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Phytoremediation of municipal wastewater for reuse using three pilot-scale HFCW under different HLR, HRT, and vegetation: a case study from Egypt

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ABSTRACT

This study investigated the effect of multivariance on the performance of three identical pilot-scale subsurface horizontal flow constructed wetlands treating real municipal wastewater for reuse. Each basin was vegetated with certain kind of plants namely *Cyperus papyrus, Canna flaccida,* and *Phragmites australis.* They were operated simultaneously with the same configuration and at three different hydraulic loading rates (HLR), 0.18, 0.10, and 0.07 m^3/m^2 d, and at corresponding hydraulic retention times (HRT), 1.8, 3.2, and 4.7 d. The flow rate in each basin was 8 m^3/d. Each basin has three identical sampling points representing a specific HLR and HRT. Results indicated that the highest removal values of organic, nutrients, and pathogens were achieved at 0.07 m^3/m^2 d HLR and 4.7 d HRT. The order of the removal efficiency of biochemical oxygen demand and chemical oxygen demand in the three basins was *P. australis* (88.6% and 89.1%) > *C. papyrus* (86.9% and 87.5%) > *C. flaccida* (83.4% and 83.5%). The higher removal efficiency of nitrogen and phosphorus was achieved by *C. papyrus* followed by *P. australis* then *C. flaccida* plant species. Removal of three logs of total coliform, fecal coliforms, and *Escherichia coli* was obtained with *C. papyrus*, while *C. flaccida* and *P. australis* removed only 2.5 logs.

Keywords: Horizontal flow constructed wetland; Plants; HLR; HRT; Wastewater treatment; Reuse

1. Introduction

Water scarcity is one of the most emerging problems around the world, and Egypt is not exempted. It is expected that there will be water poverty in the next five decades. According to World Health Organization [1], there are 2.6 billion people living without proper means of sanitation and 1.1 billion without access to improved drinking water. Shortage of drinking water supplies and sanitation services causes 2–5 million deaths per year, and it is found mostly in developing nations [2]. As the world's population continues to grow and freshwater resources continue to be used and degraded, these problems will intensify. Because of these discouraging estimates, many cities and communities around the world are starting to adopt appropriate technologies and successfully promote water reclamation and reuse to preserve the limited high-quality freshwater supplies, while helping to meet the ever-growing demand for water. A major handicap hindering progress in using treated wastewater for irrigation is the low-coverage sanitation system in developing countries. A universal crisis is rapidly aggravated due to the shortage in supply of clean freshwater and the lack of adequate sanitation facilities. The availability of freshwater per capita is decreasing rapidly. About 80 countries, representing 40% of the world's population, are experiencing water stress, and about 30 of these countries are suffering from water scarcity during a long part of the year [3]. Historically, traditional centralized sewage treatment systems have been used successfully for water pollution control in most countries [4]. However, these wastewater treatment technologies such as activated sludge process, membrane bioreactors, and membrane

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separation are rather expensive and not entirely feasible for widespread application in rural areas [5]. Furthermore, they are limited and insufficient when facing ever more stringent water and wastewater treatment standards [6]. Thus, selecting low-cost and efficient alternative technologies for wastewater treatment is significant especially in developing regions. Several types of researches have been conducted to find a solution to this problem, such as creating some low-cost decentralized treatment units. Examples of these units are the biological aerated filter, passively aerated biological filter, upflow anaerobic sludge blanket, oxidation ponds, and constructed wetlands (CWs). The technology of CWs is an effective technique in wastewater treatment, especially in developing countries. It is capable of removing organic and inorganic matters, pathogens, and nutrients; besides, it has several advantages [7]. It is cost-effective, easy to operate, and has a good landscape [8]. Although there are many types of CWs, the horizontal subsurface flow is the most commonly used [9,10]. Horizontal flow constructed wetlands (HFCWs) are an alternative option for secondary treatment prior to land application. HFCWs in tropical regions are considerably more effective than non-tropical systems and may show organic and nutrient removal rates almost a factor of 10 higher than conventional systems [8].

There are different factors affecting the performance of CWs such as hydraulic loading rate (HLR), hydraulic retention time (HRT), and vegetation. The type of macrophytes is considered as the basic constituent of CWs, and its location in the basin distinguish CWs from unplanted lagoons [11]. The performance of CWs depends on the interaction between plants, substrate, and microorganisms. Plant species make a significant contribution to the natural purification process of wastewater, even under high pollutant load conditions [12]. The plants used in HFCW stabilize the surface of the beds, provide good conditions for physical filtration, insulate the surface against frost during winter, and provide a huge surface area for attached microbial growth [13].

The most macrophytes used in CWs are cattail (*Typha* sp.), common reed (*Phragmites* sp.), rush (*Juncus* sp.), and bulrush (*Scirpus* sp.). Recently other plants such as *Canna flaccida* (red and yellow) (*C. flaccida* spp.), umbrella palm (*Cyperus alterufolius*), and woolgrass (*Scirpus cyperinus)* are used [8]. Different research works have been carried out on the effect of plant species on the performance of CWs. Hua et al. [14] did not find differences amongst different plant species in organic matter and pathogen removal or in nutrient removal. However, Maltais-Landry et al. [15] and Leto et al. [12] found a higher efficiency in nutrient removal in CW planted with *Typha* sp. in comparison with other species. Studies reported by Fraser et al. [16] indicated better removal efficiency using *Scirpus validus*, *Iris pseudacorus* [17], or *Phragmites australis*. In a study by Villaseñor Camacho et al. [17], *Juncus effusus* reached lower biochemical oxygen demand (BOD), nitrogen, and phosphorus removal efficiencies than *Typha latifolia* L.

All the abovementioned studies focused on the performance of CW planted with only one kind of plant under different operating conditions, while other works studied the effect of plant types on the performance of CW under the same operating conditions. Therefore, the main objective of this study is to investigate and evaluate the effect of HLR, HRT, and vegetation on the performance of three identical pilot-scale HFCWs treating real municipal wastewater, and they were operated at the same time and under the same operating conditions.

2. Materials and methods

2.1. Pilot plant description

To achieve the objective of this study, a pilot-scale subsurface HFCW system was designed, constructed, and put into operation within the vicinity of Abu Rawash Wastewater Treatment Plant, North Giza Governorate, Egypt. It consists of three separate and identical basins (A, B, and C). Dimensions of each basin are length = 33 m, width = 5.5 m, and depth = 0.85 m. The surface area of each basin was 181.5 m^2 with 0.7% slope along the basin. The three basins were filled with 20–25 mm diameter of gravel in the entire basin except 1 m from the beginning, and the end of the basin was filled with 40–80 mm gravel to prevent clogging. The bottom of HFCW was covered with 2 mm poly vinyl chloride (PVC) liner to prevent the seepage to the groundwater. Each basin was planted with certain kind of plant species. Basin A was planted with *C. flaccid*, Basin B was planted with *P. australis*, and Basin C was planted with *Cyperus papyrus*. Three sampling points were established across the longitudinal axis of each basin at a spacing distance of 10 m from each other to represent different operating conditions such as HLR and HRT. The first sampling point was located 13 m away from the influent feeding point of the basin. The plantation in the basin started 2 m from the influent distribution point. The three basins were fed with real pre-settled municipal wastewater at the same time using a submersible pump and PVC pipes inserted through three wide holes of diameter 4 inches at the beginning of each basin. The distance between every hole was 1.5 m. The wastewater flows horizontally below the surface of the gravels at the beginning of the basins up to the end. A schematic diagram of the engineering layout and a profile photo of the three different basins are illustrated in Figs. 1 and 2, respectively.

