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Studies on Biomass Yield from *Echinochloa pyramidalis, E. crus-pavonis* and *Leersia hexandra* in Yard-Scale Surface Flow Wetlands in Cameroon

[1]Lekeufack Martin, [1]*Fonkou Théophile, [1]Ivo Balock Sako, [2]Pamo Etienne and [3]Amougou Akoa

[1]Laboratory of Applied Botany, University of Dschang, Cameroon
[2]Department of Animal Production, Faculty of Agronomy, University of Dschang
[3]Department of Plant Biology, Faculty of Science, University of Yaoundé I

***Corresponding author, email: tfonkou@yahoo.fr**

Abstract:

In this work, yard-scale horizontal surface flow constructed wetlands were fed with primarily treated domestic wastewater to assess the growth of and biomass production from *Echinochloa pyramidalis, E. crus-pavonis* and *Leersia hexandra*. The loading rate of about 85 liters per m^2 per day was applied to each wetland for two consecutive years. Growth parameters including plant density, height of plants, diameter of stems and leaf dimensions were measured after every two weeks. The aerial parts of the plants were harvested and weighed after each season. Total biomasses ranging from 113 to 154 tons/hectare/year were estimated for *E. pyramidalis*, while for *E. crus-pavonis* and *Leersia hexandra* they were estimated to range from 74 to 79 tons/hectare/year and from 61 to 64 tons/hectare/year respectively. The biomass yield of *E. pyramidalis* was significantly higher than those of *E. crus-pavonis* and *Leersia hexandra*. In addition more biomasses were produced in the dry seasons than in the rainy seasons. The growth and biomass yields were significantly influenced by the seasons and temperature.

Keywords: constructed wetlands, wastewater, macrophytes, biomass yield.

1.0 Introduction:

Macrophytes are one of the main components in constructed wetlands (CWs) used for wastewater treatment. They provide large surfaces for microbial growth and attachment, uptake nutrients and add oxygen into the rhizosphere for biodegradation (Kadlec, 2000; Ayaz and Akça 2001; Li *et al.,* 2008). Macrophyte based CWs are used all over the world to treat wastewater as alternatives to the more widespread conventional treatment technologies with high energy inputs (Kadlec and Knight, 1996). In addition to the water quality improvement, the biomass produced by the plants in CWs is one of the added values since it can be valorized as food, medicine, biofuel, paper, organic fertilizer in compost, and reserved fodder for animals in the dry season (Polprasert, 2007; Perbangkhem and Polprasert, 2010). Although high cellulose fibers content might be a prerequisite for use of plant biomass as forage, they are considered as refractory material that might complicate the processes of converting the biomass into certain fuels (North *et al*., 1981). Understanding relationship between biological traits of aquatic macrophytes and ecological variable such as water quality can help determine plant carrying capabilities of different aquatic and wetland habitats (Mitsch *et al*., 2005; Daniel *et al*., 2006; Haury *et al*., 2006; Welch *et al*., 2006).

Macrophytes used in CWs are not ubiquitous, and therefore are not always transferable from one ecological region to another. In Africa macrophytes such as duckweeds (*Lemna equinoxialis*), water hyacinth (*Eichhornia crassipes*), and water lettuce (*Pistia stratiotes*) have been tested in macrophytic lagoons for wastewater treatment (Agendia, 1995; Ennabili *et al*., 1998). In Cameroon, recent studies have been carried to identify macrophytes in natural wetlands that could be potential candidates to be used in CWs for wastewater and fecal sludge treatment (Fonkou *et al.,* 2005). Macrophytes used in CWs should not only grow and adapt easily in anoxic/hypoxic conditions in their rhizosphere, but also to highly polluted wastewater. The plants should equally take up large amounts of nutrients and respond to nutrients enrichment with enhanced growth (Twilley *et al.,* 1985; Verhoeven *et al.,* 1999, Bakker *et al*., 2010).

Echinocloa pyramidalis, E. crus-pavonis and *Leersia hexandra* were found among the macrophyte species which grow rapidly and healthily in wetlands receiving wastes. Some of these species found in natural polluted wetlands showed high accumulation rates of pollutants especially heavy metals (Fonkou *et al.,* 2005; Dhir, 2010). In the western highlands of Cameroon, the climate is characterized by two seasons: an eight months rainy season and a four months dry season (mid-March to mid-November). The biomass produced by the plants could therefore be exploited in the dry season as animal fodder. These plants have been found to grow well in polluted wetlands in both seasons in the region (Fonkou *et al.,* 2011). The purpose of this study was to assess the growth and biomass yields of these species in CWs treating domestic wastewater.

