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Full Length Research Paper

Nutritional potential of *Echinochloa pyramidalis* (Lam.) Hitchc. & chase, a forage plant used in constructed wetlands treatment of faecal sludge and wastewater

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In Cameroon, *Echinochloa pyramidalis* used in constructed wetlands as alternative macrophytes revealed an attractive market potentials because of its quality as forage in addition to its pollutant removal capacity. The effects of salinity and the combined effects of salinity and flooding on the nutritional potential of *E. pyramidalis* under drained and flooded conditions were evaluated after 45 and 100 days of faecal sludge treatment. Leaf and stem samples were analysed for their crude protein (CP) and chemical compositions, especially in terms of cell-wall components while their apparent digestible dry matter (DDM), total digestible nutrients (TDN) and metabolizable energy (ME) were estimated. The results showed that *E. pyramidalis* is potentially suitable for cattle feeding. Thus, its use on wetland systems provides an opportunity to link sanitation stewardship to animal feed production which may contribute to sustain sanitation infrastructures and development. However, further investigations are necessary to evaluate the digestibility of most nutrients, the harvesting period and the contamination risk of *E. pyramidalis* grown in such system.

Keys words: Constructed wetlands, *Echinochloa pyramidalis*, flooding, nutritive value, salinity, treatment period.

INTRODUCTION

With regards to their high rate of primary productivity, reuse of macrophytes grown in wetland treatment systems of wastewater and faecal sludge is a needed management option in Sub-Saharan countries. High volumes of biomass are produced in such systems due to the large potential for nutrient removal by plant uptake under warm tropical and subtropical climates which are favourable to higher biological activity and productivity (Boar et al., 1999). Previous studies pointed out that there is a high biomass production in constructed wetlands (CWs) with *Cyperus papyrus, Eichhornia crassipes, Phragmites australis, Typha latifolia, Typha augustifolia* (Boar et al., 1999), whereas many others widely demonstrated that these aquatic plants are involved in almost every major function within wetland treatment systems. However, the most commonly used macrophytes such as *Typha* sp. and *Phragmites* sp. which are known to exhibit high biomass production while treating human wastes are not always locally available. In addition, their economic or energetic reuse potentials are very limited compared to other plants such as *Echinochloa pyramidalis* (Adebowale, 1988; Kengne et al., 2009). In fact, in the context of developing countries, such plants can be considered as valuable resources due to their biomass production under treatment systems.

E. pyramidalis commonly called antelope grass is an erect grass species that grows to about 3 m (rarely 4 to

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5 m) in height (Yabuno, 1968) with laterally creeping rhizomes. The plant grows best in humid environments, mainly swampy areas (Anderson and Cameron, 2006) and along rivers (River Niger in Mali, Niger). It is a perennial herb generally soil-attached, but also found floating or submerged (Adebowale, 1988). The plant is widely distributed in tropical regions of Africa, America, Asia, and Australia (Anderson and Cameron, 2006; Yabuno, 1968) and has been successfully used for wastewater and faecal sludge dewatering in Cameroon (Bojcevska and Tonderski, 2007; Kengne et al., 2008).

In Cameroon, few experimental trials have been tested with macrophytes based-systems for wastewater and faecal sludge treatments. These studies included the use of *C. papyrus* and *E. pyramidalis* (Kengne et al., 2009). The assessment of vertical-flow constructed wetlands (VFCWs) vegetated with tropical forage *E. pyramidalis* revealed its high potential to improve the quality of wastewater and human excreta, and to produce large amounts of biomass through the rapid growth rate of the plant. Indeed, Kengne et al. (2009) estimated the above-ground biomass of *E. pyramidalis* in the faecal sludge treatment beds to be about 100 to 150 dry tons/ ha on an annual three-harvest basis. This shows the necessity of harvesting and using excess plant material from management systems in order to sustain effective treatment. Many studies have shown that as plant biomass increases, the role of the vegetation in wetland treatment shifts (Thullen et al., 2002). Besides, it has been demonstrated that the most effective removal of pollutants depend upon the health and sustainability of the macrophytes in the CW systems (Thullen et al., 2005). For this reason, proper methods of biomass disposal and /or utilization are required. In this regards, several authors suggested that biomass of aquatic plants can be transformed into raw materials for paper industry, fertilizers, compost or as a feed supplement for animals (Stottmeister et al., 2003).

However, the potential for forage production in CWs is neglected, despite the fact that aquatic plants can provide a promising source of feed supplement and thus, may reduce the feed concentration needs for local farmers. Therefore, an integration of macrophyte-based wetland systems for liquid wastes treatment with forage production projects as a means of using excess macrophytes biomass could be a good alternative. This can be done by linking sanitation technology to urban food production systems in order to create a local economy based on forage production. It might help to maximise nutrient saving and resource recovery at the same time while treating liquid waste, giving an opportunity to close the nutrient loop between urban excreta and wastewater management. Although there is an excellent yielding potential of plants under CWs, this treatment process faces various problems such as variability of liquid waste compositions with high salt contents and clogging of the wetland treatment units. Given that stress conditions may affect the morphological, physiological, and biochemical components of plants, their suitability for reuse systems will depend on their nutritional quality. There is therefore a need to evaluate the nutritional potential of *E. pyramidalis* as forage grown on CWs of faecal sludge treatment under saline and saline-flooding conditions for ruminant nutrition.

