

Full Length Research Paper

# Growth and yield potential of *Echinochloa pyramidalis* (Lam.) Hitchc & Chase: A forage plant used in vertical-flow constructed wetlands in Cameroon

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This work aims at assessing growth and productivity of *Echinochloa pyramidalis* in the saline and saline-flooded processes of wetland treatment. Growth characteristics such as density, number of dead plants, height and number of new shoots and biomass production of the plant were studied using 24 laboratory-scale units of vertical-flow constructed wetlands fed with faecal sludge supernatant. Plants collected from the surrounding wetlands were subjected to four salinity levels with electrical conductivity of 2, 3, 6 and 9 dS.m<sup>-1</sup> under both drained and flooded conditions for a 100 day period. The results revealed that salinity and flooding combined with salinity stresses had similar effect on plant survival, height and density, leading to growth and biomass reduction at the higher salinity level than under natural growth conditions. Despite these stress effects, *E. pyramidalis* remained healthy with no signs of salt or saline-flooding stress injury but higher biomass production. As *E. pyramidalis* is a forage plant its high biomass production in the wetland treatment systems shows the potential of wetland systems to create a local economy based on forage production and thus the opportunity to link sanitation stewardship to food production. This may contribute to sustain sanitation infrastructures at the same time as increasing food security, especially in developing countries.

**Key words:** Constructed wetlands, *Echinochloa pyramidalis*, faecal sludge, flooding, growth, salinity, yield potential.

## INTRODUCTION

In the last decade, constructed wetlands (CWs) have been increasingly recognized as a relatively low-cost, low-technology, energy-efficient and an environmentally friendly means of treating sewage, agricultural and industrial wastes, and storm water runoff (Kadlec and Knight, 1996; Cronk, 1996; Kivaisi, 2001; Wetzel, 2001; Ciria et al., 2005). Compared with conventional treatment systems, the potential for application of this wetland technology is enormous in developing countries. With multiple benefits, CWs have a long lifetime and they can be maintained by relatively untrained personnel (Kivaisi,

2001). CWs are characterized by a high rate of primary productivity which means high volumes of biomass production, due to the large potential of nutrient removal by plant uptake (Hammer and Bastian, 1989; Brix, 1993; Neue et al., 1997; Boar et al., 1999). In CWs, wetland plants, often called macrophytes are an integral part of the systems; great biomass quantities are produced at the same time as the pollutants are removed from wastewater or excreta (Kivaisi, 2001; Brix, 1987; Tanner, 1996; Billore et al., 1999). However, the most commonly used macrophytes such as *Typha* sp., and *Phragmites* sp. known for their pollutant removal efficiencies and their ability to exhibit high biomass production while treating wastewater and human waste are not always locally available in tropical and subtropical regions, and their economic or energetic reuse potential is very limited

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as compared to other plants which can be used as forage (De Maesener, 1997). In Cameroon, an experimental study of the assessment of vertical-flow constructed wetlands (VFCWs) vegetated with tropical forage *Echinochlois pyramidalis*, revealed promising results (Kengne et al., 2009).

*E. pyramidalis* is a high value fodder plant well-known as antelope grass. It is a perennial emergent aquatic grass of the Poaceae family. Considered to be one of the most widespread and productive macrophytes in tropical and subtropical regions, the plant grows in a wide range of habitats mostly in floodplain wetlands, swampy places, paddy fields, and salt-affected humid soils (Yabuno, 1968; Rosas et al., 2006; Kengne et al., 2008). Its rapid vegetative growth, drought- and alkali- tolerance indicates that it has a high potential to be adopted as an important forage and fodder crop especially in tropical and subtropical regions. However, *E. pyramidalis*' tolerance to salinity and sometimes to mixed salt compositions in the growing media has not been explored to date although it has been shown that the plant is saline-resistant; moreover the plant's resistance threshold is still unknown. Indeed, although *E. pyramidalis* has exhibited an excellent yielding potential in vertical-flow constructed wetlands, this treatment process faces numerous problems such as the variability of liquid waste compositions and clogging of the wetland treatment units. In Cameroon, the faecal sludge contains high organic matter, macro and micronutrients; and their composition varies widely, with high concentration in salt contents that have a similar range of electrical conductivity (from 0.7 to 15 dS.m<sup>-1</sup>, with an average value of 2.7 dS.m<sup>-1</sup>) as compared to those of other Sub-Saharan countries such as Burkina-Faso, and Ghana (Koné et al., 2004; Kengne et al., 2006). It is well-known that under stress conditions, several physiological and morphological changes occur (Parida and Das, 2005), caused by direct and indirect effects of the stress; moreover, the intrinsic responses of the plants can influence the likelihood of their productivity and their feeding quality can be adversely affected. Moreover, in the case of macrophytes grown in CWs, no information is currently available on the effect of saline effluents on the productivity of *E. pyramidalis* under these conditions. For these reasons, studies on plants' growth and productivity in the wetland treatment processes are necessary in order to evaluate plant suitability for reuse systems. Therefore, the aim of the study presented here was to evaluate the salinity effect of faecal sludge on the growth and productivity of antelope grass under drained and flooded conditions.

## MATERIALS AND METHODS

### Experimental design

The investigations were conducted at the experimental field of the University of Yaoundé I, Cameroon located at 760 m above sea

level (3°45 N and 11°32 E) from December 2007 to 2008. Yaoundé has a typical equatorial Guinean climate characterised by two rainy seasons (from September to mid-November and from mid-March to June) and two dry seasons (from mid-November to mid-March and from July to August). Annual rainfall is about 1600 mm and daily temperature varies between 23 and 32°C.

