

NUTRIENT UPTAKE EFFICIENCY AND GROWTH OF TWO AQUATIC MACROPHYTE SPECIES UNDER CONSTRUCTED WETLANDS, ETHIOPIA

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ABSTRACT: This study was carried out to investigate the growth and nutrient uptake of two wetland plants from Lake Tana. Rhizomes of *Cyperus papyrus* and *Phragmites karka* were grown outdoors in four parallel-aligned horizontal sub-surface flow constructed wetland (HSSFCW) treatment beds. The treatment beds were irrigated with wastewater sourced from students' residence from January 21 to March 20, 2011. The results of the study showed that *Cyperus papyrus* had higher rate of biomass accumulation as evidenced by increase in shoot and root weights (83.93 gm) compared to *Phragmites karka*. It had also significantly ($p < 0.05$) higher root total phosphorus and leaf total nitrogen content than that of *Phragmites karka*. The mean removal efficiency of the *papyrus*-planted treatment bed was 56.37% ($\text{NO}_3\text{-N}$), 84.04% (PO_4^{3-}); the *phragmites*-planted treatment bed was 58.37% ($\text{NO}_3\text{-N}$), 65.18% (PO_4^{3-}) and the unplanted (control) treatment bed was 36.13% ($\text{NO}_3\text{-N}$) and 50.21% (PO_4^{3-}). Pollutant removal efficiency differences were statistically significant ($p < 0.05$) between planted and unplanted treatment beds for PO_4^{3-} but not for $\text{NO}_3\text{-N}$. The study also showed that the progressive increase in the plant density, shoot length and stem diameter was positively correlated with the nutrient removal efficiency of the treatment beds. The average removal efficiency of the two planted treatment beds was higher than unplanted bed. The study proved that these macrophytes had the ability to accumulate high biomass and remove nutrients and therefore have high potential in biological nutrient removal processes.

Key words/phrases: *Constructed wetlands, Cyperus papyrus, nutrient uptake, Phragmites karka, wastewater treatment*

INTRODUCTION

Excessive nutrient enrichment is one of the most serious threats to wetland ecosystems. Treatment is necessary to improve wastewater quality in such a way that the use of final disposal of the treated effluent can take place in accordance with the rules set by the relevant legislative bodies without causing adverse impacts on receiving water bodies. Constructed wetlands are nowadays considered as low-cost alternative for effective wastewater treatment (Vymazal, 2002), especially in developing countries like Ethiopia where suitable land can be available. Macrophytes are an active component of horizontal subsurface flow constructed wetlands (HSSFCW) (Vymazal, 2011). Wastewater treatment is accomplished through the integrated combination of physical, biological and chemical interactions among biotic and abiotic components of the ecosystem and macrophytes cultivated in constructed wetlands make one of the basic components in the treatment process. They influence plant-microorganisms wastewater interactions by providing microbial attachment sites, sufficient wastewater residence time, trapping and settlement of suspended wastewater

components as a result of resistance to hydraulic flow, surface area for pollutant adsorption, uptake and storage in plant tissues, and diffusion of oxygen from aerial parts to the rhizosphere (Kyambadde *et al.*, 2005).

To evaluate the potential application of a macrophyte in wastewater treatment constructed wetlands, knowledge of structural development and recruitment rates of new shoots and the general growth rate of the macrophyte in question is crucial (Hoffmann and Platzer, 2010). Lack of this knowledge has been the most frequently reported problem for the failure and poor survival of plants in treatment wetlands (Kadlec and Wallace, 2009). There are some comparative studies, for different set of species, but they are not always conclusive (Coleman *et al.*, 2001; Sim *et al.*, 2011; Lu *et al.*, 2012). Moreover, species applicability can change with latitude and local climatic condition. There are also research works in treatment wetlands in Ethiopia with different wastewater types and different plant species (Asaye Ketema, 2009; Tadesse Alemu, 2010; Girum Feleke, 2011; Kenatu Angassa, 2011). However, these studies did not investigate the frequently used macrophytes species in HSSCWs (*Phragmites*

karka and *Cyperus papyrus*) using domestic wastewater. None of these studies also investigated growth parameters and biomass production in relation to nutrient uptake of these plants. Therefore, rigorous comparison studies under controlled conditions are necessary to evaluate the potential of these species for wastewater treatment using HSSFCW system. Hence, the main objective of this study was to determine the nutrient uptake and growth characteristics of *Cyperus papyrus* and *Phragmites karka* and also assess the potential use of these macrophytes in domestic wastewater treatment using a laboratory scale HSSFCW system.

