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The potential of four tropical wetland plants for the treatment of abattoir effluent

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Abstract: We investigated morphological characteristics and treatment potential of *Cyperus papyrus*, *Typha domingensis*, *Miscanthidium violaceum*, and *Phragmites mauritianus* receiving slaughterhouse wastewater in Kampala, Uganda, in experimental mesocosms. Unplanted mesocosms acted as controls. All planted mesocosms achieved significantly higher removals for nitrogen, phosphorus and organic matter than unplanted mesocosms. Among macrophytes, *C. papyrus* depicted highest pollutant uptake. The umbel of *C. papyrus* had the highest concentration of phosphorus (3.9 mg/g dry weight); while nitrogen concentration was highest in *P. mauritianus* shoot tissue (39.70 mg/g dry weight). Plants provided the necessary conditions that aided the removal of nutrients and organics through physical and biochemical processes. *C. papyrus* attained the highest biomass (31.0 kg dry weight/m^2), compared to *T. domingensis* (7.5 kg dry weight/m^2), *P. mauritianus* (7.2 kg dry weight/m^2) and *M. violaceum* (5.0 kg dry weight/m^2). *C. papyrus* had the largest total root surface area (200,634 cm^2) in experimental mesocosms measuring 960 cm^2.

Keywords: *Cyperus papyrus*; *Miscanthidium violaceum*; nutrient removal; *Phragmites mauritianus*; slaughterhouse wastewater treatment; *Typha domingensis*; Uganda.

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1 Introduction

In Uganda, abattoir effluent is one of the major sources of pollution into Lake Victoria (Kyambadde et al., 2006). The effluent has high concentrations of soluble and insoluble organics in the ranges of 7000–16,000 mg COD l^{-1} and originates from two slaughterhouses located along Nakivubo channel in Kampala. Both abattoirs discharge over 700 m^3/day of highly recalcitrant untreated effluent into Nakivubo channel that drains into Lake Victoria at Inner Murchison Bay via Nakivubo wetland. Unfortunately, the natural ability of Nakivubo wetland to treat abattoir effluents including wastes from other industries and surface runoff from Kampala City has significantly reduced due to human activities (Kansiime et al., 2005; Kyambadde et al., 2006). This has led to deterioration of the water quality of Murchison Bay from where drinking water for Kampala City and the neighbouring towns is abstracted (Kyambadde, 2005; Haande, 2008; Larsson et al., 2009).

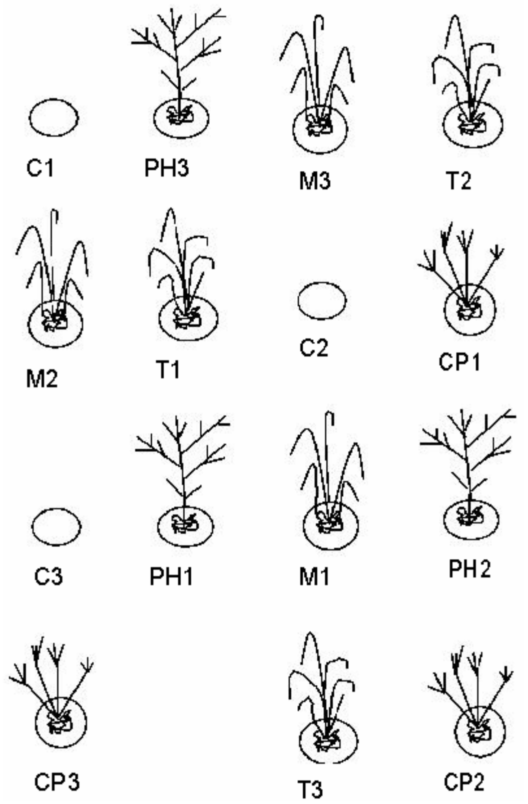
In the past two years, City Abattoir operators in collaboration with Makerere University have carried out technological studies to treat the wastewater before discharge. In this regard, an integrated system comprising of anaerobic and aerobic Sequencing Batch Reactors (SBRs) was tested under laboratory conditions with an ultimate goal of scaling up to treat slaughterhouse wastewater at the source. In the field, the integrated system would be composed of a constructed wetland as the last treatment step. In this study, we investigated different types of aquatic plants that could be used in the constructed wetland. In recent years, several wastewater treatment wetlands have been constructed in East Africa. For instance, municipal wastewater treatment using constructed wetlands has been investigated in Uganda (Sekiranda and Kiwanuka, 1998; Okurut et al., 1999; Kyambadde et al., 2004; Kyambadde et al., 2005) and in Tanzania (Mashauri et al., 2000) and has shown that constructed wetlands can be suitable for wastewater treatment under tropical conditions. In Kenya, the potential application of constructed wetlands in treatment of domestic wastewater (Nyakango and van Bruggen, 1999; Nzengy'a and Witshitemi, 2001), industrial wastewater from pulp and paper production processing (Abira et al., 2003), sugar-milling effluents (Bojcevska and Tonderski, 2006) has also been investigated. Studies on the growth characteristics and nutrient retention of selected macrophyte species using municipal and domestic wastewaters have also been conducted (Okurut et al., 1999; Kansiime et al., 2005; Kyambadde et al., 2005; Mugisha et al., 2007; Kansiime et al., 2007). This study was carried out to assess which one of the four macrophytes, namely *Cyperus papyrus*, *Miscanthidium violaceum*, *Phragmites mauritianus*, and *Typha domingensis* offers the best morphological features which influence physical and biochemical processes that remove pollutants from pre-treated abattoir wastewater. Results from this study could be used to select the wetland plant which offers the most promising morphological conditions that influence physical and biochemical processes for pollutant removal from abattoir wastewater for application in a constructed wetland receiving pre-treated abattoir effluent at City Abattoir, Kampala, Uganda.

2 Materials and methods

2.1 Experimental set-up

The mesocosms receiving pre-treated abattoir effluent were set up at City Abattoir, Kampala, Uganda, in October 2009. Five replicated treatments composed of three planted mesocosms which were randomly placed in the compound of the City Abattoir (Figure 1). The respective diameter and depth dimensions for all cells were 0.35 m and 0.48 m, respectively. Experimental mesocosms were planted with *M. violaceum*, *C. papyrus*, *P. mauritianus* and *T. domingensis*, collected from wetlands bordering Lake Victoria. The planting densities for each mesocosm corresponding to 5.2 kg fresh weight were 145, 135, 93, and 21 plants per square metre for *C. papyrus*, *M. violaceum*, *P. mauritianus*, and *T. domingensis*, respectively. Treatment 1 acted as a control and its mesocosms were not planted. Young plant clones were suspended in the water column by tying them to a network of wooden pegs before the establishment of thick-interlaced root mats that later supported the macrophytes.

