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Bacteriological Quality of a Forage Grass (*Pennisetum purpureum Schumach*) Used in Constructed Wetland Removing Domestic Wastewater Pathogenic Microorganism

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Abstract

Constructed Wetlands (CWs) are an adequate wastewater treatment system with possibility to generate income, in particular by the use of plants of economic interest. However, very few studies deal with the bacteriological quality of plants after wastewater treatment. Thermotolerant coliforms and Sulfite-reducing bacteria were investigated on the above-ground biomass of a species of forage plant (*Pennisetum purpureum*) as well as their removal in an experimental pilot consisting of four beds, for three months. Two beds were planted and two unplanted beds were used as control. Germs in the wastewater were significantly reduced in both filtrates, with higher removal efficiency of 97.4% for Thermotolerant coliforms and 87.5% for Sulfite-reducing bacteria, in the planted bed. Wastewater treatment resulted in bacteriological contamination of the above-ground plant biomass with a significant decreases in number of germs from 660 to 28 CFU/g (Thermotolerant coliforms) and from 15 to 0 CFU/g (Sulfite-reducing bacteria), when the harvest height increased from the base to the upper end of the plants. However, averages of 305 CFU/g of Thermotolerant coliforms and 5 CFU/g of Sulfite-reducing bacteria were obtained in the above-ground plant biomass which would not present any potential risks for a possible use of the plant biomass as fodder. Thus, the use of forage plant suggests good prospects for upgrading said plants for animal feed.

Keywords

Constructed Wetlands, Pathogens Microorganisms, Forage Plant, Above-Ground Plant Biomass Quality

1. Introduction

Constructed wetlands (CWs) are artificial engineered wastewater treatment systems that use natural processes involving wetland vegetation, soils substrates (*i.e.* sand, stones, clay), and their associated bacteria and invertebrates population to improve water quality [1] [2]. These systems are increasingly recognized as a reliable wastewater treatment technology due to their efficiency, low energy consumption, good landscape integration and aesthetic appearance, but above all, due to the possible valorization of the above-ground biomass of the plant species used [3] [4].

In fact, in addition to the preserving the environment by improving the quality of wastewater before discharging into the natural environment, the use of plants with economic interests would guarantee good prospects for valuing the above-ground plant biomass produced. The sale of above-ground plant biomass produced during wastewater treatment could generate income that could support maintenance costs and ensure the proper functioning of the process in the long term [4]. Thus, plants in constructed wetlands are not only useful for taking up nutrients, filtering organic matter, and creating an environment conducive to the proliferation of organisms to provide safe sanitation. The above-ground plant biomass can therefore be harvested and used for the production of food for direct use, fodder for livestock and for fuelling purposes [5].

Due to the advantages offered by CWs, namely the possible valuation of certain plant species, the scientific community should increasingly be confronted with the problem of the quality of plants of economic interest used in the process. However, this issue remains very little to be discussed and presented in fora and article publications, in contrast to those focusing on the sanitation efficiency of CWs [6] [7], the removal processes and the design [8] [9] and operational parameters that affect the removal processes in the systems [3] [10].

The same is true in sub-Saharan African countries, which however benefit from better assets for the implementation of constructed wetlands, given their tropical climate. In addition, the economic level of these countries makes the CWs the suitable alternative for the treatment of wastewater from different agglomerations. However, plants for direct consumption by humans (*i.e. Amaranthus hybridus* and *Corchorus olitorius*) have been successfully tested by Coulibaly *et al.* [11] [12], without however showing any level of potential bacteriological contamination. However, an opinion study on a sample of the population showed a refusal of consumption of said food plants because of their origin of production. The work relating to the quality of plants for indirect consumption

by humans (*i.e.* forage plants) is those of Pare *et al.* [13]. However, these are limited to the nutritional potential of the plant (*Echinochloa Pyramidalis*) such as, crude proteins (CP), digestible dry matter (DDM), total digestible nutrients (TDN) and metabolizable energy (ME).

On the other hand, wastewater from human activities contains several pollutants including nutrients (nitrogen and phosphorus), organic matter and pathogenic microorganisms. However, in most of the work, the focus is usually on organic matter and nutrients, unlike pathogenic microorganisms. However, the latter, particularly fecal pathogens also pose public health risks (*i.e.* typhoid fever, cholera, diarrhea, dysentery, ineffective hepatitis, skin and tissue infections) when they are released without adequate treatment in the receiving environment [14] [15]. Diarrhea, for example, is the third leading cause of morbidity and the sixth leading cause of mortality worldwide, and nearly 80% of diarrhea cases worldwide are due to release of untreated wastewater in environment and inadequate sanitation [16].