MATERIALS AND METHODS

Experimental design

The experimental study was conducted in a laboratory-scale HSSFCW system at Addis Ababa University, College of Natural Sciences campus. The system consists of four analogous treatment beds aligned in parallel and is designed with an average wastewater flow-rate of 26 L/d (0.026 m³/d) measured using bucket and stop watch method (EMB-DENR, 2008) and a theoretical hydraulic residence time (HRT) of 7 days. The substrate or plant growth media used in this HSSFCW system was 20 to 30mm diameter sized gravel which is the recommended gravel size by USEPA (2000). The substrate was filled to a height of 0.5 m. Fragments of rhizomes about 10 cm long carrying young shoots of *C. papyrus* and *P. karka* plants selected according to Hoffmann and Platzer (2010) were taken from the natural wetlands of Lake Tana and transplanted into their respective treatment beds with surface area of 1.3 m² at a density of 6 rhizomes /m². The first and the second treatment beds were planted with *P. karka*; the third was planted with *C. papyrus*; and the fourth treatment bed was left unplanted to serve as a control. Each treatment bed was fed with the influent wastewater with the same average flow rate from equalization tank through pipes after 3 weeks acclimation period of the liquid waste. Wastewater used in the study was collected from a small primary treatment plant (oxidative pond) receiving domestic liquid wastes from the students' residence at the College.

Data collection from the treatment beds

Measurement of the growth parameters of the plants

Plant density in the treatment bed, plant height, stem diameter and biomass were considered as

growth parameters of the young plants. Except biomass, plant density, plant height and stem diameter were measured five times at two weeks intervals. Plant density in the treatment beds was obtained by counting all the plants including each independent morphological unit arising from rhizome as an individual macrophyte as stated in Pompeo and Moschini-Carlos (1996). Shoot length and stem diameter were measured on 15 randomly selected and tagged plants in the treatment bed using a graduated rope. The aboveground and belowground biomasses were measured after harvesting selected individual plants at the end of the experiment. Plants from each cell were harvested from 30 cm × 30 cm quadrat thrown at two corners and at the center of each wetland plot. The selected plants from each cell were put in plastic bag and brought to laboratory to estimate the standing biomass following the methodology described by Silva *et al.* (2010). Plants were divided into three components before drying and dried separately in hot oven at 105°C for 24hrs until constant weight and the dry weights were determined.

Measurement of plant nutrient content

Prior to nutrient analysis; leaf, stem and root samples of each plant species were pulverized and made into fine powder. The powder of each sample was used for total nitrogen (TN) and total phosphorus (TP) analysis by Kjeldahl method as stated in Blamire (2003) and by dry-ashing method, respectively. The molybdo-vanadate method (Ammonium Vanadate-Ammonium molybdate) was used to determine the phosphorus content following the procedures of Zhu *et al.* (2011), with slight modification in such a way that prior to application of the molybdo-vanadate method, the samples were first ash-dried to get complete digestion.

Analysis and measurement of physico-chemical parameters of water quality

Samples for physico-chemical parameters of water quality were collected from five different points labelled as S1, S2, S3, S4 and S5 as shown in Figure 1. S1 represents sampling point for the influent waste water. S2, S3, S4 and S5 represent sampling points for the treated effluent coming out of treatment beds 1, 2, 3 and 4, respectively. Samples were taken over the period of six weeks after established plants grew fully and extended from January 21 to March 20, 2011.

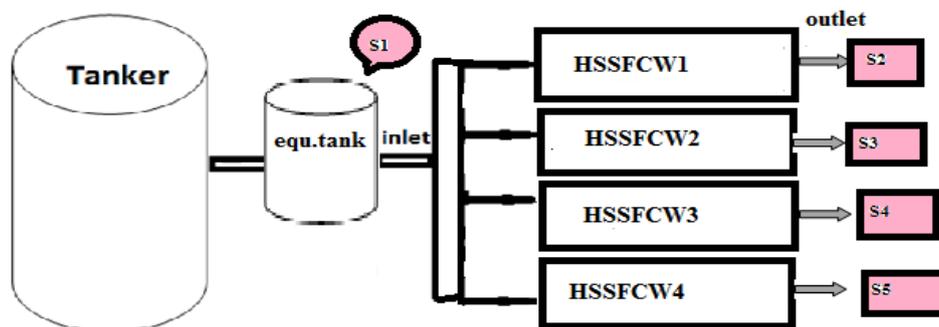


Figure 1. Setup of the constructed wetlands showing the five sampling sites.

