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Phytoremediation performance of *Acorus calamus* and *Canna indica* for the treatment of primary treated domestic sewage through vertical subsurface flow constructed wetlands: a field-scale study

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Abstract

Under the present investigation, vertical subsurface flow constructed wetlands (VSSFCWs) planted with macrophytes treated domestic sewage in an environmentally sustainable manner. Treatment of domestic sewage with wetlands is an alternative method that decreases energy consumption and economic costs involved in the treatment of environmental contaminants. This study evaluates the potential efficiency of VSSFCWs using two different macrophytes, *Acorus calamus* and *Canna indica* for the treatment of domestic sewage. To perform this study, two chambers of VSSFCWs of dimensions 2.48 m × 1.24 m × 1.54 m were built. The wetland was fed with the primary treated sewage at a hydraulic loading rate (HLR) of 0.67 m^3/h (hours) in a batch flow. Treatment of primary sewage was observed from day 1 to day 6; once a day (i.e. 24 h to 144 h). The treatment of sewage was found to be significant up to day 6 (144 h); beyond this time, no significant removal was observed. The results revealed that both the wetland setups performed significant removal of TDS, BOD_5, total nitrogen, and phosphate. The wetland planted with *Canna indica* was a better performer for the removal of TDS (22.31%), BOD_5 (81.79%), total nitrogen (60.37%), and phosphate (80%).

Key words: *Acorus calamus*, aquatic macrophytes, *Canna indica*, domestic sewage, nutrients, vertical subsurface flow constructed wetland

INTRODUCTION

In developing counties like India, geographically remote areas, villages and outskirts of towns or cities lack sewage networks or centralized wastewater collection with a treatment system. Since a conventional treatment system for sewage wastewater is very costly, it always requires highly trained operators on the site and does not work well on a small scale (EPA 1993; Mishra & Tripathi 2008). The constructed wetlands (CWs) have been proven an attractive solution for wastewater treatment (Coleman *et al.* 2001; Karathanasis *et al.* 2003; Regelsberger *et al.* 2005). The CWs are particularly useful for small communities in urban and rural areas with no access to public sewage systems (Vymazal *et al.* 1998). Constructed wetland (CW) works on natural principles of the treatment, having very low running and maintenance cost; due to this fact, the interest of researchers in the use of CW for wastewater treatment is increasing throughout the world (Vymazal 2011; Talukdar & Pal 2017; Orimoloye *et al.* 2018; Ansari & Golabi 2018). The treatment through the constructed wetland is due to various physical, chemical and biological processes similar to the processes occurring in natural wetlands (Stefanakis *et al.* 2014; Mishra *et al.* 2018).

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Wastewater generated from households leads to an enrichment of nutrients when it is discharged without proper treatment into the surrounding water bodies; it may trigger a series of adverse impacts on receiving systems such as eutrophication, hypoxia and deterioration of water quality, ultimately ending in deterioration of the aquatic ecosystems (Mohanty *et al.* 2014). Treatment of the wastewater through existing methods has its own limitations (Mishra & Tripathi 2008). Contrary to this, the treatment of wastewater through constructed wetlands offers an ecologically sustainable, cost-effective, and natural system of wastewater treatment. Vertical subsurface flow constructed wetlands have been used primarily for the treatment of sewage wastewater (Zidan *et al.* 2015) and are more efficient than the surface flow wetlands. Constructed wetland has evolved through the ages and its performance has been improved from time to time by conducting experiments. The performance of the CW mainly depends on its structure and mode of water flow (horizontal, vertical or hybrid setups), types of filter media (sand, gravel, pebbles, biochar, zeolite of different sizes) and the plant species grown on the wetland. These components are arranged in a specific way to achieve the maximum efficiency for wastewater treatment (Stefanakis *et al.* 2014; Wu *et al.* 2015; Sanmuga & Senthamil Selvan 2017).