The experimental units, comprised 24 small-scale units, each with a 0.78-m<sup>2</sup> and 50-L-strong vertical-flow constructed wetland microcosm (VFCW). They were randomly divided into two groups: flooded and drained units. The flooded condition initiated by waterlogging where only the roots were flooded was simulated to cope with the clogging situations generally faced in constructed wetlands. It consisted in allowing faecal sludge supernatant (FSS) to lay 5-cm above the surface of the substrata throughout the experiments. The drained condition was based on a normal water infiltration capacity after feeding the FSS. Prior to the experiment, the bottom of these experimental units was filled with 15-cm of large-sized gravel (15–30 mm) diameter, the middle with 10-cm of small-sized gravel (7–15 mm), and the top with 10-cm of a fine-sized sand layer (0–3 mm). This substrata arrangement was adapted from Koottatep et al. (2005). In this system, treatment occurs in substrate around plant roots. The size of this filter material was made by taking into account the best compromise between available surfaces for biofilm growth, suitability as a rooting medium and hydraulic conductivity that able to provide and support a healthy macrophyte growth and good treatment efficiency (Armstrong and Armstrong 1990; Armstrong et al., 1999; Vymazal, 2005; 2007). The wetland microcosm units were positioned in two rows at a distance of 0.5-m between the buckets in each row. A completely randomized design with three replicates for each treatment was used in these experiments. A perforated polyvinyl chloride piezometric tube of 0.60-cm in diameter was inserted vertically 50-cm into the substrata of the wetland microcosm units for sampling, because antelope grass has most of its roots and rhizomes in the top 60-cm. The units were equipped with a drainage pipe connected to a tap, allowing vertical drainage of the percolate through the media. The experimental units were covered by a 5-m high transparent and waterproof plastic roof to allow lateral air flow and avoid the dilution of salt concentration by the process of water seepage.

### Plant material and growing in the wetland system

Young shoots of *E. pyramidalis* of uniform size were collected in the surrounding natural wetlands and transplanted the same day in the 24 VFCW microcosm units. Seven plants, each with about 20-cm long rhizomes and stems, were planted in each bed. After planting, the beds were flooded with raw domestic wastewater (EC < 2 dS.m<sup>-1</sup>) to about 5-cm above the gravel layer and the plants were left to grow for eight weeks (acclimatization). They were subjected to four different treatments (T<sub>0</sub>, T<sub>1</sub>; T<sub>2</sub> and T<sub>3</sub>) corresponding to four salinity levels with an electrical conductivity (EC) of 2, 3, 6 and 9 dS.m<sup>-1</sup>, respectively in the growing medium of VFCW microcosms. Each treatment was replicated three times in a completely randomized block design. These treatments refer to the most prominent characteristics of FS effluent usually treated in CWs. Hence, to fit with the EC currently recorded and to avoid the use of unrealistic saline solution, the effluents of FSS were prepared to simulate a

similar range of composition and concentration of the major cation (Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>) and anion (Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup> and NO<sub>3</sub><sup>-</sup>) contents of the faecal sludge of Yaoundé urban areas based upon a preliminary study of its potential characteristics (Ngoutane Pare, unpublished). The saline solutions were prepared by adjusting the conductivities and dissolved mineral contents of FSS by either dilution either with domestic wastewater collected at the dormitory of the University of Yaoundé I or with addition of KCl, Ca(NO<sub>3</sub>)<sub>2</sub>, Na<sub>2</sub>SO<sub>4</sub> in a stoichiometric ratio corresponding to the composition of the faecal

**Table 1.** Compositions of the nutrient solutions (means  $\pm$  standard deviation; n=18) used in the VFCW units prepared on the basis on the faecal sludge supernatant adjusted or diluted to reach the target levels of salinity proportionally to the general composition and concentration of the major cations ( $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ) and anions ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$ ) contents of the faecal sludge of Yaoundé urban areas.

Parameter	2 dSm <sup>-1</sup> (T <sub>0</sub> )	3 dSm <sup>-1</sup> (T <sub>1</sub> )	6 dSm <sup>-1</sup> (T <sub>2</sub> )	9 dSm <sup>-1</sup> (T <sub>3</sub> )
<b>Physico-chemical parameters</b>				
TDS (mg/l)	995.6 $\pm$ 12.7	1522.5 $\pm$ 28.5	3160.8 $\pm$ 22.6	4875.5 $\pm$ 45.4
pH	6.4 $\pm$ 1.2	6.9 $\pm$ 1.2	6.83 $\pm$ 1.0	6.9 $\pm$ 0.8
T (°C)	26.5 $\pm$ 2.9	26.3 $\pm$ 2.9	26.6 $\pm$ 2.1	27.1 $\pm$ 2.7
Eh (mV)	27.6 $\pm$ 62.9	9.9 $\pm$ 69.4	9.4 $\pm$ 60.1	13.4 $\pm$ 58.2
<b>Chemical compositions (mg/l)</b>				
Mg <sup>2+</sup>	75.6 $\pm$ 144.2	86.0 $\pm$ 156.4	180.8 $\pm$ 202.3	144.3 $\pm$ 206.4
Ca <sup>2+</sup>	73.0 $\pm$ 103.0	133.6 $\pm$ 173.8	187.0 $\pm$ 218.6	518.3 $\pm$ 844.3
Na <sup>+</sup>	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.02 $\pm$ 0.03	0.03 $\pm$ 0.06
K <sup>+</sup>	0.1 $\pm$ 0.1	0.2 $\pm$ 0.6	0.23 $\pm$ 0.6	0.24 $\pm$ 0.5
Cl <sup>-</sup>	31.0 $\pm$ 39.3	47.8 $\pm$ 51.9	38.2 $\pm$ 36.4	39.2 $\pm$ 67.4
NO <sub>3</sub> <sup>-</sup>	130.9 $\pm$ 10.7	380.7 $\pm$ 11.6	270.1 $\pm$ 21.4	620.8 $\pm$ 158.8
SO <sub>4</sub> <sup>2-</sup>	601.0 $\pm$ 67.1	990.8 $\pm$ 89.1	1250.6 $\pm$ 124.1	1620.7 $\pm$ 179.4
Total N	577.4 $\pm$ 97.5	979.2 $\pm$ 177.1	1016.2 $\pm$ 95.6	1447.0 $\pm$ 142.4
PT	510.6 $\pm$ 42.9	490.7 $\pm$ 35.3	670.3 $\pm$ 32.9	450.8 $\pm$ 36.1

