

The Fate of Nitrogen and Faecal Coliform in the Lubigi Wetland in Uganda

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ABSTRACT

The capacity of the Lubigi wetland to reduce nitrogen and faecal coliform pollution entering Lake Kyoga in Uganda was investigated. Three transects with 5 sampling points in each, were established in the wetland. Wetland plants samples were collected from the specific locations along the transects, and laboratory tests and analyses were carried out for plants biomass and nitrogen contents determination. Samples of wetland sediments were also collected, and laboratory tests and analyses were done for determination of nitrogen content in the sediments. Wetland water samples were taken from the main wetland inlet and the main outlet from the wetland main study area. At the same time, wetland water pH, dissolved oxygen and temperatures were measured in-situ. Laboratory tests and analyses for ammonia-nitrogen, nitrate-nitrogen, total Kjeldahl nitrogen, total nitrogen and faecal coliforms were carried out on the wetland water samples. The results indicate that the Lubigi wetland received about 16 to 173 mg/l of total nitrogen, largely in form of organic-nitrogen (64.7%) and ammonia-nitrogen (35.2%). Nitrate-nitrogen and Nitrite-nitrite accounted for only 0.1% of the total nitrogen in the inlet. The wetland removed about 24.9% of this nitrogen, which is equivalent to removal of about 1,672 tons of nitrogen annually. About 67.5 gN/m² and 0.30 gN/kg of dry sediments were sequestered in the wetland plants biomass and the benthic layer respectively. The Lubigi wetland main study area also receives faecal coliform concentrations with the wetland inlet mean value of 653,509 CFU/100ml and its outlet mean value is 218,676 CFU/100ml. These values give a faecal coliform (FC) overall removal efficiency of approximately 66.5%. It was thus concluded that the Lubigi wetland has considerable capacity to buffer and protect Lake Kyoga, by reducing nitrogen and faecal coliform pollution entering the lake.

Key words: Faecal coliform pollution, Lubigi wetland, Nitrogen removal.

INTRODUCTION

The Lubigi wetland, which is located in the north-western part of Kampala, is one of the largest Lake Kyoga drainage basin wetlands (Kayima and Mayo, 2018). The wetland receives discharges from a canalized stream and the Nsooba-Lubigi

drainage channel with an average capacity of approximately 220,000 m³/day. The channel collects municipal and industrial wastewater, storm water run-off and sub-surface water flow from the upstream densely populated and unsewered slums. In addition, the stream receives effluent discharges from the Lubigi sewage

treatment plant, with a design capacity of 5,400 m³/day (Watebawa, 2012; National Water and Sewerage Corporation, 2015). Therefore this wetland is practically used for disposal of municipal wastewater (Kayima *et al.*, 2018a).

Natural wetlands, Lubigi inclusive, have capacities to remove toxins and nutrients from water bodies (Marwa, 2013; Kayima *et al.*, 2018a; Masawe *et al.*, 2018). Therefore they act as ecological transition zones that protect the quality of water in downstream fresh water bodies such as rivers and lakes (Terer *et al.*, 2005; Henry and Semili, 2005; Mayo *et al.*, 2018). At present, there are serious concerns that the Lubigi wetland will not only become degraded, but will also pass on pollutants from upstream discharges, to the downstream fresh water bodies of the Mayanja River and Lake Kyoga (Kayima *et al.*, 2018). This could lead to excessive concentrations of, among other pollutants, nitrogen and faecal coliforms (FC) (Kayima *et al.*, 2018) leading to serious problems for people who rely on these water bodies for various uses (Gumm, 2011).

Nitrogen is among the major pollutants found in raw or partially treated wastewater (Metcalf and Eddy Inc., 2003). Excessive concentrations of nitrogen can stimulate eutrophication (Bricker *et al.*, 2008), which can lead to production of greenhouse gases (Kinney and Valiela, 2013), blockages of receiving fresh water bodies, and interference with navigation (UNEP, 2013). The exposure to excessive concentration of ammonia can lead to toxicity to aquatic life and depletion of dissolved oxygen, which can cause the death of fish, damage coral reefs and render water septic and odorous and therefore unfit for human and animal use (Morrow, 2009; Baker *et al.*, 2013; Li *et al.*, 2014; Shin *et al.*, 2016). Excessive nitrate in drinking water is known to cause *methamoglobinaemia* (blue baby disease)

in infants and increased cancer risks in human beings (Senzia, 2003; Rogan and Brady, 2009; Karr, 2012; Brender *et al.*, 2013). On the other hand, pathogenic organisms present in wastewater can cause diseases and severe public health problems, and are considered to be responsible for most of the morbidity and mortality in developing countries (WHO, 2015).

Wetlands are not only effective for removal of chemical pollutants, but they are also effective in reduction of pathogens (Mayo and Kalibbala, 2007; Kalibbala *et al.*, 2008), which is vital for protection of human health against diseases. However, the knowledge on the fate and removal of bacteria in wetlands is still unclear owing to the complexity of influencing factors and bacterial removal mechanisms (Weber and Legge, 2008; Wu *et al.*, 2016). Wetlands receive bacterial pollution from multiple sources including inlet wastewater and animals that live in or around the wetland. Their performance is also highly dependent on the type and density of macrophytes, hydrology, hydraulic regime, retention time and various physical-chemical factors that influence natural die-off of bacteria (Kalibbala *et al.*, 2008; Weber and Legge, 2008).

While it is known that wetlands receiving wastewater have capacities to transform and remove the pollutants contained in the wastewater (Mitsch and Gosselink, 2007; Bavor and Waters, 2008; Mayo *et al.*, 2014), the performance of wetlands differ because of aforementioned factors. Therefore, it is important to investigate the fate of nitrogen and faecal coliforms in the Lubigi wetland, with a view to assessing the capacity of the wetland to reduce nitrogen and faecal coliform pollution entering the downstream Mayanja River and Lake Kyoga. To achieve this objective, it was necessary to investigate the effectiveness of Lubigi wetland in

reduction of nitrogen and faecal bacteria, which is the main objective of this research study.

