

Optimizing Processes for Biological Nitrogen Removal in Nakivubo Wetland, Uganda

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Cover photograph
Early growth of a *Cyperus papyrus*-based constructed wetland
Treating domestic wastewater in Uganda

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To my daughter *Hannah Nammanda* and her grand mama *Angella Lwanga*

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ABSTRACT

The ability of Nakivubo wetland (which has performed tertiary water treatment for Kampala city for the past 40 years) to respond to pollution and to protect the water quality of Inner Murchison Bay of Lake Victoria was investigated. The aim of this study was to assess the capacity of Nakivubo wetland to remove nitrogen from the wastewater after its recent encroachment and modification, in order to optimize biological nitrogen removal processes using sustainable and environmentally sound biological processes.

Field studies were performed to assess the hydraulic loading, stability and water quality of this wetland. The distribution and activity of ammonium-oxidizing bacteria (AOB) in Nakivubo channel and wetland were also investigated, and the significance of the different matrices in biological nitrogen transformations within the two systems elucidated. Studies to optimize nutrient removal processes were carried out at pilot scale level both in container experiments and in the field using substrate-free constructed wetlands (CWs) planted with *Cyperus papyrus* and *Miscanthidium violaceum* which were adapted to the local ecological conditions.

Results showed that Nakivubo wetland performs tertiary treatment for a large volume of wastewater from Kampala city, which is characterised by large quantities of nutrients, organic matter and to a lesser extent metals. Mass pollutant loads showed that wastewater effluent from a sewage treatment plant constituted a larger proportion of nitrogen and biochemical oxygen demand (BOD) discharged into the wetland. The upper section of Nakivubo wetland exhibited high removal efficiencies for BOD, whereas little or no ammonium-nitrogen and metals except Lead were removed by wetland. Studies further showed that nitrifying bacteria existed in the wetland but their activity was limited by oxygen depletion due to the high BOD in the wastewater and heterotrophic bacteria from the sewage treatment plant. Distributional studies indicated the presence of more AOB in surface sediments than the water column of the lower section of Nakivubo channel, an indication that nitrifiers settled with particulate matter prior to discharge into the wetland, and thus did not represent seeding of the wetland. The significant reductions in concentrations of BOD compared to ammonium and total nitrogen in the channel and wetland wastewater confirmed this finding. Whereas suspended nitrifiers upstream of Nakivubo channel equally influenced total nitrogen balance as those in surface sediments, epiphytic nitrification was more important than that of sediment/peat compartments in the wetland, and thus highlighted the detrimental impacts of wetland modification on the water quality Inner Murchison Bay and Lake Victoria as a whole.

Performance assessment of pilot-scale container experiments and field-based CWs indicated highly promising treatment efficiencies, notably in papyrus-based treatments. Plant biomass productivity, nutrient storage, and overall system treatment performance were higher in papyrus-based constructed wetlands, and resulted in effluent that met national discharge limits. Thus, papyrus-based CWs were found to be operationally efficient in removing pollutants from domestic wastewater.

Key words: Ammonium-oxidizing bacteria; Biological nitrogen removal; Coliform retention; Constructed wetlands; *Cyperus papyrus*; Macrophytes; *Miscanthidium violaceum*; Nakivubo; Nitrification activity; Nitrogen; Phosphorus; Self-purification; Tropical wetlands; Uganda; Wastewater treatment.

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LIST OF RESEARCH PAPERS APPENDED

This thesis is based on the following original research papers which in the text are referred to by their roman numerals.

- I. **Kyambadde J.**, Kansiime F., Gumaelius L., Dalhammar G., 2004. Hydraulic loading, stability and water quality of Nakivubo wetland, Uganda. *Afr. J. Aquat. Sci.* **29**(2):213–220.
- II. **Kyambadde J.**, Kansiime F., Dalhammar G., 2005. Distribution and activity of ammonium-oxidizing bacteria in Nakivubo channel and wetland systems. (Submitted to *Acta Hydrochim. Hydrobiolog.*).
- III. **Kyambadde J.**, Kansiime F., Gumaelius L., Dalhammar G., 2004. A comparative study of *Cyperus papyrus* and *Miscanthidium violaceum*-based constructed wetlands for wastewater treatment in a tropical climate. *Water Res.* **38**(2): 475–485.
- IV. **Kyambadde J.**, Kansiime F., Dalhammar G., 2005. Functional assessment of horizontal surface flow constructed wetlands receiving pre-treated domestic wastewater in Uganda. (Submitted to *Water Res.*).
- V. **Kyambadde J.**, Kansiime F., Dalhammar G., 2005. Nitrogen and phosphorus removal in substrate-free pilot constructed wetlands with horizontal surface flow in Uganda. (Accepted for Publication in *Water, Air, Soil Pollut.*).

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ACRONYMS

AOB	Ammonium-oxidizing bacteria
AOR	Ammonium-oxidation rate
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
CFU	Colony-forming unit
CW	Constructed wetland
DO	Dissolved oxygen
DON	Dissolved organic nitrogen
DW	Dry weight
FAM	Floating aquatic macrophyte
FWS	Free water surface
GoU	Government of Uganda
HSFCW	Horizontal surface flow constructed wetland
IUCN	The World Conservation Union
MDG	Millennium development goal
MCL	Maximum containment level
NEMA	National Environment Management Authority
NOB	Nitrite oxidizing bacteria
NO _x	Nitrite or nitrate
POAs	Phosphorus accumulating organisms
PON	Particulate organic nitrogen
SAV	Submerged aquatic vegetation
SSF	Subsurface flow
TN	Total nitrogen
TP	Total phosphorus
TSS	Total suspended solids
VF	Vertical flow

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1 INTRODUCTION

Provision of safe water free from contaminants to mankind is a global, regional and national priority. It is estimated that more than 1 billion people in the less developed countries lack access to safe, clean water and an estimated 1.6 million children under the age of 5 die from diarrhoeal diseases each year [1, 2]. Besides, about 2.6 billion people have no access to improved sanitation facilities [2]. In Uganda, less than 60% of the 26 million people have access to safe water supply or sanitation services [3]. Strong efforts are currently being made to provide clean water to the remaining population. However, though provision of clean water and sanitation services should ideally move hand in hand if waterborne diseases are to be prevented, little effort has been undertaken to improve or upgrade the existing and deteriorating wastewater treatment systems in urban areas of Uganda. Therefore in order to meet the anticipated millennium development goal target of reducing the population at risk of water-borne related infections from 2.6 billion to 1.9 billion by 2015, improved sanitation and affordable wastewater treatment facilities are required [2].

Conventional wastewater treatment processes are widely applied in developed countries. However, they require high energy inputs, chemicals, skilled man-power and large capital investments to build and operate [4, 5]. This makes the technology expensive and unattractive to resource-scarce developing countries like Uganda where wastewater treatment is of lower priority. Moreover, the high per capita costs for wastewater collection and treatment make these advanced biological processes far beyond the reach of rural communities and small scale enterprises whose tax-bases and profit margins are low. Consequently, untreated or partially treated wastewater is discharged into nearby surface water streams and wetlands, with eventual pollution of drinking water sources.

In Uganda, conventional biological wastewater treatment has only been installed for Kampala City and Masaka Town. Other large towns namely, Entebbe, Jinja, Mbale, Mbarara, Tororo, Lira, Gulu, Kasese, Fort Portal and Kabale treat their wastewater using stabilization pond systems [6]. In either case, the treatment systems are directed primarily to meet the needs of large towns and the wealthy class without considering the rural community majority. As in most developing countries, stabilization ponds have long been recognized by the government as the most appropriate technology for biological treatment of wastewaters because of their sustainability in terms of operation and maintenance. However, these systems have often been neglected, poorly maintained and not upgraded to cope with the ever increasing populations they serve probably due to lack of direct monetary benefits from these systems. As a result, low quality effluents are being discharged into the environment.

With the permissible discharge limits becoming more stringent [7], sustainable wastewater treatment systems that require low operation and maintenance (O&M) costs are not only desirable but a requirement to prevent the spread of waterborne diseases and prevent pollution of the environment. Since many natural wetlands that have long acted as wastewater disposal sites are rapidly being modified for agriculture and infrastructure development [7, 8], upstream treatment of wastewaters using constructed wetlands is one option that can be exploited to optimize pollutant removal in degrading natural treatment wetlands to ensure a sustainable supply of safe and clean water. These systems effectively integrate wastewater treatment and resource enhancement at a competitively lower cost (60–95%) compared to conventional mechanical treatment systems [9]. Moreover, they generate effluent that is far less damaging

to the environment since no chemicals are added during the treatment process.

1.1 Motivation

For over 4 decades, Nakivubo wetland has received and processed surface runoff and untreated domestic and industrial effluents from Kampala city and its suburbs prior to discharge into Lake Victoria at Inner Murchison Bay. In addition, the outflow for Kampala's sewage treatment works at Bugolobi flows into the same wetland. Therefore the ecological importance of this ecotone for the control of eutrophication of Lake Victoria in general, and protection of the drinking water supply for Kampala city in particular, is highly valued.

Previous studies of Nakivubo wetland indicated a high wastewater treatment potential [10–15]. In their investigations however, they did not assess the individual contributions of all the inflows into the wetland, especially for heavy metals. Though important bacteria involved in nitrogen transformation were quantified and found to be less abundant in the lower section of Nakivubo wetland [12], the underlying factors responsible for their spatial distribution in the wetland and its major inflows were not thoroughly investigated. In addition, since the above studies were performed, more than 50% of the wetland has been strongly impacted by human encroachment, notably its conversion into agricultural fields covered by cocoyam and sugar cane.

Over the last decade, Uganda has experienced a period of rapid economic growth, rehabilitation and urban expansion, with urban population growth rates of up to 5% per year [16]. In Kampala, the population has doubled to nearly 1.5 million people since 1991 [17]. Notably, the majority of these developments have taken place in Kampala and have been undertaken in the absence of proper planning and controls and implemented at the expense of wetland drainage and reclamation of which

Nakivubo wetland takes a lead [7]. Consequently, the quantities of wastewater generated are enormously high yet the existing treatment infrastructures have neither been improved nor expanded. As a result, large volumes of untreated and partially treated effluents are discharged into Nakivubo wetland which is already stressed by human activities, posing undesired pollution reverberations such as nitrate and metal contamination to downstream consumers of Inner Murchison Bay water.

In addition, the nutrients reaching Inner Murchison Bay of Lake Victoria will promote eutrophication, leading to clogging of the water supply system located in the same bay, only 4 km downstream of the wastewater discharge point at Inner Murchison Bay. In view of this situation, it was imperative to investigate the current ability of the wetland to buffer incoming pollutants and to protect the water quality of Lake Victoria. Additionally, upstream treatment of wastewater using substrate-free constructed wetlands was sought as an alternative low-cost technology to optimise nutrient removal processes in Uganda's degrading natural treatment wetlands, particularly Nakivubo wetland. This information is critical for the proper planning of the drinking water supply of Kampala and other municipalities namely, Jinja, Entebbe and Masaka which also discharge municipal and industrial effluents into wetlands surrounding their drinking water sources.

1.2 Aims and objectives of this thesis

This thesis presents an investigation of the current ability of Nakivubo wetland to respond to external pollution loads and preserve its ecological balance, here referred to as stability, and to protect the water quality of Inner Murchison Bay from where water supply to Kampala city is extracted. To establish this feature, the hydraulic loading and pollution

profiles of major inflows into the upper section of this wetland were determined. Water quality of the lower section of Nakivubo wetland bordering Inner Murchison Bay was also assessed.

A quantification of nitrifying bacteria and the corresponding nitrification activity in the different phases of Nakivubo channel and wetland was carried out to establish their spatial distribution and influence on the nitrogen balance.

Nakivubo wetland is ecologically stressed by high wastewater discharges and is on the verge of complete modification due to human encroachment, which might result in substantial loss of its wastewater treatment capacity. Therefore, pollutant removal from the incoming wastewaters is a challenge, without which the quality of drinking water extracted from Inner Murchison Bay is threatened. Due to this critical situation, this thesis aimed at methods for optimizing nitrogen removal in Nakivubo wetland through upstream treatment of influent wastewaters. The feasibility of low-cost substrate-free constructed wetlands to remove pollutants from domestic wastewater was investigated both in bucket experiments and field conditions at pilot-scale level.

The specific objectives of this thesis are summarized below. Details are presented in the individual papers I–V.

1. To assess the wastewater hydraulic loading and the ability of Nakivubo wetland to buffer incoming pollutants and to protect the water quality of Inner Murchison Bay of Lake Victoria (Paper I).
2. To investigate the spatial distribution and activity of nitrifying bacteria in Nakivubo channel and wetland in order

to estimate their influence on the nitrogen balance (Paper II).

3. To investigate the potential application of constructed wetlands in optimizing biological nitrogen removal in Nakivubo wetland. In the present study, the substrate-free constructed wetlands were planted with *Cyperus papyrus* and *Miscanthidium violaceum* which are dominant in many of Uganda's wetlands, and in particular, Nakivubo wetland. The influence of both macrophytes on wastewater treatment processes was investigated in well controlled container experiments (Paper III) and simulated field conditions (Paper IV and V) under a continuous, free water surface (FWS) flow regime.

1.3 Scope of thesis

This thesis is presented in six sections including this introduction. Section II presents an overview of the conventional biological processes applied for wastewater treatment. Natural and constructed treatment wetlands as cheaper alternatives to conventional biological methods are presented in section III. Section IV describes the processes that influence the removal, retention and cycling of nutrients in wastewater treatment wetlands. Section V presents the role of wetlands in Uganda's economy, water supply and environmental protection. The significance of Nakivubo wetland in Kampala's water supply and wastewater disposal is also highlighted in this section. Section VI outlines the present investigation. Concluding remarks, recommendations and future studies are presented in section VII of this thesis. Acknowledgements and references feature in sections VIII and IX, respectively.

2 CONVENTIONAL BIOLOGICAL WASTEWATER TREATMENT

2.1 Overview of environmental concern of nutrient-rich wastewaters

Wastewater treatment is a problem that has plagued man ever since he discovered that discharging his wastes into surface waters can lead to many additional environmental problems. Intensified industrial and agricultural practices, as well as the exponential growth of the human population and explosive urbanization in the last few decades have led to an enormous increase in the discharge of nutrients (nitrogenous and phosphorus compounds) into the environment. Severe environmental problems which are of global concern arise from these excessive loadings due to their pollution effects on the receiving ecosystems. In wastewaters, nutrients are found either as inorganic or organic compounds. Owing to microbial processes in sewers and other wastewater distribution systems, nitrogen is present in raw wastewaters mostly in reduced form as ammonia nitrogen ($\text{NH}_3\text{-N}$ or $\text{NH}_4\text{-N}$) and organic nitrogen (urea, amino acids, proteins, and nitrogen heterocyclic compounds). However, in well established treatment systems, the oxidized forms of nitrogen ($\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$) exist in wastewaters. Phosphorus in wastewaters exists both as inorganic and organic forms, with orthophosphate ($\text{o-PO}_4\text{-P}$) form that is easy to assimilate dominating raw wastewaters.

The discharge of nutrient-rich wastewaters of domestic and industrial origins can have deleterious consequences on the ecological balance and functioning of the receiving environment as well as the public health of downstream end-users of the polluted water sources. Such devastating consequences manifest as: toxicity to fish and other aquatic organisms; depletion of the dissolved oxygen in receiving water bodies as ammonia or ammonium ions are oxygen-consuming; eutrophication when nitrogen and phosphorus

are made available to aquatic plants as nutrients; and potential public health risk (methaemoglobinemia) in drinking water, especially when consumed by infants [18–20].

Methaemoglobinemia is a blood disorder caused by bacterial reduction of consumed nitrate to nitrite in the digestive system and its subsequent interaction with the haemoglobin in red blood cells. Once in the blood, nitrite oxidizes iron in the haemoglobin of red blood cells to form methaemoglobin, which lacks haemoglobin's oxygen-carrying ability. Thus the methaemoglobin formed in this interaction cannot carry sufficient oxygen to the infant's cells and tissues leading to its blue appearance (blue baby syndrome). Not only does consuming drinking water contaminated with nitrate-nitrogen above the maximum containment level (MCL) of 10 mg/L have the potential to result in methaemoglobinemia, but recent studies also have indicated a possible risk of cancer, as well as the potential to be a contributing factor in spontaneous abortions. Nitrates can react with amines or amides in the body to form nitrousamine which is known to cause cancer [19]. Due to these potential environmental and public health threats, the environmental laws are becoming more stringent concerning the residual levels of nutrients allowed in waste streams.

Nutrients often are removed within wastewater treatment systems by suspended or flocculated biomass (activated sludge) in reactors in series. Due to the slow growth rate of the micro-organisms involved (particularly those involved in the oxidation of ammonia and nitrite), long retention times and large aeration tanks are required for effective nitrogen removal. To meet the sharpened nutrient discharge limits, even larger treatment units are needed. Frequently, the funds necessary to

realize these extensions at the treatment site are not available in resource-scarce developing countries like Uganda. Thus, to reduce the undesirable impact of nutrient-rich wastewaters

on receiving ecosystems, the exploitation of environmentally sound low-cost biological treatment technologies is required to achieve low effluent concentrations of pollutants and realize a habitable environment in Uganda.

2.2 Conventional biological wastewater treatment technologies/processes

Over several decades, various configurations for biological treatment of wastewaters have been developed to meet the desired effluent standards [21, 22]. The process configurations have relied on the maintenance of high microbial densities in the system often as fixed films (biofilms) or as a suspension (activated sludge).

2.2.1 Biofilm process

Biofilm processes are among the oldest technical processes in the field of biological wastewater treatment aimed at removing carbon, nitrogen and phosphorus [22]. Trickling filters and immersed carriers were developed at the beginning of the 20th century; the first submerged fixed-bed installations appeared in 1910. Trickling filters consist of a bed of support materials over which wastewater is uniformly distributed. The wastewater percolates over the biofilm growing on the carrier material (usually gravel although recent innovations are now employing low-density plastic media such as polystyrene or polyvinyl chloride) to achieve a very high biofilm specific surface area.

In immersed carriers (biofilters), a reactor is packed with a filter medium to which micro-organisms can become attached and is operated in either upflow or downflow mode. The filter is continuously submerged in wastewater while

the wastewater is aerated from beneath the medium.

Rotating biological contactors have also been widely used for biological treatment of wastewater (carbon removal and/or nitrification). They consist of closely spaced circular plastic discs, partly submerged in wastewater and gently rotated to allow growth of the biofilm and alternately expose it to pollutants in wastewater and oxygen in the air.