Figure 1 Schematic arrangement of mesocosms in experimental set-up at City Abattoir compound, Bugolobi, Kampala, Uganda



Notes: C1: Unplanted mesocosm 1, C2: Unplanted mesocosm 2, C3: Unplanted mesocosm 3, M1: *M. violaceum* mesocosm 1, M2: *M. violaceum* mesocosm 2, M3: *M. violaceum* mesocosm 3, CP1: *C. papyrus* mesocosm 1, CP2: *C. papyrus* mesocosm 2, CP3: *C. papyrus* mesocosm 3, PH1: *P. mauritianus* mesocosm 1, PH2: *P. mauritianus* mesocosm 2, PH3: *P. mauritianus* mesocosm 3, T1: *T. domingensis* mesocosm 1, T2: *T. domingensis* mesocosm 2, T3: *T. domingensis* mesocosm 3.

2.2 Wastewater loading and water quality monitoring

All mesocosms were fed once a week with 15 l of pre-treated wastewater from anaerobic-aerobic SBRs treating slaughterhouse wastewater at City Abattoir, Kampala. Both anaerobic and aerobic SBRs were operated in four sequential phases, namely fill, react, settle, and draw. Effluent of the anaerobic SBR served as the influent to the aerobic SBR; thereafter this effluent was collected and fed into experimental mesocosms. Experimental mesocosms were topped up to the 15 l mark daily with tap water to compensate for water losses due to evapo-transpiration. Before replacing wastewater, physicochemical parameters (pH, temperature, dissolved oxygen, and electrical conductivity) were measured *in situ* and treated wastewater samples taken for nutrient and chemical analyses at the laboratory. Thereafter, plants were gently removed, treated effluent was poured away, mesocosms cleaned and 15 l of effluent wastewater from aerobic SBR added before plants were returned to the mesocosms. Dead roots, leaves, and detritus were removed from plants before placing plants back into the mesocosms.

In order to allow the plants to acclimatise, sampling was started after one month of macrophyte growth. Wastewater in all experimental units was monitored between November 2009 and April 2010 and water samples were collected from the mesocosm once a week before the next feeding regime. The *in situ* measurements for temperature, pH, electrical conductivity (EC), and dissolved oxygen (DO) were determined using HACH, HQ 30d probes. A diazotization method was used to analyse nitrite-nitrogen (NO_2-N), while nitrate-nitrogen (NO_3-N) and total nitrogen (TN) were analysed by Cadmium reduction method and TN using the same method after digestion (APHA, 1995). Ortho-phosphate (*o*-PO_4-P) and total phosphorus (TP) were analysed following the ascorbic acid method and for TP using the same method after digestion. Biochemical Oxygen Demand (BOD) and chemical oxygen demand were determined according to standard procedures (APHA, 1995). All spectrophotometric determinations were made using an Aquamate spectrophotometer (Thermo Electron Corporation, UK Model No. 300).

2.3 Plant growth characteristics and nutrient content

An overview of the rhizomatous habit and the rooting structure of the four plant species is described elsewhere (Azza et al., 2000, for *Cyperus sp.* and *Miscanthidium sp*; Deegan et al., 2007 for *Phragmites sp.* and *Typha sp.*; Tewksbury et al., 2002 for *Phragmites sp.*; and Kim et al., 2003 for *T. domingensis*). Weekly recruitment of main roots and shoots on all plants was examined and newly emerged main roots and newly recruited shoots counted. During examination, plants were carefully removed from the experimental units and weighed using a Salter Samson spring balance (Salter Abbey, West Midlands, UK). Plant weight and height was recorded at the beginning of the study (day 1) and thereafter weekly. Plant root surface area was determined as described by Kyambadde et al. (2004). To determine plant biomass and nutrient content, replicate samples were taken from each of the three mesocosms ($n = 3$, each measuring 0.096 m^2) at the end of the study period (1 April 2010). Plants were separated into below ground (roots and rhizomes) and above ground plant parts (culm, stems, leaves, and papyrus umbel) for analysis. Each sample was cut into small pieces, mixed, sun-dried for one week to reduce the moisture content and later oven dried to constant weight at 103°C for dry weight determination as described by Kyambadde et al. (2005). The biomass for each macrophyte per m^2 was calculated as an average of the values obtained from the three replicate ($n = 3$) mesocosms. Nutrient (nitrogen and phosphorus) content in plant tissues was determined as described by Novozamsky et al. (1983).

2.4 Statistical analyses

MINITAB Release 13.1 statistical software package for Windows was used, and tests included analysis of variance (ANOVA), *F*-test, Levene's test for homogeneity of variance and normality, and Tukey's multiple comparisons for differences between means. A significance level of $p \leq 0.05$ was used.

3 Results

3.1 Physicochemical parameters of influent and effluent wastewater

Changes in wastewater physicochemical variables and removal of nutrients and organics in planted and unplanted control mesocosms are shown in Table 1. Effluent wastewater was slightly acidic (pH 6.73 ± 0.23 and 6.91 ± 0.42) in mesocosms with *C. papyrus* and *T. domingensis*, respectively, while average pH values in *M. violaceum* and *P. mauritianus* were in the neutral range (7.08 ± 0.21 and 7.2 ± 0.23, respectively). Unplanted control mesocosms recorded alkaline pH value (8.41 ± 0.64). One-way ANOVA detected significant difference in water pH ($p = 0.000$) between unplanted control and planted mesocosms.

One-way ANOVA showed that unplanted control mesocosms had significantly higher dissolved oxygen (DO) values in their effluent wastewater than influent wastewater ($p = 0.000$), compared to planted mesocosms which had lower DO values in effluent wastewater. However, Tukey's multiple comparison found no significant difference in the value of DO in effluent wastewater between planted mesocosms.

One-way ANOVA showed that planted mesocosms significantly reduced the values of electrical conductivity in wastewater compared to unplanted mesocosms ($p = 0.000$). Tukey's multiple comparison showed that the reduction in the value of electrical conductivity followed the trend *C. papyrus* = *T. domingensis* > *P. mauritianus* = *M. violaceum*. Generally, there was decrease in temperature in planted and unplanted control mesocosms. One-way ANOVA did not detect significant difference in water temperature between treatments ($p = 0.78$), although planted mesocosms had relatively lower temperatures than unplanted control mesocosms (Table 1).

