Available online at http://www.journalijdr.com

IJDR

ISSN: 2230-9926

International Journal of Development Research
Vol. 08, Issue, 06, pp.21046-21055, June, 2018

ORIGINAL RESEARCH ARTICLE

OPEN ACCESS

NUTRIENT REMOVAL AND BALANCE IN VERTICAL-FLOW CONSTRUCTED WETLANDS PLANTED WITH DIFFERENT FORAGE MACROPHYTES FOR DOMESTIC WASTEWATER TREATMENT

Franck Michaël Zahui, Jean-Marie PétémanagnanOuattara, *Aman Messou and Lacina Coulibaly

Laboratory of Environment and Aquatic Biology, Department of Sciences and Environment Management,
Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire

ARTICLE INFO

Article History:
Received 17th March, 2018
Received in revised form
26th April, 2018
Accepted 24th May, 2018
Published online 30th June, 2018

Key Words:
Constructed wetland - Nutrients removal - Balance - Domestic wastewater – Forage plants.

ABSTRACT

This study investigated nutrient removal as well as nutrient balance in a vertical flow constructed wetland planted with *Andropogongayanus*, *Chrysopogonzizanioides*, *Echinochloapyramidalis*, *Pennisetumpurpureum* and *Tripsacumlaxum* for treating synthetic domestic wastewater. The experiment was conducted under pilot-scale composed of six beds constructed with bricks. Each bed was filled from the bottom to the top with 0.1 m of gravel and 0.6 m of white lagoon sand. Five beds were transplanted wile one unplanted was used as control. On each bed, 80 L of synthetic wastewater were applied (3 times/week) during six months. All plant species improved nutrient removal, but *P. purpureum* achieved the best TN, NH_4^+, NO_3^-, TP and PO_4^{3-} removal efficiencies around 84.30 %, 84.18 %, 69.32 %, 94.68 % and 97.17 % respectively. Likewise, TP and TN amount decreased in the beds' sediments from upper surface to the bottom. According to the mass balance approach, plant uptake removed 1.1–15.3% of TN and 1.6–25.3% of TP input while sediment storage contributed to 0.9–49% and 43.9–70.5% of TN and TP removal, respectively. Microbial uptake accounted for 9.3–68.1% and 1.3–20.3% of nitrogen and phosphorus removal, respectively. The results proved that sediment storage and plant uptake were the main phosphorus removal pathways while for nitrogen, plant uptake or sediment storage and microbial mechanisms were the main removal pathways in CWs treating domestic wastewater.

Citation: Franck Michaël Zahui, Jean-Marie Pétémanagnan Ouattara, Aman Messou and Lacina Coulibaly. 2018. "Nutrient removal and balance in vertical-flow constructed wetlands planted with different forage macrophytes for domestic wastewater treatment", *International Journal of Development Research*, 8, (06), 21046-21055

INTRODUCTION

In many developing countries, eutrophication has become an increasing phenomena because of the excessive nitrogen (N) and phosphorous (P) loading from domestic wastewater which is drained to natural watercourses without efficient treatment. As a result, the ecological integrity of surface waters becomes compromised, fish populations become extinct, toxic cyanobacteria blooms are abundant, and oxygen levels reduced (Nyenje *et al*., 2010; UN-Water /WWAP, 2017). Indeed, most of the conventional sanitation systems in these countries have been carried out by donors or North-South cooperation in their policy of assistance to developing countries. As a result, almost all wastewater treatment conventional systems are dysfunctional or abandoned due to their complexity and costly operation and maintenance, which cannot be ensured in the long term (CIEH, 1993; Maiga *et al*., 2006). However, most conventional wastewater treatment systems are designed to remove suspended solids and some of the dissolved solids. Many of them only partially remove the nitrogen (N) and phosphorus (P) contained in the wastewater and the rest of these nutrients are mineralized and returned to nature (Elser *et al*., 2007; Mahmood *et al*., 2013). Therefore, it seems imperative to explore other purification techniques that are flexible in their implementation, capable of operating in tropical environments, less expensive and efficient to protect natural watercourses from eutrophication by reducing their nutrient inputs. Constructed wetlands (CWs) are an efficient treatment technology which have been found to be the reasonable option because they are cost-effective, have less

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***Correspondingauthor:** Jean-Marie Pétémanagnan Ouattara
Laboratory of Environment and Aquatic Biology, Department of Sciences and Environment Management, NanguiAbrogouaUniversity, 02 BP 801 Abidjan 02, Côte d'Ivoire

operation and maintenance requirements, and have less reliance on energy inputs (Vymazal, 2006; Deeptha *et al.*, 2015). These systems have gained popularity in the last four decades as an alternative to conventional treatments such as activated-sludge systems (Vymazal, 2007). There are several types of CWs among which, that developed by Seidel (1966) (*e.g.* vertical flow CWs) proved to be the most appropriate because of the non-requirement of large areas for operating and, no risks of odors, nitrogen gas or nitrous oxide and methane emission to the atmosphere (Knight *et al.*, 2003; Mander *et al.*, 2005). Four main components make up the CWs namely, water, sediment, microbes and vegetation, which interactions are utilized to purify wastewater. Among these components, plants are considered to be the main biological component because of the important played role in wetland (Vymazal, 2007). They not only directly take up certain nutrients, but also act as intermedium for purification reactions by increasing the environmental diversity in the rhizosphere and enhancing a variety of chemical and microbial processes which promote purification efficiency in CWs (Gagnon *et al.*, 2007; Stottmeister *et al.*, 2003; Vymazal, 2011). Thus, the plants species that can withstand the harvesting and operating conditions of CWs (*e. g.,* submerged period, dry periods, high rates of organic matter and nutrients, fluctuating pollution load), and presenting abundant biomass and rapid growth are suggested (Vymazal, 2007). Even if plants, meeting these criteria can be used, little attention has been paid to using local plants species with economic interests would make the CWs more advantageous because, in addition to wastewater treatment, they can generate revenues that can support maintenance costs. Although local plant species such as *Amaranthus hybridus* and *Corchorus oliterius* (food plants) have given overall satisfactory results without any contamination of above-ground biomass (Coulibaly *et al.*, 2008a, 2008b), these plants have experienced a problem of acceptance when the population knew where they origin. However, nitrogen and phosphorus removal processes as well as their removal efficiency could vary with plant species through variation in rates of oxidation of the wetland sediment and supply of labile carbon and transpiration. In addition, plant species could also respond differently after the harvest (Greenway & Woolley, 2001; Wu *et al.*, 2011, 2013a, 2013b).