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 December 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 design comprised 24 small-scale units, each with a 0.78-m^2 and 50-L-strong vertical-flow constructed wetland (VFCW) microcosms of 50 L capacity and 0.78 m^2 sizes each, They were randomly divided into two groups: flooded and drained units. For the flooded condition initiated by waterlogging, only the roots were flooded, simulating the clogging situations generally faced in constructed wetlands by allowing faecal sludge supernatant 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 w the faecal sludge supernatant. Prior to the experiment, the bottom of these experimental units was filled with 15-cm of large-sized gravel of (15 to 30-mm) diameter, the middle with 10-cm of small-sized gravel (7 to 15-mm), and the top with 10- cm of a fine-sized sand layer (0 to 3-mm) diameter (Figure 1).This substrata arrangement was adapted from Koottatep et al. (2005). Since in such systems, 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 et al., 1990; Armstrong and Armstrong 1999; Vymazal, 2005). The wetland microcosm units were positioned in two rows with 0.5 m spacing 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 of substratum. Each unit was 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 waterproof plastic roof to allow lateral air flow and avoid the dilution of salt concentration by the process of water seepage.

Plant material and growth in the wetland system

Young shoots of *E. pyramidalis* of uniform sizes were collected in the surrounding natural wetlands and transplanted the same day in the 24 VFCW microcosm units. Seven plantlets, 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 $dS.m^{-1}$) to about 5 cm above the gravel layer and the plants

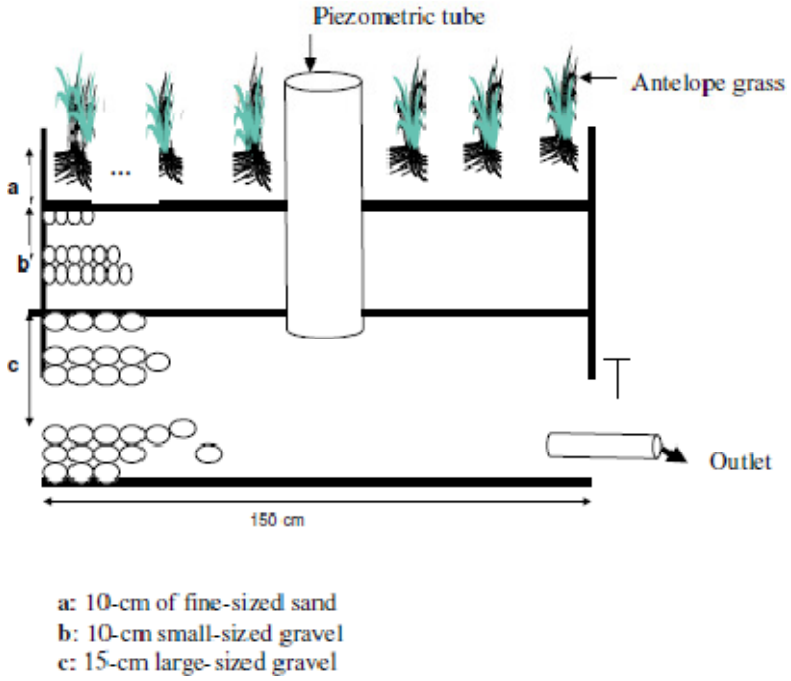

Figure 1. Schematic diagram of the vertical flow constructed wetland (VFCW) used to grow *Echinochloa pyramidalis.*

were left to grow for eight weeks (acclimatization). They were subjected to four different treatments (T_0, T_1; T_2 and T_3) corresponding to four salinity levels with an electrical conductivity (EC) of 2, 3, 6 and 9 $dS.m^{-1}$, 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^+, Ca^{2+}, Mg^{2+}, K^+) and anion (Cl^-, SO_4^{2-} and NO_3^-) 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, $Ca(NO_3)_2$, Na_2SO_4 in a stoichiometric ratio corresponding to the composition of the faecal 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^+, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, NO_3^-, Cl^-, 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_0 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, i.e. 100 days after the start of FSS application on the growing medium.

At each harvest, the plants were cut 20 cm above the sand level, their leaves separated from stems and analyzed.

Cell-wall composition analysis

All chemical analysis were carried out at the Animal Nutrition Laboratory of the Department of Animal Production, Faculty of Agronomy and Agricultural Sciences; University of Dschang-Cameroon. The leaf and stem samples of *E. pyramidalis* harvested in the experimental units were dried in a forced draft oven at 65°C for 48 h. Dried samples were ground to pass through a 1 mm sieve. To determine dry matter (DM), the samples were oven-dried at 105°C for 6 h. Ash determination was done at 550°C for 8 h. Total N was determined by Kjeldahl procedure (AOAC, 2000) and crude protein (CP) calculated as N×6.25 (AOAC, 1990). Acid detergent fibre (ADF) including ash residue was analyzed according to the standard methods of AOAC (1990). Neutral detergent fibre (NDF) including residual ash and sulphuric acid detergent lignin (ADL) were determined by the methods of van Soest et al. (1991). The content of lignin was determined by solubilization of cellulose with sulphuric acid. The ether extract (EE) was determined by soxhlet method (AOAC, 1990).

Estimation of dry matter digestibility, total digestible nutrients and metabolizable energy of the leaf samples of *E. pyramidalis*

The apparent dry matter digestibility (DMD), the total digestible nutrients (TDN) in the leaf samples were calculated using the following summative equations:

1. DMD = 0.98 × CC + NDF (1.473 - 0.789log ADF) - 12.9 (van Soest, 1967), Where CC, the cell content equals to 100 - %NDF; NDF, the neutral detergent fiber; and ADF, the acid detergent fiber.
2. TDN = DMD - ash + (1.25 × EE) (van Soest, 1994); where, DMD is the dry matter digestibility and E.E (ether extract).