The three basins were operated under the same operating conditions for 1 year during 2016–2017. The only difference was the type of vegetation. The water flow was measured by an electromagnetic flow meter. The flow rate and the runoff of the pumps were controlled using SCADA software. All the main climatic parameters affecting the HFCW hydrobalance such as temperature, wind speed and direction, humidity, and atmospheric pressure are monitored on daily basis and recorded with Solar Radiation Sensor Model DW-6450 (Davis Instruments , California 94545 USA) [18]. The design criteria and operating conditions of the three basins are shown in Table 1.

2.2. Sampling locations, collection, the frequency of sampling, and duration

Wastewater samples were collected on a biweekly basis from the influent and the three sampling points along each basin axis. The total numbers of samples were 250. Also, various parts of the plant (stem, leaves, and roots) were collected on a monthly basis for analysis. The samples were collected and analyzed for almost 1 year (2016–2017).

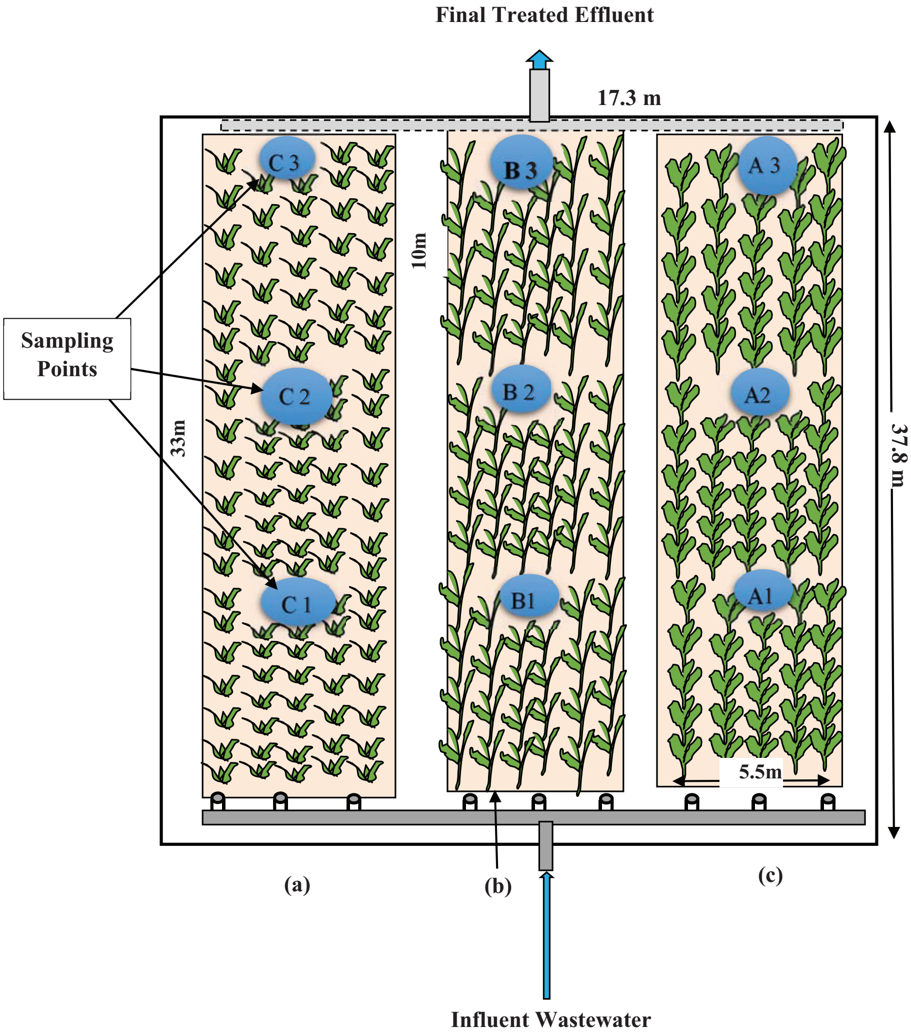

Fig. 1. Engineering layout of the HFCW: (a) *Canna flaccida*; (b) *Phragmites australis*; (c) *Cyperus papyrus*.

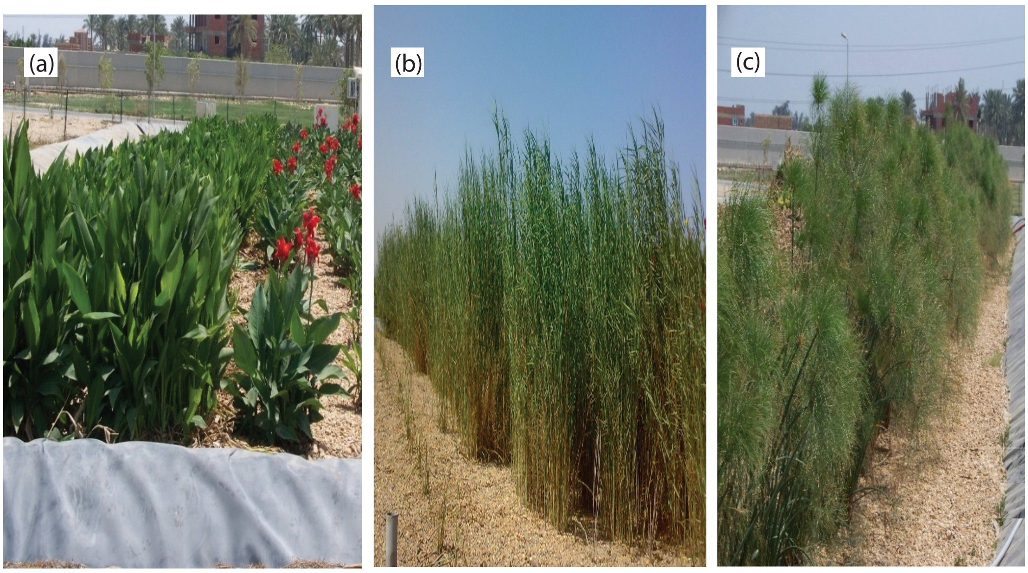

Fig. 2. Profile photos of the three basins: (a) *Canna flaccida*; (b) *Phragmites australis*; (c) *Cyperus*.