2.2.2 Activated sludge process

It was not until the 1950s that activated sludge systems were preferred to biofilm processes in practice. The activated sludge process was primarily designed for the removal of carbon, nitrogen and phosphate from wastewaters. The system relies on dense microbial populations being mixed in suspension with the wastewater under aerobic conditions. This process, which utilises a continuous or semi-continuous (fill-and-draw) operational mode, is either a four-stage (Bardenpho process for biological nitrogen removal) or five-stage (Phoredox processes for simultaneous nitrogen and phosphate removal). Both process configurations consist of a sequence of primary anoxic, primary aerated, secondary anoxic and secondary aerated zones followed by a clarifier [21] as shown in Figure 1 below.

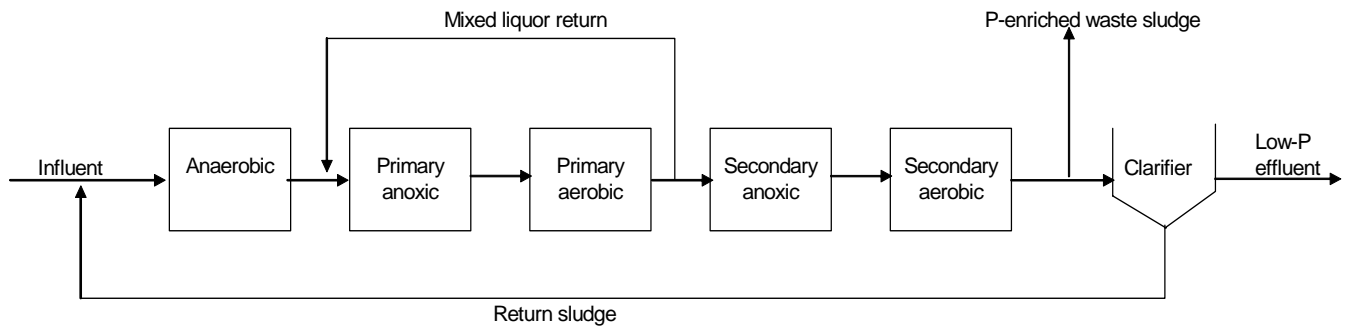


Figure 1: Schematic diagram of the five-stage Phoredox or Modified Bardenpho process for carbon, nitrogen and phosphorus removal (Adapted from Toerien et al., [23]).

Ammonia is nitrified to NO_x in the aerobic tank. The nitrified mixed liquor is returned from the aerobic zone to the anoxic basin for nitrogen removal via the denitrification process using influent wastewater as a carbon source. The incorporation of sequences anaerobic-aerobic zones (as in the Phoredox process, Figure 1) enhances the development of phosphate accumulating organisms (PAOs). Under anaerobic zone, PAOs release phosphates to obtain energy for short chain fatty acids uptake. In the next aerobic zone, the PAOs take up soluble phosphates in the bulk solution [24, 25] resulting in phosphorus removal through sludge wasting.

Although high nitrogen removal rates have been reported for activated sludge systems processing wastewaters of municipal [26] and industrial [27] origin, phosphorus removal is still problematic in most treatment designs. Whereas nitrogen removal necessitates longer sludge retention time (SRT), phosphorus removal requires a short SRT since it is removed only through sludge disposal. Thus, there is an SRT conflict between nitrogen

and phosphate removal reactions [28].

2.2.3 Fluidized bed reactor process

In recent years a clear trend towards a return to biofilm processes has been realized. So-called fluidized-bed processes, in particular, have become prevalent recently because the carriers provide a large specific surface area capable of maintaining a high cell density, significantly increasing the volume efficiency, and thus achieving high loading and specific removal rates [29, 30]. In addition, they require relatively small reactors, and may afford protection from toxic shocks and adverse temperature in cold regions which would help maintain year round treatment [30, 31]. In the fluidized-bed process, suspendable carrier material is made available for the growth of biofilm. Moreover, this material is mixed thoroughly in the reactor and held in suspension. Slow-growing organisms can be retained in the system with the carrier material which is held in suspension by the requisite process air or by agitators.

3 ALTERNATIVE TECHNOLOGIES FOR WASTEWATER TREATMENT: NATURAL AND CONSTRUCTED WETLANDS

3.1 An Overview

Over the last 10 decades, wetlands have been widely regarded as biological filters, providing protection for water resources such as streams, lakes, estuaries, and groundwater. Although naturally occurring wetlands have always served as ecological buffers [5, 32, 33], research and development of wetland treatment technology is a relatively recent phenomenon [34]. Moreover, little was known about the physico-chemical and biological principles and mechanisms underlying their ecological functioning. However, the increased demands on wastewater treatment efficiencies and the rising operation and maintenance (O&M) costs of conventional treatment systems [35] concomitantly demanded for a better understanding of wetland treatment mechanisms upon which treatment designs and configurations would be based to improve their efficiency and to meet the increasingly more stringent discharge standards [36].

Studies of the feasibility of using wetlands for wastewater treatment were initiated during the early 1950s in Germany [34] with the first operational horizontal subsurface flow constructed wetland appearing in 1974 [5]. In the United States, wastewater to wetlands research began in the late 1960s and increased dramatically in scope during the 1970s. As a result of both extensive research efforts and practical application of these technologies, our knowledge base regarding their design, performance, operation and maintenance has expanded considerably in the past three decades leading to better effluent quality. Also, the use of wetlands for water and wastewater treatment has gained considerable popularity worldwide resulting in a renaissance of treatment wetlands which subsequently became widespread almost all over the world [32, 36–38].

More than 1000 wetland treatment systems, both natural and constructed, are in use in North America [39] while over 5000 subsurface flow (SSF) systems are operational in Europe [5] with nearly 3500 systems being operated in Germany alone [40]. Often however, the primary goal of wetland treatment systems has been the removal of total suspended solids (TSS) and organic matter (BOD) with ammonia oxidation as a secondary objective [41]. Moreover, the earlier design loads were too high to leave sufficient oxygen for considerable nitrification to occur [42].

In the past 10 years however, the design criteria have been significantly changed to include nitrification and denitrification zones within a treatment wetland system to cater for biological ammonia removal [5, 43, 44; Paper III–V]. In addition, metal removal is today incorporated into treatment designs [45]. Besides domestic, municipal and agricultural effluents, a variety of industrial wastes, including pulp and paper, food processing, slaughtering and rendering, chemical manufacturing, petroleum refining, and landfill leachates are amenable to wetland treatment [4, 34, 46–48].

In Uganda, many wetlands are being subjected to wastewater discharges from municipal and industrial sources, and have received agricultural and surface mine runoff, irrigation return flows, urban storm water discharges, leachates, and other sources of water pollution. The functional role of wetlands in improving water quality has been studied only on few wetlands [12, 49, 50] and has been a compelling argument for the preservation of natural wetlands [8, 51]. However, concerns remain over the possibility of harmful effects resulting from toxic materials [Paper I] and

pathogens [12] that are present in many wastewater sources. There are also concerns over the potential for long-term degradation of natural wetlands due to the addition of nutrients, human encroachment, and changes in the natural hydrologic conditions influencing

these systems [12, 52; Paper I]. Due to such concerns, constructed wetlands for wastewater treatment are a potential alternative technology that can be exploited to mitigate further pollution of the environment while offering open space and visual amenities [53].

3.2 Constructed wetlands for wastewater treatment

Although natural wetlands have been used in Uganda to dispose of wastewater for over 40 years now, a technology that has not been well exploited is the use of constructed wetlands to supplement the degrading natural wetlands receiving wastewater discharges. Only very few investigations [54–56] have attempted to evaluate their potential application for upstream treatment of wastewater. Constructed wetlands can reduce concentrations of suspended solids, biochemical oxygen demand (BOD₅), nitrogen, phosphorus, and coliform bacteria often by up to 98% [56]. Their simplicity and scalability make them effective for treatment of waste from small communities. If constructed on suitable topography, they require little energy input, which makes them suitable for both underdeveloped and rural sites. However despite the suitability of climate in developing countries, the spread of treatment wetlands in such areas has been described as "depressingly slow" [58].

During the last decades, constructed wetlands (CW) were very successful when used for artificial treatment of wastewater and low quality water from different sources [32, 36–38, 59]. This new approach is designed based on natural processes involving complex and concerted interactions between the plants (floating or submerged), the substrata and the inherent microbial community to accomplish wastewater treatment in a more controlled and predictable manner [60] through physical, chemical and biological processes [5, 32, 44, 59, 61, 62; Paper III–V]. They are preferred systems particularly for small communities and

resource-scarce developing countries because they have low O&M demand, relying on natural processes in which plant and bacterial life cycle excess nutrients through successive seasons of plant growth, death, and decay [32, 63–66].

Constructed wetlands have been successfully applied world-wide for biological treatment of municipal and industrial wastewater [44, 47, 67–73] and agricultural wastewater [74–79] as well as surface-run-off water [32, 45, 80–82]. However, the variations in climatic conditions notably in cold regions often interfere with their treatment performance as microbial activity and plant metabolic rates are influenced by climatic conditions [38]. Due to this draw back, subsurface flow systems are preferred in temperate regions since substantial microbial activity can be maintained in the system during freezing conditions [34, 38].

Two flow regimes exist in treatment wetlands namely, free water surface (FWS) flow and subsurface flow (SSF) regimes [5, 34]. In the USA, surface-flow systems often configured either as a continuous-marsh or a marsh–pond–marsh (m–p–m) are preferred for wastewater treatment [5, 76, 83] whereas subsurface flow systems are a widely applied concept in Europe [5, 34].

3.2.1 Free water surface flow (FWS) wetlands

Free water surface (FWS) wetland technology was initiated with the ecological engineering of natural wetlands for wastewater treatment [84,

85]. The design (Figure 2a) typically mimics the hydraulic regime of natural wetlands by incorporating a shallow layer of surface water that is open to the atmosphere, flowing over impermeable synthetic materials [44, Paper III–V], or mineral (sandy) or organic (peat) soils [5, 34] in a horizontal flow regime. The vegetation often consists of marsh plants, such as *Typha* (cattails) and *Scirpus* (bulrush), but may also include floating and submerged aquatic vegetation and wetland shrubs and trees [34].

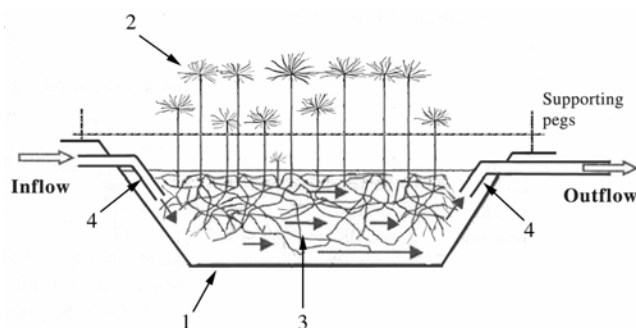


Figure 2a: Schematic of a substrate-free CW with horizontal surface flow (Adapted from Paper V, this study). 1 – Impermeable plastic liner, 2 – vegetation, 3 – root system, 4 – inflow and outflow drainage pipes

In FWS wetlands, the near-surface layer is aerobic whereas the deeper water and substrate are usually anaerobic [5, 34]. These systems effectively remove suspended solids containing BOD components, fixed forms of TN and TP as well as trace levels of metals and organics which enter the biogeochemical cycles within the water phase and surface soils of the wetland system [5, 34, 59]. Additionally, a portion of the dissolved BOD TN, TP and trace elements are sorbed by soils and active microbial and plant populations throughout the wetland

Payne and Knight [86] have considered wetlands with surface-flow emergent plants as the only likely candidate for wide scale adoption. For some treatment applications, FWS wetlands are designed and managed to encourage dominance by either floating or submerged macrophytes. Water depth is one parameter that is often controlled to discourage emergent macrophytes, thereby allowing the development of either a floating aquatic macrophyte (FAM) or submerged aquatic vegetation (SAV) system.

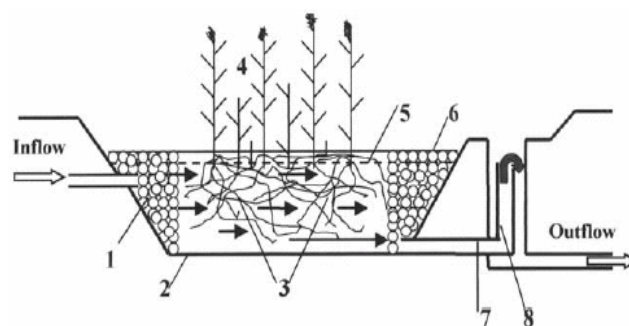


Figure 2b: Schematic of a constructed wetland with sub-surface horizontal flow. 1 – Distribution zone with large stones, 2 – impermeable liner (usually PVC or HDPE), 3 – filtration substrate (gravel or crushed rock), 4 – vegetation, 5 – water level in the bed, 6 – collection zone with large stones, 7 – collection drainage pipe, 8 – outlet structure for maintaining of water level in the bed. The arrows indicate only a general flow pattern (Adapted from Vymazal, [144].

environment. These dissolved elements also enter the overall mineral cycles of the wetland ecosystem [32]. The wetland system serves basically as an attached growth biofilter in an anaerobic contact chamber. The anaerobic micro-organisms present attach to plants, suspended particles and soil/sediment matrix and use it for support while degrading the influent organic matter into CH_4 , H_2S , and CO_2 [61]. If the organic loading to the wetland is high, and/or the wetland is of a depth that surface transfer of oxygen from the atmosphere

cannot provide aerobic conditions, anaerobic conditions will dominate the FWS wetland system. In FWS wetlands, epiphytic and suspended bacteria effect the removal of soluble BOD using oxygen from atmospheric aeration at the water surface.

Nitrification–denitrification is the major removal process for nitrogen in FWS systems [60]. Ammonia is oxidized to nitrite and nitrate by nitrifying bacteria in aerobic zones while nitrate and nitrite are reduced to molecular nitrogen or nitrous oxide in the anoxic zones by heterotrophic denitrifying bacteria. Sustainable phosphorus removal in FWS systems however occurs from adsorption, absorption, complexation and precipitation though at slower rates [5]. The major removal mechanism, precipitation, is in most cases limited by the minimal contact of wastewater with the soil substrate [5]. Although FWS systems require a large footprint compared to other treatment systems and that the water is exposed to potential human contact, their capital and operating costs are lower [9]. In addition, they are easy to construct, operate and maintain and therefore are ideal for tropical countries like Uganda with lower tax bases.

3.2.2 Subsurface flow (SSF) wetlands

Subsurface flow wetlands differ from FWS wetlands in that they incorporate a rock or gravel matrix that the wastewater is passed through in a horizontal or vertical fashion (Figure 2b). Unless the matrix clogs, the top layer of the bed in horizontal flow systems usually remains dry. The SSF configuration offers several advantages, including a decreased likelihood of odour production and no insect proliferation within the wetland as long as surface ponding is avoided.

Unlike FWS wetlands, SSF systems provide no aesthetic or recreational benefits and few, if any, benefits to wildlife [34]. The water column of SSF systems is never exposed to sunlight and does not undergo significant diurnal

variations in pH and dissolved oxygen, which together are predominant means of disinfection in natural treatment systems such as waste stabilisation ponds [87]. Compared to FWS wetlands, subsurface flow wetlands continue to provide effective treatment of most wastewater constituents through the winter in temperate climates [34]. The subsurface microbial treatment processes still function, though at a reduced rate, even when the surface vegetation has senesced or died, and the matrix surface is covered with snow and ice.

Most horizontal SSF systems are characterised by anoxic conditions due to limited atmospheric aeration, macrophytic oxygen transfer to the rhizosphere and high influent BOD concentrations, implying that there will be practically no oxygen left for nitrification of ammonia to occur [5, 34, 87]). Because of this poor performance, subsurface flow wetlands have been redesigned and operated in a vertical flow fashion to reduce matrix clogging problems and enhance certain contaminant removal processes such as nitrification [5, 34, 88].

Vertical flow (VF) systems are typically composed of a flat bed of gravel topped with sand, with reeds growing at similar densities as in horizontal flow SSF treatment wetlands. They are fairly popular systems in Europe because of their reduced footprint and their good effluent quality [36, 89–91]. Unlike horizontal flow systems, they are intermittently fed with the wastewater in large batches to flood the bed surface. As all the wastewater completely drains vertically through the bed and collected by a drainage network at the base allowing the air to refill the bed, the next flooding of the bed traps this air which together with the aeration caused by the rapid dosing on the bed lead to good oxygen transfer for better BOD decomposition and nitrification of ammonia [88]. Albeit VF treatment systems can remove BOD and nitrify, they are less good at the removal of suspended solids [57] and are therefore often followed by a horizontal SSF

system as a multistage wastewater treatment wetland [5, 57].

Because of the high cost of the gravel or rock matrix, SSF wetlands do not attain the large spatial footprint of the FWS wetlands mainly because of concerns over the matrix clogging phenomena, and the potentially high cost of renovation also limits the deployment of extremely large SSF wetlands [34]. Besides their increased use for small applications, such as small communities or single family homes in developed countries, they are hardly adoptable in resource-scarce developing countries like Uganda. Therefore, FWS treatment systems are the only low-cost wetland configuration because land is still available and relatively cheap, and the high standing plant biomass can be harvested more often for economic gains such as making crafts, mulching etc.

3.3 Role of macrophytes in wastewater treatment wetlands

Since Seidel [92] demonstrated the role of bulrushes (*Scirpus lacustris*) in wastewater treatment, a lot of research has been performed to assess the potential of different macrophyte-based systems for controlling nutrient discharges into surface wastewater [34, 93]. Macrophytes ranging from duckweed [59, 94] through water hyacinth (*Eichhornia crassipes*) [95, 96] to cattails, reeds and sedges [32] have been widely investigated for wastewater renovation potential. Duckweeds reportedly have a very low biomass but very high production rate, high protein content, low fibre content and are easy for manual harvest from the surface [59, 93]. These properties make the duckweed cost-effective and attractive for recycling as fertilizer and animal fodder [59]. However, their wastewater treatment efficiency is limited due to the fact that duckweed plants

grow only in the upper layer of the water phase where they remove nutrients from a thin layer (1–2 cm) of water [59, 93]. The water hyacinth exhibited high biomass productivity, and greater potential for removing nutrients and suspended particles. However, its potential for wastewater treatment was limited by its susceptibility to pest damage which reduces its treatment performance [97]. Besides, water hyacinth harvesting often required specialised equipment, with the harvested biomass having little product value. Today, the majority of the constructed treatment wetlands use reeds (*Phragmites australis*), cattails (*Typha* spp.) or bulrushes (*Scirpus* spp.) to treat various forms of wastewater [5, 33].

In addition to their aesthetic roles, wetland plants exhibit several properties which enhance wastewater treatment processes and thus make them an essential component of the treatment wetlands. These properties influence wastewater treatment through physical effects such as erosion control, filtration, adsorption and sedimentation, provision of surface area for the growth and attachment of micro-fauna [98; Paper III]. Metabolically, plants take up pollutants, produce organic carbon, oxygen and release sugars, amino acids and antibiotics through their root systems, thereby improving the water quality to varying extents [99–104].