The estimation of the metabolizable energy values of *E. pyramidalis* leaves was calculated according to the following equations developed from MAFF (1984).

Table 1. Compositions of the nutrient solutions (means ± 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 (Na^+, Ca^{2+}, Mg^{2+}, K^+) and anions (Cl^-, SO_4^{2-} and NO_3^-) contents of the faecal sludge of Yaoundé urban areas.

Parameter	2 dSm^{-1} (T_0)	3 dSm^{-1} (T_1)	6 dSm^{-1} (T_2)	9 dSm^{-1} (T3)
Physico-chemical parameter				
TDS (mg/l)	995.6±12.7	1522.5±28.5	3160.8±22.6	4875.5±45.4
pH	6.4±1.2	6.9±1.2	6.83±1.0	6.9±0.8
T (°C)	26.5±2.9	26.3±2.9	26.6±2.1	27.1±2.7
Eh (mV)	27.6±62.9	9.9±69.4	9.4±60.1	13.4±58.2
Chemical compositions (mg/l)				
Mg^{2+}	75.6±144.2	86.0±156.4	180.8±202.3	144.3±206.4
Ca^{2+}	73.0±103.0	133.6±173.8	187.0±218.6	518.3±844.3
Na^+	0.01±0.01	0.01±0.01	0.02±0.03	0.03±0.06
K^+	0.1±0.1	0.2±0.6	0.23±0.6	0.24±0.5
Cl^-	31.0±39.3	47.8±51.9	38.2±36.4	39.2±67.4
NO_3^-	130.9±10.7	380.7±11.6	270.1±21.4	620.8±158.8
SO_4^{2-}	601.0±67.1	990.8±89.1	1250.6±124.1	1620.7±179.4
Total N	577.4±97.5	979.2±177.1	1016.2±95.6	1447.0±142.4
Total P	510.6±42.9	490.7±35.3	670.3±32.9	450.8±36.1

3. IVDMD = 1.1 × DMD and (4) ME = 0.15146 × (IVDMD × 1.05)
Where IVDMD is *in vitro* DMD value and ME (MJ/kg DM) is metabolizable energy value of *E. pyramidalis*.

Statistical analysis

The data of chemical compositions were subjected to two-way analyses of variance (ANOVA) using SPSS 15.0 for Windows (SPSS, 2006). The factor 1 termed treatment (saline-flooded or saline-drained with 4 levels of salinity); factor 2 termed treatment period (2 periods) and an interaction termed treatment × treatment period were carried out to determine if there were differences between the chemical composition of plants produced when exposed to four levels of salinity under different growing conditions (flooded and drained conditions), and their subjected period. The one-way ANOVA analyses with factors such as period of treatment and saline-flooded/saline-drained treatment were also used to identify their individual significant differences on the nutritional qualities of plant parts.

RESULTS

Growing conditions

The plants were grown in the sunlight with a natural tropical photoperiod. The measured average temperatures inside and outside the shelter throughout the investigation at 7 and 12 a.m. as well as 6 p.m. showed amounts of 23.5 ± 1.2°C, 29.3 ± 2.8°C and 24.8 ± 1.6°C, respectively, inside a shelter and 22.9 ± 1.1°C, 28.2 ± 2.2°C and 24.25 ± 1.9°C , respectively, outside a shelter (mean ± 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 ionic compositions of the FSS applied in these experiments were dominated by Ca^{2+} > Mg^{2+} (cations) and SO_4^{2-} > NO_3^- > 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, SO_4^{2-} was the major contributor**,** followed by NO_3^- and lastly by Cl^- while the cations were at least 10-fold less represented than most of the anion - contributors (SO_4^{2-}, NO_3^-). In addition, great quantities of total nitrogen and total phosphorous were also found in the faecal sludge supernatant, suggesting their high nutrient contents.

Cell-wall components of *E. pyramidalis*

Ash contents

Under saline-flooded condition, the variations of the ash contents of leaves and stems as a function of age are shown in the Tables 2 and 3. Statistically there were no significant difference (P > 0.05) between ash contents of 45 days and those of 100 days (Table 2). The ash contents (Table 3) significantly increased (P = 0.004) with increasing level of salinity (2 to 9 dS m^{-1}). The interactive effects of salinity and period of treatment were highly significant (P = 0.01). This indicates that the difference between ash contents of leaves depend on the treatment duration on the plant. However, the ash contents of leaves grown under drained condition showed an average value of 9.5% DM at 45 days period of

Table 2. Effects of treatment duration of saline effluent of faecal sludge supernatant under flooded and drained conditions of the vertical-flow constructed wetlands on the chemical composition (%DM) of the leaves and stems of *E. pyramidalis.*