Table 1
Design criteria and operating conditions for the three HFCW basins

Design parameters	Basin A			Basin B			Basin C		
	A1	A2	A3	B1	B2	B3	C1	C2	C3
Plants	*Canna flaccida*			*Phragmites australis*			*Cyperus papyrus*		
Length (m)	13	23	33	13	23	33	13	23	33
Surface area (m^2)	71.5	126.5	181.5	71.5	126.5	181.5	71.5	126.5	181.5
Total volume (m^3)	60.7	107.5	154.2	60.7	107.5	154.2	60.7	107.5	154.2
Effective volume (m^3)	24.3	43	61.7	24.3	43	61.7	24.3	43	61.7
HRT (d)	1.8	3.2	4.7	1.8	3.2	4.7	1.8	3.2	4.7
HLR (m^3/m^2 d)	0.18	0.1	0.07	0.18	0.1	0.07	0.18	0.1	0.07
Average OLR (kg BOD/m^3 d)	0.094	0.053	0.037	0.094	0.053	0.037	0.094	0.053	0.037
Width	5.5 m								
Flow rate for each sector	8.3 m^3/d								
Depth	0.85 m								
Porosity	40%								

2.3. Physicochemical and biological analysis

In situ measurement of pH, total dissolved solids (TDS), and temperature was measured using Thermo Fisher Scientific (USA) Orion 5-Star Meter. Chemical oxygen demand (COD), nitrite (NO_2^-), and nitrate (NO_3) were measured with a Hach Company (USA) DR 6000 Spectrophotometer. Analyses of ammonia ($N–NH_4$) and total Kjeldahl nitrogen (TKN) were carried out using GERHARDT GMBH & Co. (USA) Vapodest 10sn digestion and distillation apparatus. Analyses of BOD_5, TSS, total coliforms (TCs), fecal coliforms (FCs), and *Escherichia coli* were performed according to the procedures in Standard Methods for Water and Wastewater Examinations [19]. Analysis of heavy metals namely lead (Pb), chromium (Cr), nickel (Ni), copper (Cu), cadmium (Cd), and zinc (Zn) was carried out using inductively coupled plasma atomic emission spectrometry, Agilent 5100 Synchronous Vertical (USA). The analysis of heavy metals was carried out because the municipal wastewater in Abu Rawash WWTP receives few industrial wastewaters.

2.4. Characterization of influent wastewater

Physicochemical and biological analysis of the influent wastewater indicated great variations in its strength. TSS ranged from 84 to 122 mg/L with an average value of 102.4 mg/L, while TDS ranged from 430 to 550 mg/L. The BOD_5 concentration ranged from 150 to 220 mg/L, with an average value of 175.7 mg/L. The COD value ranged from 260 to 360 mg/L. Ammonia and TKN concentrations varied from 19 to 27.1 mg/L and from 30.6 to 50.2 mg/L.

Analysis of heavy metals (Pb, Cu, Ni, Cr, Cd, and Zn) indicated the presence of very low concentrations. This was expected because the raw wastewater used in this study is mainly municipal wastewater and received few industrial wastewaters. All physicochemical analysis of influent wastewater indicated that it is a medium-strength wastewater [20].

3. Results and discussion

3.1. Effect of HLR, HRT, and plant species on the performance of HFCW

Comparative study of the three plant species at different HLR, organic loading rate (OLR), and HRT was as follows:

3.1.1. Case study 1: at sampling points A1, B1, and C1

The performance of each plant under consideration and at HLR = 0.18 m^3/m^2 d, OLR = 0.094 kg BOD/m^3 d, and HRT = 1.8 d is shown in Table 2.

3.1.1.1. Reduction of TSS, BOD, and COD Results indicated that vegetation with different plant species has a noticeable effect on the performance of the treatment system. The efficiency of three plants followed the order *P. australis* > *C. papyrus* > *C. flaccida* as shown in Fig. 3. The residual concentrations of TSS, BOD, and COD effluent in Basin B (*P. australis*) were 50, 54, and 92 mg/L, respectively, versus 53, 62, and 103 mg/L of effluent in Basin C (*C. papyrus*) and 59, 69, and 118 mg/L of effluent in Basin A (*C. flaccida*). These results are better than that recorded by Çakir et al. [21]. They obtained 41.3 mg/L TSS, 161 mg/L BOD, and 258 mg/L COD, under HLR of 0.125 m^3/m^2 d and HRT of 2.2 d. In this study, the low removal rate of BOD and COD at HLR of 0.18 m^3/m^2 d and HRT of 1.8 d may be related to the insufficient contact time within the system. Reed and Brown [22] found that BOD_5 removal in CWs is very low less than 1 d HRT and improved gradually until it reached about 7.5 d.

3.1.1.2. Nitrogen compounds removal Results depicted in Fig. 4 indicated that higher HLR (0.18 m^3/m^2 d) and lower HRT (1.8 d) had a negative effect on ammonia and TKN removals. The removal of TKN was very limited in the three basins at the beginning of the study. The average removal rate reached 16% using *C. flaccida*, 29% using *P. australis*, and 34% using *C. papyrus*. The corresponding residual concentrations were 34, 29, and 27 mg/L. Results presented

Table 2
Physicochemical characteristics of treated effluents from the three basins at sampling points A1, B1, and C1 (HLR 0.18 m^3/m^2 d, OLR 0.094 kg BOD/m^3 d, and HRT, 1.8 d)

Parameters	Average influent wastewater	*Canna flaccida* Basin A			%R	*Phragmites australis* Basin B			%R	*Cyperus papyrus* Basin C			%R
		Minimum	Maximum	Mean		Minimum	Maximum	Mean		Minimum	Maximum	Mean	
pH	7.2	7.0	7.76	7.3	–	7.0	7.6	7.3	–	6.9	7.7	7.2	–
TDS (mg/L)	498	446	623	520	–	460	616	531	–	400	600	527	–
TSS (mg/L)	102.4	42	82	59	42	38	74	50	51.1	39	75	53	48.2
BOD_5 (mg/L)	175.7	38	124	69	60.7	32	98	54	69.2	37	108	62	64.7
COD (mg/L)	304.4	70	201	118	61.2	60	169	92	69.7	61	185	103	66.1
NH_3 (mg/L)	22.7	18.1	26.2	21.8	4	13.5	24.2	18.5	18.5	13	24	17	25.1
TKN (mg/L)	41.07	26	45.2	34.7	15.5	19.5	42	30	26.9	18	39.6	28	31.8
NO_2^- (mg/L)	0.03	0.03	0.166	0.07	–	0.03	0.16	0.06	–	0.04	0.14	0.07	–
NO_3^- (mg/L)	0.7	0.14	1.5	0.97	–	0.28	1.2	0.86	–	0.22	1.22	0.9	–

in Fig. 4 indicated that ammonia concentrations steadily decreased in the three basins at starting time and increased again at the end of the study. This may be explained by the process nitrate-ammonification, that is, reduction of nitrate to ammonia under anaerobic conditions [23]. The nitrate concentration (Fig. 4) was increased steadily then decreased with the increase of ammonia concentration. These results can be explained that when the HLR increased, overfeeding and soil permeability decreased quickly to a small value resulting in anaerobic condition leading to the great elevation of NH_3 concentration in the effluent under these conditions, and accordingly, the average TKN removal efficiency decreased.

3.1.1.3. Microbial removal The results depicted in Table 3 show that despite the use of different plant species only one order of magnitude of TC, FC, and *E. coli* was achieved by all plants under investigation. These results are in agreement with that obtained by Abou-Elela et al. [10]. They studied the effect of HLR on the performance of HFCW planted with *C. papyrus* at different HLRs. They concluded that the bacterial indicators removal was directly affected with HRT and HLR. The removal of bacterial indicators at this high HLR and low HRT may be attributed to sedimentation. Several studies have found that coliforms and FCs tend to concentrate in sediments of CW at lower HRT [24]

3.1.2. Case study 2: at sampling points A2, B2, and C2

The impact of decreasing the HLR to 0.10 m^3/m^2 d and OLR to 0.053 kg BOD/m^3 d while increasing HRT to 3.2 d is recorded in Table 4.