Vertical sub-surface flow constructed wetlands (VSSFCWs) are a type of CW that allow the flow of wastewater vertically through them. Due to the vertical flow, the length of wastewater flow is increased and it facilitates the faster and efficient treatment of organic matter and reduction in the nutrients from wastewater. Therefore, vertical subsurface flow constructed wetlands (VSSFCWs) are recommended for the treatment of primary treated sewage (García-Avila *et al.* 2019). The performance of CWs enhance when they are planted with macrophytes (Zhu *et al.* 2014). Vegetation is an important factor influencing the effectiveness of VSSFCWs in the elimination of biochemical oxygen demand, chemical oxygen demand, suspended solids and ammonia under all conditions (Coleman *et al.* 2001; Abdelhakeem *et al.* 2016). The macrophyte species such as *Canna indica* and *Acorus calamus* are identified as phytoremediation plants (Bose *et al.* 2008). In the tropical and sub-tropical region, *Canna indica* is mostly found along the roads, and around ponds and agricultural fields. Some of the attributes of the plants, like fast natural growth, tolerance to the adverse climatic conditions, hyperaccumulation capacity, and long fibrous root systems make the macrophytes more suitable for phytoremediation studies. These plants, when used in CW, enhance the aerobic environment throughout the treatment bed, which in turn enhances the treatment efficiency of the wetland (Sharma *et al.* 2014).

Currently used sewage treatment plants (STPs) in India require large space for the treatment of sewage, need trained operators and electricity, etc. In contrast, VSSFCWs require less space, work naturally with minimum energy requirement, and have aesthetic values. In India, the field-scale study on VSSFCWs related to its efficiency, performance, and commercialization is lagging. Therefore, under the present study, we have constructed VSSFCWs planted with two species of macrophytes (*Canna indica* and *Acorus calamus*) at the field scale for the treatment of primary-treated domestic sewage, and its efficiency for the treatment of primary sewage was evaluated.

MATERIALS AND METHODS

Site for construction of VSSFCW

The present field-scale VSSFCW was constructed near the existing MBBR based sewage treatment plants (STP) at Indira Gandhi National Tribal University (IGNTU) campus; this is a University funded by the Government of India, located in a tribal area of Central India at geographical coordinates 22° 48′N and 081° 45′E with an altitude of 1,048 meters above sea level. IGNTU campus, Amarkantak is situated in completely rural settings surrounded by forests; the area receives an

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average annual rainfall of 123.5 cm and an annual average maximum and minimum temperature of 31.6 °C and 18.2 °C respectively (District Groundwater Information Booklet-Anuppur District 2007).

Source of primary treated sewage

The primary treated sewage was collected from the University STP, which receives the sewage from the residential buildings of the University campus. This sewage was tapped after the primary treatment and collected in the settling tank and detained for about 2 hours (h) before it was supplied to the CW. This was done in order to homogenize the quality and flow of sewage before it was supplied to the experimental wetlands.

Construction of the vertical Sub-surface flow constructed wetland

Two different units of VSSFCWs having a surface area of 3.08 m^2 (dimensions 2.48 m × 1.24 m × 1.54 m) were constructed in the Indira Gandhi National Tribal University campus (Figures 1 and 2). Each wetland unit was filled with selected filter media; that is, gravel (size 26 mm to 32 mm),