MATERIALS AND METHODS

The Main Study Area

Figure 1 shows the main study area, which is delineated in the north-east of Kampala city by the Hoima Road, with the main wastewater inlet located at latitude 00°20'48" N and longitude 32°32'28" E; and in the south-west by the Sentema Road with the main wetland outlets located at latitude 00°19'56" N and longitude 32°31'34" E. This section of the wetland covers an area of about 1.1 km², at an altitude of approximately 1,158 m above sea level, with a total catchment area of about 40 km². This is the section of the wetland, which receives direct impact of polluted wastewater from upstream

sources. This zone is closest to the wetland main wastewater inlet and is dominated by *Echinochloa pyramidalis* and *Paspalum scrobiculatum*, with abundance of other assorted types of plant species. Hence, transect T1 was established in this section about 700 m downstream of the wetland main wastewater inlet. The middle zone was observed to be dominated by *Cyperus papyrus* and *Typha capensis*, with abundance of other assorted types of plant species. Hence, transect T2 was established in this section about 1,440 m downstream of the wetland main wastewater inlet. The zone closest to the main study area outlets was observed to be dominated by *Cyperus papyrus* and *Thelypteris acuminata*, with abundance of other assorted types of plant species. Hence, transect T3 was established in this section about 1,930 m downstream of the wetland main wastewater inlet (Figure 1).

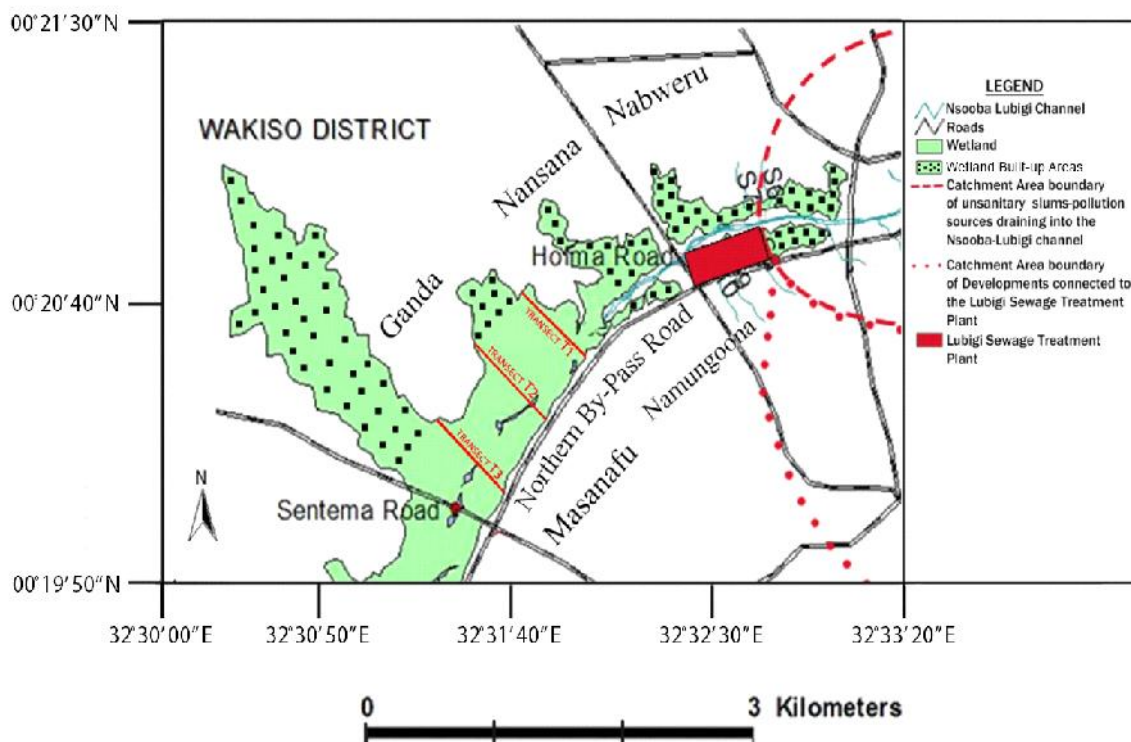


Figure 1: Map of the Lubigi Wetland Main Study Area

In each of the 3 transects, 5 sampling points were established in order to closely

follow the spatial variability across the widths of the wetland, as one moves from

the main central drainage channel away towards the edges of the wetland on either side of the channel. The transects and sampling points were geo-referenced using a Garmin Global Positioning System (GPS) device, in order to determine and record the co-ordinates of their locations. Then they were transferred to a digitized map of the area, to ensure that the same transects and points are used every time sampling is done. To facilitate movements and work within the transects, *Cyperus papyrus* culms were cut and tied in bundles which were laid down to make accessible paths. Life rafts and jackets and other safety precautions and measures, were used throughout the research field work.

Field and Laboratory Tests

Determination of plants biomass

The sampling of plants for biomass determination was done in October and November 2016. The 5 most dominant plants species namely *Cyperus papyrus*, *Echinochloa pyramidalis*, *Typha capensis*, *Rottboellia cochinchinensis* and *Oldenlandia lancifolia* were analyzed. These most dominant plant species were harvested; from the already established 1mx1m sampling quadrats in transects T1, T2 and T3. The above-ground biomass was cut and separated into leaves/umbels and stalks/culms depending on the plant type. Then these parts were weighed using a digital balance in the field, in order to obtain the total wet weight of each plant part. The below-ground biomass was removed by digging up all roots/rhizomes in the 1mx1m quadrats, and carefully washing off all the dead materials and soil/peat. The roots/rhizomes were also weighed in the field, in order to obtain their total wet weight. From the whole sample of each plant part in a 1mx1m quadrat, a sub-sample weighing 500 g was taken for sun-drying in the Makerere University Plant Sciences, Microbiology

and Biotechnology laboratory, in Uganda. The sun-dried samples of each plant part were thereafter oven-dried at 105 °C. The dry weight to wet weight ratio of the 500 g sub-sample, was used to calculate the total dry weight in the 1mx1m quadrats in the transects.

Determination of nitrogen content in the plants

Total-nitrogen (TN) was chosen as the basic element for nitrogen used up by plants. The total-nitrogen content of the dried plant parts was determined according to the methods used by Novozamsky *et al.* (1983). Fine materials out of every plant part were obtained by grinding a portion of the dried plant parts in a manual grinder. Then the fine materials were sieved through a 0.5 mm sieve in the Kawanda National Agricultural Research Organization laboratory in Uganda, and the ensuing powder was preserved following the preparation methodology devised by Muthuri and Jones (1997).

Thereafter, portions of the dried powder were scooped up, and transferred quantitatively into the destruction tubes in which digestion was done in a block, using a concentrated Sulphuric-salicylic mixture with Selenium as a catalyst. The analysis of total nitrogen in the digested samples was then carried out, in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA *et al.*, 2012). Finally, nitrogen content in plants was determined following the approach described by Muraza (2013). In this approach, the plants biomass content in kilograms of plants dry weight per square meter of wetland (kgDWm^{-2}), and the plants nitrogen content as a percentage of plants Dry Weight (%DW) were first determined. Then the plants nitrogen content in g N.m^{-2} was determined as the product of the plants biomass content and the plants nitrogen content as a percentage.