2.0 Materials and Methods:

2.1 Site of Study:

This study was conducted in a yard scale constructed wetland system in the University of Dschang campus. Dschang is located between latitudes 5°25' and 5°30' North and between longitudes 10°00' and 10°5' East in the Western Highlands region of Cameroon (figure a). The climate in this region is of equatorial type with two seasons: a 4 months dry season from mid-November to mid-March, and an 8 months rainy season from mid-March to mid-November. Annual precipitations range between 1433 mm 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^3 per day. Part of the primarily treated wastewater was channeled into a 1.3 m^3 gutter from where it was distributed to the experimental wetlands using PVC pipes.

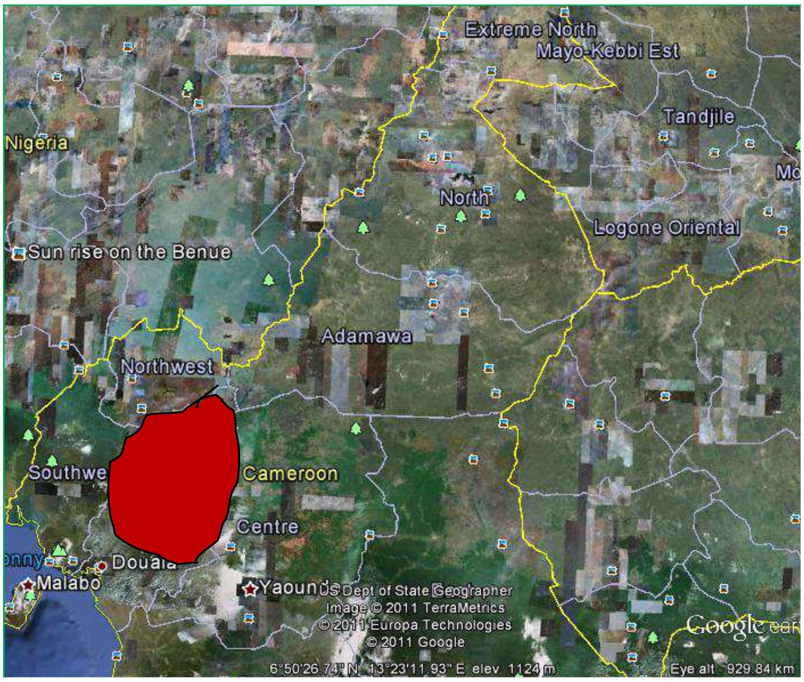


Figure a: Location of the study area (adapted from Google Earth, 16 December 2011)

2.2 Design of the experimental wetlands:

Five constructed wetlands (CW_1, CW_2, CW_3, CW_4 and CW_5) of 3 × 1 × 0.6 m^3 were constructed using cement blocks (figure b). The inside of the structures was plastered with concrete, then Cement and Lankofuge™ for water tightness. A slope of 1 % was respected on the bottom of each bed wetland to ease the circulation of water from the inlet to the outlet. Gabions of 30 cm made up of stones of 5-8 cm diameter were arranged at the inlet and outlet zones of the beds, 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. The main filter substrate was a 45 cm column of sand having particles size < 2 mm (figure c). CW3 was used as the non vegetated control bed, while the other beds were vegetated.