Item	Ash	CP	ADL	ADF	NDF	EE
Harvest time [a]			**Stems[c]**			
45	6.67	10.16	13.52	40	65.69	4.78
100	10.14	6.56	17.6	44.87	70.31	3.82
S.M.E[b]	1.7	1.8	2.0	2.4	2.3	0.5
F-ratio	13.9	5.89	9.89	3.02	5.83	1.49
F-prob.	0.002	0.029	0.007	0.104	0.03	0.242
			Stems[d]			
45	7.97	10.03	13.6	35.86	70.35	1.68
100	14.01	7.6	16.23	41.35	72.66	2.21
S.M.E[b]	3.0	1.2	1.3	2.7	1.2	0.3
F-ratio	34.99	5.22	2.33	2.64	0.71	1.82
F-prob.	0	0.038	0.149	0.126	0.411	0.198
			Leaves[c]			
45	9.12	15.35	10.56	32.87	60.58	6.87
100	9.22	10.92	12.55	36.18	64.03	4.99
S.M.E[b]	0.1	2.2	1.0	1.7	1.7	0.9
F-ratio	0.005	9.24	7.91	39.65	3.66	9.26
F-prob.	0.945	0.009	0.014	0.000	0.076	0.009
			Leaves[d]			
45	9.46	16.22	12.75	30	60.89	2.97
100	11.38	12.05	13	34.72	64.9	3.91
S.M.E[b]	1.0	2.1	0.1	2.4	2.0	0.5
F-ratio	4.47	14.4	0.23	11.54	2.92	1.51
F-prob.	0.053	0.002	0.636	0.004	0.109	0.239

[a]Day after treatment; [B]standard error of the mean; [C]stems/leaves of plant subjected to salinity under flooded conditions; [d]stems/leaves of plant subjected to salinity under drained conditions; CP: crude protein; ADL: acid detergent Lignin; ADF: acid detergent fibre; NDF: neutral detergent fibre; EE: ether extract; each data presented here are mean of eight replicates.

treatment with a significant difference from those subjected to 100 days period of treatment. Table 2 clearly showed that the level of salinity (2 to 9 dS m^{-1}) significantly affects the ash content (P = 0.009). Also, the dual effects of salinity and treatment period on the ash contents of leaves showed a significant interaction (P = 0.01), indicating that under drained conditions ash contents of leaves were highly affected by the salinity treatments (P = 0.009).

In the stem grown under saline-flooded condition (Table 2), there was no statistical difference of the mean ash contents between the two different periods of treatment. However, Table 3 showed a significant increase of the ash contents as a function of the salinity level (P = 0.004). The samples of different salinity levels significantly interacted (P = 0.01) with saline-flooding and period of treatment. Firstly, this suggests that the treatment effect depends on the plant's treatment period, and secondly, the difference between plant concentrations of ash depends on the salinity levels. In drained condition (Table 2), the significant increased (P = 0.00) of ash contents from 45- to 100- day period of treatment (about 8 to 14% DM of mean average) was observed in stems as well as the salinity stress (P = 0.028). The 100 days period reacted by increasing the ash contents of stems at all levels of salinity. Also, the interactive effects of salinity and period of treatment were highly significant (P = 0.028). However, the difference of ash concentrations depends on the level of salinity and the period of treatment.

Ether extracts (EE) contents

In the salt flooded treatment, the mean of EE contents of leaf parts decreased significantly (P = 0.009) from 6.9% DM at 45- day period to about 5% DM at 100 days period of treatment (Table 2). Salinity level application had no significant effect (P > 0.05) on the EE content (Table 3) while the interactive effect of salt-flooding and the

Table 3. Effects of the treatment process of saline effluent of faecal sludge supernatant under flooded and drained conditions of the vertical-flow constructed wetlands on the chemical composition (%DM) of *E. pyramidalis*.

EC[a]	Ash	CP	ADL	ADF	NDF	EE
			Stems[c]			
2 dS m^{-1}	10.37	12.59	12.82	45.23	66.33	2.93
3 dS m^{-1}	7.19	5.96	14.32	44.23	68.04	3.36
6 dS m^{-1}	8.03	7.53	16.66	42.09	68.16	5.51
9 dS m^{-1}	8.04	7.37	18.45	38.2	69.47	5.4
S.E.M[b]	0.7	1.5	1.2	1.6	0.6	0.7
F-ratio	1.21	5.52	3.42	1.11	0.29	5.41
F-prob.	0.348	0.013	0.052	0.382	0.828	0.014
			Stems[d]			
2 dS m^{-1}	10.15	10.99	18.65	47.62	74.93	0.86
3 dS m^{-1}	9.68	9.72	17.16	39.26	73.71	2.46
6 dS m^{-1}	12.78	8.7	13.21	35.9	70.72	2.14
9 dS m^{-1}	11.36	5.86	10.65	31.65	66.66	2.32
S.E.M[b]	0.7	1.1	1.8	3.4	1.8	0.4
F-ratio	0.51	7.7	18.91	10.5	2.38	8.23
F-prob.	0.680	0.004	0.000	0.001	0.120	0.003
			Leaves[c]			
2 dS m^{-1}	8.47	16.16	9.82	34.13	64.62	5.08
3 dS m^{-1}	8.51	15.18	11.34	34.58	62.02	6.67
6 dS m^{-1}	8.78	10.23	12.39	34.86	61.46	5.45
9 dS m^{-1}	11.04	10.97	12.66	34.53	61.13	6.53
S.E.M[b]	0.6	1.5	0.6	0.2	0.8	0.4
F- ratio	7.64	4.65	3.34	0.07	0.6	1.05
F-prob.	0.004	0.022	0.056	0.973	0.624	0.405
			Leaves[d]			
2 dS m^{-1}	10.33	15.79	12.01	29.46	59.97	2.57
3 dS m^{-1}	8.16	15.58	12.32	32.53	61.23	4.25
6 dS m^{-1}	12.43	13.11	13.4	33.52	64.03	4.17
9 dS m^{-1}	10.76	12.36	13.77	33.93	66.32	2.76
S.E.M[b]	0.9	0.9	0.4	1.0	1.4	0.4
F- ratio	6.2	1.26	5.5	1.32	1.42	1.43
F-prob.	0.009	0.332	0.013	0.312	0.285	0.281