Determination of nitrogen content in the sediments

The collection of wetland sediments was done simultaneously with the plants sampling. The sediments were collected from 3 of the 5 sampling quadrats, in each of the 3 transects T1, T2 and T3. The sediments samples were packed in cool boxes and transported to the Makerere University Plant Sciences, Microbiology and Biotechnology laboratory in Uganda, where they were oven dried at 105 °C. After drying, the samples were ground in a manual grinder and sieved through a 0.5 mm sieve size in the Kawanda National Agricultural Research Organization laboratory in Uganda, in order to obtain the dry powder.

To analyze the sediments for nitrogen contents, sub-samples of appropriate weights were taken and digested in a block, using a concentrated Sulphuric-salicylic mixture with Selenium as a catalyst. The analysis of nitrogen in the digested samples was then carried out following the total nitrogen determination procedures, in accordance with the Standard Methods for the Examination of Water and Wastewater samples (APHA *et al.*, 2012). The determination of the nitrogen content in the sediments in gm^{-2} , was done by multiplying the sediments nitrogen content in grams of nitrogen per kilogram of sediments by the density of sediments in kgm^{-3} and then by 0.5m. This was based on the consideration that the effective sediments depth for nitrogen sedimentation is 0.5m. At depths in the sediments exceeding 0.5 m, layers of stiff and almost impermeable clay soils are encountered in the wetland.

Determination of wetland water characteristics

Seventy two (72) wetland water samples were collected at bi-weekly intervals from August 2016 to February 2018 inclusive,

from the main wetland inlet and the main wetland outlet. Samples of 2.0 liters each were collected from a depth of 10 cm to 20 cm below the water surface, into sterilized plastic containers. The samples were stored in cool boxes, and then transported to the National Water and Sewerage Corporation laboratory in Uganda, and examined within a period of 4 hours. At the same time, *in situ* wetland water temperatures were measured with portable thermometer WTW 3310. The pH values were measured with a pH electrode SenTix 21 WTW, connected to a portable pH meter WTW 3310. Dissolved oxygen (DO) was measured with a dissolved oxygen electrode CellOx 325 WTW, connected to a portable DO meter WTW 3310.

Laboratory tests and analyses for ammonia-nitrogen ($\text{NH}_3\text{-N}$), nitrite-nitrogen ($\text{NO}_2\text{-N}$), nitrate-nitrogen ($\text{NO}_3\text{-N}$), Total Kjeldahl Nitrogen (TKN), Total Nitrogen (TN) and faecal coliforms (FC) were carried out on the wetland water samples, in accordance with the standard methods for the examination of water and wastewater (APHA *et al.*, 2012). Wetland water samples were kept under refrigeration at about 4 °C during storage. Chemical preservation was done by adding 1 ml of sulphuric acid (H_2SO_4) to 1 liter of the sample to achieve a pH below 2 for preservation of samples for determination of ammonia-nitrogen. Ammonia-nitrogen was analyzed using the Turbidimetric method, and the Nessler reagent. Nitrate-nitrogen ($\text{NO}_3\text{-N}$) was analyzed using the Spectrophotometer with Cadmium method, and the Nitra Version 5 reagent. TKN was analyzed using the Semi-Micro Kjeldahl method. The organic-nitrogen (Org-N) was determined by calculating the difference between TKN and ammonia-nitrogen. Faecal coliforms were enumerated using membrane filtration technique using M-FC agar base solution as the medium in accordance with Standard methods for the examination of

water and wastewater samples (APHA *et al.*, 2012).

Data processing

All data generated in this research study were preliminarily examined to check for their correctness. Those found to be obviously erroneous, by virtue of appearing to be incongruous with the rest of the data in their respective sets, were discarded. Subsequently, Microsoft EXCEL 2008 spreadsheet and MINITAB Release 15 for Windows software were used to analyze the remaining data. The Anderson-Darling test was used for testing normality. Comparisons of variables were performed using the Analysis of Variances (ANOVA) technique. Multiple comparisons were performed using Tukey’s HSD tests. Analyses of the data also included statistical correlations and statistical differences between related variables.

Spearman’s correlation tests were performed on various data sets, in order to examine the relationships between the corresponding variables. These tests assessed the linear relationships between variables/parameters, and they were

performed using Spearman’s rank-order linear relationships, by examining the coefficients of correlation R and the coefficients of determination R², which range between -1.0 and +1.0. The stronger the relationship between the variables/parameters, the higher the coefficient of correlation R and the coefficient of determination R². Coefficients of correlation R of -1.0 and +1.0 represent perfect correlation between the variables/parameters under examination. Coefficients of correlation of (-0.5) and (+0.5) were regarded as indicating variables/parameters having a strong correlation and relationship. Variables/parameters having coefficients of correlation R (-0.5) and (+0.5), were regarded as having poor correlation and relationship.

RESULTS AND DISCUSSIONS

Variation of physical-chemical characteristics

Table 1 summarizes the values of the observed Lubigi wetland water inlet and outlet nitrogen, faecal coliforms and related physical-chemical parameters.

Table 1: Variation of physical parameters in Lubigi Wetland

Parameter	Inlet Values		Outlet Values	
	Min – Max	Mean ± SD	Min – Max	Mean ± SD
pH	6.60- 8.10	7.22 ±0.34	6.80-8.00	7.24 ±0.29
Temperature (°C)	19.7-22.4	21.2 ±0.71	20.5-24.8	22.6 ±1.06
Dissolved Oxygen (mg/l)	0.06 – 2.43	0.30 ±0.37	0.07-0.64	0.23 ±0.10

Min-minimum and Max-maximum

The pH

Table 1 shows that in the wetland main inlet zone, the pH of the water ranged from 6.7 to 8.1 with a mean value of 7.22 ±0.34, while in the outlet zone, the pH varied from 6.8 to 8.0 with a mean value of 7.24 ±0.29. Statistical analyses

showed that at the 95% confidence interval (α = 0.05), there was no significant difference between the wetland inlet and outlet pH values (p = 0.143) although there is a positive linear correlation (R²= 0.391), between the wetland outlet and inlet pH values. The range of the observed outlet pH values suggests that microbial activities in the wetland are favored, since

these values fall within the optimum functioning pH range of microbes (Kadlec, 2009).

In particular, mineralization having the optimum pH range of 6.5 to 8.5 (Patrick and Wyatt, 1964), and denitrification by *Pseudomonas* bacteria having the optimum pH range of 7.0 to 8.0 (Delwiche and Bryan, 1978; Muller *et al.*, 1999) are favored to occur at appreciable rates. However, nitrification by *Nitrosomonas* bacteria having the optimum pH range of 8.5 to 8.8, and by *Nitrobacter* bacteria having the optimum pH range of 8.3 to 9.3 (Halling-Sorensen and Jorgensen, 1993), appears to be less favored to occur at high rates. The range of the observed outlet pH values, also favors ammonia-nitrogen to exist predominantly in the ionized-ammonium (NH_4^+) form, rather than in the volatilized un-ionized-ammonia gas ($\text{NH}_3(\text{g})$) form, which starts to become predominant at pH values above 9.0 (Konig *et al.*, 1987).

