

## ***Panicum maximum* density effect upon the efficiency of pilot-scale vertical flow constructed wetlands treating domestic wastewater**

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**ABSTRACT:** Plant density may have an influence on constructed wetlands (CWs) operation. In this study, the effect of plant density on the efficiency of vertical-flow CWs planted with *Panicum maximum* treating domestic wastewater was investigated. Two beds were planted with *P. maximum* at 10 plants/m<sup>2</sup> (low density) and two others at 20 plants/m<sup>2</sup> (high density). Two unplanted beds were used as control. During six months, domestic (0.05m<sup>3</sup>) raw wastewater was applied on each bed intermittently. Wastewater samples were taken once a week into the influent and the filtrate of each bed and preserved at 4°C until analysis. The results showed that pollutants were significantly more removed in the planted beds than in the controls. But, the plant densities used were not significantly impact the treatment efficiency. However, the bed planted at low density was clearly distinguished as the one that provided the higher pollutants removal rates (TSS = 91.8%, DCO = 91.6%, P = 69%, PO<sub>4</sub><sup>3-</sup> = 74.9%, NTK = 86.5%, NH<sub>4</sub><sup>+</sup> = 86.5%, *Escherichia coli* = 87.1%, *Clostridium perfringens* = 96.7%).

**KEYWORDS:** Constructed wetlands, *Panicum maximum*, plant density, wastewater treatment.

### **1 INTRODUCTION**

Nowadays, developing countries are faced with enormous environmental problems like collection and treatment of municipal wastewaters [1]. The conventional wastewater treatment systems, such as activated sludge systems which were realized in these countries, are unsuited and are difficult to operate and maintain with stable removal efficiencies [2]. These wastewaters are released to receiving waters without any treatment because of the costs of conventional technologies. Therefore, these countries are in great need of low-cost, low-maintenance wastewater management strategies that offer a good investment for such countries where funding for infrastructure building is scarce. Constructed wetlands (CW) are an inexpensive option for wastewater treatment in developing countries and can often be constructed out of locally available materials [3], [4], [5]. Conventional treatment systems are maintenance- and energy-intensive [6]. Consequently, their performance is affected when these requirements cannot be properly provided. Thus, in regions where conventional treatment technologies cannot be effectively maintained, wastewater treatment by wetland technology could result in improved water quality, benefiting the health, economy, and aesthetics of the region [7].

Many studies have been focused on engineering aspects (organic load, hydraulic retention time, plant species) of this technology [6], [8], [10]. CWs are ecosystems designed to recreate natural processes that leverage the interactions of soil, microorganisms and plants to treat a wide range of wastewater [11]. There are several types of CW among which the concept developed by [12] included beds composed of sand or gravel supporting emergent aquatic plants (vertical flow CW) proves 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 [13], [14]. Aquatic plants offer a simple, cheap, energy-efficient method for treating wastewater [15]. They require little technical back-up and are easy to maintain [16]. Certain rooted plants have bactericidal properties [17] and the ability to breakdown chemical pollutants, while submerged aquatics are important as oxygenators [16]. Moreover, their rhizosphere enhances density and activity of the CW organisms by providing root surface for their growth, source of carbon and a micro-aerobic environment via root oxygen release [18] therefore improving the CW

effectiveness. But plants species and planting density can impact on the CW organism's activities by their different potentialities to release exudates and oxygen in the beds [19], [20]. Plants canopies intercept solar rays and preserve favorable hygrometry for biological activities in the planted beds [21].

Previous study had shown that the presence of a fodder plant like *P. maximum* on the CW increases the effectiveness in removing domestic wastewater pollutants [22]. Pollutant removal was significantly superior in the planted beds than in the control. But, the density of this plant can affect CW performance, particularly, plants growth and wastewater infiltration, thus modifying the activities of organisms responsible for pollutants degradation in the beds media.

The purposes of this study were to evaluate the impact of *P. maximum* density upon to (i) plant aboveground biomass, (ii) beds hydraulic and (iii) pollutant removal efficiencies.

