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Nutrient removal efficiency by floating macrophytes; *Lemna minor* and *Azolla pinnata* in a constructed wetland

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ABSTRACT

The use of constructed wetlands for purifying pre-treated wastewater is a cost effective technology that has been found to be more appropriate for many developing countries. The technology is also environmentally friendly with the wetlands being habitats for many water birds and other aquatic organisms. This study assessed nutrient removal efficiency of two floating macrophytes (*Lemna minor* and *Azolla pinnata*). The data generated was analyzed using both descriptive and inferential statistics. The significance level was maintained at 0.05. The results showed that the wastewater physicochemical parameters did not vary during the study period. The concentrations of nitrites and nitrates increased over the experimental period in all the treatments (*Azolla pinnata*, *Lemna minor* and control), and the increase between the sampling occasions was statistically significant for the two nutrients (Nitrates: F=24.78, P= 0.00; Nitrates: F=198.26, P= 0.00). To the contrary, in all the treatments the concentrations of ammonia, total phosphorous, soluble reactive phosphorous and total nitrogen, decreased over the experimental period. The decrease in concentration for these nutrients between the sampling occasions was statistically significant (ammonia: F=195.57, *p= 0.00*; total phosphorous: F= 56.50, *p= 0.00*; soluble reactive phosphorous: F= 37.11, *p= 0.00*; total phosphorous: F= 104.025, *p= 0.00*). *Azolla pinnata* proved to be better than *Lemna minor* in the uptake of the nutrients particularly for the soluble reactive phosphorous (F= 35.18, P= 0.044). We conclude that the two macrophytes are good for wastewater treatment. It is recommended introduction and/or multiplication of *Azolla pinnata* in the constructed wetlands meant for wastewater treatment especially within the tropics.

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INTRODUCTION

Wastewater pollution is a global problem (Dhote and Dixit, 2009) with most of the cities in the world not only facing the challenge of providing tolerable hygiene facilities to their residents but also water resources that are not contaminated (Leong *et al.,* 2008). The discharge of untreated wastewater contributes to contamination of nearby water bodies and deteriorating health conditions. Poor environmental health has emerged as a major challenge in fast urbanizing world, threatening livelihoods and the health mostly of the poor (Bick *et al.,* 2012; Spinosa, 2011). Wastewater treatment is an issue of environmental concern that has plagued man for many years. In the past 20 years, significant interest has been articulated in the possible use of a variety of natural biological systems to help sanitize water in a well-ordered manner (Liu, 2007). Ponds, wetlands systems and land treatments form part of the natural biological treatment systems (Vymazal, 2010). Constructed Wetlands (CWs) present an idea aimed at combating decline of water resources of the receiving water bodies and wastewater by acting as buffers (Bick *et al*., 2012). Other than being relatively economical to construct and function and easy to sustain, constructed wetlands deliver reliable, effective, and ecologically comprehensive wastewater treatment. Constructed wetlands can also endure both small and large volumes of water together with varying pollutant levels (Wu *et al.*, 2015). If well-polished, wastewater can be reused for the intended productive purposes. Constructed wetlands are effective in the reduction of nutrients, mainly the nitrates (NO_3^-) and phosphates (PO_4^-) from wastewaters through their uptake for the buildup of wetlands vegetation biomass (Horne *et al*., 2000; Mitsch *et al*., 2001). However, in the specific case of surface flow treatment wetlands, plant harvesting can remove a significant amount of nitrate and phosphate as well as removal of suspended solids and organic matter. Other than nutrient uptake by the wetland vegetation, microbial transformation that include immobilization and denitrification of nutrients also occur in the wetlands and is mediated by macrophytes (Hernandez and Mitsch, 2006). Plants are wetland system important constituent (Kalff, 2002). Plants effectiveness in promoting CW performance is depended on numerous aspects: CW type (for instance; vertical, surface, subsurface flow, horizontal, or with or lacking recirculation), quantity and quality of the loads in the wastewater (Shelef *et al.,* 2013). Plant types and their combinations, plant management such as their harvesting regime, medium type, climate (Stottmeister *et al*., 2003) also contribute to CW nutrient removal efficiency. Also the removal efficiency of CW is controlled by the time spent by contaminants into vegetated zones (Fabris, 2013). Despite the aforementioned information from various wetland studies, there is limited data on nutrient removal efficiency by *Lemna minor* and *Azolla pinnata*. Results stated in most studies show that diverse vegetation is extra effective at nutrients uptake as compared to single-species plants (Fraser *et al.,* 2004). However, the data on the driving forces leading to this deduction is scarce. Moreover, experimental strategies have been used in various studies with different wetland plants, leading to contradictory findings. With even more disparity emerging when contrasts are made amongst different categories of CW. It is in contrast to this contextual that this study was conducted towards understanding the performance of two floating macrophytes, *Lemna minor* and *Azolla pinnata* in wastewater polishing at Egerton University's constructed wetland. This study was carried out in Egerton University in Kenya in 2018.

MATERIALS AND METHODS

This study was carried out in Egerton University in Kenya. Kenya is in African continent and is located about 25 Km South-west of Nakuru town in Nakuru County within latitude 0 15' and between longitudes 35^0 50' and 35 05'E. (Fig. 1). The institution stands on about 1580 hectares of land within the River Njoro watershed at an altitude of 1890 - 2190 metres above sea level. The institution lies in an agricultural area characterized by bimodal precipitation pattern ranging from 760 - 1270 mm per annum with the long rains falling between March and May while the short rains occur in September – November. It experiences a daily temperature range of 14.9 - 21.9˚C. The University had a population of about 18,000 people and was generating about 800 m^3 per day of wastewater which is treated in wastewater stabilization ponds (lagoons) and the constructed wetland within the University. The constructed wetland is a free-water surface wetland which covers 0.25 hectares of land. It was constructed in 2007 to

