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International Journal of Current Research
Vol. 10, Issue, 09, pp.73167-73177, September, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

USE OF DIFFERENT PLANTS SPECIES IN VERTICAL-FLOW CONSTRUCTED WETLAND FOR ORGANIC MATTER REMOVAL FROM DOMESTIC WASTEWATER

Franck Michaël Zahui, Jean-Marie Pétémanagnan Ouattara*, Moussa Balla Konaté, Lassina Sandotin Coulibaly 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 20th June, 2018
Received in revised form
17th July, 2018
Accepted 10th August, 2018
Published online 30st September, 2018

Key Words:

Organic matter removal, Masse Balance, Synthetic Domestic Wastewater, Forage Plants, Constructed Wetland, Developing Countries.

ABSTRACT

Background: In developing countries where the economic situation is less sustained, constructed wetlands might be suitable in the management of wastewater before the discharge into natural environments because of their possible generation of revenues that could support maintenance costs. However, organic matter (OM) removal in constructed wetlands using different species of plants often varies, and the contribution to organic matter removal by various pathways remains unclear. **Aims:** To demonstrate the effects of five local forage plants *(i.e Andropogon gayanus, Chrysopogon zizanioides, Echinochloa pyramidalis, Pennisetum purpureum and Tripsacum laxum),* and to determine the contributions of different pathways onto organic matter (OM) removal from synthetic domestic wastewater in vertical flow constructed wetlands (CWs). **Methods:** A pilot-scale composed of six beds constructed with bricks, and filled from bottom to top with 0.1 m gravel covered with cloth and 0.6 m white lagoon sand was used. Five beds were transplanted by local forage seedlings, while one was used as the control. 80 L of domestic synthetic wastewater was then applied on the beds intermittently over six months. **Results:** All of the five plants species grew well, improving thus organic matter removal, but those of *P. purpureum* provided a greater and fresher with 15.86 $kg.m^{-2}$ and achieved the best Total Suspended Solids (TSS), 5-day Biochemical Oxygen Demand (BOD_5), and Chemical Oxygen Demand (COD) with removal efficiencies around 93.81 %, 98.51 %, and 95.92 %, respectively. Likewise, OM amount decreased in the beds' sediments from upper surface to the bottom. According to the mass balance approach, OM removal by sediment storage and microbial uptake were 12.26–49.97 % and 43.87–85.10 %, respectively. Regarding OM removal pathways, a slight difference between species of plants of CWs was observed, but microbial uptake remained mainly OM removal pathways. Nevertheless, sediment storage is a main OM removal pathway for some plants (*e.g. C. zizanioides*). **Conclusion:** Constructed wetlands transplanted with *P. purpureum* could be a cost-effective alternative method of wastewater treatment.

Citation: ***Franck Michaël Zahui, Jean-Marie Pétémanagnan Ouattara, Moussa Balla Konaté, Lassina Sandotin Coulibaly and Lacina Coulibaly.*** **2018.** "Use of different plants species in vertical-flow constructed wetland for organic matter removal from domestic wastewater", *International Journal of Current Research*, 10, (09), 73167-73177.

INTRODUCTION

Since the independence, in most developing countries, while priority has been given to agriculture, health, and education-training, sanitation is not the case because of their low economic level (Van Minh and Nguyen-Viet, 2011). This situation results the rejection of the majority of wastewater in the environment without adequate treatment, which has adverse effects on human health, economic productivity, and quality of freshwater resources and ecosystems (Metcalf and Eddy, 1991; UN-Water/WWAP, 2017). In Côte d'Ivoire, the degradation of aquatic environments via wastewater is well illustrated by the case of lagoon in Abidjan and lakes in Yamoussoukro district, which present now a state of siltation and advanced eutrophication (Adingra and Kouassi, 2011; N'Guessan *et al.,* 2011). Even if shoreline rehabilitation of these water bodies is envisaged, it is necessary to look for upstream solutions that would consist of treating the wastewater before dumping it in these environments. However, the socio-economic situation of the country does not allow considering in the upcoming days the use of expensive

Corresponding author: **Jean-Marie Pétémanagnan Ouattara,**
Laboratory of Environment and Aquatic Biology, Department of Sciences and Environment Management, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire.

DOI: https://doi.org/10.24941/ijcr.32008.09.2018

processes (*e. g.* activated-sludge systems) to treat wastewater. Thus, it is need to turn to remediation techniques such as constructed wetlands, less expensive, easier to carry out and maintain, and adapted to the realities of developing countries (Mashauri *et al.,* 2000; Kadlec and Wallace, 2009). Constructed wetlands (CWs) are depollution technics designed to recreate natural processes that leverage the interactions of soil, microorganisms and plants to treat a wide range of wastewater (Vymazal, 2010).There are several types of CWs among which, that introduced by Seidel (1966), composed of beds containing sand or gravel supporting emergent aquatic vegetation (*e.g.* vertical flow CWs), proves to be the most appropriate.

This, 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 of the vertical flow systems (Knight *et al.*, 2003; Mander *et al.,* 2005). Vertical flow CWs, unsaturated with water, allow the oxygenation of the effluent by crossing the sediment, and create aerobic micro sites where aerobic bacteria live as well as facultative anaerobic bacteria strongly implicated in degradation of organic matter (Vymazal, 2007; Kadlec and Wallace, 2009). In addition to their accumulation capacity of certain pollutants (*e.g.,* nitrogen, phosphorus, heavy metals, etc.), plants allow through their rhizosphere, the establishment of microbial communities responsible for the biodegradation of pollutants by providing them with oxygen, a carbon source and an attachment surface (Stottmeister *et al.,* 2003; Gagnon *et al.,* 2007). Although degradation of organic matter is enhanced by the water flow regime of the CWs, it can be affected by the plant species (Stein and Hook, 2005).

Moreover, the uptake of pollutants by the plant as well as the flow of oxygen into the rhizosphere varies with the plant species (Sorrell and Armstrong, 1994; Jespersen *et al.,* 1998). As a result, microbial activity may also be impacted relatively to the plant species used in the CWs. Furthermore, the use of plant species that can withstand the harvesting and operating conditions of CWs (*i.e.* submerged period, dry periods, high rates of organic matter and nutrients, fluctuating pollution load), and presenting abundant biomass and rapid growth is suggested (Brix, 2003; Vymazal, 2011). Even if the plants meeting these criteria can be used, local plant species with economic interests would make the CWs more advantageous. In addition to wastewater depollution, they can generate revenues able to support maintenance costs (Coulibaly *et al.,* 2008a, 2008b; Ouattara *et al.,* 2008). The aims of this study were to develop a vertically flow CW using local forage plants for the efficient removal of organic matter from domestic wastewater. To effectively conduct this study, the effects of five forage plant species (*i.e. Andropogon gayanus, Chrysopogon zizanioides, Echinochloa pyramidalis, Pennisetum purpureum, and Tripsacum laxum*) on organic matter removal in vertical flow CWs were investigated. Then, effect assessments of the plant species on the hydraulics of CWs as well as contributions of different organics matter removal pathways in vertical CWs, such as sediment storage and microorganisms were performed. Finally, plant biomass production and stump diameters developed by the different species of plants were determined.