One-way ANOVA detected significant removal of nitrite-nitrogen (NO_2-N), $p = 0.000$ and nitrate-nitrogen (NO_3-N), $p = 0.000$ in planted mesocosms compared to unplanted control mesocosms in which there was increment in values of both variables. However, Tukey's multiple comparisons did not find significant difference in reduction in the values of NO_2-N and NO_3-N between planted mesocosms. One-way ANOVA indicated significant removal of total nitrogen (TN) in planted mesocosms compared to unplanted control mesocosms ($p = 0.000$). Tukey's multiple comparison showed that removal of total nitrogen (TN) among planted mesocosms followed the trend *P. mauritianus* < *C. papyrus* = *T. domingensis* = *M. violaceum*.

One-way ANOVA indicated that planted mesocosms significantly removed orthophosphate (PO_4-P) compared to unplanted controls ($p = 0.000$). Tukey's multiple comparison showed that removal of orthophosphate among planted mesocosms followed the trend *C. papyrus* > *T. domingensis* = *M. violaceum* = *P. mauritianus*. Results of one-way ANOVA showed that planted mesocosms significantly removed total phosphorus (TP) compared to unplanted control mesocosms ($p = 0.000$). Tukey's multiple comparison indicated that the reduction in the value of TP among planted mesocosms followed the trend *C. papyrus* > *P. mauritianus* = *T. domingensis* = *M. violaceum*.

One-way ANOVA detected significantly higher removal of COD and BOD in planted mesocosms compared to unplanted control mesocosms ($p = 0.000$). However, Tukey's multiple comparison did not detect significant differences in removal of COD and BOD between planted mesocosms.

Table 1 Mean ± standard error of the mean values of physicochemical parameters determined for the mesocosms experiments at City Abattoir, Kampala of three replicate mesocosms for each plant. Variables with the same upper case letters are not significantly different between treatments, while those with different upper case letters are significantly different between treatments ($p < 0.05$)

Treatment	*Parameter*	*Inflow conc.*	*Outflow conc.*	*Removal*	*%Removal*	*Significance*
Control	pH ($n = 14$)	7.48 ± 0.08	8.41 ± 0.16	n.a	n.a	A
	DO (mg/l) ($n = 13$)	3.78 ± 0.31	6.18 ± 1.48[a]	n.a	n.a	A
	EC (µS/cm) ($n = 16$)	914 ± 118	793 ± 102	287.06	31.4	A
	Temp (°C) ($n = 16$)	26.91 ± 0.55	25.16 ± 0.82	n.a	n.a	A
	NO_2-*N* (mg/l) ($n = 20$)	2.17 ± 0.42	4.42 ± 0.62[a]	–2.25	103.76	A
	NO_3-*N* (mg/l) ($n = 15$)	5.14 ± 0.81	5.89 ± 0.50[a]	–0.75	–14.58	A
	T*N* (mg/l) ($n = 9$)	63.8 ± 19.6	58.6 ± 18.70	5.23	8.19	A
	PO_4-P (mg/l) ($n = 17$)	18.88 ± 2.93	17.70 ± 3.10	1.18	6.23	A
	TP (mg/l) ($n = 13$)	33.37 ± 3.84	28.14 ± 3.72	5.23	15.68	A
	COD (mg/l) ($n = 14$)	314.1 ± 34.0	225.3 ± 31.4	88.81	28.28	A
	BOD (mg/l) ($n = 6$)	85.2 ± 11.1	77.33 ± 9.57	7.9	9.27	A
M. *violaceum*	pH ($n = 14$)	7.45 ± 0.08	7.09 ± 0.05	n.a	n.a	E
	DO (mg/l) ($n = 13$)	3.34 ± 1.05	2.06 ± 0.56	n.a	n.a	B
	EC (µS/cm) ($n = 16$)	896 ± 114	539.4 ± 64.9	356.49	39.79	E
	Temp (°C) ($n = 16$)	26.50 ± 0.50	24.06 ± 0.53	n.a	n.a	A
	NO_2-*N* (mg/l) ($n = 20$)	2.20 ± 0.44	0.28 ± 0.04	1.93	87.45	B
	NO_3-*N* (mg/l) ($n = 15$)	4.92 ± 0.77	1.20 ± 0.19	3.72	75.63	B
	T*N* (mg/l) ($n = 9$)	61.4 ± 18.10	32.4 ± 11.00	28.96	47.18	B
	PO_4-P (mg/l) ($n = 17$)	18.67 ± 3.03	12.33 ± 2.44	6.34	33.95	B
	TP (mg/l) ($n = 13$)	32.90 ± 3.43	16.18 ± 1.64	16.73	50.83	B
	COD (mg/l) ($n = 14$)	291.3 ± 28.2	93.9 ± 10.3	197.4	67.76	B
	BOD (mg/l) ($n = 6$)	78.98 ± 8.8	25.01 ± 5.86	53.97	68.34	B

Table 1 Mean ± standard error of the mean values of physicochemical parameters determined for the mesocosms experiments at City Abattoir, Kampala of three replicate mesocosms for each plant. Variables with the same upper case letters are not significantly different between treatments, while those with different upper case letters are significantly different between treatments ($p < 0.05$) (continued)

