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ACCUMULATION OF NITROGEN AND PHOSPHOROUS BY VETIVER GRASS (*CHRYSOPOGON ZIZANIOIDES*) IN A MODEL CONSTRUCTED WETLAND TREATMENT SYSTEM FOR POLISHING MUNICIPAL WASTEWATER

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ABSTRACT: Kenya is classified as water scarce country yet the existing fresh water resources are under constant threat of pollution resulting from wastewater inflows. Wastewater contains nitrates and phosphates that stimulate excessive plant growth when released into water bodies thus deteriorating their quality. The purpose of the study was to evaluate the performance of Vetiver grass in the uptake of Nitrogen and Phosphorous from the three (horizontal, vertical and hybrid subsurface flow wetland systems) model constructed wetland units for treating municipal wastewater. Nitrogen and phosphorous accumulation in the roots and shoots of the Vetiver grass was determined and the data subjected to ANOVA at 5% confidence level. Vetiver grass accumulated 18,100 mg and 35.3 mg/kg Nitrogen and Phosphorous, respectively in the hybrid system compared to 9,400 mg Nitrogen and 19 mg/kg Phosphorous, in the horizontal subsurface flow system and 10,400 Nitrogen and 18.3mg/kg Phosphorous in the vertical subsurface flow system. Accumulation of nitrogen and phosphorous by Vetiver grass in all the wetland systems were significantly different ($P \leq 0.05$). There was also significant ($P \leq 0.05$) difference of N and P accumulation in the shoots and the roots with N accumulating more in the shoots while P in the roots.

KEYWORDS: Vetiver Grass N and P uptake, constructed wetland, horizontal flow, vertical flow, hybrid system.

1 INTRODUCTION

Fresh water has increasingly become one of the rare valuable resources under the constant threat of pollution. The rapid build-up of toxic pollutants in soil and water bodies not only affects natural resources, but also causes major strains on ecosystems thereby affecting their functions [1],[2]. The deteriorations in water quality resulting from eutrophication are estimated to reduce biodiversity in water bodies and wetlands by about one-third globally [3]. Nutrients discharged into water bodies stimulates excessive plant growth which results into decreased water quality [4],[5],[6]. Inadequately treated wastewater and agricultural run-off into water bodies has contributed significantly to most of the eutrophication in water bodies seen today [7]. The use of conventional wastewater treatment system has proved to be costly and ineffective [8], [9] and this necessitates the need to develop low energy, effective and low cost technologies in developing countries like Kenya for efficient treatment.

Phyto-remediation as a green technology is one of the main environmentally friendly technologies that is gaining wider use for wastewater treatment [10], [11]. Diamond[12], defines phyto-remediation, as the use of plants and their associated microorganisms to stabilize or remove contamination in water. Plant roots exude a wide variety of organic compounds which support the microbial community and can facilitate the absorption of some heavy metals [13] that are hazardous to both human and livestock.

The use of Vetiver grass for phyto-remediation has gained wider use in the recent years as it has proved to be very effective, low cost natural methods of environmental protection [14],[15],[16]. Vetiver grass (*Chrysopogon zizanioides*) belongs to the graminae family and was first used for soil and water conservation in India in the 1980s by the World Bank [17]. Since then, its role has been successfully extended to wastewater treatment works [18],[19].In the process of wastewater treatment, the Vetiver grass absorbs essential plant nutrients such as nitrogen (N) and phosphorus (P) and stores them for other physiological uses [20],[21]. The objective of this study was to evaluate the effectiveness of Vetiver grass in the uptake and accumulation of N and P from municipal wastewater passing through horizontal, vertical and hybrid subsurface flow constructed wetland treatment systems.

2 MATERIALS AND METHODS

2.1 STUDY SITE DESCRIPTION

The study was conducted at the Gusii wastewater treatment plant located in Suneka Division in Kisii County, Kenya at latitude 0° 39' 30” S and longitude 34° 42' 30” E**.** The area has a highland equatorial climate resulting into bimodal rainfall pattern with the long rains occurring between February and June, and short rains taking place between September and December. The area receives an average annual rainfall of 1500mm [22]. The month of January and July are generally dry and the maximum temperatures in the area range between 21°C to 30°C, while the minimum temperatures ranges between 15°C to 20°C [23]. Kisii County is characterized by hilly topography and is endowed with several permanent rivers draining into Lake Victoria [22],[23].