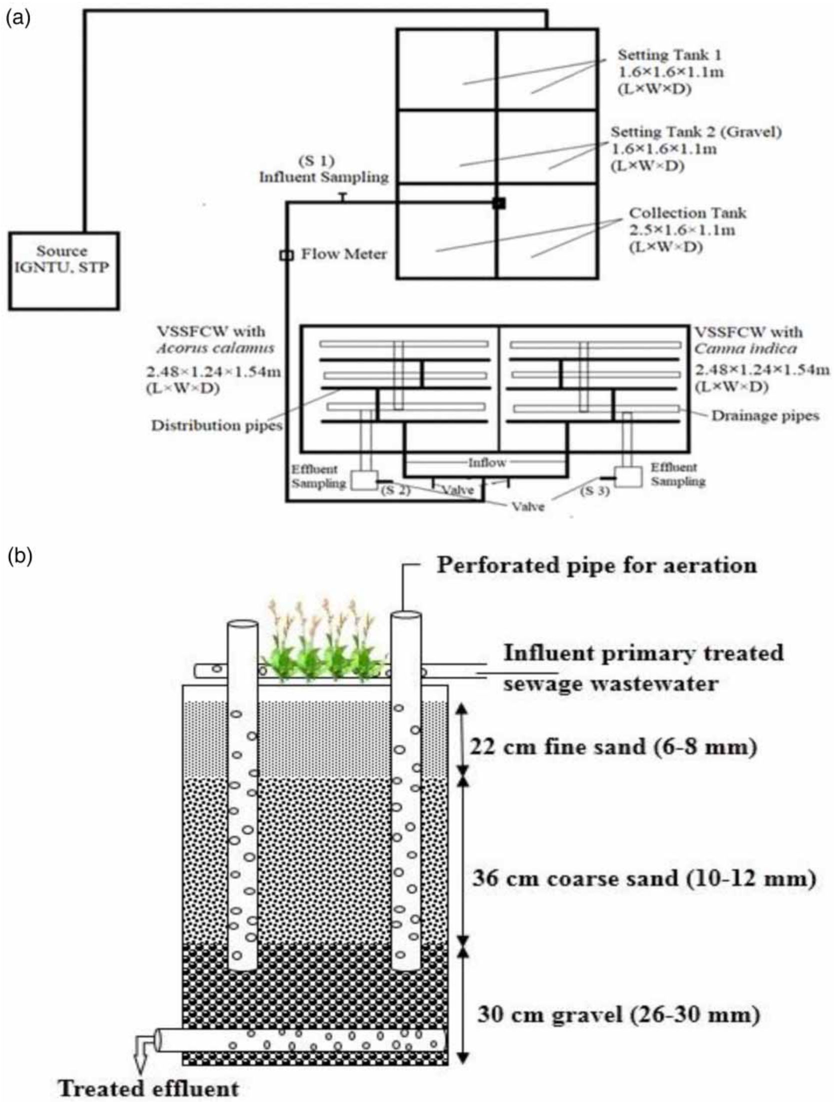

Figure 1 | (a) The schematic diagram of the experimental vertical subsurface flow constructed wetlands (VSSFCW) and sampling points (S1, S2, S3). (b) Composition of vertical layers of experimental constructed wetland.

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Figure 2 | VSSFCWs construction to field-scale (a) Filled with coarse gravel, (b) Filled with fine sand, (c) Initial growth of *Acorus calamus* (d) Initial growth of *Canna indica,* (e) *Acorus calamus* and *Canna indica* growth in the fourth month (f) A front view of constructed wetland.

coarse sand (size 8–10 mm) and fine sand (size 6–8 mm). The media was arranged in each bed with a thickness of 30 cm, 36 cm, and 22 cm of the gravel, coarse sand, and fine sand respectively (Figure 1). Each wetland unit was filled with gravel in the bottom, coarse sand in middle and fine sand at the top. The total thickness of the wetland media was 0.88 m. After the construction of the wetland chamber, a perforated PVC pipe with a diameter of 4 cm (drainage pipe) was placed at the bottom of the wetland

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with an outlet (S2 and S3 to collected treated effluent). Following this, a gravel layer of 30 cm was placed on top of the bottom PVC pipe followed by sand layers. After the filling of media, again perforated PVC pipe (feeding pipe with diameter 2 cm) was layered on top to discharge the influent wastewater. A perforated PVC pipe was inserted vertically at regular intervals for aeration. The filter media was collected and washed several times, after which it was filled in a wetland chamber. Again the constructed wetland was washed several times to clean the gravel and sand by filling and evacuating the water.

One unit of VSSFCW was planted with *Acorus calamus* and another unit with *Canna indica*. Overall, 54 young plants each of the two species were planted into two different wetlands. The young plants of *Acorus calamus* were collected from the forest area of Amarkantak, near the study site; while *Canna indica* were collected from IGNTU campus, Amarkantak. After the plantation, VSSFCW was regularly monitored for one month to check the survival and growth of the plants before starting the experiment.

Operation and monitoring of VSSFCW

The VSSFCW after the planting: the macrophytes *Acorus calamus* and *Canna indica* were subjected to a brief adjustment period of one month. During this period, the wetland was irrigated with normal groundwater and the growth was observed. After this period, the wetlands were fed with primary treated sewage in batch mode, the primary sewage was supplied vertically from the top of the constructed wetland and the treated effluent was collected from the lower surface of the constructed wetland. The hydraulic load rate (HLR) was adjusted to 0.67 m^3/h until the wetland was filled and a hydraulic retention time (HRT) of 144 hours (h) was provided. The CW was run in batch mode; for one cycle, it was operated for six days, after which the wetland was completely evacuated for the next cycle of treatment. The wetland planted with two species of macrophytes *(Canna indica and Acorus calamus)* was run for six months to evaluate the performance of the wetlands for the treatment of primary treated sewage.

Sampling points

The samples of primary treated sewage and wetland treated sewage were collected form the entry and exit point of the wetland. The samples were collected from the three different sampling points; that is, the first point (S1) was the point from where the primary treated sewage entered the constructed wetland and two other sampling points were at the outlet of each constructed wetland (S2 and S3). The samples of treated sewage were collected each day for six continuous days (from day 1 to day 6); that is, at 24 h, 48 h, 72 h, 96 h, 120 h, and 144 h from initiation.