## 2 MATERIALS AND METHODS

### 2.1 EXPERIMENTAL DEVICE

The pilot-scale vertical flow CW was composed of six rectangular beds (Length × wide × depth = 1.75 m×0.75 m×0.45 m) made of cement, giving a bed surface of 1.31 m<sup>2</sup> (Figure 1). The beds were filled from the bottom to the surface by respectively 0.1m of gravel (size = 15/25 mm), textile and 0.3 m of white lagoon sand (mean sand diameter = 513 μm, uniformity coefficient = 0.4, porosity = 59%). The sand and gravel used for beds filling were both washed to remove clay, loam and organic matter. Four beds were planted at different densities with young *P. maximum* taken from endemic adult plants: two beds at 10 roots/m<sup>2</sup> (low density) and two other beds at 20 roots/m<sup>2</sup> (high density). Two unplanted beds were used as control. The beds were taken in pair to make repetition. The advantages of using *P. maximum* are: fodder plant, growth in natural wetland, high production rate, easy for manual harvest, ammonia preference uptake and high protein content. During six months corresponding to three growth cycles of *P. maximum*, each bed was fed three times per week (Monday, Wednesday and Friday) with 0.05 m<sup>3</sup> of domestic wastewater (hydraulic loading = 16.3×10<sup>-3</sup> m/d) taken from the sewerage system in Abidjan (Côte d'Ivoire) at each feeding. Homogeneous wastewater distribution on the surface of each bed was ensured using an irrigation device. The filtrates were drained out of the beds through a PVC pipe (diameter: 0.032 m).



**Fig. 1. View of the experimental device (A) and the vertical completely drained beds planted with *P. maximum* (B); 1: bed, 2: irrigation device, 3: feeding tank, 4: *P. maximum* growing on the beds.**

### 2.2 PLANT ABOVEGROUND BIOMASS DETERMINATION

At the beginning of grains production (every two months approximately), plants were harvested according to [22] 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 biomass and stumps diameter were carried out during the experiment and data obtained in each pair of beds were used for analyses.

### 2.3 BEDS HYDRAULIC CHARACTERIZATION

Bed hydraulic was achieved by determining wastewater infiltration rate in the beds, the hydraulic residence time and the beds plugging rate according to [23]. Wastewater infiltration rate was obtained from the measurement of the infiltration time of wastewater film applied on each bed by feeding. It represents the ratio of the wastewater volume applied and the infiltration time corresponding following the relation (1).

$$Q = \frac{V_w}{t} \quad (1)$$

Q = Infiltration flow rate (m<sup>3</sup>/min),

V<sub>w</sub> = Feeding wastewater volume (m<sup>3</sup>),

t = Infiltration time (min).

The hydraulic residence time or hydraulic retention time (HRT) corresponding to the average time that makes a drop of wastewater in the beds was determined according relation (2).

$$\text{HRT} = \frac{V}{Q} = \frac{\rho * A * h}{Q} \quad (2)$$

HRT = Hydraulic retention time (d),

V = Media pore volume (m<sup>3</sup>),

ρ = Porosity,

A = Bed surface (m<sup>2</sup>),

h = Bed depth (m),

Q = Water flow rate (m<sup>3</sup>/d).

From the wastewater infiltration rate determined at the beginning of the experiment (initial infiltration rate) and those obtained after each bed feeding (instantaneous infiltration rate), the bed plugging rates were calculated according to relation (3).

$$\text{Plugging rate (\%)} = \left( \frac{Q_{\text{init.}} - Q_{\text{inst.}}}{Q_{\text{init.}}} \right) * 100 \quad (3)$$

Q<sub>init.</sub> = Initial water flow rate (m<sup>3</sup>/min),

Q<sub>inst.</sub> = Instantaneous infiltration flow rate.

When the bed plugging rate was superior to 85%, solid matters accumulated on the beds surface were removed and beds media was plough on the first 5 cm to regenerate wastewater infiltration.

