Int. J. Environ. Sci. Technol.
DOI 10.1007/s13762-017-1423-y

ORIGINAL PAPER

Potency of constructed wetlands for deportation of pathogens index from rural, urban and industrial wastewater

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Received: 17 December 2016 / Revised: 25 April 2017 / Accepted: 13 July 2017

Abstract Pathogen removal is essential for wastewater treatment and its potential reuse in agriculture. Three field-scale wastewater treatment systems consisting of free surface flow were operated around 1.5 years receiving water from urban domestic, rural domestic and industrial sources. The study was conducted to evaluate seasonal performance of constructed wetland systems in removing *Escherichia coli*, *Enterococci* and total coliforms under continuous hydraulic flow. Results displayed that all three wetlands gain recognition in removing pathogen load with high removal efficacy till water reaches output ports. Removal efficiencies were even higher, 66–93, 78–92 and 80–94% for *E. coli*, *Enterococci* and total coliforms, respectively, within constructed wetlands. Remarkably at shorter temporal scales in CW-A, greater homogeneity of pathogen concentrations was assessed at wetland outlet sites. In outlet ports, results displayed a highly effective removal of *E. coli* concentration 80–90% (June 2015), 86–92% (October 2015) and 79–92% (February 2016), *Enterococci* 80–94% (June 2015), 83–94% (October 2015) and 80–94% (February 2016) and total coliforms 85–93% (June 2015), 87–95% (October 2015) and 88–96% (February 2016). Positive correlation was observed between bacterial indicators (*E. coli*–*Enterococci*, $r = 0.038$; $p < 0.01$ and *E. coli*–total coliforms, $r = 0.142$; $p < 0.01$). Removal of bacterial indicators in constructed wetland was also displayed by PCA in which three-component analysis of variance was 98.39% and showed a clear decrease in measured parameter gradients toward samples from outlet ports. Constructed wetlands provide cost-effective treatment systems for reducing the pathogen load in wastewater in variable agro-climatic conditions and thus improve water quality.

Keywords Pathogen removal · Water reclamation · *E. coli* · *Enterococci* · Total coliforms · Principal component analysis

Introduction

Water sparsity is a critical problem experienced in the semiarid regions which has stimulated demand for reuse of treated water in agriculture. Thus, rephrase of treated wastewater (TWW) is seen as foremost substitute and necessity to meet agricultural demands when natural water resources are restricted. However, alleviating health and environmental risks from microbial pathogens in treated wastewater on crop, soil quality and consumers is another side to be considered that manifests the urgency to monitor wastewater treatment facilities (Dolan et al. 2014). With the raised scarcity of water budget, use of urban and rural wastewater in agriculture has escalated in arid and semiarid zones. Also, in many developing countries like India, irrigation with urban and rural wastewater is a fact of life in and around urban areas. Besides increase in crop yields due to the presence of nutrients in wastewater, there are several health implications. Wastewater from urban areas mainly contains water (99.9%) along with suspended and deliquesce organic solids, inorganic solids such as metals and also

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Editorial responsibility: M. Abbaspour.

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microbial pathogens (Paranychianakis et al. 2015). There is a prevailing habit for the disposal of urban and rural wastewater into lakes and rivers or nearby surface water bodies with zero or minimal treatment which creates problems for the public. Groundwater injection contributes in a very less amount to improve water quality for agricultural purpose while behaving as environmental buffers (Li et al. 2016). However, population growth and extensive economic development imposed severe pressure on groundwater environment and becomes as great social concern (Wu et al. 2013). On the other hand, effluents from industrial wastewater are fuelled by variety of contaminants such as acids, alcohols, fertilizers, salts, metals, dyes and pigment characterized with significantly higher values of chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), ammoniacal nitrogen (AN). depending upon the source of generation (Li et al. 2013). Complexity in industrial wastewater treatment arises mainly from the issues pertaining to volume of effluent, nature and concentration of pollutants and usually requires one or more processes of physical, chemical and biological methods which raised the overall treatment cost.

The presence of nutrients (nitrogen and phosphorus) in the treated wastewater boosted the plant production like natural water resources, but there is always a risk due to the presence of some microbial pathogens. The presence of pathogenic microorganisms is imperative in wastewater coming from different point sources and can allow an effective assessment of the treatment process and its reuse in agriculture. *Escherichia coli* are the common enteric bacterial genera found in wastewater and considered as major indicator of microbial pathogen along with *Enterococci* and Fecal coliforms (Ashraf 2015). Removal of these bacteria in treated wastewater does not necessarily guarantee the absence of pathogens as there may be always the chance of recycle while accumulating in the media. Thus, to impede transmission of many diseases, treatment of wastewater should comply with the microbiological quality (Lopez et al. 2010; Vivaldi et al. 2013) to avoid unfavorable impacts on crop quality and productivity (Chen et al. 2008).

