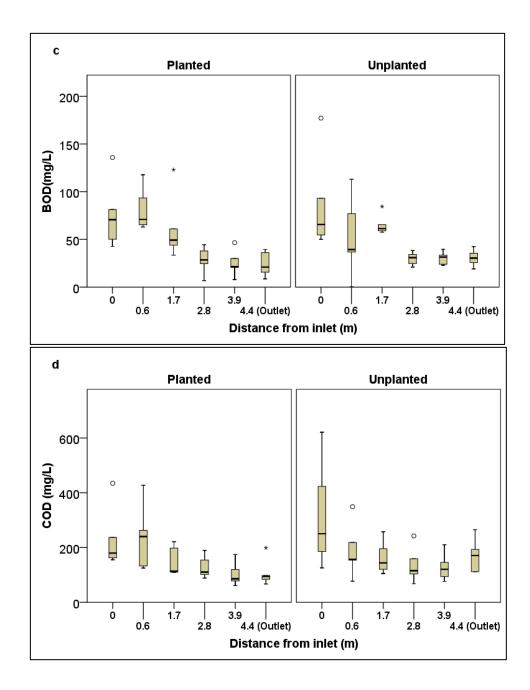
264 ** Significant difference (p < 0.05) in % reduction.

265 The mean TN, TP, BOD₅, COD and DO concentration measured for all time points in samples

taken along the length of the HSSF-CWs were plotted (Fig 2). Linear reduction in contaminant

- 267 concentrations were observed in both systems for each parameter except for TN and TP. A
- reduction of 39 % and 18 % in TN and 24 % and 26 % in TP concentrations at the sample point
- 269 3.9 m along the HSSF-CWs (sample points 8 & 9, Fig 1) were observed in the planted and
- 270 unplanted HSSF-CW systems respectively (Fig 2). However, both parameter concentrations
- were observed to increase at the outlet (sample point 10, Fig 1) relative to the concentration at
- sample points 8 and 9. This could be attributed to either re-suspension and dislodging of
- 273 suspended particles and biofilms or short-circuiting of inflows in the constructed wetlands (Fu
- et al., 2018; Geranmayeh et al., 2018). Short-circuiting leads to increased hydraulic conductivity
- in the diagonal flow path which in turn reduces the HRT thereby affecting the treatment performance of the HSSF-CW (Wang et al., 2014; Guo et al., 2015; Shih et al., 2017).





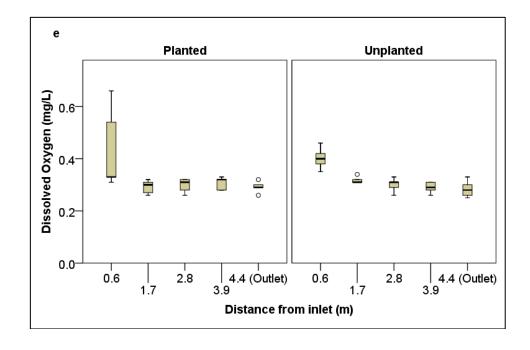


Fig 2: Variation in mean concentrations of a) TN b) TP c) BOD₅ d) COD and e) DO with distance
from the inlet. The bands show the median value for each group; bottom and top of boxes
show the first and third quartiles; and whiskers show maximum and minimum values with 1.5
of IQR of upper and lower quartiles.

287 Despite a decreasing trend across the length, an increase in TP concentration was observed at

the outlet of both systems. This could be attributed to the reappearance of phosphorus that

289 was temporarily removed and stored in the sediments via precipitation (see Fig 2). Anoxic

290 conditions along the length of CW leads to redox-sensitive mobilization and release of

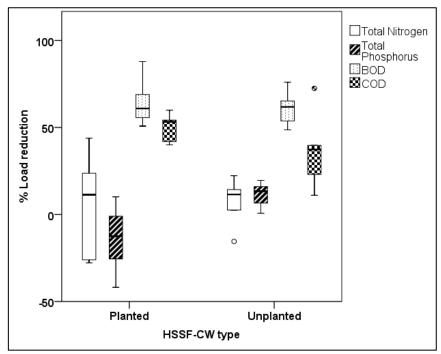
291 phosphorus thereby contributing to poor TP removal efficiencies (Fig 2 e) (Søndergaard et al.,

292 2003).

