NUTRIENTS REMOVAL BY FLOATING MACROPHYTES (*Lemna minor* and *Azolla pinnata*) FROM EGERTON UNIVERSITY GENERATED EFFLUENT, KENYA

FELIX MUVEA BONIFACE

A thesis submitted to the Graduate School in partial fulfillment for the requirements of the Master of Science degree in Environmental Science of Egerton University

EGERTON UNIVERSITY

NOVEMBER 2019

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted or presented for examination in any other university, either in part or as a whole.

Signature ………………………. Date …………………………….

Felix Muvea Boniface

NM12/14336/15

Recommendation

This thesis has been written under our supervision and submitted for examination with our approval.

Signature ……………………….. Date: ……………………....

Prof. George M. Ogendi (PhD)

Department of Environmental Science

Egerton University

Signature ………………………. Date: ……………………………

Dr. Steve Omondi (PhD)

Department of Biological Sciences

Egerton University

COPYRIGHT

DEDICATION

This work is dedicated to my loving wife Emmaculate Kalee, my daughter Madeleine Kavuu, my parents Boniface Nzwii and Jane Kavuu, my brothers Nicholas Nzwii and Joseph Kivuva and my sisters Catherine Mwende, Angela Kanini, Winfred Nthenya and Damian Mwikali.

ACKNOWLEDGEMENT

Most sincerely, I thank the Almighty God for the grace He has accorded me throughout the study. My sincere appreciation goes to Egerton University Graduate School, Faculty of Environment and Resource Development, Department of environmental Science for according me the opportunity to pursue my graduate studies. I wish to express my sincere gratitude to my research supervisors Prof. George M. Ogendi of the Department of Environmental Science, and Dr. Steve O. Omondi of the Department of Biological Sciences of Egerton University for their professional guidance, advice and tireless support all along through a thorough conceptualization of the research proposal and scientific content herein. I reserve my deepest gratitude to them. I appreciate exceedingly the contribution by my field assistant (Priscilla Wangari Mureithi) and her contribution to my work and advice during the study period. Special thanks to Biological Science department of Egerton University; the chairman of the Biological Sciences Department, Dr. Steve Omondi and Mr. Mungai a technologist for their advice and for allowing me to use their lab and equipment whenever needed. Your kindness is highly appreciated. I am thankful to Saeed Hassan for counsel and direction with the data analysis process. My special thanks are also extended to Peter Murunga whom greatly helped me when my computer developed problems and made sure that it served me well all the time. I also recognize the contribution of my fellow students especially my classmates whose contribution was significant to my work. I would wish to acknowledge the assistance received from my friends who spared their time during my interaction with them. Last but not least, I am gratefully indebted to my parents and family who provided both moral and financial support not forgetting my wife and daughter for their love, prayers and care for me. God reward you all immensely.

ABSTRACT

Floating macrophytes have the capacity to improve wastewater quality by absorbing nutrients thus purifying pre-treated wastewater and have been found to be more appropriate for effluent in developing countries. This is due to their effective and extensive root system and moderate capital investment required in their engagement in wastewater treatment. They are especially appropriate for tropical countries due to the warm climate experienced in these regions which support rapid plant growth and microbial activities that enhance uptake of nutrients and other pollutants from the wastewater. Being dependent on uptake of nutrients by the wetlands vegetation, the efficiency of wastewater treatment systems in wastewater purification is therefore influenced by the type of flora growing in them and how effective these flora are in the uptake of nutrients from the wastewater. Many of macrophytes are normally found growing together in a single wetland and the nutrient removal efficiency of individual plant species has not been effectively determined yet this is important in promotion of the effluent treatment technology. This study assessed the nutrient removal efficiency of two floating macrophytes (*Lemna minor* and *Azolla pinnata*) in Egerton University generated effluent. The key research question to be answered was; was there a significant variation in nutrients concentration in the wastewater after treatment by the selected floating macrophytes? In answering this question, randomized complete block design was used. In all cases, American Public Health Association standard protocols for sampling, sample processing and analysis were used. The data generated was analyzed using both descriptive and inferential statistics. The nutrients removal efficiency was determined using one-way ANOVA and Tukey test. In all determinations, the level of confidence was 0.05. The wastewater physicochemical parameters varied slightly during the study period. Increase in biomass for the selected macrophytes was noted suggesting that there was significant uptake of nutrients (*Azolla pinnata*: F= 621.713, P= 0.00; *Lemna minor*: F= 786.494, P= 0.00). Decreases in ammonia, total nitrogen, total phosphorous and soluble reactive phosphorous concentration were noted whereas an increase in nitrates and nitrites concentration was observed. *Azolla pinnata* proved to be better than *Lemna minor* in the uptake of soluble reactive phosphorous where the nutrient uptake was statistically significant (F= 35.183, P= 0.044). Based on the study results we can conclude that *Azolla pinnata* and *Lemna minor* are efficient in nutrient removal and therefore good in wastewater treatment. Thus we recommend increasing the population of the two floating macrophytes in the treatment of effluents especially within the tropics.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS/ACRONYMS