Treatment	*Parameter*	*Inflow conc.*	*Outflow conc.*	*Removal*	*%Removal*	*Significance*
C. *papyrus*	pH ($n = 14$)	7.42 ± 0.08	6.73 ± 0.06	n.a	n.a	B
	DO (mg/l) ($n = 13$)	3.30 ± 0.31	1.73 ± 0.19	n.a	n.a	B
	EC (μS/cm) ($n = 16$)	879 ± 115	249.9 ± 28.3	629.19	71.56	C
	Temp (°C) ($n = 16$)	26.50 ± 0.50	24.06 ± 0.53	n.a	n.a	A
	NO_2-N (mg/l) ($n = 20$)	2.44 ± 0.57	0.11 ± 0.02	2.33	95.51	B
	NO_3-N (mg/l) ($n = 15$)	4.53 ± 0.75	1.23 ± 0.45	3.3	75.87	B
	TN (mg/l) ($n = 9$)	59.10 ± 16.7	22.87 ± 7.68	36.25	61.32	C
	PO_4-P (mg/l) ($n = 17$)	18.79 ± 2.96	3.23 ± 1.56	15.56	82.8	C
	TP (mg/l) ($n = 13$)	32.82 ± 3.62	8.34 ± 0.91	24.48	74.58	C
	COD (mg/l) ($n = 14$)	323.70 ± 37.1	94.20 ± 14.5	229.55	70.92	B
	BOD (mg/l) ($n = 6$)	87.20 ± 13.0	25.6 ± 5.38	61.63	70.66	B
P. *mauritianus*	pH ($n = 14$)	7.46 ± 0.09	7.20 ± 0.06	n.a	n.a	D
	DO (mg/l) ($n = 13$)	3.31 ± 0.34	1.85 ± 0.46	n.a	n.a	B
	EC (μS/cm) ($n = 16$)	855±103	562.6±28.3	292.29	34.19	E
	Temp (°C) ($n = 16$)	26.63±0.51	24.15±0.63	n.a	n.a	A
	NO_2-N (mg/l) ($n = 20$)	2.52±0.50	0.48±0.10	2.04	80.80	B
	NO_3-N (mg/l) ($n = 15$)	5.28±0.72	2.83±0.63	2.45	46.36	B
	TN (mg/l) ($n = 9$)	61.6±16.7	31.32±7.68	30.31	49.18	B
	PO_4-P (mg/l) ($n = 17$)	19.22±2.88	12.43±2.07	6.79	35.32	B
	TP (mg/l) ($n = 13$)	33.55±3.69	18.21±4.38	15.34	45.72	B
	COD (mg/l) ($n = 14$)	350.0±47.4	87.41±9.46	262.61	75.03	B
	BOD (mg/l) ($n = 6$)	83.15±9.50	23.78±5.28	59.37	71.40	B

Table 1 Mean ± standard error of the mean values of physicochemical parameters determined for the mesocosms experiments at City Abattoir, Kampala of three replicate mesocosms for each plant. Variables with the same upper case letters are not significantly different between treatments, while those with different upper case letters are significantly different between treatments ($p < 0.05$) (continued)

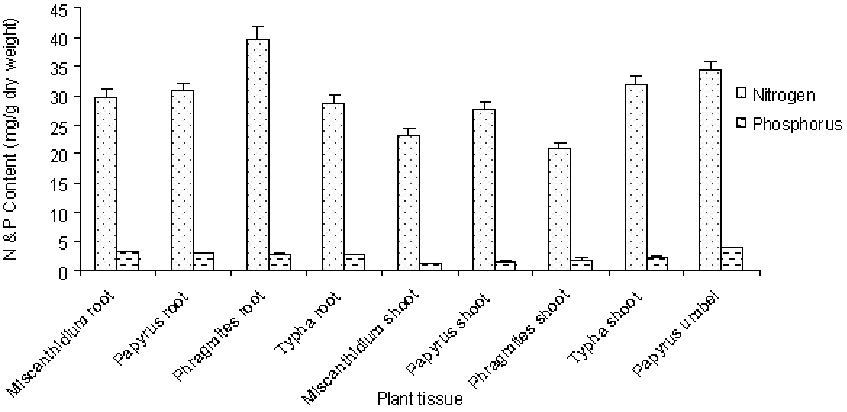
Treatment	*Parameter*	*Inflow conc.*	*Outflow conc.*	*Removal*	*%Removal*	*Significance*
T. *domingensis*	pH ($n = 14$)	7.39 ± 0.07	6.91 ± 0.11	n.a	n.a	E
	DO (mg/l) ($n = 13$)	3.31 ± 0.35	2.37 ± 0.45	n.a	n.a	B
	EC (µS/cm) ($n = 16$)	882 ± 112	397.4 ± 72.3	484.52	54.94	C
	Temp (°C) ($n = 16$)	26.45 ± 0.49	24.13 ± 0.47	n.a	n.a	A
	NO_2-*N* (mg/l) ($n = 20$)	2.43 ± 0.49	0.34 ± 0.06	2.09	86.17	B
	NO_3-*N* (mg/l) ($n = 15$)	5.02 ± 0.66	1.19 ± 0.29	3.83	76.31	B
	T*N* (mg/l) ($n = 9$)	51.7 ± 13.9	26.34 ± 7.47	25.38	49.07	B
	PO_4-P (mg/l) ($n = 17$)	18.02 ± 2.68	10.26 ± 2.27	7.77	43.09	B
	TP (mg/l) ($n = 13$)	31.25 ± 3.69	13.03 ± 1.68	18.22	58.3	B
	COD (mg/l) ($n = 14$)	393.1 ± 63.2	152.3 ± 13.7	240.8	61.25	B
	BOD (mg/l) ($n = 6$)	81.21 ± 9.04	38.64 ± 7.32	42.57	52.41	B

Notes: [a] Increment in the value of the variable. n.a: non applicable.

3.2 Nutrient uptake by macrophytes

Nitrogen and phosphorus content (measured as mg/g dry weight) in tissues of the four plant species is presented in Figure 2. One-way ANOVA did not detect significant difference in nitrogen concentration in shoot tissues of different macrophyte species ($p = 0.127$), although the concentration of nitrogen content in plant shoot tissue was highest in *P. mauritianus* (39.70 mg/g dry weight) and least in *T. domingensis* (28.70 mg/g dry weight). One-way ANOVA did not detect significant difference in nitrogen content in root tissue of different macrophyte species ($p = 0.062$).

Figure 2 Nitrogen and phosphorus content in macrophytes' shoots, roots, and *C. papyrus* umbel at the end of six months study period. Bars show standard errors of the mean value of three replicate mesocosms for each plant ($n = 3$)

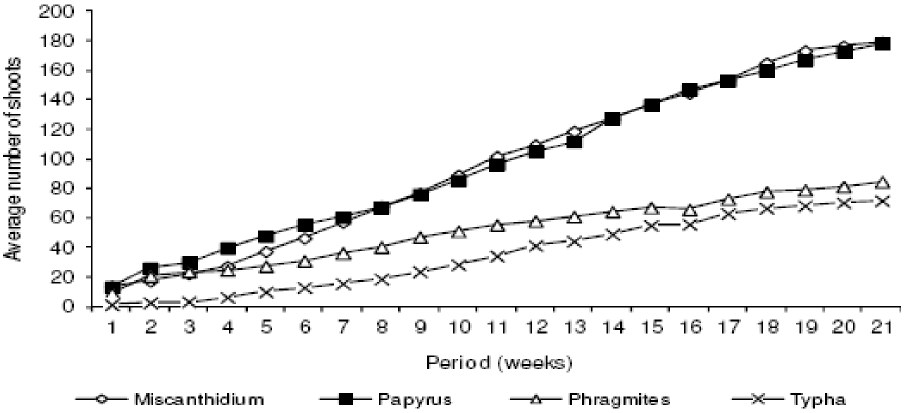


One-way ANOVA detected significant differences in the phosphorus content (measured as mg/g dry weight) in shoot tissues of the different plants ($p = 0.023$). Tukey's multiple comparison showed that phosphorus content in shoot tissues of different plants followed the trend *C. papyrus* umbel > *T. domingensis* = *P. mauritianus* = *C. papyrus shoot* = *M. violaceum*. One- way ANOVA did not detect significant difference in the phosphorus content in root tissue of different plants ($p = 0.191$).