MATERIALS AND METHODS

Experimental wetland system: The study was performed on an experimental pilot located at Nangui Abrogoua University (Abidjan, Côte d'Ivoire). The pilot was built cement and composed of six rectangular beds (length = 1.45 m, wide = 1.00 m, depth = 0.60 m and area of 1.45 m^2) according to Coulibaly *et al*. (2008a, 2008b) and Ouattara *et al.* (2009) (Fig.1). Each bed was filled from bottom to the surface with 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 of 0.032 m diameter to drain out the effluent of the bed. Each bed was equipped with irrigation devices consisted of 6 PVC pipe (length: 1.70 m; diameter; 0.008 m) containing 60 lateral holes. Five plants namely *Andropogon gayanus* (Kunth, 1833), *Chrysopogon zizanioides* (Roberty, 1960), *Echinochloa pyramidalis* (Lam.) Hitchc. & Chase (1917), *Pennisetum purpureum* (Schumach. 1827) and *Tripsacum laxum* (Nash, 1909) were experimented. These are perennial forage plants highly appreciated by agro-pastoralists for their palatability, adaptation to local climatic conditions and presence in Côte d'Ivoire (Boudet, 1991).

Composition of synthetic domestic wastewater: Synthetic domestic wastewater was used as influent of the wetlands in the experiment to avoid CWs clogging problems reported by Coulibaly *et al.* (2008a, 2008b) and Ouattara *et al.* (2008), and minimize the fluctuation of organic compound concentrations. The composition of synthetic domestic wastewater was performed according to 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 and 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.

Experimental procedure, Plant aboveground biomass, and stump diameter determination: The effect of the five specie plants on the organic pollution treatment of wastewater in the CWs was examined during three cycles for two months of plant growth. Five beds were transplanted with seedlings (*i.e.*9 plants/m²) spaced of 40 cm x 40 cm between the stems and one was used as a 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 thus 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 $\times10^{-3}$m/d hydraulic loading of synthetic domestic wastewater according to Ouattara *et al.* (2008, 2009, 2011) over 6 months. The synthetic domestic wastewater tank was cleaned before and after the feeding of beds to remove all the impurities settled. 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. The diameter of the plants' stumps was also measured at each harvesting. Three measures of plant aboveground biomass and stumps diameter were carried out during the experiment and data obtained in each bed were used for analyses.

Determination of hydraulics of beds: Monitoring of bed hydraulic consisted in determining the time elapsed at each application of synthetic wastewater in the bed, allowing to calculate flow rates according to Ouattara *et al.* (2008) following equation 1:

$$Q = \frac{Va}{T} \quad (1)$$

Where Va, the volume of influent at each feed in CWs (mL), T, the time elapsed during infiltration in the bed (s), and Q, the infiltration flow rate (mL/s).

Wastewater sampling, analysis of physico-chemical parameters and removal efficiencies: During the experiment, samples were taken once a week at inlet and outlet of each bed, stored in an ethylene bottle at 4 °C until analysis. The pH and dissolved oxygen (DO) were determined according to ISO 10523 (2008) and ISO 5814 (2012), respectively, while total suspended solids (TSS) by ISO 11923 (1997). Then, chemical oxygen demand (COD) and 5 day-biochemical oxygen demand (BOD_5) analyses were done according to ISO 6060/2 (1989) and ISO 5815/1 (2003), respectively. Finally, removal efficiencies was calculated according to Abissy and Mandi (1999) for TSS, COD, and BOD_5.

$$\text{Removal efficiency (\%)} = \frac{C_iV_i - C_eV_e}{C_iV_i} x\ 100 \quad (2)$$

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) an in the CWs.

Sediment sampling and analysis: To understand the organic matter distribution in the bed sediments, six sediment layers in the vertical profile were investigated upper surface to the bottom: [0;-10cm], [-10;-20 cm], [-20;-30 cm], [-30;-40 cm], [-40;-50 cm] and [-50;-60 cm]. After six months of wastewater application on the beds, 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). All the five samples of each layer were mixed to make the sample of this one, stored in cooling jars at 2°C during 24 hours for analysis. Thus, organic matter concentration was determined from organic carbon obtained according to the NF ISO 10694 (1995) standards as following this equation:

$$[OM] = 1.724\ x\ [OC] \quad (3)$$

Where, [OM] and [OC] are the concentrations of organic matter (mg/kg) and organic carbon (mg/kg), respectively.

Mass balance: Assuming that the input OM concentration average was same as that at the output the CWs, the OM mass balance was determined in order to quantify the contributions of different removal pathways of beds during the experiment. The factors considered were (i) amounts imported and exported from the CWs systems, (ii) amounts stored (*i.e.* adsorbed and precipitated) by the sediment, (iii) amounts assimilated by the microorganisms. A simple conceptual model for OM mass balance in CWs is shown below:

$$OM_{\text{Inlet}} = OM_{\text{(Sediment)}} + OM_{\text{(Assimilated)}} + OM_{\text{(outlet)}} \quad (4)$$

Where $OM_{(Inlet)}$ is the amount of organic matter introduced in CWs (g/m^2), $OM_{(Sediment)}$ is the amount stored by the sediment (g/m^2),

$OM_{(Assimilated)}$ is the amount assimilated by the microorganisms (g/m^2) and $OM_{(Outlet)}$ is the amount of organic matter released in CWs (g/m^2).

Calculation of OM introduced or released from the CWs: OM introduced or released from the CWs was indirectly determined from the concentrations of COD and BOD_5 according to Rodier (1996) following relationship:

$$[MO]_{(Average)} = \frac{[COD]_{(Average)} + 2\ x\ [BOD_5]_{(Average)}}{3} \quad (5)$$

Where $[OM]_{(Average)}$ is the inlet or outlet concentration average of OM (mg/L), $[COD]_{(Average)}$, is the inlet or outlet concentration average of COD (mg/L), and $[BOD_5]_{(Average)}$ is the inlet or outlet concentration average of BOD_5 (mg/L). Then, OM mass corresponding was deduced from:

$$OM_{\left(\frac{\text{Inlet}}{\text{outlet}}\right)} = \frac{[OM]_{(Average)}\ x\ TV_{(Inlet/Outlet)}}{\text{wetland area}} \quad (6)$$

Where $[MO]_{(Inlet/Outlet)}$ is the inlet or outlet amount of organic matter introduced or released from the CWs systems (g/m^2), $TV_{(Inlet/Outlet)}$ is the inlet or outlet total volume of introduced or released from the CWs systems (L), and the wetlands area is 1.45 m^2.

