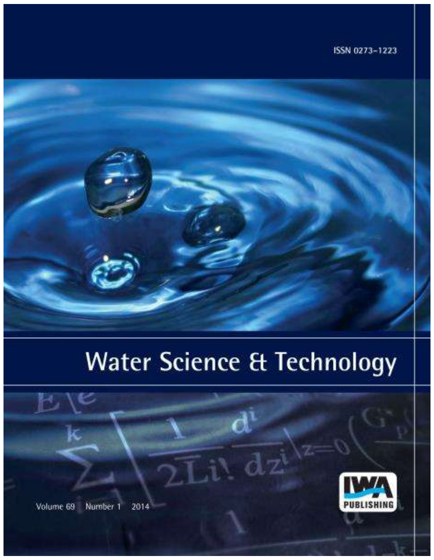
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Evaluation of *Ageratum conyzoides* in field scale constructed wetlands (CWs) for domestic wastewater treatment

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ABSTRACT

Ageratum conyzoides were evaluated in field scale subsurface flow constructed wetlands (CWs) to quantify its nitrogen (N) and phosphorus (P) uptake and compare with wetland plants (*Pistia stratiotes, Typha latifolia and Canna indica*). The two-field scale subsurface flow CWs, located in the International Crops Research Institute for Semi-Arid Tropics, received wastewater from an urban colony. The CW1 and CW2 had the same dimensions (length:10 m, width:3 m, total depth:1.5 m and sand and gravel:1 m), similar flow rates (3 m^3/d), hydraulic loading rates (HLRs-10 cm/d) and hydraulic retention time (HRT-5 days) from July 2014–August 2015. The vegetation in both CWs consisted of *Pistia stratiotes*, *Typha latifolia*, *Canna indica*, and *Ageratum conyzoides,* respectively. The CW1 (% reduction with respect to concentrations) reduced total suspended solids (TSS) (68%), NH_4-N (26%), NO_3-N (30%), soluble reactive P (SRP) (20%), chemical oxygen demand (COD) (45%) and fecal coliforms (71%), while the CW2 (%-reduction with respect to concentrations) reduced TSS (63%), NH_4-N (32%), NO_3-N (26%), SRP (35%), COD (39%) and fecal coliforms (70%). *Ageratum conyzoides* can be used in combination with *Pistia stratiotes, Typha latifolia* and *Canna indica* to enhance removal of excessive N, P and fecal coliforms from domestic wastewater.

Key words | domestic wastewater treatment, field scale constructed wetlands (CWs), *Pistia stratiotes*, *Typha latifolia*, *Canna indica*, *Ageratum conyzoides*

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INTRODUCTION

Ever increasing population growth rates, abrupt climate changes and inefficient management of water resources have led to water scarcity and a greater need for efficient management of available water resources by designing water harvesting structures, re-using wastewater, and utilizing solar energy for the operation of desalination plants, especially in developing countries (Asthana & Shukla 2014). The re-use of wastewater in agriculture is widely seen in peri-urban areas of developing countries due to close vicinity, excellent connectivity, available land for cultivation, a perennial source of nutrient-rich wastewater and urban markets for agricultural produce (Varkey *et al.* 2015). However, the peri-urban wastewater used in agriculture for irrigating crops contain harmful pathogens, bacteria, viruses, excessive N, P and heavy metals like Pb (Lead), Cd (Cadmium), Cr (Chromium) and Zn (Zinc), which are deleterious to human and animal life (Singh & Kumar 2006). Urban areas in developing countries like India are equipped with wastewater treatment plants (WTPs), but often yield partially treated wastewater (Kaur *et al.* 2012). However, the lack of WTPs in rural areas of India offers the opportunity for a decentralized wastewater treatment system (DWT) that is low cost, uses less energy and is less labour intensive. Engineered constructed wetlands (CWs) mimicking the functions of natural wetlands to treat wastewater through natural processes involving wetland vegetation, coarse sand and associated microbial populations can be designed to treat domestic wastewater (Kadlec 2009). The plants in the CWs uptake excessive N, P and heavy metals, and act as drivers for growth of the microbial populations (Kadlec 2009).

There are number of studies on CWs that quantify the N and P removal capacities of commonly used wetland plants such as *Typha latifolia*, *Schoenoplectus validus*,

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Phragmites australis, *Juncus effuses*, *Typha angustifolia* L, *Canna indica*, *Pistia stratiotes*, *Eichhornia crassipes* and duckweed (Reddy & De Busk 1985; Tanner 1996). However, none of the CWs studies (laboratory or field scale) have reported the use of *Ageratum conyzoides* as wetland vegetation for wastewater treatment. *Ageratum conyzoides* is an annual herb found in tropical and sub-tropical regions of the world, and grows in wastelands, grasslands, and corners of agricultural land, having minimal disturbance, and spreads through its stolons. The optimum plant height depending upon environmental conditions varies from 1–1.5 m, leaves and stems have white hairs and leaves are ovate shaped (Okunade 2002). The germination of *Ageratum conyzoides* seeds is influenced by light and soil temperature (20–25 °C) (Okunade 2002). *Ageratum conyzoides* has a great potential for use in CWs, due to its high growth rates, shorter life cycle and higher reproductive potential. The roots of this plant release a chemical known as an 'allelopath', which adversely affects the growth of other plants and can be potentially utilized for maintaining a monoculture plant regime in CWs. The leaf and root extracts of *Ageratum conyzoides* have medicinal properties (insecticidal and pharmacological), a potential use of harvested biomass in CWs (Okunade 2002). However, in spite of the higher growth rate, reproductive potential and medicinal properties, this plant has never been field tested in a CW for removal of excessive N, P and fecal coliforms from domestic wastewater.

The primary objective of this 14-month study (July 2014–August 2015) was to assess the wastewater treatment efficiencies of two field scale subsurface flow CWs vegetated with four types of plants including *Ageratum conyzoides*. The specific objectives of this study were: (1) Quantify the wastewater treatment efficiencies of subsurface flow CW1 and CW2 having vegetation species (*Typha latifolia, Ageratum conyzoides, Canna indica* and *Pistia stratiotes*); (2) Quantify N and P uptake of *Ageratum conyzoides*, *Canna indica, Typha latifolia* and *Pistia stratiotes*; (3) Quantify the total N, P and organic carbon (C) accumulation in coarse sand media; and (4) quantify fecal coliform removals (%) from both CWs.

MATERIALS AND METHODS

Site description

The two field-scale subsurface flows CW1 and CW2 are located at International Crops Research Institute for Semi-Arid Tropics (ICRISAT) campus in Telangana, India (17° 29′ 22.76″ N, 78° 16′ 47.38″ E). The wastewater source for these CWs is an urban residential colony. The climate at the site is semi-arid with rainfall generally occurring from June–October, winter from November–February and summer beginning from March and ending in May.

