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Nitrogen and Phosphorus removal from municipal wastewater by three wetland plant species

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Abstract

Abstract: Due to the sensitivity of municipal wastewater, further wastewater treatment is essential to ensure public health and crop production. One solution for the additional treatment of municipal wastewater is the use of surface and subsurface constructed wetlands. In order to evaluate the proportion of total nitrogen (TN) and total phosphorus (TP) removal attributed to storage in plants, three wetland plant species (Phragmitesaustralis, Typha and Rush) were planted in separate cells. For each plant, four cells were considered. After 24 weeks, the plants were sampled to determine the nutrient's accumulation rate; also, the capacity of these species to retain the nutrients in above and below-ground plant tissues was reported. The uptakes of nutrients by Rush, Common Reed and Typha from the pilot system after 24 weeks, were 58.6% TKN; 35.71% P, 56.48% TKN; 30.35% P and 32.19% TKN; 14.28% P, respectively. The results can be applied in plant species selection in the design of constructed wetlands in Isfahan as well as in optimizing the performance of these systems.

Keyword

Constructed wetland, Nutrient removal, PhragmitesAustralis,

Typha , Rush.

Introduction

A good alternative for small settlements is touse constructed wetlands (CW). The use of these systemsis becoming very popular in many countries (Kuschk et al., 2003; Akratos and Tsihrintzis, 2007).

Constructed wetlands are artificial wastewater treatment systems consisting of shallow (usually less than 1 m deep) ponds or channels which have been planted with aquatic plants, and which rely upon natural microbial, biological, physical and chemical processes to treat wastewater. They typically have impervious clay or synthetic liners, and engineered structures to control the flow direction, liquid detention time and water level. Depending on the type of system, they may or may not contain an inert porous media such as rock, gravel or sand.Constructed wetlands have been used to treat a variety of wastewaters including urban runoff, municipal, indus-trial, agricultural and acid mine drainage (EPA, 2000).

The first experiments aimed at the possibility of wastewater treatment by wetland plants were undertaken by Käthe Seidel in Germany in 1952 at the Max Planck Institute in Plön (Seidel, 1955).The constructed wetlands systems can have different flow formats, media and types of emergent vegetation planted. The basic classification is based on the type of macrophytic growth (emergent, submerged, free floating and rooted with floating leaves), further classification is usually based on the water flow regime (free water surface flow, sub-surface vertical or horizontal flow) (Sitikamariah, 2006). In horizontal sub-surface flow systems (HF CWs), the wastewater is fed in at the inlet and flows slowly through the porous media under the surface of the bed in a more or less horizontal path until it reaches the outlet zone where it is collected prior to leaving via level control arrangement at the outlet. During this passage, the wastewater will come into contact with a network of aerobic, anoxic and anaerobic zones. The aerobic zones occur around roots and rhizomes that leak oxygen into the substrate (Cooperet al., 1996; Brix, 1987; Vymazal, 2009).

Constructed wetland treatment systems utilize wetland plants and micro-organisms which are the active agents in the treatment processes (Kadlec and Knight, 1996). Most of constructed wetland systems are marshes with shallow water regions dominated by emergent marsh plants such as cattails, bulrushes, rushes and reeds.