Seventy five percent of the county has deep red volcanic soils (Nitisols) rich in organic matter, while the remaining area comprises of clay, red loams, sandy soils, black cotton soils classified as Vertisols and organic peat soils classified as Phanosols [22],[24]. Mixed farming is the main economic activity in the area and over eighty percent of the agricultural land is devoted to food and cash crops mainly maize, finger millet, sorghum, beans, sweet potatoes, tea, coffee and sugarcane [25].

2.2 EXPERIMENTAL DESIGN AND LAYOUT

The treatments consisted of horizontal, vertical and hybrid subsurface flow wetland systems with each treatment replicated four times. The horizontal subsurface flow wetland had a length of 3.2m, width of 0.8m and depth of 0.3m. The vertical subsurface flow wetland had a length of 3.2m, width of 0.8m and depth of 0.4m. The first stage of the hybrid system consisted of vertical flow system having length of 3.2m, width of 0.8m and depth of 0.4m and the second stage was a horizontal flow system of length 3.2m, width of 0.8m and depth of 0.3m. The wetland units were lined with high density polythene 0.3mm thick filled with coarse river sand with a porosity of 34.3% and silt content of 9.9% as substrate. The constructed wetland units were planted with Vetiver grass at a spacing of 10cm within rows and 15cm between rows in all the wetland units. The horizontal subsurface system was operated on a continuous basis with wastewater flow rate of $0.036m^3d^{-1}$ while the vertical system was operated with two batches per day each constituting $0.018m^3$ of wastewater and evenly spread over the surface of the vertical subsurface flow wetland units through perforated pipes. The pipes were perforated and had 6mm diameter spaced at 80mm. The flow rates at the inlets of the wetland systems were determined using a measuring cylinder and a stop watch as shown in Figure 1.