### 2.4 WATER SAMPLING AND PARAMETERS ANALYSIS

Wastewater samples were taken once a week into the influent and the filtrate of each bed in an ethylene bottle and preserved at 4°C until analysis. During the experiment, 26 water samples were taken in each bed. All analyses were completed within 24 hours of sample collection. For each water sample, total suspended solids (TSS), pH, chemical oxygen demand (COD), total nitrogen (NTK), ammonium (NH<sub>4</sub><sup>+</sup>), total phosphorus (P), orthophosphate (PO<sub>4</sub><sup>3-</sup>), *Escherichia coli* and *Clostridium perfringens* were analyzed using standard laboratory procedures and methods [24]. TSS was measured by filter method. pH was determined with a model WTW pH-meter 95. COD was measured by the potassium dichromate-boiling method. NTK was determined with by kjeldahl method after mineralization with selenium. NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup> were measured by the distillation–nesslerization and ascorbic acid-molybdate methods, respectively. *E. coli* were determined by miniaturized method by inoculation in liquid medium and *C. perfringens* by general method for incorporation in agar deep tubes. Pollutant removal was calculated using pollutant concentrations in the influent and in the bed filtrates (relation 4).

$$\text{Efficiency removal (\%)} = \left( \frac{X_{\text{inf.}} - X_{\text{fil.}}}{X_{\text{inf.}}} \right) * 100 \quad (4)$$

$X_{inf}$  = Parameter concentration into the influent (mg/l),

$X_{fil}$  = Parameter concentration into the filtrate (mg/l).

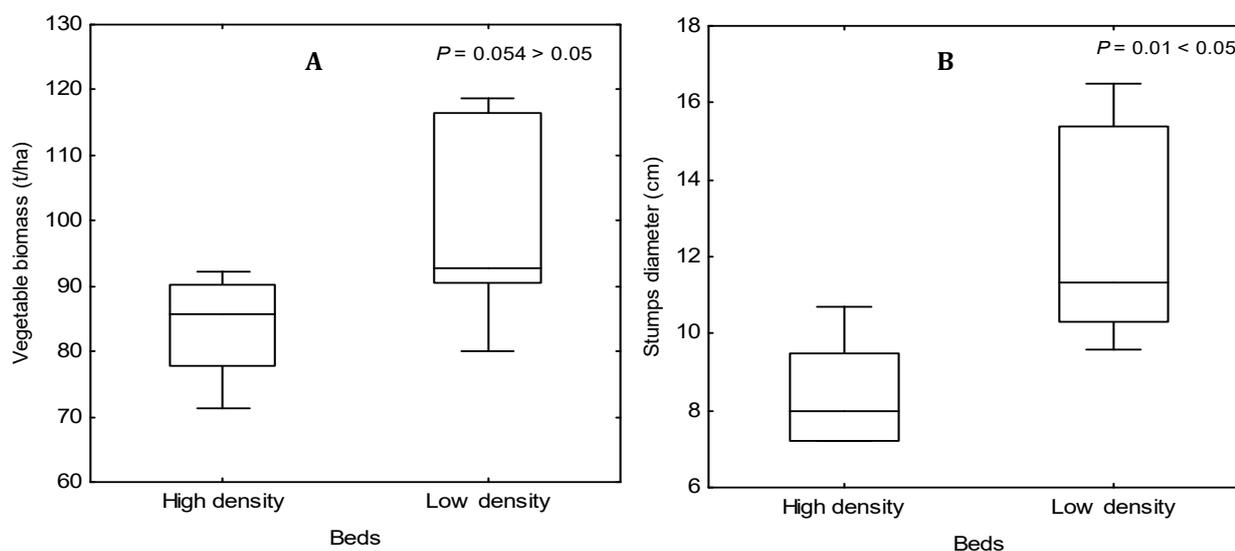
## 2.5 DATA ANALYSIS

Data of plant biomass and stumps diameter were not normally distributed (Shapiro–Wilk normality test), so Kruskal–Wallis and Mann–Whitney tests were used to make comparison. Concerning pollutant data, Mann–Whitney test showed no significant difference between data of both beds of each pair (planted beds at high density, planted beds at low density and unplanted beds). Consequently, the mean values of pollutant data for each pair have been used. These data were normally distributed (Shapiro–Wilk normality test) with homogeneous variances. Thus, ANOVAs (HSD of Tukey test) was used to compare those. In all cases, the significance level was  $p < 0.05$ . The statistical analyses were done with STATISTICA software, version 7.1.