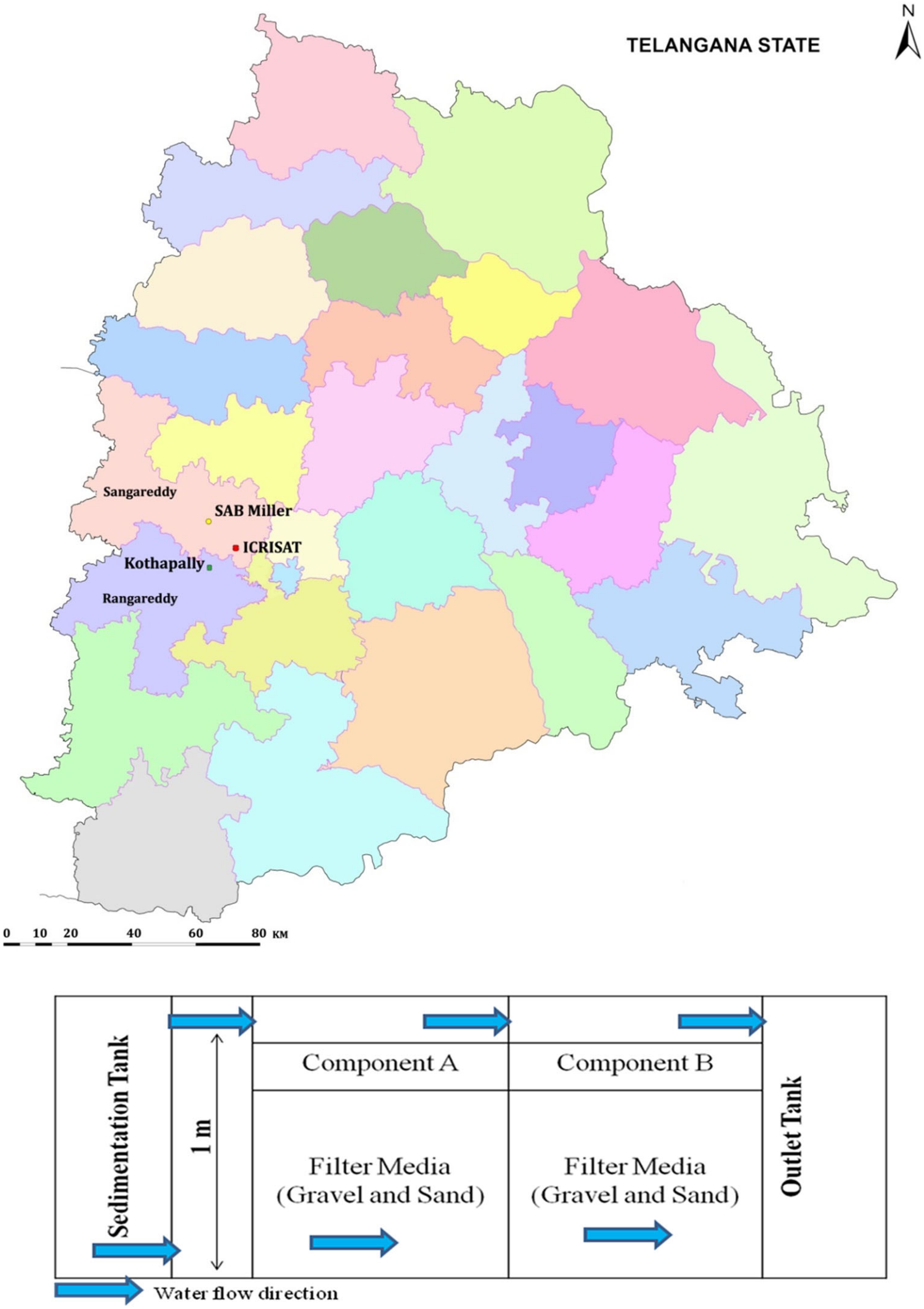
Constructed wetlands (CWs) are denoted biologically engineered architectures which are designed to mimic natural wetlands to eliminate excessive nutrients like nitrates, phosphates, pollutants, toxic substances and pathogens from industrial and municipal wastewater through interactions involving wetland substrates, plants and associated microorganisms in a cost-effective, environmental and sustainable manner (Auvinen 2016). Numerous research studies have reported CWs as an attractive on-site domestic wastewater treatment system adopted preferably in rural small communities, decentralized regions and developing countries for effective removal of organic carbon (expressed as BOD, COD and TOC), heavy elements and pathogens (Maestre-Valero et al. 2013). It has also been demonstrated that in wetlands pathogens are physically eliminated and biodegraded by multitude of mechanisms, viz. sedimentation, natural die-off, UV inactivation, biofilm sorption and predation by protozoans and bacteriophages (Kaushal et al. 2016). CWs are categorized free water surface (FWS) and subsurface flow (SSF) according to the flow of water in CWs. The latter is further categorized as subsurface vertical flow (SSVF) and subsurface horizontal flow (SSHF) constructed wetlands. Moreover, in earlier studies, utmost emphasis was given on constructed wetlands mainly used for urban sewage treatment. Also, the deportation microbial pathogens were generally qualitative and less quantitative. So this paper focuses on the three FWS wetland types receiving water from urban domestic, rural domestic and industrial sources under continuous hydraulic flow. The objectives of this investigation were directed toward seasonal detection of pathogenic bacteria and determination of caliber of CWs for the reduction of pathogens. Thus, in the present study, evaluation is extended to 1.5 years in order to better understand the long-term efficiency and potential stability of wetlands. The outcomes are useful to cater direct theoretical basis and practical experience in the feasibility application of running large-scale constructed wetland structures in the semiarid areas.