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Transformations of nitrogen in wetland systems are diverse whereas the removal pathways which literally remove nitrogen from the systemare only few such as denitrification, volatilization, and plant uptake if connectedwith harvesting. Other mechanisms, such as nitrification or ammonification,are only responsible for transformations among various nitrogen forms orothers retain nitrogen in the beds (adsorption, burial) (Vymazal and Kröpfelová, 2008). Brix and Schierup (1989) suggested that standing stock in above-groundbiomass of emergent macrophytes is approximately between 20 and 250 g N m^{-2}. Vymazal (1995)reported above-ground N standing stock in the range of 22 to 88 g N m^{-2} for29 various emergent species. Mitsch and Gosselink (2000) reported thatthe above ground stock of nitrogen in freshwater marsh plants ranges from aslow as 3 to 29 g N m^{-2}.Tanner (2001) found in combined above(including standing dead) and below-ground Scirpustabernaemontaninitrogen accumulation between 48 and 69 g N m^{-2} increasing with wetlandwastewater loading.
Kuusemets et al. (2002) and Mander et al. (2004) reported nitrogenaccumulation in plant biomass (Phragmitesaustralis and Scirpussylvaticus)growing in a HF constructed wetland at Kodijärve, Estonia to be 13.7 and67.6 g N m^{-2} in 2001 and 2002. The biomass included above andbelow ground parts and litter. Most of the nitrogen standing stock wasallocated to below-ground. Vymazal (2007)summarized that in HF constructed wetlands the nitrogen-removingmechanisms are quite limited due to thelack of oxygen in filtration beds due tocontinuous water logging of the bed.
For phosphorus, it is important to note that HF constructed wetlands are rarely built withphosphorus being the primary goal of the treatment and therefore, materials with relatively low sorption capacity but high hydraulic conductivity such as river gravel or crushed rock are commonly used. As both above ground biomass and P concentration in the above groundplant tissues are similar in natural stands and constructed wetlands, it isobvious that also P standing stocks in constructed wetlands should be withinthe range found in natural stands(Vymazal and Kröpfelová, 2008). Reddy and DeBusk (1987), suggested the P standing stock between 1.4 and 37.5 g Pm^{-2} yr^{-1} for Typha, Phragmites, Scirpus and Juncus. However, the valuesincluded also below ground standing stock which is generally not availablefor harvest and the authors indicated that usually >50% of the stock is storedin below ground biomass..Vymazal (1995)reported above ground P standing stock in the range of 0.1 to 11 g P m^{-2} $yr^{-1}$for 29 various emergent species. Tanner (2001) found mean combined above(including standing dead) and below ground Scirpustabernaemontaniphosphorus accumulation between 8.8 and 13.4 g P m^{-2} increasing withwetland wastewater loading. Kuusemets et al. (2002) reported that plants (PhragmitesaustralisandScirpussylvaticus) growing in a HF constructed wetland at Kodijärve, Estonia, assimilated on average 2.1 g P m^{-2} yr^{-1} with 1.15 g P m^{-2} yr^{-1}beingallocated below ground. Vymazal (2004) reported that P standing stock in above-ground biomassofTyphaglauca, Typha spp., Phalarisarundinacea and Phalarisarundinaceagrowing in 30 constructed wetlands in Australia, Canada, Czech Republic, Germany, Poland, The Netherlands, UnitedKingdom and USA varied between 0.2 and 10.5 g P m^{-2}.

The aim of this study is tounderstand and compare the potential capacity of thethree wetland plants for the accumulation ofnutrients and therefore, selecting the most suitable plant for application in constructed wetlands in Isfahan and possibly in the similar climates. Choice of three plants that grow in Isfahan and the plants statisticalcomparison makes this study different from similar studies. High nutrient removal efficiency in Rush and Common Reed was achieved in this study.
In following manuscript first, the preparation of cells is discussed, then planting, sampling andtesting details are presented respectively. Results consisting of wastewater quality, statistical comparison of the plants, plant growth and nutrient removal are discussed and finally, the present findings are summarized and concluded in the last section.

MATERIALS AND METHODS

Study Site

The study site is located in Isfahan University of Technology, Iran with the longitude of 51º 28’ East and the latitude of 32º 42’ North having the elevation of 1626.4 meters from the Sea level. The climate is temperate and dry, with a mean annual temperature of 10 ◦C; ranging from 22.5ºC in July to −1.8ºC in January.The average annual rainfall is approximately 120 mm.

Preparation of Cells

Four cells were considered for each plant species, .Three cells were also considered for the three plants growing using regular water (control cells), to be compared to that of treatment cells and thus the effect of plants on wastewater treatment would be determined. In order to study the effect of cells and porous media on nutrients removal, a cell without plant was considered and sampled from cell outflow at several times to obtain average concentrations of nutrients in this cell. To prepare the cells, first, the place of cells was marked into2× 0.7 m rectangles and all of them were dug to a depth of 0.5 m. Afterwards, a layer of soft soil was bestrewed upon, dense enough to achieve the slope of 1%. Then, the floor and walls of cells were insulated. Later, all cells were filled with gravel (2-6 mm). In this step, the pipes were embedded for water entrance and control of the water level in the cells. For the no plant cell, an outlet pipe was used.