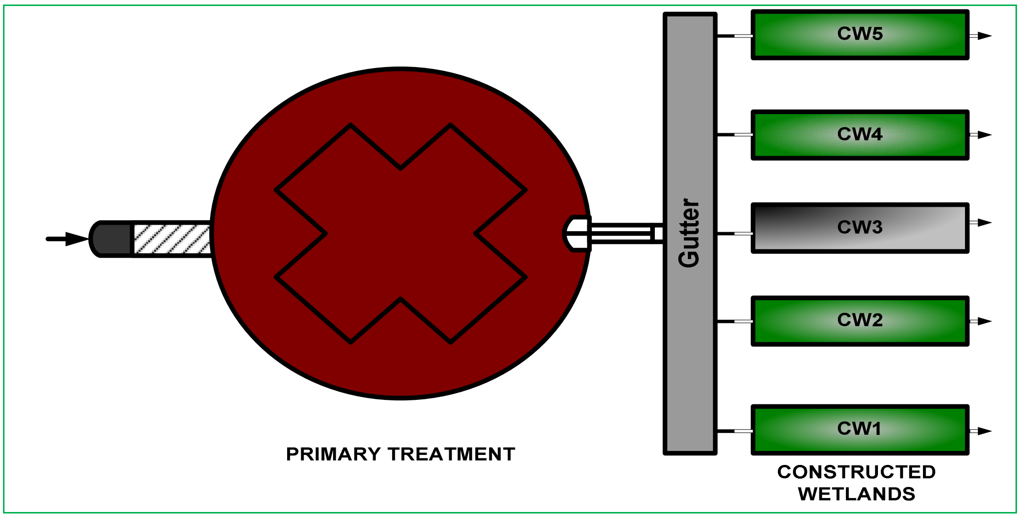


Figure b: Yard Scale Setup of the Experiment

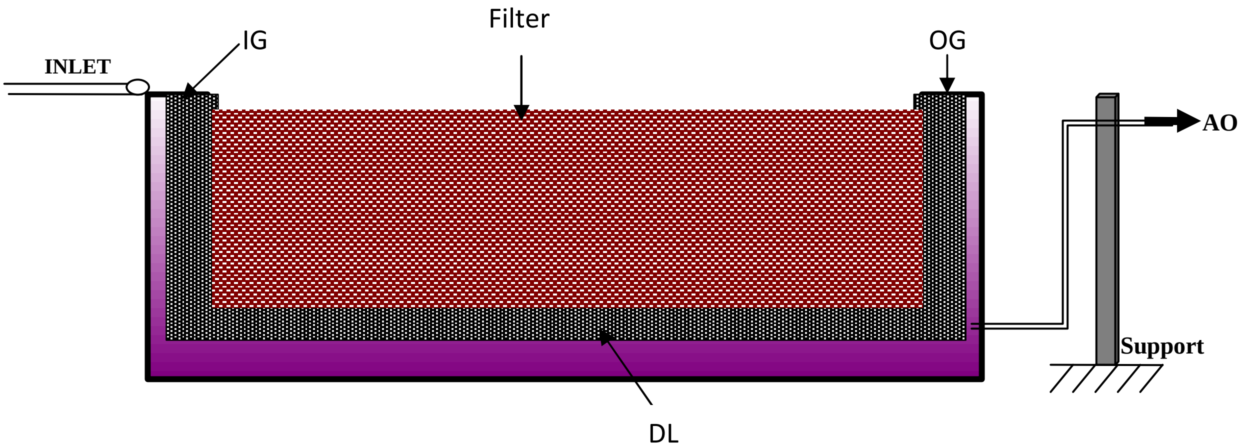


Figure c: Longitudinal Section of the Experimental Bed Configuration. (AO: Adjustable outlet, IG: inlet gabion, OG: outlet gabion, DL: drainage layer)

2.3 Setting Up of the Experiment:

Young shoots of *E. pyramidalis, E. crus-pavonis* and *Leersia hexandra* were collected from natural wetlands and washed in fresh water. After weighing the shoots, *Echinochloa pyramidalis* was planted in CW1, *E. crus-pavonis* in CW2, and *Leersia hexandra* in CW4 at densities of 14 plants/m^2. The primarily treated effluent from the conventional treatment plant was collected in a gutter and allowed to directly flow into the wetlands at a loading rate of about 35 L/m^2/day. Macrophytes were domesticated in the wetlands for one month during which they grew and had standing vegetations considered to have good biological activity (figure d). The effluent was then allowed to flow constantly into each bed at a loading rate of 85.43 Litres.m^{-2}.day^{-1} 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. Some physicochemical characteristics of the primarily treated effluent used in this work are indicated in table 2.

Figure d: Standing biomasses of the plants in the experimental site at the start of the analysis.

Table 1: Some Characteristics of the Wastewater Used in the Study (Mean±SEM, n=25)

Parameters	pH	CND (µs/cm)	TSS (mg/l)	NO_3^- (mg/l)	PO_4^{-3} (mg/l)	BOD_5 mg/l
Rainy season	22.2±0.07	3216±434	517±114	10.2±2.7	58±21	13.8±31.47
Dry season	25.4±0.08	4422±301	486±109	102±52	189±13	75±32

2.4 Measuring of Growth and Productivity Parameters:

One month after planting the young shoots, growth parameters were measured at two weeks intervals. These parameters include height of plants, stem diameter, length and width of leaves for *E. pyramidalis* and *E. crus-pavonis.* The parameters were measured using a graduated meter rule while the number of plants and leaves were obtained by direct counting. For *L. hexandra,* the height of plants, the number of leaves and the plant density in the bed were considered. The plants in each wetland bed were harvested at the end of every season after flowering and weighed to determine fresh biomass using a scale balance. The dry biomass was then obtained by drying the plant tissue samples separately in a thermo-ventilated oven at 80°C for 24 hours, and then weighing on an electronic balance.

3.0 Results and Discussions:

The total above-ground dry biomasses produced by the three species are presented table 2 below. In the dry season of the first year of study *E. pyramidalis* and *E. crus-pavonis* showed 100% survival and coverage of the bed while *Leersia hexandra* showed only about 70% survival and coverage.