The parameters electrical conductivity (EC), pH and temperature (T°) were measured on-site at the inlet and outlet using portable EC meter (YSI model 30), and pH/ T° meter (Hanna 9024), respectively. Nutrient analysis ($\text{NO}_3\text{-N}$ and PO_4^{3-}) was made after filtering the water sample through glass fiber filters (GF/F) using standard procedures as indicated in Yang *et al.* (1998) and APHA *et al.* (2005), respectively, using a spectrophotometer (Jenway 6405 UV).

Though the experiment was conducted in the four analogous parallel – lined treatment beds, the second treatment bed failed due to cracking problem; so the results and discussion refer only to the three functional treatment beds. The removal efficiency of the treatment beds for each wastewater quality parameter was calculated using the following formula:

$$\text{Removal Efficiency (\%)} = [(C_i - C_e) / C_i] \times 100$$

where,

C_i is the concentration of the waste material in the influent

C_e is the concentration of the waste material in the effluent

Statistical analysis

The data were analyzed through one-way analysis of variance (ANOVA) at 95% confidence level to compare the performance efficiency of each treatment bed with respect to removal of $\text{NO}_3\text{-N}$, PO_4^{3-} , EC and pH using Statistical Package for Social Sciences (SPSS) software, Version 15.0.

RESULTS AND DISCUSSION

Growth characteristics of the plants

From a density of 6 rhizome fragments per m^2 at the start of the study, 41 plants per m^2 were obtained after 3 weeks acclimation in the treatment

bed planted with *C. papyrus* and 16 plants in the treatment bed planted with *P. karka*. Where the system was continuously fed with primary treated wastewater; a density of 81 plants per m^2 was recorded for *C. papyrus* and 51 for *P. karka* (Fig. 2a) during the last experimental period in the treatment beds. Statistical analysis (one-way ANOVA) and independent samples test showed that there was significantly higher ($p < 0.05$) mean plant density of *C. papyrus* than that of *Phragmites* species. Plant height growth slightly increased in both species with increasing time, (Fig. 2b). There was no significant difference ($p > 0.05$) in plant height growth between the two plant species, but there was somewhat a slightly higher growth rate of *C. papyrus* with mean plant height of 21.61cm than in *P. karka* with mean plant height of 20.59cm. Stem diameter of *C. papyrus* started to increase immediately after the second week while that of *P. karka* started to increase after the fourth week (Fig. 2c). The mean diameter increment was slightly higher for *C. papyrus* ($\bar{x} = 2.28$ cm) compared to *P. karka* ($\bar{x} = 1.97$ cm), but the difference was not statistically significant ($p > 0.05$).

The results of growth response conditions in the present study indicate that these plants showed practical withstand of shock loads and possibly grow very well which is one of the criteria for selecting plants to use in constructed wetlands for wastewater treatment. The dry biomass estimates per 30 cm^2 area of the experimental treatment beds is presented in Table 1. *P. karka* had significantly higher ($p < 0.05$) leaf dry weight with a mean of 13.74 gm compared to *C. papyrus* with a mean leaf dry weight of 8.49 gm. The biomass variations of wetland plant species may be due to the plant species as well as physiological and morphological characters of the plants (Ma *et al.*, 2010; Zhu *et al.*, 2011). The larger leaf area for photosynthesis in *Phragmites karka* could be one of the reasons for

more biomass in the species. Although there was no meaningful difference ($p>0.05$) between the aboveground biomasses in the two wetland plants,

the underground biomass estimate was significantly higher for *C. papyrus* ($p<0.05$) (paired samples T-test statistics).