Analytical methods

The physicochemical analysis of the primary treated sewage influent and treated effluent collected from the VSSFCW was conducted for the following important parameters, namely temperature, pH, conductivity, acidity, alkalinity, total dissolved solids (TDS), phosphate, nitrate, total nitrogen (Total Kjeldahl nitrogen), biochemical oxygen demand (BOD), and dissolved oxygen (DO). The analysis was conducted on the same day in the research laboratory of the Department of Environmental Science, IGNTU, Amarkantak. The basic parameters, viz. temperature, pH, conductivity, and TDS, were measured on site using a calibrated digital probe (PCS Tester 35 TM Series Multi-parameter kit). Five-day Biochemical Oxygen Demand (BOD_5) was measured using the Winkler method. Nitrate (NO_3-N) was estimated by ultraviolet (UV) spectrophotometric screening and phosphate (PO_4^{3-}) was measured by the stannous chloride method. Total nitrogen was estimated by the Kjeldahl method. All

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the analyses conducted during this research followed the protocols contained in AWWA-APHA (2012). All the reagents used for the physicochemical analysis were prepared, using AR-grade chemicals prepared in double-distilled water.

Calculations

The treatment efficiency of the constructed wetland was evaluated based on percentage removal. The removal efficiency (removal percent) for each parameter was calculated as follows:

$$Removal\ efficiency(\%) = \frac{C_{inf} - C_{eff}}{C_{inf}} \times 100$$

where, C_{inf} = influent and C_{eff} = effluent concentrations

Statistical analysis

One-way ANOVA test was applied for different parameters; that is, BOD, nitrate, total nitrogen, and phosphate to check the significant differences between the influent and effluent of household sewage wastewater through different macrophytes at *p*-value 0.05. Microsoft Excel 2013 was used for the statistical test.

RESULTS AND DISCUSSION

Characteristics of influent primary treated wastewater

The domestic sewage collected from a primary treated storage tank of STP located in IGNTU was transferred into settling tank 1 and then detained in settling tank 2 (filled with coarse gravel) for 2 h and then retained in a collection tank. This practice was adopted for quality enhancement of primary sewage. The physicochemical characteristics of the primary sewage (i.e. from the collection tank) were analyzed before it entered into the constructed wetlands. The pH of the primary treated sewage was found to vary from 7.23 to 7.76 during the experimental period. Conductivity values represent all the ions dissolved in the water (Yilmaz & Koc 2014). The conductivity of primary treated sewage ranged from 862 to 941.33 μS/cm, high conductivity specified the occurrence of dissolved inorganic matter in higher concentrations. The quantity of TDS and alkalinity varied from 623.3 to 666.3 mg/L and 262 to 293.7 mg/L respectively in the primary treated sewage. DO and BOD values of the primary sewage ranged from 1.87 to 2.27 mg/L, and 83.33 to 110 mg/L respectively. Nutrients available in primary treated sewage, like nitrate, total nitrogen and phosphate, varied from 2.04 to 5.54 mg/L, 42.33 to 53.67 mg/L, and 5.92 to 7.84 mg/L, respectively. Generally, the physicochemical characteristics of primary treated sewage were of medium-strength wastewater (Techobanoglous *et al.* 2014).

Performance of the field-scale VSSFCW using different macrophytes

The physicochemical characteristics of influent (S1) and effluent (collected from S2 and S3) were observed continuously on day 1 to day 6 for six months (January-June) and their average value is shown in Table 1.

The pH value as shown in Figure 3(a) indicated a slight decrease in the pH value when primary treated sewage was passed through wetlands planted with *Canna indica* and *Acorus calamus*. The pH value decreases in the vertical flow constructed wetland (VFCW) as during the nitrification

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Table 1 | Physicochemical characteristics of primary wastewater treated with *Canna indica* and *Acorus calamus* ($N = 24$)

Parameters	Primary treated sewage (influent)	*Canna indica* (treated effluent)	*Acorus calamus* (treated effluent)
pH	7.49 ± 0.19	6.80 ± 0.03	6.82 ± 0.06
Temp. [°C]	25.97 ± 3.51	23.80 ± 4.23	24.08 ± 3.77
Conductivity [μScm^{-1}]	895.18 ± 32.07	713.25 ± 16.13	749.19 ± 5.28
TDS [mg/l]	641.87 ± 16.84	498.65 ± 10.10	520.12 ± 49.06
Alkalinity [mg/l]	298.83 ± 36.40	145.58 ± 21.85	154.28 ± 22.09
BOD_5 days [mg/l]	99.97 ± 8.26	18.20 ± 5.56	21.25 ± 4.79
DO [mg/l]	1.99 ± 0.10	5.14 ± 0.35	4.62 ± 0.28
Nitrates [mg/l]	3.34 ± 0.96	8.64 ± 0.78	9.03 ± 0.87
Phosphate [mg/l]	6.66 ± 0.48	1.23 ± 0.50	1.36 ± 0.42
Total nitrogen [mg/l]	48.25 ± 3.17	19.12 ± 1.63	21.07 ± 1.76

process H^+ ions are released (Vymazal 2007), which lowers the pH of the effluent. The effluent temperature of the constructed wetlands was between 23.80 °C and 24.08 °C.