Calculation of OM stored by the sediment: The amount of OM stored by the sediment was determined from the estimated mass of the sediment and also the average concentration of organic matter analyzed in the sediment from the relationship:

$$OM_{(sediment)} = \frac{OM_{(Analyzed)}\ x\ M_{(Sediment)}}{\text{wetland area}} \quad (7)$$

Where $OM_{(analyzed)}$ is the average concentration of OM in the sediment in g/kg, $M_{(sediment)}$ is the estimated mass of the sediment (kg) and wetlands area is 1.45 m^2.

According to the equation 5, the amount of assimilated OM by microorganisms was calculated as follows:

$$MO_{(Assimilated)} = MO_{(Inlet)} - \left(MO_{(Sediment)} + MO_{(Outlet)}\right) \quad (8)$$

Data analysis

Statistical tests used for datasets analysis included Kruskal-Wallis, Mann Whitney, ANOVA variances, and T-test after Shapiro-Wilk normality test. These statistical tests are performed using R studio 3.3.2 software (Ihaka and Gentleman, 1996). In all tests, the differences were considered statistically significant for $p< 0.05$.In addition, the hydraulic behavior of the CW beds was assessed by the linear regression lines of the instantaneous infiltration rates of wastewater in the beds. In fact, negative slopes would induce a decrease of the instant infiltration rate, while positive slopes would translate increase when wastewater was applied to beds.

RESULTS

Plant biomass production and stumps developed in CWs

Figure 2 shows plant biomass produced (Fig. 2A) and the diameter of plant stumps developed (Fig. 2B) on the beds.

Table 1. Inlet and outlet mean concentrations and percentage of removal statistics for TSS, BOD_5 and COD, and the average pH and DO values, B_{TL}: bed planted with *T. laxum*, B_{PP}: bed planted with *P. purpureum*, B_{EP}: bed planted with *E. pyramidalis*, B_{AG}: bed planted with *A. gayanus*, B_{CZ}: bed planted with *C. zizanioides* and UB: unplanted bed.

Parameters	Inlet CWs	Outlet CWs											
	Wastewater	B_{TL}		B_{AG}		B_{CZ}		B_{EP}		B_{PP}		UB	
		Filtrates	R (%)	Filtrates	R (%)	Filtrates	R (%)	Filtrates	R (%)	Filtrates	R (%)	Filtrates	R (%)
pH	6.81 ± 0.07^{a}	7.17 ± 0.30^{bc}	-	6.92 ± 0.26^{a}	-	7.05 ± 0.27^{c}	-	6.93 ±0.27^{a}	-	7.06 ±0.21^{c}	-	7.33 ± 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}	-
TSS (mg /L)	284.9 ± 11.67^{a}	28.55 ± 2.92^{b}	93.0 ± 1.05	34.37 ± 6.19^{c}	90.86 ± 1.91	39.48 ± 5.91^{d}	89.20 ± 1.82	29.53 ± 3.09^{b}	92.3 ± 1.42	25.98 ± 1.92^{e}	93.81 ± 0.80	16.66 ± 1.29^{f}	94.70 ± 0.45
COD (mg O_2/L)	611.8 ± 18.02^{a}	48.7 ±12.07^{bc}	94.46 ± 1.39	73.90 ± 15.89^{bc}	90.91 ± 1.89	63.37 ± 5.58^{b}	91.9 ± 0.90	55.3 ± 7.17^{bc}	93.38 ± 1.08	36.67 ± 3.78^{c}	95.92 ± 0.68	150.2 ± 31.87^{d}	77.7 ± 5.03
BOD_5 (mg O_2/L)	369.7 ± 4.07^{a}	14.68 ± 1.66^{b}	97.2 ± 0.46	23.30 ± 4.79^{c}	95.24 ± 1.06	21.84 ± 4.35^{c}	95.40 ± 1.01	18.36 ± 4.71^{d}	96.36 ± 1.04	8.00 ± 3.42^{e}	98.51 ± 0.74	43.80 ± 6.40^{f}	89.29 ± 1.56

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. Organic matter mass balance in the beds of CW throughout the end of the experiment trial, B_{TL}: bed planted with *T. laxum*, B_{PP}: bed planted with *P. purpureum*, B $_{EP}$: bed planted with *E. pyramidalis*, B_{AG}: bed planted with *A. gayanus*, $B_{CZ:}$ bed planted with *C. zizanioides* and UB: unplanted bed.

Parameters	Inlet CWs	Outlet CWs											
	Wastewater	B_{TL}		B_{AG}		B_{CZ}		B_{EP}		B_{PP}		UB	
		Filtrates	R (%)	Filtrates	R (%)	Filtrates	R (%)	Filtrates	R (%)	Filtrates	R (%)	Filtrates	R (%)
pH	6.81 ± 0.07^{a}	7.17 ± 0.30^{bc}	-	6.92 ± 0.26^{a}	-	7.05 ± 0.27^{c}	-	6.93 ±0.27^{a}	-	7.06 ±0.21^{c}	-	7.33 ± 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}	-
TSS (mg /L)	284.9 ± 11.67^{a}	28.55 ± 2.92^{b}	93.0 ± 1.05	34.37 ± 6.19^{c}	90.86 ± 1.91	39.48 ± 5.91^{d}	89.20 ± 1.82	29.53 ± 3.09^{b}	92.3 ± 1.42	25.98 ± 1.92^{e}	93.81 ± 0.80	16.66 ± 1.29^{f}	94.70 ± 0.45
COD (mg O_2/L)	611.8 ± 18.02^{a}	48.7 ±12.07^{bc}	94.46 ± 1.39	73.90 ± 15.89^{bc}	90.91 ± 1.89	63.37 ± 5.58^{b}	91.9 ± 0.90	55.3 ± 7.17^{bc}	93.38 ± 1.08	36.67 ± 3.78^{c}	95.92 ± 0.68	150.2 ± 31.87^{d}	77.7 ± 5.03
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Values within the same row followed by the same superscript letter (*i.e.* a, b, c ...) are not significantly different at $P < 0.05$

Fig. 1.View of the pilot-scale constructed wetlands (CWs): feeding tank (1), bed (2), water supply valve (3), irrigation device (4), and plants growing on the beds (5).