ANOVA	Analysis Of Variance
APHA	American Public Health Association
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
CW	Constructed Wetland
DO	Dissolved Oxygen
EC	Electrical Conductivity
EHS	Environmental Health and Safety
EPA	Environmental Protection Agency
pH	Hydrogen Potential
PSU	Practical Salinity Unit
SRP	Soluble Reactive Phosphorous
SSF	Sub-Surface Flow
TDS	Total Dissolved Solids
TN	Total Nitrogen
TP	Total Phosphorous
TS	Total Solids
TSS	Total Suspended Solids
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background information

Water pollution is a major problem throughout the world (Dhote and Dixit, 2009) with most of the world's cities not only facing the challenge of supplying adequate sanitation facilities to their residents but also water resources that are not contaminated (Leong *et al*., 2008). Pollutants found in wastewater can be divided into two broad categories: biological and chemical, with the major chemical pollutants being nitrogen, phosphorus, heavy metals, detergents, pesticides and hydrocarbons (Akpor, 2011). The discharge of untreated wastewater is a major contributor to deteriorating health conditions and pollution of nearby water bodies. In a rapidly urbanizing world, poor environmental sanitation has emerged as a major challenge, threatening the health and livelihoods particularly of the poor (Spinosa, 2011; Bick, *et al*., 2012).

According to Akpor *et al*, (2008), many of the waterborne microorganisms that cause human disease come from animal and human fecal wastes. These contain a wide variety of viruses, bacteria, and protozoa that may get washed into drinking water supplies or receiving water bodies. Treatment of wastewater is an issue of environmental concern that has plagued man for many years. During the past 20 years, considerable interest has been expressed in the potential use of a variety of natural biological systems to help purify water in a controlled manner (Liu, 2007). These natural biological treatment systems include various forms of ponds, land treatment and wetlands systems (Vymazal, 2010). Effluent treatment systems present a concept aimed at combating deterioration of water resources and acts as a buffer between wastewater and receiving water bodies (Bick *et al*., 2012). Many biological processes have been extensively investigated in wastewater treatment over the past decades, mostly investigations have been concentrated on bacteria (Momba, 2010). Previously, many treatment plants were designed to remove nutrients by the addition of chemicals. The nutrients removal from wastewater might be a very expansive process. Recently, the use of various aquatic macrophytes has been suggested for this purpose since chemical treatment is known to increase sludge volume and often results in sludge with poor settling and dewatering characteristics and the depression of the pH (Forni and Nicolai, 2001) and that is why biological treatment has been advocated for in the last few decades. Though some data have been generated on the uptake of nutrients by the floating macrophytes, the data has been scanty. Despite this paucity of information, the macrophytes are still recognized as an important contributor to solving pollution problems associated with nutrients currently facing water bodies. The growth rates of the macrophytes are differently influenced by the presence of nitrate and phosphate nutrients present in the wastewater under treatment. Use of plants in purification process is called phytoremediation, and it has gained attention as a suitable option for the treatment of wastewater (Daud *et al*., 2018). During the last two decades, phytoremediation has gained prominence and the discovery of plants with high nutrients uptake has made it more promising because of their ability to take up high amount of nutrients for their growth (Wu *et al*., 2015). If well treated, the wastewater can be reused for productive purposes. Most of the macrophytes meant for wastewater treatment are aesthetically pleasing and provide beautiful scenery for human enjoyment (Meyer *et al*., 2015). While nutrients are converted into biomass by wetland plants which take place during the growth of the plants, the plants may release nutrients back in to the system when they die and decompose in the treatment system (Verdegem, 2013).

Macrophytes are effective in the reduction of nutrients, mainly the nitrates and phosphates from wastewaters through their uptake for the buildup of their biomass (Kassa and Mengistou, 2014); Mitsch *et al*., 2001). Other than nutrient uptake by the wetland vegetation, microbial transformation that include immobilization and denitrification of nutrients also occur in the wetlands and is mediated by macrophytes (Zhang, 2012). Plants are an important component of wetland systems (Kalff, 2002). Plant efficiency in promoting effluent treatment depends on several factors: type of the treatment technology, quality and quantity of the wastewater loads (Shelef *et al*., 2013), plant species and their combinations, climate, medium type, and plant management, such as harvesting regime (Stottmeister *et al*., 2003). Also the nutrients removal efficiency by the plants is controlled by the time spent by contaminants into vegetated zones (Fabris, 2013). Moreover, macrophyes' contribution to the wastewater treatment is a complex of various functions that are rarely studied. An ecologically-friendly system of reducing the amounts of the nutrients released to water bodies to the given thresholds is desirable. There was need to evaluate and compare the efficiency of different macrophytes in treating the wastewater generated with respect to the macrophyte type that dominated the treatment system. Unfortunately, most studies have not explored mechanisms, and therefore most of my information is restricted to effectiveness describing the impact of the presence of *Lemna minor* and *Azolla pinnata* on water quality. Results reported in most studies indicate that mixed vegetation is more effective at nutrients removal than single-species vegetation (Fraser *et al*., 2004). But information on the driving forces leading to this conclusion is scarce. The concentration of the nutrients in the effluent over time needed to be determined as well as determining the amount of nutrients a single macrophyte can take up in a determined period of time. By utilizing nitrates and phosphates, and other nutrients, plants can reduce the concentrations of elements that would otherwise be considered pollutants. Plants can also accumulate phytotoxic elements, such as heavy metals, in vacuolar or granular compartments. Thus, phytoremediation may be an important role for plants in wastewater treatment. This study therefore assessed the nutrient removal efficiency of two floating macrophytes (*Azolla pinnata and Lemna minor*) in effluent generated at Egerton University. This would do away with the limits on the ability of decision-makers (policy-makers) from making decisions on the best macrophyte to use in effluent treatment. Macrophytes take up nutrients to build up their biomass, but how faster or slow they grow has never been assessed, this study also determined the macrophyte that grows faster and able to double its biomass faster between *Lemna minor* and *Azolla pinnata*. Due to notable increase in human population and industrial development which has exerted pressure on water bodies and the release of excessive nutrients to them, the policy makers will come up with the decision on the best macrophytes to use in phytoremediation.