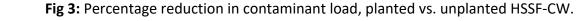
293 3.2 Impact of vegetation on contaminant load reduction

294 A mass balance approach was applied to estimate the contribution of plants to nutrient and 295 organic matter removal. During the study period, both systems were operated at similar HLR 296 (84 mm/day). The BOD₅ and COD mass loading rates were similar for each system however the 297 TN and TP mass loading rates were dissimilar (Table 2). This could be attributed to the 298 performance of the conventional septic tank and the plug flow septic tanks connected 299 upstream of the unplanted and planted systems respectively. Given the difference in mass 300 loading between the systems with respect nutrients, the pollutant removal data is normalized 301 by estimating the percentage of contaminant load removal to enable comparison between the

302 systems (Fig 3).







307 Table 2: Contaminant loading and removal rates, percentage load reduction by planted and308 unplanted HSSF-CWs.

Parameter	Planted HSSF-CW (Mean ± SD)			Unplanted HSSF-CW (Mean ± SD)		
(n = 5)	Loading rate	Removal rate	% Load	Loading rate	Removal rate	% Load
	(gm ⁻² day ⁻¹)	(gm ⁻² day ⁻¹)	removal	(gm ⁻² day ⁻¹)	(gm ⁻² day ⁻¹)	removal
TN	9.6 ± 2.9	1.7 ± 2.7	13 ± 24	12.8 ± 2.7	1.2 ± 1.6	8 ± 13
ТР	0.9 ± 0.1	-	-	1.5 ± 0.4	0.2 ± 0.1	12 ± 7
BOD₅	6.6 ± 3.3	4.6 ± 2.7	68 ± 14	7.5 ± 4.6	4.9 ± 3.9	61 ± 10
COD	19.3 ± 8.6	10.7 ± 5.5	54 ± 7	25.1 ± 14.6	11.2 ± 13.5	36 ± 22

An average of 13 % and 8 % TN load and - 6 % and 12 % TP load reduction was observed in

311 planted and unplanted systems respectively. Time series plots of the TN and TP load reduction

indicates increased nutrient load removal in the planted HSSF-CW up to 90 days from planting

the Canna lilies (Fig 4). This could be attributed to nutrient uptake by plants in the form of

ammonium, amino acids and nitrates during the growing phase (da Costa et al., 2013; Vymazal,

2007). In October 2019 i.e. after 102 days of planting *Canna indica,* yellow pustules were

observed in the leaves of the plants accompanied by premature drying and falling of the leaves.

317 The infection could have inhibited growth and development of the Canna lilies resulting in

reduced nutrient uptake and subsequent poor nutrient load reduction (Abrego et al., 1957;

319 Marycruz et al., 2018). This appears to be confirmed by reduced TP uptake and percentage load

320 reduction after 102 days in the planted system (Fig 5).

- 321 The relative contribution of plants in the removal of nutrients was estimated to be less than 5%
- 322 (Table 2). Contribution of plants to the COD and BOD₅ load removal were estimated at 18 %,
- 323 and 7% respectively suggesting that biofilms play an important role in contaminant removal
- 324 (lasur-Kruh et al., 2010; Wu et al., 2019). In terms of loading rate, 0.09 gm/m²/day of TN, 0.3
- gm/m²/day of BOD₅ and 1.9 gm/m²/day of COD was removed by plants. A similar study by da
- 326 Costa et al., 2013 reported 1.06 % TN load assimilation by the plant biomass. Proper
- 327 maintenance of wetland vegetation, which includes regular harvesting of plants, inspection for
- 328 pest infestation, weeds and pathogen infections, is essential to enhance performance of plants
- in contaminant removal.

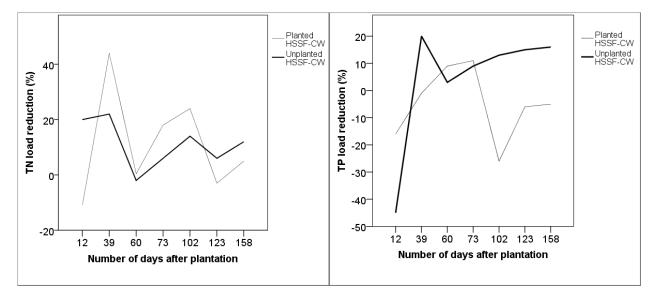




Fig 4: Percentage load reduction in nutrients with plant growth, planted vs. unplanted HSSF-CW