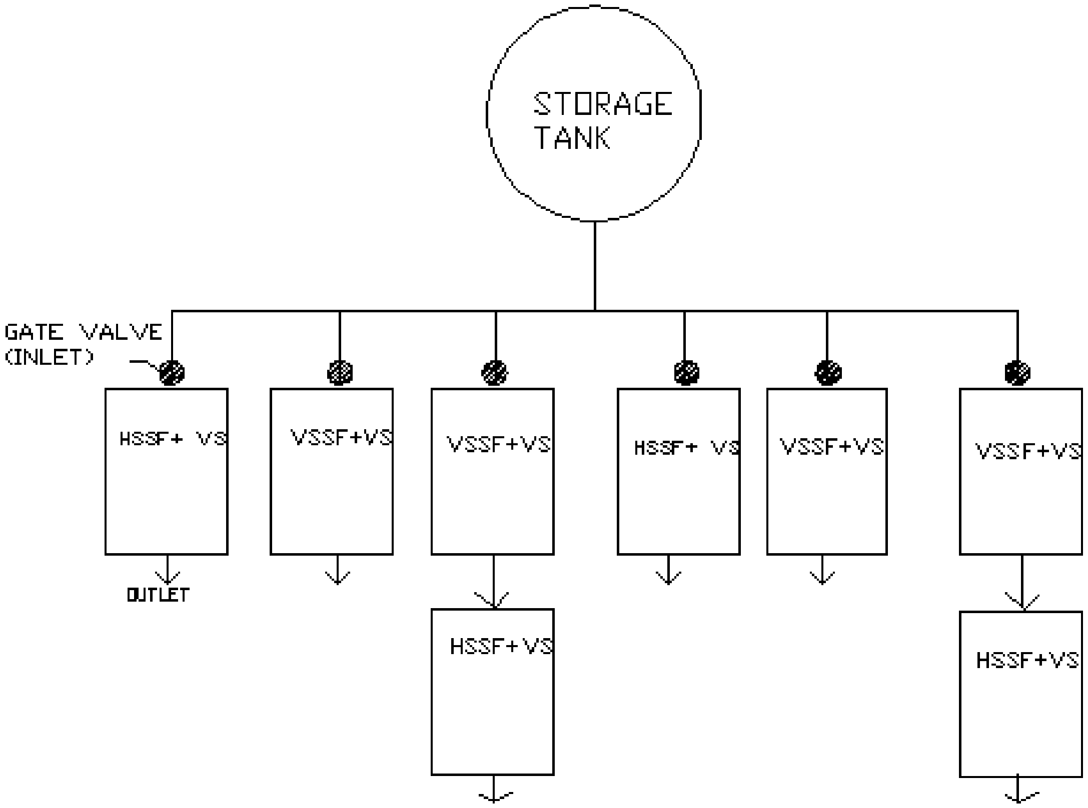

Fig. 1. Layout of the experimental units

Legend: *HSSF=Horizontal subsurface flow wetland, VSSF=Vertical subsurface flow wetland, VS= Vetiver Grass*

2.3 Planting And Establishment Of Vetiver Grass

The Vetiver grass slips of 300mm height were obtained from Kenya Agricultural and Livestock Research Organization (KALRO) in Kisii and planted at a spacing 100 mm within and 150 mm between rows in the substrate of the Horizontal, Vertical and Hybrid subsurface flow wetland systems. Diammonium Phosphate (DAP) fertilizer was used at planting to enable root establishment since the substrate had low N and P content of 1200 and 19 mg/kg, respectively. For a period of one month since planting, they were watered with fresh water and subsequently in the 2nd and 3rd month with wastewater from the maturation pond. The Vetiver grass in all the wetland units began to continuously receive wastewater based on the experimental flow rate of $0.036m^3d^{-1}$ at the beginning of the fourth month for a period of 8 weeks into the Horizontal subsurface flow system. In the Vertical subsurface systems, it was intermittently fed with two batches daily with each batch having $0.018\ m^3$. Figure 2 and 3 shows the Vetiver grass at planting and at three months period since planting, respectively.

Fig. 2. Planting Vetiver Grass (3/1/2016)

Fig. 3. Vetiver Grass at three months since planting (2/4/2016)

2.4 HARVESTING OF VETIVER GRASS AND DATA COLLECTION

Five stems of Vetiver grass were randomly harvested from each wastewater polishing unit at the 138th day after planting. The shoots and roots from each wetland unit was air dried, weighed and analyzed for N and P concentration using atomic absorption spectroscopy[26],[27].The data obtained was subjected to a two way ANOVA at 5% level of significance. Means were separated using LSD test to determine if there were significant differences between treatment pairs.

3 RESULTS

3.1 ESTABLISHMENT OF VETIVER GRASS

Figure 4 shows the variation of Vetiver grass shoot height with time in the constructed wetland systems during the monitoring period at the 96th ,110th ,124th and 138th day after planting corresponding to two weeks interval as from 7/4/2016 upto 19/5/2016. Vetiver grass achieved significantly ($P \leq 0.05$) higher mean height of 1.52m in the vertical subsurface flow system, compared to the horizontal flow system at 1.46m as from the 96th upto 138th day after planting. In the hybrid set up, growth of Vetiver grass in the first stage (Vertical subsurface flow) and in the second stage (Horizontal subsurface flow) varied significantly($P \leq 0.05$). The grass in the first stage grew taller to a mean height of 1.52m compared to the height in the second stage of 1.44m as from the 96th upto 138th day since planting. In all the wetland systems however, there was progressive increase in Vetiver grass height during the monitoring period.