Planting

In order to grow the plants in cells, late February plants with the rhizome were harvested from the areas that were alreadyidentified and were transported to thesite plan. Then, they were planted in the cells 20 cm deep and with a distance of 20 cm together. For the first three weeks, all cells were irrigated with normal water. Later, wastewater was entered into cells except for control cells.

Sampling and Testing

When the cells were ready, plants began to grow and after 24 weeks, sampled plants and above-ground and

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underground tissues of the plants were tested separately to identify and to compare the accumulation of nutrients in each plant. By calculating the nutrients' accumulation rate for the control and treatment plants, the net nutrient removal by plants can be obtained. By measuring the nutrient concentration of the no plant cell effluent, the removal rate of nutrients by porous media is determined. So the nutrient concentration in the outflow of cells is estimated according to the Equation 1 and 2.

$S_{out} = S_{in} - R_{net} - R_p$ (1)

$R_{net} = R_t - R_c$ (2)

Thus nutrient removal was calculated as follows:

$R = (S_{in} - S_{out} / S_{in}) \times 100$ (3)

Where R_{net} is net nutrient removal by plants, R_p is nutrient removal by porous media, R_t is nutrient removal by treatment plants, R_c is nutrient removal by control plants, S_{in} is inflow concentration, $S_{out}$is outflow concentration and R is removal efficiency.

The pipes embedded in the cells, were used for entering the wastewater into the cells. The volume of wastewater required to fill the cells with respect to the inflow (0.5 liters per second) and time (4 min) was calculated at 120 liters.

The water level drop in the cells was measured several times and eventually, the average value was equal to 8 cm per week, therefore, approximately 55 liters of wastewater was necessary in a week to compensate water-loss. Thus, during the test period (24 weeks), the total amount of wastewater used in each cell was calculated 1275 liter (in the first three weeks wastewater was not used in the cells).

In the no plant cell, an alternating flow was maintained for 12 weeks and during this period there were 3 times sampling from wastewater outflow and the average was recorded as the quality of wastewater outflow.

Results

Wastewater Quality

To determine the quality of wastewater inflow, they were sampled several times during the experiment and TP and TN were measured. Average concentrations were achieved 13.3 and 0.44 mg/l for TN and TP, respectively.

With regards to these concentrations and the total volume of wastewater entered into each cell calculated previously, total nitrogen and phosphorus entered into each cell after 24 weeks was calculated at 16.9575, 0.561.

To investigate plant growth in the cells, plant's stem length was measured every week. Also wet and dry biomass of plants after 24 weeks was measured. In the first three weeks, they showed to be very slow growing plants; therefore, the plants did not sprout over this period. However, the primary signs of growth were observed, at the beginning of the fourth week after planting. The reason for this behavior is the fact that Reed first begins to develop the roots and rhizomes and later shoots emerge from the rhizomes and then begin to grow.Typha was the first plant to begin above-ground growth, Common Reed being the second and lastly Rush began to grow with atwo-week delay in comparison to the other two plants. It seems that Rush needs more time for growth and development compared with Reed and Typhabecause of its denser underground tissues. Despite Typha had started its growth before the other two species, it had a lower growth rate than other species and Rush had the best growth rate. After 24 weeks, the maximum length was observed for Common Reed, Rush and Typha,respectively. Figure 1 shows the changes in the stem length of plants during 24 weeks.