[a]Electrical conductivity in deci siemens per meter (dS m^{-1}); [B]standard error of the mean; [C]stems/leaves of plant subjected to salinity under flooded conditions; [d]Stems/Leaves of plant subjected to salinity under drained conditions; Each data presented here are mean of sixteen replicates; CP: crude protein; ADL: acid detergent Lignin; ADF: acid detergent fibre; NDF: neutral detergent Fibre; EE: ether extract.

treatment period (Table 4) was significant (P = 0.030). However, statistical analysis showed no significant (P > 0.05) effect of treatment periods (Table 2) and salt-drained treatment (Table 3) within mean concentrations of EE contents. The interactive effect of salt-drainage and the treatment period (Table 4) was highly significant (P = 0.000).

For the stem parts, the 45- and 100- day period of salt-flooded and salt-drained treatments exhibited no significant effect on their EE contents (P > 0.05), while in both treatment conditions, the salinity levels consistently increased their contents (P < 0.05).

Crude protein (CP) contents

The CP contents decreased from the mean value of 15.3% DM at 45 days period to10.9% DM at 100 days period of treatment (Table 2). The analysis of variance showed a highly significant (P = 0.009) effect of treatment

Table 4. Combined effect of saline-flooded/saline-drained treatment and their duration on the chemical composition of *E. pyramidalis*.

Variable	Leaves				Stems			
	Flooded		Drained		Flooded		Drained	
	F-ratio	F-prob.	F-ratio	F-prob.	F-ratio	F-prob.	F-ratio	F-prob.
Ash								
Period	0.03	0.863	44.81	0.000	109.77	0.000	97.72	0.000
Treat	19.44	0.000	37.48	0.000	17.06	0.001	5.15	0.028
Period × Treat	7.48	0.010	6.54	0.015	17.11	0.001	5.21	0.028
CP								
Period	207.58	0.000	353.24	0.000	175.54	0.000	346.40	0.000
Treat	93.51	0.000	55.62	0.000	114.59	0.000	279.66	0.000
Period × Treat	8.58	0.007	56.14	0.000	21.76	0.000	27.11	0.000
ADL								
Period	20.42	0.002	0.37	0.556	95.96	0.000	70.97	0.000
Treat	8.58	0.002	4.43	0.041	35.65	0.000	136.78	0.000
Period × Treat	0.79	0.531	0.42	0.738	6.93	0.013	2.59	0.125
ADF								
Period	92.82	0.000	12.38	0.008	2.57	0.147	16.79	0.003
Treat	0.73	0.551	2.27	0.157	1.05	0.421	25.50	0.000
Period x Treat	7.50	0.010	0.07	0.976	0.25	0.853	1.44	0.299
NDF								
Period	3.47	0.099	2.47	0.154	3.71	0.090	0.70	0.425
Treat	0.73	0.561	1.25	0.353	0.29	0.832	1.80	0.225
Period × Treat	1.01	0.434	0.04	0.989	0.01	0.997	0.12	0.944
EE								
Period	23.34	0.001	28.33	0.001	3.86	0.085	4.76	0.061
Treat	4.07	0.050	25.60	0.000	7.69	0.010	9.26	0.006
Period × Treat	5.01	0.030	59.29	0.000	1.73	0.237	0.24	0.861

CP: Crude protein; ADL: acid detergent lignin; ADF: acid detergent fibre; NDF: neutral detergent fibre; EE: ether extract.

period and salinity treatment (P = 0.02) as well as their interactive effects on the CP contents. This indicates that as the duration of treatment increased CP contents decreased and at any level of salinity, this content is significantly smaller at the 100-days treatment period. Under drained conditions, CP contents significantly (P = 0.002) decreased from 16.2 % DM at 45 days period to12 % DM at 100- day period of treatment (Table 2). The individual effects of salinity and treatment period exhibited a highly significant decrease (P = 0.000) as well as their combined factors (Table 4).

On the other hand, the CP contents of stem samples in general decreased significantly (P = 0.029) from 10.2 % DM at 45- day period to 6.6 % DM at 100 day under saline-flooded conditions (Table 2).

Similarly, under saline-drained conditions the significant decrease (P = 0.038) was ranged from 10 % DM at 45-day period to 7.6 % DM at 100 days (Table 3). The two factors interacted significantly (P = 0.000) suggesting that at both conditions the effect of exposure period depends on the level of salinity (Table 4) and these results showed a linear relationship between the CP contents and the two different factors.

ADL contents

The ADL contents of leaves significantly increased from45- day period of treatment with the mean value of 10.6 % DM to 12.5 % DM at 100 days period of salt-flooded conditions while under drained conditions, the contents at the first period and those of the second were not significantly different. As the salinity level (2 to 9 dS m^{-1}) increases under flooded conditions, the ADL contents of leaves increased without significant difference at $P < 0.05$ (Table 3). However, the salinity under drained

condition increased significantly (12 to 13.8 % DM) with the increasing level of salinity (2 to 9 dS m^{-1}). There was an interactive effect of salinity under flooded and drained conditions and treatment duration, though their individual effects were not significantly shown.