Design of CWs

Various aspects of wastewater treatment process were investigated in two field scale subsurface flow CWs established at ICRISAT. At both the CWs, the targeted influent flow rate was 3 m^3/d to achieve a hydraulic retention time (HRT) and hydraulic loading rate (HLR) of 5 days and 10 cm/d respectively. An additional overhead tank (1.5 m above ground level) of capacity 70 m^3 maintained the continuous influent flow into both the CWs. Overhead tank is connected to influent points of each CW by PVC pipes (10 cm diameter). Each CW is divided into four sections (A, B, C and D) (Figures 1 and 2(a) and 2(b)). Sections A and D do not have any substrate media and serve as holding tank for untreated and treated wastewater respectively. Section A provides additional pre-treatment in form of sedimentation process. Section B and C contain 1 m thick filter bed consisting of three layers of gravel and sand media (each layer-0.25 m). Larger sized gravel (40 mm) was used as bottom layer, 20 mm sized gravel layer was placed above bottom layer and 10 mm sized gravel layer was placed below sand layer.

Plant species such as *Pistia stratiotes*, *Typha latifolia*, *Ageratum conyzoides*, and *Canna indica* were collected from the nearby wetland sites and planted the same day in the wetland bed. The *Pistia stratiotes* were introduced in sections A and D of both CWs from November 2014–May 2015. In CW1, the wetland vegetation in sections A, B, C and D consisted of *Pistia stratiotes*, *Typha latifolia*, *Ageratum conyzoides*, and *Pistia stratiotes* respectively. In CW2, the wetland vegetation in sections A, B, C and D consisted of *Pistia stratiotes*, *Canna indica*, *Ageratum conyzoides* and *Pistia stratiotes* respectively (Figure 2(a) and 2(b)). Influent flow rate into section A was regulated by manual valves (2.54 cm diameter) and measured by a mechanical Itron water flow meter. Hydraulic gradient in the CWs was maintained by providing an elevation difference between the water level in sections A and D. The connecting points between sections were designed to provide an inversion of the water flow path that increased the travel time and provided more opportunity for the biogeochemical treatment process. Thus, the flow directions in sections A, B, C and D are top to bottom, bottom to top, top to bottom and bottom to top respectively (Figure 1).

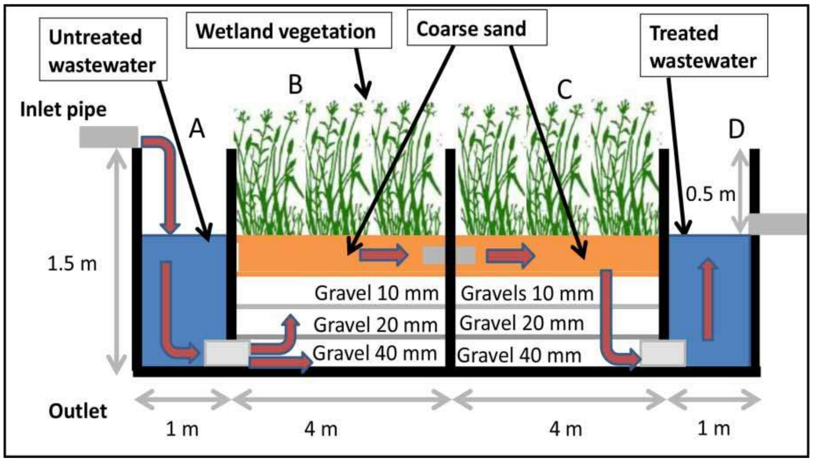

Figure 1 | Cross-section of the two subsurface flow CWs (Not to scale).

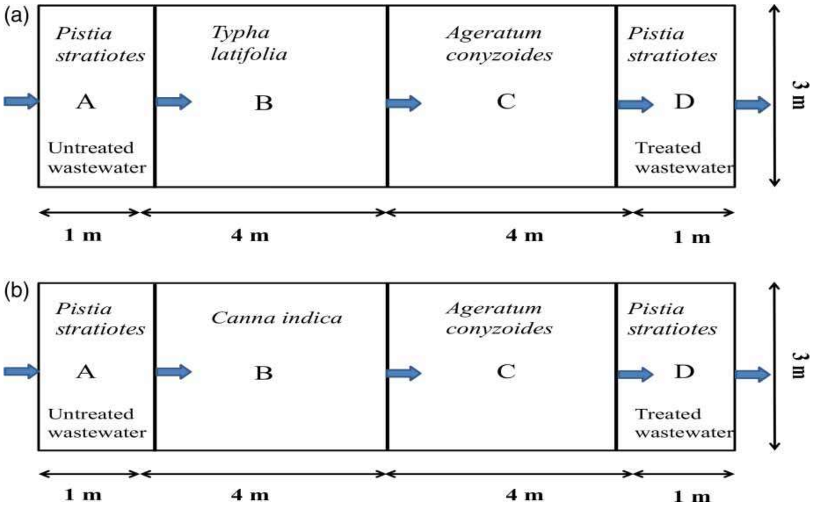

Figure 2 | Top-view of (a) CW1 and (b) CW2. *Note:* the blue arrow indicates the wastewater flow directions. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2017.119.

Water sampling and analytical methods

Influent and effluent water samples were collected weekly from both CWs. The samples were analysed for NH_4-N, NO_3-N, soluble reactive phosphorus (SRP), chemical oxygen demand (COD) and total suspended solids (TSS) using APHA (2005) standard methods 4500-NH3 F, 4500-NO3, 4500-P D, 2540-D and 5220-C; respectively.

Coarse sand sampling and analytical analysis methods

In the case of filter media, top 5 cm samples were collected from sections B and C using a T shaped auger (AIC Agro Instruments (P) Ltd, Kolkata, India). Collected samples were air dried for 2 days, and 2 mm sieved samples were analysed for total N, total P, available SRP, organic C and exchangeable calcium. The thiosulphate modification of the Kjeldahl method was used to analyse total N (Dalal *et al.* 1984). The total P and SRP were analysed using procedures given in Tandon *et al.* (1962) and Olsen & Sommers (1982) respectively. The organic C and exchangeable Ca were analysed using methods given in Nelson & Sommers (1982) and Thomas (1982) respectively.

Wetland plant sampling and analytical methods

Above ground biomass (stems, branches and leaves) of the plants was harvested from each CW at maturity stage. The above-ground biomass production was monitored for 14 months (July 2014–June 2015). From June 2015, below ground root biomass was collected for analysis. Plant samples were analysed for total N and total P using the sulphuric acid-selenium digestion method (Sahrawat *et al.* 2002).