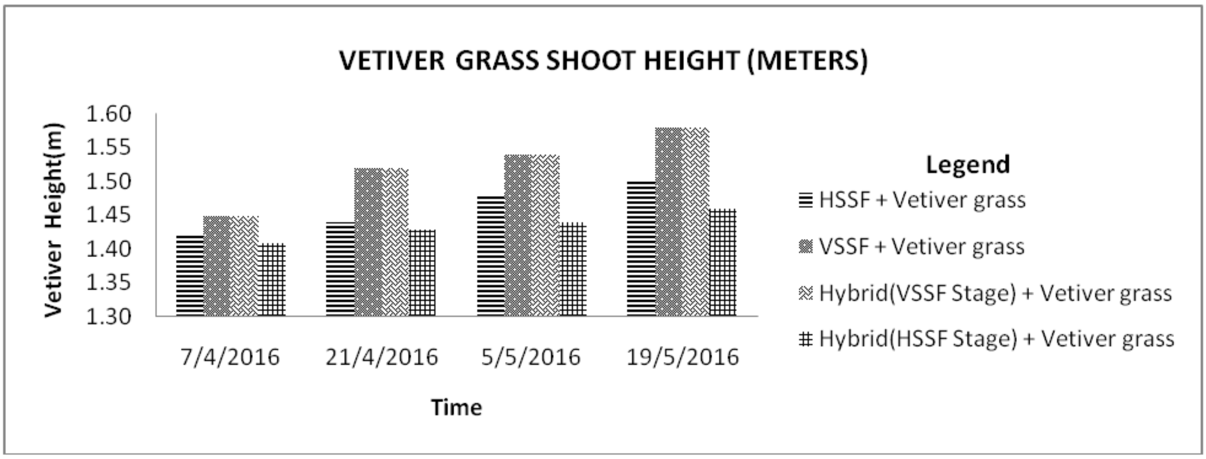

Fig. 4. Variations of Vetiver grass shoot height during the monitoring period.

3.2 NITROGEN ACCUMULATION IN THE ROOTS AND SHOOTS OF VETIVER GRASS IN THE VARIOUS TREATMENTS

Table 1 shows the accumulation of N in the roots and shoots of Vetiver grass in the Horizontal, Vertical and Hybrid subsurface flow constructed wetland systems during the monitoring period. Nitrogen accumulation in the roots and shoots of Vetiver grass in the Horizontal subsurface flow system was 4200 mg and 5200 mg/kg, respectively as at 138th day after planting. Accumulation of N in the Vertical subsurface flow constructed wetland system in the roots and shoots of Vetiver grass was 4500 mg and 5900 mg/kg, respectively as at 138th day after planting. In the hybrid system, accumulation of N in the roots and shoots was 770mg and 10400mg/kg, respectively as at 138th day after planting. Nitrogen accumulated was significantly ($P \leq 0.05$) more in the shoots than in the roots of Vetiver grass in all the systems at the end of the monitoring period which corresponded to 138th day after planting. In total, Vetiver grass accumulated significantly ($P \leq 0.05$) the highest N content of 18,100 mg/kg in the hybrid system, followed by vertical system at 10, 400 mg/kg and finally in the horizontal system at 9,400 mg/kg as at 138th day after planting.

Table 1. Nitrogen accumulation in the roots and shoots of Vetiver Grass

Wetland System	N-root (mg/kg)	N-shoot (mg/kg)	Total Accumulation (N-root + N-shoot) (mg/kg)
HSSF + VS	4200^{a}	5200^{a}	9400^{a}
VSSF + VS	4500^{b}	5900^{b}	10400^{b}
HB(VSSF stage + VS)	4500^{b}	5900^{b}	10400^{b}
HB(HSSF stage + VS)	3200^{c}	4500^{c}	7700^{c}

Legend: *HSSF=Horizontal subsurface flow system, VSSF=Vertical subsurface flow system, HB= Hybrid subsurface flow system, VS= Vetiver Grass, N-root= Nitrogen accumulation in root, N-shoot= Nitrogen accumulation in shoot, Total accumulation with the same letter (a,b,c) in the same column are not significantly different at 5% confidence level.*