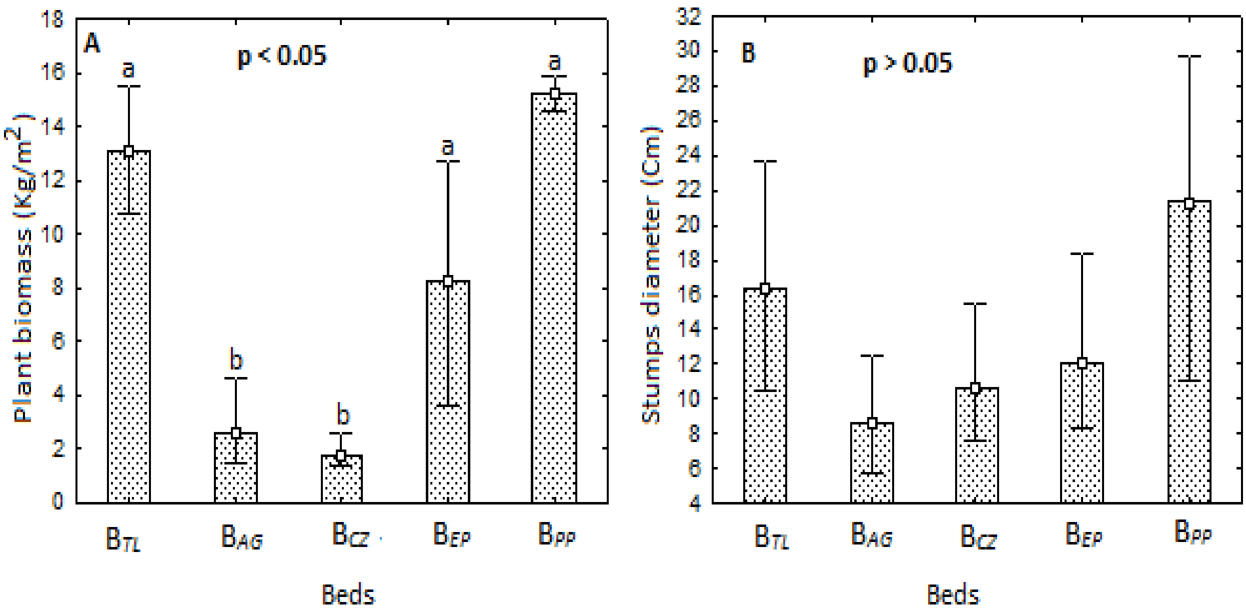

Fig. 2. Variation of plant biomass produced (A) and diameter of plant stumps (B) in the different planted beds with *T. laxum* (B_{TL}), *P. purpureum* (B_{PP}), *E. pyramidalis* (B_{EP}), *A. gayanus* (B_{AG}), *and C. zizanioides* (B_{CZ}).

Indeed, plant biomass obtained ranged from 14.55 to 15.86 kg/m^2, 10.76 to 15.52 kg/m^2, 3.59 to 12.76 kg/m^2, 1.45 to 4.67 kg/m^2, 1.45 to 2.58 kg/m^{2}on the beds transplanted with *P. purpureum*, *T. laxum*, *E. pyramidalis*, *A. gayanus* and *C. zizanioides,* respectively (See Fig. 2A). Overall, the biomasses produced on the beds planted with *E. pyramidalis*, *P. purpureum* and *T. laxum* were significantly higher than those of *A. gayanus* and *C. zizanioides* (Mann Whitney test: $p < 0.05$). Concerning the diameter of plant stumps, the obtained values varied from 11.00 to 29.77 cm, 10.48 to 23.73 cm, 8.26 to 18.39 cm, 7.60 to 15.43 cm, and 5.68 to 12.55 cm on the bed planted with *P. purpureum*, *T. laxum*, *E. pyramidalis*, *C. zizanioides, A. gayanus,* respectively (Fig. 2B). The sequence of diameter of plant stumps average values was ranked as follows: B_{PP} (21.32 cm) > B_{TL} (16.41 cm) > B_{EP} (12.08 cm) > B_{CZ} (10.68 cm) > B_{AG} (8.65 cm). However, the statistical analysis showed no significant difference between the diameters of the different plant stumps (ANOVA test: $p > 0.05$).

CWs hydraulic: Figure 3 exhibits the water infiltration rate in the bed sediments of different plants measured at the feed days during the treatment. The results show two trends according to the slope sign of the regression lines with the slopes of the planted beds positive, while that of the unplanted was negative (Fig. 3). In addition, the slope positives are ranked in increasing order depending on the plants (*i.e. A. gayanus* (0.58) < *E. pyramidalis* (0.87) < *C. zizanioides* (1.77) < *T. laxum* (1.81) < *P. purpureum* (2.79)). Finally, the statistical analysis showed a significant difference between the infiltration rates within the planted beds and that in the unplanted bed (Kruskal-Wallis test: $p < 0.05$), but no significant difference was observed among the infiltration rates of the planted beds (Mann Whitney test: $p > 0.05$).

CWs organics pollutant removal performance: Table 1 exhibits inlet and outlet mean concentrations of CWs of each parameter, percentage of removal statistics for TSS, BOD_5 and COD, and the average pH and DO values.

