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Warm season performance of horizontal subsurface flow constructed wetlands vegetated with rice treating water from an urban stream polluted with sewage

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The performance of horizontal subsurface flow constructed wetlands vegetated with rice (*Oryza sativa* L.) was investigated in Campina Grande (7° 13' 11" S; 35° 52' 31" W; 550 m above mean sea level), Paraíba state, northeast Brazil. The pilot-scale system comprised 24 circular tanks (76.80 cm diameter, 54 cm height) batch fed daily with water from a nearby urban stream polluted with sewage. Experimental units were filled with substrate of either sand or gravel and operated under hydraulic retention times of 5 and 10 days. Constructed wetlands demonstrated a very good performance in removing organic matter, fecal indicator microorganisms and nutrients from the influent representing a good alternative for the improvement of water quality of urban and peri-urban water resources. Vegetation was found to be the most important factor affecting their performances being the changes in both substrate and hydraulic retention time investigated herein of minor influence.

Key words: Urban polluted stream, urban polluted water treatment, constructed wetlands, effluent reuse.

INTRODUCTION

Unplanned urbanization did become a common feature of developina countries leading environmental to degradation and population risk of diseases particularly the fecal-oral ones. Urban and peri-urban areas in developing countries are among the most polluted of the world (Douglas, 2006) and much of such pollution is caused by either lack or inadequacy of sanitation services. Frequently, sewage is collected and discharged into water bodies contributing to their fecal contamination, nutrient overload and anti-aesthetic aspects of water quality. Natural wetlands are widely recognized as ecosystems playing important role in removing water pollutants through a combination of physical, chemical and biological processes occurring amongst sediments, vegetation and water. Constructed wetland technology is a low energy and cost alternative for treating polluted water. Their low operational and maintenance requirements have contributed to their use in rural communities in developed countries and as an appropriate technology for developina countries et al., 2000; Hunt and Poach, 2001; (Hagendorf Massoud et al., 2009; Kadlec and Wallace, 2009; Redder at al., 2010). In the last decades, throughout the world, there has been an increased interest in the application of ecological engineering systems, as constructed wetlands, to treat polluted surface waters even in countries with severe seasonal climatic conditions as reported by Vymazal (2002). In fact, the application of such a technology has been classified as a reliable, effective and feasible solution for wastewater treatment (Tsihrintzis and Gikas, 2010; Stefanakis and Tsihrintzis, 2012). In countries under rapid urbanization and industrialization processes like China, this technology has been suggested as an alternative for the treatment of wastewaters from small to moderate size cities (Zhang et al., 2009).

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Figure 1. Schematic representation of experimental system.

On the other hand, vegetated constructed wetlands can be advantageously used for growing agricultural products useful for feeding human and animals, ornamental plants and natural fibers for handicraft. Vegetation is a dominant feature of constructed wetlands and subsurface flow systems may support a variety of macrophyte species. As nutrient uptake and storing capacity differ significantly from one macrophyte to another, selection is one of the most important factors for a good performance of vegetated constructed wetlands treating polluted waters (Reed et al., 1995). In literature, the role of vegetation in removing pollutants is recognized as significantly positive even though it remains unclear, the specific effects of different plants with comparable life forms and sizes in such a removal (Akratos and Tsihrintzis, 2007; Brisson and Chazarenc, 2009).

Constructed wetland application in agriculture appears to be an excellent alternative for solving problems, such as environmental degradation, mainly soil and water pollution, and lack of financial resources for investment in sanitation and malnutrition affecting low income areas in developing countries. This paper describes a fieldwork aiming to evaluate the performance of a pilot-scale experimental system made up of a set of horizontal subsurface flow constructed wetlands vegetated with rice (*Oryza sativa* L.) using either gravel or sand as substrate media and fed with water from an urban polluted stream in the city of Campina Grande, Paraiba state, northeast Brazil.