332 3.3 Pollutant removal kinetics

- 333 Kinetics of a wetland is closely related to the system design, and modelling helps in
- understanding the contaminant removal rate and scaling up of constructed wetland systems.
- 335 Design parameters, environmental conditions and operating conditions directly affect the
- biochemical pathways within a wetland. Here, the first-order plug-flow model (equation 4)
- described by Sooknah & Wilkie, 2004 was used to understand and determine first order
- 338 contaminants decay rates in both systems. Linear regression was used to estimate the kinetic
- 339 volumetric base removal coefficient K at 25^oC for different contaminants and the
- 340 corresponding correlation co-efficient R² (Table 3) (Fig 5).

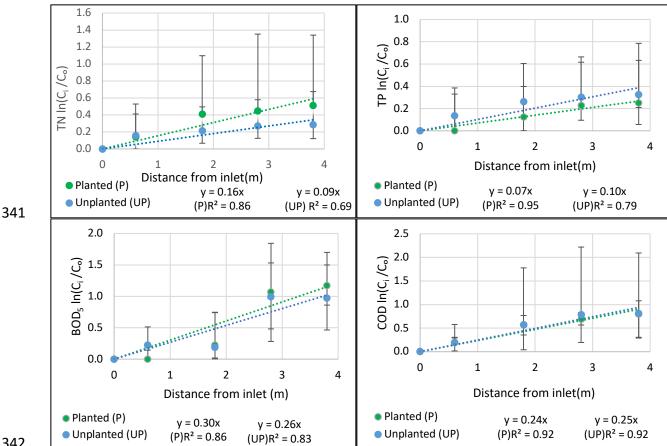


Fig 5: Linear regression of $\ln \frac{C_{inlet}}{C_{outlet}}$ of TN, TP, BOD₅, COD along planted and unplanted HSSF-343 CWs. The horizontal axis presents distance along the HSSF-CWs and vertical axis presents the 344 345 natural logarithm of the ratio of the inlet to outlet contaminant levels.

Table 3: First-order removal rate constant K (day⁻¹) obtained from linear regression at 25 °C, 346

347 correlation coefficient (R²)

Parameters	Planted I	HSSF-CW	Unplanted	Unplanted HSSF-CW		
	К	R ²	К	R ²		
TN	0.16 (K _{TN})	0.86	0.09 (K _{TN})	0.69		
ТР	0.07 (K _{TP})	0.95	0.10 (K _{TP})	0.79		
BOD ₅	0.30 (K _{BOD5})	0.86	0.27 (K _{BOD5})	0.83		
COD	0.24 (K _{COD})	0.92	0.25 (K _{COD})	0.92		

348

The TN, TP, BOD₅, and COD removal rate constants for the planted HSSF-CW were 0.16 day⁻¹, 349

0.07 day⁻¹, 0.30 day⁻¹ and 0.24 day⁻¹ respectively. Higher R² (~0.90) values were observed in the 350

data describing performance of the planted HSSF-CW suggesting greater confidence in the 351

352 application of first-order models to explain contaminant decay in the planted system. By

353 contrast, in the case of unplanted system, variable factors such uptake/release of contaminants

- by biofilm microflora, biofilm diversity/density might have affected the contaminant decay rate in the system (Jegatheesan et al., 2003; Rossman, 1999).
- 356 The contaminant decay rate depends on design parameters (HRT, HLR), inflow characteristics,
- 357 physical chemical and biological processes, and environmental conditions (temperature,
- rainfall) (Dornelas et al., 2009). Úsuga et al., 2017 reported contaminant decay rates of 0.22
- day⁻¹ for COD and 0.46 day⁻¹ for BOD₅ at 24 $^{\circ}$ C at a HLR of 91 mm day⁻¹. Table 4 presents the
- 360 volumetric rate constants reported in studies with HSSF-CW deployed in tropical regions. The
- 361 decay rates reported in the literature cited are much higher than observed in the systems
- deployed at Berambadi. This might be due to the type of wastewater being treated and higher
- 363 HLRs used in these studies. Garcia et al., 2010 reported improved organic pollutant removal
- 364 efficiencies at high HLRs in HSSF-CW. Systems deployed at Berambadi received inflows with low
- 365 contaminant loads and complex organic matter (biodegradable and non-biodegradable) from
- 366 septic tank thereby contributing to lower contaminant decay rates.
- 367 Table 4: Volumetric rate constants (K) obtained from studies on HSSF-CWs in tropical region368 (24-27 °C).