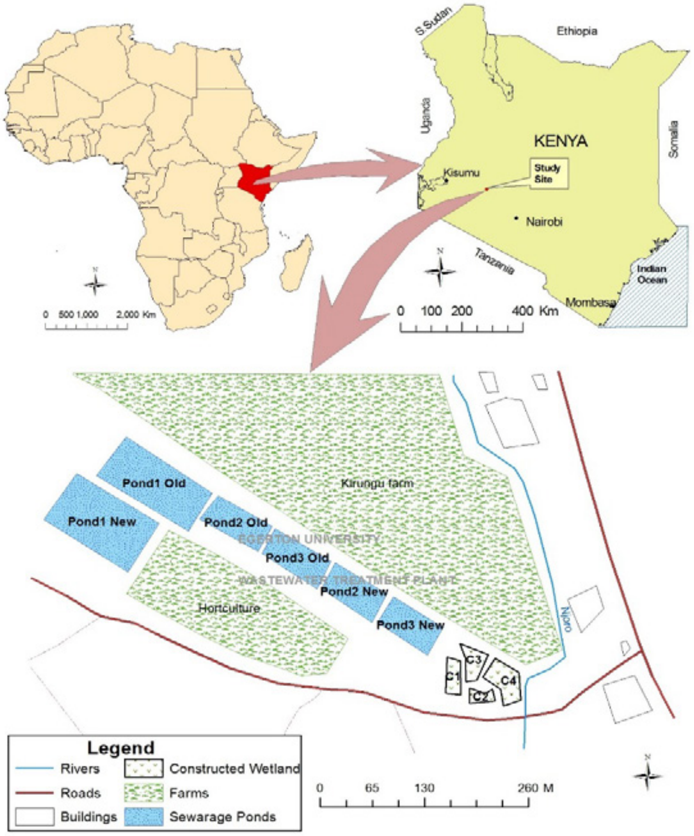

Fig. 1: Geographic location of the study area in Egerton University in Kenya

polish the pre-treated wastewater effluent from the wastewater stabilization ponds. The system consists of one vegetated sedimentation/gravel bed that had always been dominated by emergent macrophytes *Cyperus alopecuroides* and *Scirpus lacustris.* It also had some floating macrophytes that included *Pistia stratiotes* and Salvinia *auriculata.* This compartment is followed by a series of three connected, vegetated wetland cells. The dominant plant species in the first two cells has been *Eichhornia crassipes*, while the last cell was largely an open pond with few tufts of *Cyperus alopecuroides*. The vegetation in the cells had however changed with the harvesting and removal of the emergent macrophytes that used to dominate these cells. This followed introduction of *Lemna minor* and *Azolla pinnata* both of which are floating macrophytes that now dominate the cell. The system was designed to purify about 100m^3 of water per day with an approximate detention time of 10 to 14 days before discharging into River Njoro. But during this study period, the volume of the wastewater treated by the system had increased to about 800m^3 per day hence the need for this study. Again, this study extended the retention time of the wastewater that was under study since a longer hydraulic retention time would in theory be expected to have a positive effect.

Research design and sample collection

This study employed completely randomized design as the experimental design. The research design was based on understanding the effectiveness of floating macrophytes (*Lemna minor* and *Azolla pinnata*) in removal of nutrients (nitrates (NO_3), nitrites (NO_2), ammonia (NH_4), total nitrogen (TN), soluble reactive phosphorous (SRP) and total phosphorous (TP) when growing within a constructed wetland. The experiment was conducted with 45 buckets (Fig. 2) and on each sampling occasion wastewater from 9 buckets was sampled. 3 from the buckets containing *Azolla pinnata*, 3 from buckets containing *Lemna minor* treatments and 3 from the controls (with no treatment). Before the introduction of the wastewater to the buckets, baseline sampling was done to establish the status of the wastewater (nutrients concentration and physicochemical parameters of the wastewater) (Fig. 2).

The buckets were divided into 5 groups each group containing 9 buckets where wastewater of 7.5 litres (L) was put in each bucket. The predetermined wet weight of the treatments (*Lemna minor* and *Azolla pinnata*) was introduced in 30 buckets separately (15 buckets each treatment) and the rest 15 had no plants thus acting as the controls. Sampling from each group of 9 buckets (3 with *Lemna minor*, 3 with *Azolla pinnata* and 3 with no treatment that is the control) was done after every 5 days. The first group during the 5th day, the next group during the 10th day, the third during the 15th day, the fourth during the 20th day and the last group during the 25th day. In each sampling occasion, physicochemical parameters in the wastewater were measured *in situ*. Samples for total suspended solids (TSS), biological oxygen demand (BOD) and nutrients analysis were collected from the buckets in each sampling occasion and taken to the laboratory for analysis. The samples of *Lemna minor* and *Azolla pinnata* were harvested for further biomass analysis. Nutrients concentrations and change in weight of the selected macrophytes over time was determined during each sampling occasion.

Laboratory analysis

Lemna and *Azolla* biomass

At the beginning of the experiment, an initial damp weight of 10 grams (g) of *Azolla pinnata* and *Lemna minor* was determined and recorded and then introduced into their respective buckets. On the final day of each experiment (sampling occasion) *Azolla pinnata* and *Lemna minor* were sieved from each bucket using a hand sieve and muslin cloth of known damp weight. They were dried up of excess water then taken to the lab. Then later *Azolla pinnata* and *Lemna minor* were kept for five hours to dry up of the remaining water and their wet weight determined by use of a balance machine and recorded. The damp weight of the muslin cloth and the hand sieve was subtracted from the total weight to compute the total damp weight of *Azolla pinnata* and *Lemna minor*.