Microbiological analysis (fecal coliforms)

Influent and effluent samples collected each month (replicated twice) from July 2014 to August 2015 were used to determine fecal coliform concentrations. The quantitative estimation of fecal coliforms via the number of colonies (colony forming units per 100 ml) was determined. Multiple tube fermentation technique using most probable number (MPN) analysis was adopted for determining fecal coliforms concentrations (APHA 2005).

Maintenance activities for both CWs

The inlet tank (capacity-70 m^3) was cleaned every 3 months, while the inlet and the outlet pipes were manually cleaned each week. In both the CWs, monoculture plant regime was maintained and the invasive plants were removed each week. The pipes carrying wastewater from the inlet tank into both the CWs were subject to clogging. To attenuate this problem, 'U' shaped bends were installed to remove larger suspended particles by allowing them to settle and were removed manually.

RESULTS AND DISCUSSION

Wastewater flow in CW1 and CW2

In both CWs, the targeted influent flow was 3 m^3/d; however, there were deviations in the flows (daily and monthly) due to clogging of the influent pipes. The influent flow deviations were within ±20% for a 14-month period. The influent flow rate, HRT and HLR ranged from 2.7–3.3 m^3/d, 4.6–5.5 days and 9–11 cm/d respectively. The variations in HRT and HLR directly affected wastewater treatment efficiencies in terms of TSS, COD, NH_4-N, NO_3-N and SRP removals as presented in the later section. The 'U' shaped bends installed to reduce the pipe clogging reduced the TSS concentrations by 30% (average 14 months' value-difference between inlet tank wastewater TSS (average value-54.5 mg/L and wastewater TSS after passing through the U-shaped bend (average value-37.6 mg/L). The TSS value of 37.6 mg/L is the average influent value for both CWs.

Wastewater treatment efficiencies of CW1 and CW2

Removal efficiencies (with respect to concentrations) of TSS, COD and fecal coliforms

TSS in the influent samples for both CWs ranged from 24–54 mg/L (avg-37.6 mg/L), while the effluent TSS concentrations for CW1 and CW2 ranged from 4–30 mg/L (average-12 mg/L) and 4–22 mg/L (average-14 mg/L) respectively. Average TSS removal efficiency of CW1 and CW2 was 68% and 63%, respectively (Figure 3). In case of COD, influent concentrations in both CWs varied from 88–213 mg/L (average-136 mg/L) and effluent concentrations in CW1 and CW2 ranged from 40–148 mg/L (avg-75 mg/L) and 48–160 mg/L (average-84 mg/L) respectively for a 9-month period (Figure 4). The average COD removal efficiencies of CW1 and CW2 were 45% and 39% respectively over a 9-month period (December 2014–August 2015). The maximum fecal coliform concentration (MPN index of >1,600 cfu/100 ml) was recorded in the influent of both CWs each month. The treated wastewater from both the CWs consistently showed a reduction in fecal coliform concentrations each month for a 14-month period (Table 1). The coliform reduction in the CW1 ranged from 53–80% with an average reduction of 71%. In the CW2, coliform reductions ranged from 58–77% with an average reduction of 70%.

Removal efficiencies (with respect to concentrations) of NH_4-N, NO_3-N and SRP

Influent NH_4-N concentrations for both CWs ranged from 34–96 mg/L (avg-59 mg/L) with highest concentrations occurring in the winter months (November 2014–February

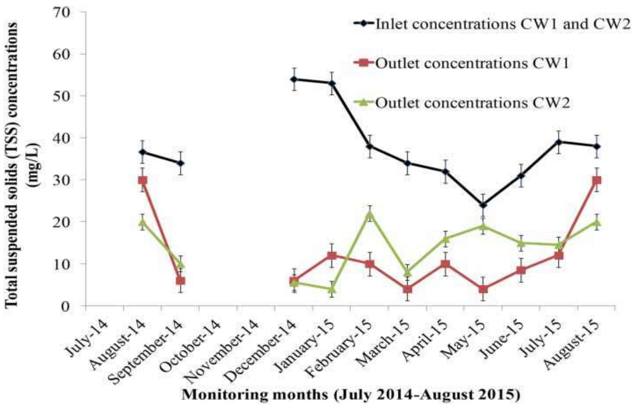

Figure 3 | Average influent and effluent TSS concentrations in CW1 and CW2 (July 2014–August 2015). No TSS data were available in July 2014 and October–November 2014.

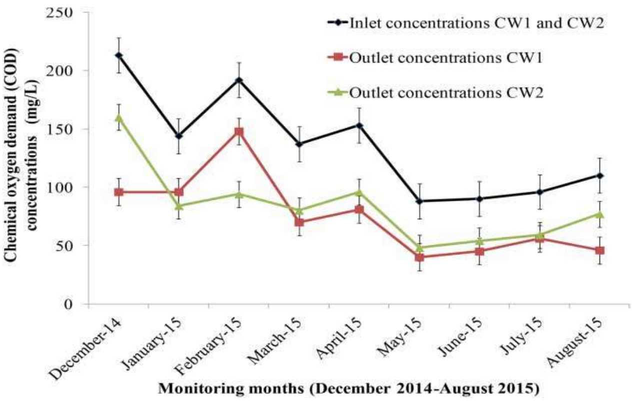

Figure 4 | Average influent and effluent COD concentrations in CW1 and CW2 (December 2014–August 2015). No COD data were available from July–November 2014.

Table 1 | Average fecal coliform removals (%) in CW1 and CW2

Year	Months	CW1	CW2
2014	July	73.62	70.54
	August	70.64	77.36
	September	80.31	72.64
	October	76.34	65.36
	November	75.63	70.42
	December	71.36	64.56
2015	January	68.32	69.38
	February	66.74	69.36
	March	78.36	74.56
	April	73.68	69.36
	May	72.85	74.87
	June	72.31	75.20
	July	60.96	68.32
	August	52.77	58.36
	Mean	71	70

2015) and March–May 2015 (Figure 5). Effluent NH_4-N concentrations from CW1 and CW2 ranged from 29–70 mg/L (average-44 mg/L) and 23–52 mg/L (average-40 mg/L) respectively. The NH_4-N treatment efficiency of CW1 was highest from June–August 2015, while the CW2 had highest NH_4-N removal efficiency from July–October 2014 and November 2014–February 2015. The average NH_4-N removal efficiencies of CW1 and CW2 for the 14-month monitoring period were 26% and 32% respectively. In case of NO_3-N, influent concentrations ranged from 2.60–3.83 mg/L (average 3.04 mg/L), while the effluent NO_3-N concentrations in CW1 and CW2 ranged from 1.76–2.86 mg/L (average 2.14 mg/L) and 1.74–3.26 mg/L (2.24 mg/L) respectively (Figure 6). The average NO_3-N removal efficiencies of CW1 and CW2 for the 14-month monitoring period were 30% and 26% respectively. Influent SRP concentrations ranged from 1.86–4.7 mg/L (average

