

Full Length Research Paper

# Removal of faecal bacteria and nutrients from domestic wastewater in a horizontal surface flow wetland vegetated with *Echinochloa pyramidalis*

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The aim of this study was to evaluate the removal of faecal bacteria and nutrients from domestic wastewater, in surface flow wetlands vegetated with *Echinochloa pyramidalis*. Horizontal surface flow (HSF) wetlands were fed with primarily treated domestic wastewater at organic loading rates varying from 20.74 to 27.15 g BOD/m<sup>2</sup>/day for two consecutive years. Water samples were collected at two weeks interval from the inflow and the outflow of the treatment wetlands for physicochemical and faecal bacteria analyses. Physicochemical parameters with highest removal rates were total suspended solids (TSS, 62-80%), Nitrates (57-73 %) and PO<sub>4</sub><sup>-3</sup> (57-84 %). Faecal indicators reductions of 1.4 and 0.3 log units (1<sup>st</sup> year) and 1.15 and 0.65 log units (2<sup>nd</sup> year) were respectively recorded in the vegetated and the control wetlands. Reductions of total coliforms, faecal coliforms and faecal streptococci were significantly higher in the vegetated wetland than in the non-vegetated control in both seasons of the first year. In the contrary, there was no significant difference in the removal rates of faecal indicators between vegetated wetland and the non-vegetated control during the dry season of the second year. Wetlands vegetated with *E. pyramidalis* was proven to be continuously efficient in the removal of faecal indicators for the two years of study, with no significant differences between the seasons of the year and between the years.

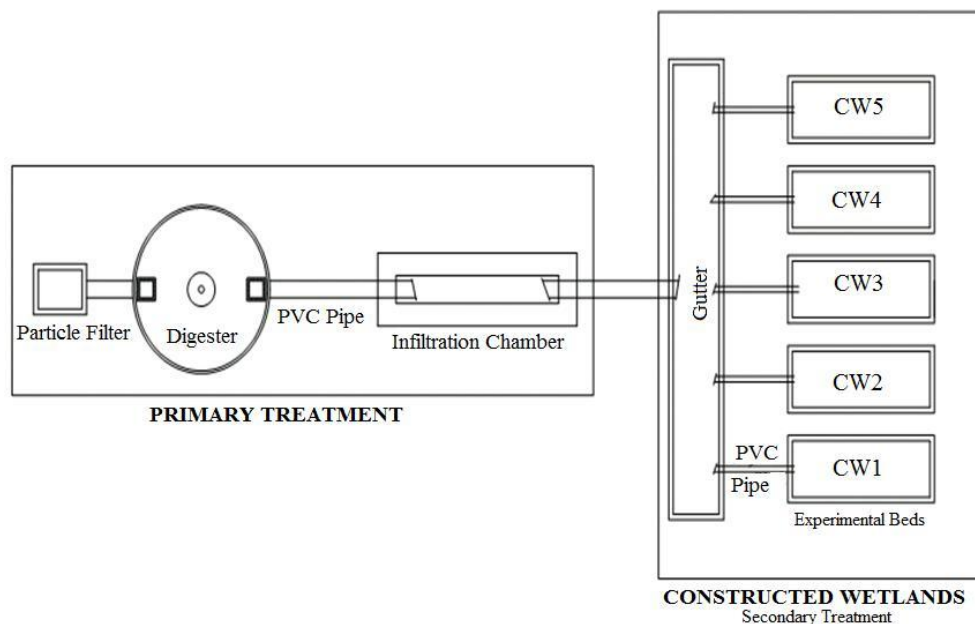
**Key words:** Wastewater, wetlands, *Echinochloa pyramidalis*, faecal bacteria removal, nutrients removal.

## INTRODUCTION

Constructed wetlands (CWs) have been used as promising wastewater treatment alternatives to the more expensive conventional systems, especially in developing countries (Kivaisi, 2001; Song et al., 2006). They produce high quality effluent at low investment cost, simple operation and with very low energy dissipation (Ayaz and Akca, 2001). They therefore help greatly to reduce the health hazards associated with the waste disposal methods in most developing countries (Gilbert et al., 1976; Mburu et al., 2008). Studies on constructed

wetland systems vegetated with macrophytes have shown that the percentage reductions of physicochemical parameters are generally high especially in tropical zones, where climatic conditions are able to sustain vegetation all the year round. Research works in the recent years have been focused on the ability of macrophytes based constructed wetlands to reduce oxygen demands and nutrients such as nitrates and phosphates (Kengne et al., 2009a; Fonkou et al., 2010). Meanwhile, the removal of faecal contaminants indicators from domestic wastewater has not gained much concern. Several studies reporting on microbial water quality improvement have shown that constructed wetlands reduce microorganism populations in wastewater at varying but significant degree of effectiveness (Paluszak

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**Figure 1.** The yard-scale setup of the experiment station.

et al., 2003; Keffala and Ghrabi, 2005; Song et al., 2006; Mburu et al., 2008; Reinoso et al., 2008). Song et al., (2006) reported 99.7% reduction of faecal coliforms in Asia, Paluszak et al. (2003) and Reinoso et al. (2008) respectively reported the removals of 99 and 96% for *Escherichia coli* and faecal streptococci in Europe while Keffala and Ghrabi (2005) and Mburu et al. (2008) also respectively reported removals of 90 and 99.9 for faecal coliforms in Africa.