sludge of Yaoundé. In addition, salinity levels in surface water and in the piezometer content of the experimental units were monitored with a Hach HQ14d conductivity meter. Before each application on the experimental units, the FSS were sampled and analyzed for physico-chemical parameters such as pH, T°C, redox potential (Eh) and total dissolved solids (TDS) using a Hach HQ14d conductivity meter moreover chemical constituent measurements such as Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>, total N and P were conducted as outlined in the standard methods for examination of water and wastewater (APHA, 1995). The plants exposed to the effluents T<sub>0</sub> were considered as the control experiment. Each experimental unit was fed twice a week with an appropriate 15-L treatment solution (Table 1). In all the experiments, the plants were harvested at 100 day treatment period that is 100 days after the start of FSS application on the growing medium.

#### Plant morphological monitoring

Before feeding with the FSS, the morphological parameters of *E. pyramidalis* such as the number of dead plants, the number of plants in experimental unit (density), plant height (5-cm above the ground) and the number of new shoots were monitored weekly, starting one day prior to FSS feeding. The status of plants with indicators such as leaf size, colour, quality (soft or rough) was also monitored by visual observation and touching of leaves.

#### Measurement of biomass production

At harvest after a 100-day treatment period, plants were separated into above ground (leaves and stems) and below-ground components. Subsequently, parts of the roots were collected from the soil and washed several times with tap water. The fresh weight (FW) of below-and above-ground parts was determined by weighing after eliminating excess water ; FW recorded for all units, then below- and above-ground parts were oven-dried at 80 °C in plotting paper for 72 h and reweighed for dry-weight determination. The above- and below-ground biomass (grams dry weight per

square meter (g DW.m<sup>-2</sup>) were recorded based on plant dry-weight (DW).

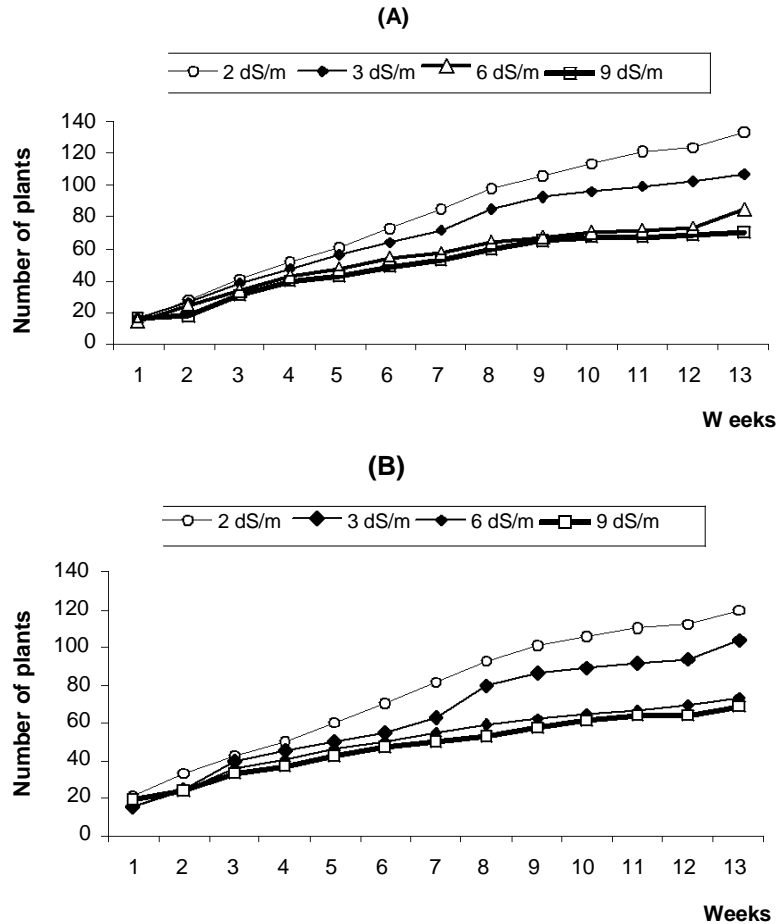
#### Statistical analysis

The data obtained were subjected to two-way analyses of variance (ANOVA) using SPSS 15.0 for Windows (SPSS, 2006). Factor 1 termed growing conditions (flooded or drained); factor 2 termed salinity treatment (4 levels) and an interaction termed growing condition (flooded or drained) x salinity. These computations were carried out to determine whether there was a difference between the plant biomass produced when exposed to four levels of salinity under different growing conditions, and what subsequent growth and biomass production responses of *E. pyramidalis* grown under these conditions may be. Each data point was the mean of three replicates (n = 3) and comparisons with P values  $\leq$ 0.05 were considered significantly different, Duncan's Multiple Range Test was used to compare their means.