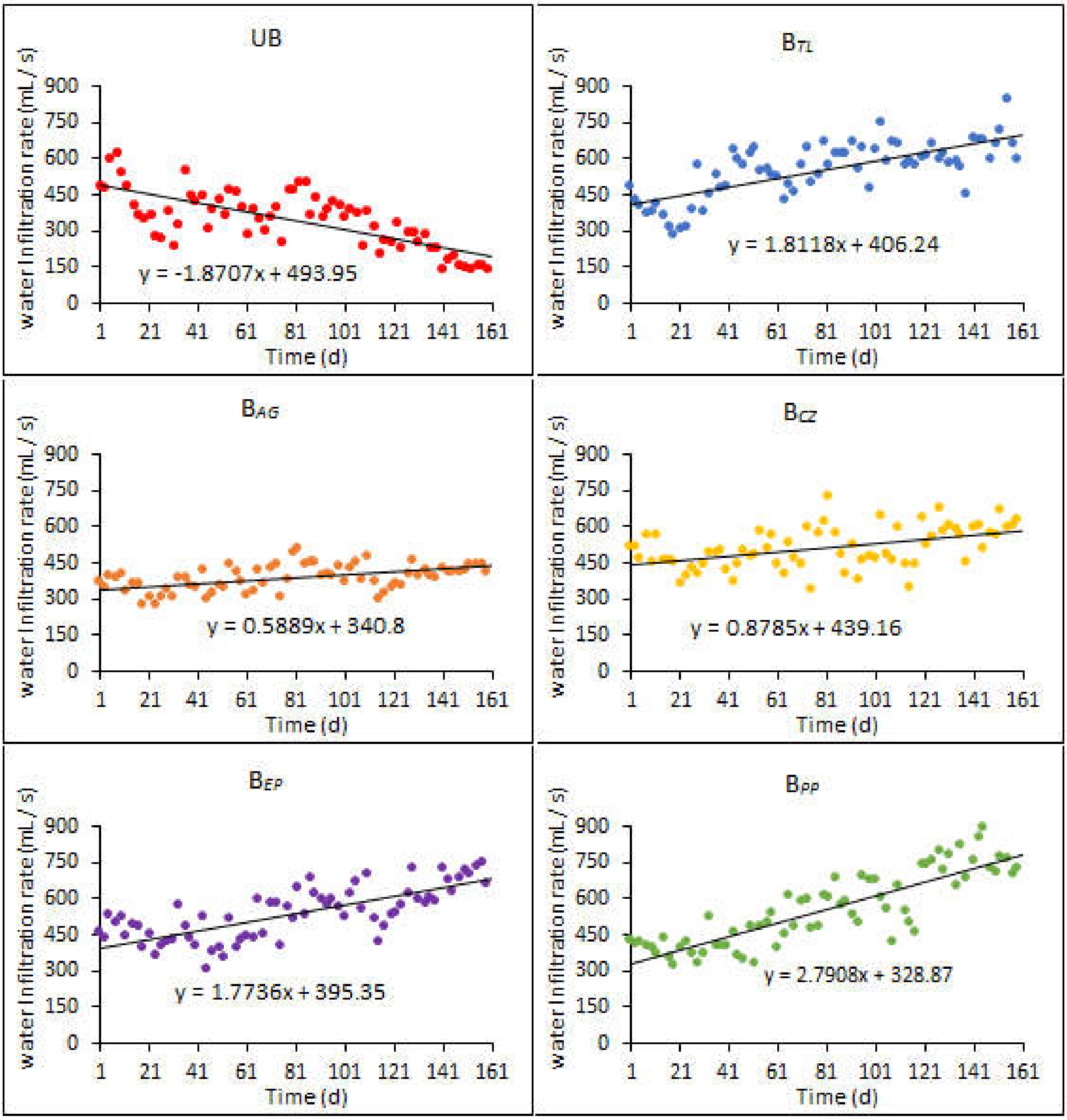

Fig. 3. Evolution of infiltration rate in the beds' sediments containing different plants during the experiment: bed planted with *T. laxum* (B_{TL}), *P. purpureum* (B_{PP}), *E. pyramidalis* (B_{EP}), *A. gayanus* (B_{AG}), *C. zizanioides* (B_{CZ}) and unplanted bed (UB).

At the exit of all beds, the average pH values of the planted beds (*i.e.* between 6.92 and 7.17) were slightly lower than those of the unplanted bed (7.32), conversely at wastewater pH (6.81). However, the sequence of pH mean values was ranked as decreasing in this order: wastewater pH (6.81) < pH B_{AG} (6.92) < pH B_{EP} (6.93) < pH B_{CZ} (7.05) < pH B_{PP} (7.06) < pH B_{TL} (7.17) < pH UB (7.32). Besides, significant differences between wastewater pH and those of planted beds, and also among them obtained in the different beds were observed (Mann Whitney test: $P < 0.05$). Regarding the DO, the mean values spent from 2.13 ± 0.55 mg/L in the wastewater to 7.53 ± 1.56 mg/L (B_{TL}), 6.50 ± 0.80 mg/L (B_{AG}), 6.70 ± 1.04 mg/L (B_{CZ}), 6.63 ± 0.98 mg/L (B_{EP}), 7.24 ± 1.06 mg/L (B_{PP}) and 5.41 ± 0.88 mg/L (UB), thereby underscoring an aeration of planted beds by increasing of dissolved oxygen. The wastewater DO values were statistically lower than those of the planted beds, whereas significant differences were noted between DO mean values of the planted beds.

Moreover, the average TSS of wastewater was significantly reduced from 284.9 ± 11.67 to 16.66 ± 1.29 mg/L (Mann Whitney test: $P < 0.05$). Otherwise, TSS concentrations in the unplanted bed were lower than those of the planted beds. Thus, we note that TSS removal efficiency are depending of plants (*i.e.* B_{PP} (93.81%) > B_{TL} (93.01%) > B_{EP} (92.36%) > B_{AG} (90.86%) > B_{CZ} (89.20%)). Concerning the organic matter removal efficiencies, the mean concentrations of BOD_5 oscillated between 8 ± 3.42and 43.80 ± 6.40 mg O_2/L with averages of 98.51 ± 0.74 and 89.29 ± 1.56 %, while those of COD varied from 36.67 ± 3.78 to 150.2 ± 31.87 mg O_2/L with means between 95.92 ± 0.68 and 77.7 ± 5.03 %, respectively. In fact, the bed planted with *P. purpureum* achieved the best performance with an average removal efficiency of 98.51 ± 0.74% for BOD_5 and 95.92 ± 0.68 % for COD. It worth note that the planted bed with *T. laxum* obtained 97.2 ± 0.46 % and 94.46 ± 1.39 %, whereas that with *E. pyramidalis* was 96.36 ± 1.04 % and 93.38 ± 1.08 % for BOD_5 and COD, respectively.