Although these plants have been used variously, few studies [12, 55, 105–110] have investigated the wastewater treatment potential of *Cyperus papyrus* and *Miscanthidium violaceum* that colonize many wetlands in Africa, particularly the Nile and Lake Victoria basin. Therefore there is need for further research on tropical plants adapted to the local ecological conditions of developing countries such as Uganda in order to supplement and optimize the treatment efficiency of degrading native natural treatment wetlands.

4 PROCESSES AND FACTORS INFLUENCING THE REMOVAL, RETENTION AND RELEASE OF NUTRIENTS IN TREATMENT WETLANDS

4.1 Nitrogen

Nitrogen (N) is a key element in wetland biogeochemical cycles. As shown in Figure 3 and 4, nitrogen entering treatment (natural or constructed) wetlands occurs in a number of different oxidation states and is present in particulate and dissolved organic and inorganic forms, the relative proportions of which depend on the type of waste and pre-treatment [111]. The primary forms of inorganic N entering constructed wetlands are: ammonium/ammonia ($\text{NH}_4^+/\text{NH}_3$), nitrite (NO_2^-), and nitrate (NO_3^-) [32, 112]. Organic N is present in wetlands in the form of amino acids, urea, uric acid, amines, purine, and pyrimidines [113]. Numerous biological and physical processes such as plant uptake, sediment/peat accumulation, adsorption of ammonium on to the organic sediments/peat, and nitrification-denitrification processes can transform N between these different forms [62, 114, 115; Paper III].

The major and more permanent removal mechanism of organic nitrogen in treatment wetlands is the sequential processes of ammonification, nitrification and denitrification (Figure 3). As part of the nitrogen cycle, the various forms of N are converted into gaseous components that are expelled into the atmosphere as nitrogen gas (N_2) or nitrous oxide (N_2O).

4.1.1 Mineralization

This process is either aerobic or anaerobic, but occurs much faster in oxygenated zones [5, 116]. The rates of mineralization are dependant on temperature, pH (optimum range of 6.5–8.5), the C:N ratio of the residue, available nutrients in the system, and soil conditions such

as texture and structure [117]. In well saturated soils, pH is buffered around neutrality but under well drained conditions, the pH value of the soil decreases due to nitrate accumulation and the production of protons (eq. 4.3) during nitrification [118]. As shown in Figure 3 and 4, organic N (plant detritus, organic sediments and peat) is mineralized to ammonia by a variety of micro-organisms that utilize organic carbon as an energy source. Organic nitrogen such as proteins and amino acids are broken down to smaller organic molecules, both particulate and dissolved, and ultimately to ammonium (NH_4^+), which is either utilized as a nutrient by the micro-organisms and plants or diffuses back into the soil or water (Figure 4).

4.1.2 Nitrification

Nitrification, the biological aerobic oxidation of reduced nitrogen (ammonia) to nitrite by ammonium-oxidizing bacteria (Nitritation; eq.4.1) or nitrate (Nitrification; eq.4.2) by nitrite-oxidizing bacteria is a pivotal chemoautotrophic process in N cycling and regulation of water quality of aquatic environments [119; Paper III, V]. The oxidation of NH_4^+ to NO_3^- (eq.4.3) is an exergonic process (Figure 3) that yields sufficient energy to synthesize new cells using CO_2 as a carbon source. Nitrification occurs in aerobic regions of the water column, soil-water interface, and root zone [111]. The oxygen required for the nitrification process is supplied by diffusion from the atmosphere and leakage from macrophyte roots [100, 120, 121]. Studies have indicated that DO levels below 1–2 mg/L in water substantially reduce nitrification [122, 123].

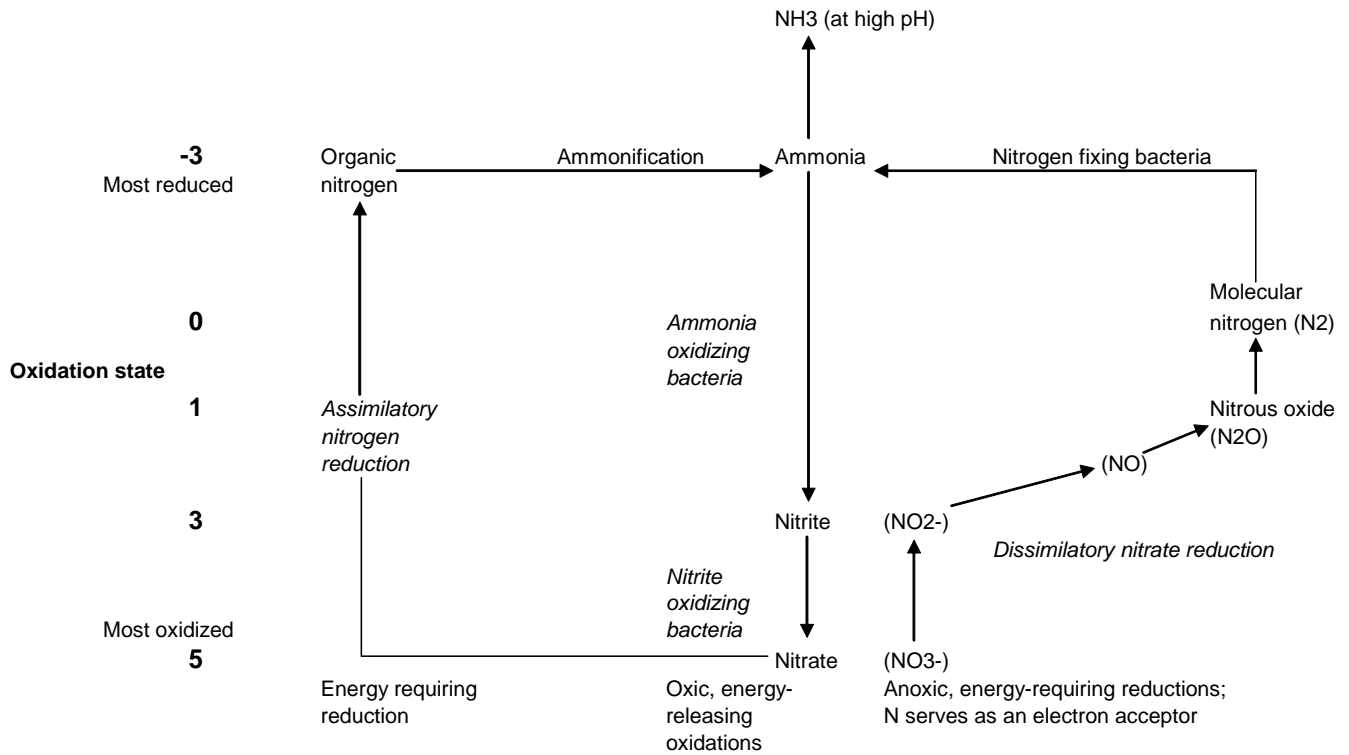
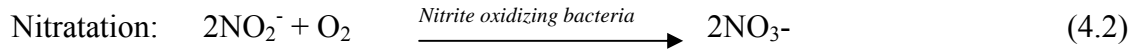
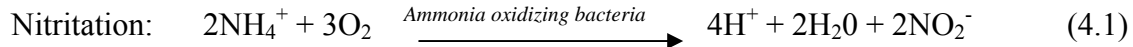


Figure 3: Microbial transformation of nitrogen in biological wastewater treatment systems (Adapted from [20]).

Nitrification is essentially carried out by two distinct groups of bacteria (ammonium and nitrite-oxidizers respectively) belonging to the family *Nitrobacteriaceae*. Strictly chemolithotrophic species oxidizing ammonium belong to the genera *Nitrospira*, *Nitrosovibrio*, *Nitrosolobus*, *Nitrosococcus*, *Nitrosomonas*, and *Nitrosocystis*. Those oxidizing nitrite to nitrate (facultative chemolithotrophs) are grouped under *Nitrobacter*, *Nitrococcus*, *Nitrospira* and *Nitrospina* [20, 124–127]. Albeit nitrification is

widely believed to be an oxic process, investigations have shown that at least ammonia oxidizers are able to oxidize ammonia under anoxic conditions [128, 129]. Various heterotrophic and lithotrophic micro-organisms, including bacteria (actinomycetes and planctomycetes), algae and fungi have also been reported to have nitrifying activity [130–137]. Since autotrophic nitrification usually occurs at higher rates than heterotrophic nitrification it is believed to play a more important role in nature [130, 131].

4.1.3 Denitrification

Denitrification is a stepwise enzymatic anoxic reduction process (eqs.4.4 and 4.5; Figure 3) in which nitrite and nitrate are reduced to molecular nitrogen or nitrogen gases by chemoorganotrophic, lithoautotrophic, and phototrophic bacteria [5, 21, 32]. In this microbial process, the nitrogen oxides (in ionic and gaseous form, Figure 3) irreversibly serve as terminal electron acceptors in the electron transport chain. The electrons are usually but not exclusively transferred from organic compounds through a series of carrier systems to a more oxidized nitrogen form (eq.4.5). The resultant free energy conserved as ATP is used

by the denitrifying organisms to support respiration [5].

Denitrification is a significant mechanism in treatment wetlands for the permanent removal of N from wastewater [122]. In treatment wetlands, the nitrification rate is usually much slower than the denitrification rate, and thus the first process affects the latter [115]. The supply of $\text{NO}_3\text{-N}$ which limits the denitrification process has often been identified as a problematic issue [70, 138, 139] and remains a challenge in treatment wetlands. Although denitrification takes place preferably under anoxic conditions, there is accumulating evidence however, that some bacteria also denitrify aerobically [140, 141].



4.1.4 Volatilization

In treatment wetlands, loss of NH_3 through volatilization is generally insignificant compared to nitrification–denitrification if the pH is below 7.5 [117]. Under high-pH conditions ($\text{pH} \geq 7.5$), the concentration of the un-ionised form of ammonia (NH_3) becomes

appreciable compared to NH_4^+ as shown in equation 4.6, and NH_3 is released to the atmosphere [5, 117, 142]. This process is not usually a major factor for N cycling in most wetlands but can lead to substantial N losses in poorly buffered waters due to the pH increase from high algal, free-floating or submerged macrophyte photosynthetic activity [5].



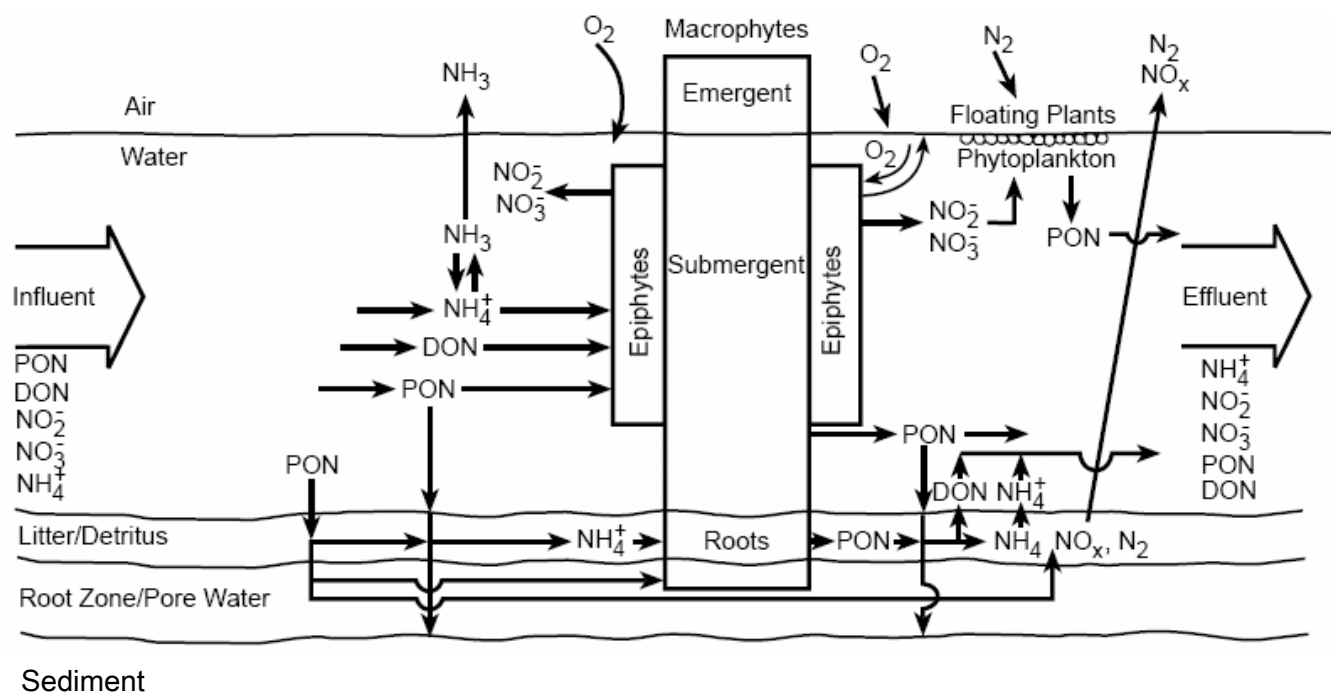


Figure 4: Nitrogen transformations in a FWS wetland with a floating emergent macrophyte mat (Adapted from Kadlec et al., [143])

4.1.5 Plant uptake and matrix adsorption (storage)

Plant uptake and matrix adsorption are other mechanisms involved in nitrogen cycling in wetland systems (Figure 4). However, this is only a temporary solution, because the wetland has a finite storage capacity, and the stored N can be re-mineralized back into solution or undergo desorption. Plants require nutrients for growth and reproduction, which in rooted macrophytes, are taken up primarily through their roots although some nutrients are taken through immersed stems and leaves from the surrounding water [5, 100]. The potential of nutrient uptake by wetland plants tends to be limited by their net productivity (growth rate) and the concentration of nutrients in plant tissues [5, 144; Paper III, V].

Nutrient storage is known to depend on plant tissue nutrient concentrations as well as the ultimate accumulation of standing crop biomass

[5; Paper III, V]. Depending on the macrophyte used, reasonable quantities of nutrients can be removed from the system through plant biomass harvesting [15, 34, 100, 144; Paper III, V]. Plant uptake and storage has been observed to account for 25–89% of nutrients removed in tropical wetlands [34, 109; Paper III, V]. However, the quantities of nutrients removed by plant harvesting are generally insignificant in comparison with the loading into the wetlands [37, 144–147; Paper V]. Harvesting is particularly important for phosphorus removal since it cannot be transformed into volatile substances as in the case of nitrogen.

In a reduced state, $\text{NH}_4\text{-N}$ is stable and can be adsorbed on active sites of the bed matrix or sediments of a wetland system [5, 114]. However, the ion exchange of $\text{NH}_4\text{-N}$ on cation-exchange sites of the matrix is not considered to be a long-term sink for $\text{NH}_4\text{-N}$ removal. Rather, sorption of $\text{NH}_4\text{-N}$ is presumed to be rapidly reversible [5]. As the

$\text{NH}_4\text{-N}$ is lost from the system via nitrification, the exchange equilibrium is expected to redistribute itself. The sorbed $\text{NH}_4\text{-N}$ in a continuous-flow system will therefore be in equilibrium with the $\text{NH}_4\text{-N}$ in solution. In the event of seasonal variations in $\text{NH}_4\text{-N}$ content of the water, alternate loading and unloading of

sorption sites sets in. Thus systems intermittently loaded with wastewaters will tend to exhibit rapid $\text{NH}_4\text{-N}$ removal by adsorption mechanisms as a manifestation of the depleted $\text{NH}_4\text{-N}$ on the sorption sites during rest periods [5].

4.2 Environmental factors influencing nitrification

The occurrence of nitrification is significantly influenced by temperature, pH, alkalinity, inorganic C source, the microbial population and concentration of $\text{NH}_4\text{-N}$, dissolved oxygen and inimical pollutant compounds [126, 142, 148–152]. Whereas nitrification occurs over a wide temperature range of 4–40°C, the optimum temperature in pure cultures ranges from 25–30°C, and 30–40°C in soils [20, 88, 126, 142]. A narrow optimum pH (7.2–8.6) exists [5, 20, 26, 126] but acclimatized systems can be operated to nitrify at a much lower pH value [5]. Nitrification is obligatorily coupled to oxygen consumption and has an effect on the decrease in wastewater alkalinity. Such a decrease in wastewater alkalinity might cause a decrease in its pH when the alkalinity of the wastewater is low or when its ammonia content is relatively high [5]. During ammonium oxidation, the wastewater alkalinity increases slightly due to CO_2 consumption for autotrophic growth whereas acidic nitrite formation results in a drop in wastewater pH. Thus if the buffer capacity of the system wastewater is weak, the pH might drop well below 6.7 preventing further autotrophic nitrification [126]. Although effective nitrification has been reported in systems with residual oxygen as low as 0.5 ml/L [21, Paper III–V], DO concentrations below 1.5 mg/L are

reported to limit the nitrification process [122, 123, 126].

Nitrifying bacteria are sensitive organisms that are extremely susceptible to a wide range of inhibitors present in wastewaters. Such inhibitory pollutants include phenolic compounds, cyanide, thiourea, anilines and heavy metals primarily originating from industrial processes. Extremely high concentrations of ammonical nitrogen and nitrous acid are reported to be inhibitory (substrate inhibition) to the nitrification process [20, 126, 148]. Similarly, high organic loading inhibits nitrification by promoting heterotrophic growth and activity which culminate in limited nitrifier growth and activity as a result of strong competition for the available oxygen and ammonia [130, 153–157, Paper I–V]. The fast growing heterotrophs tend to occupy the outer layers of the biofilm, where both substrate concentration and detachment rate are high; whereas the slow growing nitrifying bacteria stay deeper inside the biofilm. Thus the heterotrophic layer forming above the nitrifiers in the biofilm negatively influences the nitrification process through limited oxygen availability to autotrophic nitrificants as a result of consumption and resistance to mass transfer within the heterotrophic layer [158].

4.3 Environmental factors influencing denitrification

Several factors including anoxic conditions, redox potential, soil moisture, temperature, pH, presence of denitrifiers, soil type, organic matter (energy source) and the presence of overlying water are known to influence denitrification rates in aquatic systems [142]. The presence of oxygen suppresses the synthesis of the enzyme needed for the substitution of nitrogen for oxygen as the

terminal electron acceptor [88]. Moreover, the optimum pH range is reported to lie between 7.0 and 8.5 [5, 20, 22, 126]. However, alkalinity produced during the denitrification process can result in increased pH. Denitrification is also highly temperature-dependent, with reaction rates significantly decreased at temperatures below 5°C [5].

4.4 Phosphorus retention, cycling and release in wetland ecosystems

In wastewaters, phosphorus exists in various forms; as organic (in dissolved or particulate form) or inorganic [primarily in solution as orthophosphate (HPO_4^{2-}) or as phosphate-containing minerals suspended in the water column]] compounds. The different processes involved in phosphorus cyclic in free water surface wetlands are depicted in Figure 5.