3.1 Growth and Productivity of E. pyramidalis:

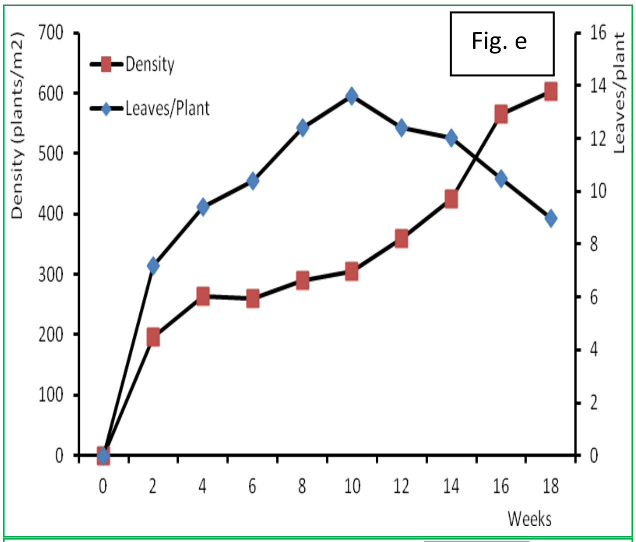
From the initial density of 14 plants/m^2 in October, *E. pyramidalis* grew rapidly and healthily, to a new density of 342 plants/m^2 at the end of the dry season in March. The plants had an average stem diameter of 0.93 cm; and about 70 % of the plants were bearing flowers. The mean number of leaves per plant varied from 8 to 20 in two months. At the end of the season, this number was reduced to 6 leaves and the total above-ground biomass was estimated at 64 tons DM/ha, which is higher than the 56.5, 52.7 and 20.1 tons DM/ha obtained from *Typha angustifolia, Phragmites australis* and *Scirpus maritimus* as reported by Ennabili *et al*., (1998). According to Delgado *et al*., (1992), water hyacinth could yield only 39.5 tons DM/ha in greenhouse experiment. The height of the plants changed from 85 cm at the start of data collection to 224 cm, without any drop as observed for the number of leaves (figure e).

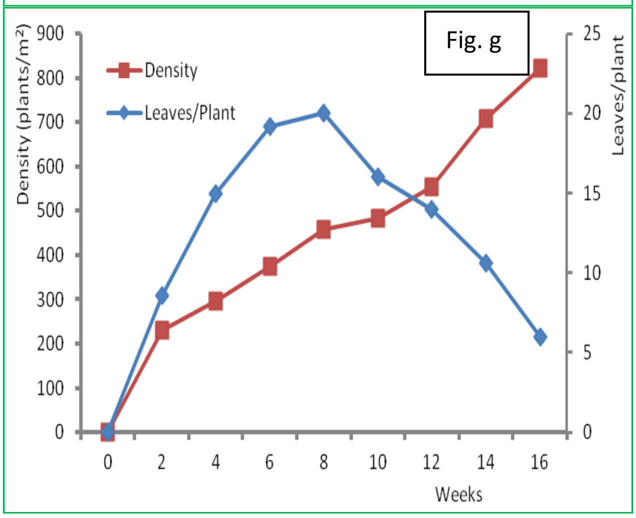
After harvesting the plants at the end of the dry season, young shoots arose at the beginning of the rainy season, grew and rapidly multiplied to form new vegetation. Six months after the dry season harvest, a total density of 250 plants/m^2 was observed. The average stem diameter of 1.04 cm was estimated and about 50% of the plants were bearing flowers. The above-ground parts of these plants produced a total biomass of 48 tons DM/ha. The number of leaves during this phase varied equally as in the dry season, increasing to 14 leaves at 2 ½ months and dropped to 9 leaves at the end of the rainy season (figure g). The plant population in this phase showed a sigmoid variation of normal population growth that would have been completed if the plants were allowed to grow and be replaced by natural processes (figure g). The plants in this season grew taller than in the dry season, but with a similar variation in height increase trend (figure h).

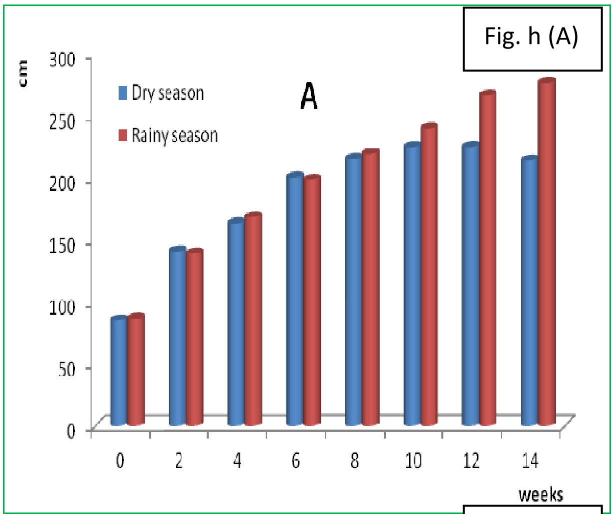
In the second year after harvesting the above ground parts of *E. pyramidalis*, the young shoots which arose grew with much vigor for the dry season. The only difference with the first year is that, these plants flowered earlier than expected. But they were allowed in the beds till the end of the season before being harvested. During this period, a total above-ground biomass of 88 tons DM/ha was obtained. The biomass produced during this dry season of the second year was more than that produced in the dry season of the first year with a difference of 24 tons DM/ha corresponding to 27.19 % (table 3). In the rainy season of the second year, the young shoots grew normally and rapidly till the end of the season where about 50% of the total species population flowered. The above-ground biomass was estimated to be 66 tons DM/ha. This biomass was also observed to be more than that produced in the rainy season of the first year with almost the same difference as in the dry season (26.65%).