3.1.2.1. Reduction of TSS, BOD, and COD By decreasing HLR from 0.18 to 0.10 m^3/m^2 d and increasing HRT to 3.2 d, the removal efficiency of the treated effluents from all basins was increased, and their removal values were related to the type of plant. The order of the average removal rates of COD and BOD was *P. australis* > *C. papyrus* > *C. flaccida*. The corresponding average removal rate of COD reached 81.2%, 77.6%, and 71.4%, respectively, while it was 80.6%, 77.2%, and 71.5% for BOD. Decreasing the HLR from 0.18 to 0.10 m^3/m^2 d increased the removal of TSS in all basins. It was 68.7% in Basin B planted with *P. australis*, 64.8% in Basin C planted with *C. papyrus*, and 57% in Basin A planted with *C. flaccida*. A comparison of residual values of TSS, BOD, and COD in the treated effluents in the three basins at A2, B2, and C2 at HLR of 0.10 m^3/m^2 d and HRT of 3.2 d is shown in Fig. 5. Al-Omari and Fayyad [25] reported that COD and BOD reduction in wetlands is achieved by aerobic bacteria attached to the media and to the plant's roots where oxygen is provided by natural reaeration and through plants roots. Also, residence time, which is a function of bed volume and flow rate, plays a significant role in the organic matter and suspended solids removal.

3.1.2.2. TKN and ammonia removal Decreasing HLR and increasing HRT to 3.2 d showed that *C. papyrus* was the most efficient plant for the removal of nutrients. The average removal of NH_3 and TKN reached 16% and 27% in *C. flaccida* basin, 33.9% and 41.5% in *P. australis* basin, and 42.7% and 46.4% in *C. papyrus* basin. The results obtained by Van

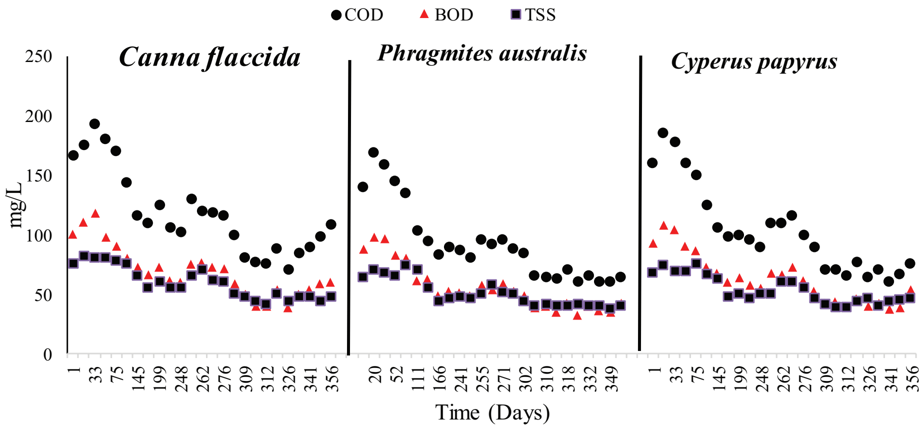

Fig. 3. Residual values of TSS, BOD, and COD for the treated effluents from the three basins at sampling points A1, B1, and C1.

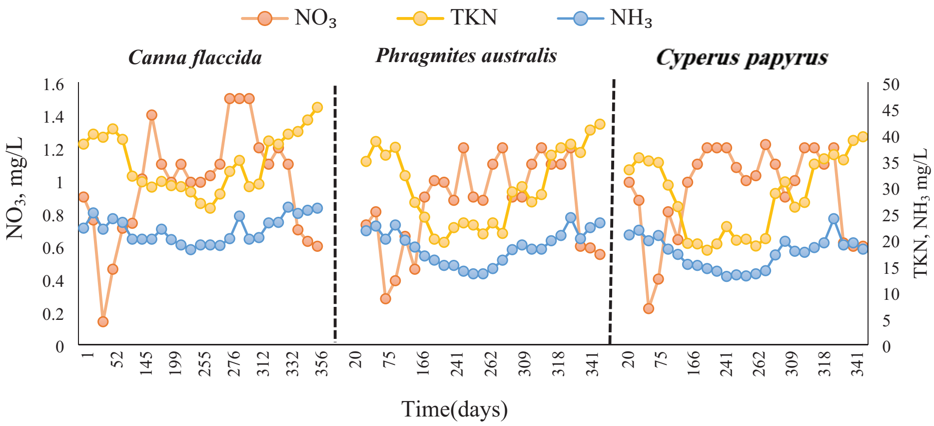

Fig. 4. Variations of TKN, NH_3, and NO_3 in the treated effluents from the three basins at sampling points A1, B1, and C1.

Table 3
Average bacterial counts of the treated effluents from the three basins at sampling points A1, B1, and C1

Parameters		Total coliform	Fecal coliform	*Escherichia coli*
Influent wastewater	Average	4.06×10^7	2.95×10^6	2.09×10^4
Canna flaccida	Minimum	1.8×10^5	5.5×10^4	1.5×10^3
	Maximum	6.3×10^7	4.6×10^6	9.4×10^3
	Average	1.4×10^7	1.0×10^6	4.3×10^3
Phragmites australis	Minimum	1.8×10^5	5.6×10^4	2.0×10^3
	Maximum	4×10^7	3.3×10^6	5.8×10^3
	Average	8.4×10^6	7.5×10^5	3.9×10^3
Cyperus papyrus	Minimum	2×10^5	3.6×10^4	1.5×10^3
	Maximum	2.5×10^7	3.1×10^6	5.8×10^3
	Average	4.5×10^6	6.23×10^5	3.0×10^3

Bruggen et al. [26] indicated that 81% of the nitrogen loaded in the wetland was taken up by *C. papyrus* basin, whereas 14% could be found in the outflow. About the ammonia removals in the HFCWs, they were higher during the first 6 months but lowered during the last 3 months (Fig. 6). Probably, during this time, the ammonia removal through their adsorption on the substrate was relevant, but once the available attachment sites were saturated, the process was reversed and other more endurable processes such as nitrification and plant uptake became more important [27].

3.1.2.3. Microbial removal The results in Table 5 show that the removal rate of bacterial indicators has been improved at the second HLR (0.10 m^3/m^2 d) and HRT (3.2 d) compared with the first load. By decreasing the HLR from 0.18 to 0.10 m^3/m^2 d, the removal efficiency of TC, FC, and *E. coli*

Table 4
Physicochemical characteristics of the treated effluents from the three basins at A2, B2, and C2 (HLR, 0.10 m^3/m^2 d)

Parameters	Average influent wastewater	*Canna flaccida*				*Phragmites australis*				*Cyperus papyrus*			
		Minimum	Maximum	Mean	%R	Minimum	Maximum	Mean	%R	Minimum	Maximum	Mean	%R
pH	7.2	7.0	7.9	7.3	–	7.0	7.7	7.3	–	7.0	7.7	7.3	–
TDS (mg/L)	498	486	625	549	–	488	640	564	–	499	650	559	–
TSS (mg/L)	102.4	28	47	44	57	18	62	32	68.7	29	62	36	64.8
BOD (mg/L)	175.7	32	98	50	71.5	24	59	34	80.6	27	68	40	77.2
COD (mg/L)	304.4	58	165	87	71.4	40	105	57	81.2	48	121	68	77.6
NH_3 (mg/L)	22.7	15	25	19	16	11.5	21	15	33.9	8	18	13	42.7
TKN (mg/L)	41.07	23	40	30	27	16	39	24	41.5	15	35	22	46.4
NO_2^- (mg/L)	0.03	0.06	0.2	0.1	–	0.06	0.11	0.08	–	0.04	0.16	0.1	–
NO_3^- (mg/L)	0.7	0.44	1.66	1.2	–	0.01	1.6	1.0	–	0.7	1.7	1.2	–