Regarding the stem parts, ADL contents were consistently affected (P = 0.013) by the interactive effect of salt-flooding and treatment duration. Although the ADL contents increased with increase in salinity level (12.8 to 18.5% DM) and no significant effect of salinity under flooded condition but the treatment period significantly increased (P = 0.007) these contents from 13.5 to 17.6 % DM (Table 3). Under drained condition, the interactive effect of salinity and treatment duration showed no significant difference although salinity significantly affected the ADL contents.

The NDF and ADF contents

The leaf NDF contents varied from an average value of 60.6 % DM at 45- day period of treatment to 64 % DM at 100- day period under salt-flooded conditions and they were not significantly (P = 0.076) affected by the treatment duration at P ≤ 0.05 (Table 2). With an average of 60.9 % DM at 45- day and 64.9 % DM at 100 day period, the treatment period did not significantly affect NDF concentrations. The salt treatment under both conditions also was not statistically pronounced on the leaf contents of NDF. The interactive effects of salinity and period of treatment under both conditions showed no significant difference (Table 4).

For the stems, the NDF concentrations under flooded condition with an average of 65.69% DM at 100 days period were significantly higher (P = 0.03) than the mean of 70.3 % DM of the 45- day period. On the other hand, under drained condition the NDF contents at 45- day period increased significantly at 100 days period. As seen statistically, the salt treatment and; the interactive effects of salinity and period of treatment under both conditions affected the stem contents of NDF.

Concerning the ADF, their highly significant increase of the leaf contents from the 45- day to the 100- day period under saline-flooded condition were noticed (P = 0.000) while the salt level did not affect these contents. The interactive effects of salinity and period of treatment under saline-flooded conditions significantly affected the ADF contents of leaves. Under saline-drained condition, the ADF concentration significantly increased from 45-day to 100-day treatment period (P = 0.004). However, no significant difference was observed among the samples taken from different salinity level treatment at all treatment periods and consequently the interactive effects of salt treatment and their exposure duration were not significant for ADF contents.

On the other hand, under flooded condition the stems ADF concentrations at 45 days period and those taken at 100 days were not significantly different. Regarding the salt effect on the ADF contents, no significant difference was clearly shown while the interactive effect of treatment and period of treatment was also not pronounced. Under drained condition, the stems ADF concentrations at 100-day period were not significantly higher at P < 0.05 than those of 45- day period. However, although statistically the salt treatment significantly affected the ADF concentrations of stems (Table 3), the interactive effects of salinity and period of treatment were not markedly obvious (Table 4).

Dry matter digestibility (DMD), total digestible nutrients (TDN) and metabolizable energy (ME) of the leaf and stem samples of *E. pyramidalis*

The effects of salinity under drained and flooded conditions and the treatment duration on the DMD, TDN and ME levels of leaves of *E. pyramidalis* are illustrated in Table 5. Statistically, the apparent digestibility of DM was slightly comparable among all levels of salinity under flooded and drained conditions (P > 0.05). Although the digestibility coefficients of the 45- day treated samples in both drained and flooded conditions decreased with the exposure duration to treatment, there was no significantly difference with those of 100 days. As treatment interval increased from 45- to 100- day, the rate of reduction of the DDM was 10.7 and 13.3 % respectively under flooded and drained conditions. The same trend for DM digestibility on the leaves was also found on the stems. As can be seen, leaf digestibility was very high-compared to-those of the stems at different salinity levels.

The TDN contents of leaves at the 45 days treatment period ranged from about 43 to 40.7 % DM under flooded conditions and 32.2 to 43.8 % DM under drained conditions whereas after 100 days treatment they varied from 38.8 to 30.3 % DM and about 35 to 28.9 % DM in the same respect order which were somewhat lower. In general, there was no difference between the TDN contents of leaves under the two treatment conditions and the two treatment periods at all salinity levels. With the TDN contents of stems almost at the lowest levels compared to the leaves, the results revealed the same pattern of concentration as those of the latter.

The ME contents of leaf samples ranged steadily around 7.6 to 7.3 MJ/kg and 8.4 to 7.1 MJ/kg from 2 to 9 dSm^{-1} of EC under flooded drained conditions respectively at the first treatment period while those of the second treatment period exhibited a slight reduction with 5.9 to 7.1 MJ/kg and 6.2 to 7.4 MJ/kg in the same order. Statistically, the significant difference (P = 0.009) was observed under flooded condition between the ME values at all salinity levels and treatment periods against no significant difference (P > 0.05) between those of drained condition and; almost between samples grown under drained and flooded conditions from 2 to 9 $dS.m^{-1}$

Table 5. Estimated values of dry matter digestibility (%DM), total digestible nutrients (%DM) and metabolizable energy (MJ/kg DM) of the leaf samples of *E. pyramidalis* grown in the VFCWs treating saline faecal sludge under flooded and drained conditions harvested at two different periods.