Conductivity is directly proportional to the concentration of dissolved solids, conductivity values of effluent collected from both the wetlands were slightly decreased when compared with the influent. The total dissolved solids (TDS) content was reduced in the wetland planted with *Canna indica* by 22.31% and in the wetland planted with *Acorus calamus* by 18.96% (Figure 3(b); Table 2). The removal efficiency of TDS was directly proportional to plant growth during the monitoring period. TDS removal may be improved by sedimentation for its assimilation by microbes developed at the roots of the plants in the wetland (Wu *et al.* 2011). The average value of alkalinity in the primary treated sewage was 298.83 mg/L, which decreased to an average of 145.58 and 154.28 mg/L for *Canna indica* and *Acorus calamus* respectively (Figure 3(c)). The decrease in alkalinity concentration indicates a decrease in pH value (García-Avila *et al.* 2019).

The removal efficiency for BOD_5 through *Canna indica* and *Acorus calamus* wetlands was 81.79% and 78.74% respectively, therefore *Canna indica* had higher efficiency than *Acorus calamus* (Table 2; Figure 3(d)). As the HRT was set for 144 hrs, BOD values might have been decreased due to the breakdown of organic matter by the microbial population attached to the roots of the macrophytes (Maina *et al.* 2011; Stefanakis *et al.* 2014). The average value of DO significantly increased from 1.99 mg/L to 5.14 mg/L in wetland planted with *Canna indica* and 4.62 mg/l in *Acorus calamus* planted wetland (Figure 3(e)). It has been observed that batch mode feeding produces better aeration conditions in the constructed wetlands than continuous feeding (Abdelhakeem *et al.* 2016).

The average concentration of nitrate in the effluent was more than in the influent for both VSSFCW units. In influent wastewater, nitrate concentration was 3.34 mg/L, after 144 hrs of HRT its concentration increased to 8.64 mg/L in wetland planted with *Canna indica*, and 9.03 mg/L in wetland planted with *Acorus calamus* (Figure 3(f)). Therefore, an increase in nitrate concentration in treated wastewater might specify the nitrification process. Since the VSSFCW was run in batch mode, aerobic conditions in the wetland have increased several times, which favours the nitrification process. Increasing nitrate concentration in treated effluent was also confirmed by several researchers in VSSFCWs (García-Avila *et al.* (2019); Abou-Elela & Hellal (2012)). Nitrification processes boosted in the VSSFCW might be because of the addition of oxygen from the atmosphere to the beds (Brix 1997). Plantation of macrophytes and the functioning of root systems in the wetlands may have also accelerated nitrification by oxygenation.

High efficiency of total nitrogen elimination was observed during the monitoring period. The initial concentration of total nitrogen was 48.25 mg/l. The removal efficiency of wetland planted with *Canna indica* was 60% and for wetland planted with *Acorus calamus* was 56% with a