3.3 PHOSPHOROUS ACCUMULATION IN THE ROOTS AND SHOOTS OF VETIVER GRASS IN THE VARIOUS TREATMENTS

Table 2 shows the P accumulation in the roots and shoots of Vetiver grass in the horizontal, vertical and hybrid subsurface flow constructed wetland systems. Phosphorous accumulation in the roots and shoots of Vetiver grass in the Horizontal subsurface flow system was 10.50 and 8.50 mg/kg, respectively compared to Vertical subsurface flow system at 9.80 and 8.50 mg/kg, respectively as at 138th day after planting.In the hybrid system, accumulation of phosphorous in the roots and shoots was 18.5 and 16.8 mg/kg, respectively as at 138th day after planting. Phosphorous accumulated significantly ($P \leq 0.05$) more in the roots than in the shoots of Vetiver grass in all the wetland systems. In total, Vetiver grass accumulated significantly ($P \leq 0.05$) the highest amount of P at 35.3 mg/kg in the hybrid system, followed by the horizontal system at 19 mg/kg and finally in the vertical system at 18.3 mg/kg as at 138th day after planting. It was also observed in this study that P accumulation in Vetiver grass in all the wetland systems were significantly ($P \leq 0.05$) lower compared to N. For instance, in the hybrid system, Vetiver grass accumulated 35.3 mg/kg P compared to 19,100 mg/kg N.

Table 2. Phosphorous accumulation in the roots and shoots of Vetiver Grass

Wetland System	P-root (mg/kg)	P-shoot (mg/kg)	Total Accumulation (P-root + P-shoot) (mg/kg)
HSSF + VS	10.50^{a}	8.50^{a}	19.00^{a}
VSSF + VS	9.80^{b}	8.50^{a}	18.30^{b}
HB(VSSF stage + VS)	9.00^{c}	8.00^{b}	17.00^{c}
HB(HSSF stage + VS)	9.50^{d}	8.80^{c}	18.30^{b}

Legend: *HSSF=Horizontal subsurface flow system, VSSF=Vertical subsurface flow system, HB= Hybrid subsurface flow system, VS= Vetiver Grass, P-root= Phosphorous accumulation in root, P-shoot= Phosphorous accumulation in shoot, Total accumulation with the same letter(a,b,c,d) in the same column are not significantly different at 5% confidence level.*

4 DISCUSSION

4.1 ESTABLISHMENT OF VETIVER GRASS

Continuous water flow in the horizontal system could have occupied the voids thereby creating waterlogged conditions thus inhibiting Vetiver grass uptake of nutrients and thereby lowering its growth. Parent et al. [28] observed that as water saturates the soil pores, gases are displaced and reduction in gas diffusion occurs which reduces photosynthesis and translocation of photoassimilates. Steffens et al. [29] made a similar observation in a study to investigate the effect of waterlogging on growth and plant nutrient concentrations where waterlogging resulted in a significant decrease of shoot dry weight production. They explained this observation that due to oxygen deficiency in the root medium of waterlogged soils, synthesis of ATP may be inhibited thus lowering energy status of the plant which consequently leads to a decrease in nutrient uptake.

Despite the saturated conditions in the horizontal subsurface flow wetland system, the progressive growth observed indicates that Vetiver grass has strong adaptation to excess moist conditions.Boonsong and Chansiri[30] using Vetiver grass cultivated with floating platform technique demonstrated its ability to thrive in waterlogged conditions.They observed that after eight weeks, in both experimental set up with highly concentrated wastewater and low concentrated wastewater, the survival percentages of Vetiver grass were ranging from 75-100%.Yeboah et al.[31] in a study on purification of industrial wastewater with Vetiver grasses grown hydroponically, also observed shoot height was increasing progressively thus demonstrating it could thrive optimally in waterlogged conditions.

The variation in shoot height in the hybrid system could be attributed to the better uptake of nutrients by Vetiver grass in the first stage(vertical subsurface flow) which is well aerated and thus wastewater that flowed to the second stage(horizontal subsurface flow) had lower nutrient content. In all the wetland systems however, the progressive increase in Vetiver grass height during the monitoring period could be attributed to the increase in the uptake of nutrients with physiological age of Vetiver grass. Xia et al. [32] similarly observed that the purifying capacity of Vetiver grass in the vertical subsurface flow wetland treating oil refined wastewater gradually increased with the gradual growth and development resulting in gradual increase of biomass.

4.2 NITROGEN ACCUMULATION IN THE ROOTS AND SHOOTS OF VETIVER GRASS IN THE VARIOUS TREATMENTS

The significantly higher N accumulation in the shoots than in the roots could be an indication that Vetiver grass has higher translocation rate of nitrogen from roots to shoots to meet its high nitrogen requirement for stem and leaf growth. Gerrard [33] observed that when Vetiver grass was grown hydroponically in raw sewage, the accumulation of N was significantly higher in the shoot at 2.35 compared to 1.54% in the roots. Akbarzadeh et al. [34] observed that Vetiver grass grown hydroponically on domestic wastewater had significantly higher total nitrogen accumulation in the shoots than in the roots. They attributed their observations to rapid growth rate and high biomass yield in the grass.