MATERIALS AND METHODS

The experimental system, illustrated in Figures 1 and 2, was installed on the campus of Federal University of Campina Grande (7º 13' 11" S; 35º 52' 31" W; 550 m above mean sea level), Paraíba state, northeast Brazil. It comprised 24 circular tanks (76.8 cm diameter, 54 cm height) batch fed once daily with water from a nearby polluted urban stream, with horizontal subsurface flow and hydraulic retention times (HRT) of either 5 or 10 days. Half of the tanks were filled with gravel (19 mm diameter) as substrate and the other half with washed sand, up to the height of 40 cm in both cases. Half of each set operated under a HRT of 5 days and the other half operated with 10 days. For each substrate and each HRT, 3 tanks were vegetated with rice (O. sativa L.) and 3 were kept without vegetation (controls). Rice seedlings were cultivated in 350 ml-disposable pots inside a greenhouse. Twenty days after seeding, eight seedlings were transplanted in each tank to be vegetated with rice.

Fieldwork comprised monitoring of two (1st and 2nd experiments) complete rice cultivation cycles both from June to September of two consecutive years. Meteorological data throughout both periods, shown in Table 1, were obtained from a meteorological station nearby monitored by The National Center of Cotton Research (CNPA) belonging to The Brazilian Enterprise for Agriculture and Cattle. In both experiments, influent and effluent water grab samples were collected twice a month and analyzed for the following parameters: temperature (T – direct reading with mercury filament thermometer), electrical conductivity (EC - potentiometric method), total alkalinity (ALK - potentiometric acid titration), pH (potentiometric method), biochemical oxygen demand (BOD5 sample dilution in standard bottles), chemical oxygen demand (COD - potassium dichromate closed reflux method), total phosphorus (Total-P - ascorbic acid spectrophotometric method following persulphate ammonium digestion), soluble orthopho-

Month	Temperature ¹ (°C)		Rain precipitation ² (mm)		Evaporation ² (mm)		Sunlit hours ² (h)	
	1 st cycle	2 nd cycle	1 st cycle	2 nd cycle	1 st cycle	2 nd cycle	1 st cycle	2 nd cycle
June	22.1	21.9	145.3	158.5	74.0	68.3	133.9	120.3
July	21.6	21.9	119.1	55.1	68.4	81.6	171.5	179.0
August	21.1	21.9	59.8	51.7	105.7	115.3	222.3	222.4
September	22.5	23.0	29.0	2.3	137.4	164.7	254.3	284.3
Total	-	-	353.2	267.6	385.5	429.9	782.0	806.0

Table 1. Atmospheric temperature, rain precipitation, standard evaporation (class A) and sunlit hours throughout both experimental investigation periods.

Source: National Center of Cotton Research (CNPA)/Embrapa. ¹Monthly mean values. ²Monthly cumulative values.



Figure 2. Front (above) and horizontal (below) representations of constructed wetland.

sphate (SoI-P – ascorbic acid spectrophotometric method following filtration through 0.45 μ m cellulose membrane), ammonia (NH₃-N – titrimetric method following distillation), nitrate (NO₃-N – sodium salicylate method), turbidity (TURB – nephelometric method), total suspended solids (TSS – gravimetric method after filtration through standard glass fiber filter paper), thermotolerant coliform (TC – filter membrane technique and incubation on m-FC broth) and fecal streptococci (FS - filter membrane technique and incubation on KS-streptococcus agar).

Except of nitrate, analyzed according to Rodier (1984), all analytical parameters were determined following procedures described in APHA (2005). As each experiment lasted for four months and the experimental system was sampled eight times, each parameter was analyzed in three sample replicates; twenty four readings were obtained from each tank as well as from the influent totaling six hundred readings for each parameter in each experiment.

RESULTS

Quality of the influent water

Results from both investigation periods are shown in Table 2. Respectively, in the first and second cultivation cycles, the influent presented a slightly alkaline pH and high content of ions, expressed by the high mean values of EC (1,146 to 1,490 µmho/cm). The organic matter concentrations in terms of mean BOD₅ (19 to 21 mg/L) and mean COD (121 to 221 mg/L) were high showing that stream water was severely polluted with domestic wastewater as confirmed by the mean results of thermotolerant coliforms $(9.60 \times 10^5 - 6.38 \times 10^5 \text{ CFU}/100 \text{ CFU})$ ml) and faecal streptococci (1.11 x 10^5 to 6.66 x 10^4 CFU/100 ml). These mean values as well as those for total phosphorus (3.34 to 3.97 mgP/L), soluble orthophosphate (2.84 to 3.55 mgP/L) and ammonia (24.6 to 27.5 mgN/L) are typical of water polluted with wastewater.