Table 2: **Biomass Dry Weight (tons/ha) Produced by the Three Species in different Seasons**

	Echinochloa Pyramidalis		*Echinochloa Crus-Pavonis*		*Leersia Hexandra*	
Seasons	**Dry Season**	**Rainy Season**	**Dry Season**	**Rainy Season**	**Dry Season**	**Rainy Season**
1st Year	64.35	48.47	44.60	35.14	31.73	29.31
2nd Year	88.38	66.08	44.03	29.71	39.71	24.73







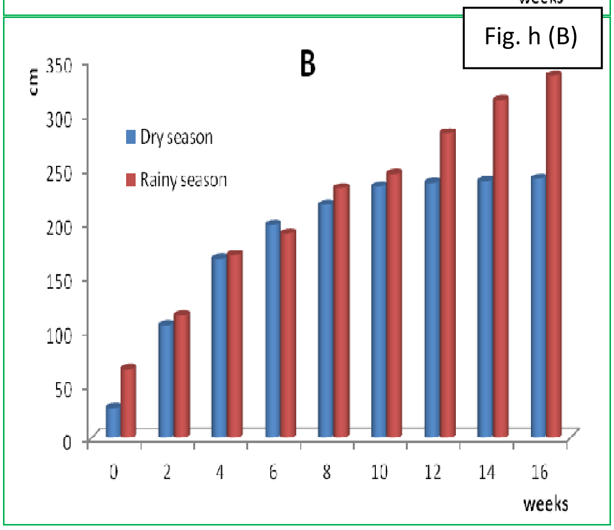


Figure e: Changes in *E. pyramidalis* Density and Number of Leaves per Plant during the Dry Season of the First Year

Figure f: Standing Biomass of *E. pyramidalis* at the End of the Dry Season of the First Year

Figure g: Changes in *E. pyramidalis* Density and Number of Leaves per Plant During the Rainy Season of the First Year

Figure h: Changes in the Height of *E. pyramidalis* With Time during the Dry and Rainy Seasons of the First Year (A) and the Second Year (B).

3.2 *Growth and Productivity of E. Crus-Pavonis:*

Thirty two young seedlings of *E. crus-pavonis* transplanted in the bed CW_2 immediately entered into the growth phase where they grew and multiplied rapidly to produce a population density of 376 plants/m^2 two months after transplanting. The species then started expressing wilt, with many leaves drying up and the plants density also reduced drastically to only 166 plants/m^2 at the end of the dry season (figure i). The total above-ground biomass of the plants produced at the end of this season was about 44 tons DM/ha. The number of leaves per plant also increased at almost the same rate from zero to 7 leaves and dropped to 2 leaves at the end of the dry season. The length of leaves increased slightly from the beginning and reduced towards the end of the season. The height of *E. crus-pavonis* like its density also increased very rapidly from 0.93 m at the start of data collection to 1.65 m at the end of the season.

Figure j: Standing Biomass and Adventitious Roots of *E. crus pavonis* During the Experiment.