In total, the significantly higher accumulation of N by the Vetiver grass planted in the hybrid system compared to the other systems could be attributed to the N uptake by Vetiver grass over a length of 6.4m in the hybrid system compared to 3.2m in both the horizontal and vertical system. However, the significantly higher accumulation of N in the vertical system than in the horizontal system could be due to the better aeration in the vertical subsurface flow system that favours oxidation of ammonia in wastewater to nitrate (NO_3^-) and ammonium (NH_4^-) that is easily taken up by Vetiver grass[35],[36].Reddy[37] in a study on N cycling in a flooded soil ecosystem planted with rice also noted that in aerobic soils where nitrification can occur, nitrate is usually the predominant form of available nitrogen that is absorbed as opposed to waterlogged conditions that inhibit the biological oxidation of ammonia. Mengel and Kirkby [38] in a study on plant nutrition, noted that ammonium accumulates in the soil when N conversion is limited or completely stopped if waterlogged soil conditions persists, further supports the findings of this study.

4.3 PHOSPHOROUS ACCUMULATION IN THE ROOTS AND SHOOTS OF VETIVER GRASS IN THE VARIOUS TREATMENTS

The significantly higher phosphorous accumulation in the roots than in the shoots in all the wetland systems could indicate that Vetiver grass utilizes more P for root development. Gerrad [33] observed that when Vetiver grass was grown hydroponically in raw sewage, the accumulation of P was significantly higher in the root at 0.41% compared to 0.29% in the shoots. Boonsong and Chansiri [30] observed that P accumulation in the shoots of Vetiver grass grown in the highly concentrated wastewater was significantly lower compared to the accumulation in the roots. They explained that phosphorous was the macronutrient required in high amounts for root development.

The higher accumulation of P by Vetiver grass in hybrid system than in the other systems could be attributed to the uptake of phosphates by Vetiver grass over a length of 6.4m in the hybrid system compared to 3.2m in both the horizontal and vertical system. However the significantly higher accumulation of P in the horizontal system than in the vertical system could be due to longer contact time between Vetiver grass roots and wastewater as opposed to vertical system whereby wastewater is uniformly spread over the whole surface area and flows downwards under gravitational influence. This influence of gravity could cause wastewater to drain out faster thereby shortening contact time with Vetiver grass roots. The observation that P accumulation by Vetiver grass was significantly lower than N in all the wetland system is a fact attributed to adsorption of P by the sandy substrate making it unavailable for Vetiver grass uptake. Holford [39] similarly noted that more than 80% of the phosphorous in soil become immobile and unavailable for plant uptake due to adsorption, precipitation or conversion to the organic form. According to Hoffman et al. [40], phosphorous removal can be achieved in constructed wetland by adsorption and precipitation and only a small amount is taken up by plant growth. Wagner et al. [41] observed that Vetiver requirement for P was not as high as for N and no growth response occurred at rates higher than 250 kg/ha/year under P supply while for N supply, the growth increased significantly upto an application rate of 6000kg/ha/year.

5 CONCLUSIONS

Vetiver grass accumulated 18,100 mg/kg and 35.3 mg/kg Nitrogen and Phosphorous, respectively in the hybrid system as compared to 9,400 Nitrogen and 19 mg/kg Phosphorous, in the horizontal subsurface flow system and 10,400 Nitrogen and 18.3mg/kg Phosphorous in the vertical subsurface flow system. Hence it can be concluded that Vetiver grass accumulates more N and P in the hybrid systems than in single systems (horizontal and vertical system) and it up takes more nitrogen from wastewater in well aerated soils in vertical subsurface flow systems than under waterlogged conditions in the horizontal subsurface flow systems. Phosphorous uptake is generally low compared to nitrogen and it is independent of substrate aeration but on the contact time between wastewater, substrate and Vetiver grass. Purifying ability of Vetiver grass also increases with time.

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