Quality of effluent and performance of constructed wetlands

All pH effluent values were alkaline and tended to be higher (7.41 to 8.43) in control tanks than in vegetated ones whose mean values varied between 7.03 and 7.58. reflecting both respiration and organic matter degradation around rhizosphere zone. Throughout fieldwork, mean BOD₅ removal efficiency varied within the range of 60 to 85%, being the constructed wetlands able to reduce influent concentration from about 20 mg/L down to 3 to 8 mg/L. Such a performance is comparable to that (75%) found by Vymazal and Kröpfelová (2008) treating wastewater with a mean BOD₅ of 140 mg/L. Akratos and Tsihrintzis (2007) obtained a mean removal of 90.5% treating synthetic wastewater with mean influent BOD₅ of 361.1 mg/L in horizontal subsurface flow constructed wetland filled with medium gravel and vegetated with Typha latifolia under temperatures above 15°C. It is important to observe that in both cases, influent BOD₅

were much higher than in this work. The efficiency obtained herein was very good mainly considering the high degree of dilution of BOD₅ in the water being treated. Any significant effect of either HRT or substrate type (sand or gravel) was not identified on the behaviour of this variable. Such a performance complies with brazilian maximum BOD₅ value of 120 mg/L permitted for discharges from sewage treatment plants, performing a minimum of 60%, into water bodies (Brasil, 2011). However, they did not reduced COD significantly being removals between 21 and 47% in control tanks and between 2 and 43% in vegetated ones which are much lower than those found by Akratos and Tsihrintzis (2007), who obtained mean removal performance of 90.8% treating synthetic wastewater with mean influent COD of 583.6 mg/L in vegetated constructed wetlands filled with gravel and temperatures above 15°C.

In this work, constructed wetlands were used for the treatment of water from an urban stream polluted with sewage but with a very diluted concentration (influent mean COD between 121 and 221 mg/L). Vegetation played an important role in reducing influent ammonia from mean concentrations in the range of 24.6 to 27.5 mgN/L down to 2.8 to 6.1 mgN/L contrasting with control units whose effluent mean concentrations were within the range of 8.1 to 19.3 mgN/L. In both cultivation cycles, control tanks filled with gravel performed better than those filled with sand being the best results obtained under HRT of 10 days. Ammonia removal in vegetated constructed wetlands was always superior to 75% which is a very good result compared to those percents (30 and 39) reported respectively by Kadlec and Wallace (2009) and Vymazal and Kröpfelová (2008) in constructed wetlands treating wastewaters with concentrations of 40 and 36 mgN/L, respectively. They reported ammonia removal mean performances of respectively 305 and 213 horizontal flow constructed wetlands operating under various conditions both climatic and operational. Akratos and Tsihrintzis (2007) obtained mean removal of 69.1% treating a mean influent concentration of 38.4 mgN/L in vegetated wetlands under the conditions already described. Dong et al. (2011) evaluating an integrated constructed wetland system, with an area of 3.25 ha, made up of five cells treating a typical domestic wastewater near Monaghan, Ireland, obtained ammonia concentration mean removals varying between 50.72% (fall) and 99.31% (spring) being this mainly attributed to high plant uptake and increased nitrification process caused by root-zone re-aeration due to the very long HRT of up to 92 days.