The main objective of this study were to develop a vertically flow CW using a local forage plant for the efficient treatment of nutrients (nitrogen and phosphorus) of domestic wastewater. Specifically, the effects of forage plants species (*i.e. Andropogon gayanus, Chrysopogon zizanioides, Echinochloa pyramidalis, Pennisetum purpureum,* and *Tripsacum laxum*) on nutrients removal in vertical flow CWs were investigated. Then, the contributions of different nutrients removal pathways such as sediment storage, plant uptake, and microbial degradation were quantify.

MATERIALS AND METHODS

Characterization of experimental wetland

The experiments were carried out on a pilot scale located at Nangui Abrogoua University (Abidjan, Côte d'Ivoire). The pilot scale was composed of six rectangular beds (vertical flow CW) (length x wide x depth = 1.45 m x 1.00 m x 0.80 m) built cement according to Coulibaly *et al.* (2008a, 2008b) and Ouattara *et al.* (2009), giving a bed surface of 1.45 m^2 (Figure1). Each bed was filled from the bottom to the surface by respectively 0.1 m gravel (5/15 mm) covered with cloth and 0.6 m white lagoon sand, previously washed to remove any clay, loam and organic matter. The bed bottom slope was 1 % oriented via PVC ($\Phi = 0.032$ m) to drain out the effluent. Each bed was equipped with irrigation devices consisted of six (6) PVC pipe (length: 1.70 m; diameter: 0.008 m) containing 60 lateral holes. Five plant species namely, *Andropogon gayanus* (Kunth, 1833), *Tripsacum laxum* (Nash, 1909), *Echinochloa pyramidalis* (Lam.) Hitchc & Chase (1917), *Pennisetum purpureum* (Schumach. 1827) and *Chrysopogon zizanioides* (Roberty, 1960) were experimented. These are perennial forage plants highly appreciated by agro-pastoralists for their palatability, their adaptation to local climatic conditions and their presence in Côte d'Ivoire. In addition, these plants can grow in a humid environment with or without wastewater and develop a very abundant aboveground and belowground biomass that can contribute to the treatment of wastewater (Boudet, 1991).

Experimental procedure

In order to minimize the variability in the experiment and address CWs clogging problems reported by Coulibaly *et al.* (2008a, 2008b) and Ouattara *et al.* (2008), synthetic domestic wastewater was employed in the experiment as the influent of the wetlands. The composition of synthetic domestic wastewater was made according Rodgers *et al.* (2006) and Healy *et al.* (2010). However, some modifications were done in order to respect the characteristics (*i.e.* nitrogen, phosphorus and carbon concentrations) of domestic wastewater encountered in developing countries according to Metcalf & Eddy (1991). The characteristics of the synthetic domestic wastewater used were: COD = 628 mg O_2/L, BOD_5 = 380 mg O_2/L, TN = 45 mg/L, TSS = 300 mg/L, TP = 12 mg/L, pH = 6.7- 8.00. Five beds were transplanted with the plants seedlings (*i.e.* 9 plants/m²) spaced of 40 cm x 40 cm between the stems and one was preserved unplanted (UB) and used as control. These young plants were collected from nurseries established near the experimental pilot and previously cut to 20 cm above the roots before the bed planting. The plants transplanted were fed with tap water for one month to allow acclimatize. After the acclimation period, each bed was intermittently fed (3 days/week) with 23.64×10^{-3}m/d hydraulic loading of synthetic domestic wastewater according to Ouattara *et al.* (2008, 2009, 2011) over 6 months. At the end of each two-month growth cycle, plants were harvested according to Ouattara *et al.* (2008) and the plant aboveground biomass produced was determined by weighing. Three measures of plant aboveground biomass were carried out during the experiment and data obtained for each bed were used for analyses.

Water sampling, analysis and removal efficiency

Wastewater samples were taken once a week at inlet (influent) and outlet (effluent) of each bed, stored in an ethylene bottle at 4 °C until analysis. A total of 24 water samples were taken in each bed during the whole period of the experiment. The pH, and dissolved oxygen (DO) were determined according to ISO 10523 (2008) and ISO 5814 (2012), respectively. Then, total nitrogen (TN), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3), total phosphorus (TP) and orthophosphate (PO_4^{3-}) were determined according to ISO 5663 (1984), ISO 7150-1 (1984), ISO 6777 (1984), ISO 7890-3 (1988) and ISO 6878 (2004) respectively. Finally, removal efficiencies was calculated according to Abissy and Mandi (1999) for TN, NH_4^+, NO_2^-, NO_3^-, TP and PO_4^{3-} as follows:

$$\text{Removal efficiency (\%)} = \frac{C_iV_i - C_eV_e}{C_iV_i} \ x\ 100 \quad \ldots\ldots\ldots.1$$

Where C_i and C_e are the inlet and outlet concentrations (mg/L), V_i and V_e are the inlet and outlet volume (L) in the CWs.

Plants and sediments sampling and analysis

During the whole harvest period, samples of each plant were taken (one at each corner (4) and one in the center (1) of the beds), then, put together to make the composite sample of this one. The samples were washed with tap water to remove any adhering sediments, rinsed with distilled water, and blotted with absorbent papers and weighed (80°C to constant dry weight). All of dried plant samples were ground separately to pass through a 40-mesh (0.425 mm screen) and wet digested and analyzed for total N and P according to NF ISO 11261 (1995) and NF ISO 6491 (1988), respectively. TN and TP contents were calculated by multiplying plant dry weight by nutrient concentration. Concerning nutrients concentration in the beds sediments of CWs, six sediment layers in the vertical profile were investigated from the upper surface to the bottom: [0;-10cm], [-10;-20 cm], [-20;-30 cm], [-30;-40 cm], [-40;-50 cm] and [-50;-60 cm] in order to understand the nutrient distribution in the bed sediments. After six month of wastewater treatment, five sediment samples were taken in each layer above-mentioned (*i.e.* one at each corner and one in the center of the beds) using PVC auger (Φ = 40 mm). The five samples of each layer were mixed to make the composite sample of this one, stored in cooling jars at 2°C during 24 hours for analysis. Thus, on each sediment sample taken, the total N was determined according to the NF ISO 11261 (1995), while total P was measured according NF ISO 11263 (1994).