The removal mechanisms of these bacteria are numerous. These include predation, natural die off, ultra violet radiations on the cell wall of bacteria, oxygen leakages from the roots of macrophytes, bactericidal substances secreted by roots as well as the antibiotic properties of some bacteria living in the rhizosphere (Green et al., 1997; Song et al., 2008). In Cameroon, many studies have been published on *Echinochloa pyramidalis* as concerns its biomass production in constructed wetlands, its ability in faecal sludge dewatering improvement and biosolids recovery, its response to salinity stress, its nutritional potentials and also its presence in natural wetlands especially the polluted ones (Kengne et al., 2009a, b; Fonkou et al., 2010; Pare et al., 2011a, b; Lekeufack et al., 2011). But there has been no concern on its ability to reduce bio-indicators of faecal contamination from wastewater, despite the continuous report of a water related diseases in developing countries.

The aim of this study was to evaluate domestic wastewater quality improvement in a horizontal surface flow (HSF) wetland configuration vegetated with *E. pyramidalis*. The removal efficiencies of faecal coliforms,

faecal streptococci and total coliforms as well as some physicochemical parameters were measured in the wetland vegetated with *E. pyramidalis* and in the non vegetated control wetland for two consecutive years.

## MATERIAL AND METHODS

This study was conducted in a constructed wetland system in the campus of the University of Dschang, located between latitudes 5°25' and 5°30' North and between longitudes 10°00' and 10°5' East in the Western Highlands of Cameroon. The climate in this region is of equatorial type with two seasons: 4 months of dry season from mid-November to mid-March, and 8 months of rainy season from mid-March to mid-November. Annual precipitations range between 1433 and 2137 mm, while annual mean temperature is estimated at 20.8°C with thermal amplitude of 2°C (Anonymous data from the local meteorological station, 1978-2008). Wastewater used in the study was collected from a small primary treatment plant receiving domestic liquid wastes from the students' residence at an inflow rate of 3 m<sup>3</sup> per day. Part of the primarily treated wastewater was channelled into a 1.3 m<sup>3</sup> gutter from where it was distributed to the experimental wetlands using PVC pipes.

### Design of the experimental wetlands

Five wetlands of 3 × 1 × 0.6 m<sup>3</sup> were constructed using cement blocks (Figure 1). The inside of the structures were plastered with concrete, then Cement and Lankofuge™ for water tightness. A 1% slope was constructed on the bottom of each wetland bed to ease the movement of water from the inlet to the outlet. Gabions of 30 cm with stones of 5-8 cm in diameter were arranged at the inlet and outlet zones of the wetlands, while a drainage layer of about 10 cm was arranged at the bottom. The outlet structures were adjustable to enable the regulation of the water level in the substrate.

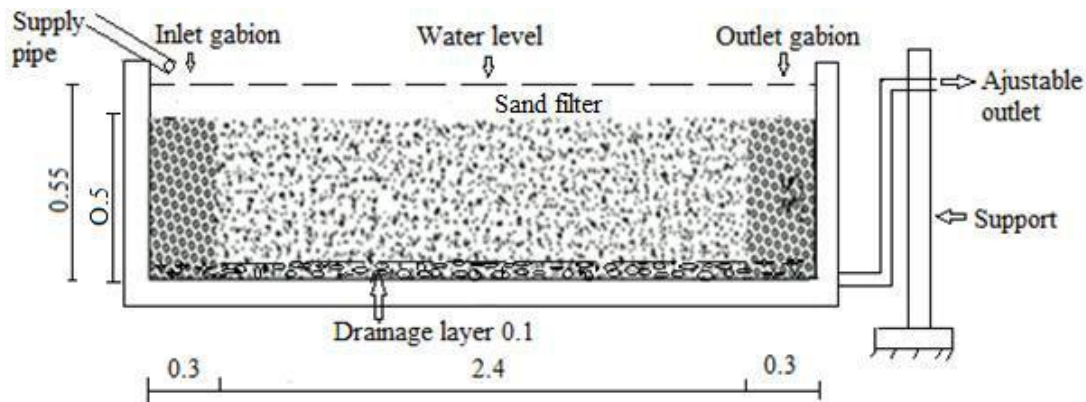


Figure 2. Longitudinal section of the experimental wetland.



Figure 3. Aspect of the vegetation in the wetland at the start of the analysis in the first year.

The main filter substrate was a 40 cm column of sand having particles size  $< 2$  mm (Figure 2). Results presented on this paper are those of CW1, that was vegetated with *E. pyramidalis* and CW3 that was used as the non vegetated control wetlands. The inflow rate was estimated at 205 litres/day. The bed capacity measured from the porosities of the gabion and the sand filter was 923 litres. The hydraulic retention time (HRT) of 4.5 days was then calculated from the equation:

$$= \frac{\text{Bed Capacity}}{\text{Inflow Rate}}$$

as stated by Lorion et al. (2001). The constructed wetlands serve as a secondary treatment associated to the existing primary treatment in which only the particle filter and the digester were still functioning.

### Setting up the experiment

Young shoots of *E. pyramidalis* were collected from a natural wetland and washed in fresh water. After weighing, shoots were

planted in CW1 at a density of 14 shoots/m<sup>2</sup>. Primarily treated effluent from a conventional treatment plant was collected in a gutter and allowed to directly flow into the wetlands at organic loading rates varying from 20.74 to 27.15 g BOD/m<sup>2</sup>/day in dry season and the rainy season of two consecutive years. Macrophyte shoots were domesticated in the wetland for one month during which they grew and had standing vegetation considered to have good biological activity (Figure 3). The wastewater was then allowed to flow constantly into each bed at a loading rate of 85.43 L m<sup>-2</sup>day<sup>-1</sup> in a horizontal surface flow (HSF) configuration for two consecutive years. At the end of every season, the plants were harvested after they have flowered.