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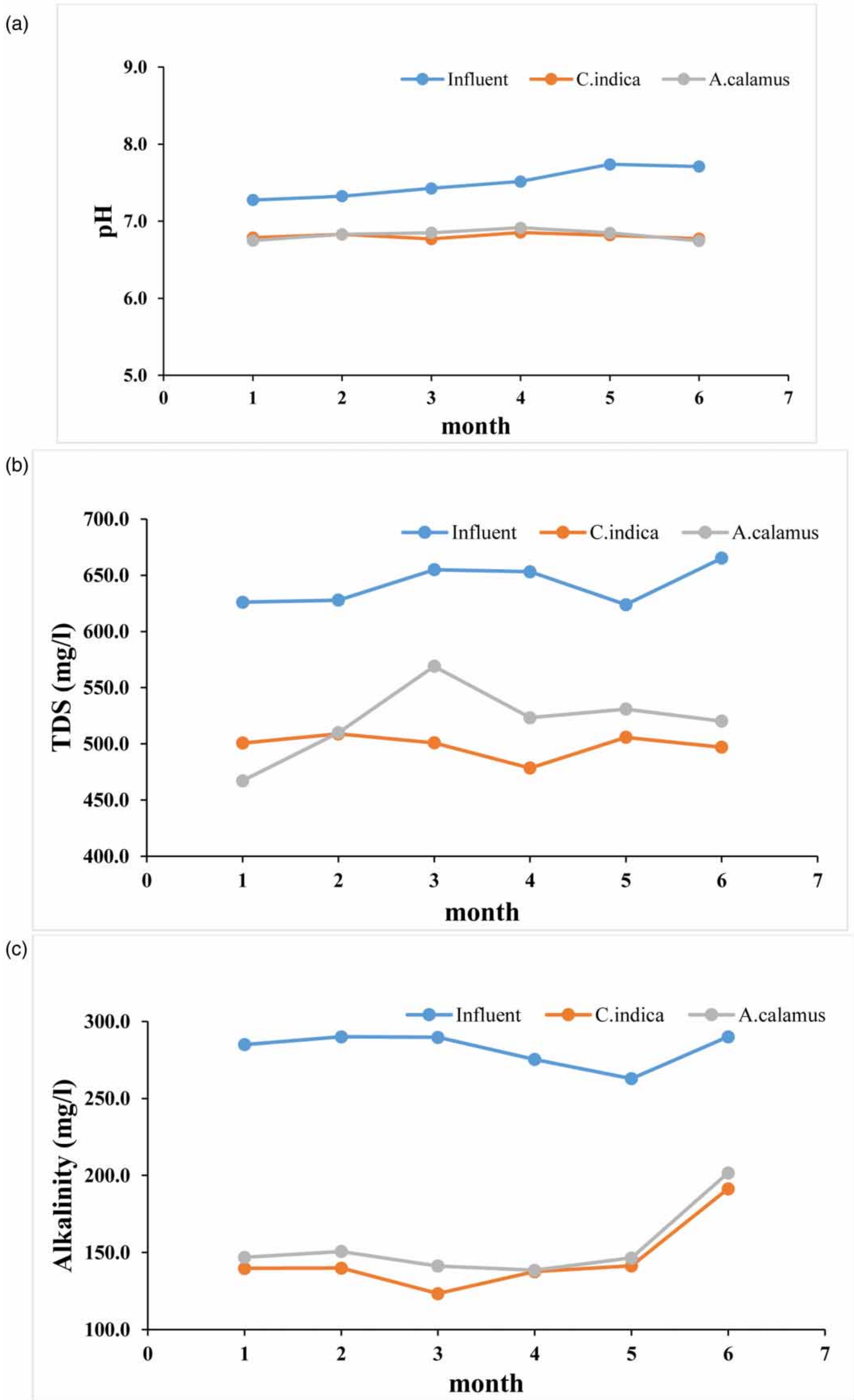

Figure 3 | Concentration of different parameters of influent and effluent in VSSFCW planted with *Canna indica* and *Acorus Calamus* during six-month operation (a) pH (b) total dissolved solids (c) alkalinity (d) BOD_5 (e) DO (f) nitrate (g) phosphate (h) total nitrogen. *(Continued.)*

residual value of 9.04 mg/l, and 13.82 mg/l respectively (Figure 3(h)). The removal of total nitrogen was higher and the performance better when compared to subsurface constructed wetland (SSCW) (Hernández *et al.* 2017).