In our study, ammonia nitrogen effluent concentration was very low indeed complying with brazilian maximum value permitted of 20 mgN/L for disposing effluents into streams. Nitrogen as nitrate was low in the influent (0.11 to 0.29 mgN/L) and effluents of all vegetated (from 0.09 to 0.22 mgN/L) and control tanks (from 0.07 to 0.41 mgN/L) not being apparently characterized differences

Variabla	Cycle	Influent -	Control				Vegetated unit			
			5G	10G	5S	10S	5GV	10GV	5SV	10SV
Temperature (°C)	1 st	20.6	17.9	19.8	18.4	19.8	18.2	19.9	18.3	19.9
	2 ^{na}	20.6	19.5	19.3	19.6	19.3	19.5	19.4	19.7	19.5
ъЦ	1 st	7.57	8.07	8.43	7.91	8.17	7.57	7.58	7.44	7.44
рп	2 nd	7.63	7.91	7.56	7.75	7.41	7.41	7.09	7.30	7.03
$BOD_{r}(ma/l)$	1 st	19	7	6	8	7	6	6	5	8
BOD₅ (mg/L)	2 nd	21	4	3	5	4	5	5	4	5
COD (mg/L)	1 st	121	64	80	70	90	96	111	69	110
	2 nd	221	130	125	175	160	167	216	174	208
	1 st	24.6	13.8	11.8	16 1	19.3	6 1	49	4.5	5.6
NH ₃ -N (mgN/L)	2 nd	27.5	14.4	8.1	16.0	12.5	4.4	2.8	2.9	3.1
	1 st	0.29	0.21	0.41	0.25	0.13	0.22	0.10	0.22	0.09
NO ₃ -N (mgN/L)	2 nd	0.11	0.13	0.11	0.07	0.10	0.10	0.14	0.10	0.15
T () D () D ()	1 st	3.34	2.64	2.66	1.90	2.08	2.00	2.46	1.01	1.36
Total-P (mgP/L)	2 nd	3.97	3.10	2.91	3.58	2.92	3.06	3.22	2.17	3.36
	1 st	2.84	2.27	1.96	1.60	1.76	1.54	1.34	0.25	0.27
Sol-P (mgP/L)	2 nd	3.55	2.67	2.30	3.01	2.64	2.71	2.58	1.77	1.77
TSS (mg/L)	1 st	19	17	11	15	9	14	26	25	34
155 (mg/L)	2 nd	15	8	5	6	8	9	8	19	15
	1 st	88,5	11.3	3.3	5.0	5.2	6.3	8.9	39.0	44.5
TURB (NTU)	2 nd	57.4	6.7	1.9	6.7	6.8	10.7	9.1	13.3	14.1
TC (CFU/100 ml)	1 st	9.60×10 ⁵	5.90×10^4	5.23×10 ⁴	2.36×10^{4}	2.10×10^{4}	4.87×10^{4}	3.02×10^{4}	7.46×10 ³	2.51×10 ⁴
	2 nd	6.38×10 ⁵	1.53×10 ⁴	1.29×10^{4}	1.18×10^{4}	2.42×10^{4}	1.68×10^{4}	8.94×10 ³	5.78×10 ³	1.46×10 ³
FS (CFU/100 ml)	1 st	1.11×10⁵	8.37×10 ³	3.63×10 ³	4.54×10 ³	3.05×10 ³	6.23×10 ³	2.33×10 ³	1.15×10 ³	2.72×10 ³
	2 nd	6.66x10 ⁴	9.18x10 ³	2.39x10 ³	2.82x10 ³	1.68x10 ³	3.04x10 ³	2.81x10 ³	8.87x10 ²	5.46x10 ²
\mathbf{D}	1 st	1,146	1,156	1,142	1,207	1,161	1,172	1,322	1,311	1,316
EC (µmno/cm)	2 nd	1,490	1,396	1,357	1,451	1,467	1,560	1,606	1,664	1,930
	1 st	365	340	307	355	345	369	397	407	412
ALK (INGUAUU ₃ /I)	2 nd	426	369	336	386	383	414	427	454	529

Table 2. Mean values of physico-chemical and microbiological parameters analysed in samples of influent and effluents of constructed wetlands in two cultivation cycles of rice (*Oryza sativa* L.).