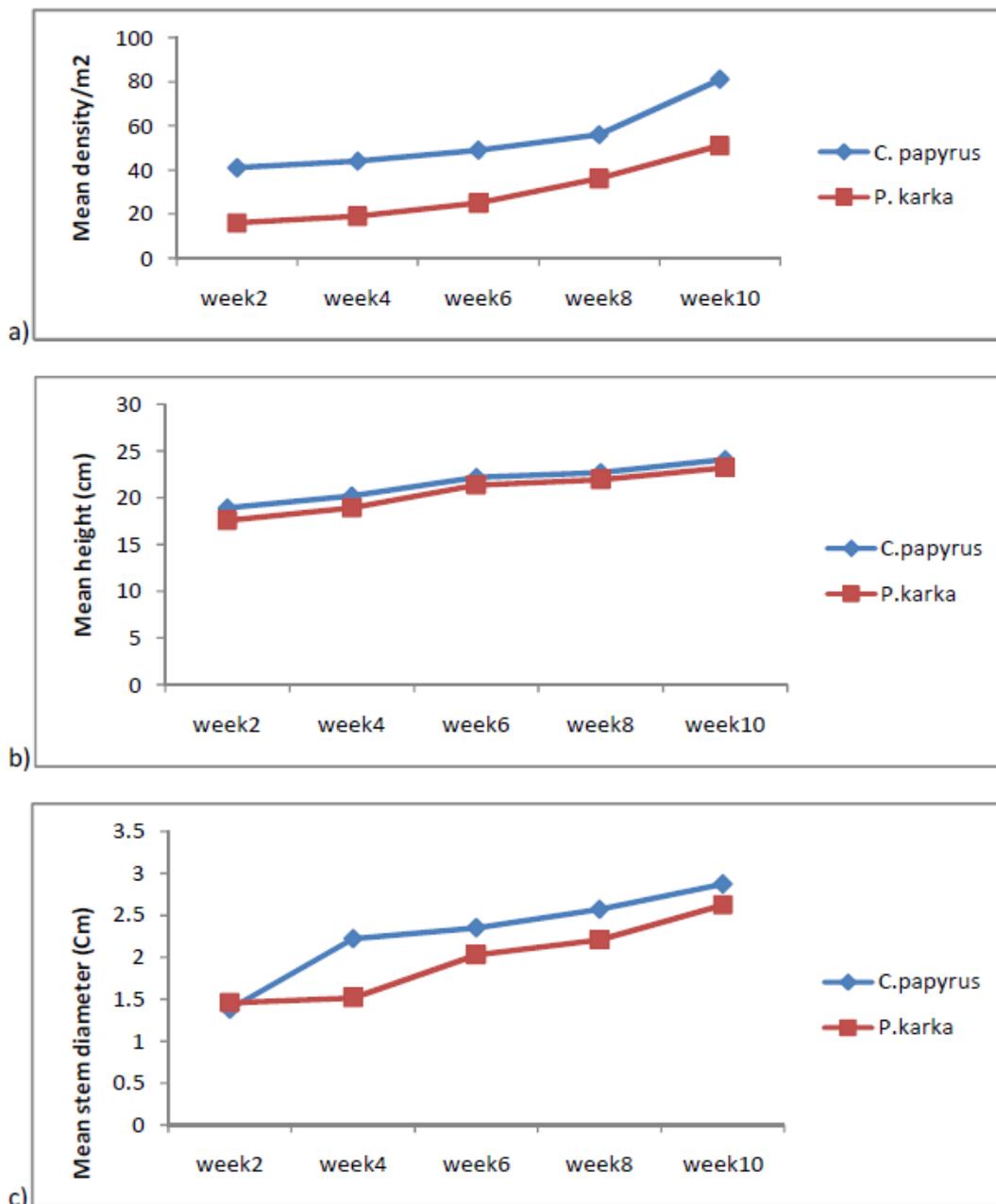


Figure 2. Growth parameters during the experimental period.

Table1. Mean Leaf, Stem and Root Biomass measurements of macrophytes in the treatment beds per 30 cm² area.

Plant part	Average Biomass (gm)	Std. Deviation	Std. Error of the Mean
<i>Papyrus</i> leaf	8.4900	0.03000	0.01732
<i>Papyrus</i> stem	38.1700	0.02000	0.01155
<i>Papyrus</i> root	37.2700	0.04000	0.02309
<i>Phragmites</i> leaf	13.7400	0.05000	0.02887
<i>Phragmites</i> stem	26.6000	0.30000	0.17321
<i>Phragmites</i> root	16.4000	0.40000	0.23094

Plant tissue nutrient analysis

Comparative studies of different parts of the two species (Fig. 3) showed that *C. papyrus* had higher leaf nitrogen content ($\bar{x} = 3.26\% \text{ N}$) compared to *P. Karka* ($\bar{x} = 2.82\% \text{ N}$). There were significant differences ($p < 0.05$) in nitrogen content within the different body parts of the two species. In many instances, nitrogen content of leaves was significantly higher ($p < 0.05$) than roots. This is consistent with studies made by several authors who have reported that nitrogen content of leaves was higher than roots (Mars *et al.*, 2003, Kyambadde *et al.*, 2005, Mugisha *et al.*, 2007). This is because photosynthetically active organs (leaves) generally have higher nitrogen content than other organs (rhizome and roots) under optimal growth conditions. There were significant differences ($p < 0.05$) in root, leaf and stem phosphorus content between the two species and among plant parts. *C. papyrus* had slightly higher root phosphorus content than *P. karka* (Fig. 3). It was found that phosphorus concentration in *C. papyrus* was highest in root followed by stem and leaf. This is because most of the phosphorus was absorbed by plant root