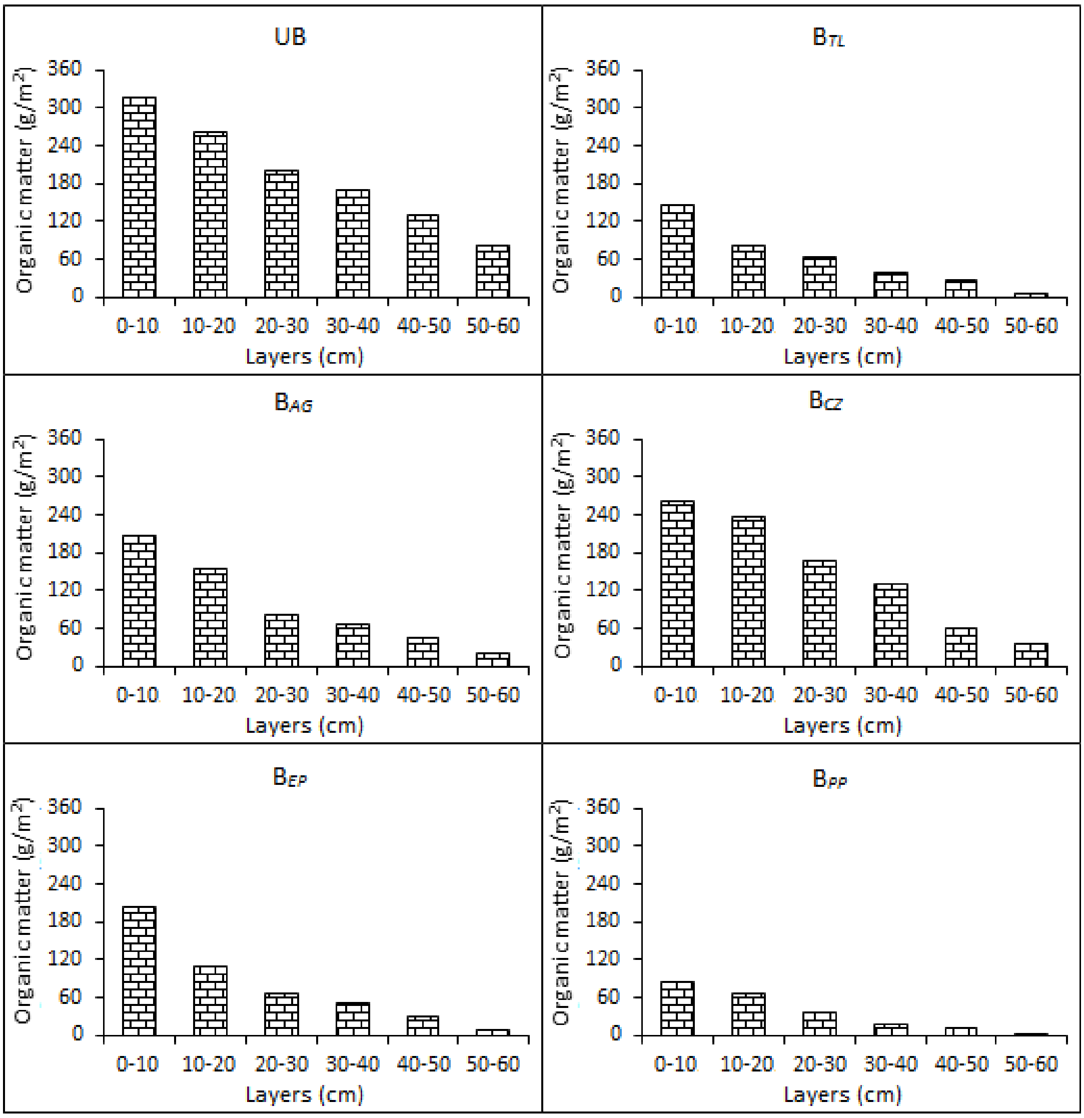

Fig. 4. Contents of organic matter (OM) in the sediment layers of the various beds of the CW at the end the experiment: B_{TL}: bed planted with *T. laxum*, B_{PP}: bed planted with *P. purpureum*, B_{EP}: bed planted with *E. pyramidalis*, B_{AG}: bed planted with *A. gayanus*, B_{CZ}: bed planted with *C. zizanioides* and UB: unplanted bed.

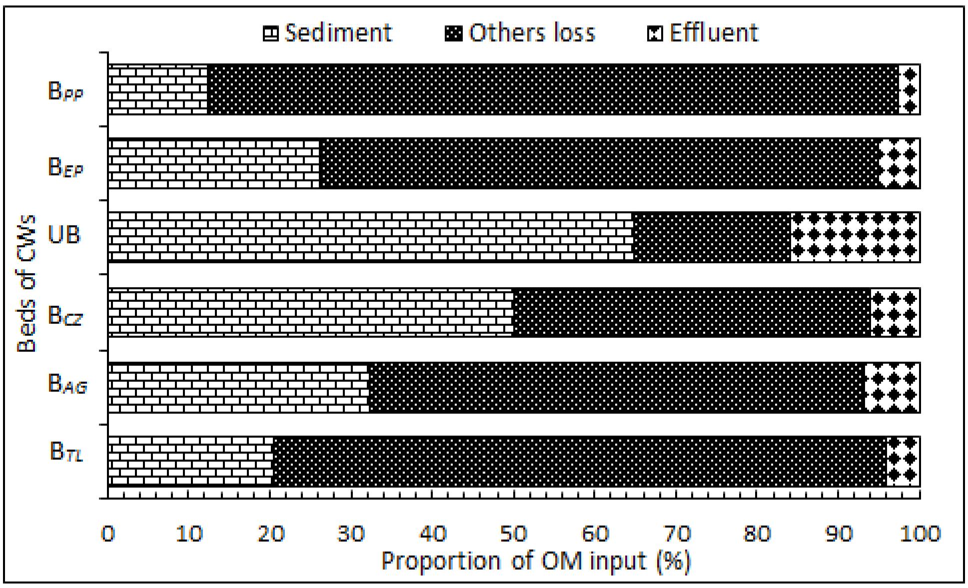

Fig. 5. Proportion of organic matter (OM) removed by different pathways among different wetlands during the experimental period: B_{TL}: bed planted with *T. laxum*, B_{AG}: bed planted with *A. gayanus*, B_{CZ}: bed planted with *C. zizanoides*; B_{EP}: bed planted with *E. pyramidalis*, B_{PP}: bed planted with *P. purpureum* and UB: unplanted bed

Unlike those of plants above-mentioned, 95.40 ± 1.01% and 91.9 ± 0.90% were obtained with the bed of *C. zizanioides*, while 95.24 ± 1.06% and 90.91 ± 1.89% with *A. gayanus* for BOD_5 and COD, respectively. Overall, the planted beds provided the best removal efficiencies of organic matter than the unplanted bed (*i.e.* the control). However, the performance of the beds was significantly different except those of beds transplanted with *C. zizanioides* and *A. gayanus* for BOD_5. Unlike for BOD_5, the difference was significant between the bed transplanted with *P. purpureum* and that transplanted with *C. zizanioides*. Finally, significant differences were found between the removal efficiencies of organic matter (Kruskal Wallis test: $p < 0.05$).

Organic matter quantification and mass balance in the CWs

Organic matter in the beds' sediments

- **Total organic matter storage in beds' sediments**

Total content of the accumulated organic matter (OM) in the beds' sediments at the end of the experiment was denoted in the Table 2. Total OM input to the bed was 1 789.4 g/m^2 and the accumulated OM in the sediment of different beds ranged from 219.4 to 1 161.1 g/m^2. However, the accumulated OM in the planted and unplanted beds differed obviously with each other. Indeed, the unplanted bed accumulated the highest OM concentration in its sediments (*i.e.* 1 161.1 g/m^2). Comparing the planted beds, the highest accumulated OM (894.2 g/m^2) was obtained with that of *C. zizanioides*, whereas those obtained in the sediments of *A. gayanus*, *E. pyramidalis*, *T. laxum* and *P. purpureum* were 578.4 g/m^2, 469.0 g/m^2, 366.7 g/m^2 and 219.4 g/m^2, respectively.