Whereas nitrogen can be lost to the atmosphere through denitrification, phosphorus is a more conservative nutrient whose removal can only be effected through plant/microbial uptake, chemical precipitation, and adsorption onto sediments or substrate media. A number of wetland studies have shown that soil/litter compartment is the major (>95%) long-term storage pool for phosphorus (P) [159–165]. Storage in organic matter due to soil adsorption and peat accretion is pivotal in controlling the long-term P sequestration in wetlands [160].

Studies indicate that sorption and P retention

in wetlands ecosystems is effected by the interaction of redox potential, pH, Fe, Al, and Ca minerals, and the amount of native soil P [162, 166]. Because P can exist in dissolved form either as organic or inorganic, it can be transferred from surface water to soil solution (porewater) and vice versa, through the process of diffusion. The driving force behind this process is the concentration gradient which is controlled by the native soil P. The adsorption of the orthophosphate ion by clays and Fe, Al, and Ca oxides (chemisorption) in the soil and precipitation of orthophosphate with Fe, and Al oxides or dissolved calcium in soils and water forms potentially very stable phosphate minerals, affording long-term storage of P. However, there is evidence that P removal due to sorption decreases over time, referred to as the “aging phenomenon” [167], due to a finite P-sorption capacity of the substrate/bed [5, 146].

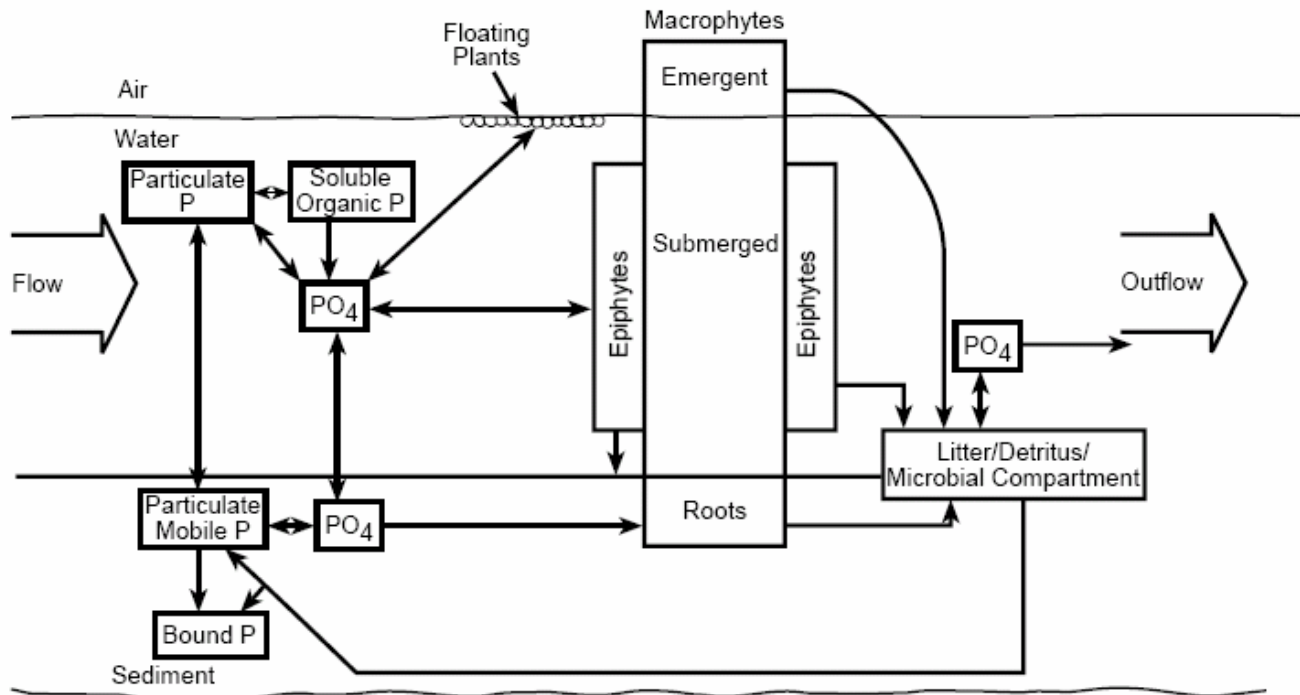


Figure 5. Phosphorus cycling in a FWS wetland with a floating emergent macrophyte mat (Adopted from Kadlec et al., [143]).

The following is a summary of other processes affecting retention, cycling and release of P in wetlands as shown in Figure 5.

- ❑ **Plant uptake:** Inorganic P, primarily orthophosphate is taken up by plants rooted in the soil or floating on the water including algae. Many studies have shown that plants contain only a small proportion of the total P that occurs in wetlands indicating that the uptake of macrophytes in wetlands is limited [100, 142, 161, 168, Paper V]. However, under well controlled conditions, P uptake and storage into plant tissues can be substantially high [44, Paper III] and thus plant harvesting can remove substantial quantities of P sequestered into plant tissues.

- ❑ **Sedimentation and decomposition (mineralization):** This process refers to directing settling of particulate matter (inorganic and/or organic sediment) entrained in the water column due to the reduced water velocity, shallow water depth and filtering action of emergent vegetation. The settled organic matter, including plant detritus, organic sediments and peat is broken down by a variety of micro-organisms that utilize organic carbon as a source of energy. The resultant organic P compounds are further broken down to smaller organic molecules, both particulate and dissolved, and ultimately to orthophosphates which may be utilized as a nutrient by the micro-organisms (epiphyton), plants, or diffuse back into the soil or water.

5 ROLE OF WETLANDS IN UGANDA'S ECONOMY, WATER SUPPLY AND ENVIRONMENTAL PROTECTION

5.1 An overview

With an estimated coverage of 30,000 square kilometres, or about 13% of Uganda's land surface, wetlands constitute an important natural resource in Uganda, both from ecological and socio-economic point of view. In Uganda, wetlands are widespread and complex. Their overall presence in the southern and western parts is in the form of an extensive low gradient drainage system in steep V-shaped valley bottoms with a permanent wetland core, and relatively narrow seasonal wetland edges. The northern parts of Uganda mainly consist of broad floodplains, whereas in the east a complex network of small, vegetated valley bottoms exist in a slightly undulating landscape [8].

The water regime of wetlands in Uganda is determined by many factors, of which rainfall is the most important. Most of Uganda has a bi-modal rainfall regime. The southern half of the country receives between 1200 and 2000 mm of rain, the drier areas in the north-east may receive up to 600 mm in one rainy season. High and relatively well distributed rainfall in the south and west of the country, result in a heavily vegetated wetland core, often covered by *Cyperus papyrus* (Figure 6), *Typha*, *Phragmites*, or swamp forest complexes. The wetland fringes, which are inundated during the wet seasons and dry out during the drier periods, may consist of grassland, sedges and small trees like *Sesbania* sp. In the north, where rainfall is less abundant and reliable, the permanently wet plains are covered with grasses like *Vossia* and *Oryza* spp, and the seasonal wetland plains consist mainly of natural grasslands.

Uganda's wetlands have intrinsic attributes, perform a wide variety of biophysical functions, produce goods and services and play a significant role in the socio-economics of the

country. Whereas some wetlands are primarily of local interest, others have regional, national, or international significance. Together, wetlands represent considerable ecological, social, and economic value. While it is generally difficult to place a specific monetary value on wetlands, data compiled by Emerton, [169, 170] has placed their values into four categories (Figure 7), which are translated and estimated to contribute 100s of million US\$ per year to the Ugandan economy [8].

Although a large proportion of this monetary value is attributed to water treatment and purification services, these benefits are small compared to the value that can be placed on the role wetlands play in water supply (Figure 8). More than five million people depend directly on wetlands for their water supply [8]. Using very conservative figures for daily consumption, estimates showed that at least 50 million litres of water are extracted daily and from commercial prices for water in rural areas, this amounts to at least US\$ 25 million a year [8].

Wetlands contribute to water supply not only to neighbouring communities, but to most of the population (Figure 3)—through groundwater recharging, and water storage and purification [171, 172]. They form the backbone of the entire drainage system in Uganda. Apart from Lake Victoria in the south, Lake Kyoga in the centre, and the Rift Valley lakes in the west, most of Uganda's surface water is absorbed and stored in its wetlands. The wetlands function as fresh water reservoirs that slowly release the water, either underground to replenish aquifers, or laterally towards the major drainage basins. The slow release of water increases water availability during the dry season for domestic use, edge cultivation and livestock watering. In addition, this keeps boreholes, shallow wells, and springs functioning. Notably, wetlands also distribute water widely throughout much of



Figure 6: A *Cyperus papyrus* dominated wetland on the shores of Lake Victoria

DIRECT VALUES	INDIRECT VALUES	OPTION VALUES	NON-USE VALUES
Production and consumption goods and services such as ... <input type="checkbox"/> fish <input type="checkbox"/> fuel wood <input type="checkbox"/> building poles <input type="checkbox"/> sand, gravel, clay <input type="checkbox"/> thatch <input type="checkbox"/> water <input type="checkbox"/> wild foods <input type="checkbox"/> medicines <input type="checkbox"/> agriculture/cultivation <input type="checkbox"/> pasture/grazing <input type="checkbox"/> transport <input type="checkbox"/> recreation	Ecosystem functions and services such as ... <input type="checkbox"/> water quality <input type="checkbox"/> water flow <input type="checkbox"/> water storage <input type="checkbox"/> water purification <input type="checkbox"/> water recharge <input type="checkbox"/> flood control <input type="checkbox"/> storm protection <input type="checkbox"/> nutrient retention <input type="checkbox"/> micro-climate regulation <input type="checkbox"/> shore stabilisation	Premium placed on possible future uses and applications such as ... <input type="checkbox"/> pharmaceutical <input type="checkbox"/> agricultural <input type="checkbox"/> industrial <input type="checkbox"/> leisure <input type="checkbox"/> water use	Intrinsic significance in terms of ... <input type="checkbox"/> cultural value <input type="checkbox"/> aesthetic value <input type="checkbox"/> heritage value <input type="checkbox"/> bequest value <input type="checkbox"/> existence value

Figure 7: Importance of wetlands to Uganda: four categories of values derived from attributes, functions, goods and services (Adapted from Emerton, L., [169]).



Figure 8: Abstraction and processing of drinking water from a natural wetland in Uganda



Figure 9: Fishing and recreational activities in one of Uganda's natural wetlands

Uganda, bringing water closer to the rural communities.

Besides provision of a continuous, reliable supply of water to a large proportion of the population (Figure 8), wetlands ensure that it is relatively clean, by trapping silt and pollutants [171, 172], thereby making an important

contribution to public health and supply of protein (Figure 9). It can henceforth be concluded that the total value of wetlands in economic terms is extremely high and that any further significant loss or continued degradation of the wetlands, and their inherent values, will be economically disastrous for Uganda.

5.2 Importance of Nakivubo wetland in Kampala water supply and wastewater disposal

5.2.1 Wetland description

Located 3.8km, south-east of Uganda's capital city-Kampala (00° 18'N, 32° 38'E) at an altitude of 1135m above sea level, Nakivubo wetland occupies the northern shore of Lake Victoria and is the largest of the twelve main wetland areas in Kampala. It covers approximately 5.3 km² with a total catchment of 40 km² [173]. This natural wetland is located downstream of a municipal wastewater treatment plant at Bugolobi which has discharged partially treated sewage effluent for more than 4 decades [14]. As shown in Figure 4, the wetland is fed by small streams that drain large parts of Kampala. Nakivubo wetland runs from the central industrial district of Kampala, carrying wastewater from the city centre, industrial area, and residential zones which it discharges into Lake Victoria at Inner Murchison Bay (Figure 10 and 11). The wetland is bisected into upper and lower Nakivubo wetland by a railway line running through central Kampala to Port Bell on Lake Victoria (Figures 10–12). Whereas the vegetation in the upper wetland has been completely modified and is dominated by cocoyam and sugar cane, approximately half of the vegetation in the lower wetland has been replaced with cocoyam [Figure 12; Paper I].

5.2.2 Wastewater disposal

Nakivubo's characteristics and location provide a uniquely important set of services to Kampala's dwellers. It functions as a buffer through which much of the city's municipal, industrial and domestic wastewater passes prior to its discharge into Lake Victoria at Inner Murchison Bay. It is estimated that about 90% of Kampala's residents are not connected to sewerage pipe systems for the centralized processing and treatment of wastewater. This implies that they generate largely organic raw sewage equivalent to over half a million people or 40% of the population of Kampala [173]. Moreover, over 33,000 persons discharge domestic wastes into the wetland, either as runoff into the surface waters which enter it or through groundwater inflows from the infiltration of rainfall on hills beside the wetland, from pit latrines, septic tanks, soak-away pits and leaking sewer pipes.

In addition, the outflow for Kampala's sewage treatment works, at Bugolobi, also runs into the wetland. Partially treated effluent from a sewage treatment plant is mixed with the untreated effluents already in Nakivubo channel before entering the wetland. Three other point sources of wastes enter the southern parts of the wetland directly, including two sewage

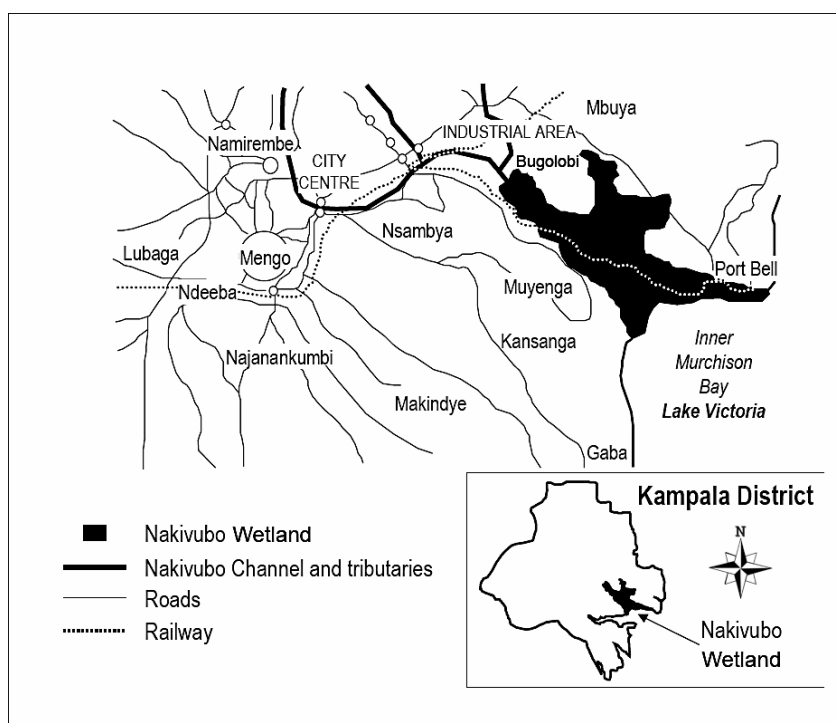


Figure 10: Location map of Nakivubo wetland and its major inflows in Kampala¹

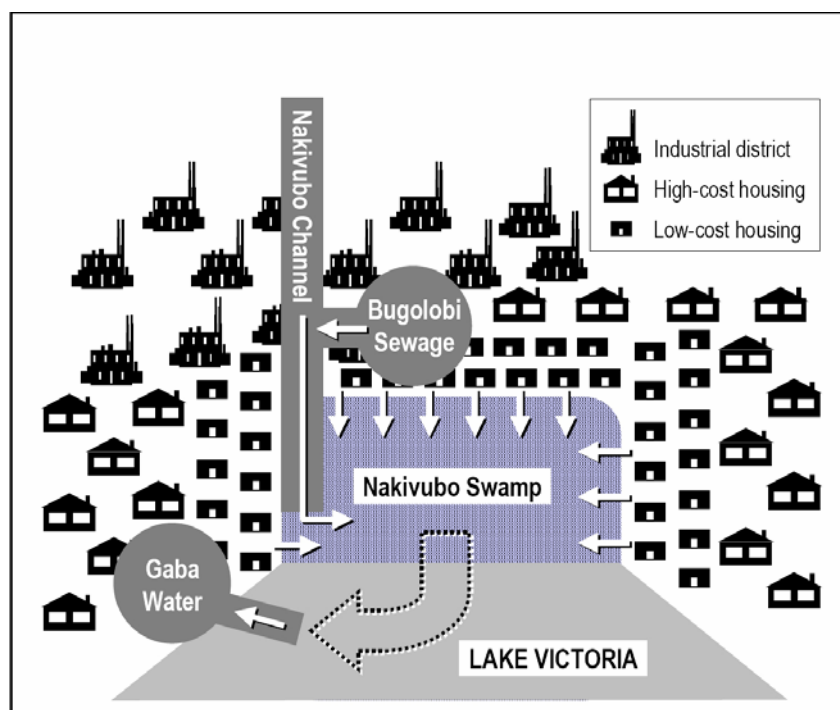


Figure 11: Kampala water supply and wastewater disposal into Nakivubo wetland¹ (¹Adapted from IUCN, 2003, Case study number 7; Nakivubo Swamp, Uganda: managing natural wetlands for their ecosystem services. Integrating Wetland Economic Values, IUCN Eastern Africa Regional Office).

outflows from Murchison Bay Prison and Uganda Breweries (Figure 10).

Nakivubo wetland also processes industrial effluents which often are discharged without any form of pre-treatment (Figure 13; Table 1). Of the 15 medium to large scale industries and factories located on its fringes, close to 40% have no pre-treatment facilities while more than 200 small-scale enterprises situated within the same industrial area of Kampala barely provide any form of on-site pre-treatment [170]. Thus they discharge high oxygen demand, nutrient-rich effluents often laden with other organic and metal pollutants to surface waters draining into the wetland.

5.2.3 Water quality protection and water supply

Besides other socio-economic direct benefits (Figure 7), Nakivubo wetland provides indirect benefits in terms of wastewater treatment and protection of Kampala's water supply. In fact, drinking water supplied to Kampala is abstracted from the same bay at Gaba, located just 4 km south-west of the wetland's outflow into Inner Murchison Bay (Figure 11). Using replacement cost and mitigation expenditure analytical methods, wastewater purification and nutrient retention services of Nakivubo wetlands are put at USD 1 million and 1.75 million a year, respectively [170]. If the costs (some USD 235,000) of managing the wetland so as to simultaneously optimise its wastewater treatment potential and maintain its ecological

functioning are considered, the net benefits that accrue to Kampala residents and industries as well as the general public sector are enormous.