and absorption through the leaves and shoot was restricted, so phosphate was easily concentrated in the belowground tissue. Generally it was noted that total nitrogen content in both plant species followed the order: Leaf > Root > Stem while total phosphorus showed the order: Root > Stem > Leaf. The study also revealed that macrophytes had higher nitrogen concentration than phosphorus and similar result was observed by Mueleman *et al.* (2002). This is a consequence of the Redfield ratio that quantifies the molar ratio of elements as follows: C/N/P = 106/16/1.

Sim *et al.* (2011) studied macrophytes in a subtropical/tropical climate and reported only slight variability between nutrient content in different plant components (root, rhizomes, stem, and leaves), with nitrogen generally highest in the leaves and phosphorus highest in roots. Similarly, a study by Mars *et al.* (2003) stated that phosphorus storage was highest in belowground components with lowest amount of phosphorus storage in leaves, suggesting that there is translocation of nutrients towards young tissues where climatic conditions are conducive to year-round growth.

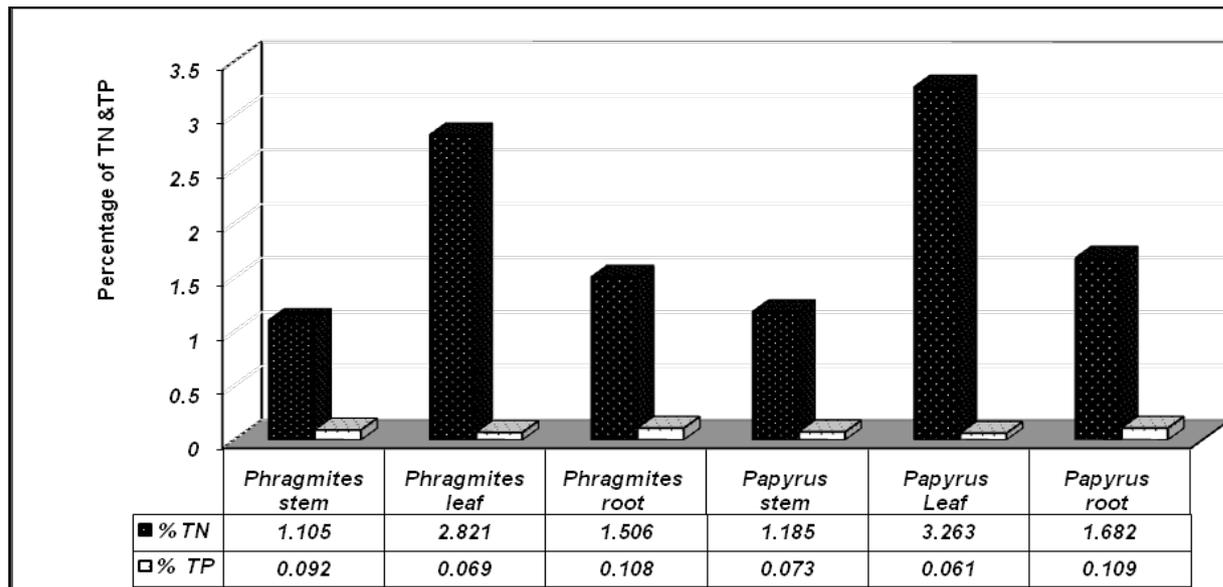


Figure 3. Comparisons of TN and TP content in plant parts.

Effluent and influent characterization of domestic wastewater

Table 2 presents a summary of the physico-chemical characteristics of the effluent monitored at the inlet and the outlets of the treatment beds. Water quality monitoring in the systems revealed that physicochemical characteristics of the effluent progressively improved because a dramatic decline was recorded from the inlets to the outlets of the experimental treatment beds. EC, PH and T° mean influent values were 667.85µS/cm, 7.63 pH units and 17.2°C, respectively. pH values were in the range of 7.51–7.66 and 7.55–7.82 in planted and control treatment, and the *papyrus*-planted treatment bed showed lowest pH value (7.51) at the end of experiment. This may be because plants take up significant amounts of sparingly soluble nutrients from the rhizosphere by acidifying the rhizosphere (Rao *et al.*, 2002) via excreting H⁺ in exchange for cat-ions and exuding organic acids and CO₂ (Hinsinger *et al.*, 2003). The control treatment bed showed slightly higher pH than planted treatment beds. Similar result was recorded by USEPA (1999), Coleman *et al.* (2001) and Lin *et al.* (2002) and could be due to algal growth which was observed at the surface of control set. The algae have the effect of absorbing CO₂ faster than it can be replaced by bacterial respiration. This has the effect of leaving excess hydroxyl ions which cause sudden rises in the pH to around 7.82 (Lin *et al.*, 2002).