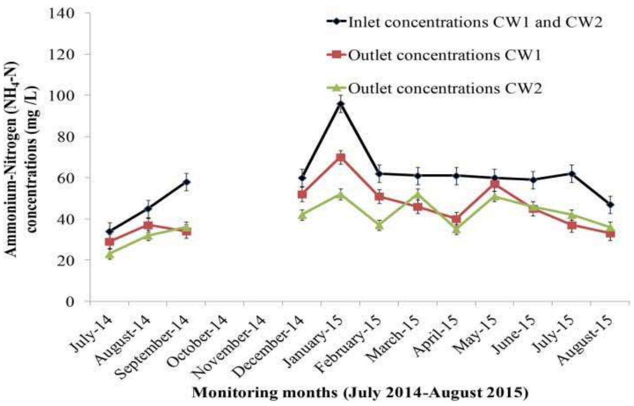

Figure 5 | Average influent and effluent NH_4-N concentrations in CW1 and CW2 (July 2014–August 2015). No NH_4-N data were available from October–November 2014.

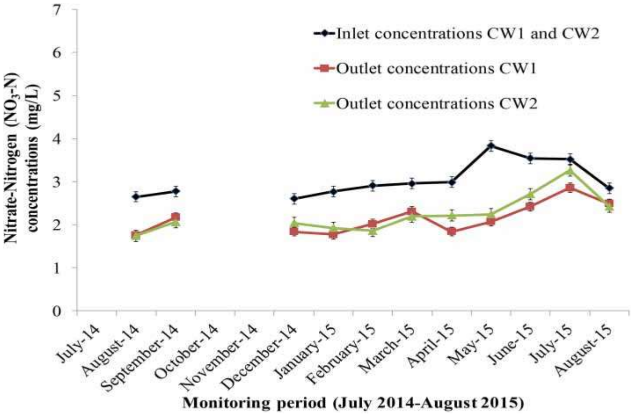

Figure 6 | Average influent and effluent NO_3-N concentrations in CW1 and CW2 (August 2014–August 2015). No NO_3-N data available in July 2014 and from October–November 2014.

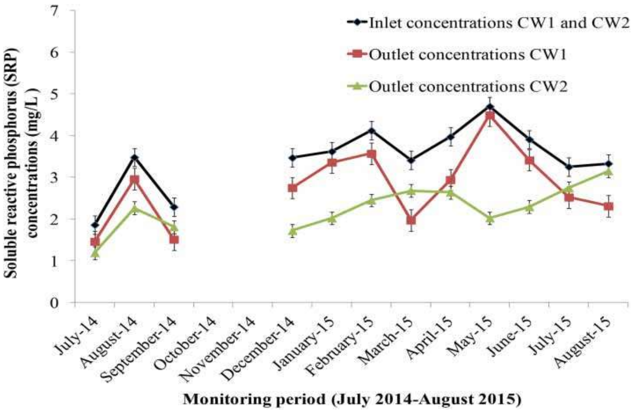

Figure 7 | Average influent and effluent SRP concentrations in CW1 and CW2 (July 2014–August 2015). No SRP data available from October–November 2014.

3.45 mg/L), while the effluent SRP concentrations in CW1 and CW2 ranged from 1.46–4.49 mg/L (average 2.77 mg/L) and 1.19–3.15 mg/L (average 2.25 mg/L) respectively (Figure 7). The average SRP removal efficiencies of CW1 and CW2 for the 14-month monitoring period were 20% and 35% respectively.

Influent and effluent loading rates and removal efficiencies (with respect to loadings) of TSS, COD, NH_4-N, NO_3-N and SRP in CW1 and CW2

The observed influent and effluent wastewater loading rates of CW1 and CW2 were quantified for four seasons, i.e. July–October 2014 (rainy season), November 2014–February 2015 (winter season), March–May 2015 (summer season) and June–August 2015 (rainy season) (Table 2). The concentrations of different wastewater parameters (TSS, COD, NH_4-N, NO_3-N and SRP) and daily flow rates from both CWs were used to compute influent and effluent loading rates based on Equations (1) and (2) (Fernanda & Lúcia 2012).

$$M_{in} = HLR \times C_{in} \tag{1}$$

$$M_{out} = HLR \times C_{out} \tag{2}$$

In Equations (1) and (2), the M_{in}, M_{out}, C_{in} and C_{out} and HLR refers to influent loading, effluent loading, influent concentrations, effluent concentrations and hydraulic loading rate of different wastewater constituents respectively. The TSS loading rates were highest from November 2014–February 2015 and June–August 2015 respectively. The TSS removal efficiencies for CW1 and CW2 were highest in November 2014–February 2015 (CW1-81% and CW2-80%) and March–May 2015 (CW1-80% and CW2-51%) compared to lower efficiencies in July–August 2014 (CW1-49% and CW2-57%) and June–August 2015 (CW1-45% and CW2-54%). The overall average TSS removal efficiency of CW1 and CW2 was 64% and 61% respectively from July 2014–August 2015. The COD removal efficiencies of CW1 and CW2 were highest from March–May 2015 (CW1-50% and CW2-41%) compared to lower removal efficiencies from November 2014–February 2015 (CW1-39% and CW2-38%) respectively. Overall, the average COD removal efficiencies in CW1 and CW2 were 46% and 38% from December 2014–August 2015 respectively.

The NH_4-N influent loadings for both CWs were highest from November 2014–February 2015, March–May 2015 and June–August 2015 respectively, compared to lower loadings from July–October 2014. The CW2 had NH_4-N removal efficiencies of 33%, 41% and 25% from July–October 2014, November 2014–February 2015 and March–May 2015 respectively compared to CW1, having removal efficiencies of 27%, 22% and 21% from July–October 2014, November 2014–February 2015 and March–May 2015, respectively. Overall the average NH_4-N removal efficiencies of CW1 and CW2 were 26% and 32% from July 2014–August 2015, respectively. The NO_3-N removal efficiencies of both CWs were higher in three seasons, i.e. from July–October 2014 (CW1-30% and CW2-32%), November 2014–February 2015 (CW1-32% and CW2-29%) and March–May 2015

Table 2 | Total influent and the effluent seasonal loadings for TSS, COD, NH_4-N, NO_3-N and SRP for CW1 and CW2 from July 2014–August 2015

		July–Oct 2014		Nov 2014–Feb 2015		March–May 2015		June–August 2015	
		Avg Q_{in}	Avg Q_{out}	Avg Q_{in}	Avg Q_{out}	Avg Q_{in}	Avg Q_{out}	Avg Q_{in}	Avg Q_{out}
Wastewater parameters	CW type	(g/m^{-2} season)							
TSS	CW1	112	57	169	32	108	22	140	77
TSS	CW2		48		34		53		64
COD	CW1	NA	NA	630	384	448	226	379	188
COD	CW2		NA		393		265		250
NH_4-N	CW1	142	104	251	197	215	170	199	129
NH_4-N	CW2		95		149		162		144
NO_3-N	CW1	8.8	6.18	9.44	6.43	11.7	7.40	11.8	9.86
NO_3-N	CW2		5.97		6.69		7.92		10.47
SRP	CW1	7.98	6.22	12.8	11	14.4	13.5	12.1	8.9
SRP	CW2		5.50		7.00		8.7		10.9

TSS-total suspended solids, COD-chemical oxygen demand, NH_4-N-ammonium nitrogen, NO_3-N-nitrate-nitrogen, SRP-soluble reactive phosphorus. The TSS, COD, NH_4-N, NO_3-N and SRP loadings for CW1 and CW2 are identical, since both wetlands had the same flow rates and inlet concentrations.