Previous studies [10–12, 14, 15, 108] showed that Nakivubo wetland was capable of renovating the influent wastewater by removing nutrients and bacteria. Kansiime and Nalubega, [12], Kansiime et al., [15] also found that the effluent discharged had resulted in increased plant growth, implying that the natural wetland was acting as a sink for nutrients. In a related study, Kansiime and Nalubega, [12, 14] further showed that the two zones in Nakivubo wetland which are dominated by *Cyperus papyrus* and *Miscanthidium violaceum* positively influenced pathogen removal from wastewater though at different levels. The differential nutrient uptake and pathogen removal were found to be related to the rooting structures of the two vegetation types [12, 14, 108]. However, concerns are now growing over the rate at which the wetland is being modified which could lead to nutrients and bacterial contamination of Inner Murchison Bay waters.

Significant contamination can cause eutrophication resulting in the need for relocation of drinking water supplies for Kampala city due to clogging problems, and the high cost of chemicals required to treat drinking water. The relocation costs involved are very large [170], and therefore there is a need for an immediate intervention for the protection and sustainable utilisation of Nakivubo wetland by adopting appropriate low-cost, environmentally sound technologies for upstream mitigation processes, particularly on-site treatment of wastewaters.

5.3 Environmental challenge and threat to Nakivubo wetland and Kampala's water supply

Despite the fact that Nakivubo wetland is a dynamic system, it has experienced severe degradation over the last decade, and is

particularly threatened by human encroachment notably the expansion of industrial and residential zones, conversion of the wetland

into agricultural fields, and wastewater discharges, all stemming from population and economic pressures. The rapid economic growth, rehabilitation and urban expansion in the last decade has resulted in a growing demand for housing and land for settlement

(Figure 12), rapid construction of commercial and industrial facilities particularly in Kampala. Nearly all these developments have been undertaken without proper planning and controls and implemented at the expense of drainage and reclamation of Nakivubo wetland [52].

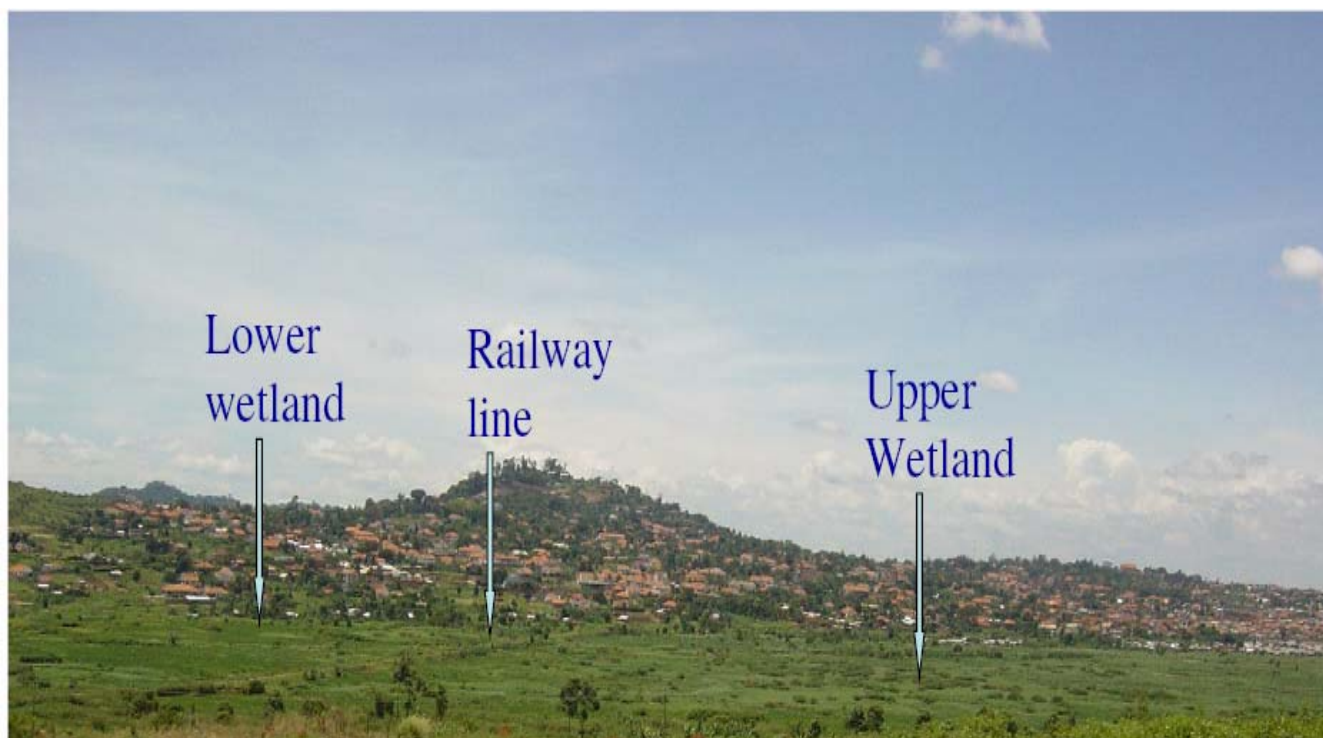


Figure 12: Upstream developments and encroachment of Nakivubo wetland in Kampala

The area around Nakivubo, including the wetland itself, are regarded as prime sites for urban development due to their proximity to the city centre and industrial area as a result of land shortage in the city, and because land is still relatively cheap compared to other parts of Kampala. While almost all of the north-western part of Nakivubo wetland above the railway line, comprising approximately half of its total area [173, 174], has been modified or reclaimed for agriculture, industry and settlement, a large proportion of its south-eastern part below the

railway line is already modified for agricultural activities [Paper I].

Presently, more than 200 large, medium and small-scale manufacturing and processing enterprises are abutting Nakivubo wetland (Figure 11). These include breweries, distillers, soft drink manufacturers, oil and soap factories, dairy producers, abattoirs and meat processors, fish processors, paint producers, tanneries, bakeries, metal works and garages, plastic and foam industries, saw mills, battery manufactures, pharmaceutical industries, shoe makers and paper makers among others. The

Table 1: Characteristics and on-site treatment process of selected industrial wastewaters discharged into Nakivubo wetland, Uganda (adapted from COWI/VKI, [173]).

Industry	Wastewater characteristics	On-site primary treatment
<i>Discharge into surface water:</i>		
Abattoir	BOD, nitrogen, phosphorus, suspended solids	None
Brewery	BOD, COD, detergents	None
Fish processing	BOD, COD, nitrogen, phosphorus, oil	Aeration pond
Meat processing	BOD, nitrogen, phosphorus, suspended solids	None
Oil and soap	BOD, COD	Oil separator,
septic tank		
<i>Discharge through sewerage system:</i>		
Battery producer	Heavy metals, oil, lubricants, acids	Neutralisation
Dairy	BOD, COD, nitrogen, phosphorus, detergents, oil	None
Galvanising	Heavy metals, oil, lubricants, acids	None
Paints	Xenobiotics, heavy metals	None
Pharmaceutical	BOD, COD, xenobiotics	None
Soft drinks	BOD, COD, detergents	Neutralisation

majority discharge their complex effluents to surface waters flowing directly into the wetland without any form of treatment (Figure 13; Table 1). As shown in Table 1 and paper I, these enterprises discharge a wide variety of wastes into the environment, primarily as liquid effluents heavily laden with organic matter, nutrients, heavy metals, oils, detergents, suspended solids and xenobiotics. In addition, the outflow for Kampala's sewage treatment works, at Bugolobi, runs into the same wetland. Therefore the overall impact of urban expansion and wastewater discharges on the ecological integrity and functioning of the receiving wetland ecosystem and Inner Murchison Bay is significant [Paper I, II] and is associated with economic costs which have distributional implications.

Even though Nakivubo wetland is legally held in trust by the government; there is a great confusion as to its boundaries, ownership and status. Nearly all the land surrounding this wetland is privately owned and used, with the exception of Murchison Bay Prison to the north east. Large parts of the wetland have been reclaimed by or allocated to, private individuals whereas some

farmers settled on wetland fringes lay claim to cultivated plots. To date, more than 100,000 people abut Nakivubo wetland, including both high-cost housing estates and low-cost, high density settlements and slums (Figure 11 and 12).

Notwithstanding the enormous economic benefits accruing from purification and treatment of Kampala's municipal, domestic and industrial wastewaters in addition to other benefits, there is a great danger that Nakivubo wetland will soon be modified and converted completely, leading to the total loss of wetland resources and services as well as their associated economic benefits. Whereas the benefits from industrial and residential infrastructure largely accrue to individual property owners and industrialists, the economic impacts associated with wetland degradation are felt and cushioned as broader, social costs. These manifest as employment losses for some of the poorest sectors of Kampala's population, as increased costs to many other residents of Kampala, and as increased public sector expenditures on the infrastructure required to replicate wetland functions or offset the effects of their loss.



Figure 13: Raw wastewater (above) and Marabou stalks (below) scavenging bones, meat and fat from effluent wastewater discharged into Nakivubo wetland by City Abattoir, Kampala.

Although well-established standards for the discharge of industrial effluents are in operation [7], and policies for the conservation and sustainable utilization of wetland resources have been enacted [51], cheaper and industrially competitive wastewater treatment processes have not been fully developed in the country. This has paved the way for industrialists to abuse wetlands using political

gates. Unless wetlands are conserved and cheaper technologies developed for the treatment of wastewaters prior to their discharge into surface waters, industrial and urban expansion will cease to be viewed as driving forces for economic development of this country but rather appear as liabilities to the national treasury as well as posing critical environmental and public health concerns.

6 PRESENT INVESTIGATIONS

The methodological aspects and findings of this research are presented in five papers, referred to in the text by their Roman numerals (I–V). The study focussed on (i) assessing the hydraulic loading, pollution profiles, stability and water quality of Nakivubo wetland to establish its ability to respond to external pollution loads and preserve ecological balance [Paper I], (ii) assessing the spatial distribution and activity of autotrophic ammonium oxidizing bacteria in the different compartments of Nakivubo channel and wetland systems to estimate their influence on biological nitrogen transformations in the two systems [Paper II], (iii) developing and evaluating a pilot-scale bioprocess planted with two macrophyte

species dominant in Nakivubo wetland to establish their ability to remove nutrients (nitrogen and phosphorus) from wastewater and investigate the factors responsible for the differential nutrient removal rates [Paper III], (iv) scaling up of pilot studies and functional assessment of horizontal surface flow constructed wetlands receiving pre-treated domestic wastewater under field conditions in Uganda to evaluate the quality of the effluent with respect to its safe discharge and reuse [Paper IV], (v) studying the influence of microbial activities, plant uptake and biomass production on nutrient removal processes in substrate-free constructed wetlands with horizontal surface flow regime. [Paper V].

6.1 Role of Nakivubo wetland in Kampala wastewater disposal and water supply (Paper I)

Previously, different studies of Nakivubo wetland [10–12, 14–15, 108] indicated a high potential for nutrient and pathogen removal from influent wastewater. In their investigations however, they did not assess the individual contributions of all the inflows into the wetland, especially for heavy metals. Besides, since these studies were performed, over 50% of the wetland has been modified and used for agriculture and infrastructure development. Here, a recent study of the hydraulic loading, pollution profiles and ability of Nakivubo wetland to respond to external pollution and protect the water quality of Inner Murchison Bay is presented [Paper I]

6.1.1 Wastewater hydraulic flow and pollutant loading rates

Nakivubo wetland is strategically located and thus has particular significance because it acts as a sink for much of Kampala's composite domestic and industrial effluents. As shown

in Table 2 below, the daily discharge of wastewater into this wetland is considerable. The average hydraulic flow of wastewater into the upper and lower Nakivubo wetland ranged from $4.13\text{--}7.66 \times 10^4$ and $3.50\text{--}10.32 \times 10^4$ m³/day respectively. Based on mass loading rates [Tables 2 and 3, Paper I], $2.6\text{--}4.4 \times 10^3$ kg BOD/day and $0.79\text{--}1.68 \times 10^3$ kg NH₄-N/day are discharged into the upper Nakivubo wetland and lower Nakivubo wetland ($0.45\text{--}0.51 \times 10^3$ kg BOD/day and $0.69\text{--}1.51 \times 10^3$ kg NH₄-N/day), respectively. Therefore, the potential environmental impact of wastewater discharges on to Nakivubo wetland is reasonably high.

This study also showed that 48.3–57.9 % of the wastewater draining into Nakivubo wetland flows from the city centre and is untreated. The discharge of secondary treated effluent from the National Water and Sewerage Corporation (NWSC) wastewater treatment plant (WWTP) at Bugolobi (Table 2) represents only 15.7–26.1 % of total hydraulic flow to the upper section of

Nakivubo wetland. Moreover, only 8–9% of Kampala's 1.28 million people [17] are connected to sewer systems [175]. The remaining 91% use on-site sanitation facilities such as septic tanks, soak-away pits and pit latrines which in many slum areas are drained

during heavy storms to dispose of the accumulated wastes. This therefore demonstrated that a large proportion (over 70%) of wastewater is discharged into Nakivubo wetland without treatment.

Table 2: Mean and standard errors of hydraulic loading rates (HLRs) for the sampling stations investigated during this study ($n = 6$, this study).

Sampling station	Channel name	Mean hydraulic loading rate (m ³ /day)	Range (m ³ /day)
S1	Owino-Kitante	28 282 ± 1994	23 933–36893
S2	NWSC	14 573 ± 2583	6480–20131
S3	Lugogo	6 019 ± 635	3 542–8294
S4	5 th Street bridge	49 063 ± 5046	39 571–71539
S5	Kibira	3 557 ± 969	432–7690
S6	Bugolobi Flats	144 ± 29	86–259
S7	Bugolobi ponds	806 ± 101	432–1210
S8	Railway embankment	59 789 ± 9349 ^a	34 819–102471
S9	Murchison Bay Prisons	302 ± 91	86–691

^aFlow measured using a suspension method. The rest were determined using the wading method

Table 3: Ranges of the loading rates of physico-chemical variables determined for the sampling stations ($n = 6$, this study).

Sampling station	pH	Temp (°C)	EC (μS/cm)	DO (kg/day)	BOD ₅ (kg/day)	NH ₄ -N (kg/day)
S1	6.6–7.7	22.9–23.4	346–392	30–144	774–1 181	235–561
S2	7.1–8.0	24.1–24.9	798–1 036	26–45	700–2 365	428–1 263
S3	6.7–7.6	21.5–23.0	301–388	8.7–38.2	94–183	62–126
S4	6.6–7.7	22.9–23.7	407–520	4.2–85.8	2 498–4 221	722–1 560
S5	6.5–7.4	24.3–26.2	572–764	0.3–12	14.8–238	8.8–77.7
S6	5.9–7.2	24.1–26.6	800–856	0.0–0.1	13.3–40.4	4.8–12.3
S7	6.2–7.1	21.1–24.9	634–819	0.1–0.2	33.7–109	17.8–37.5
S8	6.3–7.5	22.5–26.1	508–573	5.0–31	418–1 004	682–1 465
S9	6.4–7.6	23.3–27.1	743–1 050	0.0–0.6	13.7–107	4.3–44.6

As shown in Table 4 below, heavy metals are components of wastewaters discharged into Nakivubo wetland, demonstrating that pollution from these chemicals originates from factories using heavy metals but lacking any form of wastewater pre-treatment facilities [Paper I]. Moreover, substantially high levels of lead above Uganda's allowable discharge limit of 0.1mg/L were occasionally detected in influent wastewater to the wetland where agricultural activities are taking place. Surprisingly, lead loading into the wetland was higher during storm events [Figure 5,

Paper I] suggesting that its discharge depends on the activities at the sites where it is used as well as the availability of surface runoff to carry the wastewater away into the wetland. At the outlet of upper Nakivubo wetland, no lead was detected [Figure 6, Paper I], indicating that this metal was retained by the wetland.

Lead is a very toxic element, causing a variety of effects at low dose levels. Brain damage, kidney damage, and gastrointestinal distress are seen from acute (short-term) exposure to high levels of lead in humans.

Chronic (long-term) exposure to lead in humans results in effects on the central nervous system (CNS), blood pressure, kidneys, and Vitamin D metabolism [176, 177]. Children are particularly sensitive to the chronic effects of lead, with slowed cognitive development, and reduced growth [176, 177]. Additionally, reproductive effects, such as decreased sperm count in men and spontaneous abortions in women, have been associated with high lead exposure [177, 178]. The developing fetus is at particular risk from maternal lead exposure, with low birth weight and slowed postnatal neurobehavioral development effects [178]. Therefore, in the light of its potential toxicological effects, Kampala residents consuming agricultural products from this metal laden wetland may

be at risk.

Metals can be inhibitory to microbial processes; particularly nitrification [148, 179–182] which is the limiting factor to biological nitrogen removal in treatment systems [126, 183]. Under inhibitory concentrations, metals interact with intracellular functional groups thereby destroying protein structure and function [180]. Inhibition by Copper, however, appears to involve a different mode of action which encompasses the rapid loss of cell membrane integrity [182, 184, 185]. Even though the kinetics of Zn internalization have been shown to be slow and their inhibitory properties related to their intracellular fraction [182], continuous exposure can present detrimental effects on biological nutrient removal processes particularly nitrification [179–182, 184, 185].

Table 4: Ranges of metal loadings (g/day) into Nakivubo wetland by the individual sampling stations ($n = 4$, this study). Flows are as indicated in Table 5.

Sampling station	Pb	Zn	Cu	Cr	Ag	Ni	Cd
S1	–	915–7 241	305–567	499–738	–	–	–
S2	–	871–4 429	130–403	0.0–200	0.0–403	–	–
S3	–	0.0–498	0.0–83	0.0–83	–	–	–
S4	–	1 190–3 577	0.0–1 431	–	–	–	–
S5	0–1 538	415–2 585	0.0–77	42–1 846	–	–	–
S6	–	24.2–74.3	0.9–3.5	0.0–1.7	–	–	–
S7	–	13.0–62.2	–	–	–	–	–
S8	–	632–11 195	0.0–1 025	0.0–1 488	–	–	–
S9	–	4.3–55.3	0.0–6.9	0.0–13.8	–	–	–

– = not detected

As shown in Table 4 above, the presence and continuous exposure of micro-organisms to Zn, Cu and Cr could have negatively impacted the nitrogen removal processes in this wetland. Moreover, there was limited ammonium-nitrogen removal in Nakivubo wetland despite the pH and temperature (Table 3) being in the acceptable ranges of 4–9.5 and 4–40°C, respectively, for the survival of nitrifying bacteria [5, 20, 26, 32, 88, 126, 142].

6.1.2 Stability of upper Nakivubo wetland

High organic loading in wastewaters is known to inhibit nitrification by promoting

heterotrophic growth and activity over autotrophic nitrifiers [130, 153–157; Paper I–V). One theory is that the first growing heterotrophs tend to occupy the outer layers of the biofilm, where both substrate (ammonia and oxygen) concentrations and detachment rate are higher whereas the slow growing nitrifying bacteria are kept deeper inside the biofilm. Accordingly, the heterotrophic layer forming above the nitrifiers in the biofilm consumes the substrates and also confers resistance to mass transfer within the heterotrophic layer thereby limiting autotrophic nitrification [158].