Considering pollutants degradation mechanisms, wetlands use numerous symbiotic processes for concurrent removal of the different pollutants. These mechanisms involve the major components of CWs, namely plants, substrate and microorganisms living in the wetlands [3]. To understand the mechanisms that govern the degradation of pollutants from wastewater in CWs, several studies have been carried out about plants [17], substrate [18]. Studies relating to microorganisms, in particular the total aerobic and anaerobic bacteria flora living in the CWs, are poorly documented. Thus, knowledge of the ecology of these organisms could help to evaluate CWs wastewater treatment performance and to better understand the biological removal mechanisms of pathogens microorganisms that take place there.

This study aims to examine advantages of using a forage grass in a constructed wetland improving domestic wastewater pathogenic microorganism's quality. Specifically, the pathogenic microorganisms (*i.e.* Thermotolerant coliform and Sulfite reducing bacteria) removal efficiency of a CW transplanted with *Pennisetum purpureum.* Then, the pathogenic microorganism's contamination on the above-ground plant biomass. And finally, the aerobic, anaerobic and total bacteria densities as well as their vertical distributions profile within pilot-scale substrates.

2. Materials and Methods

2.1. Materials

The study was performed out on the experimental pilot of the Biotechnology and Environmental Engineering Research Unit of NANGUI ABROGOUA University (Abidjan, Côte d'Ivoire). The analyses were carried out both within the Laboratory of Environment and Aquatic Biology of NANGUI ABROGOUA University and the National Laboratory for Quality Assurance Testing, Metrology and Analysis of Côte d'Ivoire.

2.2. Methods

2.2.1. Description of the Treatment Units

The treatment units was composed of four (4) rectangular beds [vertical flow CW] (length × wide × depth = 1.45 m × 1.00 m × 0.80 m) built cement according to Coulibaly *et al.* [11] [12]. Each of the 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 (mean sand diameter = 572 µm, uniformity coefficient = 0.4, porosity = 37.5%), previously washed to remove any clay, loam and organic matter. Finally, each of them was equipped with irrigation devices consisted of six (6) polyvinyl chloride (PVC) pipe (length: 1.40 m; diameter; 0.008 m) containing 60 lateral holes to allow the homogeneous distribution of the wastewater on the surface (**Figure 1**).

The bed bottom slope was 1% oriented via PVC of 0.032 m diameter to drain out the effluent of the bed. However, the effluents were collected in a device accommodated at the outlet of the beds. As seen in **Figure 2**, the whole of the beds was equipped with a wastewater supply device (feeding tank 1000 L).

2.2.2. Plantation

Two (2) beds were transplanted with the plants seedlings (*i.e.* 9 plants/m^2) spaced of 40 cm × 40 cm between the stems and Two (2) was preserved unplanted (UB) and used as controls. The young plants (those showing the same and good vigor) were collected from nurseries established near the experimental pilot and previously cut to 20 cm above the roots before transplanting in the bed.

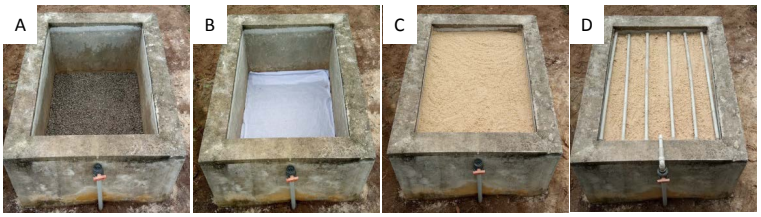

Figure 1. Stages of assembly of the bed components of the constructed wetland; (A) = lower layer of gravel; (B) = cloth separating the layers of gravel and sand, (C) = top layer of sand and (D) = irrigation device for the homogeneous distribution of wastewater on the surface of the bed made of perforated PVC pipes.

Figure 2. Overview of the experimental pilot after two (2) months of the treatment trial, (A) feeding tank, (B) plants growing on the beds.

Furthermore, *Pennisetum purpureum* was used in this study as wetland vegetation, because of the better purification yields obtained in several studies [7] [19] [20]. However, it is a perennial forage plants highly appreciated by agro-pastoralists for their palatability, adaptation to local climatic conditions and presence in Côte d'Ivoire. Thus, its above-ground biomass produced could generate income likely to cover the maintenance costs of the process.