The high electrical conductivity value could be attributed to the presence of various types of ions

in the wastewater. There were only slight differences in temperature and pH between influent and effluents from the three treatment beds, indicating the absence of any thermal pollution. Analytical results of ANOVA and least significant difference (LSD) confirmed that values of T°, pH and EC had no statistically significant differences ($p > 0.05$) between planted and unplanted treatment beds. Effluents from all the treatment beds were within the range of the Ethiopian effluent standard limits for T°, EC and pH which are 40°C, 1000µs/cm (at 20°C) and 6–9 pH units, respectively (EEPA/UNDO, 2003).

The mean influent values for NO₃-N was 26.89 mg/L and the mean effluent concentrations of all three treatment beds were in the range of 11.19 to 17.18 mg/L. The mean influent value for PO₄³⁻ was 35.35 mg/L and the mean effluent concentrations were between the ranges of 5.64 mg/L to 17.60 mg/L (Table 2). Since there are different water-using fixtures in the campus, there are different ways of looking at nutrient sources. It is obvious that the use of a garbage disposal and human waste are the main sources of nutrients in domestic wastewater. The high levels of NO₃-N obtained in the influent could be associated with the sources of the nitrogen form (NO₃-N) which could be protein hydrolysis and Urea from urine. While the high PO₄³⁻ levels obtained in the influent could be due to the use of phosphorous containing chemicals like phosphates in detergents for washing cloths and kitchen wares (Trepanier *et al.*, 2002).

Table 2. Influent and effluent characteristics of domestic wastewater and the experimental HSSFCW system.

Parameter	Mean Influent		Effluent Concentrations					
			HSSFCW 1 ^a		HSSFCW3 ^b		HSSFCW4 ^c	
	\bar{x}	std. error	\bar{x}	std. error	\bar{x}	std. error	\bar{x}	std. error
EC(µS)	667.85	244.35	371.35	130.39	242.52	74.88	372.15	124.95
pH	7.63	0.06	7.63	0.02	7.51	0.13	7.66	0.05
T(°C)	17.02	0.63	16.02	0.42	16.25	0.55	15.87	0.57
PO ₄ ³⁻ (mg/L)	35.35	2.64	12.27	1.19	5.64	3.32	17.60	2.69
NO ₃ -N(mg/L)	26.89	4.46	11.19	3.88	11.73	4.17	17.18	5.41

^a *Phragmites*-planted treatment bed;

^b *Papyrus*-planted treatment bed;

^c control bed.

N.B Treatment bed 2 is missing because of cracking problem.

Nutrient removal efficiencies of the treatment beds

The maximum NO₃-N removal was observed in treatment bed planted with *P. karka* (\bar{x} =58.37%) followed by *C. papyrus* (\bar{x} = 56.37%) and unplanted (\bar{x} = 36.13%) (Fig. 4a). Several experimental studies on nitrogen removal treatment have confirmed that unplanted treatment had lower nitrogen removal compared with planted treatment (Coleman *et al.*, 2001; Yang *et al.*, 2001; Lin *et al.*, 2002). Similar results were obtained in the study of Kenatu Angassa (2011) and Tadesse Alemu (2010) for the treatment wetlands planted with *P. karka*. In this study, the high nitrogen removal in treatment beds planted with *P. karka* may be due to its high leaf biomass content (13.74 gm/30 cm²) as compared to that of leaf of *C. papyrus* (8.49 gm/30 cm²) and its root mat, which enables the plant to take up and store more soluble inorganic nitrogen.