Mass balance

Nutrient mass (N) balance model was designed in this study in order to quantify the contributions of different removal pathways in removing nutrient in each bed during the experiment according to Wu *et al.* (2011; 2013a; 2013b). The factors considered were (i) amounts added to and exported from the CWs systems; (ii) amounts accumulated by plants; (iii) amounts stored (*e.g.* adsorbed, precipitated) in the sediment (unanalyzed); (iv) other losses involving the uptake by microbiota (bacteria, fungi, algae, etc.). The conceptual model for nutrient mass balance in CWs is shown below:

$$N_{TN/TP\ (Inlet)} = N_{TN/TP\ (Sediment)} + N_{TN/TP\ (Plant)} + N_{TN/TP\ (Other)} + N_{TN/TP\ (Outlet)} \quad \ldots\ldots(2)$$

where, $N_{TN/TP\ (Inlet)}$ is the amount of TN or TP introduced in CWs (g/m^2), $N_{TN/TP\ (Sediment)}$ is the amount of TN or TP stored in the sediment (g/m^2), $N_{TN/TP\ (Plant)}$ is the amount of TN or TP accumulated by plant (TN or TP) (g/m^2), $N_{TN/TP\ (Other)}$ is the amount of TN or TP involving the uptake by microbiota and $N_{TN/TP\ (outlet)}$ is the amount of TN or TP released in CWs (g/m^2).

Calculation of Nutrients (TN or TP) introduced in the CWs

Nutrients (TN or TP) imported in the CWs was determined from TN or TP concentrations according to the following relationship:

$$N_{TN/TP\ (Inlet)} = \frac{Ci\ x\ Vi}{\text{wetland area}} \quad \ldots\ldots\ldots\ldots(3)$$

Where, $N_{TN/TP\ (Inlet)}$ is the amount of TN or TP introduced in CWs (g/m^2), C_i is the TN or TP concentrations in g/m^2, V_i is the inlet total volume introduced in CWs system (L) and the wetland area is 1.45 m^2.

Calculation of Nutrients (TN or TP) content in Effluent

Nutrients (TN or TP) exported from the CWs was determined from TN or TP concentrations according to the following relationship:

$$N_{TN/TP\ (Outlet)} = \frac{Cf\ x\ Vf}{\text{wetland area}} \qquad (4)$$

Where, $N_{TN/TP\ (outlet)}$ is the amount of TN or TP released in CWs (g/m^2), C_f is the TN or TP concentrations in (g/m^2), V_f is the outlet total volume released from the CWs (L) and the wetland area is 1.45 m^2.

Calculation of Nutrients (TN or TP) stored in the sediments

The amount of nutrients (TN or TP) stored in the sediment was determined from the estimated mass of the sediment and also the average concentration of nutrients (TN or TP) analyzed in the sediment from the relationship:

$$N_{TN/TP\ (Sediment)} = \frac{CN_S\ x\ M_S}{\text{wetland area}} \qquad (5)$$

Where, $N_{TN/TP\ (Sediment)}$ is the amount of TN or TP stored in the sediment (g/m^2), CN_S is the average concentration of TN or TP analyzed in the sediment (g/kg), M_S is dry weight of the media per wetland (1.5 10^3 kg) and the wetland area is 1.45 m^2.

Calculation of Nutrients (TN or TP) accumulated by plants

Nutrients (TN or TP) accumulated by plants were determined from the concentration of TN or TP in the plant's biomass according to the following relationship:

$$N_{TN/TP(Plant)} = \frac{\sum_{n=1}^{3}(CBIO_n\ x\ MBIO_n)}{\text{wetland area}} \qquad (6)$$

Where, $N_{TN/TP\ (Plant)}$ is the amount of TN or TP accumulated by plants (TN or TP) (g/m^2), CBIO is the average of TN or TP concentration in plants biomass (g/kg), MBIO is the dry biomass per wetland in kg and n is the period of plants harvesting.

Calculation of Nutrients (TN or TP) involving the uptake by microbiota

From the amounts of Nutrients (TN or TP) introduced and released from the CWs systems and the amount stored in the sediment and that assimilated by plant, the amount of TN or TP, involving the uptake by microbiota was determined as follow:

$$N_{TN/TP(Other)} = N_{TN/TP(Inlet)} - (N_{TN/TP(Plant)} + N_{TN/TP(Sediment)} + N_{TN/TP(Outlet)}) \quad (7)$$

Data analysis: Statistical tests used for data analysis include Kruskal-Wallis, Mann Whitney, and ANOVA variances and T test after Shapiro-Wilk testing normality test. These statistical tests were performed using the R studio 3.3.2 software (Ihaka & Gentleman, 1996). In all tests, differences were considered statistically significant when $P < 0.05$.

RESULTS

Biomass production

Figure 2 shows the fresh and dry biomasses produced by each plant species during the three harvesting periods. Overall, only *P. purpureum* biomasses increased according to the harvesting period. Conversely, *T. laxum* and *E. pyramidalis* biomasses decreased with the harvesting. The fresh biomasses ranged between 14.55 and 15.86 kg/m^2 (*P. purpureum*), 10.76 and 15.52 kg/m^2 (*T. laxum*), 3.59 and 12.76 kg/m^2 (*E. pyramidalis*), 1.45 and 4.67 kg/m^2 (*A. gayanus*) and between 1.45 and 2.58 kg/m^2 (*C. zizanioides*) (Fig. 2A). Dry biomasses ranged from 4.97 to 5.53, from 2.10 to 4.34, from 1.10 to 2.62, from 0.37 to 0.97, and from 0.28 to 0.76 kg/m^2 respectively on the beds planted with *P. purpureum*, *T. laxum*, *E. pyramidalis*, *A. gayanus* and *C. zizanioides* (Fig. 2B). The biomasses of *E. pyramidalis*, *P. purpureum* and *T. laxum* species were significantly higher than those of *A. gayanus* and *C. zizanioides* species (Mann Whitney test: $p <0.05$).