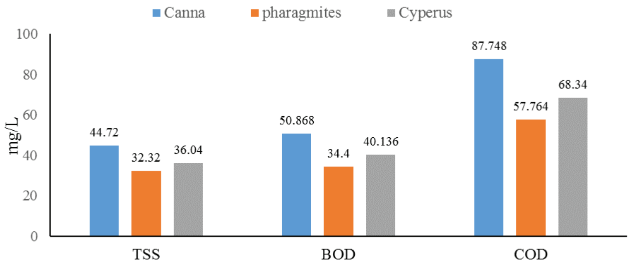

Fig. 5. Average residual values of TSS, BOD, and COD of the treated effluents from the three basins at A2, B2, and C2.

increased by 1.5, 1.5, and 0.9 log in the *C. flaccida* basin, while they were1.8, 1.6, and 1.25 log in the *P. australis* basin and reached a removal of 2.0, 1.75, and 1.25 logs in the *C. papyrus* basin. A study by García et al. [28] showed that removal of microbial indicators (FCs and somatic coliphages) in HFCW depends on HRT and size fraction of the filtration medium. They found a positive relationship between microbial inactivation and HRT. In this study, the results also indicated that *P. australis* was more efficient in the reduction of bacterial indicators. These results are also in agreement with that of Decamp and Warren [29] who reported that the presence of *P. australis* also appears to have a beneficial effect on *E. coli* removal. Higher bacteria removal in planted wetlands might be caused by the root exudates which are toxic to a range of bacteria. It is possible that the microbial attachment to the root surface reduces the settling of bacteria and viruses in CWs.

3.1.3. Case study 3: at sampling points A3, B3, and C3 (HLR 0.07 m^3/m^2 d and HRT 4.8 d)

3.1.3.1. Removal of organic matter The results depicted in Fig. 7 indicated that the three plants produced high-quality effluents in terms of BOD_5 and COD concentrations at 0.07 m^3/m^2 d (HLR) and 4.7 d (HRT). The order of the removal efficiency was *Phragmites* 88.6% > *C. papyrus* 86.9% > *C. flaccida* 83.4% for BOD. The average percentage reduction of COD reached 89.1% with *Phragmites*, 87.5% with *C. papyrus*, and 83.5% with *C. flaccida*. The corresponding average residual values were 19.2, 23.5, and 29.2 mg/L for BOD and 33, 38.5, and 50.2 mg/L for COD, respectively. The results obtained were complying with the Egyptian regulatory standards for the discharge of treated wastewater into surface water bodies (Ministerial Decree 92/2013) [30]. The limits of discharge are 80 mg/L for COD and 60 mg/L for BOD. Also, the results were complying with the Egyptian Code of practice (ECP 51/2015) [31] for the reuse of treated wastewater in unrestricted irrigation (the limits of BOD is 30 mg/L).

The mechanism of removal of organic matter as presented by COD and BOD_5 depends on the combination between physical and microbial mechanisms. The organic solids could be filtered and trapped in the bed of CWs for a long time due to the physical filtration mechanism and low porosity of the substrate media [32]. Also, organic matter can be reduced and consumed by bacteria and other microorganisms living on and around the plant root system through both aerobically and anaerobically processes [33]. In this study, the order of

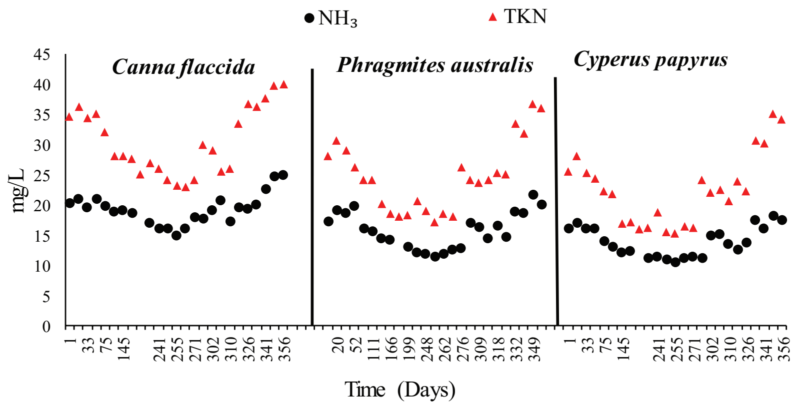

Fig. 6. Residual values of NH_3 and TKN in the treated effluents from the three basins at A2, B2, and C2.

Table 5
Average bacterial counts of the treated effluents from the three basins at sampling point A2, B2, and C2

Parameters		Total coliform	Fecal coliform	*Escherichia coli*
Influent wastewater	Average	4.06×10^7	2.95×10^6	2.09×10^4
Canna flaccida	Minimum	1.1×10^5	5.5×10^3	1.2×10^3
	Maximum	5.8×10^6	4.1×10^5	4.8×10^3
	Average	1.2×10^6	1.0×10^5	2.7×10^3
Phragmites australis	Minimum	8.2×10^3	4.0×10^2	2.1×10^2
	Maximum	3.1×10^6	2.7×10^5	3.1×10^3
	Average	5.9×10^5	7.2×10^4	1.2×10^3
Cyperus papyrus	Minimum	3.7×10^3	3.6×10^2	1.7×10^2
	Maximum	1.7×10^6	2.1×10^5	3.1×10^3
	Average	4.0×10^5	5.2×10^4	1.5×10^3

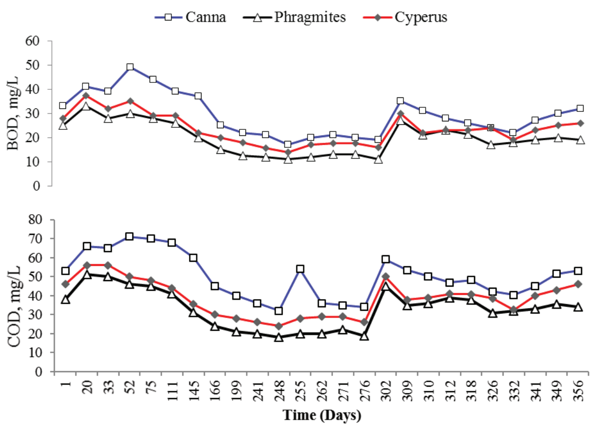

Fig. 7. Variations of residual values of COD and BOD in treated effluents using different plant species at A3, B3, and C3.

removal efficiencies was *P. australis* followed by *Cyperus* and then *C. flaccida*. These results are in agreement with that found by Calheiros et al. [34]. The choice of different plant species should consider some factors such as the rooting depth, plant productivity, and tolerance to high loads of wastewater. When the microorganisms are established on aquatic plant roots, they form a symbiotic relationship in most cases with the higher plants and produce a synergistic effect resulting in increased degradation rates and removal of organic compounds from the wastewater surrounding the plant root systems [35].