Dissolved oxygen

From Table 1, the dissolved oxygen concentrations in the main inlet zone, ranged from 0.06 to 2.43 mg/l, with a mean value of 0.32 mg/l, whereas in the outlet zone, the dissolved oxygen concentrations ranged from 0.07 to 0.64 mg/l, with a mean value of 0.24 mg/l at the 95% confidence interval ($\alpha = 0.05$). Further statistical analyses showed that there is a statistically significant difference between the inlet and outlet dissolved oxygen concentrations ($p = 0.014$) and a very weak and positive linear correlation ($R = 0.23$), between the wetland outlet and inlet pH values. The observed dissolved oxygen (DO) concentration values, suggest that the Lubigi wetland has very low levels of aeration and oxygenation, and it is therefore by and large anoxic. The dissolved oxygen concentrations decreased by 26.7% in the wetland, suggesting that the water is significantly de-oxygenated by

the high Biochemical Oxygen Demand (BOD) related to the biogeochemical activities taking place in the wetland, including nitrification and the biodegradation by microorganisms of organic matter in the wastewater, and the decaying plant litter/detritus.

Algal photosynthesis, which contributes to the oxygenation of wetlands, does not appear to be playing a major role in the Lubigi wetland although wetland received 469 to 578 $\text{cal/m}^2/\text{d}$ of solar intensity (Mean = 525 $\text{cal/m}^2/\text{d}$). It is worth to report that the average plant density in Lubigi wetland is 10.2 plants/ m^2 (Kayima and Mayo, 2018), which is about three times higher than 3.2 plants/ m^2 in Mara River Basin wetlands in Tanzania (Muraza *et al.*, 2014). The thick canopy of the dense emergent macrophytes prevalent in the Lubigi wetland has a sheltering and shadowing effect, obstructing sunlight from reaching the wetland water column and thus curtailing the growth of algae, photosynthesis and oxygen production. In addition, dense emergent macrophytes tend to reduce wind velocities and the related physical re-aeration and oxygenation actions on the wetland waters. Hence in general, nitrification by *Nitrosomonas* bacteria having the optimum dissolved oxygen concentration range of 2.0 to 7.5 mg/l, and *Nitrobacter* bacteria having the optimum dissolved oxygen concentration of 4.0 to 7.5 mg/l (Knowles *et al.*, 1965; Senzia, 2003) is not favored to occur at high rates. However, denitrification by *Pseudomonas* bacteria having the optimum dissolved oxygen concentration range of less than 0.5 mg/l (Halling-Sorensen and Jorgensen, 1993) is favored to occur at appreciable rates.

Water temperatures

From Table 1, water temperatures in the main inlet zone range from 19.7°C to 22.4°C, with a mean value of 21.2 \pm 0.71 °C, whereas in the outlet zone, water

temperatures range from 20.5°C to 24.8°C, with a mean value of 22.6 ± 1.06 °C. Figure 2 shows that there is a positive, but low linear correlation ($R= 0.49$), between outlet and inlet water temperatures, which suggests that wetland temperature is relatively independent of inlet temperature. On average, wetland temperature increased by 1.4°C, although statistical analysis at the 95% confidence interval ($\alpha = 0.05$), suggests that there is no significant difference between the inlet and outlet temperatures ($p = 0.161$). The observed water temperature values suggest that

mineralization having a limiting minimum temperature of about 11°C to 14°C (U.S. Environmental Protection Agency, 2000) is favored to occur at appreciable rates. However, nitrification by *Nitrosomonas* bacteria having the optimum temperature range of 28°C to 36°C, and *Nitrobacter* bacteria having the optimum temperature range of 28°C to 42°C (Painter, 1970), is not favored to occur at a high rate. On the other hand, denitrification by *Pseudomonas* bacteria having the optimum temperature range of 3°C to 35°C can proceed at appreciable rates.

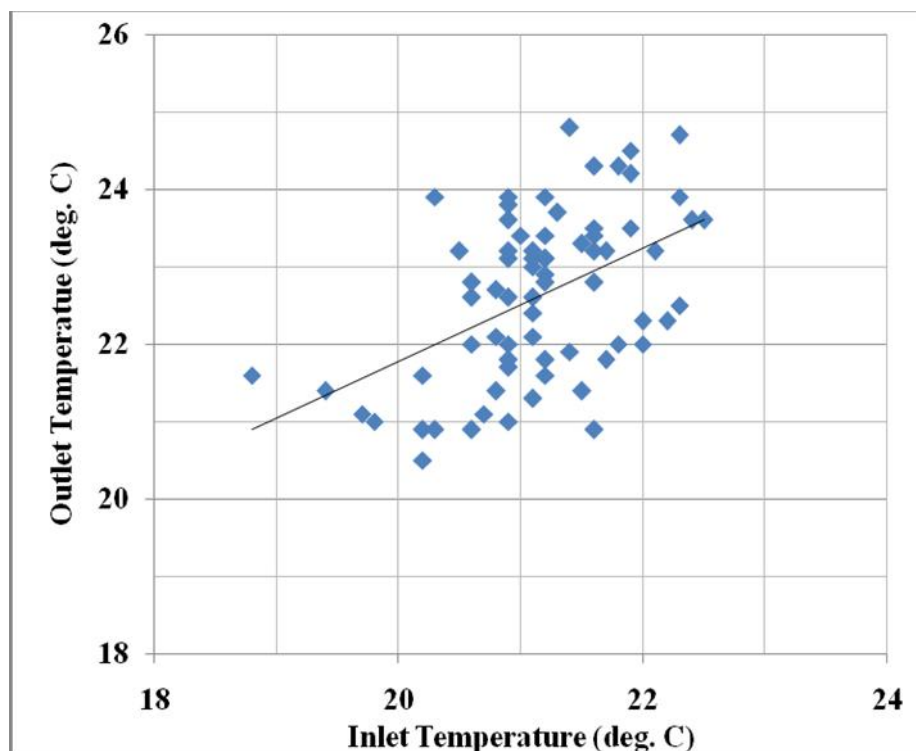


Figure 2: Correlation between wetland outlet and inlet water temperatures

Ammonia-nitrogen in the wetland

The concentration of ammonia-nitrogen in the inlet ranged from 1.23 mg/l to 73.6 mg/l, with a mean value of 29.1 ± 18.3 mg/l. However, the concentration of ammonia-nitrogen in the wetland outlet varied from 0.71 to 61.0 mg/l with a mean value of 21.9 ± 14.0 mg/l. Further statistical analyses showed that there is a statistically significant difference between the wetland inlet and outlet ammonia-

nitrogen concentrations ($p = 0.037$). These high ammonia-nitrogen concentration values can be attributed to the presence of wastewater discharged into the Lubigi wetland. In particular, the process of mineralization of organic-nitrogen to ammonia-nitrogen appears to be proceeding at significant rates due to the pH and temperature conditions which favor this process, as already discussed. There is also the indication that the originally settled organic-nitrogen is being

regenerated back into the water column as ammonia-nitrogen, especially during the rainy and flash floods periods when conditions of hydraulic turbulence are set in motion.