### Sample collection and microbiological analyses

Water samples were collected at two weeks interval throughout the research period at the inflow and the outflow of the treatment wetlands, from December 2009 to October 2011. 500 ml of each were kept in sterile laboratory glass bottles and transported in a cooler to the laboratory where they were immediately analyzed.

In the laboratory, manipulations were carried out in strict conditions of sterility. In aseptic conditions, 1 ml of homogenous raw sample was measured and added into 9 ml of sterile distilled water to have 1:10 dilution. This same operation was repeated from the first dilution until the desired dilution was obtained (1:10, 1:100, 1:1000, 1:10 000, etc). The pipette was always rinsed between dilutions to avoid contamination. The distilled water was sterilized by autoclaving in sealed sterile glass bottles for 15 min at 121°C.

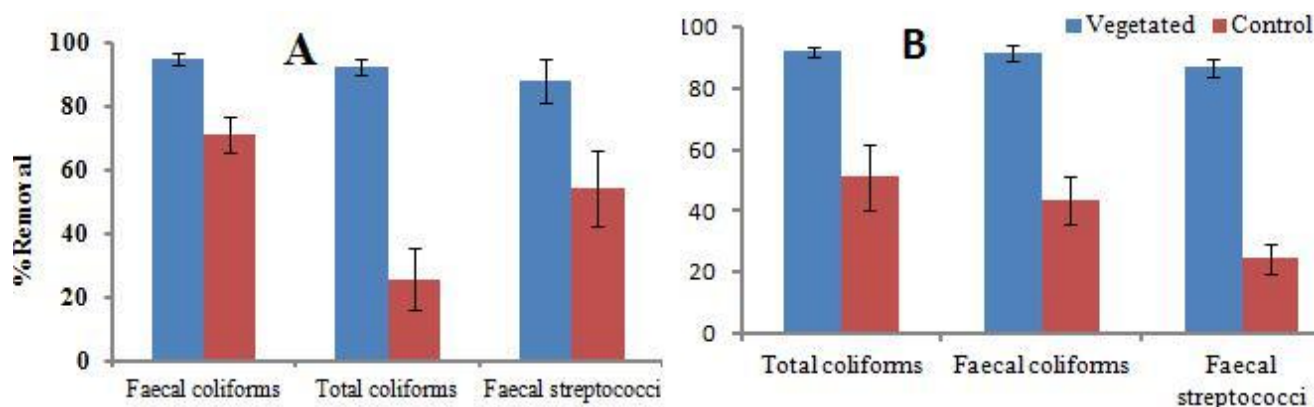
Total coliforms, faecal coliforms and faecal streptococci were detected by the membrane filtration following standard methods (APHA, 1998; CEAE, 2005). AC Cellulose Membranes Filters<sup>TM</sup> with pore-size 0.45 µm were used on a WHEATON filtering Funnel<sup>TM</sup> attached to a CM 1500 vacuum pump<sup>TM</sup>.

Appropriate sample volumes, in three different dilutions ( $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$ ) for effluent or ( $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$ ) for influent were filtered and incubated for each parameter. This was to ensure having at least a plate with colony counts ranging between 20 to 100 CFU (Hench et al., 2003). Samples for faecal coliforms were incubated on Difco<sup>TM</sup> mFC prepared Agar in Petri dishes at 44.5°C for 24 h (Karathanasis et al., 2003). Thereafter, all characteristically blue and central white hollowed blue colonies were counted as faecal coliforms.

Samples for faecal streptococci and total coliforms were respectively incubated on BBL<sup>TM</sup> Bile Esculin and Tergitol<sup>®</sup> 7 Agars at 35°C for 48 h (Karathanasis et al., 2003). Thereafter all characteristically black and yellow colonies were respectively

**Table 1.** Average counts ( $\log_{10}$  units CFU/100 ml) of faecal contaminant indicators (Total coliforms, TC; Faecal coliforms, FC; and Faecal streptococci, FS) in the inflow and outflow of the vegetated and the control wetlands during the dry and rainy seasons of the first year.

Parameter	Dry season			Rainy season		
	Inflow	Outflow Vegetated	Outflow Control	Inflow	Outflow Vegetated	Outflow Control
T.C.	7,36±0,22	6,10±0,43	7,18±0,39	7,48±0,27	6,36±0,28	7,10±0,25
F.C.	7,27±0,34	5,21±1,55	6,77±0,41	7,57±0,35	6,25±0,73	7,34±0,47
F.S.	7,76±0,43	6,32±1,60	7,51±0,45	7,38±0,56	6,37±0,76	7,25±0,62



**Figure 4.** Percentage removals of faecal indicators in the vegetated and in the control wetlands in the dry season (A) and rainy season (B) of the first year.

counted for faecal streptococci and total coliforms. Each result was expressed as number of Colony Forming Units per 100 ml (CFU/100ml) of sample.

#### Measurement of physicochemical parameters

The physicochemical parameters including total suspended solids (TSS), Nitrates, Orthophosphates, Chemical Oxygen Demand (COD), and 5-days Biochemical Oxygen Demand (BOD<sub>5</sub>) were measured according to procedures described by Hach (2004).

#### Statistical analyses

The analysis of variance ANOVA in Prism.4 software at 0.05% probability level was used to test parameter differences between the inlet and the outlets of the wetlands. Means were then separated using Newman-Keuls Multiple Comparison Test under windows (Fonkou et al., 2010). As concerns microbial population density, data were  $\log_{10}$  transformed to obtain the geometric means.