1.2 Statement of the problem

In the period 1997 to 2007, Egerton University wastewater treatment lagoons experienced an increase in wastewater load due to an increase in staff and student population. To counter the likely negative effects on human health, a constructed wetland was constructed in 2007 to act as a tertiary wastewater treatment unit for polishing the wastewater. The wastewater, if not well treated, can expose the local downstream communities to water-related disease pathogens and heavy nutrients pollution. Inadequately treated wastewater threatens the Njoro River ecosystem downstream of the discharge point as well as Lake Nakuru ecosystem where the river discharges its waters. Lake Nakuru is an important biodiversity conservation area, being a Ramsar site that hosts a large number of aquatic and terrestrial wildlife including the lesser flamingoes (*Phoeniconaias minor*) and their food *Arthrospira fusiformis*. The vegetation in the treatment system changes between the emergent macrophytes and the floating macrophytes based on their growth requirements and the conditions prevailing in the wetland. The nutrient removal efficiencies of individual macrophytes for this wetland are not known and yet they are important in making improvements on their use and promotion. Thus there was need for this study meant to establish how efficient the different types of macrophytes were in nutrient removal from the university generated effluent. Previous studies have focused on the removal of heavy metals and nutrients by the mixed macrophytes and the changes in the physical and chemical characteristics of the wastewater after treatment in the wetland. Currently, the wastewater treatment system at Egerton University has few rooted and emergent macrophytes and a dominance of two floating macrophytes (*Lemna minor* and *Azolla pinnata*). It is against this background that a study was conceived to understand the nutrient removal efficiencies of the two dominant floating macrophytes.

1.3 Research objectives

1.3.1 Broad objective

The main objective of this study was to assess nutrients removal efficiency by the floating macrophytes (*Lemna minor* and *Azolla pinnata*) to identify the best macrophyte for nutrients removal from wastewater to improve on wastewater pollution removal.

1.3.2 Specific objectives

The specific objectives were:

i). To determine the physico-chemical parameters of the wastewater in an experimental setup.

ii). To assess the temporal variation in nutrients concentration in the wastewater containing *Lemna minor, Azolla pinnata* and the control in an experimental setup.

iii). To assess the temporal variation in the biomass of *Lemna minor* and *Azolla pinnata* grown in the wastewater in an experimental setup.

iv). To compare the nutrients removal efficiencies between the two floating macrophytes (*Lemna minor* and *Azolla pinnata*) grown in an experimental setup.

1.4 Research hypotheses

i). There was no significant variation in the physico-chemical water parameters in the wastewater experimental setup.

ii). There was no significant variation in the temporal concentration of nutrients in the wastewater experimental setup containing *Lemna minor*, *Azolla pinnata* and the control.

iii). There was no significant temporal variation in the biomass of *Lemna minor* and *Azolla pinnata* grown in the wastewater in the experimental unit.

iv). There was no significant variation in the removal of nutrients from the wastewater in the experimental unit between the two floating macrophytes (*Lemna minor* and *Azolla pinnata*).

1.5 Justification of the study

Egerton University releases its wastewater into River Njoro, an important source of water for the local population living along the river as well as Lake Nakuru. Lake Nakuru is a very important conservation area, being a Ramsar site and home to many aquatic birds, especially the lesser flamingos. Njoro River is the only permanent river that flows into the lake even though its water is used for watering livestock, irrigation, and other domestic purposes. Despite all these positive attributes, the river’s water quality had been impaired due to the rapid urbanization along its catchment, increased disposal of wastes into the river from the households and wastewater from Egerton University. The growth of the macrophytes is driven by the nutrients in the water and therefore where they grow extensively would suggest that there could be high rate of pollution removal from the wastewater hence wastewater treatment. This study was therefore important due to its contribution towards the achievement of Kenya’s vision 2030 on the social pillar for the Water and Sanitation which is to ensure that improved water and sanitation are available and accessible to all in both rural and urban areas hence improving health status of the citizens. It is also contributing towards the achievement of some of the targets of sustainable development goal (SDG) six on access to improved drinking water sources and contribute to preventing water and sanitation-related diarrheal diseases as well as contributing to reduction of the percentage of wastewater released in to the environment without pollution removal. This study identified the ideal vegetation between *Lemna minor* and *Azolla pinnata* for use in wastewater treatment so as to protect, preserve and conserve the environment and specifically the wetlands. This information is useful to Egerton University, County governments and other institutions that use CWs in wastewater treatment. This information will also be useful for policy/decision makers on the use and promotion of the constructed wetlands technologies for improved wastewater treatment and protection of human health.