(CW1-37% and CW2-32%) respectively compared to June–August 2015 (CW1-16% and CW2-11%) respectively. Overall the average NO_3-N removal efficiencies of CW1 and CW2 were 29% and 26%, respectively. The SRP removals in CW1 were higher in July–October 2014 (22%) and June–August 2015 (26%) compared to November 2014–February 2015 (14%) and March–May 2015 (6.2%) respectively. The SRP removals in CW2 were higher in November 2014–February 2015 (45%) and March–May 2015 (40%) compared to July–October 2014 (31%) and June–August 2015 (9.9%). Overall, the average SRP removals were higher in CW2 (32%) compared to CW1 (17%).

Mechanisms of TSS, COD, NH_4-N, NO_3-N and SRP removals in CW1 and CW2

The major mechanisms of TSS and COD removal in CWs are physical settling, entrapment and deposition in the void pores of the sand and gravel media. TSS and COD removal may be enhanced by increasing the HRT, thus providing more opportunity for settlement of suspended solids and for wetland plants to uptake excessive N and P (Kadlec 2009). Besides physical removal mechanisms, organics such as biochemical oxygen demand (BOD) and COD are reduced by aerobic and anaerobic microbial processes. The oxygen required for aerobic degradation is supplied from atmospheric diffusion and oxygen leakage by the roots of *Typha latifolia* and other wetland plants (Vymazal & Kröpfelová 2009). Organics such as BOD can be complex mixtures of proteins, lipids, carbohydrates (labile and easily degradable by microbes) and lignin and hemicellulose (not labile and not easily degradable by microbes). Aerobic degradation in the presence of oxygen is carried out by aerobic heterotrophic bacteria, while anaerobic degradation in the absence of dissolved oxygen is carried out by facultative or obligate anaerobic bacteria (Vyzamal & Kröpfelová 2009). The major mechanism of fecal coliform removal is the strong interaction of wastewater with the sand and gravel media and its attachment to the root matrix, adhesion to biofilms, oxidation, predation, natural die-off, temperature and influent water quality resulting in its enhanced removal efficiency (Kadlec 2009).

The higher NH_4-N removal efficiency of CW2 compared to CW1 was probably due to the greater amount of N retention in the wetland plants and an unaccounted microbial process of denitrification (Figure 8). Ammonia volatilization in both CWs was probably not significant in terms of overall N reduction, since the wastewater pH was never greater than 8.1 during the entire monitoring period. The ammonia losses are insignificant for pH from 7.5–9 and for $pH > 9.3$, the ratio of ammonia and ammonium ions is 1:1 and losses through volatilization can be significant (Reddy & DeLaune 2008). In both the CWs, NO_3-N might have been lost to denitrification as it requires NO_3-N as the electron acceptor, organic C, the absence of oxygen, the presence of denitrifiers and a suitable redox potential (*Eh*) ranging from +100–350 mV, and optimum pH 6–8 (Paul & Clark 1996). In CW1 and CW2, measured pH and *Eh* values for a 14-month period ranged from 6.6–8.1 and 132–182 mV, respectively and were in the optimum range for denitrification. The wastewater present in the bottom layer of sections B and C in both CWs may have become anaerobic/anoxic, providing favourable conditions for denitrification. Organic C was also present in coarse sand with available NO_3-N needed for denitrification. Although denitrification was not quantified in both CWs, it might be a major N loss mechanism. Variations in SRP removal may be linked to vegetation uptake and the

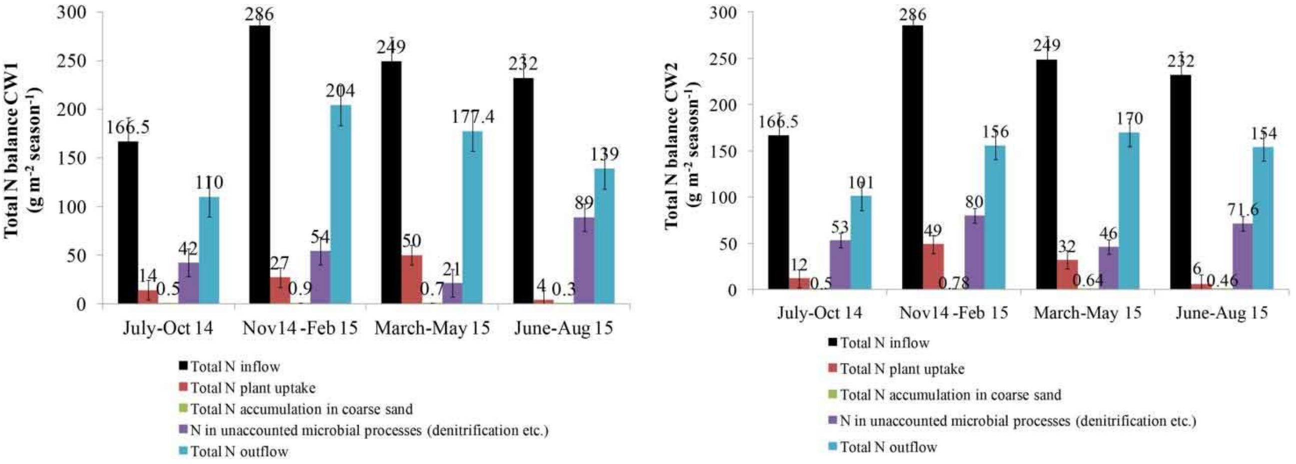

Figure 8 | Total N mass balance of CW1 and CW2 for four seasons (July 2014–August 2015).

formation of insoluble Ca-P at alkaline pH (Reddy & DeLaune 2008). The higher SRP removals in CW2 compared to CW1 were due to the higher total P uptake of all wetland plants in CW2 (0.1391 g/m^{-2} d) compared to total P uptake of all wetland plants in CW1 (0.1276 g/m^{-2} d) respectively.