Materials and methods

Site description and constructed wetlands characterization

All the three constructed wetlands are located in southern part of Telangana state and belong to semiarid regions of southeastern India (Fig. 1). The mean annual temperature is 26.7 °C, the highest average temperature is 33.0 °C (in the month of May), and the lowest average is 21.6 °C (in the month of December). Average annual rainfall is 803 mm; August has the highest precipitation (163 mm), and December the lowest (3 mm). Constructed wetland A is located in RL-35 field of ICRISAT

Fig. 1 Study area, sampling locations and basic structure of constructed wetlands



Cross-section of constructed wetlands

campus (17°29′22.10″N latitude 78°16′47.30″E longitude; elevation 548.64 m). It receives wastewater from nearby urban housing colony of approximately 500 households. Constructed wetland B is located in Kothapally village (17°22′34.92″N latitude 78°07′16.86″E longitude; elevation 607.16 m). It has approximately 1800 inhabitants, arable and livestock smallholdings being the main economic ventures. Constructed wetland C is located in Medak district (17°39′47.23″N latitude 78°07′08.82″E longitude; elevation 500.78 m). It

Table 1 General characteristics of the three constructed wetlands

Constructed wetland	ICRISAT campus (domestic wastewater—urban) CW-A	Kothapally (domestic wastewater—village) CW-B	Sabmiller (industrial wastewater) CW-C
Age (years)	2	1	2
Vegetation type	*Typha latifolia, Canna Indica*	*Typha latifolia, Canna Indica*	*Canna Indica, Napier*
Vegetation coverage (%)	~80–90%	~60–70%	~70–80%
Design	Open water	Open water	Open water
Column water depth (m)	~0.5–1	~0.5–1	~0.5–1
Hydraulic flow	Continuous	Continuous	Continuous
Hydraulic residence time (days)	4	2.5	2.5
Contributing farm land (ha)	1.5	1	1.5

receives wastewater from a beer manufacturing industry (Sabmiller India Pvt. Ltd.). This study examined the comparison of three free surface flows (FSF), viz. constructed wetlands (CWs) A (receiving domestic wastewater from urban), B (receiving domestic wastewater from rural) and C (receiving wastewater from industrial) for removal efficacy and periodic changes of pathogens. All the three CWs are receiving continuous wastewater flow with hydrologic residence time (HRT) ranging from about 3–5 days and differed in various parameters such as age, shape and catchment area (Table 1). Wetlands A and B have two components arranged horizontally; each represented monoculture systems with emergent vegetation of *Typha latifolia* and *Canna indica*. However, wetland C has single component with multispecies systems of *Napier* and *C. indica* as the dominant vegetation. The inflow, outflow and vegetation densities were similar at all three CWs; the influent and effluent flow rates were relatively constant.

Sampling

Sampling frequency was at 15-day interval for CWs A–C between periods of (November 2014–April 2016). Eleven samples were collected in each interval: 4 from CW-A and 3 each from CW-B and CW-C. The sampling locations are shown in Fig. 1. Grab water samples were collected from inlet and outlet in sterile 1-L plastic cans and transported to laboratory where they were processed by gravity sedimentation.

General water quality characterization and microbiological analysis

All these samples were analyzed for pH, EC, total suspended solids (TSS), total nitrogen (TN), total phosphorus (TP), nitrate—N, dissolved oxygen (DO) and water quality pathogen indicators (total coliforms, *E. coli* and *Enterococci*). The physicochemical parameters were examined by standard methods given in APHA (2005). Various factors considered for the characterization of pathogens in wastewater include virulence, pathogenicity, survival and multiplicity of the microorganisms, resistance to environmental control measures, host specificity, portal of entry in water, potential for secondary spread as well as taxonomy and species variations. The challenges in the determination of pathogens in polluted water are the physical differences between the major pathogen groups; low concentration in a large quantity of water requires enrichment and concentration of the samples prior to detection processing, culture-independent detection method, as well as the detection of the host origin of pathogens. At present, there is no specified method to encompass the collection and analysis of a water sample for determination of all pathogenic microorganisms of interest. Even though culture-dependent methods are widely used for pathogens detection, these methods are limited by their low sensitivity and the excessive time needed to obtain reliable results. Membrane filtration technique (APHA 2005) was used, the most widely accepted method for determination of pathogens in wastewater including total coliforms, *Enterococci* and *E. coli*. The cells recovered can be directly cultured on differential and selective media so as to determine and assay the recovered bacteria by enrichment or development of colonies which further are characterized to confirm their identity. These pathogen detection methods are relatively simple and inexpensive, also without the demerits of not providing information on host origin. Processing of samples began within 2 h of collection and was analyzed for total coliforms, *E. coli* and *Enterococci* (colony forming units

Table 2 Water quality parameters from three studied constructed wetlands; mean ± standard deviation; $n = 4$ per location

Constructed wetland parameters	ICRISAT campus (domestic wastewater—urban) CW-A	Kothapally (domestic wastewater—village) CW-B	Sabmiller (industrial wastewater) CW-C
pH	7.2 ± 0.2	7.8 ± 0.6	8.2 ± 0.6
EC (dS/m)	1.3 ± 0.4	1.2 ± 0.1	1.5 ± 0.1
TSS (mg/l)	212.4 ± 102.7	208.6 ± 113.7	252.7 ± 119.8
TN (mg/l)	6.9 ± 2.3	7.6 ± 1.9	8.2 ± 2.2
TP (mg/l)	0.7 ± 0.2	0.9 ± 0.2	0.5 ± 0.1
Nitrate—N (mg/l)	8.2 ± 0.8	9.6 ± 0.6	10.2 ± 0.7
Dissolved oxygen (mg/l)	6.9 ± 1.2	7.7 ± 1.4	5.2 ± 0.9
E. coli (cfu/100 ml)	602 ± 212	619 ± 112	312 ± 202
Enterococci (cfu/100 ml)	2194 ± 369	3012 ± 324	2268 ± 264
Total coliforms (cfu/100 ml)	5698 ± 658	6289 ± 435	4321 ± 567

(cfu) per 100 ml) by membrane filtration technique (APHA 2005). Appropriately diluted (10^{-3}–10^{-7}) sample (100 ml in volume) volumes, in triplicate, were filtered and data presented are the mean of three independent determinations. To examine shorter-term temporal variability (days and weeks), total coliforms, *E. coli* and *Enterococci* were determined on samples taken over five consecutive days for CW-C. These samples were collected and examined following the methods described above. Data for differences in water quality parameters between influent and effluent for each CW were analyzed statistically.