5G: gravel substrate with a HRT of 5 days; 5S: sand substrate with a HRT of 5 days; 10G: gravel substrate with a HRT of 10 days; 10S: sand substrate with a HRT of 10 days; 5GV: vegetated gravel substrate with a HRT of 5 days; 5SV: vegetated sand substrate with a HRT of 5 days; 10GV: vegetated gravel substrate with a HRT of 10 days; 10SV: vegetated sand substrate with a HRT of 10 days.

among mean concentrations. In the first cultivation cycle, vegetation contributed to reduce influent

total phosphorus mean concentration from 3.34 mgP/L down to the range of 1.01 to 1.36 mgP/L in

tanks filled with sand and down to 2.00 to 2.46 mgP/L in those filled with gravel. In fact,

vegetation contribution was very important for the overall removal of total-P mainly under HRT of five days. With sand as substrate, vegetation increased percent efficiency from 43.1 up to 69.8, under HRT of 5 days, and from 37.7 up to 59.3, under HRT of 10 days, while with gravel as substrate the contribution of vegetation on percent efficiency was more modest, from 21.0 up to 40.1, with 5 days, and from 20.4 up to 26.3, with 10 days. In the second cultivation cycle, total-P was reduced from 3.97 mgP/L down to the range of 2.17 to 3.36 mgP/L, with sand and down to 3.06 to 3.22 mgP/L with gravel as substrate.

In this cultivation cycle, both substrates (controls) fairly removed total phosphorus being percent removals between 21.9 (HRT of five days) and 26.7 (HRT of 10 days) for gravel and between 9.8 (five days) and 26.4 (10 days) for sand. Vegetation improved overall percent removal of total-P only under HRT of five days mainly with sand as substrate. Even the worst performances found in this work were better than that (20%) reported by Gauss (2008) for total phosphorus mean removals in horizontal flow constructed wetlands operating in several Latin American countries which in its turn, is a performance lower than that (50%) found by Vymazal and Kröpfelová (2008) in Europe.

Akratos and Tsihrintzis (2007) reported a mean percent removal of 70.1 for constructed wetlands filled with gravel being operational conditions described in preceding paragraphs. During the first cultivation cycle, soluble orthophosphate influent mean concentration of 2.84 mgP/L was reduced down to 2.27 mgP/L (five days) and down to 1.96 mgP/L (10 days) in gravel control tanks with effluent concentrations in vegetated tanks of 1.54 mgP/L (five days) and 1.34 mgP/L (10 days) with the respective overall percent removals of about 46 and 53. Effluent concentrations of sand control tanks were less than those of the respective gravel controls being very expressive the role played by vegetation that reduced influent concentrations down to values less than 0.3 mgP/L and increased overall removals up to around 91%, under both HRT. In the second cycle, mean influent concentration of 3.55 mgP/L was reduced down to the range of 2.30 mgP/L (10 days) to 2.67 mgP/L (five days), in gravel controls, and down to the range of 2.64 mgP/L (10 days) to 3.01 mgP/L (5 days), in sand controls. Vegetation slightly increased effluent concentrations in gravel substrate under both HRT and reduced effluent concentrations down to around 1.8 mgP/L under both HRT, in tanks filled with sand, being the overall performance of around 50%. Akratos and Tsihrintzis (2007) found variable mean percent removals being this variation significantly dependent on the type of vegetation similarly to what was verified to total phosphorus and ammonia nitrogen.

Brazilian water resources legislation regulates both total phosphorus and orthophosphate for surface water classification (Brasil, 2005); but not for discharges of

effluents of wastewater treatment plants into water receiving bodies (Brasil, 2011). In the whole monitoring period, control tanks reduced influent mean concentrations of suspended solids being their performances under HRT of 10 days much better than those under five days. Vegetation affected negatively removals of suspended solids, mainly in tanks filled with sand in which negative overall removal efficiencies predominated. According to Vymazal (2010), suspended solids removal can achieve a very high degree (75%) in horizontal subsurface flow constructed wetlands with gravel as substrate. The author attributed such a performance to the application of high mean inflow concentration. In this work, as the influent suspended solids mean concentrations were indeed very low, effluent suspended materials were more probably due to degradation of roots and rhizomes. In both cultivation cycles under both HRT thermotolerant coliforms were predominantly reduced from more than 6 \times 10⁵ CFU/100 ml down to less than 2.5 \times 10⁴ CFU/100 ml in vegetated tanks filled with sand being overall removal efficiencies of more than 97% (between 1.58 and 2.64 log units); while vegetated tanks filled with gravel reached the range of 94.9 to 98.6% (between 1.29 and 1.85 log units) with effluent mean counts in between 8,940 to 48,700 CFU/100 ml. These performances are comparable to that (1.7 log) reported by Gauss (2008) for a pilot-scale plant based on subsurface horizontal flow constructed wetland filled with gravel at Masaya, Nicaragua.