3.3 Growth characteristics, biomass and root surface area of macrophytes

Among the four plants studied, *C. papyrus* acclimatised very well in slaughterhouse wastewater and grew luxuriantly in experimental mesocosms. Roots and culms of *C. papyrus* were tolerant to high concentrations of slaughterhouse wastewater and did not rot. *P. mauritianus* acclimatised slowly in slaughterhouse wastewater and its leaves were vulnerable to infestation by aphids, causing them to often wither. The culms of both *M. violaceum* and *T. domingensis* were prone to rotting when submerged in slaughterhouse wastewater. However, when the plants where placed in such a manner that their culms where just above wastewater level, both plants acclimatised and grew well.

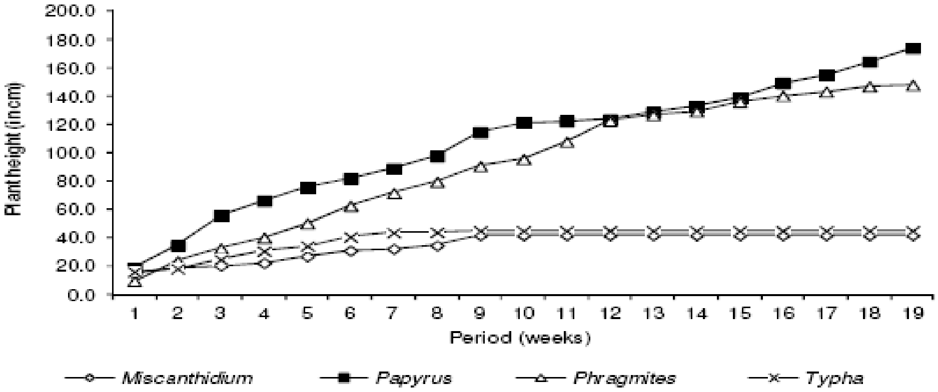
Figure 3 Average weekly recruitment of shoots by *C. papyrus, M. violaceum, P. mauritianus* and *T. domingensis*



Weekly shoot recruitment by the four plant species is presented in Figure 3. One-way ANOVA detected a significant difference in weekly shoot recruitment rate (number of new shoots added per week) ($p = 0.000$) between the four plant species. According to results of Tukey's multiple comparison, the weekly shoot recruitment rate followed the trend *C. papyrus* = *M. violaceum* > *T. domingensis* = *P. mauritianus*.

The average weekly increment in plant height (the weekly growth in height in centimetres) is presented in Figure 4. One-way ANOVA detected a significant difference in increment in height between plant species ($p = 0.000$). Tukey's multiple comparison showed that the trend in weekly increment in plant height was as follows *C. papyrus* = *P. mauritianus* > *M. violaceum* = *T. domingensis*.

Figure 4 Average weekly increment in height for *Cyperus papyrus, Miscanthidium violaceum, Phragmites mauritianus* and *Typha domingensis*



Weekly increment in fresh weight in plants (weight in kilograms added per week) is shown in Figure 5. One-way ANOVA detected a significant difference in weekly increment in fresh weight between the four plant species ($p = 0.000$). Tukey's multiple comparison showed that the weekly rate in increment in fresh weight of plants followed the trend *C. papyrus* = *T. domingensis* > *P. mauritianus* = *M. violaceum*. At the end of 22 weeks of experiments, *C. papyrus* had the highest plant biomass (31.04 kg dry weight/m^2), followed by *T. domingensis* (7.5 kg dry weight/m^2), *P. mauritianus* (7.19 kg dry weight/m^2) and *M. violaceum* (5 kg dry weight/m^2) (Figure 6). However, *P. mauritianus* had the highest dry weight to fresh weight ratio (1:3.57), followed by *C. papyrus* (1:4.46), *M. violaceum* (1:5.77) and *T. domingensis* (1:9.45).

Figure 5 Average weekly increment in fresh weight in *C. papyrus, M. violaceum*, *P. mauritianus* and *T. domingensis*

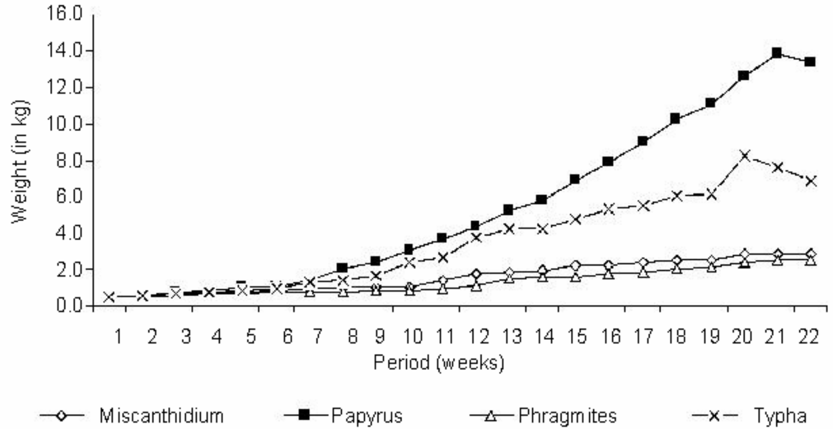
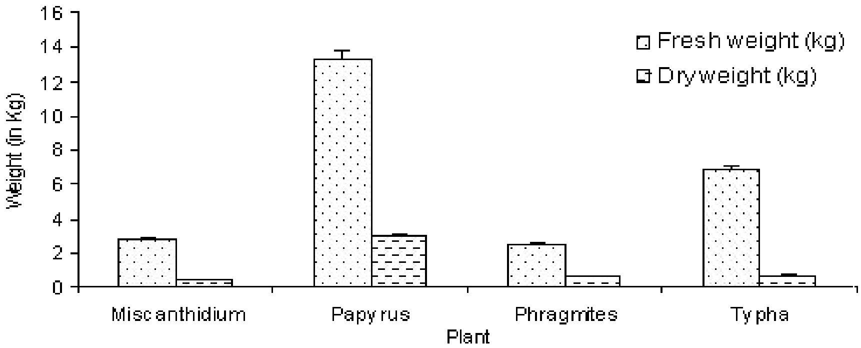