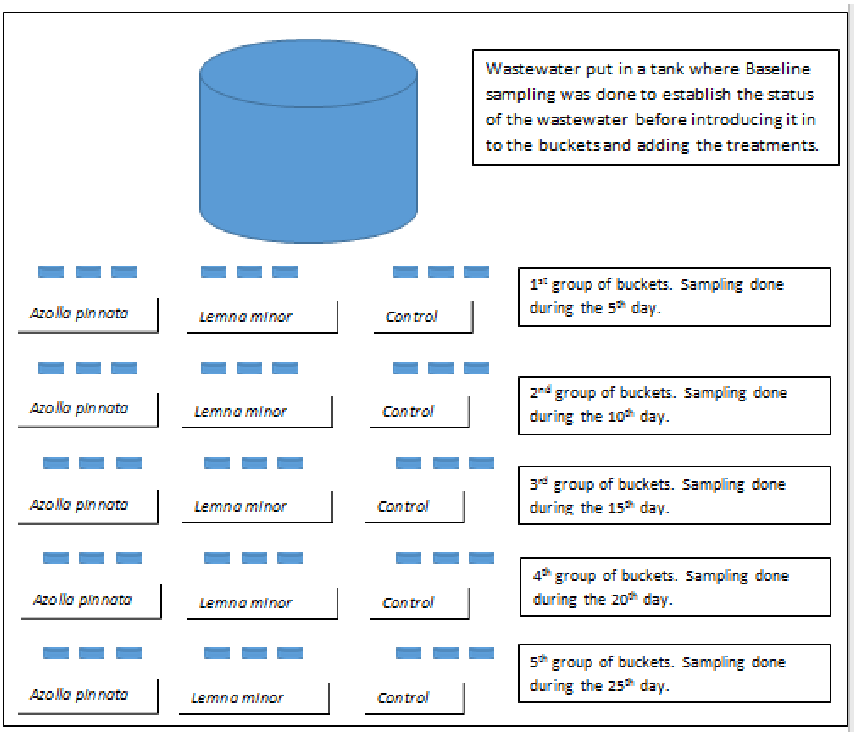

Fig 2: A diagram representing the experimental set up and the sampling sessions

Data analysis

All data on physico-chemical water quality and temporal nutrients concentration in wastewater was statistically analysed using descriptive and inferential statistics. The inferential statistics included one-way ANOVA, Multiple Linear Regression and Tukey Test to determine if there were any significant differences in nutrients and biomass amongst the treatments and plants. In all calculations, significance level was kept at 0.05. Data analysis methods are presented in Table 1.

RESULTS AND DISCUSSION

Physicochemical parameters

The physicochemical parameters of the wastewater studied were carried out before and after the introduction of *Azolla pinnata* and *Lemna minor* and the results of the same are shown in Table 2. The mean pH and temperature values noted in wastewater were very comparable during the study period in the three systems (Table 2). Mean temperature, dissolved oxygen and pH varied considerably between the treatments and the sampling occasions (Table 2). The mean dissolved oxygen (DO) concentration and the BOD_5 values kept on fluctuating during the study period (Table 2). The first sampling occasion recorded the highest mean temperature (26.80±0.048˚C) which was uniform for all the treatments (*Azolla pinnata, Lemna minor* and control). While the twenty days sampling occasion recorded the lowest mean temperature for *Lemna minor* (17.77±0.0˚C 5) and *Azolla pinnata* (18.30±0.05˚C) and twenty fifth day recorded the lowest for the control (18.70±0.05˚C). The presence of *Lemna minor* and *Azolla pinnata* in the wastewater showed a negligible influence on BOD

Table 1: showing data analytical methods

Parameter	Analytical Method	Reference Source
Ammonia	semi-automated colorimetry method	O'Dell, (1993)
Total Nitrogen	semi-micro Kjeldahl method	APHA, (2005)
Nitrates	sodium-salycilate method	APHA, (2005)
Nitrites	Colorimetric method	APHA, (2005)
Soluble Reactive Phosphorous	ascorbic acid method	APHA, (2005)
Total Phosphorous	nitric acid-sulphuric acid method	APHA, (2005)
Biological Oxygen Demand	"Five Day BOD"	Delzer and McKenzie, (2003)
Total Suspended Solids (TSS)	Gravimetric method	APHA, (2005)

Table 2: Showing physicochemical parameters (means ±SE) of the wastewater in different sampling occasions

Physicochemical parameters		Electrical Conductivity (µS/cm)	Wastewater Temperature (˚C)	Wastewater pH	Wastewater Dissolved Oxygen (mg/L)	Total suspended solids (mg/L)	Biological oxygen demand (mg/L)
Sampling occasion	Plants/control						
Baseline/ zero days	*Azolla pinnata.*	839.67±2.61	26.80±0.048	8.55±0.02	5.65±0.05	48.33±11.85	50.37±0.65
	Lemna minor.	839.67±2.61	26.80±0.048	8.55±0.02	5.65±0.05	48.33±11.85	50.37±0.65
	control	839.67±2.61	26.80±0.048	8.55±0.02	5.65±0.05	48.33±11.85	50.37±0.65
Five days	*Azolla pinnata.*	668.33±2.61	21.17±0.048	8.54±0.02	17.00±0.05	50.00±11.85	175.78±0.65
	Lemna minor.	655.00±2.61	22.40±0.048	9.32±0.02	17.85±0.05	45.51±11.85	182.66±0.65
	Control	638.33±2.61	24.97±0.048	9.57±0.02	20.60±0.05	37.78±11.85	214.18±0.65
Ten days	*Azolla pinnata.*	478.33±2.61	19.20±0.05	7.15±0.02	11.06±0.05	51.67±11.85	111.44±0.65
	Lemna minor.	514.00±2.61	19.07±0.05	7.02±0.02	11.76±0.05	51.57±11.85	119.15±0.65
	Control	540.67±2.61	19.67±0.05	7.05±0.02	10.19±0.05	45.86±11.85	99.96±0.65
Fifteen days	*Azolla pinnata.*	483.00±2.61	18.90±0.05	7.21±0.02	10.46±0.05	51.74±11.85	95.26±0.65
	Lemna minor.	350.00±2.61	18.53±0.05	7.14±0.02	8.91±0.05	22.21±11.85	87.67±0.65
	Control	395.00±2.61	19.60±0.05	7.05±0.02	11.85±0.05	35.56±11.85	120.08±0.65
Twenty days	*Azolla pinnata.*	603.33±2.61	18.30±0.05	9.55±0.02	12.42±0.05	44.44±11.85	124.63±0.65
	Lemna minor.	631.00±2.61	17.77±0.05	10.00±0.02	11.89±0.05	53.34±11.85	111.78±0.65
	Control.	665.33±2.61	20.00±0.05	10.62±0.02	14.60±0.05	28.89±11.85	148.74±0.65
Twenty five days	*Azolla pinnata.*	613.67±2.61	18.63±0.05	6.90±0.02	10.81±0.05	68.89±11.85	100.18±0.65
	Lemna minor.	650.33±2.61	18.60±0.05	7.05±0.02	9.43±0.05	68.89±11.85	85.63±0.65
	control	700.67±2.61	18.70±0.05	7.28±0.02	11.82±0.05	31.11±11.85	118.29±0.65
df= 5	F statistic	169.351	249.809	433.929	344.705	1.906	326.282
	P value	0.00	0.00	0.00	0.00	0.100	0.00