The wastewater discharged into Nakivubo wetland was characterized by high BOD

[Table 3; Paper II]. Mass balance calculations showed high removal efficiency for BOD, ranging from 77.4–86.3% (Figure 14) in the upper wetland, indicating high self-purification efficiency. As can be seen from paper I (Table 2, station S8), the effluent BOD concentration from the upper wetland was below the national discharge limit of 30 mg/L recommended for immediate discharge to land and surface waters [7]. However, the reduction of ammonium-nitrogen in the upper wetland was quite low and varied between -66.1% and 33.1% (Figure 14), suggesting limitations to key processes such as nitrification. Moreover, the agricultural

activities drain the wetland thereby limiting plant-wastewater interactions that are critical to periphyton attachment and materials transformations [186–187; Paper II, III].

Therefore the low nitrogen removal efficiency in upper Nakivubo wetland is a manifestation of high biodegradable organic matter input favoring heterotrophic growth and activity; wetland modification by farmers, and possibly metal inhibition of key metabolic processes of the vital microbial biomass [Paper I, II]. The leaching of ammonium from decomposing organic matter in the wetland may also explain its low removal rates.

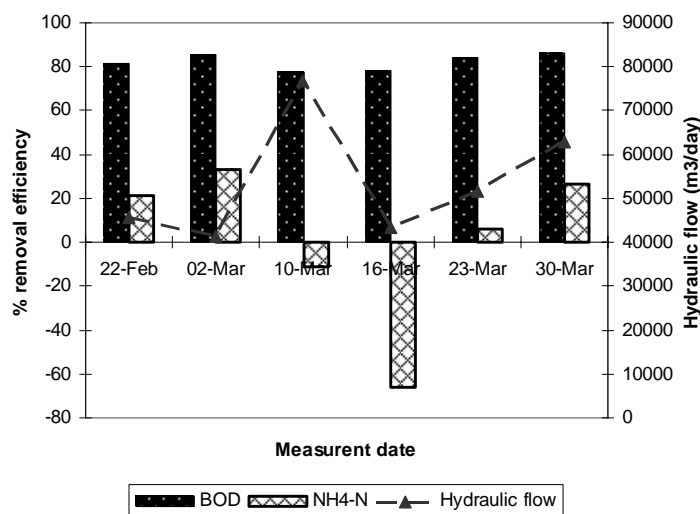


Figure 14: Changes in percentage removal efficiency for BOD and NH₄-N with hydraulic flow at the inlet of upper Nakivubo wetland (n = 6, this study).

The lower part of Nakivubo wetland exhibited significant differences in pH, and dissolved oxygen concentrations between the two vegetated zones. Dissolved oxygen concentrations higher than those detected in the *Miscanthidium* zone were recorded for the papyrus zone and are attributed to differential abilities of the two macrophytes to transfer oxygen from the aerial parts to the rhizosphere [37, 101, 120]. Additionally, the permeable root mat structure of papyrus easily allows wastewater to interact with

atmospheric oxygen unlike *Miscanthidium*, whose root mat structure is thick and compact [108].

Even though the two vegetated zones had different pH and DO levels, results indicated that their temperature, conductivity, biochemical oxygen demand and ammonium-N concentrations were not statistically different. One could also infer from the results that with the exception of ammonium-nitrogen, the water quality parameters in lower Nakivubo wetland were generally

within the limits permitted by the NEMA statute on effluent standards, but the water quality will deteriorate if the discharge of untreated effluents, and the agricultural and infrastructural developments within the wetland remain unabated [Paper I, II]. Discharge of wastewaters rich in organic matter and nitrogen compounds has deleterious effects on the ecology of Inner Murchison Bay which manifest as

eutrophication, dissolved oxygen depletion and toxicity of reduced and oxidized nitrogen compounds to aquatic life forms as well as public health [19, 20, 136]. The increased eutrophication and pathogen contamination respectively, will result into clogging problems and increased costs in terms of chemical consumption to process drinking water at Gaba water treatment plant.

6.2 Distribution and activity of ammonium-oxidizing bacteria (AOB) in Nakivubo channel and wetland systems (Paper II)

Removal of ammonia from wastewater is important because its discharge into receiving waters may lead to ammonia toxicity, oxygen depletion, and eutrophication of surface waters [20, 136]. The key process in ammonia removal during wastewater treatment is the two-step oxidation of ammonia to nitrate via a microbial-mediated nitrification [119, 126, 188]. Biological oxidation of ammonia to nitrate occurs primarily through the coordination of two distinct chemolithotrophic groups of bacteria: ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Nitrifying bacteria are characterised by slow growth rates and sensitivity to environmental factors including temperature, pH, oxygen concentration and organic matter. These factors influence the minimum bacterial density and activity required to establish stable nitrification during wastewater treatment [189]. Because treatment wetlands are particularly characterised by low oxygen concentrations, the density and activity of nitrifying bacteria are critical for the proper functioning and maintenance of the system [5, 70, 190–192].

In this study, the pollution profiles and spatial distribution of AOB, and the nitrification activities along Nakivubo channel and wetland are presented. The study focused on monitoring changes in physico-chemical and biochemical parameters which would influence the nitrification process. In

addition, the numbers and activity of AOB in the different phases (water, sediment and epiphyton) were quantified in order to estimate their influence on nitrification and nitrogen bioconversions in general, and to determine the factors influencing their distribution in the two systems.

6.2.1 Wastewater characteristics

As shown in paper II (Table 1), environmental conditions (pH, temperature, DO) and substrate ($\text{NH}_4\text{-N}$) concentrations at all sampling stations could favour growth of nitrifiers and hence nitrification [5, 21, 32, 126]. Products of nitrification were detected at several sampling stations. However, a clearly defined longitudinal build-up of nitrite and nitrate was never detected despite the favourable environmental conditions and availability of $\text{NH}_4\text{-N}$ in the channel and wetland. Substantial nitrification could have occurred as was evidenced by fairly large numbers of AOB (Figure 15) and correspondingly high potential nitrification activities (Figures 3a–c, Paper II), but was possibly masked by denitrification [43]. Alternatively, the reduction in ammonical and total nitrogen concentrations at the outlet of upper Nakivubo wetlands could be attributed to adsorption of ammonium to sediments and settleable particulate matter [43] or anaerobic ammonium oxidation (Anammox) reactions [188, 193].

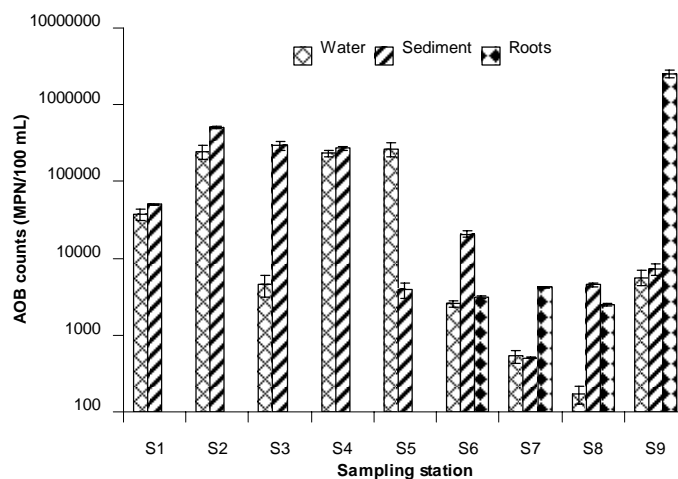


Figure 15: MPN counts of ammonium-oxidizing bacteria (AOB) present in the water, sediment and epiphyton at the different sampling station investigated in this study (this study).

One can also infer from the results that three levels of oxygen depletion existed along the channel and wetland. This is a manifestation of the high oxygen-demanding wastewaters discharged into surface waters, especially from the WWTP, sewage stabilization ponds, and slaughterhouse. The heterotrophic bacteria discharged by the sewage wastewater treatment plant [136, 194] at Bugolobi, together with Nakivubo channel wastewater which is rich in biodegradable organics, consumed much of the residual oxygen in the wastewater prior to its discharge into upper Nakivubo wetland.

Certainly, this inference was supported by the lower concentrations of BOD in wastewater at station S6 compared to S4 (Table 1), thus explaining the relatively high residual ammonium concentrations reaching the wetland [Paper I]. Most notably, more nitrifying bacteria colonized the epiphyton of the wetland system as shown in Figure 15, station S9) resulting in oxygen depletion and hence the characteristic hypoxic–anoxic conditions of the wetland.

This study further showed that more nitrifying micro-organisms thrived downstream of upper Nakivubo wetland. This

trend in proliferation of nitrifying bacteria can be explained with heterotrophic activity and sedimentation processes in Nakivubo channel, which lowered the organic matter content of the wastewater reaching the wetland, thus reducing competition between heterotrophs and nitrificants. Even though epiphytic AOB density increased downstream of the wetland, the levels of nitrification products remained very low and are attributed to the denitrification process bearing in mind the hypoxic-anoxic environment of this system [Paper I].

The results of this study show that a good population of nitrifying organisms exists in the vegetated section of the wetland but their metabolic activity and subsequently, proliferation, are impaired by limited residual oxygen [195; Paper I]. As earlier observed [Paper I], the concentrations of BOD recorded in this study for the upper Nakivubo wetland were below the national discharge limit of 30 mg/L [7], an indication that most of the residual oxygen in the wetland was preferentially used to lower BOD to acceptable levels rather than nitrogen removal [Paper I].

6.2.2 Distribution and activity of AOB in the different ecomorphological compartments of Nakivubo channel and wetland

Different hydroecomorphological factors are responsible for the physical, chemical and biological processes and thus influence the self-purification of flowing water systems [187, 196]. In order to determine the influence of these factors on the spatial distribution of nitrifying bacteria, and to establish their importance for self-purification of Nakivubo channel and wetland systems respectively, a quantitative estimation of AOB and the corresponding potential nitrification activities of the different compartments were investigated.

As shown in Figure 15 and paper II (Figure 3a–c), the upstream water and sediment compartments of Nakivubo channel (stations S1–S5) exhibited higher numbers and activity of AOB compared to their downstream counterparts (station S6), and the wetland (station S9). The longitudinal decline in numbers and activity of AOB despite a continuous influx of nitrifying bacteria from the WWTP and other non-point sources is attributed to the high input of biodegradable organic matter, notably from raw abattoir wastewater discharges (Figure 13; Table 1), with which heterotrophic bacteria suppress nitrificants [43, 157–158, 187; Papers I–V].

In addition to heterotrophic activity and inhibitory factors such as metals which have been detected in wastewaters discharged into Nakivubo channel [Paper I], the high variability of width and slowly flowed water sections (0.368–0.448 m/s and 0.177–0.286 m/s at station S1 and S4 respectively) favoured silt deposition, and probably bacteria sedimentation with suspended particulate matter in the channel waters [187]. In fact, nitrifying bacteria were significantly more numerous in the surface sediment than the water and epiphytic compartments at station S6 as opposed to stations S1 and S4 (Figure 15), indicating that sedimentation was a major factor in Nakivubo brook.

In the wetland however, the association of more nitrifying bacteria and nitrification activity with plant roots than the water and sediment phases at station S9 highlighted the importance of macrophytes in providing the surface for periphyton attachment and material transformation [Paper III, V]. Other studies [101, 196–199] also demonstrated the role of macrophytes in providing attachment sites for bacteria involved in nitrogen removal from wastewaters.

Therefore, it was deduced that the epiphyton had a larger influence on wetland nitrification relative to the water and sediment compartments, which is in concordance with observations reported for constructed wetlands treating sewage wastewater [Paper III, V]. Moreover, the epiphytic AOB enumerated were the same order of magnitude as those we obtained in pilot papyrus-based constructed wetlands [44; Paper III].

From this study, it could also be concluded that such high numbers of nitrifying bacteria as in the water and stream bed of Nakivubo channel and its inflow tributaries, such as Kibira channel are characteristic of ammonia-rich environments. Nevertheless, the majority of nitrifiers in the channel and wetland originate, either suspended or attached to particulate matter, from the sewage WWTP at Bugolobi. Numbers of AOB in the sediment and water phases were boosted 5-fold and 6-fold, respectively, after the discharge of WWTP effluent; a finding which demonstrates that the trickling filters and activated sludge processes at Bugolobi WWTP supported growth of ammonium-oxidizers.

The fact that the Nakivubo wetland system experiences low self-purification efficiency for nitrogen can be explained by the exposure of nitrifying bacteria to toxic and inhibitory factors present in wastewaters [Paper I], the prevalence of high organic matter favouring heterotrophic growth while suppressing nitrificants; the minimal DO concentrations, and sedimentation of nitrifiers in the channel bed which reduces seeding of the wetland with nitrifying bacteria.

Additionally, a comparison of the influence of the different phases on nitrification indicated that epiphytic nitrification was more important than that of sediment and water compartments of the wetland. However, the sediment and suspended nitrifiers were equally important for the nitrification process upstream of Nakivubo channel. Therefore, the modification of Nakivubo wetland by

replacing the original macrophytes with cocoyam and sugar cane and/or its development into commercial and residential zones negatively impacts the treatment efficiency of this wetland; and is detrimental to the ecological integrity of Inner Murchison Bay and the quality of drinking water supplies for Kampala city.

6.3 Optimization of processes for biological nitrogen removal in Nakivubo wetland system, Uganda (Papers III-V)

In Uganda, wastewater treatment by natural wetlands has been in use for several decades [Paper V]. As described in paper I and II, Nakivubo wetland, which has performed tertiary wastewater treatment for Kampala city for the past four decades is ecologically stressed by agricultural and infrastructural developments, besides the high-strength raw and/or partially treated wastewaters it receives. This implies that the wetland's economic and environmental significance in protecting the water quality of Murchison Bay from where the water supply for Kampala city is abstracted will decline if human encroachment on the wetland remains unabated [Paper I]. Additionally, there is an

urgent need to develop environmentally sound low-cost and easily applicable technologies that utilise natural processes for upstream treatment of wastewaters of small communities and industries (such as foods and beverages, meat and fish processing enterprises) where the main pollutant load is organic in nature. In order to abate further environmental degradation of Nakivubo wetland and to protect the water quality of Lake Victoria, constructed wetlands planted with macrophyte species adapted to the local ecological conditions were investigated for their potential application in wastewater treatment.

6.3.1 A pilot scale constructed wetland process (Paper III)

Pollutant removal in wetland ecosystems is effected through a number of complex natural mechanisms involving physical, chemical and biological processes such as sedimentation, filtration, precipitation, sorption, adsorption, plant uptake, and microbial bio-conversions and uptake [Papers I–V]. Whereas microbial bio-conversion processes are dependant on environmental conditions which influence microbial proliferation and activity [Paper II], plant uptake is influenced by the ability of the macrophytes to develop sufficient root systems and to translate nutrients into biomass production, which can later be harvested for nutrient removal [Paper V]. Macrophyte roots influence both nutrient

uptake and bio-film development including provision of attachment sites for nitrogen transforming bacteria [101, 196–199; Paper II–V]. Because these factors lend a strong support to pollutant removal processes in treatment wetlands, two macrophyte species were investigated for biomass production, nutrient storage in plant tissues, root development and surface area, and attachment and activity of nitrifying bacteria as part of the overall evaluation of their wastewater treatment potential [Paper III–V].

As described in paper III, the small-scale pilot constructed wetlands (CWs) designed to evaluate the potential of *Cyperus papyrus* and *Miscanthidium violaceum* for wastewater

treatment were setup at Makerere University botanical garden in December 2001. The two macrophytes (Figure 16 and 20) are the dominant species in Nakivubo and other

ecologically important wetlands in Uganda. A view of the schematics of the pilot process CWs is shown in Paper III, and Figure 16 below.

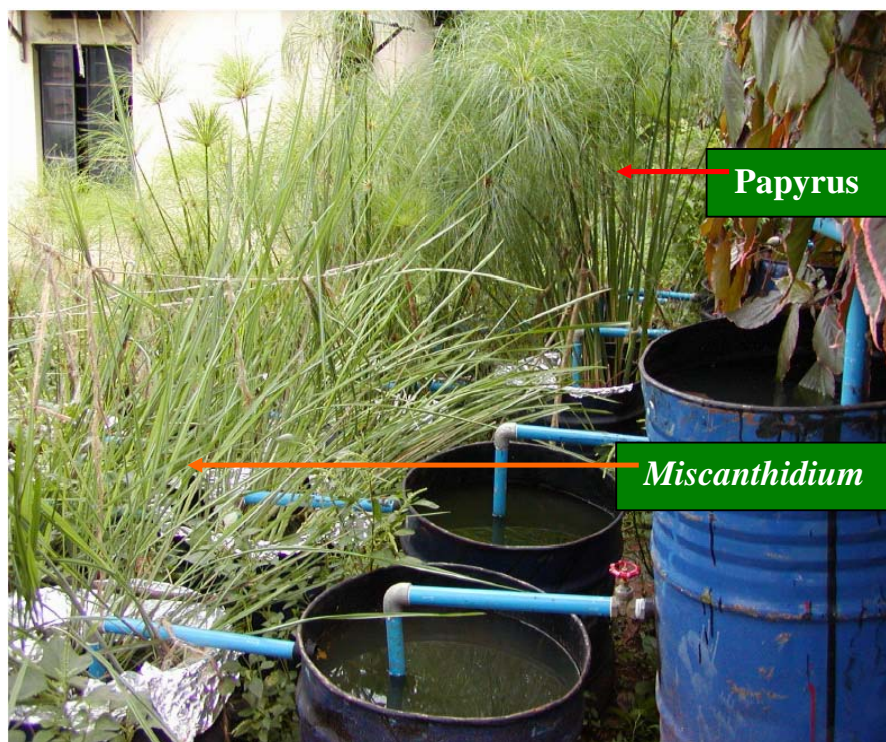


Figure 16: A view of the pilot constructed wetlands which were installed at Makerere University botanical garden, Uganda (this study).

6.3.1.1 Pilot constructed wetlands treatment efficiency

The influent wastewater to the pilot constructed wetlands was characterised by high electrical conductivity, low dissolved oxygen, and high nutrient concentrations (Figure 17). Despite high influent perturbations, reductions in electrical conductivity and the concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, TN (total nitrogen) and TRP (total reactive phosphorus) were recorded for the effluent wastewater from all treatments (Figure 17). As seen from Figure 17, reduction in these quantified water quality parameters was well demonstrated in planted

CWs and was generally higher in papyrus than *Miscanthidium*-based CWs. Specifically, the effluent TRP concentrations below the Uganda regulatory discharge limit of 10 mg/L [7] were registered for both vegetated treatments, with papyrus-based treatments exhibiting much lower concentrations of up to 2.6 ± 1.1 mg/L (Figure 17).