Figure 6 Fresh and dry weight of the four plant species at the end of six months study period. Bars show standard errors of the mean value of three replicate mesocosms for each macrophyte ($n = 3$)



Among the four macrophytes, *C. papyrus* had the largest root surface area in experimental mesocosms (200,634 cm^2) (Table 2). One-way ANOVA showed significant difference in root surface area (cm^2) between plants ($p = 0.000$). Tukey's multiple comparison showed that root surface area in plants followed the trend *C. papyrus* > *P. mauritianus* = *T. domingensis* = *M. violaceum*.

Table 2 Variables used to estimate root surface area of plants, each plant had three replicate mesocosms (n = 3). The root surface area was determined at the end of the experiments, when all plants were removed, roots cut off; and counting and measurements of the different parameters of roots carried out

Parameter	*C. Papyrus*	*M. violaceum*	*P. mauritianus*	*T. domingensis*
Mean number of main roots	5283.7 ± 889.4	1039.7 ± 721.3	395.7 ± 35.1	1052.0 ± 352.0
Mean length of main roots (cm)	24.33 ± 4.041	16.8 ± 1.59	28.67 ± 3.51	20.67 ± 2.52
Mean length of adventitious roots (cm)	3.50 ± 1.0	1.97 ± 0.16	3.0 ± 0.5	2.60 ± 0.53
Mean number of adventitious roots/main root	117.33 ± 7.51	82.67 ± 23.69	103.33 ± 10.41	81.67 ± 81.67
Mean radius of main roots (cm)	0.25 ± 0.05	0.11 ± 0.082	0.32 ± 0.08	0.19 ± 0.06
Mean radius of adventitious roots (cm)	0.04 ± 0.031	0.04 ± 0.017	0.087 ± 0.03	0.06 ± 0.01
Average roots surface area (cm^2)	200,634	14,981	23,282	24,463

4 Discussion

Abattoir wastewater is characterised by high inorganic and organic pollutants in the ranges of 250–5000, 1000–20,000, 150–10,000 and 27–217 (mg/l) for total suspended solids (TSS), COD, TN, and TP, respectively (Massé and Masse, 2000; Mittal, 2006; Li et al., 2008). Discharge of such high-strength wastewater into wetlands without pre-treatment has significant impact on the health of plant systems. When discharged into wetlands which are mostly oxygen-limited, anaerobic processes prevail resulting in acidic fermentation products that affect plant growth (Gersberg et al., 1986; Coleman et al., 2001). In addition, the little available oxygen is used to break down the high quantities of organic matter and thus depriving the system of the required oxygen for nitrification processes and aerobic metabolism of plants (Brix, 1994; Steinberg and Coonrod, 1994; Tanner et al., 1995). Therefore, such high-strength wastewater must be pre-treated in biological systems such as SBRs to reduce the pollutant loads before final cleansing of the wastewater in wetland systems.

Previously, most studies in Uganda focused on testing the feasibility of macrophytes such as *C. papyrus*, *M. violaceum* and *P. mauritianus* for treating domestic and municipal wastewaters (Okurut et al., 1999; Kyambadde et al., 2004; Kansiime et al., 2005). The use of these macrophytes for treating industrial wastewaters, such as slaughterhouse wastewater, was not investigated. The potential for using *T. domingensis* for treating both domestic and slaughterhouse wastewaters had not previously been investigated. Furthermore, no previous studies had attempted to investigate and compare the morphological characteristics of the macrophytes *P. mauritianus*, *C. papyrus*, *M. violaceum* and *T. domingensis* simultaneously to test their ability to remove nutrients and organic matter from slaughterhouse wastewater.

For all quantified physicochemical variables (Table 1), the four plant species performed better in reducing the level of pollution in slaughterhouse wastewater than unplanted control mesocosms. Nutrient and organic matter removal in unplanted control mesocosms, albeit comparably low, was attributable to uptake by photosynthetic algae and settling at the bottom of mesocosms with particulate matter (Sekiranda and Kiwanuka, 1998) in addition to microbial uptake, and nitrification-denitrification processes. In contrast, the high removal of nutrients and organics in planted mesocosms is attributed to the macrophytes which aided the removal of nutrients and organic matter through plant uptake, adsorption and retention of nutrients and organics onto roots, and providing attachment sites for microbial agents responsible for degradation and uptake of organics and nutrients (Kyambadde, 2005).

A slightly acidic pH value (mean 6.73) was recorded in *C. papyrus* planted mesocosms, while *M. violaceum* had neutral pH value (mean 7.08). These values agree with findings of Kyambadde et al. (2004) who recorded pH values in the range of 6.6–6.8 and 7.0–7.1, respectively, for *C. papyrus* and *M. violaceum* planted constructed wetland mesocosms. However, Kipkemboi et al. (2002) found an acidic pH value (5.0) in *M. violaceum* dominated wetlands on the northern shores of Lake Victoria. Slightly acidic pH values recorded in *C. papyrus* mesocosms are attributable to decomposition of wastewater and dead plant materials. The pH in *P. mauritianus* and *T. domingensis* planted mesocosms were in the neutral range (7.0 and 6.91, respectively). Neutral pH range in *P. mauritianus* and *T. domingensis* planted mesocosms agreed with findings of Sekiranda and Kiwanuka (1998) and Finlayson and Chick (1983), respectively.

Evapo-transpiration is a common phenomenon in experimental mesocosms particularly in the tropics. However, water loss in such mesocosms does not lead to loss of nutrients such as nitrogen and phosphorus, but rather concentrates them. Therefore, to compensate for the volume of water lost during the process of evapo-transpiration and reflect the actual concentration of pollutants in the mesocosms, it is essential to add water on a daily basis. This study showed that reduced electrical conductivity was found in planted mesocosms, especially in *C. papyrus* (65%) and *T. domingensis* (55%), compared to unplanted controls (27.3%) was attributable to uptake of ions by macrophytes and precipitation as insoluble compounds (Reddy et al., 1983).