The average removal efficiency for PO₄³⁻ of the treatment beds was 84.05% for *C. papyrus*, 65.29% for *P. karka*, and 50.20% for the control (unplanted) (Fig. 4b). ANOVA and Post Hoc LSD tests indicated statistically significant differences (p<0.05) in removal of PO₄³⁻ between planted and

control treatment bed and also among planted treatment beds. It can be seen that all of the planted treatment beds have better efficiency in the removal of PO₄³⁻ compared to unplanted treatment bed. Similar results have been reported by Zhang *et al.* (2010) with *Phragmites australis* in treatment beds used for saline wastewater treatment for both NO₃-N and PO₄³⁻. The removal efficiency of the plants may be due to a combination of mechanisms favoured by the plants and adsorption of certain nutrients. The most important effects of the macrophytes in relation to the wastewater treatment processes are the physical effects of the plant tissues that give rise to filtration effect and provide surface area for attached microorganisms. The pollutants removal of macrophytes by plant uptake and storage affects the wastewater treatment processes in different ways. The Ethiopian domestic effluent standard limits are 20 mg/L for NO₃-N and 5 mg/L for PO₄³⁻ (EEPA/UNIDO, 2003). The mean PO₄³⁻ from all the three treatment beds were out of the range of the standard, while the mean NO₃-N from all the three treatment beds was within the range of the standard.

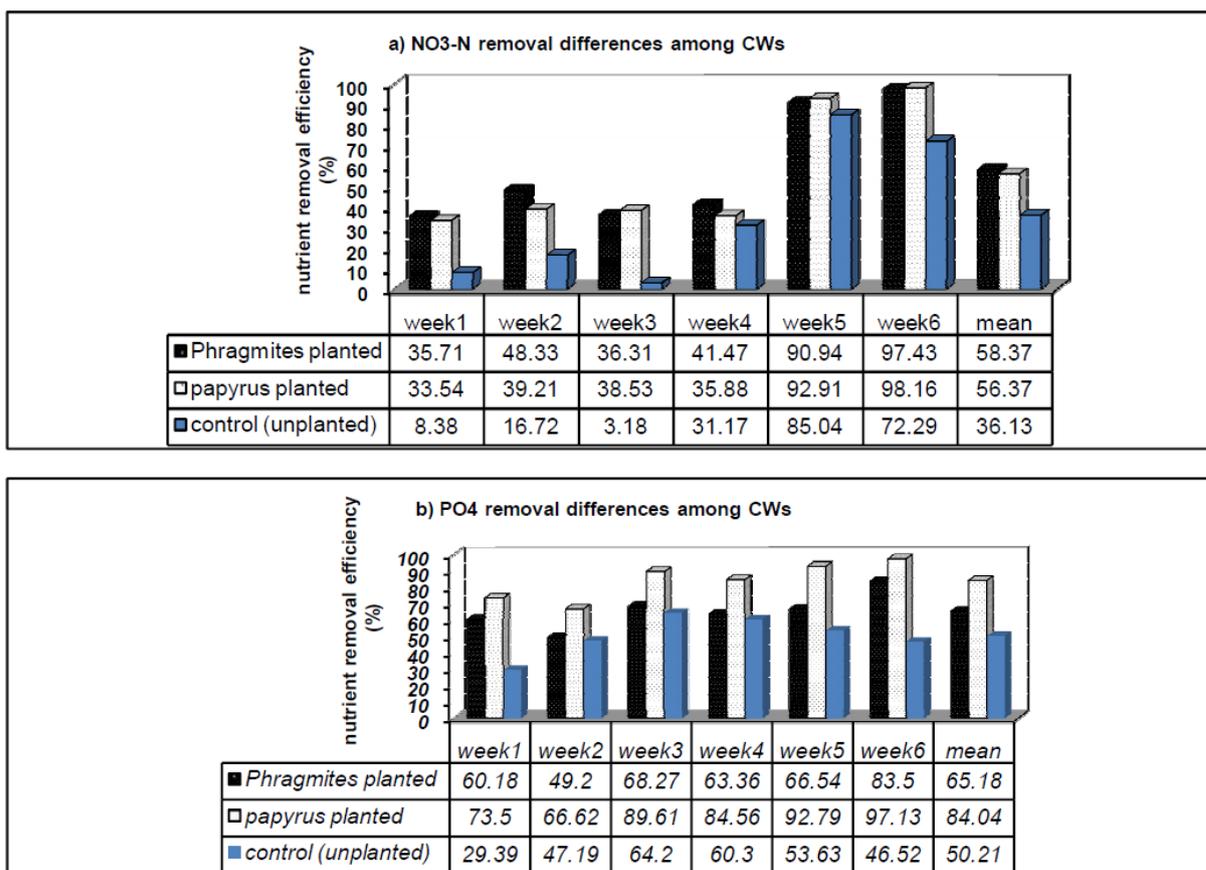


Figure 4. Nutrient removal efficiency of the three treatment beds along the experimental period.