and TSS of the wastewater. The mean temperature variation in all the treatments during the study period was not statistically significant ($p<0.05$). It ranged from a minimum of 17.77±0.05˚C to a maximum of 26.80±0.048˚C (Table 2). Temperature variations could be attributable to the influence of the vegetation and the environmental temperature conditions and had a significant role in the experimental set up. Fluctuating trend of mean temperature was observed and it was noted that there was no significant difference between the temperature of *Azolla pinnata, Lemna minor* and control ($F= 2.17$, $p= 0.12$). Again the difference in temperature variations amongst the sampling occasions was statistically significant ($F=249.809$; $p=0.00$) (Table 2). The temperature variation favoured the action of the macrophytes on the wastewater. This is supported by Yuan *et al*., (2013) who did a similar study. Their results showed that once the temperature was around 33˚C, the elimination efficiencies of $NH^{3-}N$, TN, COD and TP in wastewater were 97.1%, 85.0%, 98.3% and 96.0%, respectively. During my study, the wastewater temperature varied considerably and this helped in terms of achieving high quality performance of the macrophytes. Guo-feng *et al*., (2000) conducted a study where the water temperature ranged from 13˚C to a maximum of 32˚C and found that the macrophytes were effective in the treatment of wastewater. Shah *et al*., (2014) observed maximum performance of macrophytes at a temperature range of 15˚C-38˚C which was favorable for the macrophytes' treatment of wastewater. In this study, temperature range of between 17.77±0.05˚C to 26.80±0.048˚C (Table 2) was considered suitable for the uptake of the nutrients by the aquatic plants and for their growth. The highest pH value (10.62±0.02) was recorded in the control at twenty days sampling occasion while the lowest pH value (6.90±0.02) was recorded at twenty five days sampling occasion in *Azolla pinnata*. The pH range during phytoremediation is an important factor to be considered because it is essential to maintain acidic or basic conditions for the plants' (macrophytes) growth for maximum uptake of the nutrients (Mesania Rizwana, 2014). The pH range for the maximum nutrients uptake by *Lemna minor* and *Azolla pinnata* is between 5.0 – 7.5 (Xu and Shen, 2011). These conditions are essential because they can stop growing of the plants by changing the structure of the enzymes. Most microorganisms will do well between a pH of 6.5 to 8.5. The pH range of 6 - 9 favors microbial action to decrease COD and BOD in the wastewater (Dipu Sukumaran, 2011). pH in the presence of plants could also be related with the imbalances between nitrification and denitrification (Coleman *et al*, 2001). The value of pH forms part of the significant parameters that influence the performance of wetland systems. The pH in various stages of wastewater purification depends mainly upon the equilibria of carbonic acid (Viehl, 1932). This suggests that the relationship between pH and the concentrations of carbon IV oxide, bicarbonate and carbonate can be formulated. During the experimental period, PH particularly for 15-20-25 days changed from 7 to 10 then to 7 respectively. This can be attributed to several biochemical and physical processes that occurred during the biological purification of the wastewater and the stability in the buffer capacity of the wastewater. Priya *et al*., (2012) performed a similar study and noted that any increase in pH in the treatment system was due to the photosynthetic activities of the plants in the wastewater. An upturn in pH of the control indicates that there was algal growth, the photosynthetic activities of which resulted in the increase of pH in the wastewater made for the control purpose. Ammonia oxidation again contributed to the increase of pH from 7 to 10. Gustin and Marinsek-Logar, (2011) noted that dissolved ammonia raises the pH of wastewater to above 11 with a strong base and can pose inhibitory effects on a variety of microorganisms involved in different biological wastewater treatment process. According to Buchauer, (1998), a high pH value will be harmful to the various biochemical processes in wastewater treatment. He also states that the upper limit for biological purification lies at pH 12. During this period, it is suspected that there were high rates of respiration by the selected plants quantitatively releasing carbon IV oxide into the wastewater leading to the decrease in pH from 10 to 7 because carbon IV oxide is much more soluble in water than is oxygen. The highest electrical conductivity value (839.67±2.61 µS/cm) was recorded in zero days/baseline sampling occasion in all the treatments, while the lowest value (350.00±2.61 µS/cm) was recorded during the fifteen days sampling occasion in *Lemna minor*. Highest electrical conductivity (EC) was recorded in the absence of aquatic plants (during baseline sampling occasion) as compared to the presence of *Azolla pinnata* and *Lemna minor* (Table 2).