CWs performance

The mean values of the physico-chemical parameters at the inlet and outlet of all the beds (planted and unplanted) are consigned in Table 1. pH of influent increased from 6.81 to 7.33 in the beds effluents . The sequence of pH mean values was: Influent (6.81) < effluent of B_{AG} (6.92) < effluent of B_{EP} (6.93) < effluent of B_{CZ} (7.05) < effluent of B_{PP} (7.06) < effluent of B_{TL} (7.17) < effluent of UB (7.32).The statistical analysis carried out some differences for values pH among the influent and the effluents and that between the effluents of the different beds (Mann Whitney test: $P <0.05$). Also, the DO values increased in all beds effluents from 2.13± 0.55 mg/L in the influent to values ranging from 5.41 to 7.53 mg/L. However, the DO values of the effluents of the planted beds were greater than those of the unplanted bed (Mann Whitney test: $P < 0.05$). However, some significant differences were noted among those of the planted beds effluents (Mann Whitney test: $P < 0.05$).

The influents TN, NH_4^+, TP and PO_4^{3-} concentrations were significantly reduced in the planted beds than in the control (Mann Whitney test: $p < 0.05$). The concentrations of these parameters decreased from 41.45 ± 2.24 to 9.60 ± 5.22, from 33.37 ± 1.24 to 7.79 ± 4.16, from 10.93 ± 0.49 to 0.86 ± 0.31 and from 7.38 ± 0.34 to 0.31 ± 0.42, respectively. Considering the planted beds, the overall order of performance is as follows: performance of B_{PP} > performance of B_{TL} > performance of B_{EP} > performance of B_{AG} > performance of B_{CZ}. However, some significant differences were found between the beds performances (Kruskal-Wallis test, $p < 0.05$). Overall, the performances of the beds planted with *E. pyramidalis*, *A. gayanus* and *C. zizanioides* were not significantly different, as were those of beds planted with *P. purpureum* and *T. laxum* (Mann Whitney test: $p > 0,05$). However, the beds planted with *P. purpureum* and *T. laxum* were significantly efficient than those planted with *E. pyramidalis*, *A. gayanus* and *C. zizanioides* (Whitney test: $p < 0.05$). For NO_3^-, except the effluents of the bed planted with *P. purpureum* (0.79 ± 0.57 mg/L), the average concentrations recorded in the effluents (between 1.92 ± 1.98 and 5.06 ± 1.97 mg/L) were greater than those of the influent (1.75 ± 0.21 mg/L). The rate of NO_3^- removal were 69.32% in the bed planted with *P. purpureum*. However, the mass of NO_3^- contained in the influent was reduced by 23.85% only in the effluent of the bed planted with *T. Laxum.* Concerning NO_2^-, the influent concentrations decreased from 0.73± 0.54 to 0.17± 0.13 mg/L in the effluents. Among the NO_2^-concentrations, those determined in influent and unplanted bed effluent (0.67± 0.97 mg/L) were not statistically different (Whitney test: $p > 0.05$). However, these are significantly higher than NO_2^- concentrations in the planted beds effluents (Whitney test: $p < 0.05$). But, no significant difference were found among effluents of the planted beds ($p > 0.05$).The sequence of NO_2^- removal rate was: B_{PP} (83.81 ± 32.33%) > B_{AG} (73.68 ± 56.19%) > B_{TL} (70.77 ± 138.12%) >B_{CZ} (66.77 ± 39.28%) >B_{EP} (54.44 ± 136.55%) > UB (16.64 ± 180%).

Nutrient (N and P) quantification and balance in the CWs

N and P uptake by plants

The amounts of N and P assimilated by the biomass of the plants tested are shown in table 2. As is shown, the total nutrient accumulated was varied from 1.75 to 25.18 g N/m^2 and from 0.71 to 10.99 g P/m^2. Comparing the plants uptake of N and P, the highest amount of N and P assimilated were 25.18 g N/m^2 and 10.99 g P/m^2 for *P. purpureum* and was followed respectively by 16.00 g N/m^2 and 6.18 g P/m^2 for *T. laxum*, 6.96 g N/m^2 and 4.01 g P/m^2 for *E. pyramidalis*, 2.33 g N/m^2 and 3.42 g P/m^2 for *A. gayanus* and 1.75 g N/m^2 and 0.71 g P/m^2 for *C. zizanioides*.

N and P in the beds sediment

Total N and total P stored in the beds sediment

Total N and total P stored *(e.g.,* adsorbed and precipitated) in the sediment of CWs at the end of the experimental period were determined (Tab. 2). N and P accumulated in the sediment ranged from 1.54 to 80.76 g N/m^2 and from 19.05 to 30.59 g P/m^2. The unplanted bed achieved the highest N accumulation and the lower P accumulation of all the beds. However, in the planted beds, the highest nutrients amount was noted in bed planted with *C. zizanioides* and the lower in that planted with *P. purpureum*. The sequence of nutrient amount trapped in the planted beds was: B_{CZ} (65.94 g N/m^2 and 30.59 g P/m^2) > B_{AG} (59.72 g N/m^2 and 30.32 g P/m^2) > B_{EP} (44.66 g N/m^2 and 29.99 g P/m^2) > B_{TL} (24.64 g N/m^2 and 27.13 g P/m^2) > B_{PP} (1.54 g N/m^2 and 21.32 g P/m^2).

Vertical distribution of N and P in the beds sediment

Nutrients varied in the upper layer (0-10 cm) between 6.70 and 9.10 g P/m^2 and between 0.57 and 31.03 g N/m^2 while, fluctuated from 0.01 to 0.35 g P/m^2 and from 0.01 to 3.04 g N/m^2 in the bottom layer (50-60 cm) (Tab.3). As is shown, the sequences of P content in each layer of the beds were: P g/m^2 (B_{CZ}) > P g/m^2 (B_{AG}) > P g/m^2 (B_{EP})> P g/m^2 (B_{TL}) > P g/m^2 (B_{PP}) >P g/m^2 (UB). However the total P stored in the different layers of all the beds did not differ significantly (ANOVA test: $p > 0.05$). As for the total N obtained, in any layer the amounts stored in unplanted bed appeared the highest and was followed respectively by those of the beds planted with *C. zizanioides*, *A. gayanus*, *E. pyramidalis, T. laxum* and *P. purpureum*.