In the experimental treatment beds planted with both *P. karka* and *C. papyrus*, the rate of removal of nutrients increased progressively with time (Figs 4a and 4b). The study showed that there was positive and significant correlations ($p < 0.05$) between the removal of nitrate and phosphate and changes in the plant density ($\rho = 0.806$ and $\rho = 0.648$,

respectively) (Table 3a). Moreover, changes in plant height was positively and significantly correlated ($\rho = 0.661$) with that of removal efficiency of Nitrogen (Table 3b) whereas changes in diameter was positively and significantly correlated ($p < 0.05$) with removal efficiency of phosphorus ($\rho = 0.648$) (Table 3c).

Table 3. Correlations of growth parameters and nutrient removal efficiency.

a) Correlations of density with removal efficiency of nitrogen and phosphorus.

			REN	REP	density
Spearman's rho	REN	Correlation Coefficient	1.000	0.321	0.648(*)
		Sig. (2-tailed)	.	0.365	0.043
		N	10	10	10
	REP	Correlation Coefficient	0.321	1.000	0.806(**)
		Sig. (2-tailed)	0.365	.	0.005
		N	10	10	10
	density	Correlation Coefficient	0.648(*)	0.806(**)	1.000
		Sig. (2-tailed)	0.043	0.005	.
		N	10	10	10

b) Correlations of plant height with removal efficiency of nitrogen and phosphorus.

			REN	REP	height
Spearman's rho	REN	Correlation Coefficient	1.000	0.321	0.661(*)
		Sig. (2-tailed)	.	0.365	0.038
		N	10	10	10
	REP	Correlation Coefficient	0.321	1.000	0.600
		Sig. (2-tailed)	0.365	.	0.067
		N	10	10	10
	height	Correlation Coefficient	0.661(*)	0.600	1.000
		Sig. (2-tailed)	0.038	0.067	.
		N	10	10	10

c) Correlations of diameter with removal efficiency of nitrogen and phosphorus.

			REN	REP	diameter
Spearman's rho	REN	Correlation Coefficient	1.000	0.321	0.600
		Sig. (2-tailed)	.	0.365	0.067
		N	10	10	10
	REP	Correlation Coefficient	0.321	1.000	0.648(*)
		Sig. (2-tailed)	0.365	.	0.043
		N	10	10	10
	diameter	Correlation Coefficient	0.600	0.648(*)	1.000
		Sig. (2-tailed)	0.067	0.043	.
		N	10	10	10

*Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

REN: Nitrate removal efficiency

REP: Phosphate removal efficiency

Conventional plant growth analysis also indicated that improvements in water quality per unit time by high population density resulted directly from increased plant presence. Zhu *et al.* (2011) studied the growth characteristics, plant aboveground and belowground biomass of seven wetland plants. They suggested that a greater ratio of plant biomass to wetland volume can enhance the contact between plant roots and wastewater resulting in a greater nutrient removal. Similar conclusion was reached by Sushil (2012) and Lu *et al.* (2012). In general, plant morphology such as height of plants and the shape of leaves also affects the overall nutrients in aquatic plant treatment systems (Kyambadde *et al.*, 2005).

CONCLUSION

In this study, it was observed that *Cyperus papyrus* and *Phragmites karka* plants from the wetlands of Lake Tana significantly influenced the rate of removal of nutrients in domestic wastewater. Progressive increase in the plant density, shoot length and stem diameter were positively correlated with the nutrient removal efficiency of these plants. *Cyperus papyrus*-planted treatment beds had markedly higher phosphorus removal efficiency with higher total biomass and nutrient levels (leaf N and root P) in plant tissues in comparison to *Phragmites karka*. Similarly, more shoots were developed by *C. papyrus* than *P. karka*, possibly indicating differences in nutrient uptake. The result also showed that all of the effluent concentration values (except for PO_4^{3-}) were within the Ethiopian effluent standard discharge limit values. Therefore, the data generated from this study give some insight for the potential use of these plants for nutrient removal in constructed wetlands and their application as an alternative wastewater treatment system. Hence, the development of this experimental system into a large-scale pilot unit offers an attractive alternative for low-level income countries to reduce nutrient pollution and protect the environment. *Cyperus papyrus* and *Phragmites karka* occur locally in tropical regions like Ethiopia. The study suggests that these macrophytes possess high biomass production and remove nutrients, thus making constructed treatment wetlands incorporating these macro-phytes vegetation is a promising wastewater treatment option for wider application.

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