## RESULTS

### Growing conditions

The plants were grown in the sunlight with a natural tropical photoperiod. The measured average temperatures inside and outside a shelter throughout the investigation at 7 a.m., 12 p.m. and 6 p.m. showed amounts of 23.5  $\pm$  1.2°C, 29.3  $\pm$  2.8°C and 24.8  $\pm$  1.6°C, respectively, inside a shelter and 22.9  $\pm$  1.1°C, 28.2  $\pm$  2.2°C and 24.25  $\pm$  1.9°C , respectively, outside a shelter (mean  $\pm$  standard deviation, n = 100). The saline condition of this study showed a wide variation of different kinds and levels of salts applied in the growing medium, thus reflecting the mixed salt compositions. The



**Figure 1.** Effect of salinity on the plant density of *E. pyramidalis* under flooded (A) and drained (B) conditions.

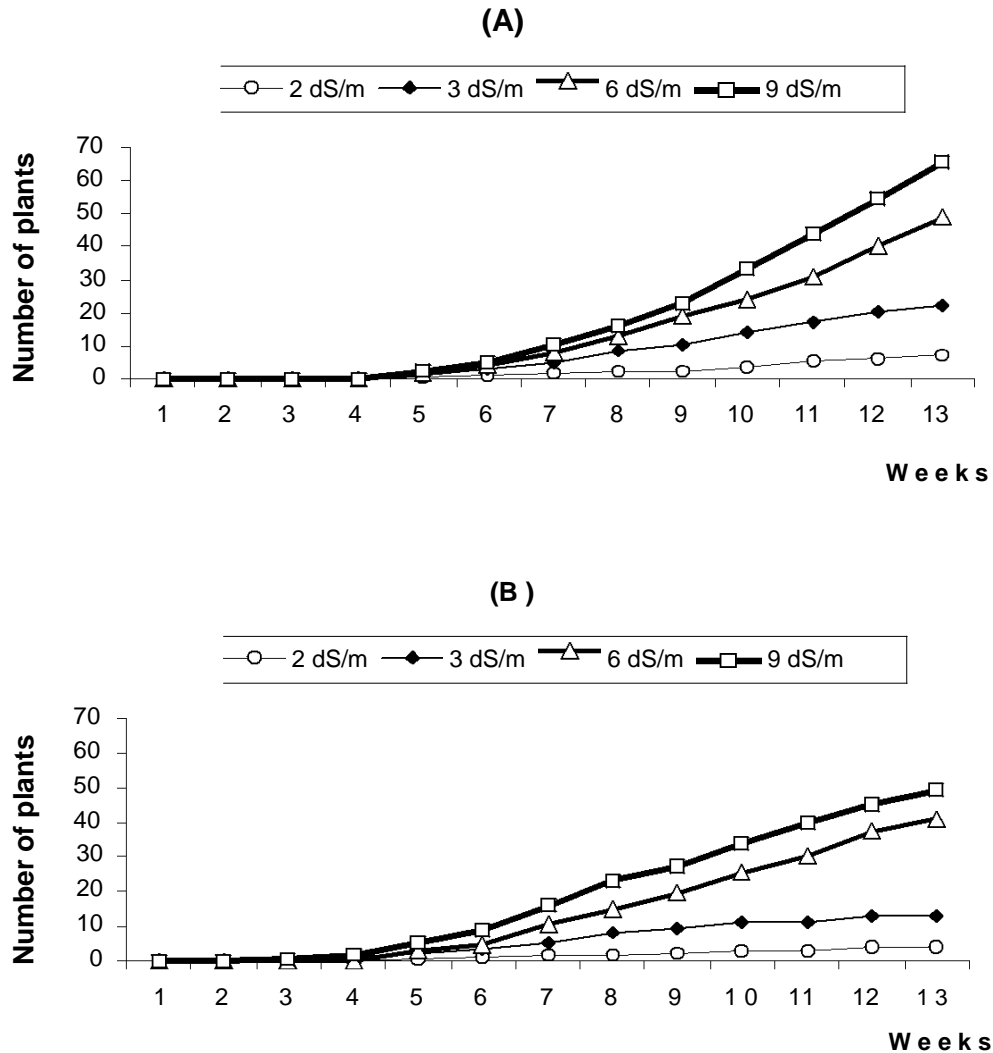
ionic compositions of the FSS applied in these experiments were dominated by  $\text{Ca}^{2+} > \text{Mg}^{2+}$  (cations) and  $\text{SO}_4^{2-} > \text{NO}_3^- > \text{Cl}^-$  (anions) respectively in the decreasing order of applied quantities which varied according to the level of salinity, indicating their level of contribution to the salinising process of the growing media (Table 1). Among the anions,  $\text{SO}_4^{2-}$  was the major contributor, followed by  $\text{NO}_3^-$  and lastly by  $\text{Cl}^-$  while the cations were at least 10 fold less represented than most of the anions contributors ( $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ). In addition, great quantities of total nitrogen and total phosphorous were also found in the FSS.

### Morphological development of *E. pyramidalis* under experimental conditions

Weekly monitoring of the morphological parameters was used to assess the growth of plants under salt stress and under the combined effect of salinity and flooding conditions. At the third week of treatment, the densities of

plants in the VFCW were the same (Figure 1). At 4 weeks of treatment, almost all plants were still alive; the decline began at the end of the fourth week (Figure 1). Although, this decline occurred gradually with increasing time and level of salinity, the plants' leaves remained larger, light green in color and relatively soft at any level of salinity until they started drying. The number of dead plants varied from 7 to 66 and 4 to 49 individuals. $\text{m}^{-2}$  respectively, under flooded and drained conditions indicating 98-64% and 98-72% survival, respectively (Figure 2). Under drained condition, by week 13 the density of plants had decreased from 134 to 69 plants/ $\text{m}^2$  respectively for 2 to 9 dS. $\text{m}^{-1}$  respectively, representing an overall approximate reduction of 48%. The density in the flooded VFCW units also decreased from 119 to 69 plants/ $\text{m}^2$  for 2 to 9 dS. $\text{m}^{-1}$ , respectively. There was no significant difference between saline and saline-flooded treatments ( $P \leq 0.05$ ).