Results and discussion

Water characteristics

Average water quality parameters of three constructed wetlands are shown in Table 2. pH and EC ranged from 7.2–8.2 and 1.2–1.5 dS/m, respectively. Solids (TSS) concentration ranged from 208 to 252 mg/l primarily removed through the method of settling and interception (Jenkins et al. 2015). Aggregation, sedimentation as well as size of wetland also influence the removal of suspended solids. Mean values at inlet and outlet ranged from 6.9 to 8.2 mg/l (TN) and 8.2 to 10.2 mg/l (nitrate—N) which are influenced by the microbial-mediated nitrification process. Average concentration of TP ranged from 0.5 to 0.9 mg/l, and removal process includes biomass accretion and also particle size distribution in wetland media. Average dissolved oxygen ranged from 5.5 to 7.2 mg/l with significant removal in all the three wetlands.

Microbiological analysis

E. coli

Escherichia coli concentration of input and output locations of three constructed wetlands is shown in Fig. 1. *Escherichia coli* concentration varied widely for a period of 18 months with no clear temporal trends. *Escherichia coli* concentration in wetland inputs ranged from 90 to 1430 cfu/100 ml. *Escherichia coli* concentrations in wetland outflows ranged from 28 to 320 cfu/100 ml with average 67–242 cfu/100 ml (Fig. 2). CW-A had highest (798 cfu/100 ml) and lowest (410 cfu/100 ml) concentrations of *E. coli* inputs with a removal 93% (highest) and 82%, respectively, until water reached outlet ports. Although CW-A also acquires lower removal efficacy of 77% with an average of 87% during whole sampling period. CW-B receives highest average *E. coli* input concentration (1189 cfu/100 ml), while CW-C had lowest average input (241 cfu/100 ml). However, removal efficiencies in both ranged from 75 to 83% (CW-B) and 66–77% (CW-C). In CW-A, CW-B and CW-C, possible sources of bacteria indicate shedding from human feces, live stocks and wildlife (Diaz et al. 2010). Significant reduction ($p < 0.05$) of *E. coli* concentration in output was observed as compared to input in all the three constructed wetlands during whole

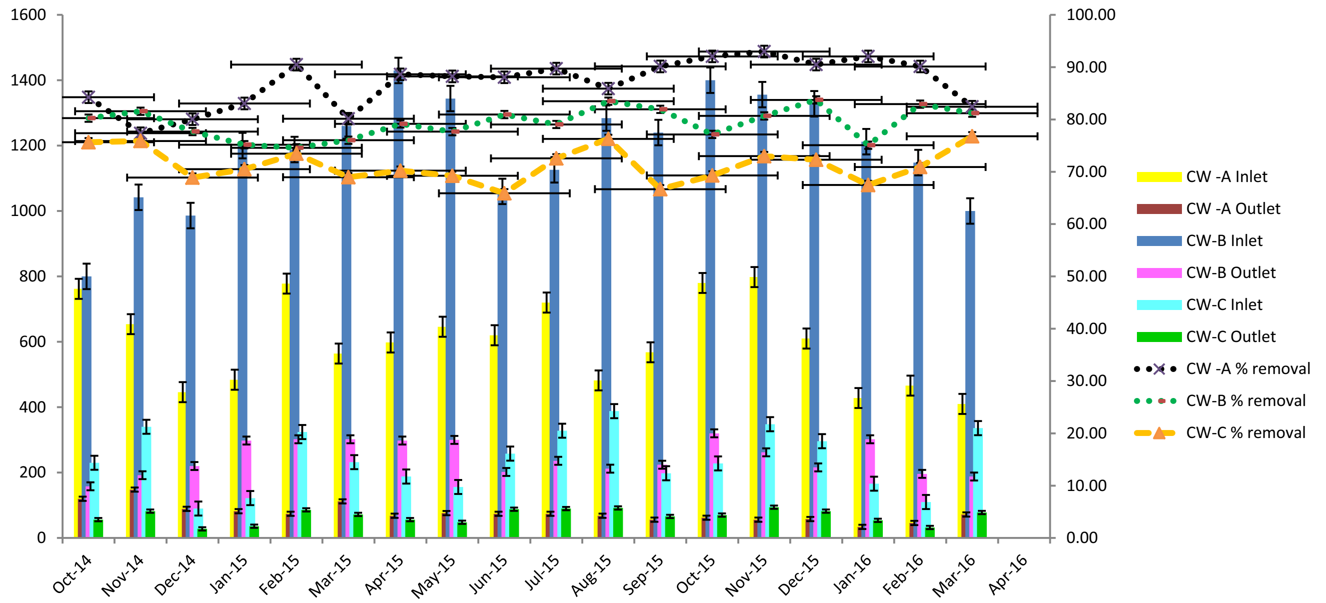


Fig. 2 *E. coli* concentration of three constructed wetlands at input and output sites with percent removal efficacy