Decrease in temperature in planted mesocosms is attributable to the shading effects of macrophytes. However in contrast to findings of Kyambadde et al. (2004), we did not find significant differences in water temperature between *C. papyrus* and *M. violaceum* planted mesocosms.

High DO concentrations in unplanted control mesocosms were attributable to algae carrying out photosynthesis due to exposure to sunlight since they had open surfaces compared to planted mesocosms (Kyambadde et al., 2004). Decrease in DO values in planted mesocosms was due to its consumption during nitrification and aerobic decomposition of wastewater components and detritus by aerobic bacteria. Whereas macrophytes transfer oxygen from shoots to roots, most of this oxygen is limited to a thin layer of rhizosphere and does not significantly diffuse into the deeper waters (Sekiranda and Kiwanuka, 1998). Alkaline pH (above 7.5) conditions in unplanted control mesocosms might have contributed to nitrogen removal through volatisation, which shifts concentration of ammonium ions (NH_4^+) to ammonia (NH_3) (Sekiranda and Kiwanuka, 1998; Kyambadde, 2005). Nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) removal in planted mesocosms is attributable to nitrification-denitrification processes and plant uptake (Kyambadde et al., 2004). These processes in turn contributed to overall total nitrogen (TN) removal in planted mesocosms. In unplanted control mesocosms, nitrification driven by high concentrations of DO (average of 6.18 mg/l) and conducive temperature (average of 25.1°C) was responsible for high concentration of NO_2-N and NO_3-N observed (Finlayson and Chick, 1983; Vymazal, 1997; Sekiranda and Kiwanuka, 1998). Furthermore, the high concentration of NO_2-N and NO_3-N in unplanted control mesocosms was probably due to the absence of denitrifiers to reduce these ions to nitrogen gas, and lack of macrophytes to uptake them. Higher concentrations of nitrites in unplanted control mesocosms compared to nitrate suggests that nitrification did not fully proceed to nitrates, due to inhibition caused by high-nitrite concentrations (Anthonisen et al., 1976). All four macrophyte species achieved significant removal of TN, the highest removal (63.92%) being observed in *C. papyrus*, which significantly removed TN compared to *P. mauritianus*. However, no significant difference in percentage removal of TN was detected between *C. papyrus*, *M. violaceum* and *T. domingensis*, suggesting that besides plant uptake, environmental conditions offered by plants such as attachment sites for periphyton (e.g. nitrifiers and denitrifiers) facilitate nitrogen removal (Anthonisen et al., 1976). Removal of TN in a *T. domingensis* planted mesocosms (47.85%) was comparable to findings of Finlayson and Chick (1983) who reported 42% removal for TN in *T. domingensis* wetland unit treating poultry slaughterhouse wastewater. However, we achieved comparatively less removal for TN in *P. mauritianus* planted mesocosms (44.56%) (attributable to floating conditions of our experiments); while Finlayson and Chick (1983) achieved 62% removal under subsurface conditions.

Nitrogen removal in constructed wetlands is partly attributable to filtering of organic matter by gravel and subsequent decomposition and recycling, resulting in a lower TN value in the final effluent (Finlayson and Chick, 1983). Macrophytes translocate oxygen from shoots to the rhizosphere, thus supplying nitrifiers and heterotrophs with their oxygen requirements for metabolism during nitrification processes and organic matter degradation (Finlayson and Chick, 1983). Further, mineralised constituents of organic matter are taken up by both periphyton and macrophytes. Root surface area and structure influence microbial attachment, retention of suspended solids, residence time, adsorption, nutrient transformation and organic matter decay in wetlands (Gersberg et al., 1986; Coleman et al., 2001). Discharge of substances such as sugars, amino acids, and organic carbon by roots in wetland units may also increase microbial activity hence wastewater degradation (Coleman et al., 2001).

Average values of effluent TP (8.34 mg/l) and PO_4-P (3.23 mg/l) from *C. papyrus* planted mesocosms were below the discharge limit of 10 mg/l and 5 mg/l, respectively, allowable by Uganda's National Environment Management Authority (NEMA, 1999). Although the other three macrophyte species, attained high percentage removal of TP, the value in the effluent was higher than the accepted discharge limit. The removal of TP (72.4%) in *C. papyrus* planted mesocosms was comparatively lower than that achieved (83.2%) in experiments by Kyambadde et al. (2004) treating secondary effluent from the municipal sewerage treatment plant in Kampala. The percentage TP removal in *P. mauritianus* (38.23%) was comparable to that achieved (37%) by Finlayson and Chick (1983) in planted gravel trenches treating poultry slaughterhouse wastewater. Removal of TP and PO_4-P in our planted mesocosms could have been through precipitation, adsorption onto plant roots, plant uptake and microbial uptake (Okurut et al., 1999; Kyambadde et al., 2004). Since our experimental design was a floating type, phosphorus removal processes by precipitation onto the substratum containing ions (such as Fe^{2+}

and Mg^{2+}) could not have influenced removal (Okurut et al., 1999; Kyambadde et al., 2004). In unplanted controls, removal of TP and PO_4-P might have taken place through immobilisation by microorganisms and settling with particulate matter onto the mesocosm bottom (Sekiranda and Kiwanuka, 1998).

We found higher concentrations of nitrogen than phosphorus in plant tissue, confirming findings of van Dam et al. (2007). The highest nitrogen content in plant tissues was found in roots of *P. mauritianus*, while the *C. papyrus* umbel was found to have the highest phosphorus content (Figure 2). Mugisha et al. (2007) found high concentrations of nitrogen and phosphorus in *C. papyrus* umbel, whereas Muthuri and Jones (1997) found the highest concentration of phosphorus in *C. papyrus* rhizomes rather than the umbel. Since *C. papyrus* umbels are both photosynthetic and inflorescence organs, they have higher metabolism than other plant parts (such as culms and roots), hence requiring more nutrients (Mugisha et al., 2007). *C. papyrus* is more efficient in utilising nitrogen, compared to other macrophytes, because of the presence of a C_4 photosynthetic metabolism, which leads to very high rates of productivity (Jones and Muthuri, 1997). Furthermore, the nitrogen concentrations in plant tissue are found to be higher in younger growing plants and to decrease with increasing age (Muthuri and Jones, 1997).