The plant's height (Figure 3) ranged from 184 to 245 cm under flooded condition and 174 to 215 cm under drained condition. A significant increase of plant height



**Figure 2.** Effect of salinity on the plant survival (number of dead plant) under flooded (A) and drained (B) conditions.

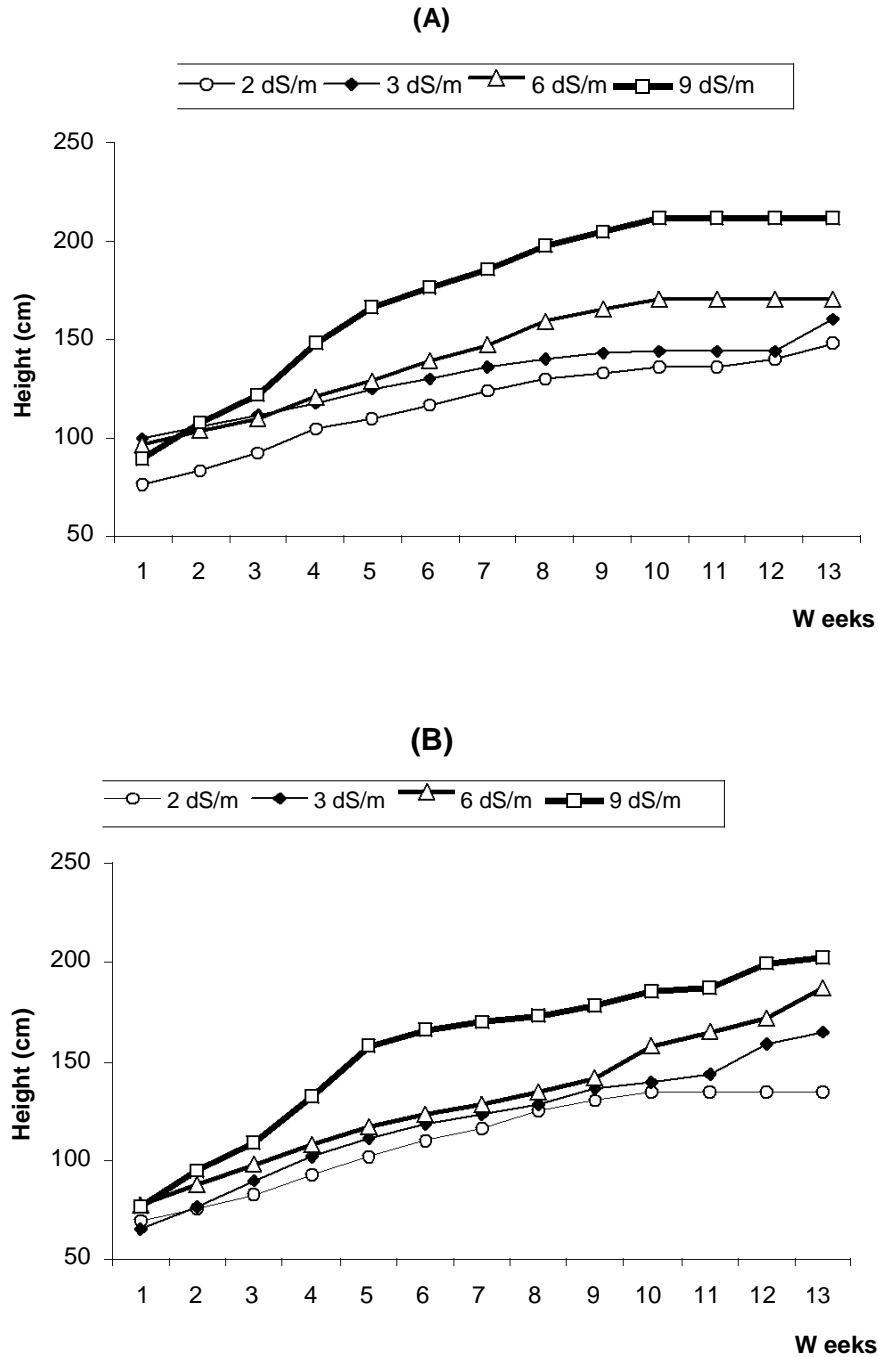
was noticed between the different salt levels ( $P \leq 0.05$ ). However no significant difference was observed between flooded and drained conditions.

#### **Effect of saline and saline-flooded treatments on *E. pyramidalis* productivity**

The below- and above-ground biomasses in terms of dry weight, % of fresh weight were all significantly reduced with the increasing level of salinity in both flooded and drained conditions. The biomass reduction of both plant parts was compensated by an increase in water content, shown here by the % of fresh weight (Table 2).

Responding to salinity under drained conditions, the above-ground productions of dry-weight biomass ranged from 1662.5 to 369.7 g DW.m<sup>-2</sup> while those of

below-ground parts lied between 2139.4 and 205.4 g DW.m<sup>-2</sup> (Figure 4). Accordingly, plants subjected to salinity under drained conditions responded by reducing their below- and above-ground biomass biomasses respectively by 49% and 22% at 9 dS.m<sup>-1</sup> compared to those of the control (Table 2). Under saline conditions, the above-ground production of dry-weight biomass in response to waterlogging ranged from 4207.14 to 1338.59 g DW.m<sup>-2</sup> while the under-ground biomass lied between 2071 and 1207.3 g DW.m<sup>-2</sup> (Figure 4). This drop in biomass production by 58% and 32% respectively for the below- and above-ground biomass at high salinity (9 dS.m<sup>-1</sup>) compared to those of the control (Table 2) showed the apparent additional effect of flooding. The tests of ANOVA two-way showed a high significant interaction effect ( $P \leq 0.000$ ) between flooding and salinity on the %FW of the above-ground biomass, suggesting



**Figure 3.** Effect of salinity on the plant height of *E. pyramidalis* under flooded (A) and drained (B) conditions.

that the flooding effect was strongly linked to the levels of salinity. Thus, the %FW of the above-ground biomass was higher under saline flooded than saline drained conditions, this difference was not however significant at  $3 \text{ dS}\cdot\text{m}^{-1}$ . The interaction between salinity and flooding on the above-ground biomass in terms of DW was highly

significant ( $P < 0.000$ ). Indeed, at any level of salinity the above-ground biomass produced under saline waterlogged conditions remained consistently higher than those of plants grown under the only effect of salinity stress. These results also showed a linear relationship between flooding and salinity.