Variable		Treatment period I[a]				Treatment period II[b]					
		Level of salinity (Electrical conductivity)									
		T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	F-value	F-prob.
DDM[c]	Flooded	43.57	43.02	41.82	41.47	33.90	37.71	39.42	40.63	2.581	0.104
	Drained	48.05	44.9	43.28	40.39	42.32	39.55	35.87	35.3	2.508	0.111
	F-value	6.351	1.387	0.585	0.027	1.514	0.276	1.088	2.022		
	F-prob.	0.128	0.36	0.524	0.885	0.001	0.652	0.406	0.291		
TDN[c]	Flooded	42.97	41.56	42.33	40.66	30.28	38.81	34.98	35.66	3.961	0.036
	Drained	42.86	43.78	34.78	32.2	33.29	34.98	29.94	28.86	3.385	0.054
	F-value	0.003	10.583	9.03	16.398	21.546	0.996	1.607	3.586		
	F-prob.	0.964	0.083	0.095	0.056	0.043	0.423	0.333	0.199		
ME[c]	Flooded	7.62	7.53	7.32	7.25	5.93	6.6	6.9	7.11	2.711	0.09
	Drained	8.41	7.86	7.57	7.07	7.4	6.92	6.27	6.17	2.534	0.11
	F-value	6.368	1.374	0.566	0.024	0.566	0.273	1.127	2.084		
	F-prob.	0.128	0.362	0.53	0.89	0.53	0.653	0.4	0.286		
DDM[d]	Flooded	34.25	32.83	34.27	37.04	28.03	27.69	29.81	28.42	1.888	0.196
	Drained	25.29	31.62	34.17	44.03	20.51	26.12	32.22	34.64	4.727	0.02
	F-value	450.767	0.294	0.007	1.553	0.784	0.127	0.628	36.464		
	F-prob.	0.002	0.642	0.941	0.339	0.469	0.755	0.511	0.026		
TDN[d]	Flooded	30.74	30.77	34.97	39.14	18.14	23.8	26.83	23.74	5.171	0.017
	Drained	20.17	25.93	26.34	39.18	7.47	18.62	19.84	22.57	9.835	0.002
	F-value	322.531	4.634	8.844	0.00	1.733	1.175	6.496	0.115		
	F-prob.	0.003	0.164	0.097	1.00	0.319	0.759	0.503	0.026		
ME[d]	Flooded	5.99	5.74	6.00	6.48	4.9	4.84	5.21	4.97	1.899	0.194
	Drained	4.42	5.53	5.98	7.70	3.59	4.57	5.64	6.06	4.721	0.022
	F-value	474.019	0.287	0.009	1.54	0.777	0.123	0.657	36.67		
	F-prob.	0.002	0.646	0.931	0.34	0.471	0.759	0.503	0.026		

[a]45-day period of treatment; [b]100-day period of treatment; [c]leaf parts; [d]stem parts; DMD; dry matter digestibility calculated using van Soest (1967) summative equation as follows: DMD = 0.98 ×. CC + NDF (1.473-0.789log ADF)-12.9; TDN (total digestible nutrients) = DMD - ash + (1.25 × E.E.) (van Soest, 1994); ME (metabolizable energy of leaves and stems respectively (MJ/kg DM)) based on in vitro dry matter digestibility (IVDMD) according to the equations developed from MAFF (1984).T_0= 2 dS.m^{-1}, T_1 = 3 dS.m^{-1}, T_2 = 6 dS.m^{-1}, T_3 = 9 dS.m^{-1}.

of EC. Contrary to the stem samples, with the lower ME values compared to leaves, there was a significant difference between samples grown at different salinity levels and periods under drained condition versus no statistical difference under drained conditions.

DISCUSSION

Chemical composition of *E. pyramidalis*

The chemical compositions of antelope grass (NDF, ADF, and ADL) varied within a wide range accordingly to the treatment and treatment period. The NDF, ADF, ADL contents increased from 45 to 100 days periods of treatment under drained and flooded conditions. The results clearly showed that the period of exposure to salt stress or to the combination of salinity with flooding stress affected the ADL, NDF, ADF contents of the different plant parts (Table 2). However, these chemical values are generally comparable to other tropical grasses (Minson, 1990). ADF concentration increased with increasing treatment period which represented about 50 % of NDF concentration. This suggested the highest level of hemi cellulose contents of forage. However, high values of ADF have been reported for *E. pyramidalis* at

different stages of growth in natural environments (Adebowale, 1988).

At 45- to 100 day period, the concentration of NDF of stems of plants grown under the two treatment conditions (65.7 to 72 % DM) surpassed the admissible limit in most ruminant feeding systems whereas those of leaves were within the range of 60 to 65 % DM suggested as the critical threshold level above which efficient utilization of tropical forages by ruminants would be impaired (van Soest, 1982). This indicates that cell wall components were very much higher in stems than leaves and it is probably due to the generally higher proportion of cell wall and the greater lignification of stems (Aman, 1993). The highest fibre concentrations in stems could be explained in part by their anatomical features containing more structural and conducting tissues than leaves compared to the thin-walled mesophyll cells in the leaves. However, the leaf concentrations of fibre were almost higher and this could be also attributed to the level of leaf maturity, since the time of sampling was based on the height of the plant and salt treatment duration rather than on the edible parts. Moreover, like all tropical and C4 photosynthetic pathway forage, *E. pyramidalis* has thinner leaves, more bundle sheaths and smaller interveinal distance with higher hemicelluloses and lignin content (Heckathorn et al., 1999).

In general, higher level of cell wall contents might have resulted from the effect of maturity than on both saline / saline-flooded treatments. This is consistent with findings of previous studies (Suyama, 2007). Many studies have shown that fibre concentrations increased as plants matured (Buxton and Redfearn, 1997). The maturity stage appeared to have induced lignification in the plant. Lignification of plant cell wall at maturity has long been discussed in numerous studies (Buxton and Russell, 1988).