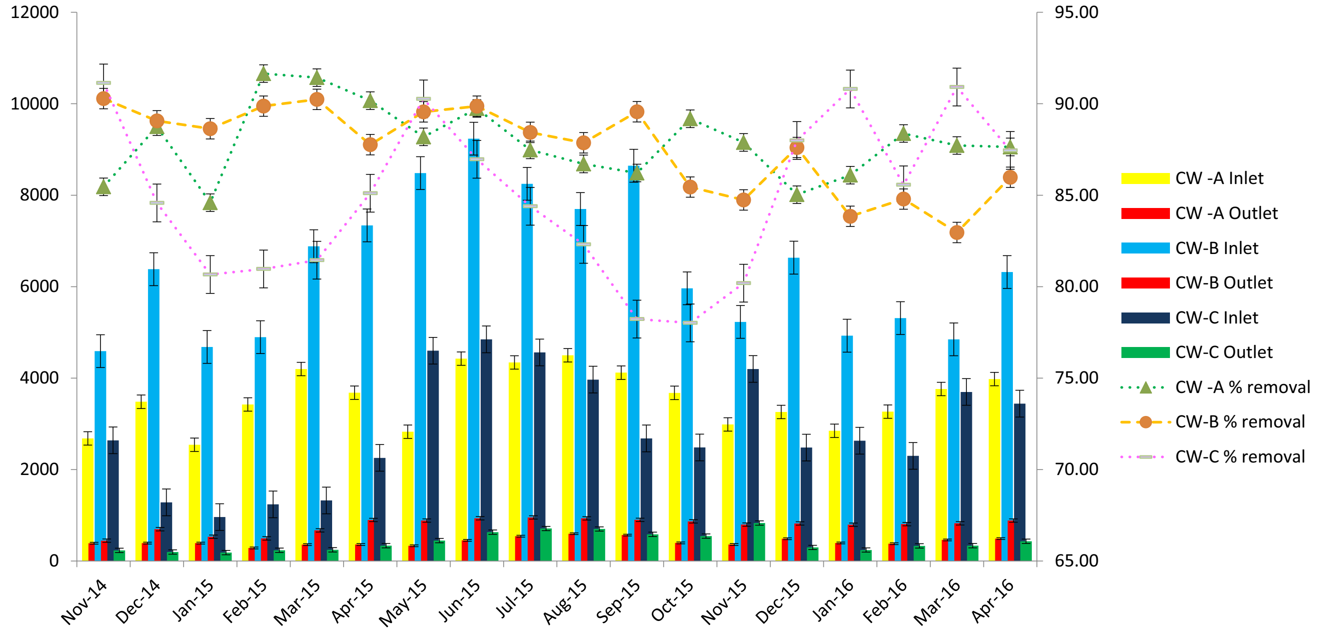


Fig. 3 *Enterococci* concentration of three constructed wetlands at input and output sites with percent removal efficacy

sampling period. Considering input and output concentrations, *E. coli* removal efficiencies ranged from 68 to 93% with an average of 87% (CW-A), 79% (CW-B) and 71% (CW-C) which are in agreement with previous studies (Harmel et al. 2013) where removal efficiency was 80%.

Enterococci

Constructed wetlands receive *Enterococci* concentration that ranged from 962 to 9236 cfu/100 ml with highest average of 6462 cfu/100 ml (CW-B); however, CW-C had lowest average input (2867 cfu/100 ml). *Enterococci*

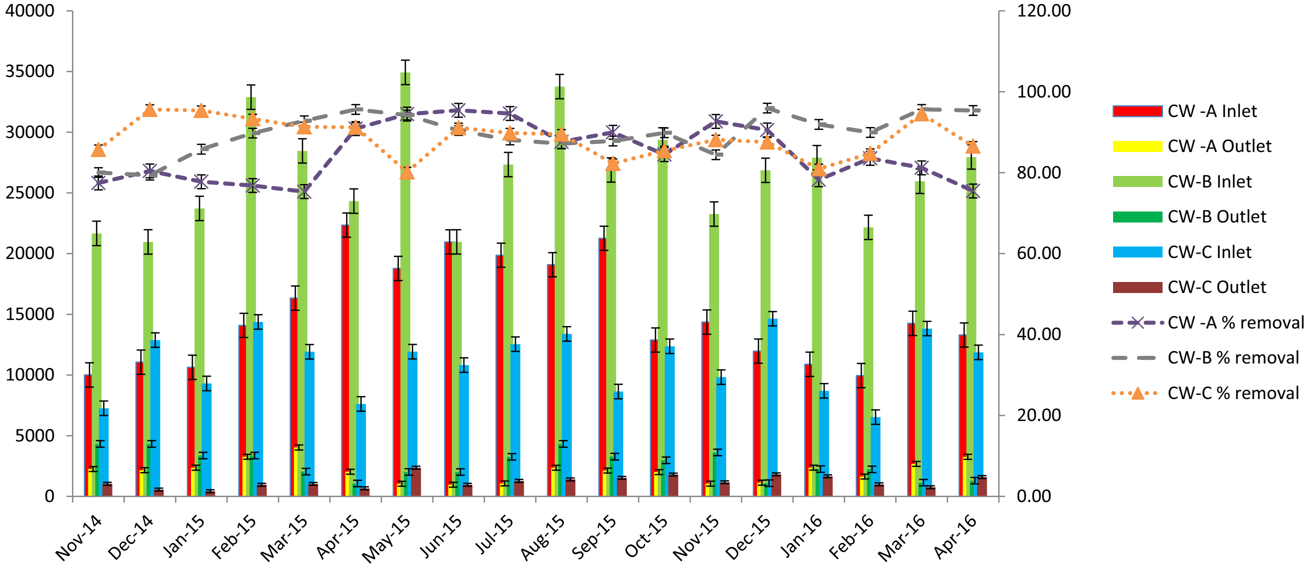


Fig. 4 Total coliforms concentration of three constructed wetlands at input and output sites with percent removal efficacy

concentrations in wetland outflows ranged from 198 to 954 cfu/100 ml with average 418 to 786 cfu/100 ml (Fig. 3). Similar to *E. coli*, significant reduction ($p < 0.05$) of *Enterococci* concentration in output was observed with removal efficiencies that ranged from 85 to 92% (CW-A), 83 to 90% (CW-B) and 78 to 91% (CW-C). Average removal efficiency in CW-A and CW-B is equal (88%), however CW-C had an average reduction of 85%. These results are in confirmation with those reported in earlier studies which also showed greater (80%) removal efficiencies of *Enterococci* (Vymazal 2005).