2.2.3. Experimental Procedure

The study was carried out over three (3) months with an intermittently feeding water volume of 120 L (3 days/week), corresponding to the hydraulic loading of 3.55 $cm \cdot d^{-1}$. However, after transplanting the young stems of the plant into the beds, they were fed with tap water for one (1) month to allow them to acclimatize to the substrate. After the acclimation period, each bed was intermittently supplied with domestic wastewater over two (2) months.

The domestic wastewater used was taken from the wastewater network of NANGUI ABROGOUA University because of its proximity to the experimental site, but above all to alleviate any supply problems and ensure the actual origin of wastewater. It was taken two (2) times a week from the network, using an emptying vehicle, then discharged into the cubitainers, from which the beds were supplied.

During the treatment trial, samples of wastewater, above-ground plant biomass and substrates were taken, each according to its own method, for analyzes of the parameters monitored.

2.2.4. Wastewater 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. The pH, and dissolved oxygen (DO) were determined according to ISO 10523 [21] and ISO 5814 [22], respectively. Then, Thermotolerant coliform and Sulfite reducing bacteria were determined according to ISO 9308-1 [23] and ISO 6461-2 [24] respectively.

Finally, removal efficiencies was calculated according to Abissy and Mandi [25] for Thermotolerant coliform and Sulfite reducing bacteria as follows:

$$\text{Removal Efficiency} = \frac{C_i V_i - C_o V_o}{C_i V_i} \times 100 \quad (1)$$

where C_i and C_o are the inlet and outlet concentrations (mg/L), V_i and V_o are the inlet and outlet volume (L) an in the CWs.

2.2.5. Above-Ground Plant Biomass Sampling and Analysis

In order to determine the bacteriological quality of the above-ground plant biomass in the CWs, five (5) plant tufts were retained at the rate of one at each corner (4) and one in the center (1) of the beds. From each of the tufts considered, composite leaf samples were taken from the base to the upper ends of plants between 20 and 70 cm, 70 and 120 cm, 120 and 170 cm and between 170 cm and the upper end.

Thus, twenty (20) composite samples were collected per planted bed at the rate five (5) composite samples at each height (*i.e.* [20; 70 cm], [70; 120 cm], [120; 170 cm] and [170 cm; $+_{\infty}$]) of the tufts considered. These samples were packaged in sterile bags and taken to the laboratory for analysis. The Thermotolerant coliforms and Sulfite-reducing bacteria were determined by colony counting techniques, obtained according to ISO 4832 [26] and ISO 15213 [27], respectively.

2.2.6. Substrate Sampling and Analysis

Substrate sampling for bacteria analysis was performed by coring with PVC pipe (Φ = 16 mm), in six substrate layers, from upper surface to the bottom of the beds (*i.e.* [0; −10 cm], [−10; −20 cm], [−20; −30 cm], [−30; −40 cm], [−40; −50 cm] and [−50; −60 cm]), according to Puigagut *et al.* [28]. The surface of the beds was divided into three (3) equal sections for a better taken account of the bacteria distribution within the beds. In each section, three sampling points (one at each extremity of the bed, and one at the center) were uniformly distributed over the width of the reactors from which a composite sample of the substrate layer under consideration was formed. Thus, the samples were stored in jars at 2˚C until analysis.

The analysis of the bacteria was carried out according to the technique of germs inoculation in Plate Count Agar (PCA) [29]. In fact, 5 g of the substrate sample were suspended in a sterile saline solution (0.85% NaCl) of 50 mL and inoculated in triplicate onto PCA after stirring and sedimentation at room temperature. The aerobic germs were grown in a single layer of agar, whereas the anaerobic germs were within a double layer of agar. These germs were incubated at 37˚C for 48 h, and then the number of colonies formed were counted according to the international standard ISO 6222 [30]. The total number of bacteria in each sample was determined by adding the numbers of aerobic and anaerobic bacteria.

2.2.7. Data Analysis

All data analysis were performed using R studio 3.3.2 software, including Kruskal-Wallis, Mann Whitney, ANOVA variances, and T-test after Shapiro-Wilk test [31]. The Shapiro-Wilk test was used to determine the normality of the data, followed by ANOVA test or that of Kruskal-Wallis depending on whether the data followed a normal distribution or not. Then, in the event of a significant difference with the latters, analyzes were refined respectively by the T-test and that of Mann Whitney.