Table 1. Mean average and standard deviations of physico-chemical parameters inlet (influent) and outlet (effluents) the beds and average removal

Parameters	Inlet	Outlet											
	Influent	B_{TL}		B_{AG}		B_{CZ}		B_{EP}		B_{PP}		UB	
		Effluent	R (%)	Effluent	R (%)	Effluent	R (%)	Effluent	R (%)	Effluent	R (%)	Effluent	R (%)
pH	6.81 ± 0.07^{a}	7.17 ± 0.30^{cb}	-	6.92 ± 0.26^{a}	-	7.05 ± 0.27^{c}	-	6.93 ± 0.27^{a}	-	7.06 ± 0.21^{c}	-	7.32 ± 0.30^{b}	-
DO (mg/L)	2.13 ± 0.55^{a}	7.53 ± 1.56^{c}	-	6.50 ±0.80^{d}	-	6.70 ± 1.04^{d}	-	6.63 ± 0.98^{d}	-	7.24 ± 1.06^{c}	-	5.41 ± 0.88^{b}	-
NT (mg/L)	41.45 ± 2.24	10.98 ± 4.23	81.59 ± 8,41^{a}	12.83 ± 5.38	76.66 ± 11.16^{b}	13.59 ±4.02	74.48 ±10,01^{b}	13.28 ± 5.15	76.54 ±11.67^{b}	9.60 ± 5.22	84.30 ± 9.56^{a}	19.40 ± 5.27	57.66 ± 14.98
NH_4^+ (mg/L)	33.37 ± 1.24	8.98 ± 3.23	81.30 ± 6.80^{a}	9.56 ± 3.83	78.39 ± 9.13^{b}	10.19 ± 3.57	76.23 ± 9.00^{b}	9.78 ± 4.05	78.54 ± 9.49^{b}	7.79 ± 4.16	84.18 ± 8.98^{a}	13.43 ± 3.08	63.43 ± 8.72
NO_3^- (mg/L)	1.75 ± 0.21^{b}	1.92 ± 1.98^{b}	23.85 ± 76.86	4.44 ± 3.05^{c}	-	4.31 ± 2.53^{c}	-	4.04 ± 3.00^{c}	-	0.79 ± 0.57^{a}	69.32 ± 29.09	5.06 ± 1.97^{c}	-
NO_2^- (mg/L)	0.73 ± 0.54^{a}	0.31 ± 0.62^{b}	70.77 ± 138.1	0.25 ± 0.25^{b}	73.68 ± 56.19	0.31 ± 0.39^{b}	66.77 ± 39.28	0.45 ± 0.47^{b}	54.44 ± 136.5	0.17 ± 0.13^{b}	83.81 ± 32.33	0.67 ± 0.97^{a}	16.64 ± 180-
PT (mg/L)	10.93 ± 0.49	1.53 ± 0.41	90.26 ± 2.90^{a}	2.61 ± 1.09	81.97 ± 8.01^{b}	3.71 ± 0.66	73.56 ± 5.01^{b}	1.99 ± 1.14	86.64 ± 8.39^{b}	0.86 ± 0.31	94.68 ± 2.10^{a}	6.62 ± 0.34	45.24 ± 3.91
PO_4^{3-}(mg/L)	7.38 ± 0.34	0.56 ± 0.47	94.74 ± 4.8^{a}	1.97 ± 0.89	79.91 ± 8.98^{b}	2.15 ± 0.68	77.35 ± 6.72^{b}	1.73 ± 1.68	82.85 ±11.48^{b}	0.31 ± 0.42	97.17 ± 4.61^{a}	3.81 ± 0.41	53.36 ± 5.42

Values within the same row followed by the same superscript letter (*i.e.* a, b, c ...) are not significantly different at $P < 0.05$

Table 2. Nitrogen and phosphorus mass balance in the wetland through the experimental period, B_{TL} = bed planted with *T. laxum*, B_{AG} = bed planted with *A. gayanus*, B_{CZ} = bed planted with *C. Zizanioides*, B_{EP} = bed planted with *E. Pyramidalis*, B_{PP} = bed planted with *P. purpureum* and UB = unplanted bed

Parameter	Beds	Inlet	Inside			Outlet
		Influent (g/m^2)	Sediment (g/m^2)	Plant uptake (g/m^2)	Other loss (g/m^2)	Effluent (g/m^2)
TN	UB	164.66	80.76	-	15.27	68.63
	B_{TL}		24.64	16.00	93.71	30.31
	B_{AG}		59.72	2.33	64.16	38.45
	B_{CZ}		65.94	1.75	54.97	42.00
	B_{EP}		44.66	6.96	74.41	38.63
	B_{PP}		1.54	25.18	112.08	25.86
TP	UB	43.42	19.05	-	0.58	23.79
	B_{TL}		27.13	6.18	5.88	4.23
	B_{AG}		30.32	3.42	1.86	7.82
	B_{CZ}		30.59	0.71	0.65	11.47
	B_{EP}		29.99	4.01	3.63	5.79
	B_{PP}		21.32	10.99	8.79	2.32

Table 3. Vertical distribution of total phosphorus (TP) and total nitrogen (TN) in the sediments layers of the various CWs beds; B_{TL} = bed planted with *T. laxum*, B_{AG} = bed planted with *A. gayanus*, B_{CZ} = bed planted with *C. Zizanioides*, B_{EP} = bed planted with *E. pyramidalis*, B_{PP} = bed planted with *P. purpureum* and UB = unplanted bed

Layers (cm)	TP (g/m^2)						TN (g/m^2)					
	UB	B_{TL}	B_{AG}	B_{CZ}	B_{EP}	B_{PP}	UB	B_{TL}	B_{AG}	B_{CZ}	B_{EP}	B_{PP}
0-10	6.70	8.32	9.05	9.10	9.00	7.48	31.03	8.59	23.24	24.48	15.28	0.57
10-20	5.31	6.84	7.01	7.05	7.00	5.97	19.31	5.98	15.69	17.24	12.76	0.48
20-30	3.67	6.25	6.41	6.46	6.39	4.12	13.10	5.66	10.99	11.90	8.45	0.34
30-40	2.29	3.71	5.06	5.15	4.85	2.55	8.28	2.41	6.28	7.45	5.21	0.10
40-50	1.07	1.84	2.46	2.48	2.45	1.10	6.00	1.83	2.50	3.10	2.20	0.04
50-60	0.01	0.17	0.33	0.35	0.30	0.10	3.04	0.17	1.02	1.77	0.76	0.01