Total coliforms

Total coliforms concentration ranged from 6530 to 34,926 cfu/100 ml in three constructed wetland inflows (Fig. 4). Inlet water load of total coliforms was correlated with outflow, and observed significant removal ($p < 0.05$) ranged from 432 to 4326 cfu/100 ml. CW-A inlet load ranged from 9954 having 84% removal efficiency to 22,348, whereas 91% removal was observed in outlet samples. CW-B inlet ports had higher number with peak value of 34,926 cfu/100 ml, whereas 94% removal takes place in outflow; however, lowest inlet was 20,962 cfu/100 ml conferring removal of 80% total coliforms. CW-C receives highest (14,635 cfu/100 ml) and lowest (6530 cfu/100 ml) with reduction of 88 and 85%, respectively. Percentage removal efficiencies varied from 75 to 96% with average that ranged from 85 (CW-A) to 90% (CW-C) due to sedimentation which is considered as important mechanism for removal of coliforms in ecological treatment systems (Wahyuni 2015). Also suspended matters play a critical role in retaining and attaching about 25% coliforms in wastewater treatment effluents (Weinrich et al. 2010).

Despite high variability of inlet microbial load (*E. coli*, *Enterococci* and total coliforms) in CW-A, CW-B and CW-C, it was interestingly observed that inlet contamination does not influence the frequency of reduction at outlet ports. Therefore, at low inlet concentration of microbial load, a high reduction is possible and vice versa. However, results showed that all the three constructed wetlands assessed in this study confirmed removal efficiency of microbial loads at acceptable limits despite the types of wastewater (rural and urban domestic, industrial) they receive.

Concentration variability of bacterial indicators

In CW-A (located in ICRISAT campus), more intensive water sampling was carried out (daily) to assess concentration variability of bacterial indicators at shorter temporal scales (Fig. 5). Over 5-day period, minimum and maximum *E. coli* concentrations at input sites ranged from 398 to 528 cfu/100 ml (June 2015), 368 to 582 cfu/100 ml (October 2015) and 298 to 422 cfu/100 ml (February 2016). In outlet ports, results displayed

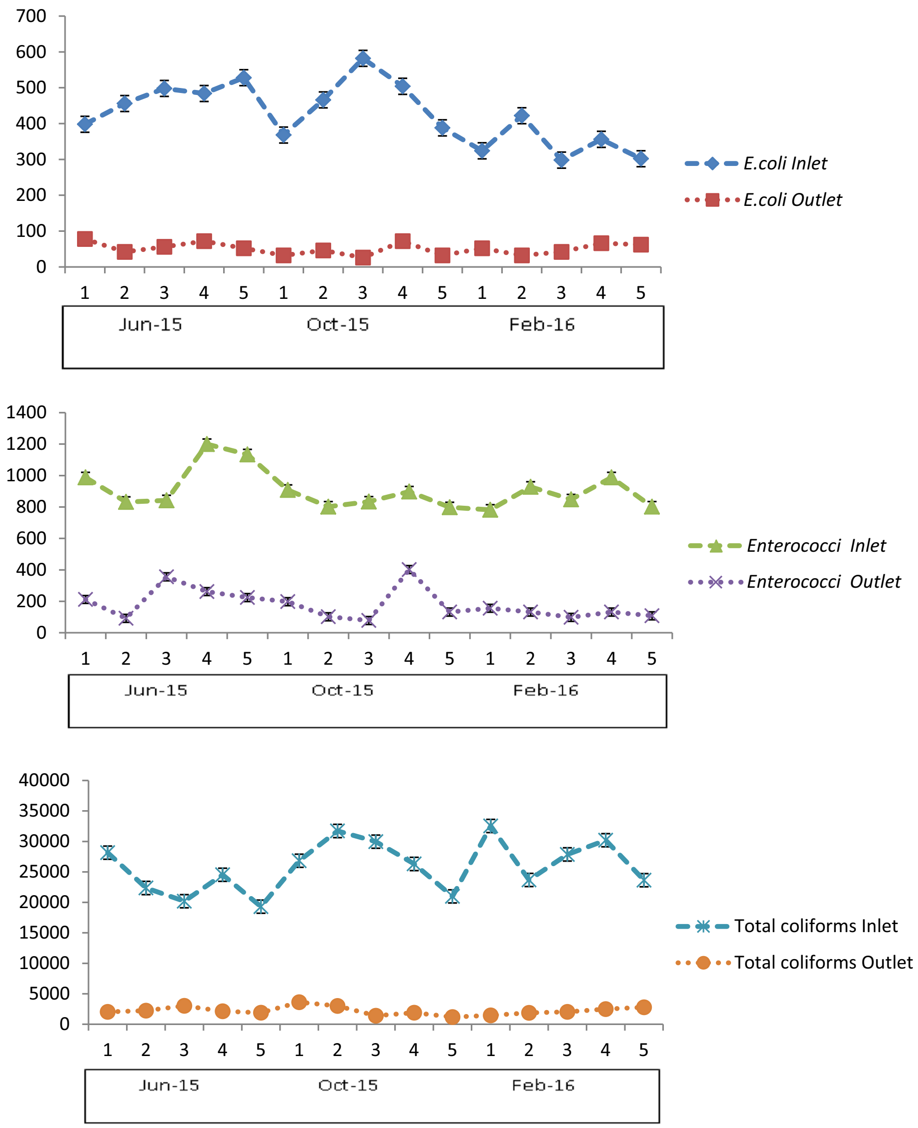


Fig. 5 Changes in *E. coli, Enterococci* and total coliforms concentration over 5 days for three different sampling periods (during June 2015, October 2015 and February 2016)