## RESULTS

### Removal of faecal bacteria

Table 1 presents mean microbial densities in the inflow and outflows of the wetland during the first year of

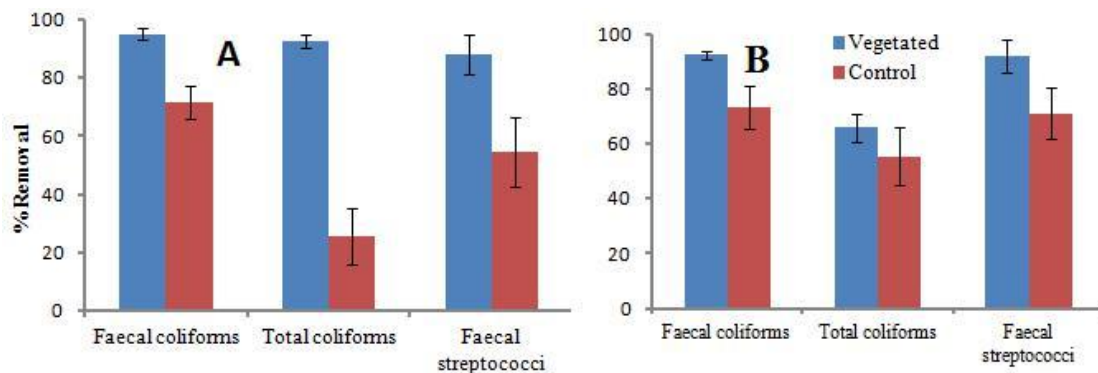
monitoring. The mean concentrations of faecal indicators varied generally from 7.3 to 7.8 log units at the inflow, 6.8 to 7.5 and 5.2 to 6.4 log units respectively at the outflows of the control and vegetated wetlands. Compared with the inlet, average reductions of 1.6 and 0.35 log units were respectively observed at the outlets of the vegetated and non vegetated control wetlands during the dry season and 1.2 and 0.27 log units during the rainy season. These corresponded to percentage removals ranging from 87 to 95% and from 24 to 71% respectively. From statistical analyses, the concentrations of faecal bacteria at the outflow of the vegetated wetland were in general significantly different ( $P < 0.05$ ) from the outflow in both seasons.

The most reduced parameter in the vegetated wetland was faecal coliforms in both seasons, while least reduced was faecal streptococci. In the control wetland, the most reduced parameter in both seasons was total coliforms and faecal streptococci were still the least reduced. Figure 4 presents the mean removal rates of faecal contaminant indicators in the wetlands during the first year. Removal efficiencies ranging from 88 to 95% in the vegetated wetland and from 26 to 71% in the control were recorded in the dry season. During the rainy season, 86 to 92% and 24 to 51% removal rates were recorded in the vegetated and control wetlands



**Table 2.** Average counts ( $\log_{10}$  units CFU/100 ml) of faecal contaminant indicators (Total coliforms, TC; Faecal coliforms, FC; and Faecal streptococci, FS) in the inflow and outflow of the vegetated and the control wetlands during the dry and rainy seasons of the second year.

Parameter	Dry season			Rainy season		
	Inflow	Outflow Vegetated	Outflow Control	Inflow	Outflow Vegetated	Outflow Control
T.C	7,65±0,44	6,59±0,55	7,05±0,26	7,60±0,27	7,08±0,31	7,14±0,38
F.C	7,50±0,65	6,35±0,44	6,94±0,54	7,50±0,65	6,32±0,90	6,81±0,99
F.S	8,00±0,38	6,89±0,77	7,59±0,45	8,19±0,35	6,74±0,55	7,41±0,67



**Figure 5.** Percentage removals of faecal indicators in the vegetated and control wetlands in the dry season (A) and rainy season (B) of the second year.

respectively. The wetlands were all more efficient in the dry season than in the rainy season, but there was no significant difference between the seasons. In this year, the outflow of all the wetlands were significantly different from the inflow for all the parameters, but the outflow of the vegetated wetland was significantly more efficient ( $P < 0.05$ ) than that of the control in the removal of total coliforms in the dry season, and all the three parameters in the rainy season. The outflow of the control wetland was not different from the inflow ( $P > 0.05$ ) in the rainy season for faecal coliforms and faecal streptococci.

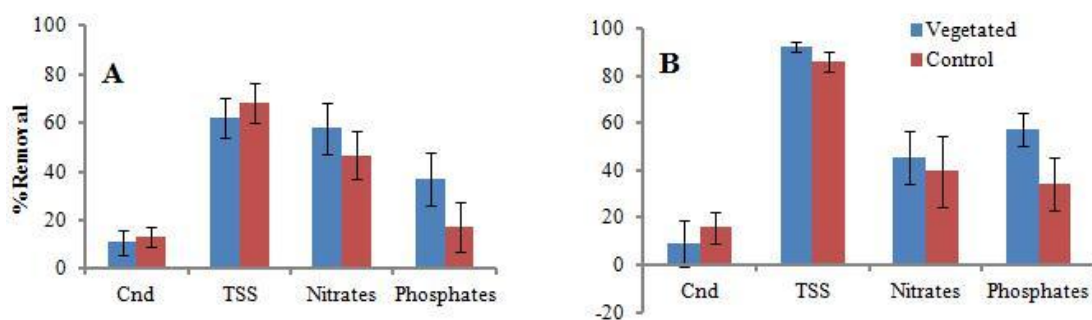
In the second year, the mean concentrations of faecal indicators at the outflows of the wetlands ranged generally from 5.6 to 7.1 and 6.0 to 7.6 log units in the vegetated and non vegetated control wetlands respectively (Table 2). This gives average removal efficiencies of 1.2 and 0.6 log units at outflows of planted and non vegetated control wetlands respectively in the dry season, and 1.09 and 0.69 log units respectively in the rainy season.