The results clearly revealed reduction in electrical conductivity in the presence of the aquatic macrophytes. The range of electrical conductivity mostly depends on the concentration of various types of soluble salts in wastewater (Dipu *et al.*, 2013). The decrease in electrical conductivity during phytoremediation indicates the heavy uptake of the nutrients by the macrophytes. In addition, electrical conductivity also indicates the content of the mineral ion of the wastewater but the parameter does not however give a clue of which ions might be in existence (Lepcha, 2016). A wide variety of mineral ions would be indicated by High levels of electrical conductivity in the wastewater that could be a problem during treatment (Dalu and Ndamba 2003). There is limited data correlating EC with duckweed uptake of nutrients. Iqbal *et a*l., (2017) conducted experiments correlating EC with *Lemna minor* growth. They reported that after 25 days of retention time of *Lemna minor*, maximum removal of nutrients and growth was observed at 1,000 µS/cm EC. Wang *et al*., (2010) also contacted similar study and noted that growth rate and nutrient removal efficiency by *Lemna minor* and *Azolla pinnata* decreased with an increase in EC. Dissolved oxygen (DO) ranged from 5.65±0.05 mg/L to 20.60±0.05 mg/L (Table 2) during zero days and five days sampling occasions respectively. The availability of vegetation in wastewater can diminish dissolved carbon IV oxide (CO_2) during the time of high photosynthetic activities (Ugya and Imam, 2015). These photosynthetic activities increase the DO of water, and this creates aerobic conditions in wastewater hence favoring the aerobic bacterial activities and this reduces the COD BOD (Rizwana and Nilesh, 2014). In the study the lowest dissolved oxygen was recorded during the initial sampling then after introduction of the macrophytes the dissolved oxygen rose from 5.65±0.05 mg/L to 20.60±0.05 mg/L (Table 2). But for the other sampling occasions the value was at least 10 mg/L (Table 2). There was no significant difference ($p>0.05$) in Dissolved oxygen between the treatments containing the macrophytes and the control. This implied that in the control, the presence of the algae and the other microbes may have contributed to the production of oxygen. But generally, according to Sirage *et al.*, (2017), supply of oxygen through the plant roots is much higher than atmospheric diffusion. This could stimulate oxygen consuming reactions in the system and hence leading to more depleted and anoxic microenvironments. The highest total suspended solids value was recorded during the sixth sampling occasion (68.89±11.85 mg/L) (Table 2) in both *Azolla pinnata* and *Lemna minor*. The lowest value (22.21±11.85 mg/L (Table 2)) was recorded in *Lemna minor* during the 4th sampling occasion however, the total suspended solids values varied throughout the sampling occasions. The removal processes of TSS in the wastewater are mainly attributed to the filtration and sedimentation. Some other factors such as the hydraulic behavior of the system and microbiological features contribute to TSS reduction (Ugya and Imam, 2015). The TSS value of the wastewater was significantly low for this study simply because there are waste stabilization ponds meant for solids sedimentation installed just before the wastewater is allowed to flow into the constructed wetland. The TSS removal mechanism can be mainly attributed to the physical processes. The TSS removal is mostly a physical separation course than microbiological and is slightly effected by the retention period (Saraiva *et al*., 2018). Further TSS removal mechanism is also influenced by the properties of substrate media used (Lepcha, 2016). As per Dordio and Carvalho, (2013) findings, sand, gravel, gravel and soil are the most materials used as substrate media. In contrast, either by the size of their pores or possible wear the placement of these filter media affords a fast clogging over the operational time (Pedescoll *et a*l., 2009). The stable hydraulic appearances of the substrate in these medias, greater treating capacity available and the variability in pores sizes effects the creating of a considerable filtering media capable of removing huge quantities of suspended solids (Davies and Cottingham, 1994). The gravel bed performs better than any of the soil or gravel and soil beds (Manios *et al*., 2003; Saraiva *et al*., 2018). The better physical–mechanical arrangement of the substrate is the main cause for such substantial performance of the gravel based media filters (Saraiva *et al*., 2018). Soil, sand, and soil and sand substrates can simply be altered when pressure is applied to them because they are compactable materials with the first outsized porosity (Passeport *et al*., 2009). Gravel offers a more stable and predictable outcome because of its less compactible nature. According to the past studies done, it needs less cautious handling and offers the system with an extended lifetime by reducing obstructive pores. The total suspended solid values were low and could not have any detrimental effects in wastewater treatment process. Biochemical oxygen demand (BOD) is the amount of the dissolved oxygen demanded by aerobic biological organisms to break down the organic matter existing in a given water sample at certain temperature over a specific time period. Suspended and Attached microbial development is accountable for the removal of solvable BOD. In this study, the lowest value of BOD was observed where *Azolla pinnata* was present, so the BOD value can be reduced a lot by treating wastewater with *Azolla pinnata*. This is in contrary to the study carried out by Sooknah and Wilkie, (2004) who found out that the wastewater with *Lemna minor* showed a much greater amount of decrease in BOD. They attributed this to the fact that there was more aerobic BOD removal that was taking place because the oxygen supply by diffusion from the air was sufficient and there was no surface cover of the treatment system. Organic contaminants including both BOD and COD are connected with the amount of DO in the wastewater. The BOD concentration in the wastewater before the treatment was 50.37±0.65 mg/L which was the lowest in all the treatments and the highest was 214.18±0.65 mg/L in the control during the second sampling occasion. Significant decrease in BOD was detected in the treatments with the aquatic macrophytes as compared to the control unit which had no macrophytes. This can be attributed to the fact that plants perform an indirect but substantial part in decreasing organic matter during the treatment course. This happens by plants offering habitat for numerous decomposing microorganisms in the root region (Sehar *et al*., 2015) and by transporting oxygen to their roots zones and rhizomes. In all the experimental units, maximum decline in BOD was noted at fifteenth day hydraulic retention time (HRT). Further increase in hydraulic retention time showed no prominent improvement in BOD reduction since the BOD levels were fluctuating throughout the experimental period. The BOD removal efficiency of the floating macrophytes for the biodegradable organic matter (OM) varied slightly in all the treatments. The BOD removal efficiency by the two macrophytes was better than the control and there was a statistically significant difference between them and the control (F= 326.282, *p= 0.00*). Sirage *et al*., (2017) noted that floating macrophytes perform better than emergent macrophytes at low and high organic matter load. However, performance variability was evident for these floating macrophytes particularly in the case of *Lemna minor* and *Azolla pinnata*. They also noted that the higher BOD removal in the floating macrophytes suggests that the presence of the plants have an added value for enhanced organic biodegradation. The high-performance variability in the floating macrophytes could be due to the influence of the rapid growth rate and dieback.

Variation in nutrients concentration over time

It was observed that nitrites (NO_2) and nitrates (NO_3) increased in concentration from day zero to the twenty fifth day and that there were significant differences amongst sampling occasions (Table 3; F=24.780, *p= 0.00*; F=198.26, *p= 0.00* respectively). To the contrary, the concentrations of ammonia (NH_4), soluble reactive phosphorous (SRP), total phosphorous (TP), and total nitrogen (TN), decreased from day zero to the twenty fifth day (Table 3). The temporal variations in these nutrients were statistically significant (F=195.572, p=0.00; F= 56.500, p=0.00; F= 37.11, p=0.00; and F= 104.025, p=0.00, respectively). Based on the current study results, uptake of nutrients by the macrophytes (*Lemna minor* and *Azolla pinnata*) led to significant reductions in the studied nutrients during the study period. Macrophytes are expected to take up nutrients to build up their biomass over time, which is why nitrates and nitrites concentration were expected to reduce over the study period. However, their concentrations increased and this was attributed to mineralization of ammonia and nitrogen and reaction of nitrogen with dissolved oxygen in the wastewater (Lee *et al*., 2009). Similar observations were made in the control pointing to the role of algae growth in the control wastewater thus producing oxygen that could actively transform organically bound nitrogen to nitrite and nitrate. This lead to the increase in dissolved oxygen over the sampling period. Since ammonia is known to be volatile, the portion that was not taken up by the macrophytes probably was released to the atmosphere by joined nitrification-denitrification and the rest ended up in the sediments (Tang *et al*, 2017). Zhang *et al*. (2013) did a similar study and noted that along the growing period, nitrates concentrations amplified under high loading of nutrients. A parallel trend was observed with respect to the concentrations of