Also, with relation to fecal streptococci vegetated constructed wetlands filled with sand reached performances of around 98% being effluent mean counts less than 3000 CFU/100 ml, and those filled with gravel showed overall performances of more than 94% being mean effluent CFU between 2000 and 6500 per 100 ml. Both substrates showed a very good performance in reducing influent turbidity. In control tanks, mean turbidity was reduced from about 88 NTU during the first cultivation cycle and from around 57 NTU in the second cycle down to the range of 1.9 to 11.3 NTU. Compared with control tanks, vegetated ones had higher mean turbidities remarkably in tanks filled with sand (39.0 to 44.5 NTU) during the first cultivation cycle. As a result of the evapotranspiration electrical conductivity as well as total alkalinity tended to increase more in vegetated units. Two-way analysis of variance (Sokal and Rohlf, 1995), at a level of significance of 0.05, was applied for unplanned multiple comparisons of results from both cultivation cycles. Table 3 presents' p-values allowing identification of significant differences ($p \le 0.05$) among groups of same variable related to water quality. Figure 3 illustrates mean values and their respective comparison intervals based on GT-2 graphic method for the selected parameters total phosphorus, soluble orthophosphate, ammonia, total suspended solids, BOD₅, and thermotolerant coliforms useful in evaluating both eutrophication and faecal pollution in water receiving bodies.

Variable	MS among	MS within	F	p-value
Т	0.003416	0.0024398	1.40013	0.139903693
рН	1.585344	0.0954776	16.60435*	4.05074E-29
BOD₅	0.393897	0.0272597	14.44978*	5.64966E-26
COD	0.28936	0.0902966	3.204548*	4.71466E-05
NH ₃ -N	1.197207	0.0706154	16.95392*	1.31301E-29
NO3-N	0.009673	0.0020527	4.712351*	3.50445E-08
Total-P	6.763333	0.7867718	8.596308*	4.19396E-16
Sol-P	0.257946	0.0134866	19.12615*	1.57436E-32
TSS	0.467942	0.055236	8.471692*	1.44564E-15
TURB	1.39028	0.0737348	18.85513*	3.555E-32
тс	4.985387	0.2235258	22.30341*	2.35387E-35
FS	3.555702	0.1882705	18.88614*	2.70932E-31

Table 3. p-values (*p \leq 0.05) among groups of a same water quality variable.



Figure 3. Graphical multiple planned comparisons of selected variable mean values based on GT-2 method. 1: 1^{st} period, 2: 2^{nd} period, 5: HRT of 5 days, 10: HRT of 10 days, Inf: Influent, G: gravel substrate, S: sand substrate; V: vegetated. So, 5GV1: effluent of vegetated unit filled with gravel substrate with a HRT of 5 days in the 1^{st} period, 10S2: effluent of control unit filled with sand substrate with a HRT of 10 days in the 2^{nd} period.

Each graphic allows a rapid and simultaneous comparison among all mean values of a particular parameter under different operational factors such as substrate (gravel and sand), HRT (5 and 10 days), either presence or absence of vegetation in both cultivation cycles. Statistically significant differences (significance level of 0.05) among means are indicated by non-intercept of comparison intervals.

Conclusions

Results indicated that constructed wetland systems associated with biomass production as investigated herein may represent a simple and low cost technology for a sustainable management of water resources in periurban areas of developing countries of tropical regions. Vegetation was the main factor influencing reduction of organic matter, fecal indicator microorganisms and nutrients from surface water polluted with sewage. No significant influence could be attributed to either substrate or hydraulic retention time on the operational performance of the pilot-scale constructed wetlands vegetated with *O. sativa* L., treating water from the urban stream polluted with sewage.