Tissue N and P concentrations in wetland vegetation in CW1 and CW2

Recall that the *Pistia stratiotes* were introduced in sections A and D of both CWs from November 2014–May 2015. In the CW1 and CW2, N and P tissue concentrations averaged from 32–45 g/kg and 10.4–11.2 g/kg respectively (Table 3). Lu *et al.* (2010) evaluated the efficiency of *Pistia stratiotes* in removing N and P from storm-water discharging into a CW. Results showed that N and P tissue concentrations averaged 17 g/kg and 3 g/kg respectively. Polomski *et al.* (2009) conducted a laboratory scale study to quantify the N and P uptake capacity of *Pistia stratiotes* treating nursery runoff water into a CW. The average N and P tissue concentrations were 16.2 g/kg and 2.3 g/kg respectively.

Currently there is no data in the literature reporting above and below ground biomass N and P tissue concentrations of *Ageratum conyzoides* in a CW. In CW1 and CW2, the above-ground N and P tissue concentrations averaged 32–35 g/kg and 3.9–4.15 g/kg respectively over a 14-month period. The below-ground N and P tissue concentrations in CW1 and CW2 averaged 12–13 g/kg and 1.48–1.62 g/kg respectively over a three-month period. The above-ground N and P tissue concentrations of *Typha latifolia* in CW1 averaged 31.5 g/kg and 3.9 g/kg respectively for a 14-month period, while the below-ground N and P tissue concentrations averaged 14 g/kg and 3.6 g/kg respectively over a three-month period. Zingelwa & Wooldridge (2009) evaluated N and P uptake of *Typha latifolia* in a CW receiving winery wastewater; the N and P accumulation in above-ground biomass averaged 11.7 g/kg and 0.31 g/kg respectively. In the CW2, the above-ground N and P tissue concentrations of *Canna indica* averaged 30.3 g/kg and 3.5 g/kg respectively for a 14-month period. The below-ground N and P tissue concentrations in *Canna indica* averaged 8 g/kg and 1.84 g/kg respectively over a three-month period (Table 3 and Table 4). Zhang *et al.* (2007b) evaluated the N and P removal capacity of *Canna indica* for tertiary purification of wastewater in a CW. The results showed N accumulation in above-ground and below-ground biomass averaged 15 ± 0.5 g/kg and 11.5 ± 0.45 g/kg respectively. The total P accumulation in above and below-ground biomass averaged 4.2 ± 0.1 g/kg and 3.25 ± 0.15 g/kg respectively (Zhang *et al.* 2007b).

N and P uptake by vegetation in CW1 and CW2 and comparison with literature

In terms of average removal rates of different vegetation species per unit area, *Pistia stratiotes* was the highest accumulator of N followed by the *Ageratum conyzoides* (CW2), *Canna indica* (CW2), *Ageratum conyzoides* (CW1) and

Table 3 | Average N and P contents (g/kg) in above-ground biomass of *Pistia stratiotes*, *Typha latifolia*, *Ageratum conyzoides* and *Canna indica*

	June–Oct 2014		**Nov 2014–Feb 2015**		**March–May 2015**		**June–August 2015**	
	Average above-ground plant tissue concentrations (g/kg)							
Wetland plants	**Total N**	**Total P**	**Total N**	**Total P**	**Total N**	**Total P**	**Total N**	**Total P**
CW1								
Pistia stratiotes	NA	NA	48	12	42	8.7	NA	NA
Typha latifolia	33	3.2	29	2.9	30	4.9	34	4.6
Ageratum conyzoides	30	3.5	25	2.8	37	5.3	36	5.0
Pistia stratiotes	NA	NA	38	12.3	32	9.1	NA	NA
CW2								
Pistia stratiotes	NA	NA	46	11.8	43	9.2	NA	NA
Canna indica	27	3.1	29	4.5	30	3.4	35	2.9
Ageratum conyzoides	36	2.5	29	3.9	38	5.4	37	3.7
Pistia stratiotes	NA	NA	34	12.5	30.6	9.8	NA	NA

NA-not available.

Table 4 | Average N and P contents (g/kg) in below-ground biomass of *Typha latifolia*, *Ageratum conyzoides* and *Canna indica*

	Average below-ground tissue concentrations (g/kg)	
	June–August 2015	
Wetland plants	Total N	Total P
Typha latifolia (CW1)	14	3.6
Ageratum conyzoides (CW1)	12	1.48
Canna indica (CW2)	8	1.84
Ageratum conyzoides (CW2)	13	1.62

Typha latifolia (CW1) over a 14-month period. In terms of P uptake, *Pistia stratiotes* was the highest accumulator, followed by *Ageratum conyzoides* (CW2), *Canna indica* (CW2) and *Typha latifolia* (CW1) over a 14-month period. The individual above-ground N and P uptake of each wetland plant each season (four seasons) and individual below-ground N and P uptake of all wetland plants for one season are shown in Tables 5 and 6 respectively. This is the first field scale study to quantify the N and P uptake (above-ground and below-ground) of *Ageratum conyzoides* in CWs treating domestic wastewater. A microcosm study receiving agricultural drainage effluents evaluated N and P uptake of four macrophytes commonly used in CWs such as pennywort (*Hydrocotyle umbellata* L.), water hyacinth (*Eichhornia crassipes*), cattails (*Typha latifolia* L.) and elodea (*Egeria densa* P.) and control (no macrophytes) (Reddy 1983). Results showed that total N and P uptake of *Typha latifolia* averaged 0.05 g/m^{-2} d and 0.001 g/m^{-2} d respectively (Reddy 1983). Zhang *et al.* (2007a) evaluated total N and P uptake of *Canna indica* along with 12 other ornamental species in a wetland microcosm receiving secondary treated municipal wastewater. Results showed that total N and P uptake of *Canna indica* averaged 0.0125 g/m^{-2} d and 0.0022 g/m^{-2} d respectively. The N and P uptake of *Typha latifolia* and *Canna indica* achieved in this field scale study are comparable with the literature studies.