Fig. 1.View of the experimental wetland system, 1: feeding tank, 2: bed, 3: irrigation device, 4: plants growing on the beds after one month and 5: plants growing on the beds after two months

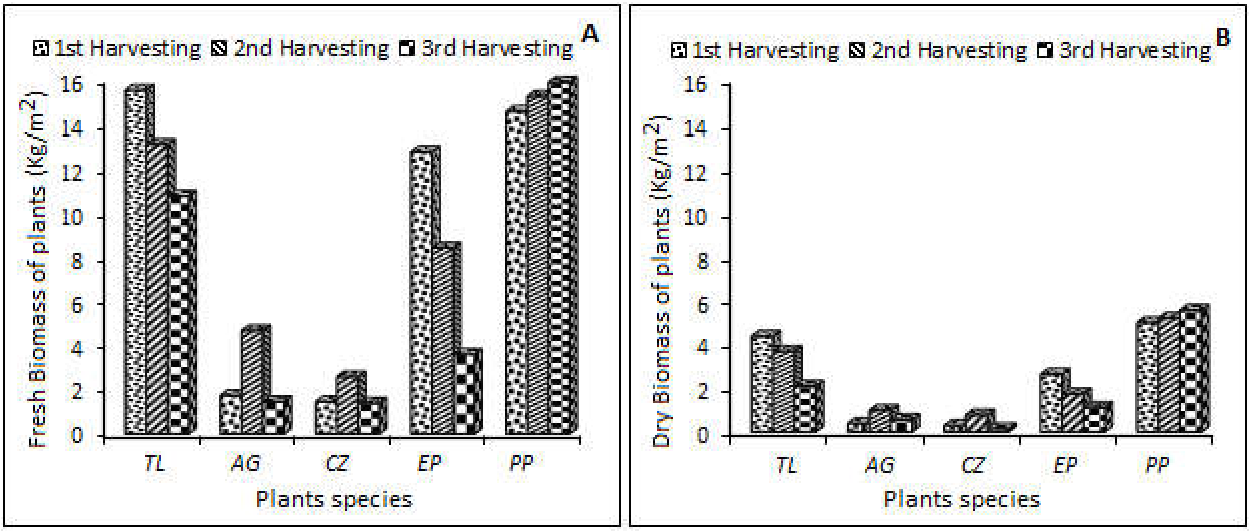

Fig.2. Fresh (A) and dry (B) aboveground plant biomasses produced in two months during three harvesting periods of the different studied plant species; TL = *T. laxum* ; AG = *A. gayanus* ; CZ = C. *zizanioides* ; EP = *E. pyramidalis*and PP = *P. purpureum*

However, only the total N stored in the layers of the bed planted with *P. purpureum* was significantly lower than those of all the beds (t test: $p < 0.05$).

N and P content in the beds effluents

Analysis of CWs effluents revealed a total nutrient contents varying between 25.86 and 68.63 g N/m^2 and between 2.32 and 23.79 g P/m^2 for 164.66 N g/m^2 and 43.42 P g/m^2 inputs to the wetland, respectively (Tab. 2). Overall, it was found that the unplanted bed achieved the highest amounts of nutrient while the bed planted with *C. zizanioides* released the highest amounts of total N (42.00 g /m^2) and total P (11.47 g /m^2) between the effluents. However, the effluents of the beds planted with *T. laxum* and *P. purpureum* were occurred the least amount of N and P.

Other losses of N and P

The other losses of total N and total P recorded in the planted beds were much greater than that of the control (15.27 g N/m^2 and 0.58 g P/m^2) (Tab. 2).This fraction of nutrient varied from 15.27 to 112.08 g N/m^2 and from 0.58 to 8.79 g P/m^2. The total nutrient lost calculated was 112.08 g N/m^2 and 8.79 g P/m^2 for bed planted with *P. purpureum*, 93.71 g N/m^2 and 5.88 g P/m^2 for bed planted with *T. laxum*, 74.41 g N/m^2 and 3.63 g P/m^2 for bed planted with *E. pyramidalis*, 64.16 g N/m^2 and 1.86 g P/m^2 for bed planted with *A. gayanus*, and 54.97 g N/m^2 and 0.65 g P/m^2 for deb planted with *C. zizanioides*. It thus appeared that the beds planted with *P. purpureum* and *C. zizanioides* contained respectively the largest and the smallest amount of these amount total nutrients.

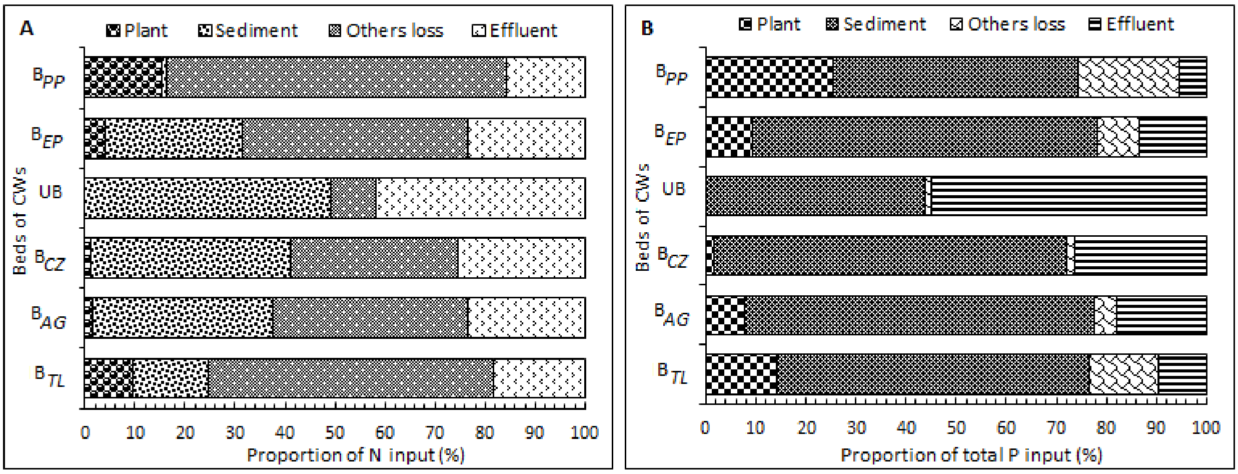

Fig.3. Proportion of nitrogen (A) and phosphorus (B) removed by different pathways among different wetlands during the experimental period; B_{TL} = bed planted with *T. laxum*, B_{AG} = bed planted with *A. gayanus*, B_{CZ} = bed planted with *C. zizanoides*; B_{EP} = bed planted with *E. pyramidalis*, B_{PP} = bed planted with *P. purpureum*and UB = unplanted bed (control)