REFERENCES

- APHA; AWWA; WEF. American Public Health Association; American Water Works Association; Water Environment Federation (2005). Standard Methods for the Examination of Water and Wastewater. Washington D.C.: APHA; AWWA; WEF.
- Akratos ČS, Tsihrintzis VA (2007). Effect of temperature, HRT, vegetation and porous media on removal efficiency of polot-scale horizontal subsurface flow constructed wetlands. Ecol. Eng. 29:173-191.
- Brasil. Brazilian Ministry of Environment. National Council of Environment (2005). Resolução 357 (Resolution 357) [in portuguese]. Brasil. Brazilian Ministry of Environment. National Council of
- Environment (2011). Resolução 430 (Resolution 430) [in portuguese]. Brisson J, Chazarenc F (2009). Maximizing pollutant removal in constructed wetlands: Should we pay more attention to macrophyte species selection? Sci Total Environ. 407:3923-3930.
- Dong Y, Wiliriski PR, Dzakpasu M, Scholz M (2011). Impact of Hydraulic Loading Rate and Season on Water Contaminant Reductions Within Integrated Constructed Wetlands. Wetlands. 31:499-509.

- Douglas I (2006). Peri-urban ecosystems and societies. In: McGregor D, Simon D, Thompson D (eds.). The Peri-urban interface: approaches to sustainable natural and human resources use. Earthscan, London. pp. 18-27.
- Gauss M (2008). Constructed wetlands: a promising wastewater treatment system for small localities. Word Bank Office, Lima, Peru.
- Hagendorf U, Diehl K, Feuerpfeil I, Hummel A, Szewzyzk R (2000). Retention of microbiological organisms in constructed wetlands. In: Proceeding 7th International conference on Wetland Systems for water Pollution Control, Lake Buena Vista, Florida, USA. pp. 391-397.
- Hunt PG, Poach ME (2001). State of the art for animal wastewater treatment in constructed wetlands. Water Sci. Technol. 44:19-25.
- Kadlec RH, Wallace, SD (2009) Treatment Wetlands. CRC Press: Boca Raton, FL, USA.
- Massoud M, Tarhini A, Nasr JA (2009). Decentralized approaches to wastewater treatment and management: Applicability in developing countries. J. Environ Manage. 90:652-659.
- Redder A, Dürr M, Daeschlein G, Baeder-Bederski O, Koch C, Müller R, Exner M, Borneff-Lipp M (2010). Constructed wetlands-Are they safe in reducing protozoan parasites? Int. J. Hyg. Environ. Health. 213(1):72-77.
- Reed S, Crites RW, Middlebrooks EJ (1995). Natural systems for waste management and treatment. McGraw Hill Co, New York, USA.
- Rodier J (1984). L'analyse de l'eau: eaux naturelles, eaux résiduaires, eau de mer (The analysis of water: natural waters, wastewaters, sea water). Bordas, Paris, France [in french].
- Sokal RR, Rohlf FJ (1995). Biometry: the principles and practice of statistics in biological research. Freeman and Company, New York, USA.
- Stefanakis AI, Tsihrintzis VA (2012). Effects of loading, resting period, temperature, porous media, vegetation and aeration on performance of pilot-scale vertical flow constructed wetlands. Chem. Eng. J. 181:416-430.
- Tsihrintzis VA, Gikas GD (2010). Constructed wetlands for wastewater and activated sludge treatment in North Greece: a Review. Water Sci. Technol. 61: 2653-2672.
- Vymazal J (2002). The use of sub-surface constructed wetlands for wastewater treatment in the Czech Republic: 10 years experience. Ecol. Eng. 18:633-646.
- Vymazal J (2010). Constructed wetlands for wastewater treatment. Water. 2:530-549.
- Vymazal J, Kröpfelová L (2008). Wastewater treatment in constructed wetlands with horizontal sub-surface flow. Springer press, Dordrecht, The Netherlands.
- Zhang D, Gersberg RM, Keat TS (2009). Constructed wetlands in China. Ecol. Eng. 35:1367-1378.