3.1.3.2. Suspended solids removal Variation of TSS residual values in all basins is shown in Fig. 8. Immediate reduction of TSS took place once the experiments are started. The average reduction of TSS reached 75.5% with *C. flaccida*, 84.3% with *P. australis*, and 80.4% with *C. papyrus*. Their corresponding residual values were 25, 16, and 20 mg/L. These results are complying with the discharge limits of TSS (50 mg/L) as stated in Ministerial Decree 92/2013 [30] and with ECP 501/2015 (30 mg/L) for reuse in irrigation [31]. Sedimentation and filtration are the main removal mechanisms of TSS followed by anaerobic and aerobic microbial degradation of the substrate reduction [36]. Vymazal and Masa [37] recorded that most of the suspended solids are retained within the first several meters along the bed.

3.1.3.3. TKN and ammonia removal During the monitoring period, a great variation of the TKN removal was observed (Fig. 9). The average reduction of TKN reached 41.8% with *C. flaccida* in Basin A, 57.6% with *Phragmits* in Basin B, and 62.2% with *Cyperus* in Basin C. The organic nitrogen in wastewater includes both soluble and particulate forms. The soluble form is mainly urea and amino acids. Its removal from CWs occurs by ammonification process [38].

The results in Fig. 10 show great variability in removal of ammonia–nitrogen during the study period. The average reduction of NH_3 reached 29.5% with *C. flaccida* in Basin A, 62.5% *Phragmites* in Basin B, and 68.7% with *Cyperus* in Basin C. Their corresponding residual values were 16, 8.5, and 7.1 mg/L. Results show that the best removal rate of ammonia was achieved using *C. papyrus* compared with other plant species. These results agree with those found by Kyambadde et al. [39]. The high treatment efficiency can refer to the root structures which provided more surface area for pollutant adsorption, more microbial attachment sites, enough wastewater residence time, trapping and settlement of suspended particles, uptake, assimilation in plant tissues, and oxygen for organic and inorganic matter oxidation in the rhizosphere. In addition, *C. papyrus* exhibited a significantly large number of adventitious roots. Nitrifying bacteria attached to *C. papyrus* and consequently nitrification activities were consistent with this finding. The results of this study indicated a good nitrification process, especially with *C. papyrus*. Oxygen secretion in the macrophyte roots could remarkably increase the number of aerobic and facultative aerobic bacteria in the root microenvironment. Wang et al. [40] stated that root zone provides continuous aerobic, anoxic, and anaerobic conditions in soil, allowing simultaneous nitrification and denitrification. Also, Abou-Elela et al. [41] concluded that nitrifying bacteria enhancement can be achieved in the presence of oxygen. The nitrifying bacteria and ammonia-oxidizing bacteria can use oxygen-produced root system to transform ammonia into nitrate and nitrite. Therefore, nitrogen removal efficiency is usually higher in CWs compared with general sewage treatment systems.

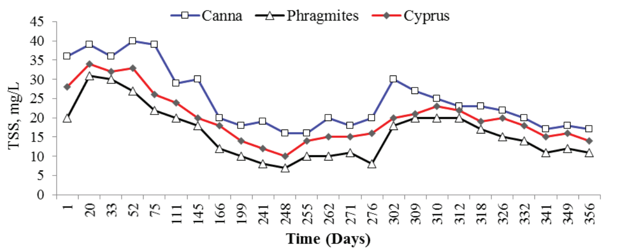

Fig. 8. Variations of residual values of TSS in the treated effluents using different plants at A3, B3, and C3.

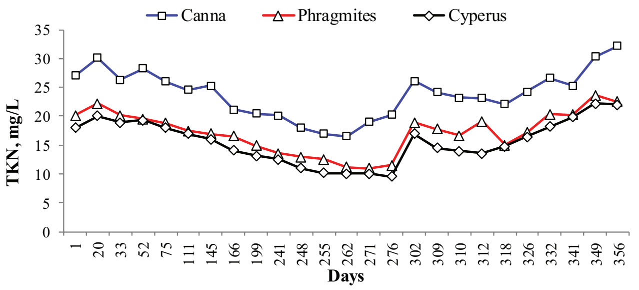

Fig. 9. Variations of residual values of TKN in treated effluents using different plant species at A3, B3, and C3.

3.1.3.4. Reduction of bacterial indicators of pollution Table 6 shows that *C. papyrus* was more effective for the removal of TC, FC, and *E. coli* than *C. flaccida* and *P. australis*. *C. papyrus* removed three logs of TC, FC, and *E. coli* in the treated effluent with an average removal value of 99.99%. Removal of about 2.5 logs of bacterial indicators was achieved using *C. flaccida* and *P. australis*. In a survey of 26 subsurface-flow CWs, Vymazal [35] found an average removal of 88%, while Zurita et al. [27] reported TCs removal from 92% to 93% in HFCW planted with three species. Abou-Elela et al [10] found that 2–3 logs of bacterial indicators were removed using HFCW planted with *C. papyrus*. Decamp and Warren [29] have demonstrated that anaerobic conditions prolong the survival of coliform in CWs. In spite of the high removal rates in all basins, the residual pathogens are not complying with the ECP 501/2015 for wastewater reuse in irrigation which stated a limit of 1,000 MPN/100 mL of FC for reuse [31]. Accordingly, disinfection of residual pathogens is required.

3.2. Plants uptake and biomass production

Because the aim of this study was to obtain the highest removal values of different pollutants, biomasses were harvested. The dry biomass of *P. australis* was 4.46 kg/m². This biomass yield was greater than that reported for the same plant by Ennabili et al. [42]. A maximum dry biomass of 2–3 kg/m² was reported by them. The *C. flaccida* yield in

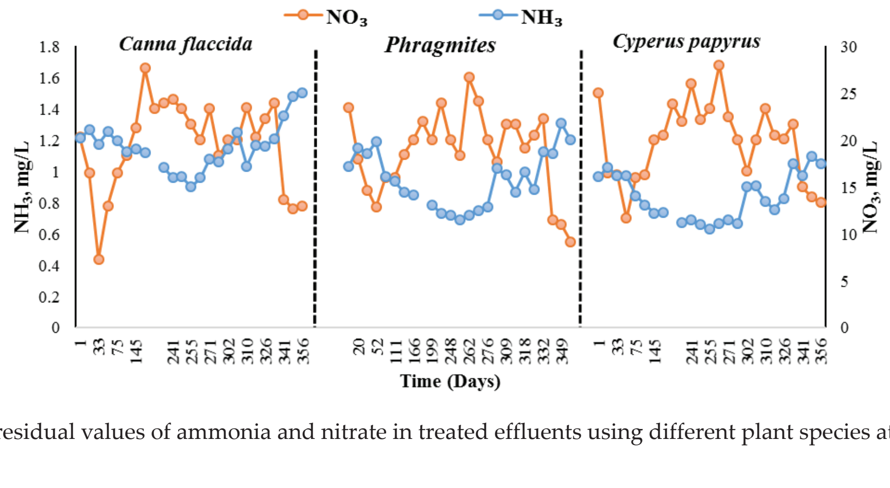

Fig. 10. Variations of residual values of ammonia and nitrate in treated effluents using different plant species at A3, B3, and C3.