It was observed that the average ammonia-nitrogen removal efficiency was 24.7%. This relatively low overall removal efficiency appears to be due to low rates of nitrification as a result of unfavorable dissolved oxygen levels. It is possible that due to the high plants densities, plant uptake may be the major process governing ammonia-nitrogen removal in the wetland. Figure 3 shows that there is a

very strong and positive linear correlation/relationship ($R = 0.88$), between the wetland outlet and inlet ammonia-nitrogen. The observed mean value of outlet ammonia-nitrogen of 21.9 mg/l is more than double the recommended Uganda National Effluent Discharge Standard of 10.0 mg/l (Uganda National Environmental Management Authority (NEMA), 2016). This implies that the overall ammonia-nitrogen ($\text{NH}_3\text{-N}$) removal capacity of the Lubigi wetland main study area, does not meet the recommended standards, before disposing effluents into the environment.

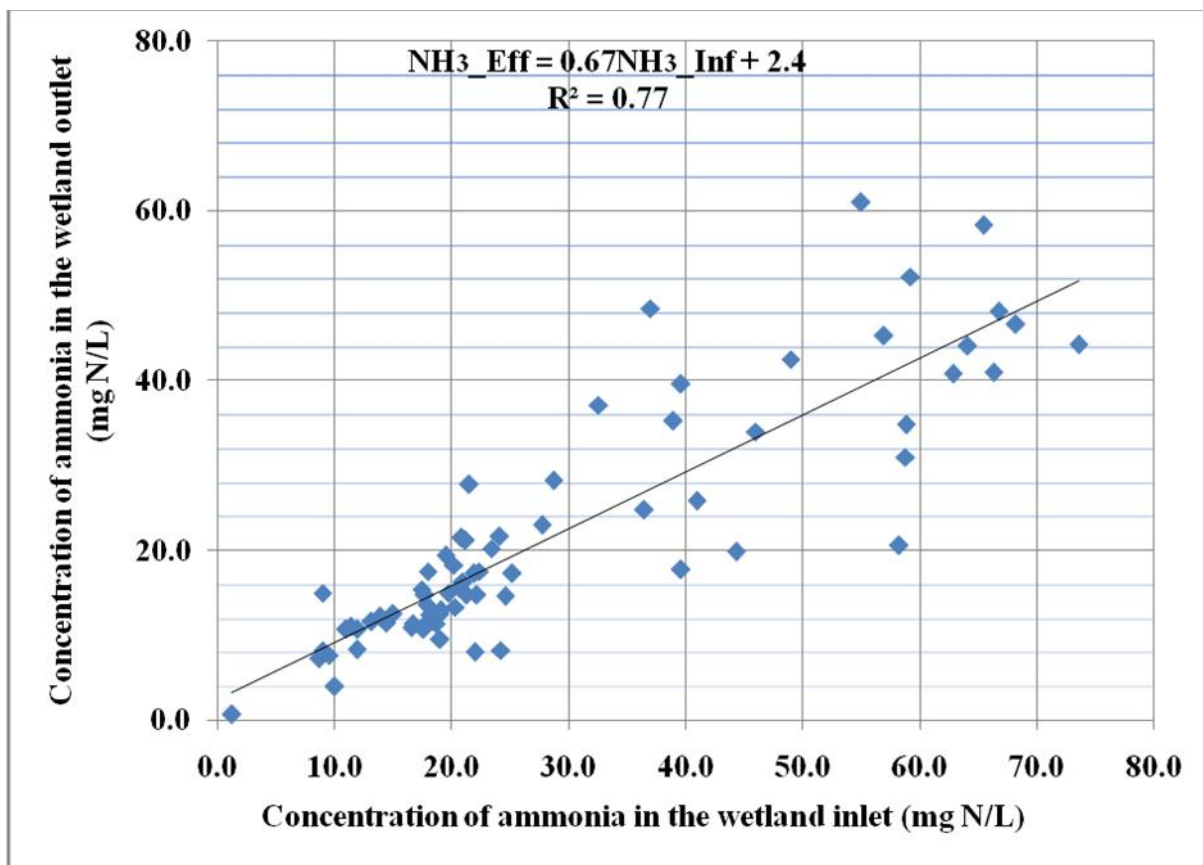


Figure 3: Correlation between wetland outlet $\text{NH}_3\text{-N}$ and inlet $\text{NH}_3\text{-N}$

Nitrate-nitrogen in the Lubigi Wetland

Figure 4 shows that there is a very strong linear correlation/relationship ($R = 0.74$) between the wetland outlet and inlet nitrate-nitrogen. The concentration of nitrate-nitrogen ($\text{NO}_3\text{-N}$) in the wetland

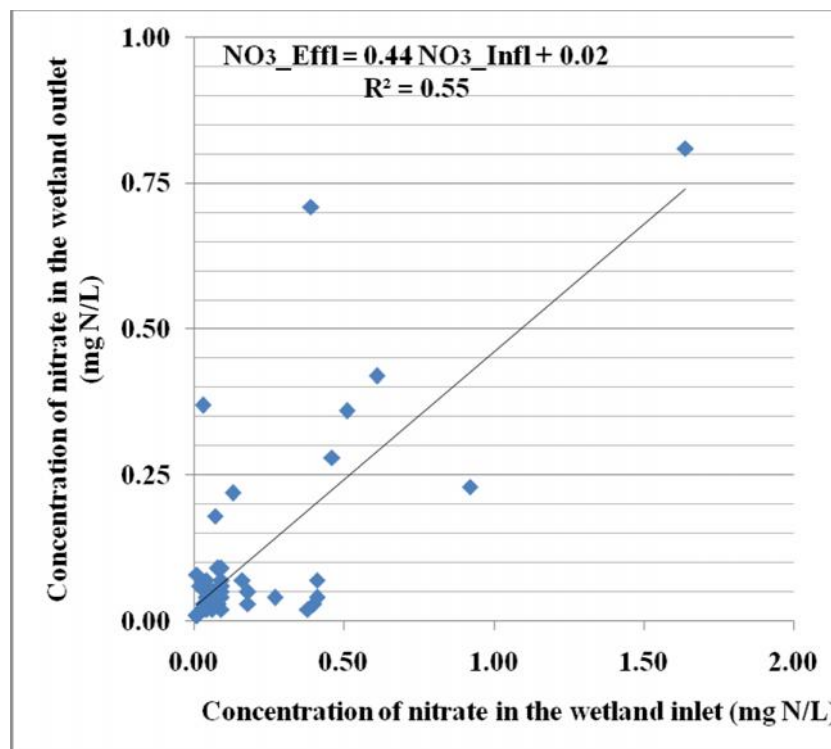
inlet ranged from 0.01 to 1.64 mg/l, with a mean value of 0.15 ± 0.20 mg/l, and in the outlet it varied from 0.01 to 0.81 mg/l, with a mean value of 0.09 ± 0.14 mg/l. Statistical analysis revealed that there is a significant difference between the inlet and outlet ammonia-nitrogen concentrations (p