1.6 Operationalization of terms

Biofilm – Is any group of microorganisms in which cells stick to each other and often also to a surface.

Lagoons – are ponds designed to receive, hold, and treat wastewater for a predetermined period of time.

Macrophytes – An aquatic plant large enough to be seen by the naked eye.

Media filter – is the gravel bed at the end of the constructed wetland cells.

Nutrient – A substance that provides nourishment to plants essential for the maintenance of life and for their growth.

Physicochemical parameters – are both physical and chemical properties of the wastewater.

Phytoremediation – a process of decontaminating soil or water by using plants and trees to absorb or break down pollutants.

Polish – is the tertiary and final effluent wastewater treatment stage before the wastewater can eventually be discharged into natural water bodies.

Removal efficiency – a percentage that represents the amount of nutrients removed or destroyed in wastewater treatment system by macrophytes relative to the amount of nutrients that entered the system.

Senescence – the condition or process of deterioration with age; loss of a cell's power of division and growth.

Total solids – is a measurement that includes the combination of total dissolved solids and total suspended solids.

Total Suspended Solids – is the dry-weight of suspended particles that are not dissolved, in a sample of water that can be trapped by a filter that is analyzed using a filtration apparatus.

Wastewater – is used water from any combination of domestic, industrial, commercial or agricultural activities, surface runoff or storm water, and any sewer inflow or sewer infiltration.

Wastewater treatment – is a process used to convert wastewater which is no longer needed or suitable for its most recent use into an effluent that can be either returned to the water cycle with minimal environmental issues or reused.

1.7 Scope/Limitations/Assumptions

1.7.1 Scope

This study focused only on the physico-chemical characteristics of wastewater, nutrients (total nitrogen, ammonia, nitrates, nitrites, total phosphorous and soluble reactive phosphorous) and floating macrophytes (*Lemna minor* and *Azolla pinnata*). This study was also limited to three months; from January to end of March 2018 based on comparing wastewater nutrients variations over time.

1.7.2 Limitations of the study

Drastic changes in the physico-chemical parameters of the wastewater in the experimental setup thus affecting the action of the macrophytes (*Lemna minor* and *Azolla pinnata*) on the nutrients. This was overcome by making sure that the wastewater was well aerated and transparent buckets were used to allow maximum light penetration into the wastewater.

1.7.3 Assumptions

i). That the water quality would not change drastically to the point of affecting the growth and performance of the floating macrophytes in the buckets.

ii). That there was to be continuous aeration of the wastewater in the buckets to enable the floating macrophytes perform optimally.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Wastewater treatment refers to the process of removing pollutants from wastewater. It includes physical, chemical, and biological processes of removing organic, inorganic and biological pollutants. Wastewater or sewage is the byproduct of many uses of water. Household uses include showering, dishwashing, laundry and, of course, flushing the toilet. Again, wastewater from industries is a byproduct of many purposes including processing, production and cleaning or rinsing of parts. After the water has been used, it enters the wastewater stream, and it flows to the wastewater treatment plant. The typical composition of municipal wastewater (after pretreatment) most often treated in CWs contains suspended solids, organic matter, and in some instances, nutrients (especially phosphorous and nitrogen forms) and heavy metals. Domestic wastewater typically contains 200 mg of suspended solids, 200mg biochemical oxygen demands, 35mg nitrogen, and 7mg phosphorus per liter (Admasu, 2007). Depending on its source, wastewater has contents that may include organic substances such as carbon, nitrogen, phosphorus, sulphur in organic matter which needs to be broken down by oxidation (Bani, 2011) Nutrients such as nitrogen and phosphorous from wastewater enrich water bodies and render it eutrophic leading to the growth of algae and other aquatic plants. (Droste and Gehr, 2018). Contaminants in wastewater are categorized into physical, chemical and biological and they need to be removed to protect the environment and protect public health (Corcoran, 2010). If left untreated, these pollutants would negatively affect aquatic ecosystems.

2.2 Physico-Chemical Parameters of Wastewater

The use to which a given water may be put is determined by its physico-chemical characteristics. Selection of parameters for testing of water is solely depended on the purpose for which the water is to be used. Water does contain different types of floating, dissolved, suspended and microbiological impurities. Increase in biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) and total suspended solids (TSS) in aquatic environment is caused by high levels of organic pollutants. Some wastewater, especially those from industries or laboratories may also have toxic metals such as cadmium, chromium, nickel and lead while those from domestic sources are likely to be contaminated with faecal coliform and hence make such water unsuitable for many human activities (Raji *et al*., 2015). Other physical tests commonly performed include temperature state, color, odour and turbidity while chemical tests include pH, dissolved oxygen content, alkalinity and hardness. These physico-chemical parameters are tested regularly for monitoring the quality of water (Chapman, 1996).