Table 6
Average concentration of pathogens in the final effluent wastewater from the three basins

Parameters		Total coliform	Fecal coliform	*Escherichia* coli
Influent wastewater	Average	4.06×10^7	2.95×10^6	2.09×10^4
Canna flaccida	Minimum	8.1×10^3	6×10^2	2.8×10^2
	Maximum	6.3×10^5	3.3×10^4	1.7×10^3
	Average	1.5×10^5	9.5×10^3	1.0×10^3
Phragmites australis	Minimum	3.6×10^2	1.8×10^2	7×10^1
	Maximum	2.4×10^5	1.2×10^4	1.4×10^3
	Average	5.7×10^4	3.1×10^3	5.7×10^2
Cyperus papyrus	Minimum	1.8×10^2	1.8×10^2	6.3×10^1
	Maximum	1.7×10^5	6.1×10^3	1.5×10^3
	Average	3.1×10^4	1.6×10^3	4.4×10^2

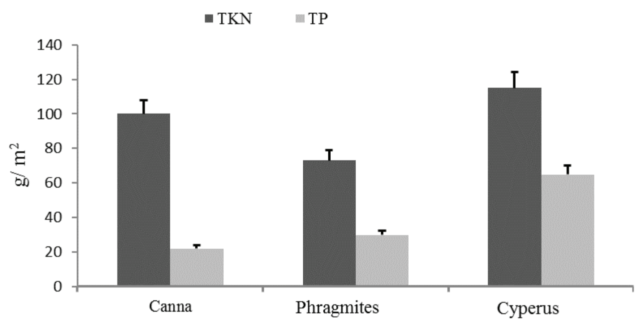

Fig. 11. Average plant uptake of nitrogen and phosphorus in the three plants.

this study reached 5.18 kg/m^2 compared with 3.125 kg/m^2 obtained by Konnerup et al. [43] for the same plant. The dry biomass in *C. papyrus* was 7.7 kg/m^2. The plant nitrogen and phosphorus uptake in HFCW are shown in Fig. 11. The results showed that *C.* papyrus was better than *C. flaccida* and *Phragmites* in nitrogen and phosphorus uptake. This may be attributed to the fact that *Cyperus* exhibited a significantly large number of adventitious roots as mentioned before.

These results are in agreement with those of Kuusemets and Lohmus [44] and Vohla et al. [45]. They reported that nitrogen accumulation in *P. australis* and *Scirpus sylvaticus* growing in an HFCW at Kodijärve, Estonia, was 37.6 g N/m^2. Brix et al. [46] suggested that standing stock in aboveground biomass of emergent macrophytes, and which is thus available for harvesting, is roughly between 20 and 250 g N m^{-2}. Vymazal [47] reported that the above-ground N standing stock in the range of 22–88 g N m^{-2} for 29 various emergent species.

4. Conclusion

Due to water scarcity, there is a great need for renewable water resources. One of the approaches of great potential is the reuse of treated wastewater for irrigation. The CW is considered one of the promising green technologies and low-cost wastewater treatments for reuse. The success of CW depends primarily on the different operating conditions which produced the optimal removal of organic and inorganic matters and pathogens. This study investigated the effect of plant species, HLR, and HRT, on the performance of three identical subsurface HFCW vegetated with different plant species for municipal wastewater treatment and reuse. Results proved that the type of plant species as well as the HLR and HRT play a significant role in the performance of HFCW. The plant species is considered the major key parameter for the nitrification process and must not be considered of secondary importance insofar. In this study, very satisfactory results were obtained at the optimum HRT of 4.8 d and HLR of 0.07 m^3/m^2 d. Comparative study of the three emergent plants (*Phragmites*, *C. papyrus*, and *C. flaccida*) grown under identical operating conditions indicated that although the three basins produced good-quality effluents in terms of TSS, BOD, and COD, the best removal efficiency was achieved by *Phragmites* then *C. papyrus* and then *C. flaccida*. The plants uptake indicated that *C. papyrus* was more efficient in nitrogen removal than *C. flaccida* and *Phragmites*. The order of removal of nitrogen compounds (NH_3 and TKN) was *C. papyrus* > *Phragmites* > *C. flaccida*. This may be referred to the fact that *Cyperus* exhibited a significantly large number of adventitious roots which assist the nitrification process. Based on the results obtained in this study, HFCW planted with *C. papyrus* at HRT of 4.7 d is recommended for the treatment and reuse of wastewater treatment. Physicochemical analysis of the treated effluent was very satisfactory for reuse in agricultural purposes according to the Egyptian Code of Practice ECP 501/2015 for treated wastewater reuse. However, for safe reuse, disinfection is recommended.

References

[1] WHO/UNICEF, Water for Life: Making It Happen, Joint Monitoring Programme, Water Sanitation Hygiene, Vol. 3, World Health Organization, WHO Press, Geneva, Switzerland, 2005.

[2] P. Gleick, R.W. Miller, The world's water: the biennial report on freshwater resources 2002–2003, Electron. Green J., (2003), p. 2. doi: 10.1080/14486563.2012.677216.

[3] P. Gleick, D. Johnson, The World's Water 2006–2007: The Biennial Report on Freshwater Resources, Envir. Prac., 10 (2008), p. 129.

[4] Y. Li, G. Zhu, W.J. Ng, S.K. Tan, A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: design, performance and mechanism, Sci. Total Environ. 468–469 (2014) 908–932.

[5] Y. Chen, Y. Wen, Q. Zhou, J. Vymazal, Effects of plant biomass on denitrifying genes in subsurface-flow constructed wetlands, Bioresour. Technol., 157 (2014) 341–345.