3. Results

3.1. Assessment of Physical Parameters

The minimum, maximum and average values of pH, dissolver oxygen (DO) and water volume at the inlet and outlet of all the beds (planted and unplanted) are reported in **Table 1**. Regarding the pH, the values measured in the outlet of the

Table 1. Average, maximum, minimum value of different parameters and removal efficiencies (RE) within outlet of the planted and unplanted beds.

Treatment and parameter		DO (mg/L)	pH	Volume (L)	Thermotolerant coliform (10^6 CFU/100 mL)		Sulfite reducing bacteria (10^4 CFU/100 mL)	
		Value	Value	Value	Value	% RE	Value	% RE
Wastewater	Average	**1.04^a**	**6.91^a**	**120^a**	**1.17^a**		**4.82^a**	
	Max	1.36	7.03	120	1.42		8.13	
	Min	0.82	6.75	120	1.00		1.45	
Planted bed (*PB*)	Average	**2.31^b**	**7.11^b**	**98.4^b**	**0.04^b**	**97.41^a**	**0.74^b**	**87.50^a**
	Max	2.84	7.24	107.3	0.08	98.79	1.22	91.35
	Min	2.03	7.04	90.3	0.02	94.93	0.24	80.13
Unplanted bed (*UB*)	Average	**1.90^c**	**7.00^a**	**107.9^c**	**0.10^c**	**92.21^b**	**0.83^c**	**84.56^b**
	Max	2.06	7.19	113.8	0.13	96.57	1.28	87.87
	Min	1.65	6.83	101.5	0.04	89.64	0.30	76.88

Values within the same column followed by the same superscript letter (*i.e.* a, b, c) are not significantly different at $P < 0.05$, Max: maximum, Min: minimum.

beds (in the filtrates) remain overall higher than those in wastewater (raw water). The sequence of the pH mean values after the experimental trial was: filtrate of planted bed (7.11) > filtrate of control (7.00) > raw wastewater (6.91). The pH values recorded in the filtrate of planted bed (7.04 - 7.17) differ markedly from those of the unplanted bed filtrate and raw water (6.75 - 7.03) [ANOVA t test: $p < 0.05$]. However, the pH of the unplanted bed (6.98 - 7.19) filtrate and that of the raw wastewater are of the same order of magnitude ($p > 0.05$).

Dissolved oxygen (DO) concentration was higher in filtrates than in raw water. Also, the filtrates from the planted bed had higher DO values than those from the filtrates from the unplanted bed. However, the measured DO values differed significantly from each other (ANOVA t test: $p < 0.05$). The sequence of DO importance was as follows: 1.04 mg/L (raw water) < 1.90 mg/L (unplanted bed) < 2.31 mg/L (planted bed).

As regards the volumes of treated water collected outlet the beds, they were lower than the applied volumes of wastewater (120 L). Compared to the planted bed, the unplanted one returns the highest volume of water (ANOVA t test: $p < 0.05$). The volumes of water collected varied from 90.3 to 107.3 L outlet the planted bed and from 101.5 to 113.75 L outlet the unplanted one, with respective averages of 98.4 L and 107.9 L.

3.2. Pathogenic Microorganism Treatment in CWs

The minimum, maximum and average numbers of Thermotolerant coliforms and Sulfite-reducing bacteria in raw water and filtrate from planted and unplanted beds are also presented in Table 1. In raw water colonies of Thermotolerant coliforms varied from 10^6 to 1.42×10^6 CFU/100 mL and that of Sulfite-reducing bacteria, from 1.45×10^4 to 8.12×10^4 CFU/100 mL, with respec-

tive means numbers of 1.17×10^6 CFU/100 mL and 4.8×10^4 CFU/100 mL.

In the filtrates, the number of Thermotolerant coliforms and that of Sulfite-reducing bacteria decreased significantly (ANOVA t test: $p < 0.05$). However, Thermotolerant coliforms were much more reduced in the planted beds (0.02×10^6 to 0.08×10^6 CFU/100 mL) than in the unplanted control (0.04×10^6 to 0.13×10^6 CFU/100 mL) [ANOVA t test: $p < 0.05$]. The average removal efficiencies of Thermotolerant coliforms in the beds were 97.4% (PB) and 92.2% (UB).

As for Sulfite-reducing bacteria, the numbers obtained ranged between 0.2×10^4 and 1.5×10^4 CFU/100 mL and between 0.3×10^4 and 1.3×10^4 CFU/100 mL, respectively in the filtrates of the planted and unplanted beds. The average removal efficiency were in the planted bed (87.5%) differed markedly from that in the unplanted bed (84.6%) [ANOVA t test: $p < 0.05$].