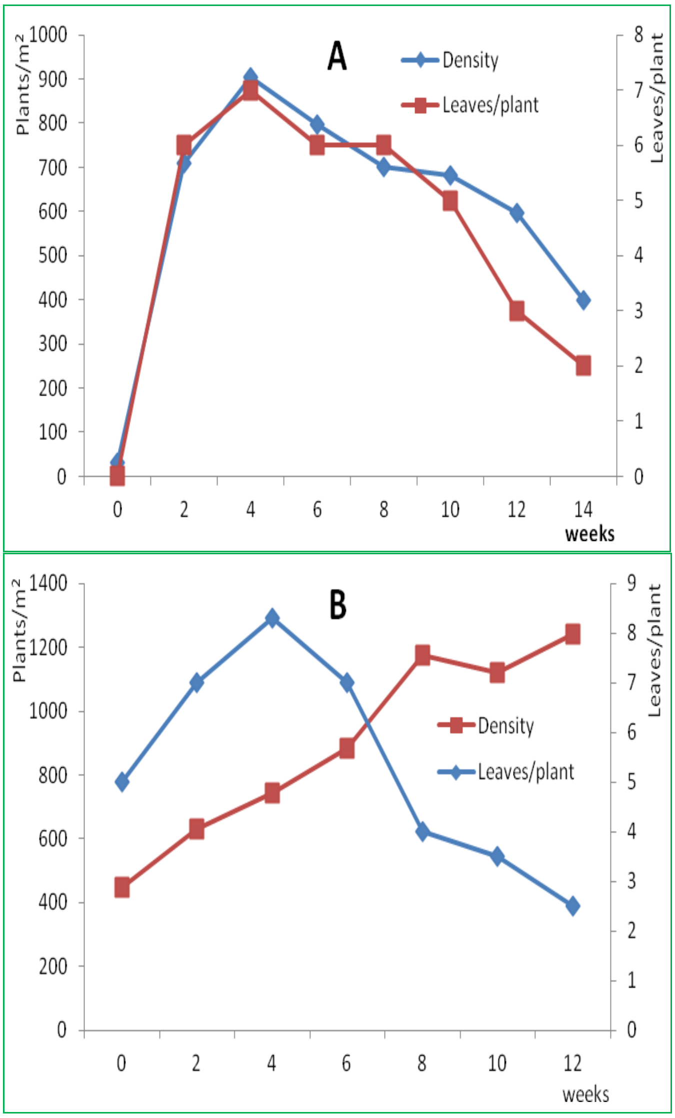
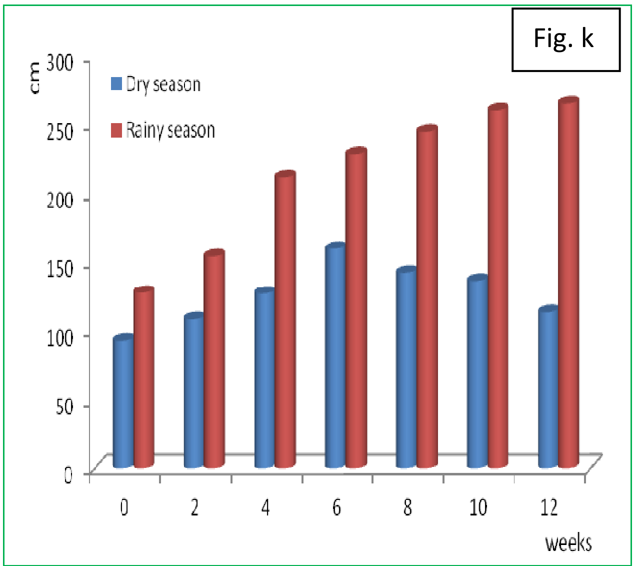


Figure i: Change in *E. crus-pavonis* Density and Number of Leaves per Plant during the Dry Season of the First Year (A) of the Second Year (B).

In the rainy season, the young shoots which arose grew rapidly without any inconveniency and covered the entire bed. These shoots showed healthy growth with the tallest plants reaching the height of 2.65 m at the time that they started flowering. The total biomass produced in this season was estimated to be about 35 tons DM/ha. The leaf size as well as height of the plants during this phase were larger compared with those of the dry season (figure k).

After harvesting *E. crus-pavonis* at the end of the rainy season for the first year, numerous new shoots arose and grew very rapidly and healthily. Plants did not express any sign of wilting like in the first year. Some individuals especially those found near the inlet gabion of the bed showed stunted growth such that, they flowered at very low heights ranging from 15-40 cm only. Records of plant growth parameters including the number of leaves per plants, leaf length and width and density in bed were generally higher compared to data recorded during the dry season of the first year (figure l). Plants here started flowering after two months of growth and by the end of the season almost all the plants in the bed had flowered. At the end of this season the total above ground biomass was estimated at 44 tons DM/ha