- **Vertical distribution of organic matter in the bed of CW**

Figure 4 shows the vertical distribution of organic matter in the different sediment layers of the CW beds. Indeed, accumulated OM decreased from upper surface towards the bottom the beds' sediments (*i.e.* 0-60 cm) as shown in Fig. 4. It ranged from 146.2 to 6.9 g/m^2, 206.9 to 22.1 g/m^2, 262.1 to 34.9 g/m^2, 203.5 to 9 g/m^2, 86.2 to 4 g/m^2 and 316.3 to 82.8 g/m^2 in beds transplanted with *T. laxum*, *A. gayanus*, *C. zizanioides*, *E. pyramidalis*, *P. purpureum* and in the control, respectively. The variance ANOVA analysis showed a significant difference between the amounts of OM trapped in the different beds ($p < 0.05$). In fact, accumulated OM determined in the control was the same order of magnitude as that obtained in the beds planted with *C. zizanioides* and *A. gayanus* (t-test: $p > 0.05$) and significantly higher than those measured in beds transplanted with *T. laxum*, *E. pyramidalis* and *P. purpureum*. At the exception of the beds planted with *C. zizanioides* and *P. purpureum*, accumulated OM in the planted beds was of an approximately same order of magnitude (t-test: $p < 0.05$).

Organics matter at the CWs outlet: The amounts of OM released by the CW beds throughout the treatment trial are shown in Table 2. It fluctuated between 47.3 and 284.9 g/m^2 at the outlet of beds with higher OM amount rejected by the unplanted bed of compared to the planted beds. About the planted beds, *P. purpureum* bed rejected the lowest amount of OM. The sequence of OM concentration of beds was as follows: 284.9 g/m^2 (UB) > 120.4 g/m^2 (B_{AG}) > 110.3 g/m^2 (B_{CZ}) > 89.3 g/m^2 (B_{EP}) > 71.9 g/m^2 (B_{TL}) > 47.3 g/m^2 (B_{PP}).

Other losses of organic matter in the CWs: The OM estimated microbial uptake in the beds ranged between 343.5 and 1 522.8 g/m^2 (Table 2). The amounts obtained in the planted beds were all greater than that of the control (*i.e.* 343.5 g/m^2). However, beds transplanted with *P. purpureum* showed the highest amount of OM (1 522.8 g/m^2), while beds planted with *T. laxum*, *E. pyramidalis*, *A. gayanus* and *C. zizanioides* were 1 350.9 g/m^2, 1 231.2 g/m^2, 1 090.6 g/m^2 and 784.9 g/m^2, respectively.

Organic matter proportion of the different removal pathways: Figure 5 shows the proportions of OM removed by different pathways in CWs during the experimental trial. OM removal by sediment storage was 12.26–64.89 %, while microbial processes removed about 19.19–85.10 % of the total OM input. We noted a difference between the main OM removal pathway in the planted and unplanted CWs. In the unplanted bed, sediment storage (*i.e.* 64.89 %) was the main pathway of OM removal, while in most planted beds with *T. laxum*, *A. gayanus*, *E. pyramidalis* and *P. purpureum*, the main OM removal pathway was microbial processes with 75.49 %, 60.95 %, 68.80 % and 85.10 %, respectively, excepted for *C. zizanioides*. Unlike the planted beds above-mentioned, sediment storage in the planted beds with *C. zizanioides* (*i.e.* about 49.97 %) was slightly over microbial uptake that removed 43.87 % of the OM input.

DISCUSSION

Plant biomass and stump developed: The plant species (*i.e. A. gayanus*, *C. zizanioides*, *E. pyramidalis*, *P. purpureum* and *T. laxum*) had good growth in the beds of the developed constructed wetland. The maximum fresh biomasses of all the plants were the same order of magnitude as those estimated reported in the literature (Talineau, 1968; Sefiétou *et al.,* 2005). The production of these large biomasses could be due to the quality of the applied wastewater and the regular harvesting of the aboveground biomass produced during the experiment (Ouattara *et al.,* 2008; Yang *et al.,* 2016). For these authors, the harvesting of plants would allow a greater removal of the nutrients contained in the wastewater used, showing thus the correlation between the biomasses produced and the diameter of the stumps developed by most plants. According to Truong *et al.* (2009), the opposite observed in *C. zizanioides* for having provided the lowest biomass despite the higher *stumps* than *A. gayanus* would be due to its physiology.

CW hydraulics: The hydraulic behavior of the CW beds assessed by the linear regression lines of the instantaneous infiltration rates of wastewater in the beds indicated two trends according to the slope sign of the regression lines. Indeed, the slope of the planted beds was positive, while that of the unplanted was negative. Thus, we are noting higher infiltration rates of wastewater in planted beds compared to the unplanted bed (*i.e.* control). These results could be explained by the generation of cavities in the sediments of beds planted contrary to the control, increasing the rate of infiltration of wastewater in beds planted (Molle *et al.,* 2004; Mbuligwe, 2005; Coulibaly *et al*., 2008a, 2008b). Moreover, the difference between infiltration rates observed in the planted beds could be related to the thickness dissimilar of the stumps of plant species (Molle *et al.,* 2004).

Organic matter removal: During the experiments, the pH and dissolved oxygen in the outlet were higher than that of the wastewater, whereas, unlike dissolved oxygen, the pH of the uncultivated bed remained higher than that of the planted beds. The increase of pH could be due to the activity of denitrifying bacteria in the deep layers of the CWs. Finlayson and Chick (1983) and Koné *et al*. (2011) also reported an increasing pH in the planted beds. Moreover, the lower pH values observed in the planted beds compared with those filtrate of control could be attributed to the action of plants (Shelef *et al.,* 2013).The increase in DO in the planted bed would result from the aeration CW beds employed. Besides, amounts of oxygen from atmospheric are rejected at the apex of rootlets of plants that could contribute to higher oxygen levels in the planted beds (Pérez *et al.,* 2014).

The difference noted between DO in the beds would be likely due to a variation in the amount of oxygen released by the plants' rhizosphere in the CW depending their physiology (Stottmeister *et al.,* 2003; Gagnon *et al*., 2007). Furthermore, the considerable reduction of TSS concentration in the beds could be explained mainly by filtration phenomena (Ouattara *et al*., 2008). Indeed, sediment acts as a filter largely retaining all relatively coarse materials (RMC water agency, 1999). Moreover, the finer materials penetrate deeply and are retained by phenomena of inter-porosity blocking, interception, and fixation on sediment (Coulibaly *et al.,* 2008a, 2008b). However, the TSS was lower in the control than the planted beds. This result disagreed with those of Pillai and Vijayan (2013) who have obtained the lowest TSS removal efficiency (65%) in the control against 89 % and 93 % in the beds planted with *Panicum maximum,* and *Pennisetum purpureum* combined of *Pennisetum typhoides*, respectively, due to the use of gravel, sand and coconut fibers as filtration materials. It is noteworthy that the result of the present study was in agreement with those of Ouattara *et al.* (2008) and Coulibaly *et al*. (2008a, 2008b), who attributed it to more severe clogging in the control bed due to a large decrease in the infiltration rate its sediment.