3.3. Above-Ground Plant Biomass Quality

Figure 3 shows the variation in number of Thermotolerant coliforms and Sulfite-reducing bacteria on the above-ground plant biomass produced in the constructed wetland according to the harvest height of the plants. Overall, the number of germs decreases significantly when the harvest height of the above-ground plant biomass increases (ANOVA t test: $p < 0.05$).

The averages of 660 ± 139 CFU/g, 393 ± 86 CFU/g, 140 ± 42 CFU/g and 28 ± 17 CFU/g of Thermotolerant coliforms were obtained, respectively at harvest heights ranging from 20 to 70 cm, from 70 to 120 cm, from 120 to 170 cm and beyond 170 cm. This corresponded to an average number of Thermotolerant coliforms equal to 305 CFU/g in the above-ground biomass of *Pennisetum purpureum* produced in the CW.

Regarding Sulfite-reducing bacteria, the number obtained was 15 ± 1 CFU/g in the harvest height interval of [20 - 70 cm], 4 ± 3 CFU/g in the harvest height interval of [70 - 120 cm] and 1 CFU/g in the harvest height of [120 - 170 cm]. No germ of Sulfite-reducing bacteria was found at the upper end of the plant (beyond 170 cm). Thus, an average of 5 CFU/g of Sulfite-reducing bacteria was obtained in the above-ground biomass of *P. purpureum*.

3.4. Assessment of Bacterial Density in CWs Substrate

3.4.1. Total Bacterial Densities

The densities of total bacteria as well as aerobic and anaerobic bacteria in the substrate of CWs beds at the end of the treatment trial are shown in Figure 4. We note that the densities of total bacteria and aerobic bacteria in the planted bed were greater than those in the unplanted bed. There were respectively 5.4×10^6 and 5×10^6 CFU/g in the planted bed against 1.5×10^6 and 0.9×10^6 CFU/g in the control. On the other hand, the density of anaerobic bacteria (0.6×10^6 CFU/g) counted in the unplanted bed remains high than that obtained in the planted bed (0.4×10^6 CFU/g). However, in the planted bed as in the unplanted one, the density of aerobic bacteria remains higher than that of anaerobic bacteria.

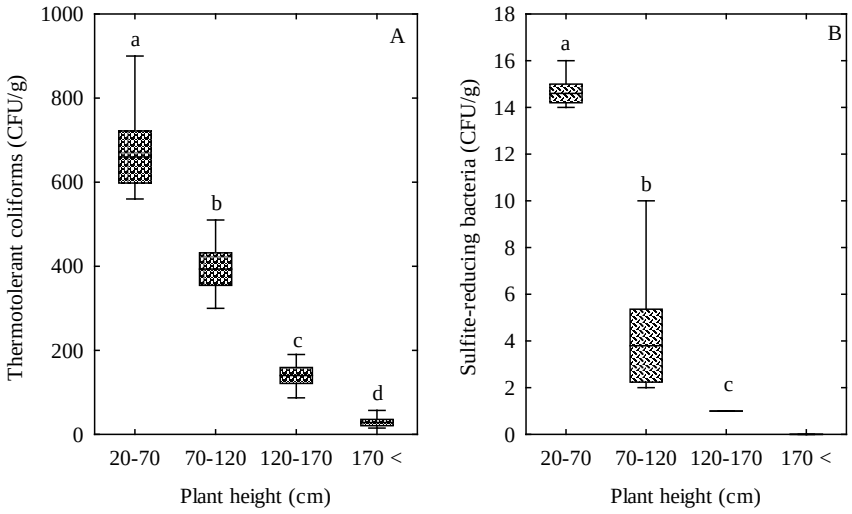

Figure 3. Variation in the number of Thermotolerant coliforms (A) and Sulfite-reducing bacteria (B) found on the above-ground biomass of Pennisetum purpureum in the constructed wetland, according to the harvest height. Box-plots no bearing one alphabetical letter-identical are significantly different (ANOVA test; $p < 0.05$).