N and P proportion of the different removal pathways: The proportions of N and P removed by different pathways among different wetlands during the experimental period are shown on figure 3. Concerning N (Fig. 3a), plant uptake removed 1.1–15.3 % of the total N input, while N removal by sediment storage was 0.9–49 %. Other N removal processes such as microbial uptake removed around 9.3–68.1 % of influent N. Overall, the main N removal pathway in the planted and unplanted beds had differences. Indeed, in unplanted bed, N removal by sediment storage (49 %) was the main pathway of N removal. On the other hand, two main N removal pathways were observed in the planted beds, which also differed from one group of beds to another. Sediment storage and microbial uptake were the main N removal pathways with respectively 15 % and 56.9 % for the bed planted with *T. laxum,* 36.3 % and 39 % for the bed planted with *A. gayanus*, 40 % and 33.4 % for the bed planted with *C. zizanioides* and, 27.1 %, and 45.2 % for the bed planted with *E. pyramidalis*. However, plant and microbial uptake were the main pathways for N removal at rates of 15.3 % and 68.1 %, respectively in bed planted with *P. purpureum*. About P (Fig. 3B), the amount removed by plant uptake accounted for 1.6–25.3 % of the total P input. P removed by sediment storage was 43.9–70.5 %. The percentage of other P losses such as microbial uptake was estimated to be 1.3–20.3 %. Analysis of the different P removal pathways in all the beds indicated that the main P removal pathway was sediment storage, and microbial uptake had the smallest contribution of P removal. However, in the planted beds, plant uptake could be identified as the second main P removal pathway for significant rates around of 25.3 % in beds planted with *P. purpureum*.

DISCUSSION

The five experimented plants developed very well on the CW beds despite differences observed between the biomasses produced by each of them during the treatment trial. Overall, these biomasses were of the same order of magnitude as estimated by Talineau (1968) and Sefiétou *et al*. (2005), who obtained in the wild mean fresh plant biomass of 20.0 kg/m^2 of *P. purpureum*, 12.0 kg/m^2 of *T. laxum*, 12.0 kg/m^2 of *E. pyramidalis*, 3.1 kg/m^2 of *C. zizanioides* and 3.0 kg/m^2 of *A. gayanus*. The production of such biomasses in the CW could be explained by the nutrients content (N and P) and organic matter of the applied wastewater and by the climatic conditions of the study area (tropical climate) (Ouattara *et al.*, 2008). However, *P. purpureum* appears to be the best adapted plant species to the culture medium for having provide highest biomass from one harvesting to another during the experience. In fact, this plant has long been investigated as a species that provides a significantly age-related yield of dry matter that is adapted to the tropics and can be grown and harvested all year round for agro-industrial purposes (Ferraris & Sinclair, 1980). With respect to physicochemical parameters, the pH and DO of the effluent from all beds were higher than those of the influent. In addition, unlike DO, the pH of the control effluent appeared greater than those of the planted beds. The increase of pH values in the effluents could be explained by the activity of denitrifying bacteria in the deep layers of the developed vertical flow CWs. The work of Finlayson and Chick (1983) and Koné *et al.* (2011) also report increasing pH in the planted beds effluents. In addition, the highest pH values observed in the control filtrate compared with those of planted beds could due to the action of plants (Shelef *et al.*, 2013).

The increase in DO in the effluents would result from the aeration of the untreated water during its application to the vertical flow CW beds used in this study. In addition, small amounts of oxygen from the aerial parts are rejected at the apex of rootlets of plants that could contribute to higher oxygen levels in the influents of planted beds (Poulet *et al.*, 2004; Pérez *et al.,* 2014). However, the different noted between beds effluent DO would be due to a likely variation in the amount of oxygen released into the plant's rhizosphere in the CW, depending on the plant's physiology (Stottmeister *et al.*, 2003; Gagnon *et al.*, 2007). Overall, TN, NH_4^+, TP and PO_4^3 removing was greater in planted beds than the control. This result was due to the removal process in the CWs in particular, the assimilation by macrophytes, the microbial processes, the precipitation or the dissolution and the adsorption or the desorption in the sediment (Vymazal, 2006). Indeed, the degradation of these nutrients requires organisms consisting of bacteria and macro invertebrates (Ouattara *et al.*, 2009) for which, the planted beds would confer more favorable ecological conditions unlike the unplanted bed (Gagnon *et al.*, 2007). In addition to the assimilation of nutrients for their growth, plants create within the sediment, an aerobic micro-habitats (in the upper layer) and anaerobic (in the bottom layer) conducive to the growth of organisms (Kroer *et al.*, 1998). Moreover, plants constitute a source of carbon supply necessary for the renewal of the energy of the organisms through secreted exudates and maintain, thanks to the shading they provide, hygrometry essential to the good development of the fauna (Andrews & Harris, 2000, Karjalainen *et al.*, 2001). The following overall order of beds performance as: Bed planted with *P. purpureum* > Bed planted with *T. laxum* > Bed planted with *E. pyramidalis* > Bed planted with *A. gayanus* > Bed planted with *C. zizanioides* was similar to plant biomass production. Thus, the higher values of biomass found in the *P. purpureum* plant species would have allowed a more abundant development of bacteria that would have made the contribution of this plant the best (Stottmeister *et al.*, 2003 ;Vymazal, 2007). However, the mean concentrations of TN and TP in the beds effluent are below the limit values (50 mg TN / L and 15 mg TP/ L) allowed in the regulation of wastewater discharges in Côte d'Ivoire (Ministry of Environment, Water and Forests, 2008).