a highly effective removal of *E. coli* concentration, i.e., 80–90% (June 2015), 86–92% (October 2015) and 79–92% (February 2016). Similar results were obtained with *Enterococci* where removal efficacies at output sites ranged from 80 to 94% (June 2015), 83–94% (October 2015) and 80–94% (February 2016). Daily minimum and maximum removal efficiencies of total coliforms concentration over 5-day period at the output sites ranged from 85 to 93% removal efficiency (June 2015), 87 to 95% removal efficiency (October 2015) and 88 to 96% removal efficiency during February 2016. Bacterial diversity in wastewater coming from potential sources of urban colony in CW-A resulted in variability at input ports. Greater homogeneity of bacterial concentration was assessed at wetland outlet sites due to removal processes and mixing of waters across the wetlands (Jenkins et al. 2012). Results also demonstrated establishment of adequate sampling frequency to precisely characterize the removal of bacterial indicators in constructed wetlands.

Relationship between bacterial indicators (*E. coli*, Enterococci and total coliforms)

PCA was used for analysis of ordination for the measured bacterial indicators. Conclusions derived from three-dimensional figure represent ordination of samples in which three-component analysis of variance was 98.39%, a rather high value. High variability in water quality parameters demonstrated the ordination of samples at inlet ports to form heterogeneous group. Differences in outlet samples might be due to dissimilarities in wetland, displayed efficiency in treatment to homogenize water quality. Removal of bacterial indicators in constructed wetland was also displayed by PCA results (Fig. 6), which showed a clear decrease in measured parameter gradients toward samples from outlet ports.

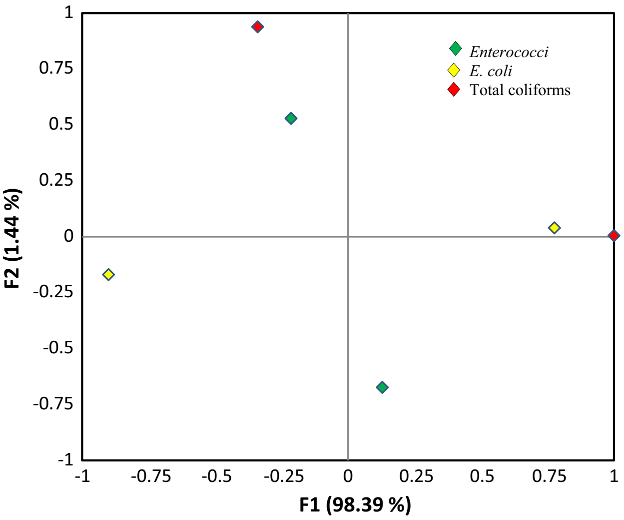


Fig. 6 Water sample ordination from input and output locations of three different wetlands using PCA

Data computed from correlation matrix revealed that only two bacterial indicators were significantly correlated (Table 3). Positive correlation was observed between bacterial indicators (*E. coli–Enterococci*, $r = 0.038$; $p < 0.01$ and *E. coli*–total coliforms, $r = 0.142$; $p < 0.01$) suggesting similar survival characteristics, source of origin and transport dynamics in wastewater. Bacterial indicators displaying positive correlations might due to the enrichment of nutrients playing hardball in the survival of microorganisms in wastewater (Wu et al. 2014; Rozema et al. 2016).

However, faster settle out of bacterial indicators occurs which are associated with suspended particles in wastewater, then free-floating ones (Spiller et al. 2015). Moreover, results also suggested that in constructed wetlands, bacterial indicators behave as free-floating cells suspended in wastewater rather than sediment particles in water columns. The mechanisms of pathogen removal may be grouped in three broad categories, viz. physical, chemical and biological all operating to bring about pathogen removal and die-off. Various factors responsible for removal of pathogens in wastewater include sedimentation, sunlight, attachment, filtration, predation and severe environmental conditions such as extreme dissolved oxygen and dissolved solids (Kaushal et al. 2016). *Escherichia coli* and coliforms belong to the same family Enterobacteriaceae with evolutionary adaptation to the intestinal tract. *Escherichia coli* was more susceptible to high pH, can be eliminated in a few days, and thus used as an indicator for

Table 3 Pearson correlation coefficients (r), p values and coefficients of determination (R^2) between *E. coli, Enterococci* and total coliforms concentration at constructed wetlands input sites

Variables	*E. coli*	*Enterococci*	Total coliforms
Pearson correlation coefficients (r)			
E. coli	**1**	0.038	−0.142
Enterococci	0.038	**1**	−0.208
Total coliforms	−0.142	−0.208	**1**
p values			
E. coli	**0**	0.881	0.575
Enterococci	0.881	**0**	0.408
Total coliforms	0.575	0.408	**0**
Coefficients of determination (R^2)			
E. coli	**1**	0.001	0.020
Enterococci	0.001	**1**	0.043
Total coliforms	0.020	0.043	**1**