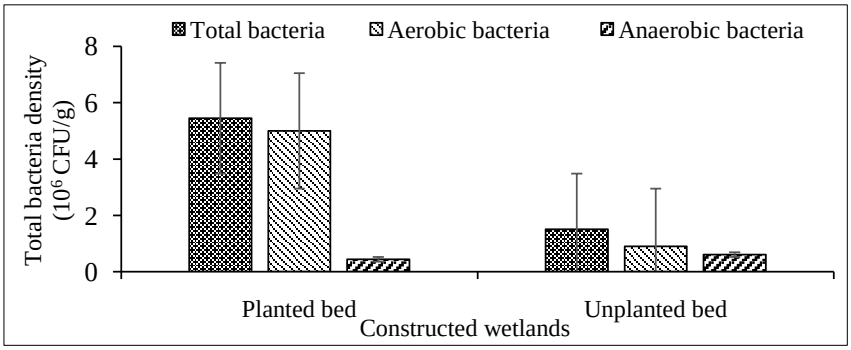

Figure 4. Densities of total bacteria and aerobic and anaerobic bacteria in the planted and unplanted constructed wetlands beds substrate.

3.4.2. Bacterial Densities Profile in the CWs Substrate

The vertical distributions of bacteria, anaerobic, aerobic and total bacterial densities from the upper layer (0 - 10 cm) to the bottom layer (50 - 60 cm) of the bed substrate are denoted in Table 2. Overall, total bacteria and aerobic bacteria densities decreased from the upper layer to the bottom layer, while that of anaerobic bacteria increased.

Specifically, total bacteria densities varied from 9.7×10^6 CFU/g (10 - 20 cm) to 2.1×10^6 CFU/g (50 - 60 cm) in the planted bed, and from 2×10^6 CFU/g (10 - 20 cm) to 1.1×10^6 CFU/g (50 - 60 cm), in the unplanted bed. Statistical analysis indicated significant difference between the substrate layers bacterial densities of the planted bed (Kruskal Wallis test: $p < 0.05$). In the unplanted bed, bacterial densities in the first two upper layers (0 - 10 cm and 10 - 20 cm) were clearly distinguished from those in the layers lower than these (Mann Whitney test: $p < 0.05$). Considering the bacterial densities in the homologous layers of the two types of CWs, those of the planted bed were largely high ($p < 0.05$).

Table 2. Vertical distribution of aerobic, anaerobic and total bacterial densities (median densities) in the substrate layers of unplanted and planted beds.

Substrate layers (cm)	Bacterial density (10^6 CFU/g)					
	Aerobic bacteria		Anaerobic bacteria		Total bacteria	
	Planted bed	Unplanted bed	Planted bed	Unplanted bed	Planted bed	Unplanted bed
0 - 10	9.6^a	1.9^a	0.1^a	0.2^a	9.7^a	2.0^a
10 - 20	7.4^b	1.2^d	0.3^b	0.4^b	7.7^b	1.6^b
20 - 30	5.1^c	1.0^c	0.4^c	0.5^c	5.5^c	1.5^c
30 - 40	3.7^d	0.7^d	0.5^d	0.7^d	4.2^d	1.4^c
40 - 50	3.0^e	0.4^e	0.7^e	0.9^e	3.7^e	1.3^c
50 - 60	1.4^f	0.1^f	0.7^f	1.0^f	2.1^f	1.1^c

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

As for the aerobic bacteria, their densities oscillated between 1.4×10^6 and 9.6×10^6 CFU/g and between 0.1×10^6 and 1.9×10^6 CFU/g, respectively in the layers of the planted bed and unplanted bed. Overall, the densities of aerobic bacteria differed significantly from layer to layer in both types of planted and unplanted beds (Kruskal Wallis test: $p < 0.05$). Likewise, the difference was clear between the bacterial densities of the homologous horizons of the planted bed and of the control. They were significantly higher in the layers of the planted bed than in those of the control (Mann Whitney test: $p < 0.05$).

The densities of anaerobic bacteria differed significantly like those of aerobic bacteria from one substrate layer to another, in the planted bed and the unplanted control (Kruskal Wallis test: $p < 0.05$). Anaerobic bacteria density increased from 0.1×10^6 to 0.7×10^6 CFU/g (planted bed) and from 0.2×10^6 to 1×10^6 CFU/g (unplanted bed), from the upper layer (0 - 10 cm) to that of the bottom (50 - 60 cm). Statistical analysis did not show any difference between the bacterial densities of the two (2) types of bed, in the three (3) upper layers (0 - 10 cm, 10 - 20 cm and 20 - 30 cm) (Mann Whitney test: $p > 0.05$), although, the bacterial densities of the unplanted bed remain higher. However, at the level of the lower layers (30 - 40 cm, 40 - 50 cm and 50 - 60 cm), the densities of anaerobic bacteria obtained in the unplanted bed were much higher than those recorded in the planted bed ($p < 0.05$).