Likewise, the best TSS reduction noted within the bed planted with *P. purpureum* could be attributed to the root system of this species. Unlike of the other species, it was found during the demolition of the beds at the end of the treatment trial that the roots of *P. purpureum* had formed a kind of mat much denser at the bottom of the bed, playing the role of a second filter at the bottom of the bed which would contribute to decrease more the TSS. Finally, the TSS mean concentrations from the planted beds outlet all comply with the national standards in force in Côte d'Ivoire (*i.e.* 50 mg/L) (Ministry of the Environment, Waters and Forests, 2008). The carbon pollutants of the wastewater were significantly reduced in the filtrate. The reduction of organic matter (*i.e.* BOD_5 and COD) in the planted beds outlet would due to, on the one hand, a good colonization of the beds' sediments by the purifying microorganisms, and on the other hand, a good oxygenation of the sediments during the rest phase as observed Molle *et al*. (2005), Ouattara *et al.* (2008) and Koné *et al.* (2011). In fact, the rest phases of the beds favor a higher recharge of the bulk of oxygen used for the metabolism of the bacteria during the biodegradation of the organic matter. The difference showed between the BOD_5 and COD values of the planted beds and those of the control could be explained by the presence of plants that would stimulate processes of degradation of organic pollutants through the secretion of root exudates. The latter would serve as a source of energy for the activity of purifying microorganisms (Corgie *et al.,* 2004, Kirk *et al.,* 2005). However, the bed planted with *P. purpureum* provided the best removal of BOD_5 (98.51%) and COD (95.92%) followed by those of *T. laxum* (97.23 and 94.46%), *E. pyramidalis* (96.36 and 93.38%), *C. zizanioides* (95.40 and 91.93 %) and *A. gayanus* (95.24 and 90.91%). This difference would result from above-ground biomass and stumps developed by the plant (Brix, 1997; Stottmeister *et al.,* 2003; Ouattara *et al.,* 2011). Indeed, during the treatment of wastewater, the species *P. purpureum* provided the most abundant biomasses followed by *T. laxum*, *E. pyramidalis*, *A. gayanus* and *C. zizanioides*. In addition, it was substantially the same for stump diameters developed by the different plants. Thus, the abundance of plant cover and clumps of plants would have created a microclimate favoring a greater proliferation of purifying organisms (macro and micro-organisms) improving the treatment. However, the BOD_5 and COD concentrations from the beds planted with *T. laxum* (14.68 and 48.74 mg O_2/L), *A. gayanus* (23.30 and 73.90 mg O_2/L), *C. zizanioides* (21.80 and 63.37 mg O_2/L), *E. pyramidalis* (18.36 and 55.33 mg O_2/L), and with *P. Purpureum* (8 and 36.67 mg O_2/L) were significantly lower than the limit value according to the regulation of wastewater discharges in Côte d'Ivoire which is 100 mg O_2/L for BOD_5 and 300 mg O_2/L for COD (Ministry of the Environment, Waters and Forests, 2008).

Organic matter quantification and balance: The total amount of OM stored in the beds' sediments was different in function of the plants and decreased from the upper surface to the depth of the beds. The amount measured in the control was higher than those of the planted beds, while between the planted beds, the sediment of the bed transplanted with *C. zizanioides* contained the largest amount, followed by those planted with *A. gayanus*, *E. pyramidalis*, *T. laxum*and *P. purpureum*. The decreasing of organic matter would likely due to the filtration of organic particles in the layers of the bed's sediments, from the surface to depth and the different pathways of organic matter degradation would justify the difference between the planted beds (Tanja *et al.,* 2006, Xu *et al.,* 2016). Indeed, the degradation of OM requires organisms consisted of bacteria and macro-invertebrates (Ouattara *et al.,* 2009) for which, planted beds would confer more favorable ecological conditions conversely unplanted bed (Gagnon *et al.,* 2007).

This would also probably justify higher microbial activity in the planted beds than in the control and the same sequence noted in the comparison of produced biomasses on the planted beds. During plant growth, they generate aerobic micro-habitats within the substrate that would conducive to the growth of organisms (Kroer *et al.,* 1998). In addition, the plants would constitute a source of carbon necessary for the renewal of the energy of the organisms through secreted exudates and would maintain the shading favoring a hygrometry essential to the good development of the fauna (Andrews and Harris, 2000; Karjalainen *et al.,* 2001). Generally, the total amount OM was much stored in unplanted bed sediment about 64.89 %. Excepted bed transplanted with *C. zizanioides* (49.97 %), OM input to the beds was more uptake by microorganism at the proportions of 75.49 %, 60.95 %, 68.80 % and 85.10 % in the beds transplanted with *T. laxum*, *A. gayanus*, *E. pyramidalis* and with *P purpureum, respectively*. Despite the high amount of OM obtained in the bed sediment of C zizanioides (49.97%), we note the same order of magnitude as the amount uptake by microorganisms (43.87%). These results would be explained by the mechanisms of organic matter elimination in constructed wetlands, whose sediments and microbial organisms would be remained the main respective removal pathways in unplanted and planted beds (Gagnon *et al.,* 2007, Ouattara *et al.,* 2009).

Higher organic matter amount stored in the sediment of *C. Zizanioides* would be related to the specificities of this plant due to its more use in phytosatabilisation the soils (Chen *et al.,* 2004; Truong *et al.,* 2009).

Conclusion

In this study, all the five plant species grew well and their presence enhanced globally organic matter removal and then increased infiltration into the beds. Average concentrations of TSS, DOB_5, and COD in the all planted bed outlets were below the current standards of wastewater discharges in Ivory Coast with 50 mg/L, 100 mg O_2/L, and 300 mg O_2/L, respectively. Moreover, organic matter amount decreased in the sediment of the beds from upper surface to the bottom. Its removal by sediment storage and microbial uptake was 12.26–49.97% and 43.87–85.10%, respectively. The OM removal pathways differ slightly from species to species of plants. The main pathways of OM removal remain microbial uptake. Nevertheless, sediment storage could a main OM removal pathway for *C. zizanioides*. The overall performance was in this order: $B_{PP} > B_{TL} > B_{EP} > B_{AG} > B_{CZ}$. As *P purpureum* favored the best performance, constructed wetlands with these plants could be a cost-effective alternative and sustainable method of wastewater treatment.

Acknowledgment

This study has benefited from the technical support of the Laboratory of National Laboratory of Testing, Quality, Metrology and Analysis (LANEMA) of Côte d'Ivoire. We thank Mr. AIE Yapi Clément, the General Director, Mr. YAO Laurent, and the Assistant Director for their collaboration. We also thank all the researchers of the Research Unit in Biotechnology and Environmental Engineering of the Nangui Abrogoua University (Abidjan, Côte d'Ivoire) for their criticisms and observations.

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