= 0.024). It was noted that the mean outlet nitrate-nitrogen concentration of 0.09 mg/l was well below the recommended Uganda National Effluent Discharge Standard of 10.0 mg/l (Uganda NEMA, 2016), implying that the overall nitrate-nitrogen removal capacity of the Lubigi wetland main study area, meets the recommended standards, before disposing effluents into the environment.

The noticeably low nitrate-nitrogen concentration values appear to confirm the earlier observations and discussions, that the process of nitrification to form nitrate-nitrogen is constrained by the dissolved oxygen level in the wetland. Furthermore, this may be confirming the earlier observations that denitrification may be proceeding and removing nitrate-nitrogen at considerable rates due to favorable pH,

dissolved oxygen and temperature conditions in the wetland. There is also indication that due to the high plants densities, the process of plant uptake is also playing a significant role in the removal of nitrate-nitrogen.

The average nitrate-nitrogen removal efficiency of 40.0% provides further evidence that denitrification may be proceeding and removing nitrate-nitrogen, at considerable rates. This denitrification would be well supported, especially due to the presence of the abundant labile organic carbon sources in the wastewater and the decaying plant detritus, acting as electron donors in favor of this process. In addition, it is possible that plant uptake is also playing a significant role in the removal of nitrate-nitrogen.



value of 40.1 ± 33.8 mg/l. It was further observed that there is a statistically significant difference between the wetland inlet and outlet ammonia-nitrogen concentrations ($p = 0.013$). Figure 5 also shows that there is a strong linear correlation/relationship ($R = 0.78$) between the wetland outlet and inlet organic-nitrogen. The observed mean value of outlet organic-nitrogen of 53.6 mg/l, is more than double the recommended Uganda National Effluent Discharge Standard of 20.0 mg/l (Uganda NEMA, 2016), implying that the overall organic-nitrogen removal capacity of the Lubigi wetland main study area, does not meet the recommended standards, before disposing effluents into the environment.

The organic-nitrogen concentration in the wetland inlet was noticeably high and can be attributed to the presence of wastewater discharged into the Lubigi wetland. Furthermore, there is suggestion that due to the high plant densities, the leaching of the abundant decaying dead plant detritus makes a significant contribution to the recycling of organic-nitrogen (Org-N) back into the water column. The average organic-nitrogen removal efficiency was 25.1%, which might have been attributed to the process of mineralization of the Org-N to $\text{NH}_3\text{-N}$. In addition, the sedimentation of organic-nitrogen into the wetland benthic layers, may also be contributing significantly to the overall removal of organic-nitrogen from the water column.

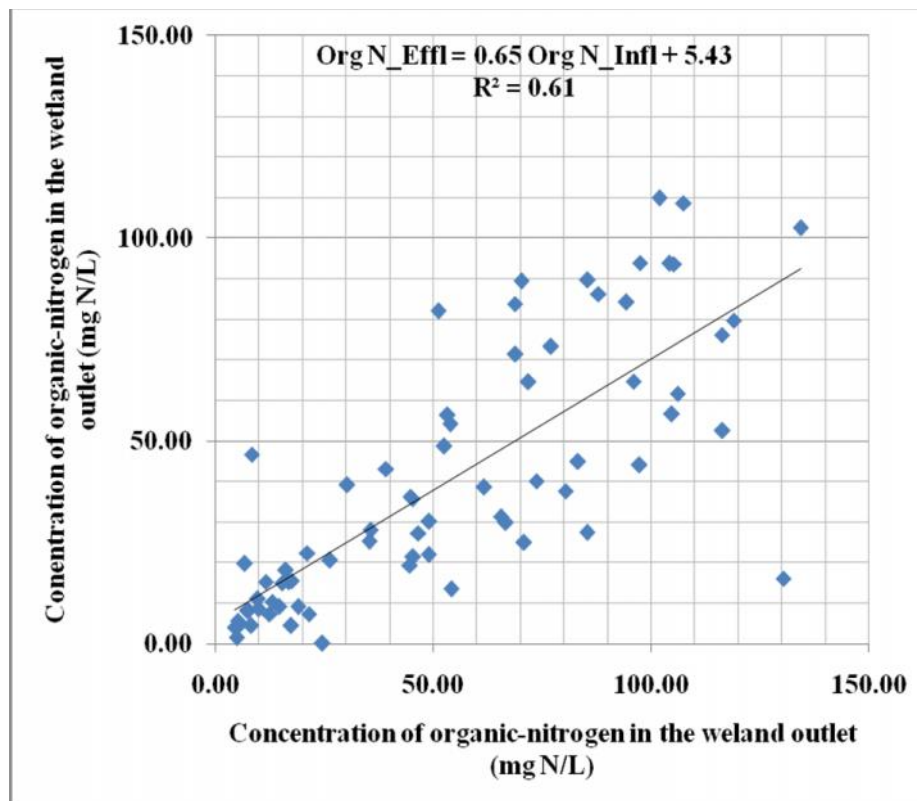


Figure 5: Correlation between wetland outlet Org-N and inlet Org-N

Total nitrogen in the Lubigi wetland

In accordance with the data collected, the concentration of total-nitrogen varied from 16 to 173 mg/l, with a mean value of 83.1 ± 44.7 mg/l in the wetland inlet zone,

and from 10 to 167 mg/l, with a mean value of 62.4 ± 36.8 mg/l in the outlet zone. Further statistical analyses show that there is a statistically significant difference between the wetland inlet and outlet ammonia-nitrogen concentrations ($p =$

0.035). The average total-nitrogen removal efficiency was 24.9%, which is an appreciable amount considering that the mean hydraulic residence time is only about 2.0 days (Kayima *et al.*, 2018b). This removal is largely through net-loss by sedimentation, plants uptake and denitrification.