4. Discussion

4.1. CWs Performance

The volume of treated water (filtrate) collected outlet the beds were much lower than the applied volumes of wastewater. This result could be related to the phenomena of evaporation in beds and evapotranspiration in plants as well as to the retention of a fraction of water in the substrate of the beds [3]. Kengne *et al.* [32] made the same observation at the outlet of the beds of constructed wetland

transplanted with *Echinochloa pyramidalis*.

Relative the purification performance of the beds, the results revealed higher values of pH and dissolved oxygen (DO) in the filtrates compared to raw wastewater. In addition, the pH and DO in the planted bed filtrate appeared to be higher than that of the control. This situation, as regards the pH, could be due to the biodegradation of the organic matter. Indeed, the CO_2 resulting from the biodegradation of organic matter acidifies the environment, in the presence of water. Thus, the calcium and magnesium hydrogen carbonate contained in the wastewater, partially adsorbed in the substrate bed, could be bring back into solution, the mineralization of which would have raised the pH of the medium [33]. In addition, according to Wegner [34] [35], the absorption of nitrate ions through the roots of the plant is against the countercurrent of a transport of hydroxide ions (HO^-) from the plant to the outside or a co-transport of hydronium ions (H_3O^+ or H^+) inside plant cells. Thus, the release of OH^- ions into the medium during the reactions would also have raised the pH of the planted bed filtrate. However, the pH values recorded in the bed filtrates remain favorable for the biodegradation of organic matter and/or the metabolism of nutrients [3]. The increase in DO in bed filtrates results from the aeration of raw water during its application to the vertical flow wetland beds used and oxygen released at the apex of the rootlets of the plants [36] [37]. However, the DO concentrations in the filtrate of the planted beds (2 - 3 mg/L) are favorable the development of heterotrophic bacteria involved in the removal of organic matter, nitrogen and phosphorus from wastewater in the CW [38].

Pathogens (Thermotolerant coliforms and Sulfite-reducing bacteria) contained in the wastewater were significantly reduced in both the filtrates from the planted bed and those from the control unplanted. This result is justified by the mechanisms that govern the removal of pathogenic microorganisms in Constructed Wetlands. Indeed, removal of pathogenic microorganisms is mainly accomplished through physical (*e.g.,* filtration, sedimentation) and biological (*e.g.,* predation, antibiosis, etc.) mechanisms [39] [40] [41]. Thus, the lagoon sand 0.6 m thick and of uniform particle size, used as a substrate in the two (2) types of beds, whether or not planted, would have favored the significant reduction of pathogenic microorganisms. However, this reduction was greater in the planted bed than in the control, probably due to the exudates secreted by plants in the substrate of constructed wetlands, which would further neutralize pathogens [41] [42]. In addition, the higher density of the total bacterial flora in the substrate of the bed planted in the present study, would highlight the processes of antagonisms of the microorganisms which would have greatly contributed to the elimination of pathogenic microorganisms.

4.2. Above-Ground Plant Biomass Quality

The treatment of domestic wastewater resulted in bacteriological contamination of the above-ground biomass of *Pennisetum purpureum* produced in the con-

structed wetland. Analyzes revealed the presence of colonies of Thermotolerant coliforms and Sulfite-reducing bacteria, the number of which decreased with increasing harvest height of the above-ground plant biomass. These pathogenic microorganisms would come from the wastewater applied to the beds of the CWs during the treatment trial. Indeed, during the treatment of wastewater, the pathogenic microorganisms retained by filtration on the surface and in the substrate [40] would have migrated from the surface of the CWs to reach the above-ground biomass of the plant [11] [12]. This, all the more so since the Thermotolerant coliforms and Sulfite-reducing bacteria contained in the raw water were reduced to 97.41% and 87.50% respectively in the planted bed. However, a very small proportion of the pathogenic microorganisms could come from the feces of higher animals (*e.g.* birds) attracted to plants.

However, the migration of pathogenic germs is reduced with harvest height of the above-ground plant biomass; which would justify the decrease in the number of germ colonies from the surface of the bed to the ends of the plants. These results are in agreement with those obtained on the above-ground biomasses of *Amaranthus hybridus* [11] and *Corchorus olitorius* [12] in constructed wetlands with vertical flow, similar to ours. However, the average concentrations of Thermotolerant coliforms (305 CFU/g) and Sulfite-reducing bacteria (5 CFU/g) obtained remain below the respective values of 1000 CFU/g and 10 CFU/g, indicated in the WHO guidelines [43]. Consequently, the germs obtained in the biomass of *P. purpureum* in the present study would not present any potential risks for a possible use of this biomass as fodder.