[6] H. Wu, J. Zhang, C. Li, J. Fan, Y. Zou, Mass balance study on phosphorus removal in constructed wetland microcosms treating polluted river water, Clean Soil Air Water, 41 (2013) 844–850.
[7] J. Vymazal, Emergent plants used in free water surface constructed wetlands: a review, Ecol. Eng., 61 (2013) 582–592.
[8] S.I. Abou-Elela, G. Golinelli, A.S. El-Tabl, M.S. Hellal, Treatment of municipal wastewater using horizontal flow constructed wetlands in Egypt, Water Sci. Technol., 69 (2014) 38–47.
[9] J. Vymazal, Constructed wetlands for wastewater treatment, Water, 2 (2010) 530–549.
[10] S.I. Abou-Elela, M.A. Elekhnawy, M.T. Khalil, M.S. Hellal, Factors affecting the performance of horizontal flow constructed treatment wetland vegetated with *Cyperus papyrus* for municipal wastewater treatment, Int. J. Phytorem., 19 (2017) 1023–1028.
[11] H. Brix, Do macrophytes play a role in constructed treatment wetlands?, Water Sci. Technol., 35 (1997) 11–17.
[12] C. Leto, T. Tuttolomondo, S. La Bella, R. Leone, M. Licata, Effects of plant species in a horizontal subsurface flow constructed wetland – phytoremediation of treated urban wastewater with *Cyperus alternifolius* L. and *Typha latifolia* L. in the West of Sicily (Italy), Ecol. Eng., 61 (2013) 282–291.
[13] H. Guasch, A. Serra, N. Corcoll, B. Bonet, M. Leira, Metal ecotoxicology in fluvial biofilms: potential influence of water scarcity, Handb. Environ. Chem., 8 (2010) 41–53.
[14] Y. Hua, L. Peng, S. Zhang, K.V. Heal, J. Zhao, D. Zhu, Effects of plants and temperature on nitrogen removal and microbiology in pilot-scale horizontal subsurface flow constructed wetlands treating domestic wastewater, Ecol. Eng., 108 (2017) 70–77.
[15] G. Maltais-Landry, R. Maranger, J. Brisson, F. Chazarenc, Nitrogen transformations and retention in planted and artificially aerated constructed wetlands, Water Res., 43 (2009) 535–545.
[16] L.H. Fraser, S.M. Carty, D. Steer, A test of four plant species to reduce total nitrogen and total phosphorus from soil leachate in subsurface wetland microcosms, Bioresour. Technol., 94 (2004) 185–192.
[17] J. Villaseñor Camacho, A. De Lucas Martínez, R. Gómez Gómez, J. Mena Sanz, A comparative study of five horizontal subsurface flow constructed wetlands using different plant species for domestic wastewater treatment, Environ. Technol., 28 (2007) 1333–1343.
[18] S.I. Abou-Elela, G. Golinielli, E.M. Abou-Taleb, M.S. Hellal, Municipal wastewater treatment in horizontal and vertical flows constructed wetlands, Ecol. Eng., 61 (2013) 460–468.
[19] American Public Health Association (APHA), Standard methods for the examination of water and wastewater (22nd ed.), Washington (DC): United Book Press, 2012.
[20] G. Techobanoglous, F.L. Burton, H.D. Stensel, Wastewater Engineering: Treatment and Reuse, 5th ed., New York (NY): McGraw Hill, 2014.
[21] R. Cakir, A .Gidirislioglu, U. Çebi, A study on the effects of different hydraulic loading rates (HLR) on pollutant removal efficiency of subsurface horizontal-flow constructed wetlands used for treatment of domestic wastewaters, J. Environ. Mange., 164 (2015) 121–128.
[22] S.C. Reed, D.S. Brown, Subsurface flow wetlands: a performance evaluation, Water Environ. Res., 67 (1995) 244–248.
[23] J. Vymazal, Constructed wetlands for wastewater treatment: five decades of experience, Environ. Sci. Technol., 45 (2011) 61–69.
[24] M.R. Karim, F.D. Manshadi, M.M. Karpiscak, C.P. Gerba, The persistence and removal of enteric pathogens in constructed wetlands, Water Res., 38 (2004) 1831–1837.
[25] A. Al-Omari, M. Fayyad, Treatment of domestic wastewater by subsurface flow constructed wetlands in Jordan, Desalination, 155 (2003) 27–39.
[26] J.J.A. van Bruggen, O.T. Okia, F. Kansiime, Wastewater Purification by the Tropical Macrophyte *Cyperus papyrus* in a Segmented Channel in the Greenhouse, Proc. 4th International Conference on Wetland Systems for Water Pollution Control, Guangzhou, China, 1994.
[27] F. Zurita, J. De Anda, M.A. Belmont, Treatment of domestic wastewater and production of commercial flowers in vertical and horizontal subsurface-flow constructed wetlands, Ecol. Eng., 35 (2009) 861–869.
[28] J. García, J. Vivar, M. Aromir, R. Mujeriego, Role of hydraulic retention time and granular medium in microbial removal in tertiary treatment reed beds, Water Res., 37 (2003) 2645–2653.
[29] O. Decamp, A. Warren, Investigation of *Escherichia coli* removal in various designs of subsurface flow wetlands used for wastewater treatment, Ecol. Eng., 14 (2000) 293–299.
[30] Ministry of Irrigation, Ministerial Decree 92/2013 for Discharge Limits of Treated Wastewater on Surface Water, Al-Ameria Press, Cairo, Egypt, 2013.
[31] Egyptian Code of Practice 501/2005 for Treated Wastewater Reuse in Agriculture, Ministry of Housing, Utilities and Urban Development, Al Gomhoria Press, Cairo, Egypt, 2015.
[32] D. Zhang, R.M. Gersberg, T.S. Keat, Constructed wetlands in China, Ecol. Eng., 35 (2009) 1367–1378.
[33] USEPA, Wastewater Technology Fact Sheet Wetlands: Subsurface Flow, Environmental Protection Agency, Pennsylvania Avenue, NW Washington, D.C, 2000, pp. 1–7.
[34] C.S.C. Calheiros, A.O.S.S. Rangel, P.M.L. Castro, Constructed wetland systems vegetated with different plants applied to the treatment of tannery wastewater, Water Res., 41 (2007) 1790–1798.
[35] J. Vymazal, Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment, Ecol. Eng., 25 (2005) 478–490.
[36] R.H. Kadlec, L. Knight, Treatment Wetlands, CRC Press/Lewis Publishers, Boca Raton, FL, 1996.
[37] J. Vymazal, M. Masa. Horizontal sub-surface flow constructed wetland with pulsing water level, Water Sci. Technol., 48 (2003) 143–148.
[38] T. Saeed, G. Sun, A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: dependency on environmental parameters, operating conditions and supporting media, J. Environ. Manage., 112 (2012) 429–448.
[39] J. Kyambadde, F. Kansiime, L. Gumaelius, G. Dalhammar, A comparative study of *Cyperus papyrus* and *Miscanthidium violaceum*-based constructed wetlands for wastewater treatment in a tropical climate, Water Res., 38 (2004) 475–485.
[40] Q. Wang, Y. Hu, H. Xie, Z. Yang, Constructed wetlands: a review on the role of radial oxygen loss in the rhizosphere by macrophytes, Water, 10 (2018) 1–11.
[41] S.I. Abou-Elela, M.S. Hellal, O.H. Aly, S.A. Abo-Elenin, Decentralized wastewater treatment using passively aerated biological filter, Environ. Technol., 40 (2019) 250–260.
[42] A. Ennabili, M. Ater, M. Radoux, Biomass production and NPK retention in macrophytes from wetlands of the Tingitan Peninsula, Aquat. Bot. 62 (1998) 45–56.
[43] D. Konnerup, T. Koottatep, H. Brix, Treatment of domestic wastewater in tropical, subsurface flow constructed wetlands planted with *Canna* and *Heliconia*, Ecol. Eng., 35 (2009) 248–257.
[44] V. Kuusemets, K. Lohmus, Nitrogen and phosphorus accumulation and biomass production by *Scirpus sylvaticus* and *Phragmiotes australis* in a horizontal subsurface flow constructed wetland, J. Environ. Sci. Health Part A Toxic/Hazard. Subst. Environ. Eng., 40 (2005) 1167–1175.
[45] C. Vohla, R. Alas, K. Nurk, S. Baatz, U. Mander, Dynamics of phosphorus, nitrogen and carbon removal in a horizontal subsurface flow constructed wetland, Sci. Total Environ., 380 (2007) 66–74.
[46] H. Brix, H. Schierup, J. Bourke, S. Wroe, K. Moreno, C. McHenry, P. Clausen, L. Aller, J. Lehr, R. Petty, T. Bennett, A.R. Abbas, K. Wolslegel, D. Seshasayee, Z. Modrusan, H.F. Clark, Use of constructed wetlands in water pollution control: historical development, present status, and future perspectives, PLoS One, 3 (1994) 209–223.
[47] J. Vymazal, Algae and Element Cycling in Wetlands, Lewis Publishers Inc., Boca Raton, USA, 1996.