2.2.1 Temperature

Temperature is an important parameter that affects certain chemical and biological reactions. The temperature state in water varies and depends upon the season and time of the day. The water discharged from domestic and industrial sources having a high temperature above 40°C may adversely affect the growth of aquatic organisms (Sahu and Chaudhari, 2015).Water temperature also affects fish growth, reproduction and immunity. Drastic temperature changes can be fatal to aquatic life (Chapman, 1996). In the case of the floating macrophytes, optimum temperature for maximum growth lies between 17.5°C and 30°C (Culley *et al*., 1981). Although some species can tolerate low temperatures, growth rate declines at lower temperatures generally. Below 17°C some duckweeds show a decreasing rate of growth (Culley *et al*., 1981). Most species of the floating macrophytes seem to die if the water temperature rises above 40°C. This suggests that the floating macrophytes will remove nutrients efficiently at temperatures between 17.5°C and 30°C in wastewater treatment systems. Again, macrophytes are sensitive to temperature and shows no growth and pollutant removal at a temperature below 10°C (Shah *et al*., 2014). Almost all the aquatic species cease to survive at this temperature. As the growth of species is negligible at temperature below 10°C (Sipaúba-Tavares *et al*., 2002) therefore, there can be no uptake of nutrients by the aquatic plants.

2.2.2 pH

pH is one of the important abiotic factors that serves as an index of pollution. The wide variation in the pH value of effluent can affect the rate of biological reaction and survival of microorganisms (Samuel, 2011). pH can also be used in determining the corrosive nature of water since a number of chemical reactions are dependent on pH state of the medium. The lower the pH value the higher is the corrosive nature of water (Smith *et al*., 1999). Reduced rate of photosynthetic activity, assimilation of carbon dioxide and bicarbonates are responsible for increase in pH in wastewater (Samuel, 2011). Various factors which bring about changes in the pH of water are organic material present in the water and plant growth (Sivasanthi and Pandian, 2012). Decomposed material releases carbon dioxide, which in turn lowers the pH levels in the water. Duckweeds are generally considered to have a wide range of tolerance for pH. They survive well from pH 5 to 9 (Culley *et al*., 1981). However, pH tolerance limits of the various species differ. Körner *et al*, (2001) noted that duckweed display optimum growth in a medium of pH 5 to 7. Generally, duckweeds grow best over the pH 6.5 to 7.5 range.

2.2.3 Electrical Conductivity (EC)

Electrical Conductivity (EC) is a measure of ions present in the aquatic ecosystem and is measured in micro- or millisiemens per centimeter (uS/cm or mS/cm). The conductivity of industrial wastewaters, treatment plant effluents and polluted water is due to the presence of ionic solutes (Brix, 1994). Ions that cause conductivity are hydrogen (H^+), hydroxide (OH^-), chloride, sodium, potassium. Conductivity shows significant correlation with a number of parameters such as temperature, pH value, alkalinity, total hardness, calcium, total solids, total dissolved solids, chemical oxygen demand, and concentration of water. The hydrogen and hydroxide ion contribution to conductivity is a function of pH (Levlin, 2010).The main processes that reduce conductivity in wastewater treatment are biological nutrient removal. Conductivity is a general indicator of water quality, especially a function of the amount of dissolved salt, and can be used to monitor processes in the wastewater treatment that causes changes in total salt concentration and thus changes the conductivity (Levlin, 2010). Many regulatory bodies require that industries and similar enterprises which discharges effluent measure conductivity and that it is not allowed to be higher than the given threshold. High values of electrical conductivity show that inorganic ions are present in reasonable concentrations in the wastewater, such ions have major influence on the conductivity of water. The more ions that are present, the higher the conductivity of water (Uwidia *et al*., 2013) likewise, the fewer ions that are in the water, the less conductive it is. Electrical conductivity of water depends on the water temperature: the higher the temperature, the higher the electrical conductivity would be. The electrical conductivity of water increases by 2-3% for an increase of 1 degree Celsius of water temperature. A sudden increase or decrease in conductivity in water can indicate pollution. Agricultural runoff or a sewage will increase conductivity due to the additional chloride, phosphate and nitrate ions (Environmental, 2014). Absence of aquatic plants in wastewater treatment records high electrical conductivity as compared to the presence of aquatic plants that will reveal reduced EC as well as with dilution of wastewater (Nair and Kani, 2016). The range of EC mostly depends on the concentration of various Types of soluble salts in wastewater. The decrease in EC during phytoremediation indicates the heavy uptake of these salts by root system of the aquatic plants. Nair and Kani, (2016) noted that *Azolla* can be better used for reducing the turbidity of dairy wastewater compared to water hyacinth. The roots of most aquatic plants are capable of retaining both coarse and fine particulate organic materials present in the water on which they are growing. This is mainly achieved through the electrical charges associated with the root hairs, which reacts with the opposite charges on colloidal particles (Dipu Sukumaran 2013).