Table 3: Showing nutrients concentrations (means ±SE) in wastewater in different sampling occasions

Physicochemical parameters							
Sampling occasion	Plants/ control	Nitrites (NO_2)	Nitrates (NO_3)	Ammonia (NH_4)	Total phosphorous (TP)	Soluble Reactive Phosphorous (SRP)	Total nitrogen (TN)
Baseline/Zero days	*Azolla pinnata*	0.02±0.002	0.17±0.048	1.65±0.087	1.42±0.049	0.68±0.018	8.87±0.111
	Lemna minor	0.02±0.002	0.17±0.048	1.65±0.087	1.42±0.049	0.68±0.018	8.87±0.111
	control	0.02±0.002	0.17±0.048	1.65±0.087	1.42±0.049	0.68±0.018	8.87±0.111
5 days	*Azolla pinnata*	0.03±0.002	0.65±0.048	0.84±0.087	0.76±0.049	0.55±0.018	6.00±0.111
	Lemna minor	0.03±0.002	0.54±0.048	0.98±0.087	1.09±0.049	0.64±0.018	6.95±0.111
	Control	0.02±0.002	0.25±0.048	1.13±0.087	1.43±0.049	0.70±0.018	7.92±0.111
10 days	*Azolla pinnata*	0.03±0.002	0.70±0.048	0.57±0.087	0.54±0.049	0.30±0.018	4.89±0.111
	Lemna minor	0.03±0.002	0.73±0.048	0.59±0.087	0.66±0.049	0.51±0.018	6.74±0.111
	Control	0.02±0.002	0.63±0.048	0.96±0.087	1.26±0.049	0.66±0.018	7.12±0.111
15 days	*Azolla pinnata*	0.04±0.002	0.95±0.048	0.37±0.087	0.35±0.049	0.18±0.018	2.83±0.111
	Lemna minor	0.04±0.002	0.86±0.048	0.49±0.087	0.55±0.049	0.24±0.018	3.70±0.111
	Control	0.02±0.002	0.68±0.048	0.73±0.087	0.75±0.049	0.61±0.018	6.24±0.111
20 days	*Azolla pinnata*	0.04±0.002	1.06±0.048	0.11±0.087	0.08±0.049	0.13±0.018	2.26±0.111
	Lemna minor	0.04±0.002	0.90±0.048	0.26±0.087	0.27±0.049	0.18±0.018	3.30±0.111
	Control	0.02±0.002	0.83±0.048	0.47±0.087	0.69±0.049	0.54±0.018	5.58±0.111
25 days	*Azolla pinnata.*	0.04±0.002	1.18±0.048	0.07±0.087	0.08±0.049	0.02±0.018	2.16±0.111
	Lemna minor	0.04±0.002	0.97±0.048	0.40±0.087	0.30±0.049	0.08±0.018	2.31±0.111
	control	0.02±0.002	0.86±0.048	0.34±0.087	0.74±0.049	0.49±0.018	4.43±0.111
df= 5	F statistic	24.780	198.261	195.572	56.500	37.11	104.025
	P value	0.00	0.00	0.00	0.00	0.00	0.00

nitrites. This increase may be due to a combination of reasons such as nutrient leaching due to senescing plants, low plant uptake and low denitrification rates due to lower temperatures. It is known that in wastewater excessive nitrogen is bound organically and nitrate is normally released through biological transformation. Therefore, the high rate of organic nitrogen transformation through mineralization and nitrification was the key factor that explains the increase in nitrate and nitrite concentration in the wastewater (Sirage *et al*., 2017). Zhang *et al*. (2013) also attests that *Lemna minor* preferentially takes up more ammonia than nitrite and nitrate. This is because nitrogen in form of ammonia is converted directly in to plant protein, rather than being subsequently reduced, as is the case with nitrate once assimilated (El-Shafai *et al*. 2007). Nitrification process may explain the increase in nitrate and nitrite concentration.