## 3 RESULTS

### 3.1 PLANT BIOMASS PRODUCTION

Figure 2 shows the variations of plant biomass produced by the planted beds (Figure 2A) and the diameter of plant stumps (Figure 2B). Plant biomass produced varied between 77.9 and 92.2 t/ha and between 80.2 and 116.6 t/ha, respectively on the planted beds at high and low densities. Concerning the diameter of plant stumps, they oscillated between 7.2 and 10.7 cm on the beds planted at high density and between 9.6 and 16.5 cm on those planted at low density. Globally, plant biomass and stump diameter of the beds planted at low density were superior to those planted at high density. However, the difference between plant biomasses of these planted beds was not significant (Mann–Whitney tests:  $U = 6$ ,  $Z = -1.92$ ,  $p > 0.05$ ). But plant stumps diameter was significantly different (Mann–Whitney tests:  $U = 2$ ,  $Z = -2.56$ ,  $p < 0.05$ ) between the planted beds at high and low densities.



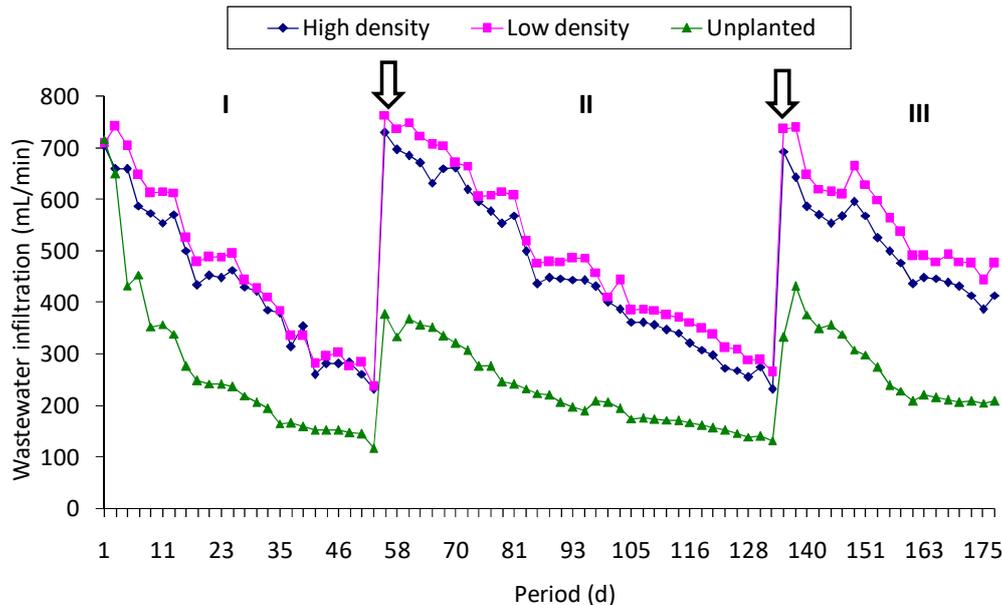
**Fig. 2.** Variation of plant biomass produced (A) and diameter of plant stumps (B) in the planted beds at high and low density.