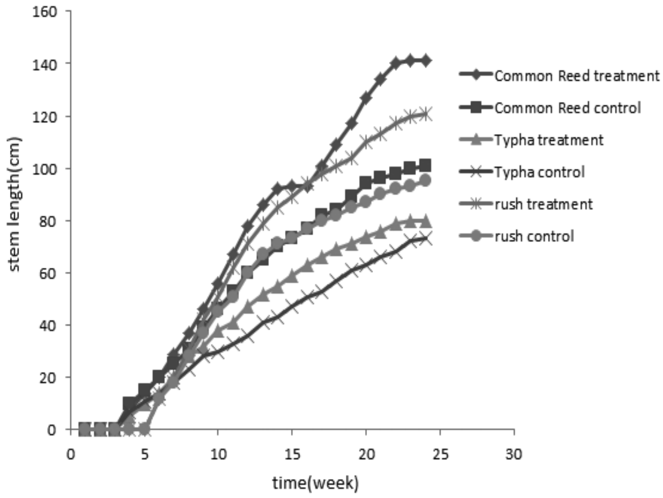

Fig. 1. Growth of Common Reed, Typha and Rush stems in treatment and control cells

After 24 weeks, the growth rate and the total harvested biomass of the three treated wetland plants were higher than those in the control cells. The fresh biomass per cell of Common Reed was 1.9 and 0.83 kg, respectively, whereas for Typha the fresh biomass per cell was 1.65 and 0.63 kg, respectively and for Rush the fresh biomass per cell was 2.15 and 1.3 kg, respectively for plant samples harvested from the treatment and control cells (Table 1 and Fig. 1).

Nutrient contents in the samples of the Rush (18.05 g kg-1 N; 0.36 g kg-1 P) were higher than those in samples of the Common Reed (17.59g kg-1 N; 0.29 g kg-1 P) and they were higher than those in the samples of the Typha (11.17 g kg-1 N; 0.16 g kg-1 P).

After 24 weeks, nutrient contents in below-ground biomass were higher than those in above -ground biomass in the three plants (Table 2).

The total net nutrient accumulations in the plant biomass of Rush were 9.94 g N and 0.2g P per cell and for Common Reed were 9.58g N and 0.17g P per cell and Typha showed a lower nutrient contents at 5.46g N and 0.08g P per cell for a period of 24 weeks (Table 3). The total net nutrient accumulations in the plant biomass of Rush were 9.94 g N and 0.2g P per cell and for Common Reed were 9.58g N and 0.17g P per cell and Typha showed a lower nutrient contents at 5.46g N and 0.08g P per cell for a period of 24 weeks (Table 3).

Rush has the highest and Typha has the least nutrient absorption and storage capacity. Total nitrogen accumulations in the Rush treatment plant samples were

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0.042 gm^{-2} day^{-1} , compared to 0.04 gm^{-2} day^{-1} in Common Reed and 0.023 gm^{-2} day^{-1} in Typha whereas total phosphate accumulation in the Rush treatment plant samples were 0.00085 gm^{-2} day^{-1} and 0.00072 gm^{-2} day^{-1} in Common Reed and 0.00034 gm^{-2} day^{-1} in Typha (Table 3). Nutrient removal through uptakes by the Rush, Common Reed and Typha were 58.6% N; 35.71% P and 56.48% N; 30.35% P and 32.19% N; 14.28% P, respectively (Table 3).

To calculate the nutrient's removal by the cells, first the concentration of nutrients in the outflow of cells should be estimated. In this regard, the outflow concentration of nutrients in the control cell was measured and presented in Table 4.

For the experimental period of 24 weeks,1275 liters of wastewater was applied to each of the treatment cells. Nitrogen and phosphorous removal rates by porous media were calculated at 2.55 gr and 0.115 gr, respectively.

The removal efficiencies in Rush, Common Reed and Typha treatment cells were 73.64% TN; 56.25% TP and 71.52% TN; 50.89% TP and 47.23% TN; 34.82% TP, respectively, throughout the experimental period (Table 5).

Discussion

The wetland plants have a large above and below biomass and these sub-surface plant tissues grow horizontally and vertically, and create a large surface area for the uptake of nutrients and ions (Cooper et al., 1996). Each wetland plant species shows differential accumulation and release of N and P and may influence the overall potential of a treatment wetland (Kao et al., 2003).