Findings by Alexia Mackey, (2017) did not support this hypothesis. In their study, as ammonia concentrations decreased, nitrite and nitrate concentrations increased, indicating that nitrification occurred. Changes in conditions throughout the day could explain variations, as conditions like temperature affect nitrification and denitrification processes. NH^{4+} removal was also done by means of nitrification pathway which gave rise to the upturn of NO^{3-}N and NO^{2-}N concentrations as has been reported by Xu and Shen, (2011) and Zhao, (2014). *Lemna minor* was observed to have a negative removal efficiency of ammonia (Table 4). This was also observed by Sayadi *et al.*, (2012) who carried out hybrid constructed wetland treatment systems study. According to their results, removal efficiency for all pollutants was high especially for NH^{4+} nutrient in the domestic wastewater treatment under different loading rates. Though, in terms of nutrient components removal, the efficiencies depend on system properties and operational conditions of the treatment system. Ammonia is as well known to be volatile because its presence in wastewater can be found in two forms, namely, ammonia gas and ammonium ions (kinidi *et al*., 2018). Limoli *et al*., (2016) also noted that relative concentrations of ammonium ions and ammonia gas are subject to the pH and the temperature of wastewater. This suggests that formation of ammonia gas is favored by increasing the pH of the wastewater, which shifts the chemical equilibrium to the right, thus inducing the formation of ammonia gas hence evaporation of ammonia. Minus ammonia nutrients removal efficiency can as well be attributed to this characteristic of ammonia. But as observed during the study period, all the other nutrients were decreasing suggesting that there was enhanced uptake by the floating macrophytes. Solano *et al*., (2004) concluded that constructed wetlands could be a suitable solution for wastewater treatment as a stand-alone treatment for domestic wastewater, although a pretreatment in order to remove grit, heavy solids, and floatable materials would be necessary. This also happens where this study was done since there are wastewater stabilization ponds installed before the wastewater gets to the constructed wetland to reduce the amount of sediments. The treatment efficiency of the selected plants was found to be favorable. The nutrients removal in the wastewater meant for control purpose may be attributed to the uptake by algae and the growth of microbes that utilize nutrients during their growth. Srivastava *et al*., (2008) noted that the decrease of nutrients from the control was due to uptake by microorganisms and other biological activities taking place. Vermaat and Hanif, (1998) performed several batch growth of macrophyte plants that lasted for 12 days using domestic wastewater. Their results showed that *Lemna minor* and *Azolla pinnata* were responsible for around 56% and 18% uptake of total phosphorus, respectively. Their outcome demonstrated that under experimental conditions, *Lemna minor* has a higher capability to remove nutrients which is contrary to the results of the current study. Again Srivastava *et al.,* (2008) performed similar study on *Lemna minor* uptake of phosphorous and nitrogen from wastewater and demonstrated that *lemna minor* wastewater stabilization pond system achieved 74% and 77% removal of nitrogen and phosphorus, respectively. The uptake of nutrients by these floating macrophytes has not yet been widely studied that is why our study focused on determining the best plant in phytoremediation between the two. Ferdoushi *et al*., (2008) results indicated that both plants had good potential to reduce various pollutants, however, *Lemna* was far better than *Azolla* for removing the pollutants. They concluded that *Azolla pinnata* and *Lemna minor* can serve the purpose of wastewater treatment in municipal areas which are easily manageable. Our study results are concurring with Azarpira *et al*., (2013) results who performed a similar study and found out that *Azolla pinnata* had high growth rate and productivity and seemed to be very promising in improving treated wastewater quality. *Azolla pinnata* removed nutrients more efficiently than *Lemna minor* hence the nutrients content in the wastewater was significantly lowered in presence of *Azolla pinnata* than in the presence of *Lemna minor*. The nutrient removal efficiencies for two floating macrophytes are shown on Table 4. In constructed wetland systems, pollutant removal mechanisms include both anaerobic and aerobic microbiological conversions, sedimentation, chemical transformations, sorption and volatilization. Changes in macrophytes' biomass was attributed to nutrients uptake from the wastewater. This study was contacted in buckets with stagnant water as opposed to a constructed wetland where there is always flow of the wastewater as it is treated by the plants. In a constructed wetland, macrophytes are always affected by water flow through direct effects (stretching, uprooting, breakage) and indirect effects (changes in uprooting, gas exchange, bed material distribution, sediment suspension (Han *et al*., 2018). During this study, there was no wastewater flow hence there was no effect of flow rate on the reduction of the nutrients using the selected plants. Water flow inhibits the growth of the macrophytes and also alters the vertical distribution of water velocity. Levi *et al*., (2015) noted that flow turbulence could inhibit plant

Table 4: Nutrients removal efficiency by the macrophytes

Nutrient	Macrophyte's nutrient removal efficiency (%)			
	Lemna minor		*Azolla pinnata*	
	Lemna minor – Control	Efficiency	*Azolla pinnata* - Control	Efficiency
Nitrite	105.25 - 21.05	84.2	84.21 - 21.05	63.16
Nitrate	479.76 - 411.90	67.86	601.79 - 411.90	189.89
Ammonia	76.08 - 79.35	-3.27	95.64 - 79.35	16.29
Total phosphorous	78.61 - 47.92	30.69	94.37 - 47.92	46.45
Soluble reactive Phosphorous	88.02 - 27.81	60.21	97.47 - 27.81	69.66
Total nitrogen	73.99 - 50.02	23.97	75.59 - 50.02	25.57
Average		43.61		68.67

growth, induce oxidant stress and photosynthetic efficiency and reduce the carbon content in the tissue of the macrophytes. But the case in this study was different since there was no flow of the wastewater from the start to the end of the study.

The study showed that both *Lemna minor* and *Azolla pinnata* can be successfully utilized to enhance dissolved nutrient uptake in treatment systems. Though both plants have shown to be effective in removing nutrients from wastewater, *Azolla pinnata* showed the highest efficiency in nutrient removal than *Lemna minor* especially for soluble reactive phosphorous. Forni *et al*., (2001) encouraged interest in using *Azolla pinnata* for the purpose of decontamination of wastewater in low cost wastewater treatment systems and also documented *Azolla pinnata* as the macrophyte with the ability to purify wastewater by removing nitrogen and phosphorous nutrients which are the elements responsible for eutrophication. This is not the case with Azarpira *et al*., (2014) who conducted a similar study where *Lemna minor* showed slightly better performance in removing all nutrients though they used wastewater with 75 % dilution. From our results it can be concluded that *Azolla pinnata* will be highly preferred candidate for phytotreatment of wastewater in a constructed wetland. Nutrients removal efficiency was different for all the nutrients by each plant and the reason for this may be attributed to the fact that different methods in phytoremediation are involved in nutrient components removal. For example organic nitrogen, nitrite and ammonia are oxidized initially by rhizoremediation to nitrate. Therefore, the extraction of the latter may take longer while the oxidation of the former may be accomplished in shorter retention intervals (Ghosh and Gopal, 2010). Our results indicate that both macrophytes (*Lemna minor* and *Azolla pinnata*) play a very important part in the soluble reactive phosphorous (SRP) removal from the wastewater. Both micro-organisms and Plants utilize SRP as a crucial nutrient and their tissues have phosphorous (Shah *et al*., 2015). Total phosphorous removal during the study in the macrophytes treatment was 46.45% for *Azolla pinnata*, and 30.69% for *Lemna minor (able 4)*. Both *Lemna minor* and *Azolla pinnata* demonstrated potential for removing of nutrients in wastewater in a constructed wetland but some nitrification was detected during the experiment in all the treatments. Solano *et al*., (2004) observed much decrease in the level of nutrients in the wastewater as nutrients are required for the growth of the macrophytes. Since the aquatic macrophytes' uptake of nutrients is depended on their biomass production (Table 5) and thus on their photosynthesis, the nutrients uptake would happen optimally only in the growing period of the macrophytes (Crispim *et al*., 2009). Macrophytes senescence may have contributed to low nutrients uptake hence the concentrations reducing significantly towards the end of the experiment. This was also observed in the macrophytes since their growth or biomass increase also reduced significantly during the last days of the study period as compared to the initial stages of the experiment where their growth was fast (Table 5).