Unlike total-N, the effluent concentrations of $\text{NH}_4\text{-N}$ below the national discharge limit of 10 mg/L were achieved only in papyrus based CWs. Longitudinally, better $\text{NH}_4\text{-N}$ and TRP removal rates, ranging from 48–83.2%, were obtained in macrophyte-based treatment systems compared to less than 30% in the unplanted controls. Moreover, higher

NH₄-N and TRP removal was achieved in systems planted with papyrus (75.3% and 83.2% respectively) relative to 61.5 and

48.4% respectively for *Miscanthidium*-based treatment systems.

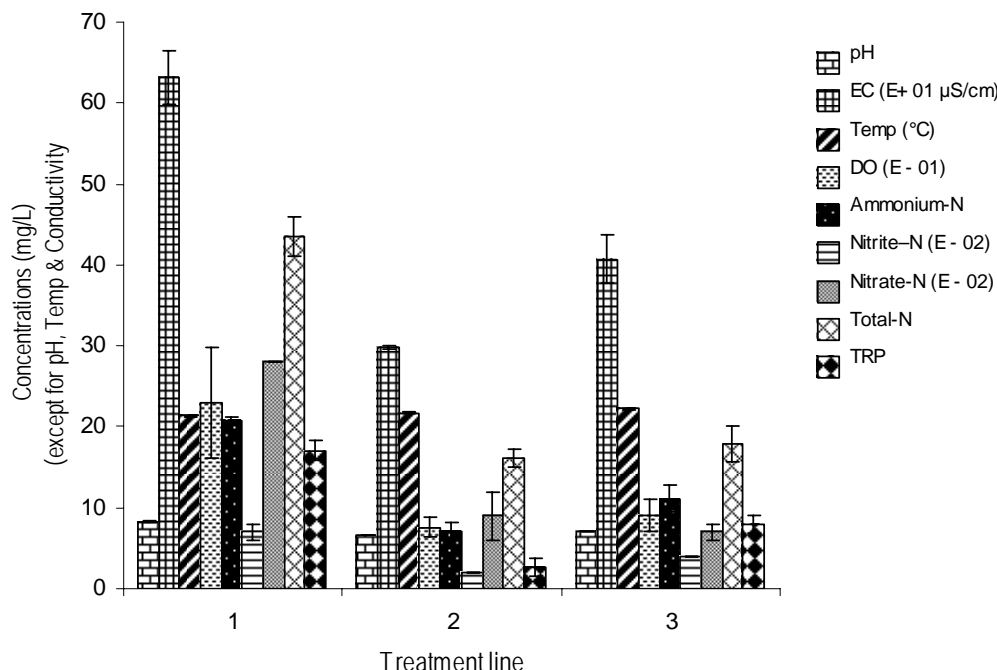


Figure 17: Effluent values of the different parameters determined for the pilot constructed wetlands which were installed at Makerere University (this study). Treatment line 1 is unplanted control, 2 and 3 are papyrus and *Miscanthidium*-based treatments, respectively.

6.3.1.2 Role of macrophytes in wastewater treatment

Good performance of wetlands for wastewater treatment depends on the growth potential and ability of macrophytes to develop sufficient root systems for microbial attachment and material transformations [187, 196, 198], and to translate nutrients into plant biomass that can be subsequently harvested for nutrient removal [5, Paper V]. Moreover, wetland plants transfer photosynthetic oxygen to the rhizosphere thus boosting oxygen concentration in the water column [100, 101, 120, 121]. In the present study, the differences in root structure, surface area and recruitment rates of the two macrophytes depicted important consequences for the degradation of wastewater components and uptake of nutrients. As shown in paper III,

Table 2, papyrus exhibited a larger number of adventitious root structures which conferred it a 3-fold larger root surface area compared to *Miscanthidium*. In addition to better uptake of nutrients, this might have availed more oxygen to the rhizosphere thereby reducing competition between heterotrophs and nitrifiers [Paper III–V].

Furthermore, apart from providing attachment sites and diffusible oxygen to the bacteria, root mats increase wastewater residence time and retention of suspended organic particles, which upon degradation avail nutrients to bacteria and plants [Paper II–V]. In wastewater treatment systems, bacteria attached as biofilms are usually more numerous and active than those living freely [200]. The activities within biofilms are regulated by inward diffusion of nutrients and internal material transformations within the

biofilm [201]. Moreover, the characteristics of the surfaces on which biofilms develop are reported to influence the development of the microbial communities [200], and thus may lend a strong support for biological transformation of nitrogen. As shown in paper III (Figure 4), nitrifying bacteria were more numerous in the papyrus root-mat compared to that of *Miscanthidium*, demonstrating that more attachment sites, better development of the biofilm, and easy accessibility of nutrients existed in within the papyrus root mat [200, 201].

Similar to field observations [Paper II, V], the water phase, in this study, showed lower activity and numbers of nitrifying organisms implying that the nitrogen transforming bacteria were removed from the water column by attachment to macrophytes, plant litter, settling particles, and algae [199]. Additionally, the findings of this study showed a 5-fold higher nitrification activity in the epiphyton than in the water and sediment compartments, which tallied with MPN numbers, except for the peat samples. Therefore one could comfortably conclude that epiphytic nitrifiers were more important for nitrification in the pilot-constructed wetlands.

Contrary to Nakivubo channel sediments [Paper II]; nitrifying bacteria in the peat phase of the CWs appeared to have been inactive in the treatment system probably due to limited oxygen and thus may not have contributed much to the nitrification process. However, under favourable laboratory conditions, re-growth and activity were regained. Moreover, as seen in the field [Paper II], nitrifiers settled with suspended particles in the treatment systems [199; Paper V] thus accounting for the high bacterial MPN counts for peat.

Microbial attachment and root development positively influenced nutrient uptake of the two macrophytes. From Figure 2 and 3 (Paper III), one can infer that the recruitment rates of roots and shoots as well as the increment in plant fresh weight were higher for papyrus than *Miscanthidium*, an

indication that papyrus assimilated more nutrients than *Miscanthidium*.

Even though only differences in weight increment between the two macrophytes were statistically significant, studies of nutrient (N & P) storage (Figure 18) revealed higher concentrations of both nutrient variables in papyrus tissues relative to *Miscanthidium* tissues. More interestingly, the nutrient storage ability was significantly higher in papyrus than in *Miscanthidium*, a factor which confers a comparative advantage to papyrus in regard to wastewater treatment [Paper III–V].

Mass balance calculations indicated that plant uptake and storage was the major mechanism responsible for N and P removal in systems planted with papyrus, where it contributed 69.5% N and 88.8% P of the total N and P removed. It however accounted for only 15.8% N and 30.7% P of the total N and P removed by treatment line 3, indicating that processes such as nitrification-denitrification and adsorption of soluble phosphorus to roots and peat were more important for N and P removal in *Miscanthidium violaceum*-based treatment wetlands.

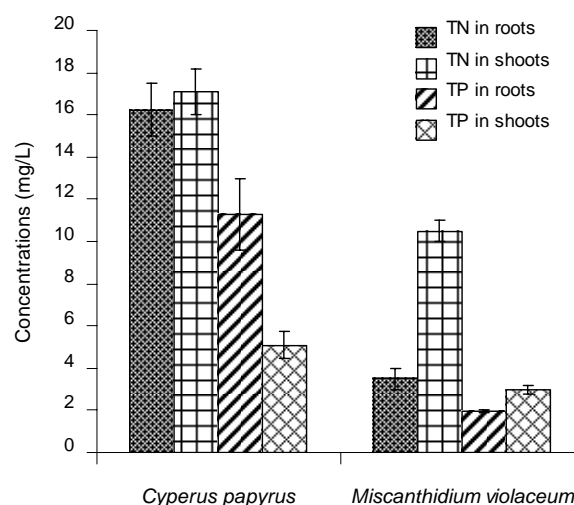


Figure 18: Nitrogen and phosphorus content in root and shoot tissues of *Cyperus papyrus* and *Miscanthidium violaceum* (this study).

Therefore in order to evaluate the potential application of a macrophyte in wastewater treatment constructed wetlands, knowledge of structural development and recruitment rates of roots and the general growth rate of the macrophyte in question is crucial. These factors influence plant-micro-organisms-wastewater interactions by providing microbial attachment sites, sufficient wastewater residence time, trapping and settlement of suspended wastewater components as a result of resistance to hydraulic flow, surface area for pollutant

adsorption, uptake and storage in plant tissues, and diffusion of oxygen from aerial parts to the rhizosphere.

The findings of this study demonstrated a positive influence of both macrophyte species on nutrient removal processes in the treatment wetlands. However, the better performance of papyrus-based treatment wetlands is a manifestation of its high root recruitment rates, larger root surface area which facilitates epiphyton attachment and material transformations, higher nutrient uptake and consequently higher biomass yield.

6.3.2 Biological nutrient removal in substrate-free pilot constructed wetlands in Uganda: Field investigations (Paper IV, V)

Small-scale pilot investigations [Paper III] showed a high wastewater treatment potential of the two tropical macrophytes, notably *Cyperus papyrus*. To slake our quest of developing low-cost, easy to adopt, environmentally sound processes for wastewater treatment in resource-scarce tropical countries like Uganda, the two macrophytes were subjected to further analysis on a larger scale under field conditions as presented in this section.

6.3.2.1 Constructed wetlands design

Different designs of treatment CWs have been applied else where including vertical and horizontal sub-surface flow treatment systems [5]. Tracer dye studies have shown that in horizontal sub-surface flow constructed wetlands the wastewater-root zone contact is reduced due to root biomass that fills the pore

spaces of the gravel and directs the flow to deeper wetland media [202]. In addition, it is recognized that: (1) the litter formed by decomposing vegetation remains on the surface of the substrate and thus does not interact with the wastewater, and (2) the substrate media usually used does not contain sufficient concentrations of Ca, Fe, or Al to actively adsorb P [144]. The plants investigated in this study have special features since they thrive either as floating or as rooted mats in aquatic environments, thereby rendering soil or gravel substrate requirements unconditional. To this effect, a substrate-free design was employed to allow sufficient mixing of wastewater and optimal interactions between wastewater, micro-organisms and macrophyte root systems. A schematic representation and view of the constructed wetlands under field conditions are shown in Figure 19 and 20 below.

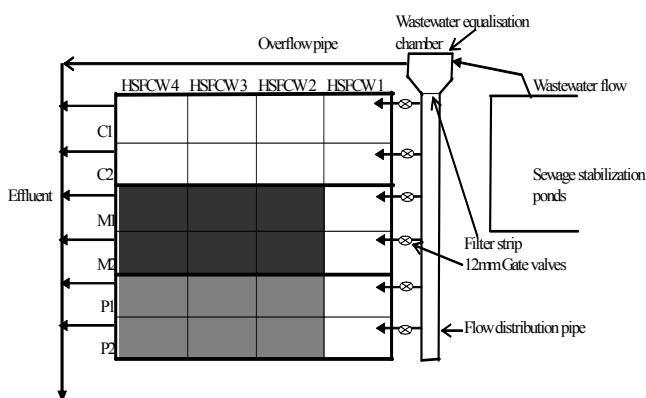


Figure 19a: Arrangement of the treatment wetlands. Treatment lines C1 and C2 are unplanted controls; M1, M2 and P1, P2 are *Miscanthidium* and papyrus-based treatments, respectively. The shaded constructed wetlands (HSFCW 2–4) are vegetated.

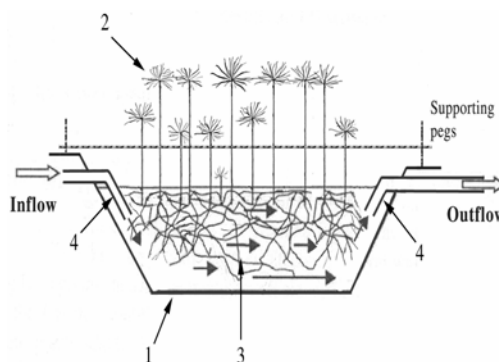


Figure 19b: Schematic representation of a substrate-free CW cell with horizontal surface flow. 1 – Impermeable plastic liner, 2 – vegetation, 3 – root system, 4 – inflow and outflow drainage pipes.

6.3.2.2 System treatment performance

Significant differences in biomass productivity existed between the two macrophyte species (Figure 21 below) and depicted important consequences for the degradation of wastewater components and uptake of nutrients. Higher aerial and below ground biomass productivity was attained in papyrus CWs in comparison to *Miscanthidium*-based counterparts. Similarly, more nutrients were sequestered in the aerial and below ground biomass of papyrus compared to the average quantities stored in *Miscanthidium* (Figure 21). Further analysis indicated that the total-N bound into papyrus umbels was significantly higher than that sequestered in *Miscanthidium* leaves (Figure 22a and 22b). Moreover, despite the fact that the N and P content of papyrus culms and roots/rhizomes portions was not significantly different from that of *Miscanthidium* stalks and roots, calculations from area-based biomass productivity demonstrated that more nutrients were bound into papyrus compared to *Miscanthidium* tissues. This shows that papyrus removed more nutrients per unit area of the CWs, which were translocated to the growing aerial parts resulting in higher aerial

biomass productivity. Consequently, more nutrients were assimilated into plant biomass thus explaining the differences in biomass productivity of the two macrophytes. Moreover, more N was detected in papyrus umbels compared to the leaves of *Miscanthidium*.

Research has shown that wetland plants transfer photosynthetic oxygen to the rhizosphere at different rates, which influences root development [165, 203]. The two macrophytes have different growth and root development properties [Paper III, 108] and consequently release oxygen to the rhizosphere at different rates. As shown in Figure 23 below, the influent wastewater to the CWs was characterised by high BOD perturbations and therefore exerted an oxygen demand at varying degrees to plant roots. This also explains the observed differences in below ground biomass production and nutrient uptake by the two macrophytes.

Even though the average below ground biomass value obtained for papyrus was two-fold lower than its aerial biomass, it was in the range reported for floating papyrus swamps [110]. Besides, papyrus has been reported to exhibit rates of aerial primary production as high as 6607 g DW/m²/year

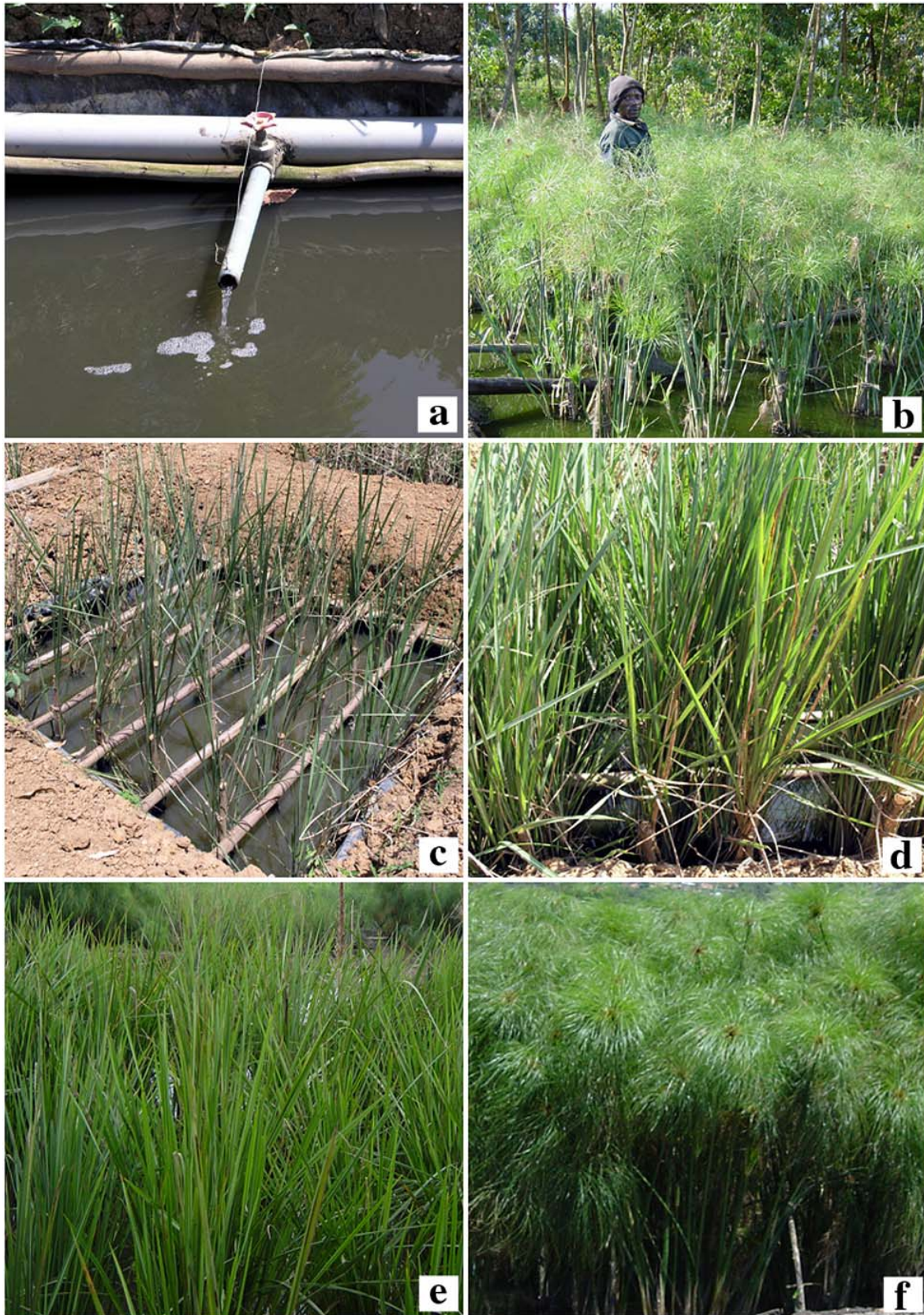


Figure 20: Representation of constructed treatment wetlands at Bugolobi, Uganda (this study) during the different phases of growth (Paper IV, V). **a** – Feeding of wastewater to CWs by flow distribution pipe, **b** and **c** – papyrus and *Miscanthidium* CWs one month after planting, and **d** – **f**, six months after planting.