The most reduced parameter in the planted bed during the second year was faecal coliforms in the dry season and total coliforms in the rainy season. The least reduced parameter was faecal streptococci in both seasons. In the non vegetated control the most reduced parameter was faecal coliforms in both seasons and the least reduced was faecal streptococci.

Figure 5 presents the average percentage removals of faecal contaminants indicators in the wetlands during the second year. Removal efficiencies ranging from 81 to 91% in the vegetated wetland and from 55 to 63% at in the control were recorded in the dry season. In the rainy season, 66 to 92% and 55 to 71% were recorded in the vegetated and non vegetated control wetlands respectively. Statistical analysis revealed no significant differences between the two wetlands, although the outflows were always significantly different from the inflows. The removals of faecal coliforms and faecal streptococci in the vegetated wetland during the dry season were significantly higher than those recorded in the non vegetated control. The wetlands seemed more efficient in the dry season than in the rainy season but the difference between the two seasons was not significant ( $P > 0.05$ ). The vegetated and the control wetlands also seemed more efficient in the removal of faecal indicators in the first year than in the second year, even though there was statistically no significant difference ( $P > 0.05$ ) between the two years.

### Reduction rates of physicochemical parameters

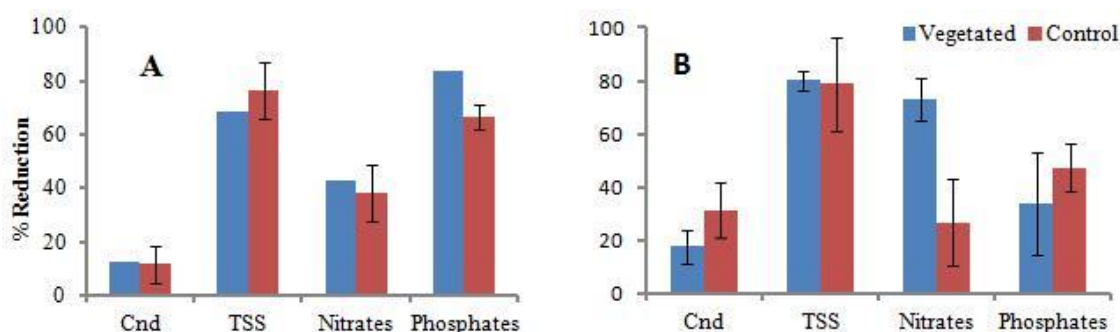
Figure 6 presents the removal efficiencies of the treatment beds during the first year as calculated from



**Figure 6.** Percentage reductions of physicochemical parameters in the vegetated and control wetlands in the dry season (A) and rainy season (B) during the first year (Cnd, conductivity; TSS, Total Suspended Solids).

**Table 3.** Average physicochemical characteristics of water at the inflow and outflow of the wetlands during the dry and the rainy seasons of the first year

Parameter	Dry season			Rainy season		
	Inflow	Outflow Vegetated	Outflow Control	Inflow	Outflow Vegetated	Outflow Control
CND ( $\mu\text{s}/\text{cm}$ )	3815 $\pm$ 288	3440 $\pm$ 239	3344 $\pm$ 295	1880 $\pm$ 322	1694 $\pm$ 309	1467 $\pm$ 221
TSS (mg/l)	399 $\pm$ 72	103 $\pm$ 14	104 $\pm$ 15	1513 $\pm$ 676	56 $\pm$ 11	100 $\pm$ 19
Turbidity (FTU)	437 $\pm$ 71	198 $\pm$ 17	176 $\pm$ 26	464 $\pm$ 89	80 $\pm$ 22	105 $\pm$ 30
Colour (PtCo)	1533 $\pm$ 186	911 $\pm$ 85	803 $\pm$ 93	1049 $\pm$ 184	386 $\pm$ 107	348 $\pm$ 68
NO <sub>3</sub> <sup>-</sup> (mg/l)	55 $\pm$ 24	35 $\pm$ 18	48 $\pm$ 18	5 $\pm$ 1	1.8 $\pm$ 0.39	2.26 $\pm$ 0.64
PO <sub>4</sub> <sup>-3</sup> (mg/l)	186 $\pm$ 43	133 $\pm$ 49	150 $\pm$ 31	69 $\pm$ 22	31 $\pm$ 15	34 $\pm$ 11
COD (mg/l)	702 $\pm$ 63	360 $\pm$ 42	376 $\pm$ 44	788 $\pm$ 97	288 $\pm$ 37	496 $\pm$ 59
BOD (Mg/l)	219 $\pm$ 38	112 $\pm$ 16	130 $\pm$ 20	317 $\pm$ 28	120 $\pm$ 9	206 $\pm$ 23



**Figure 7.** Percentage removals of physicochemical parameters in the vegetated and control wetlands in the dry season (A) and rainy season (B) of the second year (Cnd, conductivity; TSS, Total Suspended Solids).

the data presented in Table 3. The TSS was the parameter with the highest reduction rate in the vegetated wetland during the dry season (62%) and during the rainy season (92%). Percentage reduction of 58% was recorded for nitrates during the dry season and 57% for phosphates in the rainy season. Throughout this first year, conductivity and TSS had higher reduction rates in the non vegetated control than in the vegetated wetland, but there was no significant difference between the two

wetlands ( $P > 0.05$ ). The vegetated wetland had higher removal efficiencies for nitrates and phosphates than the non vegetated control in both seasons.