Accumulated nutrients in the coarse sand in CW1 and CW2

Total N, total P, available P, organic C and exchangeable Ca were measured in the pre-treatment coarse sand (control) and the post-treatment coarse sand (Table 7). In CW1, average total N accumulation in sections B and C increased by 95% and 98% respectively compared to the pre-treatment over a 14-month period. The average total P accumulation in sections B and C increased by 41% and 55%, respectively; while the average available P increased by 78% and 83%, respectively compared to the pre-treatment coarse sand over a 14-month period. In CW2, the average total N accumulation in sections B and C increased by 96% and 95% respectively, while the average total P accumulation in sections B and C increased by 61% and 55% respectively compared to the pre-treatment coarse sand over a 14-month period. The average available P, an

Table 5 | Average seasonal rate of N and P accumulation (g/m^{-2} d) in above-ground biomass of wetland plants in CW1 and CW2

	Seasons							
	June–Oct 2014		Nov 2014–Feb 2015		March–May 2015		June–August 2015	
	Average seasonal nutrient uptake (g/m^{-2} d)							
Wetland plants	Total N	Total P	Total N	Total P	Total N	Total P	Total N	Total P
CW1								
Pistia stratiotes	NA	NA	0.126	0.032	0.47	0.096	NA	NA
Typha latifolia	0.042	0.004	0.041	0.0041	0.032	0.0051	0.027	0.0037
Ageratum conyzoides	0.069	0.008	0.051	0.0055	0.064	0.0092	0.034	0.0046
Pistia stratiotes	NA	NA	0.086	0.086	0.26	0.073	NA	NA
CW2								
Pistia stratiotes	NA	NA	0.23	0.056	0.21	0.043	NA	NA
Canna indica	0.049	0.0051	0.077	0.011	0.066	0.0075	0.039	0.0033
Ageratum conyzoides	0.078	0.0054	0.065	0.0091	0.091	0.013	0.06	0.006
Pistia stratiotes	NA	NA	0.17	0.063	0.156	0.05	NA	NA

Table 6 | Average rate of N and P accumulation (g/m^{-2} d) in below-ground biomass of wetland plants in CW1 and CW2

	One season below-ground uptake (g/m^{-2} d)	
	June–August 2015	
Wetland plants	**Total N**	**Total P**
Typha latifolia (CW1)	0.00769	0.00198
Ageratum conyzoides (CW1)	0.00778	0.00096
Canna indica (CW2)	0.00650	0.00151
Ageratum conyzoides (CW2)	0.00808	0.00099

important nutrient for plant growth, increased in sections B and C by 84% and 85% respectively compared to the pre-treatment coarse sand over a 14-month period. In CW1, the exchangeable Ca in the post-treatment coarse sand compared to the pre-treatment coarse sand increased by 29%, 18% and 22% in section B and 33%, 19% and 24% in section C during June–October 2014, November 2014–February 2015 and March–May 2015 respectively. In the CW2, exchangeable Ca in post treatment coarse sand compared to pre-treatment coarse sand increased by 34%, 14% and 24% in section B and 35%, 21% and 23% in section C during June–October 2014, November 2014–February 2015 and March–May 2015 respectively. Results showed that N and P accumulation in the post-treatment coarse sand was greater compared to the pre-treatment coarse sand in both CWs.

The C: N ratio of the pre-treatment sand was 11, while in CW1, the C: N ratio in sections B and C ranged from 1.57–5.87 (average value-4.14) and 1.60–7.29 (average value-4.33) respectively. In CW2, the C: N ratio in sections B and C ranged from 4.08–5.57 (average value-4.84) and 6.27–10.15 (average value-7.92) respectively. Generally, the C: N ratio qualitatively indicates the potential for mineralization, i.e. conversion of organic N to NH_4-N or immobilization (inorganic N into the organic form) (Reddy & DeLaune 2008). A C: N ratio less than 25 qualitatively indicates mineralization and a C: N ratio greater than 25 indicates immobilization (Reddy & DeLaune 2008). In this study, the C: N ratio was less than 25 in both the CWs, which indicated mineralization, i.e. additional NH_4-N production inside both the CWs besides NH_4-N input from the influent wastewater entering both the CWs.

Total N mass balance in each season (four seasons) in CW1 and CW2

It is a well-known fact that total N in wastewater consists of organic and inorganic contents. The inorganic N content consists primarily of NH_4-N, NO_3-N and nitrite. In this study, the total organic N content was measured and

Table 7 | Average seasonal accumulation of N, P, organic C and exchangeable calcium (g/kg) in coarse sand

	TN		**TP**		**AP**		**OC**		**Ex Ca**	
	(all parameter units g/kg)									
Background coarse sand concentration	**0.015**		**0.066**		**0.0024**		**0.167**		**0.770**	
Post treatment coarse sand	**Section B**					**Section C**				
Seasons	**TN**	**TP**	**AP**	**OC**	**Ex Ca**	**TN**	**TP**	**AP**	**OC**	**Ex Ca**
CW1 (all parameter units-g/kg)										
June–Oct 2014	0.16	0.12	0.004	0.78	1.08	0.26	0.16	0.005	1.88	1.14
Nov 2014–Feb 2015	0.28	0.18	0.009	1.21	0.94	0.71	0.12	0.015	1.19	0.95
March–May 2015	0.39	0.11	0.014	0.62	0.99	0.93	0.18	0.019	1.49	1.01
June–August 2015	0.22	0.12	0.017	1.31	NA	0.35	0.13	0.019	2.34	NA
CW2 (all parameter units-g/kg)										
June–Oct 2014	0.27	0.17	0.009	1.33	1.17	0.14	0.14	0.005	1.17	1.18
Nov 2014–Feb 2015	0.38	0.19	0.013	1.86	0.89	0.33	0.15	0.013	2.24	0.97
March–May 2015	0.39	0.14	0.017	1.57	1.01	0.41	0.16	0.027	4.11	1.00
June–August 2015	0.30	0.17	0.020	1.68	NA	0.28	0.14	0.018	1.71	NA

N = 14; same coarse sand (background concentration) in sections B and C in CW1 and CW2. TN-total N; TP-total P; AP-available P; OC-organic carbon; Exc Ca-exchangeable calcium.

accounted for 10 percent of the inorganic N loading. The major inorganic N loading into both CWs was NH_4-N and NO_3-N for a 14-month period (July 2014–August 2015). The total N mass balance of both CWs accounted for total influent N loading (organic+ inorganic), total effluent N loading, total N accumulation in coarse sand, total N uptake by wetland plants (above-ground biomass) and unaccounted processes (not measured in this study) such as denitrification. The total N mass balance in this study is presented for four periods, i.e. July–October 2014, November 2014–February 2015, March–May 2015 and June–August 2015 respectively (Figure 8).

In CW1, the average data for four seasons showed that the total N influent and total N effluent loading was 233 g/m^{-2} and 156 g/m^{-2} respectively, with retention/storage of 77 g/m^{-2}. The microbial unaccounted processes, plant N uptake and N accumulation in coarse sand, accounted for 68%, 31% and 1% respectively of the average total N retained/stored in the CW1 over a 14-month period. In CW2, the average data for four seasons showed that the total N influent and total N effluent loading was 233 g/m^{-2} and 145 g/m^{-2} respectively, with retention/storage of 88 g/m^{-2}. The microbial unaccounted processes, plant N uptake and N accumulation in coarse sand, accounted for 71%, 28% and 1% respectively of the average total N retained in the CW2 over a 14-month period.