2.2.4 Alkalinity

The alkalinity of water is a measure of its capacity to neutralize acids. It also refers to the buffering capacity, or the capacity to resist a change in pH. For wastewater operations, alkalinity is measured and reported in terms of equivalent calcium carbonate ($CaCO_3$). Its values is however strongly dependent on the carbonate and bicarbonate content of the given water. It is common practice to express alkalinity measured to a certain pH. For wastewater, the measurement is total alkalinity which is measured to a pH of 4.5 SU (Gibson and Maniocha, 2004). Even though pH and alkalinity are related, there are distinct differences between these two parameters, and how they affect plant operations in wastewater treatment. During the course of treatment, plants consume a variety of chemicals. In some treatment plants, an alkali is used to provide the alkalinity required to maintain effective biological activity and for pH control. Alkalinity, pH and hardness affect the toxicity of many substances in water (Luklema, 1969). At a pH below 5 macrophytes performance in pollutant removal in almost zero. This is mainly due to highly acidic nature of the wastewater. On the other hand when pH gradually increase to 7.5, performance of the aquatic plants improves and by further increase in pH (alkalinity) again start retarding macrophytes performance in pollutant removal. At a pH of 10 (at high Alkalinity) the performance of macrophytes will again decrease to zero. Therefore, a high alkalinity is not suitable for macrophytes performance (Shah *et al*., 2014). The pH values directly influences alkalinity and bicarbonate dominance in any wastewater treatment system. Alkalinity of a given wastewater can vary over the pollutant removal period by the macrophytes and is directly associated with the pH. High alkalinity in any wastewater can again be associated with low free CO_2 available in that wastewater, which obviates changes in pH buffering of the medium (Sipaúba-Tavares *et al*., 2002). According to Maine *et al*. (2007), water with high alkalinity might limit the growth of macrophytes hence impairing the macrophytes' performance in wastewater treatment. High alkaline in aquatic environment is related to high presence of calcium compounds, because according to Mayes *et al*. (2009) the hydrolysis of calcium compounds produces the hydroxyl ion, which elevates solution's pH and releases Calcium ions in in the water.

2.2.5 Dissolved Oxygen (DO)

Dissolved oxygen is one of the most important parameters in water quality assessment as it is an index of physical and biological processes in water. Amount of dissolved oxygen is essential to maintain the variety of forms of biological life in water, and the effect of water discharge into a water body is largely determined by oxygen balance within the system, non-polluted surface water remaining normally saturated with dissolved oxygen (Saxena and Madan, 2012). Dissolved oxygen enters water through the air or as a photosynthesis byproduct. From the air, oxygen can slowly diffuse across the water’s surface from the surrounding atmosphere, or be mixed in quickly through aeration, whether natural or man-made (Wilén, 2010). It can be rapidly removed from the water by discharge of oxygen demanding waste. Inorganic reducing agents such as hydrogen sulfates, ammonia nitrites, and ferrous ions and certain available oxidizable substance also tend to decrease the oxygen in water, (Zhang et al., 2009).).

2.2.6 Biochemical Oxygen Demand (BOD)

Biological oxygen demand (BOD) is the amount of oxygen required by microorganism while stabilizing biological decomposable organic matter. The biological oxidation is a very slow process during which organic pollutants are oxidized by certain microorganism into carbon dioxide and water. Hence reduction in dissolved oxygen value gives a measure of BOD (APHA 2005). The biological oxygen demand is an important parameter that indicates the magnitude of water pollution, by the oxidizable organic matter and the oxygen used to oxidize inorganic materials such as sulfides and ferrous ions (Maruthi and Rao, 2011). The main focus of wastewater treatment plants is to reduce the BOD in the effluent discharged to the treatment system. Wastewater treatment plants are designed to function as bacteria farms, where bacteria degrade organic waste while using oxygen. If effluent with high BOD levels is discharged into a water body, it will accelerate bacterial growth in the water and consume the oxygen in the water. The oxygen may diminish to levels that are lethal for the survival of aquatic organisms. As the water re-aerates due to atmospheric mixing and as algal and aquatic plants photosynthesis adds oxygen to the water, the oxygen levels will slowly increase. The reduction in pH favors microbial action to decrease biological oxygen demand in the wastewater (Dipu Sukumaran, 2011). In wastewater treatment, BOD reduction is observed in the presence of the aquatic plants. Attached and suspended microbial growth is responsible for removal of soluble BOD_5. So the BOD value can be reduced a lot by treating wastewater with macrophytes especially *Azolla* (Mesania Rizwana 2014). However, the growth and nutrient removal potential of the macrophytes are affected by many other factors such as temperature, water salinity, and physiological limitations of the plants (Nair and kani, 2016).

2.2.7 Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is another measure of organic material contamination in water as it indicates the amount of dissolved oxygen required to cause chemical oxidation of the organic material in water completely to CO_2, H_2O and NH_3. Both BOD and COD are key indicators of the environmental health of a surface water supply. They are commonly used in wastewater treatment but rarely in general water treatment (Wilén, 2010). According to Siddiqui and Waseem, (2012) COD is a test used to measure pollution of domestic and industrial waste in terms of quantity of oxygen required for oxidation of organic matter to produce carbon dioxide and water. COD is an important water quality parameter because, similar to BOD, it provides an index to assess the effect that discharged wastewater will have on the receiving environment. Higher COD levels means presence of higher amount of oxidizable organic matter in the sample, which will reduce dissolved oxygen (DO) levels. A reduction in DO can lead to anaerobic conditions, which is deleterious to higher aquatic life forms (Yao, 2014). Usually, for industrial wastewaters, COD is higher than BOD because many organic substances, which are difficult to oxidise biologically can be oxidised chemically. If the COD value is much bigger than the BOD value, the organic compounds in wastewater are slowly biodegradable. The conjugation of BOD test, with COD test is helpful in indication of toxic conditions and the presence of biological resistance (Saxena and Madan, 2012). In wastewater treatment, the additional surface area provided by the roots of the aquatic plants and the consequent increase in microbial activity that the plants provides, enhances COD and BOD reduction (Nair and kani, 2016). Van de Moortel *et al*. (2010) observed a 53% COD reduction in municipal wastewater treatment with macrophytes, compared to 33% in the control for the same volume without the macrophytes. The growth of macrophytes in wastewater treatment shows high performance in removal of COD mainly because of well-developed root system of the plants. Similarly a major part of the degradation of COD in wastewater with macrophytes is attributed to micro-organisms which establishes a symbiotic relationship with the aquatic plants in the wastewater (Shah *et al*., 2015).