In this study, Rush, Common Reed and Typha had biomass growth of 2.15 ,1.9 and 1.65 kg per cell, respectively (Table 2), and a maximum growth of stem length of 11, 11 and 8 cm per week, respectively (Fig. 1). The growth rates of Rush, Common Reed and Typha (above-ground biomass) were 0.0036, 0.0034 and 0.0031 kgm^{-2} day^{-1}, respectively, compared to other wetland plant species such as Typhadomingensis, Schoenoplectusvalidus and Eleocharis spp. (0.002–0.006 kgm^{-2} day^{-1}) in a pilot wetland system in Cairns, Australia (Greenway and Woolley, 2001). However, Rush, with an extensive root system and a higher below-ground biomass achieved a higher plant uptake in comparison with Common Reed and Typha.Nutrient removals through plant uptake by Rush, Common Reed and Typha in this pilot study were 58.6% N; 35.71% P, 56.48% N; 30.35% P and 32.19% N; 14.28% P, respectively. According to previous studies, the numbers is in an appropriate range.In three species of plants nitrogen removal percentage was more than from phosphorus removal. This result is consistent with previous research in this field. It is important to note that in constructed wetlands in order to achieve high phosphorus removal it is necessary to select materials with high P adsorption capacity phosphorus.

Table 6 shows the nutrient removal efficiencies of the studies of plant species in constructed wetlands. Plant uptake by soft-stem bulrush Schoenoplectustabernaemontani and Baumeaarticulata accounted for around 11–26% of the N and 3–29% of the P removal rates (Tanner et al., 1995). Breen (1990) and Rogers et al. (1991) reported the nitrogen plant uptake of 55% for cattails and 85% for Schoenoplectusvalidus.A study by Lim et al. (2001) in Malaysia showed that about 50% of the nitrogen was stored in the leaves of cattail plants whereas a maximum rate of total nitrogen accumulation in plant biomass at 80% was recorded by Greenway and Woolley (2001). One of the major sink for phosphorus in most wetlands is the soil (Kadlec and Knight, 1996). Most of the Phosphorus component may fix within the soil media in the tanks (Brix, 1987). However, the nutrient stored in the substrate in this pilot study was not significant, probably due to the short experimental period.

Conclusions

Wastewater use in various applications is one of the strategies for sustainable management of water resources and the use of appropriate methods for wastewater treatment appears to be essential for reducing wastewater pollution and health concerns.

With regard to the results, it can be concluded that the constructed wetland (CW) systems and in general using the wetland plants for wastewater treatment is an appropriate solution. Theoverall nutrient removal efficiency in cells was satisfactory and it was highest in Rush and then Common Reed and Typha, respectively.Nitrogen removal was more than phosphorus removal by all of the three plants. Accumulation of nutrients in the below-ground biomass wasmore than those in above-ground biomass for the three plants. According to previous studies, these results was predicted. The study confirms that the selected plants are suitable for a treatment wetland in a temperate and dry climate. However, long term monitoring and maintenance are crucial to ensure the performance of the wetlands.

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Tab.1. Fresh and dry biomass of the plants in the treatment and control cells after 24 weeks

Plant / Parameter	Common Reed (Treatment)	Common Reed (Control)	Typha (Treatment)	Typha (Control)	Rush (Treatment)	Rush (Control)
Total above-ground fresh biomass (kg)	0.8	0.22	0.72	0.25	0.85	0.23
Total below-ground fresh biomass (kg)	1.1	0.61	0.93	0.38	1.3	0.8
Above-ground biomass/below-ground biomass ratio	0.72	0.36	0.77	0.66	0.65	0.29
Water content in above-ground biomass (%)	42.4	42.4	55.5	55.5	58	58
Water content in below-ground biomass (%)	70.1	70.1	60.8	60.8	61.9	61.9
Total above-ground dry biomass (kg)	0.56	0.15	0.46	0.16	0.52	0.14
Total below - ground dry biomass (kg)	0.65	0.36	0.58	0.23	0.8	0.49