When comparing the highest salinity level ( $9 \text{ dS}\cdot\text{m}^{-1}$ ) to

**Table 2.** Means and standard deviation of above-and under-ground biomass, percentage of fresh weight and: under-to above-ground biomass ratio (U/A) of plant produced in wetland treatment at different saline level of sludge supernatant during the 100 day treatment period. Two-way analysis of variance for statistical analysis between treatments (\*\*\*) =  $P < 0.05$  and ns= not significant). For each variable in the column, means followed by the same letter are not significantly different at 5% (Duncan's Multiple Range Test).

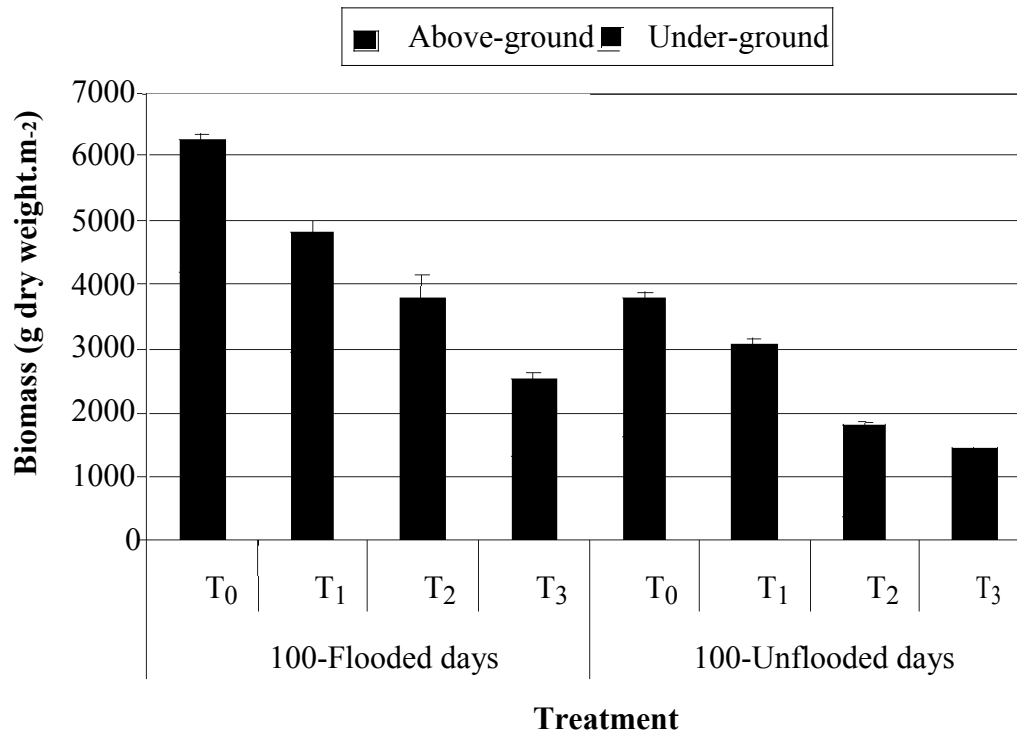
Variable	EC (dS.m <sup>-1</sup> )	Under-ground biomass		Above-ground biomass		U/A
		(%FW)	(g DW.m <sup>-2</sup> )	(%FW)	(g DW.m <sup>-2</sup> )	
<b>Unflooding or free drainage (individual effect of salinity)</b>						
	2	80.57±1.57 <sup>bc</sup>	2139.40±71.35 <sup>e</sup>	62.03±0.69 <sup>c</sup>	1662.48±63.44 <sup>c</sup>	1.29±0.09 <sup>b</sup>
	3	81.06±3.00 <sup>bc</sup>	1716.88±80.35 <sup>cd</sup>	63.21±4.63 <sup>c</sup>	1353.52±172.26 <sup>b</sup>	1.28±0.11 <sup>b</sup>
	6	83.67±3.27 <sup>c</sup>	1445.77±16.58 <sup>bc</sup>	36.90±5.88 <sup>a</sup>	370.73±80.53 <sup>a</sup>	4.02±0.81 <sup>d</sup>
	9	80.76±1.31 <sup>bc</sup>	1046.28±23.09 <sup>a</sup>	49.49 ±2.20 <sup>b</sup>	369.74±33.64 <sup>a</sup>	2.85±0.29 <sup>c</sup>
<b>Flooding (combined effect of salinity and flooding)</b>						
	2	65.58±1.99 <sup>a</sup>	2071.54±85.22 <sup>e</sup>	69.53±2.13 <sup>d</sup>	4207.14±141.19 <sup>f</sup>	0.49±0.03 <sup>a</sup>
	3	76.37±2.93 <sup>b</sup>	1909.57±143.43 <sup>de</sup>	65.69±2.01 <sup>cd</sup>	2939.74±194.20 <sup>e</sup>	0.65±0.09 <sup>a</sup>
	6	78.38±3.44 <sup>bc</sup>	1606.71±359.20 <sup>c</sup>	64.38±3.00 <sup>cd</sup>	2164.10±322.45 <sup>d</sup>	0.77±0.26 <sup>ab</sup>
	9	79.77±7.14 <sup>bc</sup>	1207.26±102.57 <sup>ab</sup>	60.92±1.20 <sup>c</sup>	1338.59±154.71 <sup>b</sup>	0.92±0.18 <sup>ab</sup>
Condition	F-ratio	89.25	628.28	20.36	3.33	150.83
	F-prob.	0.000 (***)	0.000 (***)	0.000 (***)	0.087 (ns)	0.000 (***)
Treatment	F-ratio	31.87	182.38	6.23	46.55	29.43
	F-prob.	0.000 (***)	0.000 (***)	0.005 (***)	0.000 (***)	0.000 (***)
Condition x treatment	F-ratio	17.45	22.34	4.31	0.972	20.32
	F-prob.	0.000 (***)	0.000 (***)	0.021 (***)	0.430 (ns)	0.000 (***)