2.2.8 Total Dissolved Solids (TDS)

Total dissolved solids (TDS) is a measure of the concentration of dissolved constituents in water, which commonly include carbonate, bicarbonate, chloride, sulphate, phosphate, nitrate, calcium, magnesium, sodium, organic ions, and other ions. In general, the total dissolved solids concentration is the sum of the cations (positively charged ions) and anions (negatively charged ions) in the water. Therefore, the total dissolved solids test provides a quantitative measure of the amount of dissolved ions, but does not tell us the specific types of ions in the water or the relationships between ions. In addition, the test does not provide insight into specific water quality issues, such as hardness, saltiness or corrosiveness. The total dissolved solids test is used as an indicator to determine the general quality of the water. A certain concentration of ions in water is necessary to provide nutrients essential to support aquatic life. Changes in TDS concentrations can be harmful to aquatic organisms because they affect the density of water. Excessive TDS can reduce water clarity, hinder photosynthesis, and lead to increased water temperatures (WHO, 1993). Decrease in TDS reflects improvement in quality of wastewater due to phytoremediation. The reduction of TDS in wastewater treatment is due to the retaining of coarse and fine particulate organic materials present in the treatment system supporting the growth of the aquatic plants' root system (Yadav *et al*., 2011).

2.2.9 Total Suspended Solids (TSS)

Total suspended solids (TSS) include all particles suspended in water that will not pass through a filter. Abundant suspended solids such as clay and silt, fine particles of organic matter, inorganic particulates (such as iron), soluble coloured compounds and phytoplankton can result in: Decreased light penetration in water, reducing photosynthesis of aquatic plants, decreased water depth due to sediment build up, smothering of aquatic vegetation, habitat and food, smothering of macro and micro-organisms, larva, eggs and the clogging of fish gills, reduced efficiency of predation by visual hunters, and increased absorption of heat by the water which results in lowering dissolved oxygen, facilitating parasite and disease growth and increasing the toxicity of ammonia. Elevated TSS levels can be the leading cause of blockages occurring throughout distribution and drainage systems in subsurface flow wetlands because of the tendency of porous media to clog (U.S.EPA, 1998). Wastewater treatment in low wetland water velocities and appropriate composition of influent solids, total suspended solids will settle from the water column within the wetland. Sediment resuspension not only releases pollutants from the sediments, but also increases the turbidity and reduces light penetration (Yadav *et al*., 2011). The removal of total suspended solids is an important function of natural and artificial treatment systems. In natural and artificial treatment with low water velocity combined with the presence of macrophyte shoots and litter provides an ideal environment for the settling and interception of solids (Bunting, 2013). Many pollutants, such as metals and organic compounds, are associated with suspended solids as they adsorb strongly to particulates (Kadlec and Li, 1990). A study by Ijaz *et al*. (2015) displayed that macrophytes can significantly reduce total suspended solids in the water column. This reduction is caused by the physical entrapment of particulate matter within the roots and the eventual settling within the benthic sediment.

2.3 Nutrients concentration

Nitrogen and phosphorous are two main nutrients found in wastewater in high quantities. Nitrogen is mostly found in the form of nitrates, nitrites and ammonia. Some of the problems associated with high levels of nitrates in drinking water or surface water are: serious health effects in humans like interfering with the ability of red blood cells to transport oxygen, make infants turn bluish and to have difficulty in breathing as well as eutrophication in lakes and ponds. High levels of Phosphorus in surface water can also cause eutrophication in lakes. Wastewater from agriculture and sewage contains high levels of these nutrients and effluent treatment systems are capable of reducing their levels (Khanijo, 2002).

2.3.1 Nitrogen compounds

Nitrogen is essential for living organisms as an important constituent of proteins, including genetic material. Plants and micro-organisms convert inorganic nitrogen to organic forms. In the environment, inorganic nitrogen occurs in a range of oxidation states as nitrate (NO_3^-) and nitrite (NO_2^-), the ammonium ion (NH_4^+) and molecular nitrogen (N_2). It undergoes biological and non-biological transformations in the environment as part of the nitrogen cycle. The major non-biological processes involve phase transformations such as volatilization, sorption and sedimentation (Bastviken, 2006). The biological transformations consist of: Assimilation of inorganic forms (ammonia and nitrate) by plants and micro-organisms to form organic nitrogen. For example amino acids, Reduction of nitrogen gas to ammonia and organic nitrogen by micro-organisms, Complex heterotrophic conversions from one organism to another, Oxidation of ammonia to nitrate and nitrite (nitrification), Ammonification of organic nitrogen to produce ammonia during decomposition of organic matter, and Bacterial reduction of nitrate to nitrous oxide (N_2O) and molecular nitrogen (N_2) under anoxic conditions (denitrification).