Tab.2. Nutrient content in above-ground and below-ground plant biomass of the plants in the treatment and control cells after 24 weeks

Plant / Parameter	Common Reed (Treatment)	Common Reed (Control)	Typha (Treatment)	Typha (Control)	Rush (Treatment)	Rush (Control)
Total Nitrogen content in above-ground biomass (g kg-1)	6.82	2.5	2.97	1.15	4	1.7
Total Nitrogen content in below-ground biomass (g kg-1)	10.77	2.4	8.2	2.1	14.05	6.4
Total Phosphorous content in above-ground biomass (g kg-1)	0.08	0.01	0.04	0.009	0.12	0.02
Total Phosphorous content in below-ground biomass (g kg-1)	0.21	0.03	0.12	0.03	0.24	0.1

Tab.3. Nutrient uptakes of the plants in the cells

Plant	Common Reed		Typha		Rush	
Parameter	N	P	N	P	N	P
Total net nutrient uptake by plant per cell (g)	9.58	0.17	5.46	0.08	9.94	0.2
Total nutrient applied per treatment cell (g)	16.96	0.56	16.96	0.56	16.96	0.56
Percentage of net plant uptake	56.48	30.35	32.19	14.28	58.6	35.71
Total net nutrient accumulation rate (gm^{-2} day^{-1})	0.04	0.00072	0.023	0.00034	0.042	0.00085

Tab.4. Nutrients removal using the control cell

Parameter	Inflow concentration (mg/l)	Outflow concentration (mg/l)	Removal rate (%)
TN	13.3	11.3	15
TP	0.44	0.35	19

Tab.5. Nutrient removal efficiency by cells

Plant	Common Reed		Typha		Rush	
Parameter	N	P	N	P	N	P

Input nutrients per cell (g)	16.96	0.56	16.96	0.56	16.96	0.56
Nutrient uptake by plant (g)	9.58	0.17	5.46	0.08	9.94	0.2
Nutrient uptake by porous media (g)	2.55	0.115	2.55	0.115	2.55	0.115
Outflow nutrients per cell (g)	4.83	0.275	8.95	0.365	4.47	0.245
Nutrient removal efficiency (%)	71.52	50.89	47.23	34.82	73.64	56.25

Tab.6. Nutrient removal efficiencies of the studies on plant species in constructed wetlands [HuaSim et al., 2008]

No	Studies	Nutrient removal efficiencies (kg ha-1 day-1)		Types of wastewaters	Plant species used	Nutrient plant uptake in plant biomass
		N	P			
1	HuaSimet al. (2003)	3.44	0.24	Nutrient solution	Phragmiteskarka	42.12% N; 28.92% P
2	HuaSim et al. (2003)	1.56	0.23	Nutrient solution	Lepironiaarticulata	17.43% N; 26.08% P
3	Headley (2004)			Nursery runoff	Phragmitesaustralis	41–54% N; 36–63% P in above-ground biomass, 24–30% N; 36–39% P in below-ground biomass
4	Toet (2003)	0.53	0.082	Sewage effluent	Phragmitesaustralis	37–42% N; 22–40% P
5	Browning and Greenway (2003)	0.8–7.3			Baumeaarticulata, Carexfascicularis, Philydrum lanuginosum and Schoenoplectusmucronatus	11% N; 3% P
6	Greenway and Woolley (2001)	0.72–1.93	0.22–0.68	Secondary effluent	Typhadomingensis, Schoenoplectusvalidus, Eleochrais equisetina, Eleocharissphacelata	14.5–80% N, 24–80% P
7	Kantawanichkul et al. (2001)	11.2		Livestock effluent	Cyperusflabelliformis	7–9% N 0.414–0.491 gNm^{-2} day^{-1} in aboveground biomass 0.197 gNm^{-2} day^{-1} in below-ground biomass
8	Lim et al. (2001)	4.5		Septic tank effluent	Cattail Typha sp.	2.6 kg ha^{-1} day^{-1} (50% N)
9	Tanner (2001)	3.0	1.0	Dairy farm wastewaters	Soft-stem bulrush Schoenoplectustabernaemontani	
10	Okurut (2000)	7.1	0.24	Septic tank effluent	Cyperus papyrus	14.95–21.91 mgg^{-1} N; 5.61–5.95mgg^{-1} TP in above-ground tissue
11	Okurut (2000)	10.4	0.26	Septic tank effluent	Phragmitesaustralis	19.96–22.16 mgg^{-1} TN; 7.90–10.05mg g^{-1} TP in below-ground biomass
12	Koottatep and Polprasert (1997)	3.0		Septic tank effluent	Cattail Typhaangustifolia	43% TN (31% in leaf, 10% in stem, 2% in root)