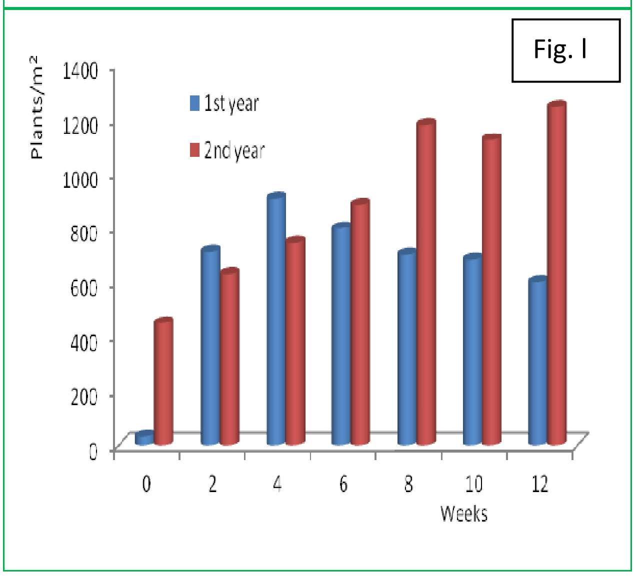
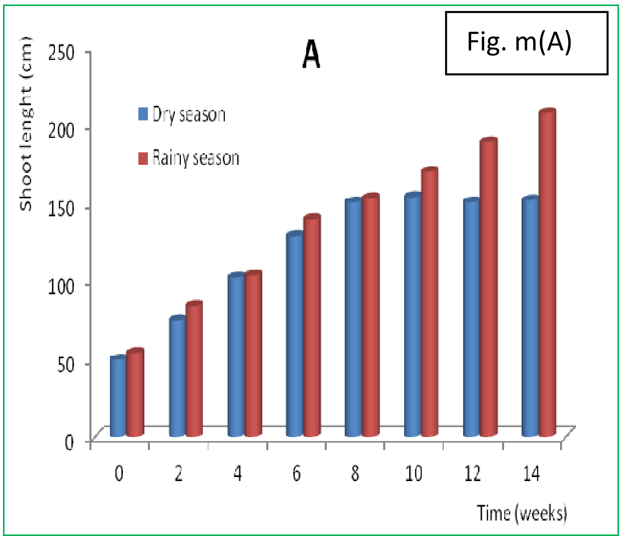


Figure k: Changes in the Height of *E. crus-pavonis* Plants during the Dry and the Rainy Seasons of the First Year

Figure l: Changes in *E. crus-pavonis* Density in the Dry Season of the First and Second Year of Experiment

In the rainy season of the second year, new shoots which arose after the harvest of the dry season plant population were numerous and showed very rapid and healthy growth. By the time that the plants were harvested, the tallest plant had reached the height of 244 cm. The species produced a total biomass dry weight estimated at about 30 tons/ha. The leaf size, the height and the density of plants in this season were larger than in the dry season.



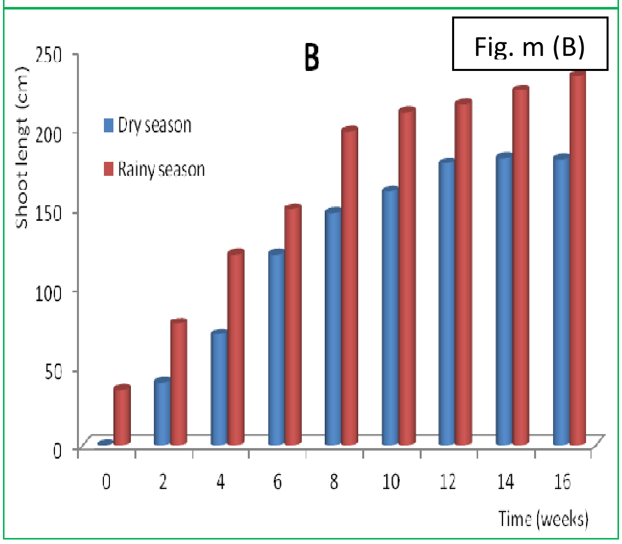


Figure m: Variation of the Shoot Length of *L. hexandra* During the Two Seasons of the First Year (A) and the Second Year (B) in the Wetlands.

3.3 Growth and Productivity of Leersia hexandra:

The 32 seedlings transplanted in CW_4 showed 87.5 % survival, grew and multiplied rapidly to a density of 1593 plants/m^2 after two months. These plants continued to grow and multiply and at the end of the dry season when they flowered, the tallest plant reached the length of 2 meters. During the dry season of the first year, this species produced a total above ground biomass of 32 tons DM/ha. The number of leaves per plant and the height of plants varied almost in the same trend. In the rainy season of the first year, the numerous new shoots which arose after harvesting the dry season plants grew and multiplied very drastically and just after two weeks of growth, the bed had a population density of 938 plants/m^2. At the end of the season, the bed was completely covered and congested, with the longest plant measuring 2.1 m. The total biomass produced during this season was estimated at 35 tons DM/ha. The shoots length in the rainy season as well as in the dry season had almost a linear variation (figure m), from where it was constant after three months of growth in the dry season.

The plants were harvested in October which marks the beginning of the dry season. The young shoots which arose grew rapidly with the coverage of the entire wetland. One month after this harvest the bed had a density of 6311 plants/m^2. The total biomass at the end this season was estimated at about 40 tons DM/ha which is more than that of the first year's dry season.

Figure n: Standing Biomass and Root System of *L. hexandra* in the Bed at Harvest.