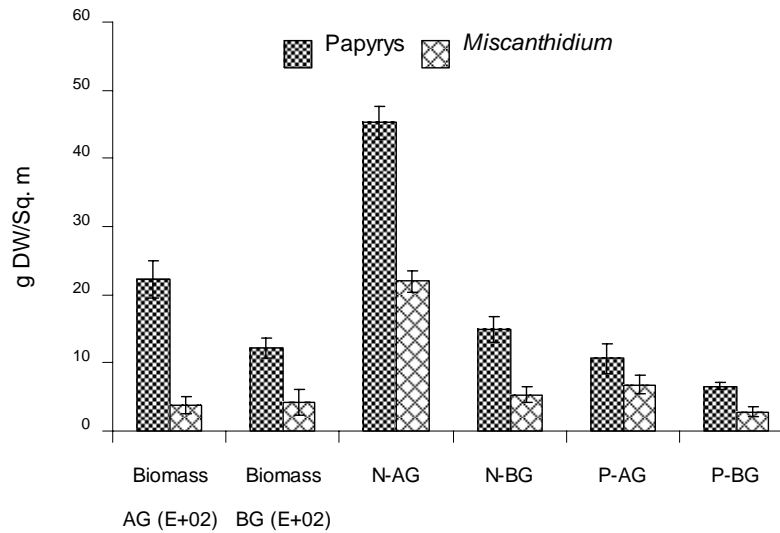


Figure 21: Above ground (AG) and below ground (BG) biomass productivity and nutrient (N & P) content of *Cyperus papyrus* and *Miscanthidium violaceum* (this study).

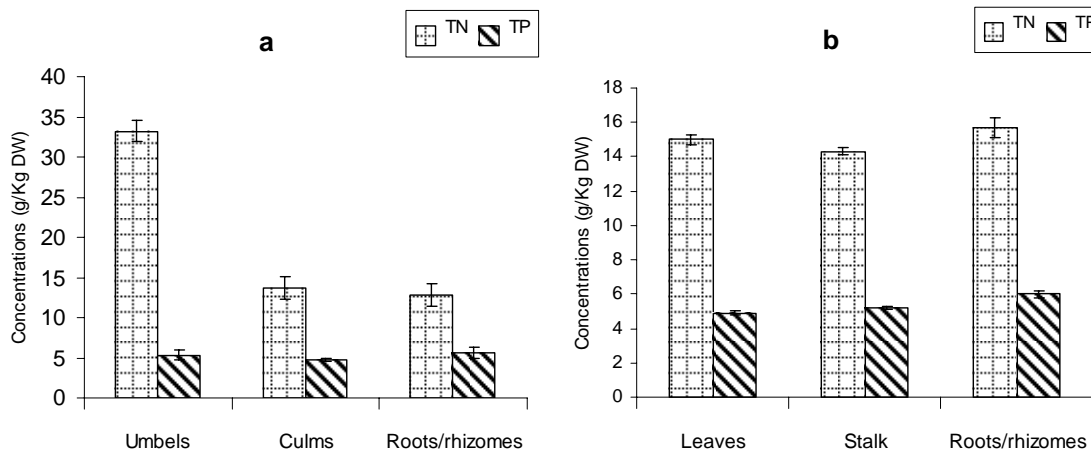


Figure 22: Concentrations of TN and TP in (a) *Cyperus papyrus* and (b) *Miscanthidium violaceum* parts.

under natural conditions (Muthuri et al., [106]) which is attributed to its high photosynthetic activity resulting from its characteristic C_4 photosynthesis [204].

Comparatively, the levels of phosphorus in standing plant stocks recorded in this study were 2–5 times higher than literature values for horizontal subsurface flow CWs planted with *Phalaris arundinacea* [146] and *Phragmites* [147, 205] which suggests better nutrient uptake and storage performance of the two macrophytes in our CWs. The presence of higher levels of bound N than P in

the aerial biomass of both plants species was in concordance with pilot container experiments [Paper III], demonstrating active translocation and storage of nutrients to sites where they are needed for primary growth (e.g synthesis of amino acids and enzymes).

Using average nutrient concentrations and area-based productivity of the aerial plant biomass, calculations showed that plant uptake and storage of nutrients contributed 28.5% N and 11.2% P of the total nitrogen and phosphorus removed in papyrus-based treatments. Similar calculations indicated

plant uptake and storage contributions of 15.7% N and 9.3% P of the total nitrogen and phosphorus removed by *Miscanthidium*-based treatments. The P removed due to plant uptake and storage appeared to be lower than 33% reported by Okurut et al., [55] for CWs in which papyrus was grown as a floating mat. This appeared so due to the high P concentrations of the influent wastewater (19.1 ± 0.6 mg o-PO₄-P/L, Figure 23) compared to 3.7 ± 0.8 mg o-PO₄-P/L for Okurut et al., [55]. In addition, differences in

hydraulic flow regimes (continuous flow, in this study, versus intermittent feeding in their study), HRTs, and general design of the wetlands existed between the two studies.

Therefore, we could infer from these findings that better nutrient uptake and storage performance of the two macrophytes was obtained in our CWs and presents a fairly good potential of the two macrophytes, especially papyrus, for biological nutrient removal from wastewater through plant harvesting.

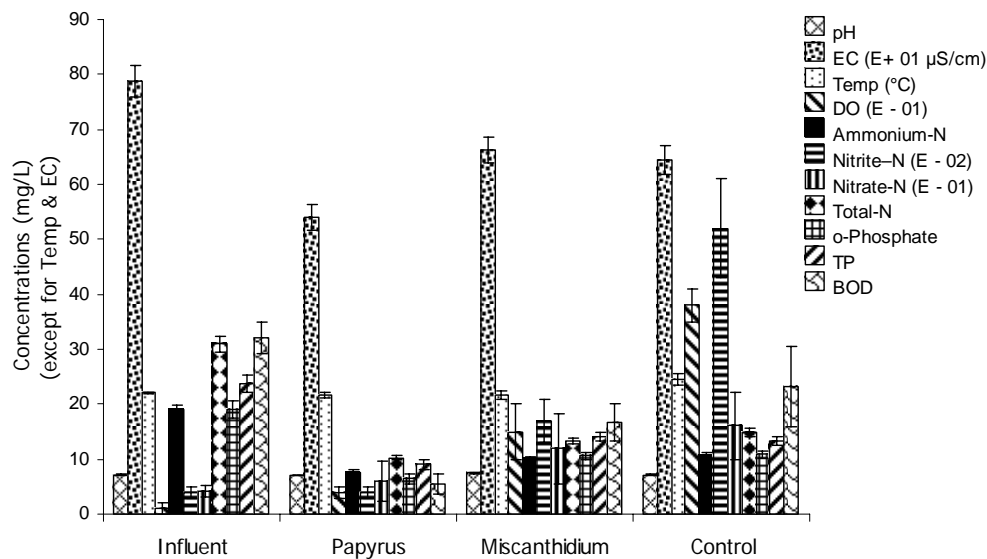


Figure 23: Changes in influent and effluent concentrations of the different water quality parameters determined for the unplanted controls, papyrus and *Miscanthidium*-based constructed wetlands at Bugolobi, Uganda (this study).

Water balance analyses showed that the practical hydraulic retention times (HRTs) were 2.85 ± 0.17 , 3.02 ± 0.13 , and 3.44 ± 0.11 days for unplanted controls, *Miscanthidium* and papyrus respectively. The differences in retention times are explained by evapotranspiration and evaporation effects which were higher in planted treatments. The higher biomass productivity of papyrus [Paper III, V]) accompanied by its high transpiration rate resulted in higher water losses compared to *Miscanthidium* and unplanted controls thus, accounting for the increased wastewater retention time of papyrus CWs. In addition, the higher transpiration and shading effect of

papyrus (Figure 21) maintained lower water temperatures and minimal algal growth compared to *Miscanthidium* and unplanted controls [206].

Similarly, water pH, electrical conductivity and DO in papyrus-based treatments were always lower than in the controls and *Miscanthidium*-based treatments (Figure 23). As explained in paper V, CO₂ production due to heterotrophic decomposition of plant litter and other wastewater components trapped in the papyrus root mat [161, 206], acid production during nitrification of ammonia [20, 126, 207], and minimal algal growth are contributory factors to the lower water pH of

the papyrus CWs. Despite the influent wastewater being practically deoxygenated (Figure 23); the outflows of all treatments contained substantial levels of oxygen (≥ 0.4 mg/L).

Dissolved oxygen levels below 1–2 mg/L in water are reported to substantially reduce nitrification [20, 122, 123, 126, 208]. On the other hand, effective nitrification has been reported in systems with DO levels below 0.5 mg/L [21]. The rise in $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ loading in the effluent wastewater compared to the influent wastewater was a manifestation of active system nitrification since temperature and pH were not limiting [21, 26, 32, 148]. Lower effluent loading of $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ was recorded for vegetated treatments compared to the controls due to the

limited atmospheric aeration, oxygen supply from algal photosynthesis, high heterotrophic competition for oxygen with nitrifiers [130, 157, 158], plant uptake of $\text{NO}_3\text{-N}$ [5, 34], and denitrification [5, 34, 122].

As in small-scale pilot investigations [Paper III], higher removal efficiencies were obtained in planted systems (Figure 23; Table 5). Systems planted with papyrus showed substantially high treatment efficiency ranging from 68.6–99.1% for nutrients, BOD, and indicator organisms [Paper IV, V]. Okurut et al., [55] also showed higher treatment performance of *Cyperus papyrus*-based systems when compared with *phragmites mauritianus* grown on a gravel substratum under tropical conditions.

Table 5: Bacterial concentrations (CFU/100 mL) and the corresponding percentage removal efficiencies in the constructed wetlands (this study).

Treatment line	Wetland unit	Water source	Bacterial concentration (CFU/100mL)		% Removal efficiency	
			FC	TC	FC	TC
C	HSFCW1	Influent	35.0×10^4	24.5×10^5	92.9	86.5
	HSFCW4	Effluent	2.5×10^4	3.3×10^5		
M	HSFCW1	Influent	35.0×10^4	24.5×10^5	93.7	89.0
	HSFCW4	Effluent	2.2×10^4	2.7×10^5		
P	HSFCW1	Influent	35.0×10^4	24.5×10^5	99.1	96.3
	HSFCW4	Effluent	0.3×10^4	0.9×10^5		

From Figure 23 and Table 5, water quality amelioration by papyrus-based CWs was satisfactory. Specifically, the outflow concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, TN, $\text{o-PO}_4\text{-P}$ and TP, FC (faecal coliform) and TC (total coliform) were in accordance with the national discharge limits adopted for sewage effluents [7]. However, despite high BOD removal efficiency for papyrus-based treatments (86.5%), the effluent concentrations were still above the permissible limit (30 mg BOD/L) for discharge to surface waters in Uganda [7]. The removal efficiencies of FC (99.1%) were

below the discharge limit of 5000 CFU/100 mL [7] but higher than values recommended for the safe reuse of wastewater for recreational and irrigational purposes [1000 CFU/100 mL; 209].

The bacterial removal efficiencies obtained in the present investigation were in the range reported for treatment wetlands [32, 59] but higher than values reported by Kaseva, [210] for a sub-surface flow CW polishing pre-treated domestic wastewater in Tanzania. As shown in paper IV, several factors including differences in hydrological retention times [211, 212], reduction in the organic matter

content [93], plant litter formation and sedimentation, as well as macrophyte root-mat structure and surface area [14] are responsible for the observed differences in bacterial removal efficiencies between the two macrophyte-based treatments. These factors influence processes namely, adsorption to organic matter, sedimentation, aggregation and filtration; all of which affect the retention of pathogens in surface-flow treatment wetlands.

6.3.2.3 System nitrification potential

Nitrifying bacteria are slow growing organisms and acclimatize over time with an increase in population size [20, 21, 126]. Nitrification studies were conducted as part of the overall performance assessment of the pilot substrate-free constructed treatment wetlands [Paper V]. Different methods [12, 207, 213] including isotope-dilution procedures [199, 214–218] have been employed in potential nitrification studies. The method exploited in this study is simple and does not require sophisticated equipment to generate good results. Nitrification measurements of the different phases of the CWs were conducted six months later, after system start-up to evaluate whether the system could nitrify under such high influent BOD loading perturbations (Figure 23).

As presented in paper V (Figures 6a–c), incubations of the root mat phase of treatment M showed significantly higher ammonium oxidation rate (2.96 ± 0.2 mg $\text{NO}_2\text{-N/L/h}$) than treatment P (0.20 ± 0.02 mg $\text{NO}_2\text{-N/L/h}$). A comparison of peat samples from the three treatments indicated that the controls had significantly higher activity (0.44 ± 0.18 mg $\text{NO}_2\text{-N/L/h}$) compared to *Miscanthidium* (0.32 ± 0.01 mg $\text{NO}_2\text{-N/L/h}$) and papyrus CWs (0.21 ± 0.01 mg $\text{NO}_2\text{-N/L/h}$). However, the water phase of the controls and treatments planted with *Miscanthidium* showed similar ammonium-oxidation rates (0.029 ± 0.09 and 0.027 ± 0.002 mg $\text{NO}_2\text{-N/L/h}$, respectively) which were higher than the activity detected

in the water column of papyrus CWs.

In treatment wetlands, surfaces on which nitrifying micro-organisms attach include litter, suspended particles, macrophytes and algae all of which interact with the flowing wastewater [199]. The control and *Miscanthidium*-based treatment wetlands were often characterized by algae growth which probably maintained higher populations of nitrifiers in suspension relative to papyrus CWs where the shading effect limited growth of algae. This observation therefore might explain the lower nitrification activity of the water phase in papyrus CWs.

Furthermore, the significantly higher activity detected in peat compared to the water column in all treatments was due to attachment and sedimentation of nitrifiers with suspended particles and/or plant litter [44, 101, 196, 199]. Nitrifying micro-organisms have an obligate requirement for oxygen and inhibition occurs under anoxic conditions [21, 126, 158, 219]. Therefore, in addition settlement with particulate matter, the relatively high DO (above 0.5 mg/L) in *Miscanthidium* and unplanted control treatments supported their metabolic requirements [21], thus explaining the high nitrification activity detected in peat.

The two macrophyte species have differing growth and root development properties [Paper III], and hence different abilities to maintain an oxygen supply to their roots in order to create a locally aerobic environment [120, 203, 220]. Using specific-activity values for various species of *Nitrosomonas* (0.023 pmol of $\text{NO}_2\text{-N}$ produced/cell/h; Belser and Mays, [221]) and the potential nitrification activities measured for the different root mat phases, calculations showed that papyrus root mats harbored $1.7 \times 10^8 \pm 6.3 \times 10^7$ cells/g DW compared to $2.8 \times 10^9 \pm 1.4 \times 10^8$ cells/g DW thriving in the *Miscanthidium* root mat. Furthermore, by considering the average below ground biomass of papyrus (1227 ± 147 g DW/m²) and *Miscanthidium* (425 ± 190 g DW/m²; Fig. 21), nitrifying bacteria in the papyrus root mat ($2.1 \times 10^{11} \pm 9.3 \times 10^9$ cells/m²) appeared to be significantly lower (*p*

= 0.000) than those in the root mat of *Miscanthidium* ($1.2 \times 10^{12} \pm 2.7 \times 10^{10}$ cells/m²). This implies that the high biomass productivity (see Figure 20) and photosynthetic activity of papyrus accompanied by the larger root surface area [Paper III] provided more oxygen and attachment sites for the proliferation of heterotrophic bacteria in the papyrus root mat than that of *Miscanthidium*.

In addition, the dense vegetation cover of papyrus limited atmospheric aeration and oxygen production by algal photosynthesis. Furthermore, the extensively interlaced but permeable root mat of papyrus effectively retained suspended organic particles which provided sufficient substrates for the

proliferation of heterotrophic bacteria [108]. In fact, the BOD removal rate was much higher in papyrus than *Miscanthidium*-based treatments (Figure 23). Therefore, all these observations support the inference that the papyrus root mat experienced a stronger heterotrophic competition for the little available oxygen with autotrophic nitrifying bacteria, and hence explain the dissimilarities in nitrification activities observed between the two macrophyte root mats. The nitrification activity values recorded in this study were higher than values obtained for pilot-scale investigations [Paper III], and therefore represented an important component of biological nitrogen removal in our CWs treatment systems.

7. CONCLUDING REMARKS, RECOMMENDATIONS AND FUTURE STUDIES

- ❑ This work has shown that Nakivubo wetland effects tertiary treatment for a large volume of wastewater from Kampala city. Its high self-purification efficiency for BOD and some metals such as Pb clearly demonstrated its pivotal role in protecting the water quality of Inner Murchison Bay from where the water supply for the city is extracted.
- ❑ The lower self-purification efficiency for nitrogen found in the upper section of this wetland is linked to the high organic matter input that favours heterotrophic growth and activity over nitrification. Besides, the metals detected in the wetland could be detrimental to critical biological processes for nutrient removal, such as nitrification-denitrification.
- ❑ Macrophytes with high nutrient uptake, biomass productivity and surface area for periphyton attachment have been replaced with agricultural crops. This does not only impact negatively on the water quality of Inner Murchison Bay but also poses health risks to Kampala dwellers consuming products from this metal laden wetland.
- ❑ Despite the continuous discharge of substantial numbers of AOB into Nakivubo channel which drains into Nakivubo wetland, a large proportion of these bacteria sedimented in the channel thereby limiting seeding of the wetland. Moreover, wastewater rich in organic matter as that from a slaughter house and other industries abutting Nakivubo channel, and the heterotrophic bacteria from a sewage treatment plant at Bugolobi limited the nitrification process.
- ❑ The study further showed that suspended nitrifiers in the Nakivubo channel equally influenced nitrogen balance as those in surface sediments. However, their influence on transformations in the wetland nitrogen was low compared to that of epiphytic nitrifiers, highlighting the significance of wetland macrophytes in nutrient stripping. Therefore, further modification of the wetland for agricultural and infrastructural development will deteriorate the water quality of Inner Murchison Bay leading to economic costs with unwanted distributional implications.
- ❑ The substrate-free pilot constructed wetland treatment processes developed and used in this study were found to be operationally efficient for high strength domestic wastewater where the main pollutant load is organic in nature. This process excludes the use of expensive substrate media such as gravel yet it enables optimal interaction of wastewater components with plants and micro-organisms. Albeit high BOD, nutrients and indicator organisms were loaded into the system, the removal efficiencies achieved in papyrus-based constructed wetlands were high; yielding effluent quality that meets national discharge limits.
- ❑ The system was capable of maintaining substantial nitrification activity which led to significant N losses. Compared to literature values, high nutrients were sequestered into plant tissues resulting into high biomass productivity. This demonstrated a high potential of this system for biological nutrient removal from wastewaters in resource-scarce tropical countries like Uganda.

□ The progress made during this study lends strong support for the development of integrated low-cost biological systems that utilise natural processes to treat wastewaters of small communities and industries where the main pollutant load is organic in nature (such as foods and beverages, the meat and fish processing industry, slaughterhouses). Such upstream or onsite treatment procedures will not only minimize environmental degradation but will also protect downstream end-users of the waters from pollution related health hazards.

Therefore, further evaluation of the two macrophytes in a polyculture system to establish their relative competitiveness in nature as well as their complementary roles in wastewater treatment remains to be done at pilot small-scale and field application levels.

□ In order to protect the residents of Kampala from contracting metal-related medical disorders, a thorough quantification of the metals in the water, sediments, and food crops cultivated in Nakivubo wetland is part of our next research focus.

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