Reductions of both BOD and COD values in planted mesocosms were attributable to degradation of organic matter by microorganisms associated with roots. Percentage COD removal for both *C. papyrus* and *P. mauritianus* (> 70%) was in the range reported by Okurut et al. (1999). Except *T. domingensis*, all macrophyte species achieved reductions of COD values below the Uganda Government's allowable discharge limit of 100 mg/l (NEMA, 1999). All four macrophyte species attained BOD removal rates below the allowable discharge limit of 50 mg/l (NEMA, 1999). High dissolved oxygen concentration in unplanted control mesocosms enabled microorganisms to decompose and reduce COD and BOD by 26% and 9%, respectively, but both values (225.3 mg/l COD and 77.33 mg/l BOD) were higher than allowable discharge limits.

Of the four macrophyte species investigated, *C. papyrus* exhibited superior growth characteristics with regard to increment in weight and height, and recruitment of roots and shoots (Figures 3–5 and Table 2). Also, *C. papyrus* easily acclimatised in experimental mesocosms, was least vulnerable to high nutrient and organics concentration and aesthetically looked most appealing (green and luxuriant). Within a period of 12 weeks, *C. papyrus* had filled the 30 l experimental mesocosms and it had the highest biomass (31.04 kg dry weight/m^2) at the end of the 21 weeks of study. The biomass of *C. papyrus* in our study (31.04 kg dry weight/m^2) was higher than the findings of Boar and Harper (1999) (11.54 ± 3.02 kg dry weight/m^2) and Jones and Muthuri (1997) (7.8 kg dry weight/m^2) in natural wetlands fringing Lake Naivasha, Kenya. Furthermore, *C. papyrus* is found to be the most dominant macrophyte, covering most areas of natural wetlands of Lake Victoria (Jones and Muthuri, 1997; Muthuri and Jones, 1997). L. Victoria riparian communities harvest and use papyrus culms for several purposes including as an energy (fuel) source (Jones, 1983), for handicrafts such as mats and baskets, and for fibres (ropes), thatching roofs, and potentially it may be used for making paper and as animal fodder (Owino and Ryan, 2007). Ecologically papyrus wetlands are net carbon sinks (Jones and Muthuri, 1997) and serve as habitat for various fauna, especially birds besides offering beautiful scenery.

In contrast to the other three plants, *C. papyrus* supports a highly branched inflorescence (umbel), which is also photosynthetic. The umbel therefore offers additional sites for photosynthesis and removal of nitrogen and phosphorus compared to *T. domingensis*, *P. mauritianus* and *M. violaceum*. Increment in weight in *T. domingensis* closely followed that of *C. papyrus* and by week 12 of the study, the plant had also filled experimental mesocosms. *T. domingensis* had very large fleshy culms and long lush leaves, but in contrast to *C. papyrus*'s maximum recorded culms height of 1.74 m, *T. domingensis* maximum culms height was only 0.45 m. Similar to *T. domingensis*, *M. violaceum* had a short culm height (0.42 m). The rate of shoot recruitment in *M. violaceum* was high and comparable to that observed in *C. papyrus*. However, because *M. violaceum* culms are thinner and grass-like, its biomass (5 kg dry weight/m^2) in planted mesocosms was low in comparison to *C. papyrus* (31.04 kg dry weight/m^2).

Increment in culm height in *P. mauritianus* was high and comparable to that observed in *C. papyrus*. However, *P. mauritianus* took long to establish in experimental mesocosms and its shoot recruitment rate was low; hence it attained comparably lower weight than *C. papyrus*. Several environmental stressors, such as low oxygen supply to roots and rhizomes, high ammonium ion concentrations, and fluctuations in water levels, are reported to seriously affect growth of *P. mauritianus* (Dinka et al., 2008). When submersed completely in wastewater, roots of *C. papyrus* proliferated easily and were not prone to rotting under high nutrient and organic matter concentration. In contrast, *M. violaceum*, *P. mauritianus* and *T. domingensis* roots often got rotten when fully submersed in wastewater; thus these macrophytes had to be suspended using strings so that their roots hung just below the water level.

Among the four macrophyte species, *C. papyrus* had the highest root surface area (Table 2). The root network in *C. papyrus comprises* rhizomes, main and adventitious roots, which were much intertwined and completely filled the bottom of the mesocosms. According to Kipkemboi et al. (2002), under high nutrient concentrations, *M. violaceum* plants possess numerous and short roots, but have fewer and longer roots under low nutrient conditions. In contrast, *C. papyrus* does not show any significant variation in root length and numbers under different nutrient concentrations (Azza et al., 2000; Kipkemboi et al., 2002).

5 Conclusions and recommendations

All four macrophyte species (*C. papyrus*, *T. domingensis*, *M. violaceum* and *P. mauritianus*) studied have high potential in improving the quality of secondary treated slaughterhouse wastewater. Decrease in nutrients in effluent wastewater is attributable to plant uptake, nitrification-denitrification processes (for nitrogen) and adsorption onto plant roots (for phosphorus). Overall, *C. papyrus* achieved the highest rate of removal for all variables, due to its superior growth characteristics such as high biomass and root surface area and possessing inflorescent and photosynthetic umbel. Morphologically, *C. papyrus* and *T. domingensis* looked most luxuriant and aesthetically pleasing. Although *M. violaceum* had a superior rate of shoot recruitment, it did not lead to superior fresh weight increment because its shoots are grass-like and do not grow very high. *P. mauritianus* established at a slow rate in experimental mesocosms and it had a comparatively low rate of weight increment.

Whereas it is evident from our investigations that *C. papyrus* performs best in improving quality of pre-treated slaughterhouse wastewater, further experiments to determine its most optimal operating conditions such as hydraulic loading rate and role of gravel in the treatment process still have to be investigated. There is an urgent need to scale up the findings of this study so as to treat slaughterhouse wastewaters (and related agro-based wastewaters) in constructed wetlands using *C. papyrus* as a final treatment step. The Government of Uganda and relevant agencies such as the National Environment Management Authority (NEMA) should encourage proprietors of agro-based industries to take advantage of the potential of *C. papyrus* in the treatment of their wastewaters. This would lead to reduction in pollution in Murchison Bay, Lake Victoria caused by discharge of untreated wastewaters from agro-based industries in the precincts of Nakivubo wetlands.

Besides the primary role *C. papyrus* would serve in treating agro-based industrial wastewaters, when harvested, their culms would serve other useful roles including as material for making handcrafts (such as mats and baskets) for thatching roofs, fuel in cooking, beautiful scenery, and habitat for fauna, especially birds.

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