The harvest of plants to mark the end of the dry season was in April. In the rainy season, the young shoots which arose grew and multiplied very drastically. Two weeks after the harvest, the plant density in the bed was 3244 plants/m^2. They grew and multiplied healthily and became highly congested in the bed three months after with the longest plant measuring 1.68 m. At the end of four months when the plants were harvested, the density in the bed was 7378 plants/m^2 with the longest plant measuring 2.4 m. The total biomass in this season was estimated at 25 tons DM/ha. The change in height of the plants during this second year was similar to that of the first year. The total biomass produced from *E. pyramidalis* was significantly higher (P<0.05) than those produced from *E. crus-pavonis* and by *Leersia hexandra*. Kengne *et al.,* (2009) and Fonkou *et al.,* (2010) reported of the luxurious growth of *E. pyramidalis* in highly polluted domestic wastewaters. Although the number of leaves/plant was more and even larger in the rainy season than in the dry season, the dry biomass was instead higher in the dry season with *E. pyramidalis*. Similar results were obtained with *E. crus-pavonis* and *L. hexandra.* This difference in biomass between the seasons could be attributed to the highly diluted influent due to rainfall and low temperatures in the rainy season. Thus, much of the water absorbed by the plants is accumulated in their tissues and constitutes the greater part of the fresh biomass. In the dry season, the environmental temperature is higher than in the rainy season such that the wastewater entering the system seems to be very concentrated due to higher evaporation rates. The plants are also subjected to the high atmospheric temperature and consequently have high transpiration rates such that much of their fresh biomass is due to plant productivity and not accumulated water as earlier suggested by Perbangkhem and Polprasert (2010). This productivity could be significantly related to nutrient availability, especially N and P (Robin and Kalff, 1988; Ennabili *et al*., 1998; Twilley *et al*., 1998; Carr *et al*., 2003).

The significant differences between the densities as well as the number of leaves per plant during the dry and rainy seasons might have also been due to temperature difference affecting the varying concentrations of the wastewater entering the different CWs. During the first year, the number of plants as well as the number of leaves started reducing drastically after a few months of growth of *E. crus-pavonis*. This might have been due to the highly concentrated wastewater instead of congestion because, the latter will cause natural die-off and the survivors will still be growing healthily instead of showing signs of infection, die-off and rotting of the entire bed's vegetation. The height of plants in both seasons after a certain period of growth became constant but started dropping with time. This gives an indication of optimum plant growth and the need for plants harvesting, giving chance for more biomass to be produced in the bed. The plants grew taller in the second year compared to the first. This could be attributed to the fact that the root systems of the plants are already well established and are in good symbiotic relationship with the micro-organisms for biodegradation and absorption of minerals in the rhizosphere region (Wolverton, 1987; Kadlec, 1995; Chang *et al.,* 2010; Vymazal, 2010; Hoffmann *et al*., 2011; Yadav *et al*., 2011). While the aerial part of the emerging macrophytes dies every year, their root system lives for a number of years, with the ratio of the root biomass to the rest of the plant being close to unity (Voinov and Tonkikh, 1987).

This higher plant growth during the second year is expressed by the more biomass produced at the different seasons except for *E. crus-pavonis* whose biomasses are less than those of the first year. These species do not only produce large quantities of biomass comparable of what is reported by Perbankhem and Polprassert (2010) for *Cyperus papyrus*, but they also perform greatly in the reduction of physicochemical characteristics of domestic and industrial wastewaters (Fonkou *et al.,* 2010; Fonkou *et al.,* 2011). In order to have high yields of biomasses and maintain them from these species, suitable management programs should be implemented to obtain the optimum period for harvesting of the plants. In this experiment the species flowered and were ready for harvest faster in the dry season (4 months of growth) than in the rainy season (6 months of growth). Although the plants flowered later in the rainy season than in the dry season, the vegetation was already unmanageable after four months of growth and needed to be harvested. The high biomass produced could be dried and stored for use as fodder in the dry season; it could also be composted to produce organic fertilizer.

4.0 Conclusions:

Gowth of *Echinochloa pyramidalis, E. crus-pavonis* and *Leersia hexandra* and their biomass yields in constructed wetlands were found to be influenced by season and temperature. In both dry and rainy reasons, biomasses yielded by *Echinochloa pyramidalis* were significantly higher than those produced by *E. crus-pavonis* and *Leersia hexandra*. All the three plants studied however yielded more biomass in the second year as compared to the first, indicating the influence of species duration in the site. Studies are now focused on the abilities of these species to perform in the reduction of physicochemical and microbiological characteristics of wastewaters. The results presented in this paper are expected to be exploited in macrophytes' management programs, in order to have high yields of biomass, optimal resource recovery and better performances of the constructed wetlands in water quality improvement.

5.0 Acknowledgment:

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