With regard to NO_2^- and NO_3^-, results indicates that the effluents of the planted bed with *P. Purpureum* and *T. laxum* had a relatively low concentration of these two nitrogenous compounds compared with the influent and the effluent of the control. This result could be explained, on the one hand, by the processes of nitrification linked to the oxygen diffusion in the different layers of these beds, and on the other hand, to the absorption of NO_3^- by these plants. Indeed, the upper layers of the beds containing these plants would be well oxygenated, which would have promoted a significant nitrification due to aerobic bacteria. The transport of these compounds to the bottom where anaerobic conditions prevail would have allowed deep denitrification. A similar removal of nitrate in a vertical flow constructed wetlands was observed by Pillai and Vijayan (2013) with a rate of about 88%. According to Koné *et al.* (2011), the reduction of NO_3^- in the effluent of CW could also be explained by a sufficient quantity of available carbon after the removal of the COD; which would favor a higher denitrification of nitrogen by heterotrophic bacteria. However, the highest concentrations of NO_3^- and NO_2^- measured in the filtrates of the beds planted with *A. gayanus, E. pyramidalis* and *C. zizanioides* were consistent with those of Koné *et al.* (2011) for *A. gayanus* and *Vétiveria nigritana* (a species of the same family as *C. zizanioides*) and Kengne *et al.* (2014) for *E. pyramidalis*. Concerning the amounts of nutrient (N and P) removed by the various components of CW, the results showed a decreasing of these nutrients in the beds sediment from upper surface to the bottom which could be attributed to the filtration of the nitrogen and the phenomena of adsorption and precipitation of phosphorus through the layers of the beds sediment (Tanja *et al.*, 2006; Wu *et al.*, 2011).

In addition, the highest amount of N recorded in the control compared to the planted beds would be justified by a much higher bacterial degradation in the planted beds to which was added the assimilation by the plants which would reduce the N amount in the planted beds (Ouattara *et al.*, 2009). On the other hand, the low value of P stored in the control compared to the planted beds in this study is in agreement with the work of Wu *et al.* (2013b). This difference would have been favored by the root development in the planted beds, which would constitute a specific surface fixation and precipitation more of P, outside the bed sediment (Poulet *et al.*, 2004). The analysis of the amounts of nitrogen and phosphorus assimilated by the plants shows proportionality between these and the plant biomass produced. This would due to the fact that phosphorus and nitrogen are essential nutrients for the growth and development of plants (Razaq *et al.*, 2017). However, the difference observed between the plants studied would be related to the physiological properties specific to each plant species investigated (Schachtman *et al.*, 1998; Richardson *et al.*, 2009). With regard to the amounts of degraded nutrients in bed sediments, the large quantities recorded in the planted beds relative to the control would be dependent on a greater presence of organisms that would have been favored by the plants. The biomasses developed by each of the plants would said to be at the origin of the difference noted between planted beds in the CWs (Kroer *et al.*, 1998; Gagnon *et al.*, 2007, Ouattara *et al.*, 2009). Proportions of N and P removed by different pathways among different wetlands during the experience trial were different. With respect to N, differences were noted between the control and planted beds and between planted beds. Indeed, in the control, N was more removed by storage in the sediment (49 %). In contrast, N was essentially eliminated by storage in the sediment (27–40 %) and by microbial uptake (33.4–56.9 %) in the beds planted with *T. laxum, A. gayanus*, *C. zizanioides* and *E. pyramidalis*. Moreover, in the beds planted with *P. purpureum*, N was mainly eliminated by plant (15.3 %) and microbial uptake (68.1 %). These differences would related to the fact that, in the absence of plants, the control bed would not have favored the conditions for a more extensive development of microbial organisms generally responsible for the degradation of nitrogen in CWs (Gagnon *et al.*, 2007; Vymazal, 2007).

This would justify the large proportion of bacterial uptake in the removal of N from the planted beds. Similar results were obtained in biomass (8.4–34.3%) and in bed sediment (5.9 – 26.6%) by Wu *et al.* (2013a) as well as recently by Yang *et al.* (2016) around 40.63 % in the biomasses harvested in a CW. In addition, Greenway and Woolley (2001) and Zhang *et al.* (2008) reported respective proportions of N from 15 to 80 % and 19 to 42 % in the biomass of plants and from 20.5 to 34.4 % in the beds sediment. These authors attributed these results to the mechanisms of nitrogen transformation (*i. e.,* ammonification, nitrification, denitrification) in CWs and to the rapid biomass growth and influent quality. Concerning phosphorus, the total amount in the influent was generally more stored in the beds sediments between 43.9 and 70.5 %, and the proportion uptake by organisms was found to be the lowest. However, in the beds planted with *P. purpureum*, the proportions of 25.3 % assimilated in the plant biomass appeared to be significant. This result could be explained by the mechanisms of elimination of phosphorus in constructed wetlands, of which sediments, plants and microbial organisms would be the essential actors (Drizo *et al.*, 2002). However, the low levels of phosphorus removal by microorganisms in this study were consistent with the work of Wu *et al.*, (2013b).

Conclusion

It was found that all plants grew well and their presence improved globally nutrients removal. Average concentrations of TN and TP in the effluents of all planted beds were below the limit values (50 mg TN / L and 15 mg TP/ L) allowed in the regulation of wastewater discharges in Côte d'Ivoire. However, *P purpureum* is probably the preferred species in constructed wetlands for treating domestic wastewater.

Nutrients amount decreased in the sediment of the beds from upper surface to the bottom, their removal by plant uptake, sediment storage and microbial uptake was 1.6–25.3 %, 43.9–70.5 % and 1.3–20.3 % for P respectively, and 1.1–15.3 %, 0.9–49 % and 9.3–68.1 % of N input respectively. The main N removal pathways differed from species to species of plants. Plant uptake or sediment storage could be the main N removal pathways with microbial mechanisms in CWs for treating domestic wastewater. Sediment storage and plant uptake were the main P removal pathways in CWs for treating domestics wastewater

Acknowledgments

This study was carried out with the technical support of Côte d'Ivoire National Laboratory of Testing, Quality, Metrology and Analysis (LANEMA). We would want to thank its Director General, Mr. AIE Yapi Clement, and Mr. YAO Laurent for their assistance. We also thank all researchers at the Biotechnology and Environmental Engineering Research Unit of Nangui Abrogoua University (Abidjan, Ivory Coast) for their criticism.

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