4.3. Bacterial Density in CWs

Bacteria (aerobic and anaerobic) in wetland beds were dominated by aerobic bacteria. This is probably due to the type of constructed wetlands with vertical flow used in this study. In this type of constructed wetland, wastewater is applied intermittently, involving resting phases that promote aeration of the substrate [44]. In addition, according to the author previously cited, during the application of raw water in the constructed wetland, the latter infiltrates and carries oxygen through the substrate to a drainage network located at the bottom of the bed. All this would have made the environment more favorable to the proliferation of aerobic bacteria compared to anaerobic bacteria.

The results showed that the planted bed was teeming with more aerobic organisms (5×10^6 CFU/g) than the unplanted one (1.5×10^6 CFU/g). In contrast, less anaerobic bacteria (0.4×10^6 CFU/g) were recorded in the planted bed than in the unplanted one (0.6×10^6 CFU/g). However, the total number of bacteria recorded in the planted bed was significantly higher than that in the unplanted bed. This is probably due to the action of the plant. In fact, in addition to the oxygen supplied to the substrate of the beds of the wetland by plants via the rhizosphere, the latter is likely to secrete exudates which would constitute sources of energy for bacterial proliferation [45]. According to Gagnon *et al.* [46] these

exudates are in particular amylases, phenolases, phosphatases, proteases or various metabolites. In addition, root biomass also constitutes additive anchoring surfaces for microorganisms in the substrate of planted beds, which would improve the density of said organisms [46]. The total bacterial densities obtained (5.4×10^6 CFU/g in planted bed, 1.5×10^6 CFU/g in unplanted bed) remain lower than those of Münch *et al.* [47] (3.2×10^9 CFU/g in planted beds and 2.5×10^8 CFU/g, in unplanted beds), probably due to the difference between the plant species used, the mode of operation and the type of wetland developed.

In planted and unplanted beds, the density of total bacteria gradually decreased from the surface to the depth of the substrate. However, aerobic and anaerobic bacterial populations evolved inversely along the vertical profile in the beds. The number of aerobic bacteria decreased while that of anaerobic bacteria increased with depth. This situation could be related to the reduction of the amount of oxygen in the bed substrate, from the surface to the bottom. Indeed, the upper layers of the beds being more aerated [47] [48], the aerobic bacteria there develop more favorably and swarm with respect to the anaerobic bacteria. On the other hand, in the bottom layer oxygenation is relatively low, which would further promote the growth of anaerobic bacteria. However, the decrease in the total bacterial density from the surface to the depth of the substrate of planted and unplanted beds could be related to the type of vertical flow wetland used in the present study, which due to its operation, promotes more aeration of the beds surface layers [4] [48].

5. Conclusion

Thermotolerant coliforms and Sulfite-reducing bacteria contained in the wastewater were significantly reduced in both the filtrates, with removal efficiencies greater in the planted bed than in the control unplanted. Treatment of domestic wastewater resulted in bacteriological contamination of the above-ground biomass of *Pennisetum purpureum* in the CW, with significant decreases in number of germs, when the harvest height of plant biomass increased. However, average of 305 CFU/g of Thermotolerant coliforms and 5 CFU/g of Sulfite-reducing bacteria obtained in the above-ground plant biomass would not present any potential risks for a possible use of the plant biomass as fodder. Bacteria in beds substrate were dominated by aerobic bacteria. However, the planted bed was teeming with more aerobic bacteria than the unplanted one. In contrast, less anaerobic bacteria were recorded in the planted bed than in the unplanted one. However, the total number of bacteria recorded in the planted bed (5.4×10^6 CFU/g) was significantly higher than that in the unplanted bed (1.5×10^6 CFU/g). From the upper to the bottom layers in the wetlands substrates, the density of total bacteria gradually decreased. However, the number of aerobic bacteria decreased, while that of anaerobic bacteria increased. This study dispels a point of veil on the possibility of valorization of forage plants after use in constructed wetlands. However, additional studies relating to the nutritional poten-

tial of *P. purpureum* deserve to be carried out. This would remove any ambiguity related to the quality of this plant after its use in constructed wetlands. In addition, the study of the balance of pathogenic organisms would make it possible to understand the degradation mechanisms that most govern their elimination in constructed wetlands.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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