Increase in biomass of the selected macrophytes

The initial biomass of the selected plants used in this study was 10g. The biomass production by the plants revealed a lag phase for the first five days followed by an exponential growth until fifteenth day, beyond which changes in growth were negligible. Similar results were found by Yin *et al*., (2015) obtaining the maximum biomass production at day 12.

Solano *et al*., (2004) suggested that for more nutrients removal, regular harvesting of the

Table 5: Macrophytes biomass increase (mean ±SE) in grams

Selected plants Sampling occasions		*Azolla pinnata* (mean±SE) in grams (g)	*Lemna minor* (mean±SE) in grams (g)
Baseline/Zero days		10.00±0.00	10.00±0.00
5 days		15.43±0.30	15.40±0.26
10 days		40.33±0.41	39.97±0.29
15 days		60.17±0.80	49.30±0.29
20 days		66.07±1.27	52.60±0.75
25 days		71.20±2.01	55.43±0.38
Df = 5	F statistic	621.713	786.494
	P value	0.00	0.00

macrophytes is necessary. Biomass yields of small-leaf floating macrophytes are quite lower than for large-leaf floating aquatic macrophyte such as *Eichhornia crassipes* or *Pistia stratiotes* (Pena *et al.*, 2017). The ability of *Lemna minor* to assimilate nutrients from culture medium has been reported by different authors as comparable (Xu and Shen, 2011; Zhao, 2014). Macrophytes have a key function in relation to wastewater purification by provisioning a surface area for attached microorganisms, pollutant uptake, enhancing filtration, and releasing oxygen; however, the role of the vegetation still requires quantification in terms of nutrients uptake over time (Zhang *et al.*, 2009). In this study, the comparison between the two macrophytes has shown obvious difference in nutrients uptake efficiencies (Table 4) indicating the positive role of the macrophytes in the process of phytoremediation. The use of *Azolla pinnata* and *Lemna minor* is a vital practice in phytoremediation, because they have very worthy potential for removal of pollutants, restoring polluted aquatic resources (Sood *et al.*, 2012). They have ability for altering water quality by regulating oxygen balance and nutrient cycles. *Azolla pinnata* proved to be more efficient in nutrients uptake than *Lemna minor*, translating to more biomass increase than the *Lemna minor* which had a low biomass build up while both were exposed to similar conditions (Table 4). The biomass produced by *Azolla pinnata* can as well be used for inoculating paddy fields or for other applications and wastewaters can be reused for irrigation purposes (Arora and Saxena, 2005). This is also supported by Zhang *et al.*, (2008) who found out that *Azolla pinnata* has distinct advantages as it has high biomass productivity coupled with high rate of nitrogen fixation, ability to grow in varied environments and multiple applications in biomonitoring, animal feed, biofilter, biofertilizer, and its ability to concentrate nutrients from wastewaters. The study on the growth aspects of macrophytes clearly indicated that the wastewater had no detrimental effects on the plants. This is because none of the plant introduced in wastewater died, and despite the nutrients uptake difference, the biomass for both plants increased over the experimental period. Moreover, longer hydraulic retention time increased the action of the selected floating macrophytes on the wastewater. Removal of the nutrients by the selected plants was strongly correlated to retention time. Thus, the efficiency of the tested macrophytes could be improved by adjusting the technical methods and increasing the hydraulic retention time (Merino-Solís *et al.*, 2015). Sehar *et al.*, (2013) found out that After 20 days' retention time, the treated wastewater was free of almost all nutrients and microbial pollutants. Hence, increasing hydraulic retention time was found to ameliorate the operational competence of a constructed wetland.

CONCLUSION

The physicochemical parameters were within the optimum range for growth of the floating macrophytes, and were stable throughout the study period. Based on current study results, *Azolla pinnata* proved to be better than *Lemna minor* in terms of growth and rates of nutrients removal from the wastewater. This conclusion is premised on the fact that in the buckets with *Azolla pinnata* nutrients were decreasing faster than where we had *Lemna minor* and the control. We recommend introduction and/or multiplication of *Azolla pinnata* in the constructed wetlands meant for wastewater treatment especially within the tropics. We also recommend that there be a scheduled periodic removal of dead macrophytes and excess biomass in the constructed wetlands. This is to avoid decomposition of the dead macrophytes which contribute to nutrients increase in the wastewater. The hydraulic retention time (HRT) should be prolonged to 20 days so as to meet the desired efficiency of the selected plants. Substantial nutrients removal can probably be achieved at HRTs longer than 15 days because nutrient removal efficiencies in the experimental set-up were enhanced by increasing the Hydraulic Retention Time. This is very important both technically and ecologically. Longer retention times mean a longer time for microbiological decay of wastewater and macrophytes' uptake of the nutrients.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

ABBREVIATIONS AND CHEMICAL SYMBOLS

ANOVA	Analysis of variance
APHA	American Public Health Association
BOD	Biological oxygen demand
CO_2	Carbon dioxide
COD	Chemical oxygen demand
CRC	Chemical rubber company
CW	Constructed wetland
^{0}	Degrees
˚C	Degrees Celsius
DEWATS	Decentralized wastewater treatment system
df	Degrees of freedom
DO	Dissolved oxygen
E	Eastings
EC	Electrical conductivity
Fig.	Figure
g	Grams
HSSF	Horizontal subsurface flow
HRT	Hydraulic retention time
KM	Kilometers
L	Litres
‘	Minutes
M	Metres
Mm	Millimeters
mg/L	Milligrams per litre
$NH_4/NH^{3-}N/NH^{4+}$	Ammonia
$NO_2/NO^{2-}N$	Nitrites
$NO_3/NO^{3-}N$	Nitrates
OM	Organic matter
%	Percent
pH	Potential hydrogen
TN	Total nitrogen
TP	Total phosphorous
SE	Standard error
SRP	Soluble reactive phosphorous
TSS	Total suspended solids
USA	United States of America
µS/cm	Micro Siemens per Centimeter
VSSF	Vertical subsurface Flow

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