### 3.2 EFFECTS OF PLANT DENSITY ON THE BEDS HYDRAULIC

The evolution of wastewater infiltration rate in the beds is showed on the figure 3. The profiles of infiltration rate in these beds presented three distinct phases (I, II and III) characterized by two periods of the removal of solids retained on the beds surface and cooling in 5 cm of sand surface at the days 53 and 133. Overall, one observes a decrease in the wastewater infiltration rate with time for the three phases. In the bed planted at high density, infiltration rate decreases from 704.7 to 232.3 mL/min (Phase I), from 730.5 to 212.6 mL/min (Phase II) and from 694 to 414.7 mL/min (Phase III). On the bed planted at low density, infiltration rate varies from 709.6 to 236.7 mL/min in Phase I, from 763.8 to 265.1 mL/min in phase II and 736.8

to 476.6 mL/min in Phase III. In the unplanted bed (control), the infiltration rate decrease from 717 to 118 mL/min, from 378.3 to 132.4 mL/min and from 334 to 208.7 mL/min respectively during phases I, II and III. During the three phases, the rate of wastewater infiltration is significantly higher in planted beds than in the control (Tukey HSD test:  $p < 0.05$ ). Considering both types of planted beds, the infiltration rate in the planted bed at low density is relatively higher (LD ( $503.5 \pm 146$  mL/min) > HD ( $465.1 \pm 135.6$  mL/min)). However, the difference in infiltration rate between the two planted beds is not significant ( $p > 0.05$ ).

Unlike the infiltration rate of the wastewater, the percentage of beds fouling and the hydraulic retention time (HRT) are significantly lower in planted beds than in the control (Tukey HSD test:  $p < 0.05$ ). However, planted beds (low and high density) have values of the same order of magnitude. For example, during Phase I, the percentage of beds fouling was 67%, 66.6% and 83.5%, respectively in the planted beds at high and low densities, and in the control. As for HRT, it is 18 h 30 min in the bed planted at the high density, 18 h 10 min in the bed planted at the low density and 36 h 26 min in the control.



**Fig. 3.** Evolution of wastewater infiltration rate in the beds; arrows indicate the two periods of the removal of solids retained on the beds surface and cooling in 5 cm of sand surface; FD = density; BD = low density; NP = not planted; I = first phase; II = second phase; III = third phase.

### 3.3 PLANT DENSITY EFFECTS ON CW PERFORMANCE

The mean values of the physico-chemical parameters of the raw wastewater and the bed filtrates are consigned in Table 1. pH of the influent decreased from 7.6 to 6.2 in the bed filtrates. The sequence of pH mean values was: influent (7.6) > filtrate of planted bed with *P. maximum* at high density (6.7) > filtrate of planted bed with *P. maximum* at low density (6.6) > filtrate of unplanted bed (6.2). These values were not significantly different (HSD of Tukey test:  $p > 0.05$ ). Concerning COD, NTK,  $\text{NH}_4^+$ , P and  $\text{PO}_4^{3-}$ , they were more removed in the filtrates of the planted beds than in the control one. Furthermore, these pollutants were more removed in the filtrates of the bed planted at low density than in those of the bed planted at high density. The sequence of pollutant removal in the CW beds was:

- For COD: bed planted at low density (91.4 %) < bed planted at high density (86.3 %) < unplanted bed (72.2 %).
- For NTK: bed planted at low density (65.4 %) < bed planted at high density (57.5 %) < unplanted bed (52.7 %).
- For  $\text{NH}_4^+$ : bed planted at low density (86.5 %) < bed planted at high density (79.3 %) < unplanted bed (65.9 %).
- For P: bed planted at low density (69 %) < bed planted at high density (63.7 %) < unplanted bed (36.4 %).
- For  $\text{PO}_4^{3-}$ : bed planted at low density (69 %) < bed planted at high density (63.5 %) < unplanted bed (49.7 %).

Except for TSS, the aforesaid wastewater pollutant parameters were significantly more removed in the planted beds than in the control (HSD of Tukey test:  $p < 0.05$ ). But between the planted beds at low and high densities, the treatment efficiency

was not significantly different ( $p > 0.05$ ). Globally, the treatment efficiency of the planted beds with *P. maximum* at low density was superior to that of the planted bed at high density.