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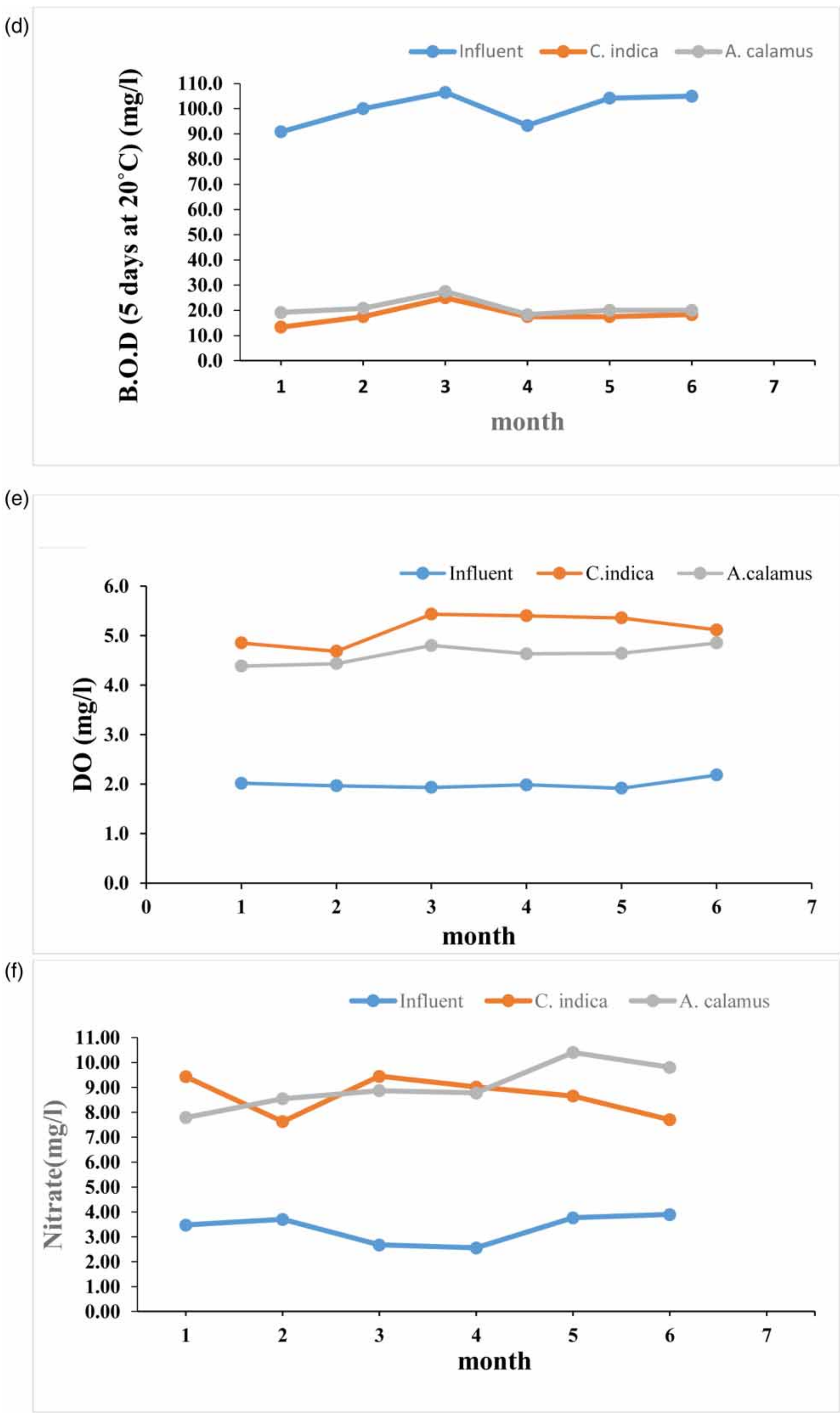

Figure 3 | Continued.

Initial phosphate concentration was 6.6 mg/l in influent wastewater, which was reduced to 1.23 mg/l in wetland planted with *Canna indica* and 1.36 mg/l in wetland planted with *Acorus calamus*. The phosphate values of the treated effluent shown that the removal efficiency of phosphate was effective using the two plants, results were very similar for both the experimental macrophytes, *Canna indica* 81% and *Acorus calamus* 79% (Figure 3(g)). However, Paruch *et al.* (2016) treated domestic wastewater and achieved phosphate removal efficiency up to 90% in HSSFCW.