References

1. ***Akratos, C.S.,and Tsihrintzis, V.A***.,*Effect of temperature, HRT, vegetation and porous mediaon removal efficiency of pilot-scale horizontal subsurfaceflow constructed wetlands.Ecol.Eng. No.29,2007, pp.173–191*

2. ***Breen, P.F.,*** *A mass balance methods for assessing the potential of artificial wetlands. Water Res., No.24, 1990, pp.689–697.*

3. ***Brix, H.,*** *Treatment of wastewater in the rhizosphere of wetlands plants—the root-zone method. Water Sci. & Technol. No.19,1987, pp. 107–118.*

4. ***Browning, K., and Greenway, M.,*** *Nutrient removal and plant biomass in a subsurface flow constructed wetland in Brisbane, Australia. Water Sci. & Technol., No. 48, pp. 183–189.*

5. ***Cooper, P.F. , Job, G.D., Green, M.B., Shutes, R.B.E.,*** *Reed Bedsand Constructed Wetlands for Wastewater Treatment, UK: Medmenham, Marlow,1996.*

6. ***Greenway, M., and Woolley, A.,*** *Changes in plant biomass and nutrient removal over 3 years in a constructed wetland in Cairns, Australia. Water Sci. & Technol, No. 44, 2001, pp.303–310.*

7. ***HuaSim, C., Yusoff, M.K., Shutes, B., Chye Ho, S., Mansor, M.,*** *Nutrient removal in a pilot and full scale constructed wetland. Environ. Manag. No. 88, 2008, pp. 307-317.*

8. ***Kadlec, R.H., and Knight, R.L.***, *Treatment Wetlands, USA, 1996.*

9. ***Kao, J.T., Titus, J.E., Zhu, W.X.*** , *Differential nitrogen and phosphorus retention by five wetland plant species. Wetlands, No.23, 2003, pp. 979–987.*

10. ***Kuschk, P., Wiebner, A., Kappelmeyer, U., Weibbrodt, E., Kastner,M., Stottmeister, U.,*** *Annual cycle of nitrogen removal by a pilot-scale subsurface horizontal flow in a constructed wetland under moderate climate. Water Resour., No.37, 2003, pp. 4236–4242.*

11. ***Lim, P.E., Wong, T.F., Lim, D.V.***, *Oxygen demand, nitrogen and copper removal by free water surface and subsurface flow constructed wetlands under tropical conditions. Environ. Int, No.26, 2001, pp. 425–431.*

12. ***Rogers, K.H., Breen, P.F., Chick, A.J.***, *Nitrogen removal in experimental wetland treatment systems—evidence for the role of aquatic plants.Water Pollut. Control. No.63, 1991, pp.934–941.*

13. ***Tanner, C.C., Clayton, J.S., Upsdell, M.P.,*** *Effect of loading rate and planting on treatment of dairy farm wastewaters in constructed wetlands—I. Removal of oxygen demand, suspended solids and faecal coliforms. Water Res,. No.29,1995, pp. 17–26.*

14. ***Vymazal, J.***, *The use constructed wetlands with horizontal sub-surface flow for various types of wastewater. Ecol. Eng., No.35,2009, pp. 1–17.*

15. ***Vymazal, J.,and Kröpfelová, L.,*** Wastewater Treatment in Constructed Wetlands with Horizontal Sub-Surface Flow, New York,2008.