**Table 1. Mean averages and standard deviations of physico-chemical and bacteriological parameters of the domestic wastewater and the beds filtrates and average removal rates (N = sample number)**

Parameters	N	Raw wastewater	bed planted at high density		bed planted at low density		Unplanted bed	
			Filtrates	Removal rate (%)	Filtrates	Removal rate (%)	Filtrates	Removal rate (%)
pH	32	7.6 ± 0.5	6.7 ± 0.6	–	5.6 ± 0.4	–	6.2 ± 0.4	–
TSS (mg/L)	32	1 518.9 ± 247	191.2 ± 173.9	86.8 ± 13.3	206.7 ± 168.7	85.6 ± 13.2	177.9 ± 177	87.7 ± 13.3
COD (mg O <sub>2</sub> /L) <sup>a</sup>	26	1 256 ± 571.5	154.9 ± 54.3	86.3 ± 5.7	99.9 ± 60.5	91.4 ± 5.1	328.1 ± 119.1	72.2 ± 6
NTK (mg/L) <sup>a</sup>	26	306 ± 101.1	126 ± 50	57.5 ± 13.7	106 ± 41.5	65.4 ± 7.9	140.8 ± 47.4	52.7 ± 15.3
NH <sub>4</sub> <sup>+</sup> (mg/L) <sup>a</sup>	26	128.1 ± 57.6	20.9 ± 10.1	79.3 ± 12.3	15.6 ± 12.4	86.5 ± 8.6	42.1 ± 21.7	65.9 ± 11
P (mg/L) <sup>a</sup>	26	8.8 ± 2.8	2.8 ± 1	63.7 ± 7.3	2.4 ± 0.9	69 ± 6.4	5.1 ± 2.2	36.4 ± 10.1
PO <sub>4</sub> <sup>3-</sup> (mg/L) <sup>a</sup>	26	6.2 ± 2.2	2.1 ± 0.8	63.5 ± 15.1	2.4 ± 0.9	69 ± 6.4	3.1 ± 1.6	49.7 ± 20.8
<i>E. coli</i> (UFC/100 mL)	26	94 033.69	3 280.08	96.5 ± 3.4	2 182.04	97.7 ± 1.8	9 547.69	89.8 ± 16.6
<i>C. perfringens</i> UFC/100 mL	26	6 411.54	834.62	87 ± 8.6	869.46	86.4 ± 12.6	789.15	87.7 ± 15.2

<sup>a</sup> High significant different (HSD of Tukey test:  $p < 0.05$ ) between the average removal rates of the planted and unplanted beds

Relating to *Escherichia coli* and *Clostridium perfringens*, the number was considerably reduced in the filtrates of the planted beds and the control. This reduction was similar in the planted beds and in the unplanted bed. In the control it was 89.8 % for *E. coli* and 87.7 % for *C. perfringens*. In the planted beds, 97.7 % and 96.5 % of *E. coli* removal, and 86.4 % and 87 % of *C. perfringens* removal were obtained respectively in the filtrates of the beds planted at low and high densities. The statistical analysis did not show a significant difference (HSD of Tukey test:  $p > 0.05$ ) between pathogens removal in the CW beds.

#### 4 DISCUSSION

The study showed more or less significant effects of *Panicum maximum* density on its growth and its biomass produced, as well as on the beds hydraulic and the CW performance. With regard to plant growth, the results obtained indicate that plants were well adapted to the environment whatever the plants density used. This good plant growth could be due to the characteristics of domestic wastewater used which were loaded with organic and nutritious matters. However, the plant biomass and the stump diameter of the beds planted at low density were superior to those planted at high density. This difference was likely due to the competition phenomena of the plants which would be more important in the bed planted at high density. In fact, more the plants density increase, more plants tend to satisfy their nutritional needs rather than their growth [25]. This could reduce the plant biomass production.