Figure 6 also shows that there is a strong linear correlation ($R= 0.81$) between the wetland outlet and inlet total-nitrogen. The observed mean value of outlet total-nitrogen of 83.1 mg/l, is more than double the recommended Uganda National Effluent Discharge Standard of 20.0 mg/l (Uganda NEMA, 2016), implying that the overall total-nitrogen removal capacity of the Lubigi wetland main study area, does not meet the recommended standards, before disposing effluents into the environment.

This section of the wetland has removed 20.7 mg/l of nitrogen. With the average flow rate of 221,357 m³/d (Kayima *et al.*, 2018), the wetland has a capacity to remove 4,582 kgN/d, which is equivalent to 1,672 tons/year. Although substantial amount of nitrogen is removed in this section of the wetland, about 5,040 tons/day of nitrogen is discharged at the outlet end of the wetland, which suggests that significant quantity of nitrogen received from the upstream is discharged at the downstream end without removal. This could lead to pollution of water bodies downstream of Lubigi wetland.

Nitrogen content in wetland sediments

Sediment samples indicate that at transect T3 the concentration of nitrogen was 0.60±0.22 gN/kg sediments. However, at transects T1 and T2, the samples concentration of nitrogen was 0.16±0.12 and 0.14±0.12 gN/kg sediments, respectively. The average nitrogen content

in the sediments was 0.30±0.15 gN/kg sediments, which translates to 157.5 gN.m⁻² of wetland. It is evident that there is a general trend of more nitrogen accumulating in the sediments as the wetland water flows from wetland inlet to the outlet. This can be attributed to the simultaneous trend of increase in plants densities from wetland upstream zones towards the downstream ends. Table 2 summarizes the results of the Lubigi wetland plants biomass and nitrogen contents. The corresponding increase in the below-ground roots and rhizomes structures of these plants is responsible for the trapping of increasing sediments quantities, which in turn determine the increasing quantities of nitrogen trapped in the sediments.

Nitrogen content in wetland sediments

Sediment samples indicate that at transect T3 the concentration of nitrogen was 0.60±0.22 gN/kg sediments. However, at transects T1 and T2, the samples concentration of nitrogen was 0.16±0.12 and 0.14±0.12 gN/kg sediments, respectively. The average nitrogen content in the sediments was 0.30±0.15 gN/kg sediments, which translates to 157.5 gN.m⁻² of wetland. It is evident that there is a general trend of more nitrogen accumulating in the sediments as the wetland water flows from wetland inlet to the outlet. This can be attributed to the simultaneous trend of increase in plants densities from wetland upstream zones towards the downstream ends. Table 2 summarizes the results of the Lubigi wetland plants biomass and nitrogen contents. The corresponding increase in the below-ground roots and rhizomes structures of these plants is responsible for the trapping of increasing sediments quantities, which in turn determine the increasing quantities of nitrogen trapped in the sediments.

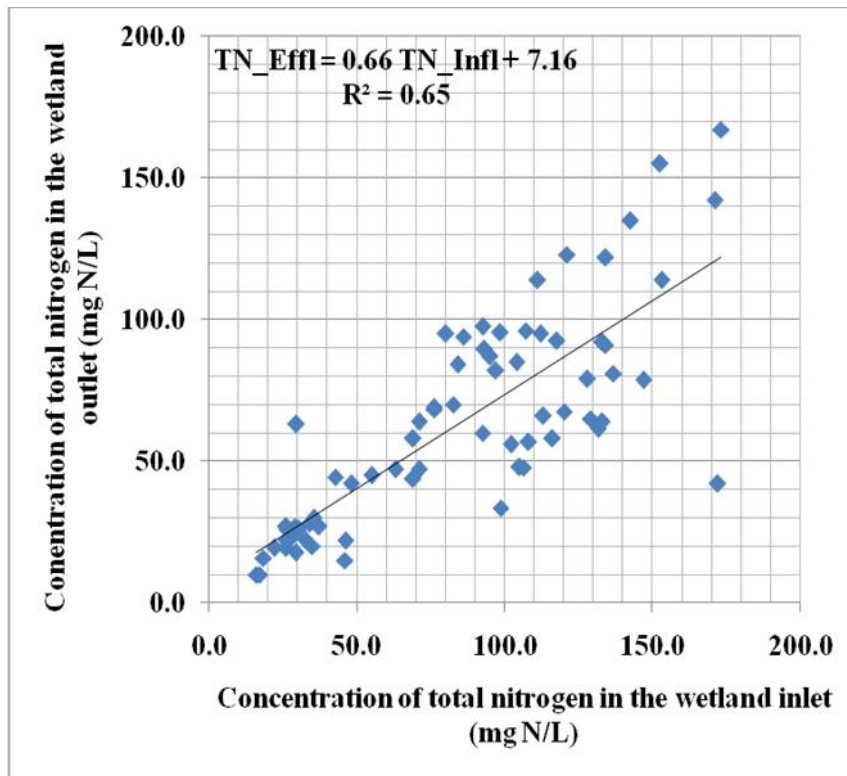


Figure 6: Correlation between wetland outlet TN and inlet TN

Table 2: Lubigi Wetland Plants Biomass and Nitrogen Contents

Item		<i>Cyperus Papyrus</i>	<i>Echinochloa pyramidalis</i>	<i>Typha capensis</i>	<i>Rottboellia cochinchinensis</i>	<i>Oldenlandia lancifolia</i>	Average±SD
Biomass (kgDW/m ²)	AG	0.55±0.48	0.14±0.10	0.21±0.16	0.023±0.015	0.28±0.28	0.24±0.09
	BG	0.46±0.23	0.03±0.02	0.05±0.03	0.005±0.003	0.09±0.09	0.13±0.08
	Total	1.01±0.05	0.17±0.06	0.26±0.08	0.028±0.009	0.37±0.01	0.37±0.17
Nitrogen Contents (%DW)	AG	0.22±0.14	0.56±0.31	0.17±0.10	0.038±0.038	0.03±0.03	0.20±0.09
	BG	0.19±0.11	0.1±0.09	0.04±0.02	0.004±0.004	0.01±0.01	0.07±0.03
	Total	0.41±0.12	0.66±0.23	0.21±0.07	0.042±0.017	0.04±0.01	0.27±0.12
Nitrogen Contents (gm ⁻²)	AG	121.0	78.4	35.7	0.9	8.4	48.89±22.57
	BG	87.4	3.0	2.0	0.0	0.9	18.66±17.19
	Total	208.4	81.4	37.7	0.9	9.3	67.54±37.91

BG= Below-Ground plant organs; AG= Above-Ground plant organs; DW = Dry Weight; SD = Standard deviation

Faecal Coliforms in the Lubigi Wetland

The concentration of faecal coliform in the wetland inlet ranged from 60 to 4,800,000 cfu/100ml with a mean value of 653,509 ± 2,182 cfu/100ml. In the wetland outlet zone, faecal coliform counts varied from 20 to 740,000 cfu/100ml, with a mean value of 218,676 ± 157 cfu/100ml. Therefore, this gives an average faecal coliform removal efficiency of 66.5%.