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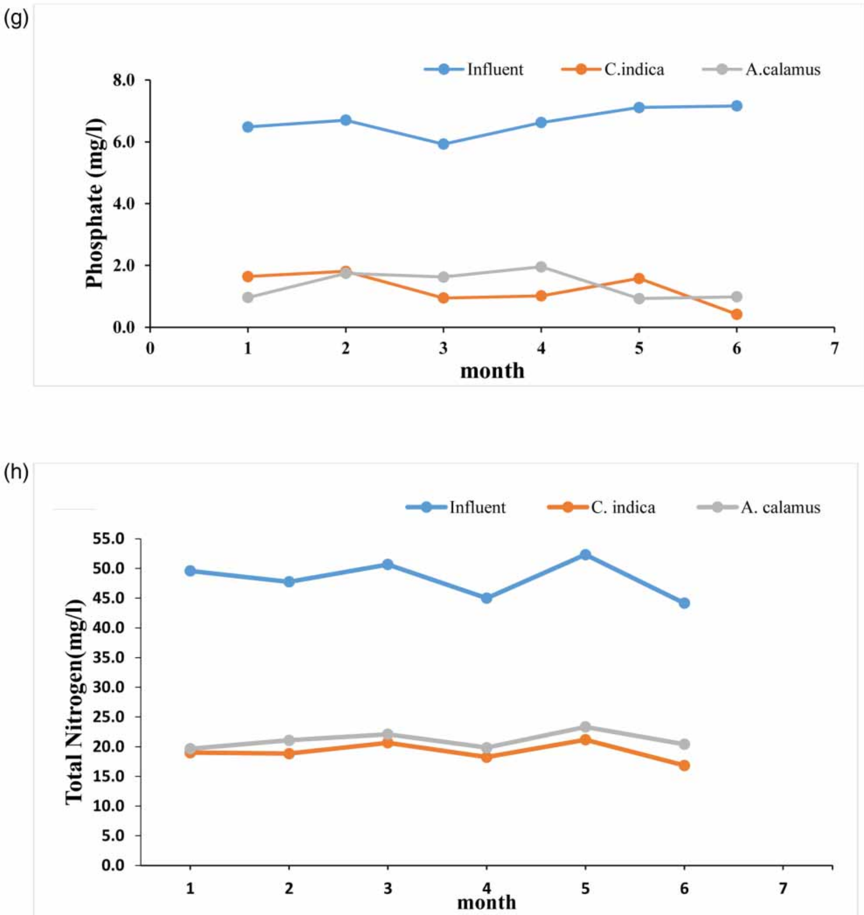

Figure 3 | Continued.

Table 2 | Removal efficiency of different pollutants from primary treated wastewater through *Canna indica* and *Acorus calamus*

Parameters	*Canna indica* (%)	*Acorus calamus* (%)
TDS	22.31	18.96
Nitrate	−61.34	−63.01
Phosphate	81.53	79.57
BOD	81.79	78.74
Total nitrogen	60.37	56.33

The average values of pH, conductivity, TDS, alkalinity, BOD_5, DO, nitrates, phosphate, and TN were observed throughout the monitored period in the VSSFCWs. A significant decrease was observed in concentrations of various contaminants during the six months of operation of the VSSFCW. To check the significance level for the data of various parameters, one-way ANOVA was applied and it was found that the results were significant at $p = 0.05$ (Table 3). In this operation, local plant species *Canna indica* and *Acorus calamus* endured very well the treatment condition in the VSSFCWs. Vegetation with high growth and biomass attest to the capability and progress in the sewage purification. Thus, *Canna indica* and *Acorus calamus* play a significant role in the purification of primary treated sewage by absorbing nutrients and degrading organic matter.

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Table 3 | Result of one-way ANOVA for selected parameters (i.e. BOD, nitrate, total nitrogen, and phosphate) of primary treated wastewater treated through *Canna indica* and *Acorus calamus* at *p*-value 0.05

	Canna indica		*Acorus calamus*		
Parameter	**F**	***P*-value**	**F**	***P*-value**	**F critical**
BOD	383.0055	4.34E-25	357.7822	1.95E-24	
Nitrate	168.2812	1.3E-17	179.1104	3.75E-18	4.03431
Total nitrogen	201.5262	3.60E-19	170.4916	9.84E-18	
Phosphate	367.0139	1.12E-24	357.9558	1.93E-24	

CONCLUSIONS

The treatment of sewage by constructed wetlands is a technically simple, eco-friendly and sustainable option as compared to the existing traditional sewage treatment technologies. Beside this, CW has low construction cost, and requires a minimum maintenance and operation cost. The VSSFCW utilized under the present study has proven quite efficient and successful in removing some major parameters of sewage like BOD, phosphate, total nitrogen, and so on. The removal efficiency of parameters like TDS, BOD_5, total nitrogen, phosphate, and alkalinity was as high as 22.31%, 81.79%, 60.37%, 81.53% and 51.28% for the wetland planted with *Canna indica* and 18.96%, 78.74%, 56.33%, 79.57% and 48.37% for wetland planted with *Acorus calamus* respectively, under fully established conditions. These values were comparable to many of the previously conducted studies. Thus, *Canna indica* was more effective in removing these parameters, whilst *Acorus calamus* was slightly less effective. Some other benefits of *Canna indica* are that it can bear temperatures from 18 to 30 °C; that is, it can adapt to subtropical areas; furthermore, by its competence of yield, it performed fast removal compared to the *Acorus calamus*, and was vital for its ornamental value. Based on the results of this study, VSSFCW planted with *Canna indica* can be recommended for the treatment of domestic sewage in the Indian subcontinent.

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