During the second year, nutrients with the highest removal rate (84%) in the vegetated wetland were phosphates, while nitrates were reduced at 73% in the rainy season. TSS in this bed was respectively reduced at 68 and 80% in the dry season and rainy season (Figure 7). The highest reduction rates in the control wetland were

**Table 4.** Average physicochemical characteristics of water at the inflow and outflow of the wetlands during the dry and the rainy seasons of the second year.

Parameter	Dry season			Rainy season		
	Inflow	Outflow Vegetated	Outflow Control	Inflow	Outflow Vegetated	Outflow Control
CND ( $\mu\text{s}/\text{cm}$ )	3705 $\pm$ 383	3311 $\pm$ 427	3183 $\pm$ 383	2293 $\pm$ 354	1936 $\pm$ 316	1414 $\pm$ 259
TSS (mg/l)	584 $\pm$ 90	151 $\pm$ 21	117 $\pm$ 38	616 $\pm$ 120	83 $\pm$ 6.2	86 $\pm$ 15
Turbidity (FTU)	265 $\pm$ 29	100 $\pm$ 14	60 $\pm$ 12	311 $\pm$ 71	58 $\pm$ 4.02	65 $\pm$ 21
Colour (PtCo)	696 $\pm$ 62	372 $\pm$ 73	232 $\pm$ 47	424 $\pm$ 58	213 $\pm$ 22	149 $\pm$ 21
NO <sub>3</sub> <sup>-</sup> (mg/l)	8 $\pm$ 1.95	4 $\pm$ 0.6	4,19 $\pm$ 0.64	5 $\pm$ 1	1,33 $\pm$ 0.39	2,52 $\pm$ 0.42
PO <sub>4</sub> <sup>-3</sup> (mg/l)	112 $\pm$ 18	17 $\pm$ 3	33 $\pm$ 4	94 $\pm$ 32	30 $\pm$ 5	29 $\pm$ 6
COD (mg/l)	545 $\pm$ 10	278 $\pm$ 15	429 $\pm$ 30	582 $\pm$ 21	213 $\pm$ 27	426 $\pm$ 43
BOD (Mg/l)	229 $\pm$ 4	117 $\pm$ 6	164 $\pm$ 22	244 $\pm$ 9	90 $\pm$ 11	173 $\pm$ 20

recorded for TSS (76%) and (78%) in the dry and rainy seasons respectively. Conductivity had the least reduction (11%) in the dry season while nitrates had the least reduction (26%) in the rainy season. The vegetated wetland was significantly more efficient ( $P < 0.05$ ) than the non-vegetated control in the removal of nitrates in this rainy season. BOD and COD removals rates during the dry season of the first year (44 and 42% respectively) were lower but not significantly different from those recorded in the rainy season (55 and 57%). This trend was not respected in the second year, since the reduction rate of 49% recorded for COD and BOD during the dry season was low and significantly different from the 64% (BOD) and 63% (COD) obtained in the rainy season. It is noticed that the reduction of oxygen demands was always  $< 50\%$  in the dry seasons and always  $> 50\%$  in the rainy seasons. As indicated in Tables 3 and 4, the quality of the water in the outflow of the wetlands was still very poor, even though high percentage reductions of several parameters were observed.

## DISCUSSION

The reductions of 1.4 and 0.3 log units observed in the vegetated and control wetlands respectively in the first year are similar to those observed by Karathanasis et al. (2003) and Torrens et al. (2009) in polycultured systems. These results are also found within the range of removals reported for constructed wetlands treating domestic wastewater in similar conditions (Greenway, 2005; Morsy et al., 2007; Reinoso et al., 2008).

The wetland vegetated with *E. pyramidalis* was significantly more efficient ( $P < 0.05$ ) than the non vegetated control in the reduction of parameters such as total coliforms in the dry season and all the three parameters in the rainy season. These differences may explain the role played by plants roots in the substratum-root matrix, and the difference in substrate's microbial composition due to presence of plants as the wastewater passes through. Kaseva (2004) emitted a similar reasoning when

he found that his vegetated systems were more efficient than the non vegetated control. The results of the present research are different from those reported by Mburu et al. (2008) where the non vegetated control bed was in the average more efficient than the vegetated wetland in a subsurface horizontal flow system.

The high but non significant removals of faecal indicators in the non vegetated control in the dry season of the first year could also be attributed to the various physical, chemical and biological mechanisms for bacterial removal in constructed wetlands (Williams et al., 1995; Green et al., 1997; Stevik et al., 2004). Photolysis (due to ultra-violet radiations) is surely the predominant mechanism because in the rainy season the vegetated wetland was more efficient in the removal of bacteria than the non vegetated control.

The second year presented a similar variability of bacteria concentrations as in the first year. Although no significant differences existed between the two years, the first year appeared to be more efficient in faecal bacteria removal than the second. This can be attributed to the high plant density observed in the vegetated wetland in the second year, which may block the U.V radiations from having effect on the bacteria. This observation is contrary to the observations found in literature where the age of the wetlands is suppose to have greater efficiency in the second year as the plants are well established in their roots network with a stable symbiotic relationship with the substrate microorganisms, thus promoting the different removal mechanisms in the wetlands (Tchobanoglous, 1987; Lee et al., 2009). Other mechanisms involved include filtration, sedimentation, adsorption, oxidation, antibiosis, predation and die-off from exposure to toxins of plants and other bacteria (Green et al., 1997; Kaseva, 2004). The differences between the non vegetated control and the vegetated wetlands may be attributed to the influence of plants. The multiplication of substrate microorganisms and their coordination in the removal processes in the second year is not to be also neglected. The better performances of the vegetated wetland could also be attributed to tropical climate conditions that are

known to be suitable all year-round for rapid biological growth, which influence the treatment processes (Kaseva, 2004). The wetland vegetated with *E. pyramidalis* had more effect on the removal of faecal coliforms and was least efficient on faecal streptococci removal. This is thought that, the bactericidal substances which may be produced by this species roots, mixed with the toxic effects of substrate microorganisms have less effect on faecal streptococci, but more on faecal and total coliforms.