However, the quality of effluent is far above the recommended Uganda National Effluent Discharge Standard of 5,000 CFU/100ml (Uganda National Environmental Management Authority (NEMA), 2016). This implies that the wetland outlet faecal coliform (FC) concentrations of the Lubigi wetland main study area, do not meet the recommended faecal coliform (FC) standards. However, when the logarithm values of the wetland inlet and outlet faecal coliform was

plotted, a strong correlation was observed ($R = 0.95$). The high faecal coliform (FC) concentration in the inlet can be attributed to the wastewater discharged into the Lubigi wetland from the open channel drains and from Lubigi sewage treatment plant. Although treatment of wastewater is

done in Lubigi sewage treatment plant the effluent quality is still poor. In several occasions raw sewage is directly by-passed into the wetland when the plant is overloaded or when it is undergoing maintenance and repairs.

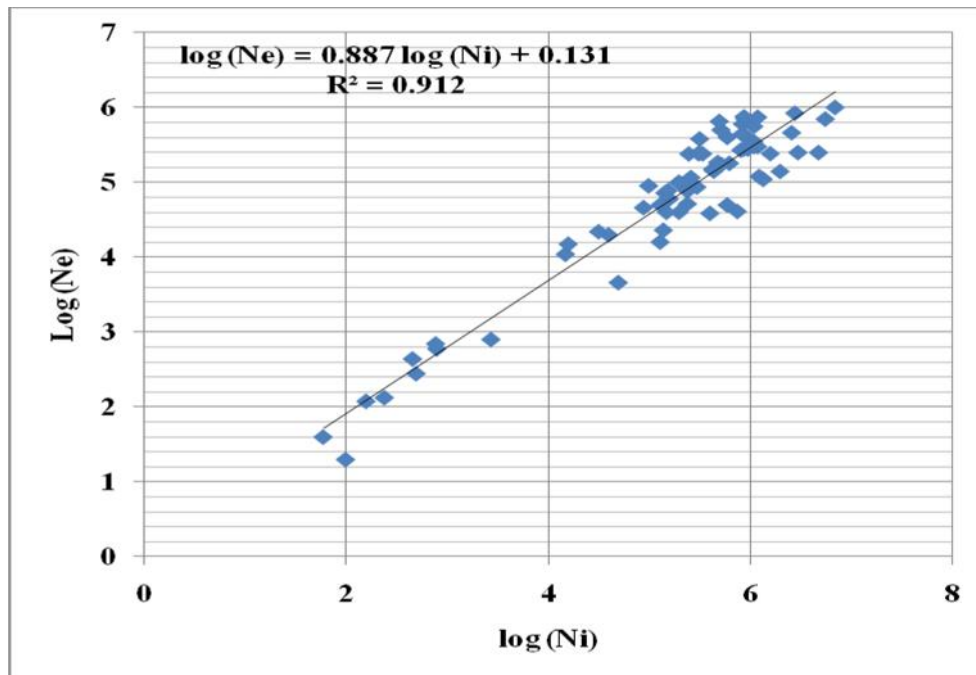


Figure 7: Correlation between the wetland outlet FC and inlet FC

In a study carried out by Kansime and Nalubega (1999) in the Nakivubo swamp in Uganda, a relatively higher faecal coliform removal efficiency of 90.9% was reported. This wetland receives wastewater from the Nakivubo storm water channel and effluents from the Bugolobi sewage treatment plant was reported to contain mean faecal coliform (FC) concentrations of $110,000 \pm 26,000$ cfu/100 ml in the inlet and $10,000 \pm 2,400$ cfu/100 ml in the wetland outlet zones. In another study by Mayo and Kalibbala (2007) in constructed water hyacinth ponds in the University of Dar es Salaam in Tanzania, which received sewage effluents from the waste stabilization ponds, faecal coliform (FC) concentrations was reduced from $136,000 \pm 16,600$ cfu/100ml in the wetland inlet zone, to a mean value of $83,900 \pm 10,200$ cfu/100ml in the outlet

zone. This reduction is equivalent to a mean faecal coliform removal efficiency of 38.3%. For comparison, Kalunde (2007) reported removal efficiency of faecal coliform of 96.0% in a horizontal sub-surface flow constructed wetland at the University of Dar es Salaam wastewater treatment facility. It is evident that faecal coliform removal efficiency varies from one system to another depending on the type of wetland and variations of physical, environmental and forcing functions affecting each system.

CONCLUSIONS

From the foregoing results and discussion, it is evident that the Lubigi wetland receives varying concentration of total nitrogen varying from 16 to 173 mg/l, largely in form of organic-nitrogen (64.7%) and ammonia-nitrogen (35.2%). The wetland removed about 24.9% of this nitrogen largely through net-loss by plants uptake, sedimentation and denitrification. The Lubigi wetland main study area has total-nitrogen (TN) mean values of approximately 83.1 mg/l and 62.4 mg/l, in the wetland inlet and outlet zones, respectively. The mean effluent discharge is approximately 221,357 m³/d, and the total area of the whole wetland is approximately 2.98 km². Hence, the whole wetland removes approximately 1,672 tons of nitrogen per year, which is equivalent to about 5,610 kg/ha/year. The analyses of the plants nitrogen contents, also indicate that significant amounts of nitrogen amounting to about 675.4 kg/ha, which is equivalent to approximately 202 tons, are contained in the plants biomass, and are thus considered to be removed by the wetland. In addition, the Lubigi wetland main study area also receives faecal coliform concentrations with inlet mean value of 653,509 CFU/100ml and its outlet mean value is 218,676 CFU/100ml. These values give a faecal coliform (FC) overall removal efficiency of approximately 66.5%. These are substantial amounts of nitrogen and faecal coliforms, which have been prevented from entering the Mayanja River and Lake Kyoga. Evidently, the Lubigi wetland has considerable capacity to buffer and protect Lake Kyoga by reducing nitrogen and faecal pollution entering the lake. The nutrients retained in the wetland has sustained habitat for diverse and endemic flora and fauna, which is important to the ecosystem. It is further recommended to develop tools for prediction and management of nutrient dynamics in the wetland.

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