Considering the beds hydraulic, there is a decrease in the wastewater infiltration rate into the beds with time. This situation could be attributed to the reduction of the pore space of the beds media by the coarse and colloidal matters contained in the wastewater used. According to [26], during wastewater infiltration, the coarse matters are retained on the surface and the thinnest in the pores, which obstructs them. As a result, beds hydraulic decreases. This hypothesis is confirmed by the increase in the infiltration rate on days 56 and 133 due to the scraping of the solids crust formed on the surface of the reactors and the cooling of the first five centimeters of the media.

In comparison with the unplanted bed, significantly higher values of wastewater infiltration rate recorded in planted beds were likely related to *P. maximum* root development. Indeed, this plant has a dense root system, and the rhizomes could create galleries in the planted beds to maintain a relatively high porosity and hydraulic conductivity [27], [28]. However, the two plant densities used were not significantly affecting the wastewater infiltration rate. But, the low density (10 roots/m<sup>2</sup>) preserves an infiltration rate superior than that of the high density (20 roots/m<sup>2</sup>). This plant density (10 roots/m<sup>2</sup>) is more favorable to maintain a low clogging of the CW beds.

During wastewater treatment, the values of all parameters measured were decreased considerably in the bed filtrates compared to the raw wastewater regardless of the planting density used. The pH decrease in the bed filtrates could be explained by the oxidation of wastewater organic matters, the nitrification of nitrogen containing compounds [29] and the adsorption of alkalis in the beds [6]. That could also be due to CO<sub>2</sub> production which may form in aqueous environment carbonic acids and reduce the pH [30]. Concerning TSS removal in the CW, this could be explained by physical mechanisms and chemical reactions in the bed sediments [31], [32]. The removal of TSS in the planted beds was lower than that in the unplanted one. This result could be due to the plant roots that create tunnels in the sediments of planted beds through which wastewater TSS

can migrate. Compared to the control, the best removal of COD, NTK,  $\text{NH}_4^+$ , P and  $\text{PO}_4^{3-}$  in the planted beds could be due to the both plants uptake and microbial oxidation [5], [33]. In regard to nutriment removal, the high performances obtained were probably enhanced by the high *P. maximum* uptake for nutriment (11.2 % for nitrogen and 13.5% for phosphorus) [22]. Generally, the treatment efficiency was not significantly different between the planted beds. However, it was better in planted bed at low density than in planted bed at high density. The higher efficiency of the planted bed at low density could be explained by an abundant oxygen transfer in the bed when plant density was 10 roots/m<sup>2</sup> (low density). Indeed, plants at low density grow and extend faster than those at high density. Consequently, there could be a higher quantity of oxygen produced that could optimize microbial activities for pollutants degradation in the planted bed at low density.

Concerning *Escherichia coli* and *Clostridium perfringens*, their removal in beds filtrates could be explained by a physical retention and their adsorption in the bed media. The reduction of these human pathogens could be also supported by biological mechanisms such as competition, parasitism and predation between microorganisms which grow in the beds media [30]. The mean values of *E coli* and *C perfringens* removal in the different beds filtrates were similar. However, *E coli* removal was slightly important in the planted beds filtrates compared to the control filtrate. This result was probably due to the action of some exudates secreted by *P. maximum* which would contribute to reduce *E coli* [34]. The two plant densities used were not significantly affecting the efficiency of the constructed wetland developed. But, the planting at 10 roots/m<sup>2</sup> improved globally the CW efficiency.

## 5 CONCLUSION

The study showed that the constructed wetland planted with *Panicum maximum* was suitable for domestic wastewater treatment. *P. maximum* planted at low and high densities was well adapted to the environment and produced optimal vegetable biomass. Biomass production decreased with the increase of plants density. The bed hydraulic study showed that CW developed could operate without clogging problem for two months despite the high concentration of wastewater TSS. The constructed wetland planted with *P. maximum* was effective in removing domestic wastewater pollutants (TSS, COD, NTK,  $\text{NH}_4^+$ , P and  $\text{PO}_4^{3-}$ ). Pollutant removal, except for TSS, was significantly superior in the planted beds than in the control. Plant density did not impact significantly on the CW efficiency. However, the bed planted at 10 roots/m<sup>2</sup> provided the highest performance.

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