The passage of wastewater through the non vegetated control wetland resulted in the reduction of several contaminants to considerable levels in different seasons; however, the presence of vegetation further improved the treatment efficiencies except in few cases. The significant removal of most physicochemical parameters was regardless of the presence of vegetation in the treatment wetland as earlier observed by Hench et al. (2003). This may be attributed to the shallow depth of the treatment wetlands which may have minimal effect on the treatment processes (Coleman et al., 2001). The reduction of BOD<sub>5</sub>, COD and PO<sub>4</sub><sup>-3</sup> was regardless of the presence of vegetation; however there were significant differences (P<0.05) between their removals in the vegetated wetland and the control in the second year and not in the first. Their concentrations in both the vegetated and the non vegetated control were significantly different from the inflow in both years. The efficiencies of the wetlands in the removal of physicochemical parameters compared to the microbial parameters were higher in the second year than those of the first year. It can be understood that the removal mechanisms for bacteria and those of physicochemical parameters are not the same. In the second year, the massive treatment is richer in substrate bacteria for biodegradation and the root network of the macrophyte is well established in the wetland. Secondly, since in the first year, plant roots had not yet reached maximum depth, the treatment processes will likely become higher during the second year of study as the roots would have reached sufficient depth (Kern and Idler, 1999).

In all, the vegetated wetland was generally more efficient in the reduction of parameters. This, in addition to uptake, may also be thanks to their additional aeration at the root zone to facilitate the processes of degradation and detoxification by aerobic microorganisms (Lin et al., 2002). In this study the macrophyte bed repeatedly reduced nitrates more than the non vegetated control. This may be due to the functions of plants in constructed wetlands which include uptake of nitrates, supplying organic carbon for denitrification and providing attachment sites for denitrifying bacteria (Kadlec and Knight, 1995; Lin et al., 2002; Thullen et al., 2002; Lee et al., 2009). COD reductions that were consistently higher in the vegetated wetland could be attributed to the fact that macrophytes and microorganisms depend on nutrients in wastewater for survival. In contrast, their metabolites

provide important N and P sources for micro-organisms which are very important mechanisms (Ji et al., 2002).

## Conclusion

The constructed wetland vegetated with *E. pyramidalis* in a horizontal surface flow configuration (HSF) was continuously efficient in the removal of faecal indicator bacteria from domestic wastewater for the two years, with no significant differences existing between the seasons of the year and between the years. The age of the wetland generally had a negative influence in the removal of faecal indicators; but this difference was not significant. The constructed wetland vegetated with *E. pyramidalis* was most efficient in the removal of faecal coliforms followed by total coliforms, but was least efficient in the removal of faecal streptococci as compared to the non vegetated control. The contribution of *E. pyramidalis* in the removal of faecal indicators and physicochemical parameters was quite high, suggesting this macrophyte to be recommended in wetland technology for domestic wastewater treatment.

## ACKNOWLEDGEMENT

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## REFERENCES

- American Public Health Association APHA, American Water Works Association, Environmental Protection Agency (1998). Standard Methods for the Examination of Water and Wastewater: 20th edition. Washington, DC, USA.
- Ayaz SC, Akca L (2001). Treatment of wastewater by natural systems: Environ. Int. 26:189-195.
- Centre d'Expertise en Analyse Environnementale du Québec (2005). Recherche et dénombrement des coliformes totaux, entérocoques, coliformes fécaux (thermotolérants) et confirmations à l'espèce *Escherichia coli* : méthode par filtration sur membrane, Edition 2003-12-05 et Révision 2005-12-15 (2).
- Coleman J, Hench K, Garbutt K, Sexstone A, Bissonnette G, Skousen J (2001). Treatment of domestic wastewater by three plant species in constructed wetlands. Water Air Soil Pollut. 128:283-295.
- Fonkou T, Fonteh MF, Kanouo MD, Amougou A (2010). Performances des filtres plantés de *Echinochloa pyramidalis* dans l'épuration des eaux usées de distillerie en Afrique subsaharienne. Tropicultura 28(2):69-76.
- Gilbert RG, Gerba CP, Rice RC, Bouwer H, Wallis C, Melnick JL (1976). Virus and Bacteria Removal from Wastewater by Land Treatment. Appl. Environ. Microbiol. 32(3):333-338.
- Green MB, Griffin P, Seabridge JK, Dohobie D (1997). Removal of bacteria in subsurface flow wetlands. Water Sci. Technol. 35(5):109-116.
- Greenway M (2005). The role of constructed wetlands in secondary effluent treatment and water reuse in subtropical and arid Australia. Ecol. Eng. 25:501-509.
- Hach (2004). The handbook. DR/2500 laboratory spectrophotometer. Hach Company®, Be Right™ Loveland, Colorado, USA p.1309.
- Hench KR, Bissonnette GK, Sexstone AJ, Coleman JG, Garbutt K,