Wastewater type & location	Pre- treatment	Plant species	HLR (mm day ⁻¹) & HRT (days)	K (day⁻¹)	Reference
Blackwater, India	Septic tanks	Canna indica	84 3.7	K _{TN} : 0.16 K _{TP} : 0.07 K _{BOD} : 0.30 K _{COD} : 0.24	This study (Planted system)
Domestic sewage, India	Settling tank	Canna indica	386 1.0	K_{TP} : 0.66 K_{BOD} : 1.92 K_{COD} : 1.33	(Lekshmi et al., 2020)
Greywater, Sri Lanka	Settling tanks with grease trap	Typha latifolia	165 2.0	K_{TP} : 0.40 K_{BOD} : 0.76 K_{COD} : 0.56	(Karunaratn et al., 2011
Domestic sewage, Thailand	Settling tank	<i>Canna</i> spp.	55 - 440 0.5 - 4.0	K _{TN} : 0.07 K _{TP} : 0.06 K _{COD} : 1.18	(Konnerup e al., 2009)
Landfill leachate, Colombia	None	Phragmites australis	91 2.2	K _{BOD} : 0.46 K _{COD} : 0.22	(Úsuga et al 2017)

370 4. Conclusion

371 Constructed wetlands serve as a good choice for secondary treatment of wastewater. However,

the design parameters, vegetation and environmental conditions influence the performance of

the constructed wetlands. We observed a significant reduction in organic matter levels and the

effluents from both systems met the discharge standards for TSS, BOD₅ COD and nitrate -N set

- by regulatory agencies in India. Similar BOD₅ and TN removal efficiency were observed in both
- 376 planted and unplanted HSSF-CWs. Unplanted HSSF-CW provided higher TP removal efficiency
- 377 (11%) as compared to planted HSSF-CW (-7%). The first-order model confidently explains the
- 378 organic matter and nutrient degradation in the planted HSSF-CW. Low R² values observed for
- 379 unplanted system could be due to the influence of other factors such as initial contaminant
- 380 levels, biofilm thickness, microflora diversity etc. on contaminant degradation. Maximum
- 381 nutrient uptake/removal is observed during the growth phase of plants in CW. Canna rust
- infection appeared to affect the contaminant removal especially the TP and TSS removal
- 383 efficiency of planted HSSF-CW.
- 384 Our data demonstrates that planting is effective in improving treatment efficiency of
- 385 constructed wetlands with respect TN and organics removal. In this case the effectiveness of
- planted system over unplanted system was limited by the development of rust infections in
- 387 Canna indica plants. Therefore, maintenance of healthy vegetation is critical to achieve and
- 388 sustain improved/longterm performance of constructed wetlands. The data suggests that
- 389 adaptability to the local climatic conditions and susceptibility towards infections should be
- 390 considered while selecting plant species for phytoremediation.
- 391 We can conclude that the planted HSSF-CW (*when maintained properly with plants being
- 392 harvested at regular intervals) can be effectively deployed to treat inflows
- 393 from anaerobic wastewater treatment systems. Importantly however the data from this study
- demonstrates that an unplanted system, which requires less maintenance than the planted
- 395 system (*Canna indica*), can also improve septic tank effluents to achieve discharge consent. This
- is an important finding with respect to the scaling up of HSSF-CW deployment in southern India,
- 397 as mitigating the requirement for planting and plant maintenance reduces system cost and ease
- 398 of use for the community/householders.

399 CRediT authorship contribution statement

- 400 Priyanka Jamwal: conceptualization, data curation and analysis, review and editing, Funding
- 401 acquisition, supervision formal analysis. Anjali V Raj: Sample analysis, data curation and
- 402 analysis, Writing review & editing. Lakshmi Raveendran: Fieldwork and sample collection and
- 403 analysis. Shahana Shirin: Fieldwork and sample collection and analysis. Stephanie Connelly:
- 404 Writing review & editing, Funding acquisition. Jagadeesh Yeluripati: Writing review & editing,
- 405 Funding acquisition. Samia Richards: Writing review & editing, Funding acquisition.
- 406 Lakshminarayana Rao: Writing review & editing, Funding acquisition. Rachel Helliwell: review
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