Nutritional quality of *E. pyramidalis*

The CP content showed a consistent decline from 45- to 100 day period of treatment, whereas at different salinity levels, this latter was very much higher in leaves than stems. A similar trend was observed by many authors (Moreira et al., 2004; Tang et al., 2008). This result can be explained by high soluble cell and less cell wall contents of leaves compared to stems. However, the higher CP values at the first period of treatment exposure (45 days) compared to those of the 2nd period (100 days) could probably be due to the fact that the plant parts at the first period were younger compared to those of the 2nd exposure period. This indicates that the treatment exposure period acting as plant age has a pronounced effect on the CP contents of plant parts. It has been shown by many authors that the plant's or plant part's advancing maturity induces reduction of CP concentration and enrichment of their cell wall with the increasing content levels of fibre components (Bayble et al., 2007; Peiretti, 2009). An earlier study on nutritive evaluation of *E. pyramidalis* indicated the decline of CP contents as plant matures with the contents ranging from 9.3 % DM at 3-weeks old to 7.2 % DM at 12 weeks (Adebowale, 1988).

In this study the general decrease of CP contents of plant parts can not be attributed to salinity or saline-flooded treatment effects. The salinity treatment which in our investigations was referred to the treatment under drained condition showed a slight decrease of CP contents with increasing salt concentration. This CP contents (leaf part) decrease was most of the time independent on the degree of salinity since no significant reduction (P = 0.332) of these contents was observed among the plants treated with different salt concentrations. This observation demonstrates that salinity alone does not consistently influence the CP contents of leaves even at 9 dS/m as they were maintained above minimum critical levels (6 to 8 % DM) required for livestock production and proper rumen-bacteria functioning (van Soest, 1994). These results corroborate previous results with Robinson et al. (2004) and Hussain et al. (1995) who demonstrated that there was not direct influence of salinity level on the CP contents of many forage plants (legumes, grasses) that grow under saline conditions.

Contrary to the lone effect of salinity, salinity treatment under flooded condition presented here with *E. pyramidalis* exposed to a combination of salinity (2 - 9 dS m^{-1}) and constant waterlogging appeared to decrease the CP contents of plants significantly. It was obvious that they still maintained their concentrations above minimum requirements of ruminant nutrition as those grown under the lone effect of salinity (van Soest, 1994). This suggested that the dual effect of salinity and waterlogging might have interacted to cause the maximum CP reduction of that caused by salinity alone.

Predicted quality of *E. pyramidalis*

This work showed that *E. pyramidalis* subjected to salinity (2 - 9 dS m^{-1}) and a combination of salinity and waterlogging was not different in terms of nutritive properties such as apparent digestible dry matter (DDM), total digestible nutrients (TDN) and metabolizable energy (ME) between all plant parts (leaves and stems) grown at 2, 3, 6 dS m^{-1} and those grown at 9 dS m^{-1}. However, the overall estimated values of DDM, TDN, ME of plant parts (stem and leaf) for each of the two harvesting periods (45- and 100-days) differed significantly (P = 0.000) among salinity and saline-flooded treatments. In general, these nutritive quality parameters in leaves and stems decrease as treatment period of exposure increases.

The DDM values were similar in both treatment cases (saline drained and saline flooded conditions) but appreciably higher (P = 0.000) at 45 day than at 100 days

period. The DDM of leaves varied from 40.4 to 48 % DM at 45 day while the corresponding value for 100 days period of exposure to treatment ranged from 33.4 to 42.3 % DM. Thus, some values of DDM appear to be slightly less than estimated requirements as DDM values above 45 % DM are considered adequate for high animal performance on pasture (Holt, 1977). At the first treatment period, the TDN contents of leaves ranged from 32.2 to 43.8 % DM while those of the 2nd period significantly decreased from 29.9 to 38.8% DM (P = 0.000). However, TDN appears to be slightly less than estimated requirements for dairy cattle (NRC, 1988).

The estimated ME contents of leaves at the 45 day period ranged from 7.1 to 8.4 MJ/kg DM making them acceptable feeds for beef, cattle, sheep and some classes of dairy cattle. While the ME values of leaves ranged from 6 to 7.4 MJ/kg DM, in general the ME values below 7 MJ/kg DM is considered to be unacceptable for cattle and goats (NRC, 1996). These ME values could be comparable to those (6.9 to 7.6 MJ/kg DM) reported by Al-Masri (2006) for some range plants such as *Enodium cicutarium, Schismus arabiscus, Alhagi camelorum and Salsola vermiculata.*

On the other hand, the stem digestibility of DM, total digestible nutrients (TDN) and metabolizable energy (ME) were lower than for leaves and as the maturity increases. This result is also supported by the fact that leaves have a low content of lignocellulosic materials and are usually more digestible than stems. The overall results corroborated the findings of Lee et al. (1991) and Park et al. (1994) which indicated that the digestibility of DM, total digestible nutrients (TDN) and metabolizable energy (ME) of grasses were higher at the earlier stages of growth and decreased as the plant approached maturity.

Conclusions

These preliminary results indicated that *E. pyramidalis* used in CWs has reuse potentials as forage for cattle production, suggesting the CW capability of producing high quality forage additionally to sanitation treatment and thus, closing nutrient loops. Therefore, the challenge lies in the way of obtaining the reliable forage with minimum health risks and maximum available nutrients for safe reuse in the livestock production. In the socio-economic context of developing countries, the biomass production of *E. pyramidalis* in CWs could guarantee substantial revenue; reducing investment, operational and maintenance costs of sanitation infrastructures and thus, linking sanitation to business opportunities. However, further investigations are necessary to evaluate the digestibility of most nutrients; the harvesting period and the contamination risk of this forage.

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