Effect of vegetation on wastewater quality in CW1 and CW2

In both the CWs, wastewater was only sampled from the inlet (section A) and the outlet (section D) during the 14-month monitoring period. However, wastewater sampling from the individual sections was not carried out, i.e. wastewater moving from section A into section B, section B into section C and section C into section D. Wastewater sampling from each section would have helped to quantify its individual treatment capacity, e.g. the reduction potential of CW1 (section B and C) containing *Typha latifolia* and sand media and *Ageratum conyzoides* and sand media respectively, and CW2 (section B and C) containing *Canna indica* and sand media and *Ageratum conyzoides* and sand media respectively. However, the flow patterns and difficulties in sampling wastewater prevented the quantification of individual treatment capacities of sections A, B and D (Figure 1). The treatment capacity of section C was measurable but was not quantified.

Even though the individual treatment capacities of sections A, B, C and D were not quantified, measured plant N uptake and C and N accumulation in sand media provided an approximate estimate of individual treatment capacities, especially of sections B and C in both CWs. The average N uptake of *Ageratum conyzoides* in CW1 (section C) and CW2 (section C) was 0.0545 g/m^{-2} d and 0.073 g/m^{-2} d respectively. The average N uptake of *Typha latifolia* in CW1 (section B) and *Canna indica* in CW2 (section B) was 0.0355 g/m^{-2} d and 0.578 g/m^{-2} d respectively. This showed that the N uptake of *Ageratum conyzoides* was very much comparable to *Canna indica* and higher than *Typha latifolia* for the monitoring period. In CW1 (section C), the organic C and total N accumulation in sand media averaged 1.72 g/kg and 0.56 g/kg respectively. In CW2 (section C), the organic C and total N accumulation in sand media averaged 2.31 g/kg and 0.29 g/kg respectively. In CW1 (section B), the organic C and total N accumulation in sand media averaged 0.98 g/kg and 0.26 g/kg respectively. In CW2 (section B), the organic C and total N accumulation in sand media averaged 1.61 g/kg and 0.33 g/kg respectively. This showed that the organic C and total N accumulation in sand media having *Ageratum conyzoides* (CW1 and CW2, section C) was higher compared to organic C and total N accumulation in sand media having *Typha latifolia* (CW1, section B) and *Canna indica* (CW2, section B) respectively. However, we are aware of the different influent conditions for *Ageratum conyzoides* in section C of both CWs due to the different vegetation type in section B. For future researchers and environmental land managers, when the desired objective of designing subsurface flow CWs is removal of excessive NH_4-N and SRP, a combination of *Pistia stratiotes*, *Canna indica*, and *Ageratum conyzoides* can be utilized as was the case in this study. If the objective of designing subsurface flow CWs is removal of excessive NO_3-N, then a combination of *Pistia stratiotes*, *Typha latifolia*, and *Ageratum conyzoides* can be utilized.

The plant N uptake and the C and N accumulation in sand media are dependent upon a number of factors such as the concentration of nutrients entering the CWs, the amount of dissolved NH_4-N and NO_3-N, the frequency and time of harvesting vegetation, the air temperatures required for plant growth, the dynamic, changing pH and redox conditions and the ubiquitous presence of microbial communities (Kadlec 2009). We monitored the concentration of the nutrients entering both the CWs (once every week for the 14-month monitoring period) and computed daily loadings using daily flows. However, we are aware that the frequency of monitoring nutrients was low and needs to be increased to gain greater accuracy in computing wastewater treatment efficiencies. There is also a need to monitor the amount of dissolved NH_4-N and NO_3-N entering each individual section (A-D) of both the CWs. The wetland vegetation was harvested upon maturity by removing its above-ground biomass. During field investigations, it was observed that *Ageratum conyzoides* needed 35–40 days to regrow to its optimum height of 1–1.5 m upon harvesting. The *Typha latifolia* and *Canna indica* needed 45–50 days to regrow to their original height before harvesting. During the 14-month monitoring period, the *Ageratum conyzoides*, *Typha latifolia* and *Canna indica* were harvested 11 times, 8 times and 8 times respectively. The weather in Hyderabad, India, never experiences air temperatures dropping below 0 °C, which meant that air temperatures were probably not a hindrance for growth of wetland vegetation. The pH and the redox values were measured in sections A, B, C and D, but these measurements were point-scale readings taken at a particular time in a day. The pH and redox measurements were conducted once every week for the 14-month monitoring period. There is a greater need for installation of data loggers for continuous nutrients, pH and redox measurements in all the sections of both CWs.

Scope for further research

This study provided preliminary evidence that *Ageratum conyzoides*, when used in combination with *Pistia stratiotes*, *Canna indica* and *Typha latifolia* in subsurface flow CWs, can remove excessive N, P and fecal coliforms from domestic wastewater. This study also quantified that the N and P uptake (g/m^{-2} d) of *Ageratum conyzoides* was very much comparable with the N and P uptake (g/m^{-2} d) of *Pistia stratiotes*, *Canna indica*, and *Typha latifolia*. However, there is a great scope for further research in using *Ageratum conyzoides* in subsurface flow CWs as a stand-alone wetland plant instead of using it in combination with *Pistia stratiotes*, *Canna indica* and *Typha latifolia*. Further research can also explore and quantify C and N cycling, microbial diversity and denitrification potential in sand media having *Ageratum conyzoides* as a stand-alone wetland vegetation.

CONCLUSIONS

The most important N retention processes in both CWs were the unaccounted (not measured in this study) microbial processes (68–71%) and plant N uptake (28–30%). The N and P accumulation in the coarse sand in both CWs was very minimal and accounted for 1%. The N and P tissue concentrations (g/kg) and uptake capacity (g/m^{-2} d) of *Ageratum conyzoides* were lower than *Pistia stratiotes* but very much comparable with the N and P uptake capacities (g/m^{-2} d) of *Canna indica* and *Typha latifolia* respectively. CW2, having *Pistia stratiotes*, *Canna indica* and *Ageratum conyzoides*, had greater NH_4-N and SRP removals (% reduction with respect to concentrations) compared to CW1, having *Pistia stratiotes*, *Typha latifolia* and *Ageratum conyzoides*, over a 14-month period. However CW1 was more efficient in NO_3-N, TSS and COD removal (% reduction with respect to concentrations) compared to CW2, and both CWs had similar removal rates (% reduction with respect to concentrations) for fecal coliforms over a 14-month period. For future researchers and environmental land managers, when the desired objective of designing subsurface flow CWs is the removal of excessive NH_4-N and SRP, a combination of *Pistia stratiotes*, *Canna indica* and *Ageratum conyzoides* can be utilized, as was the case in this study. If the objective of designing subsurface flow CWs is concentrated on removal of excessive NO_3-N, then a combination of *Pistia stratiotes*, *Typha latifolia*, and *Ageratum conyzoides* can be utilized.

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