that of the control, the under-ground biomass decreased between 51% and 42% for drained to saline waterlogged conditions, this influence was not significant. However, the above-ground biomass decreased significantly from 78% to 68%, suggesting that they were more sensitive than the under-ground biomass and their sensitivity was more pronounced with salinity alone than the combined effect of salinity with flooding. In the other hand, the above-ground biomass and under-ground biomass decreased significantly with the increasing level of salinity in both conditions (Table 2). The interaction between

growing conditions and salinity on the under-ground biomass showed a significant effect ( $P < 0.021$ ) on the %FW indicating that the effect of salinity depends on the flooding or drained condition, vice-versa. The flooding condition significantly ( $P < 0.000$ ) affected the %FW at 2 dS.m<sup>-1</sup>. There was a linear relationship between the %FW of under-ground biomass and growing conditions.

Besides, the interaction between the growing condition and salinity on the under-ground biomass in terms of dry matters revealed no significant effect. However, the Pearson

correlation was significant ( $P < 0.001$ ) with a relatively strong coefficient ( $R^2 = 0.648$ ). This positive coefficient showed a linear relationship between the above- and below-ground biomasses. Under the combined salinity and flooding effect, the highly perceptible linear relationship ( $R^2 = 0.775$ ) also confirmed a positive correlation indicating that both biomasses (above- and below-ground) grow significantly ( $P < 0.003$ ) in the same direction. The individual effect of the salinity revealed a highly significant ( $P < 0.000$ ) linear relationship with a strong positive correlation ( $R^2 = 0.905$ ) between the above-and



**Figure 4.** Impact of saline faecal sludge supernatant on biomass production of *E. pyramidalis* over 100-day treatment period under flooded and drained conditions. Data are mean of three replications  $\pm$  SD (for this and subsequent figures; T<sub>0</sub> = 2 dS.m<sup>-1</sup>; T<sub>1</sub> = 3 dS.m<sup>-1</sup>; T<sub>2</sub> = 6 dS.m<sup>-1</sup>; T<sub>3</sub> = 9 dS.m<sup>-1</sup>).

below-ground biomasses. Below- to above-ground dry weight ratios (U/A) were higher in saline-drained treatment compared to saline-flooded treatment. Thus, it appeared that the under-ground biomass represents 33%, 39%, 43% and 47% of the total biomass grown under saline flooded conditions versus 56%, 56%, 80% and 74% under the saline drained treatment respectively at 2, 3, 6 and 9 dS.m<sup>-1</sup>. By comparing the two treatments the results indicated that the above-ground biomasses were higher when subjected to the combined actions of salinity and flooding and lower under the only effect of salinity treatment (drained condition). The interaction between the growing conditions and salinity displayed a very significant effect ( $P < 0.000$ ) on the U/A ratio, suggesting that at any salt level of treatment, the flooded condition reacted by decreasing the U/A ratio.

## DISCUSSION

It has been shown by many authors that salinity and clogging (waterlogging) are two major stress factors of constructed wetland (Turon et al., 2009; Klomjek and Nitisoravut, 2005). As plants are essential components of this system, the ability to overcome these stresses is of great importance for plant growth and survival, also for the efficient treatment as well as the reuse possibility. Plants respond differently to abiotic stress at the level of

individual leaves as well as, the whole plant level and thus developing various strategies. This investigation evaluates the response of *E. pyramidalis* grown under interactive effects of salinity and flooding (waterlogging) stresses, and under the individual effect of salinity.

The results showed that growth of both vegetative and under-ground parts as well as the fresh and dry weights of *E. pyramidalis* decreased significantly with increasing level of salinity under flooded and drained conditions. These biomass reductions induced the decrease of the total biomass, expressed the adverse effect of salinity and/or saline-waterlogging on the biomass production of *E. pyramidalis*. Plants were more resistant to the individual effect of salinity and the combined effect of salinity and flooding stresses at the first six-week period of treatment.

Initially, we hypothesised that above- and below-ground biomass productions would be a function of overall sensitivity to the treatment. Our results indicated that under the two different stress conditions, the largest reduction occurred in shoot production (leaves and stems) while the lowest reduction occurred in root biomass production. Consequently, the above-ground parts of *E. pyramidalis* were highly sensitive while the below-ground parts were the most resistant to salinity and saline-flooded stress, suggesting that these organs showed varying degrees of salinity resistance. These results are not in agreement with Maas and Hoffman



(1977); Munns (2002); Ramoliya et al. (2006) who reported that salinity suppresses shoot growth more than root growth. However, this greatest reduction of above-ground compared to below-ground biomass production of plants might be probably due to the leaf senescence and shedding, and translocation of assimilates from the leaves to the other part of plants as well as to hormonal signals generated by roots. The increased reduction of above-ground biomass with respect to below-ground when subjected to salinity could be explained by the upsetting of the “functional equilibrium” between the roots and the stems of plants. This probably induced the reduction of physiological activity of the above-ground parts of stressed plants and the increasing need of these under-ground parts to search out in terms of minerals with greater allocation for under-ground parts growth than to above-ground parts. Furthermore, Short and Colmer (1999) showed that under saline conditions, the water potential gradient between the external media and xylem is lower, inhibiting the uptake of water by roots and leading to internal water deficits. This means that the overall reduction of biomass can be attributed to the decrease of osmotic potential of the growing media which might have induced plant’s mineral and water uptake limitation (Grattan and Grieve, 1992; Munns, 2002; Suyama et al., 2007). However, these biomass reductions are typical responses of most plants when subjected to increasing salinities. Similar results have been reported with some salt tolerant plants (Khan and Gulzar, 2003; Naidoo et al., 2008).