- Skousen JG (2003). Fate of physical, chemical, and microbial contaminants in domestic wastewater following treatment by small constructed wetlands. *Water Res.* 37:921-927.
- Ji G, Sun T, Zhou Q, Sui X, Chang S, Li P (2002). Constructed subsurface flow wetland for treating heavy oil-produced water of the Liaohe Oilfield in China. *Ecol. Eng.* 18:459-465.
- Kadlec RH, Knight RL (1995). *Treatment Wetlands*. Lewis Publishers, New York.
- Karathanasis AD, Potter CL, Coyne MS (2003). Vegetation effects on faecal bacteria, BOD, and suspended solid removal in constructed wetlands treating domestic wastewater. *Ecol. Eng.* 20:157-169.
- Kaseva ME (2004). Performance of a sub-surface flow constructed wetland in polishing pre-treated wastewater: a tropical case study. *Water Res.* 38:681-687.
- Keffala C, Ghrabi A (2005). Nitrogen and Bacterial removal in constructed wetlands treating domestic wastewater. *Desalination* 185:383-389.
- Kengne IM, Amougou A, Kone D (2009b). Recovery of biosolids from constructed wetlands used for faecal sludge dewatering in tropical regions. *Environ. Sci. Technol.* 43:6816-6821.
- Kengne IM, Dodane PH, Amougou A, Kone D (2009a). Vertical-flow constructed wetlands as sustainable sanitation approach for faecal sludge dewatering in developing countries. *Desalination* 248:291-297.
- Kern J, Idler C (1999). Treatment of domestic and agricultural wastewater by reed bed systems. *Ecol. Eng.* 12:13-25.
- Kivaisi AK (2001). The potential for constructed wetlands for wastewater treatment and reuse in developing countries: a review. *Ecol. Eng.* 16:545-560.
- Lee CG, Tim D, Sun FG (2009). Nitrogen removal in constructed wetland systems. A review: *Eng. Life Sci.* 9(1):11-22.
- Lekeufack M, Fonkou T, Sako IB, Pamo E, Amougou A (2011). Studies on biomass yield from *Echinochloa pyramidalis*, *E. crus-pavonis* and *Leersia hexandra* in yard-scale surface flow wetlands in Cameroon. *Univ. J. Environ. Res. Technol.* 1(4):476-485.
- Lin YF, Jing SR, Wang TW and Lee DY (2002). Effects of macrophytes and external carbon sources on nitrate removal from groundwater in constructed wetlands. *Environ. Polut.* 119:413-420.
- Lorion R (2001). Constructed wetlands: passive systems for wastewater treatment. Technology status report for prepared for the USEPA. Technology innovation office p.24.
- Mburu N, Thumbi GM, Mayabi AO (2008). Removal of Bacteria Pathogens from Domestic Wastewater in a Tropical Subsurface Horizontal Flow Constructed Wetland. In: Senupta M, Dalwani R (eds), *Proceedings of Taal2007: The 12<sup>th</sup> World Lake Conference* pp.1010-1015.
- Morsy EA, Al-Herrawy AZ, Ali MA (2007) Assessment of *Cryptosporidium* removal from domestic wastewater via constructed wetland systems. *Water Air Soil Pollut.* 179:207-15.
- Paluszak Z, Ligocka A, Breza-Boruta B (2003). Effectiveness of Sewage Treatment Based on Selected Bacteria Elimination in Municipal Wastewater Treatment Plant in Toruń. *Pol. J. Environ. Stud.* 12(3):345-349.
- Pare MMN, Koné D, Kengne IM, Akoa A (2011a). Growth and yield potential of *Echinochloa pyramidalis* (Lam.) Hitchc & Chase: A forage plant used in vertical flow constructed wetlands in Cameroon. *Afr. J. Environ. Sci. Tech.* 5(8):622-632.
- Pare MMN, Koné C, Kengne IM, Dongo K, Akoa A (2011b). Nutritional potential of *Echinochloa pyramidalis* (Lam.) Hitchc. & chase, a forage plant used in constructed wetlands treatment of faecal sludge and wastewater. *Afr. J. Agric. Res.* 6(18):4397-4408.
- Perkins J, Hunter C (2000). Removal of enteric bacteria in a constructed wetland in Yorkshire-England. *Water Res.* 34(6):1941-1947.
- Reinoso R, Torres LA, Bécares E (2008) Efficiency of natural systems for removal of bacteria and pathogenic parasites from wastewater: *Sci. Total Environ.* 395:80-86.
- Song ZW, Zheng ZP, Li J (2006). Seasonal and annual performance of a full-scale constructed wetlands system for sewage treatment in China. *Ecol Eng* 26:272-282.
- Song ZW, Wu L, Yang G, Xu M, Wen SP (2008). Indicator microorganisms and pathogens removal function performed by copepods in constructed wetlands: *Bull Environ Contam. Toxicol.* 81:459-463.
- Stevik TK, Kari A, Ausland G, Hanssen JF (2004). Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. *Water Res.* 38:1355-1367.
- Tchobanoglous G (1987). Aquatic plant systems for wastewater treatment: engineering considerations. In: Reddy KR, Smith WH (eds) *Aquatic plants for water treatment and resource recovery*. Magnolian Publishing Inc., orlando pp.27-48.
- Thullen JS, Sartoris JJ, Walton W (2002) Effects of vegetation in constructed wetland treating cells on water quality and mosquito production. *Ecol. Eng.* 18:441-454.
- Torrens A, Molle P, Boutin C, Salgot M (2009). Removal of bacterial and viral indicators in vertical flow constructed wetlands and intermittent sand filters. *Desalination* 247:170-179.
- Williams J, Bahgat M, May E, Ford M, Butler J (1995). Mineralization and pathogenic removal in gravel bed Hydroponics constructed wetlands for wastewater treatment. *Water Sci. Technol.* 32(3):49-58.