Values in bold are different from 0 with a significance level alpha = 0.05

treatment efficiencies. Also, natural die-off rates were higher for *E. coli* than that of total coliforms. Thus, it is recommended that the total coliforms test could be better than using *E. coli* alone as a pathogen indicator in wastewater. On the other hand, *Enterococci* removal also is subjected to natural die-off due to longer retention time of water in constructed wetland system. However, increment in *E. coli* and total coliforms as observed for some months in present investigation may be due to bacterial growth, but the possible discharge of affixed bacteria (devoting to higher numbers) cannot be ruled out. Sunlight has a lethal effect on total coliforms, and the rate of die-off is proportional to the intensity of sunlight (Kaushal et al. 2016) because of detrimental effect of ultraviolet (UV) radiations. However, the die-off of bacteria can slow down with increase in depth of influents as sunlight cannot penetrate deeply enough. Pathogenic microorganisms were removed by filtration which consists of adhesion of smaller particles to large ones. Filtration by the medium (sand, gravel) and attached biofilm has been implied as one of the mechanisms involved in pathogen exclusion in wetlands. Pathogens get adhered (by flagella and fimbriae or pili) on the surface of plants (host tissues) such as *Typha latifolia* with extensive root system as well as on the inner walls of constructed wetlands and to solid matter like gravel that sinks to the bottom as sediments. Followed by adherence, removal of pathogens occurs by sedimentation as weight of pathogens raised that are attached with suspended solids which finally sink to the bottom resulting in the formation of biofilm. Sedimentation is the most dominating factor responsible for maximum pathogen removal in wastewater due to dormant conditions created by plant roots coverage and released exudates. Predation and competition for nutrients by other heterotrophic bacterial community can also influence pathogens die-off rates.

Constructed wetlands are a cost-effective, environmentally sensitive and technically expedient path to provide *reliable and ecologically sound* wastewater treatment of urban, rural and industrial areas besides seasonal constraints. Wetlands are simple to construct; operation (gravity operated) and maintenance expenses are also low and required only periodically. In addition to fluctuations tolerance (hydraulic and contaminant loading rates), wetlands also facilitate groundwater recharge, water reuse and recycling contributing a good wildlife habitat and the esthetic intensification of degraded territories. Besides this, CWs are also proved to be highly efficient in removing organic material, pesticides, heavy metals, BOD, TSS, N, P and waterborne pathogens. However, there are certain constraints associated with CWs treatment technologies as they generally crave larger land areas (depending on the design) and require larger retention time than conventional systems. Seasonal responses greatly influence wetland treatment efficiencies, including heavy rainfall and drought. In present study, clogging occurred eventually in all the three CWs which becomes major problem and needs to be fixed on regular basis. Also, *improperly designed and implemented CWs may expose the odor of the wastewater. Sometimes p*hosphorus removal seems to be notably poor in many CWs system due to reduced oxygenation in root zone of slow-moving waters. Besides all, these CWs may also accrue many pollutants in addition to sediments that have a natural affinity for solids such as heavy metals.

Though, wastewater treatment technologies using CWs are drawing ample attention worldwide, especially in developing countries where conventional techniques are not practicable. Depending on the structure of CWs and its configuration, obstacles may emerge that can be held in further research progress until a satisfactory discharge quality is achieved, so that short-lived disorders do not invent severe problems with the removal mechanisms. Future research should also be progressed that must comply with applicable discharge standards of wastewater from different sources. Besides, this future research should also focus on removal of pathogens in water that is recirculated, biochemical effect by microorganisms to treat wastewater with heavy metal concentrations, controlling hydraulic conditions and restoration of technology. Long-term performance of CWs should also be limelight in future investigation as there is not much information available. Recreation of concentrated hazardous materials in CWs is also pinpoint at the end of its useful life as it is not an inert element of the environment but rather a hazardous waste disposal spot.

Conclusion

Constructed wetlands, an economical and environmental-friendly technology, are sole source and capable of treating wastewater (rural, urban and industrial) through inexpensive means and reduced impact on the environment. These complex systems involve variable physical, chemical and biological transformations taking place in ostensibly random or systemized tone. Despite low cost, constructed wetlands are also highly efficient treatment systems which work round the hour irrespective of seasonal and other climatic variations.

In present investigation, three bacterial (*E. coli*, *Enterococci* and total coliforms) indicator organisms were considered in all types of wastewater irrespective of their point of origin. Results displayed that all the wastewater sources (rural, urban and industrial) contain high levels of bacterial indicators. All the three constructed wetlands (CW-A, CW-B and CW-C) produced significantly higher bacterial indicator (*E. coli*, *Enterococci* and total coliforms) removal throughout the study period (varies from 66 to 94%). During shorter temporal scales in CW-C, outlet ports displayed a highly effective removal of *E. coli* concentration (79–92%), *Enterococci* (80–94%) and total coliforms (85–96%) over 5-day period of investigations. Positive correlations between bacterial indicators were also observed despite seasonal variations and other environmental conditions. Promise of removing pathogens with esthetically pleasing environment was offered by all three wetlands, but possibility of re-entering the pathogens in water table was always there for which work can be done on the survival of microbiological contaminants in wetlands.

Thus, our constructed wetlands are proven to be efficient treatment substitute for diminishing and inactivation of pathogens in wastewaters originating from rural, urban and industrial sources. Moreover, our constructed wetlands works largely on rule of thumb for demonstrating highly effective results during whole year against the variable temperatures and other seasonal circumstances. The overall study therefore appeals the use for constructed wetlands for treatment of rural, urban and industrial wastewaters and can assist the restoration of our multifunctional ecological systems.

Acknowledgements Authors acknowledge Water4Crops (EU) and Department of Biotechnology (DBT), Govt. of India, ‘integrating biotreated wastewater reuse and valorization with enhanced water use efficiency to support the Green Economy in Europe and India’ for financial support to carry out this work.

Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

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