Despite the effect of salinity and flooding on the productivity, *E. pyramidalis* remained higher compare to the productivity of some macrophytes commonly used in constructed wetlands treating wastewater and sludge without salinity and flooding stresses. For examples, the study on the plant growth in constructed wetlands for wastewater treatment revealed that the maximum above-ground biomass production of 5.1 kg DW.m<sup>-2</sup> for *Phragmites australis* and 1.9 kg DW.m<sup>-2</sup> for *Phalaris arundinacea* were reached only after three or four growing seasons (Vymazal and Kropfelová, 2005). Also, the growth behavior of the ornamental plants such as *Canna generalis* and *Heliconia psittacorum* in constructed wetlands, treating the domestic wastewater, showed that those plants exhibited high removal capacity with the above-ground biomass production of 3.1 kg m<sup>-2</sup> y<sup>-1</sup> and 0.55 kg m<sup>-2</sup> y<sup>-1</sup> respectively and they can potentially be used in constructed wetlands (Konnerup et al., 2009).

In addition, we predicted the highest reduction of the biomass production of plants subjected to salinity under flooding conditions than those plants grown at similar salinity levels under drained conditions. Plants subjected to salinity under flooded conditions responded by increasing the reduction rate of their total biomass by 10% more than those grown at similar salinity levels under drained conditions. Henceforth, the possible

additional stress effect imposed by the constant flooding (5-cm above the substrate surface) might have intensified the response of plant at every tested salt level. Indeed, plants subjected to salinity treatment under flooded condition probably experienced the dual effect of salinity and flooding; and these effects affected the biomass more than the individual effect of salinity. However, to the extent of our knowledge, there is no information available in the literature on the effect of salinity or saline-flooding stress on the antelope grass biomass production, but similar phenomenon were also observed with other macrophytes. Many studies have reported that the interaction of salinity with waterlogging are more detrimental than the individual effect of each of them (Barrett-Lennard, 2003; Howard and Rafferty, 2006; Colmer and Flowers, 2008). Even though roots presumably seemed to be the most vulnerable part as they are directly exposed to salinity or saline-flooded environment; our data indicated the contrary. Under flooding stress simulated in our study, *E. pyramidalis* behave against low external oxygen concentration and adapted their growth over the short and long-term flooding. It seems that the saline-flooding stress was associated with specific structural changes that probably prevented water balance from becoming detrimental to plant growth and survival. Hence, there were different reactions of plants accordingly to their organs and type of stress such as the morphological changes of flooded plants which include the development of adventitious and concentrated upwards roots on the surface of the growth medium and the rapid lengthening. These reorientations of growing parameters especially the formation of adventitious roots induced by the flood-treated plants has been reported by many authors, especially for flood-tolerant species. It is frequently postulated that this phenomenon is tightly connected to adaptive mechanisms for plant against flooding as they may serve to escape the anoxic or hypoxic condition in the root zone caused by flooding (Jackson et al., 2003; Gibbs and Greenway, 2003 and Malik et al., 2009).

Surprisingly, the below-ground biomass was greater than the above-ground parts when subjected to the only effect of salinity while the dual effect of salinity and flooding stresses caused less production of the below-ground biomass than the above-ground. Although the rapid development of the adventitious roots on plants growing under saline-flooded stresses were noticed in this study, the root biomass was more reduced under saline-flooded treatment than under saline conditions, probably because of relatively low energy production due to the reduction of oxygen supply in response to flooding. It can be also hypothesized in this case that the reduction of the below-ground biomass under saline-flooded treatment may be due to the interference of salinity with flooding resulting to a possibly death of the roots and /or development inhibition of roots, even if the root respiratory capacity of flooded plants which is certainly an

indicator of the viability of the root system and the respiratory unit was not tested in our study. Furthermore, this low root development could be also justified by the low pH (6.4 - 6.9) that resulted in nutritive solutions (faecal sludge supernatant) used in our study. De Graaf et al. (1997) found that low pH in the hydro culture study, resulted in aluminium toxicity that lead to poor root development, yellowish leaves and reduced  $Mg^{2+}$  contents in plants. Similar results have also been reported by Akhtar et al. (1994); Saqib et al. (1999; 2004). However, even though the highest level of salinity was not tested in this study, our data showed that *E. pyramidalis* were able to complete their life cycle at all studied level of salinity.

## Conclusion

This study showed the ability of *E. pyramidalis* to grow and produce large quantity of biomass, under salinity independently of the flooded or drained condition in wetlands treatment. As *E. pyramidalis* are forage plants, bringing these biomass resources into sustainable productive use will offer opportunities to reduce the feed concentrate needs of the local farmers as regards their high biomass production potential which can thus increase food security, especially in developing countries. Hence, an integration of macrophyte-based wetland systems for wastewater and excreta treatment with forage production projects as means of using excess macrophytes biomass could be a good alternative. It is expected to link sanitation technology to urban food production systems by developing a wetland system vegetated with forage plants of commercial interest in order to create a local economy based on forage production. However, further investigations are necessary to evaluate the mineral status of this forage and the potential effects of wetland treatment on the forage intake, digestibility and hygienic qualities.

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