The process of nitrogen removal by bacterial conversions in wetlands follows a series of reactions as in a nitrogen cycle. The nitrogen cycle has 3 main processes. Ammonification which is the conversion of organic N to NH^{4+}. Nitrification which is a two-step process – conversion of NH^{4+} to Nitrite and conversion of nitrite to nitrate. The third process is denitrification – where nitrates convert to nitrites and conversion of nitrites to organic N (Khanijo, 2002). In general, temperature and sunlight control duckweed growth more than nutrient concentrations in the water. At high temperatures, duckweed can grow rapidly down to trace levels of phosphorus and nitrogen (Culley *et al*., 1981). Aquatic macrophytes play a significant role in maintaining water quality. Their presence may enhance water quality due to their ability to absorb nutrients. *Lemna minor* and *Azolla pinnata* can therefore improve water quality in the wastewater treatment.

2.3.2 Phosphorus compounds

Phosphorus is an essential nutrient for living organisms and exists in water bodies as both dissolved and particulate species. It is generally the limiting nutrient for algal growth and, therefore, controls the primary productivity of a water body. Artificial increases in phosphorous concentration due to human activities are the principal cause of eutrophication of natural waters. In wastewaters, phosphorus occurs mostly as dissolved orthophosphates and polyphosphates, and organically bound phosphates. Changes between these forms occur continuously due to decomposition and synthesis of organically bound forms and oxidised inorganic forms (Khanijo, 2002).

Natural sources of phosphorus are mainly the weathering of phosphorus-bearing rocks and the decomposition of organic matter. Domestic wastewaters (particularly those containing detergents), industrial effluents and fertilizer runoff contribute to elevated levels in surface waters. Phosphorus associated with organic and mineral constituents of sediments in water bodies can also be mobilized by bacteria and released to the water column. Phosphorus is rarely found in high concentrations in freshwaters as it is actively taken up by plants. As a result there can be considerable seasonal fluctuations in concentrations in surface waters. In most natural surface waters, phosphorus concentration ranges from 0.005 to 0.020 mg/l (Brix *et al*., 2001). Concentrations as low as 0.001 mg/l may be found in some pristine waters and as high as 200 mg/l in some enclosed saline waters. Average groundwater levels are about 0.02 mg/l (Brix *et al*., 2001).

As phosphorus is an essential component of the biological cycle in water bodies, it is often included in basic water quality surveys or background monitoring programmes. High concentrations of phosphates can indicate the presence of pollutants and are largely responsible for eutrophic conditions (Karczmarczyk and Renman, 2011).

Poor water quality can have many unpleasant consequences. Phosphorous as a pollutant leads to nuisance algal blooms that yield unpleasant odor and appearance that reduce the aesthetic appeal of water bodies (Hanna, 2008). As algae die and decompose, the process consumes oxygen. Submerged plants without sunlight die, decompose and consume more oxygen. Without enough dissolved oxygen in the water, fish and other organisms suffer and die (Hey *et al*., 2007). This gives a good reason why phosphorous needs to be removed in the wastewater to avoid such problems in the receiving rivers and lakes. Phytodegradation, which is also referred to as phytotransformation entails the destruction of a contaminant through uptake by plants as nutrient. In some cases, certain plants that are used have the ability of taking up toxic compounds, detoxifying and metabolizing them as nutrients (Kiepper, 2013). A typical application of phytodegradation in wastewater is the use of macrophytes to reduce the nutrient content in wastewater. Macrophytes have the ability to assimilate nutrients into their cells (Sha *et al*., 2015). The wastewater pollutants that have reportedly been phytodegraded in wastewater include chlorinated solvents, herbicides, and insecticides and inorganic nutrients (Hanna, 2008).

While the dominant removal processes for nitrogen and phosphorus are different, both nutrients are utilized by wetland biota. Wetland plants take up inorganic nitrogen and phosphorus forms (that is; nitrate, ammonia, and soluble reactive phosphate) through their roots and convert them into biomass. However, this only provides temporary storage of the nutrients. The majority of these assimilated nutrients are released back into the water and soils when plants senescence and decompose. A small amount of the nutrients (10–20%) does remain stored in hard-to-decompose plant litter and becomes incorporated in wetland soils, but this is relatively minor compared to other removal processes (Hey *et al*., 2007).

2.4 Biology and Ecology of *Lemna minor*

Duckweed *(Lemna minor)* is a small, free floating aquatic plant belonging to Lemnaceae family (Cheng *et al*., 2002). Duckweed is well known for its high productivity and high protein content in temperate climates. They are green and have a small size (1-3mm). They also have short but dense roots (1-3cm) (Ozengin and Elmaci, 2007). Duckweed fronds grow in colonies that, in particular growing conditions, form a dense and uniform surface mat (Hasar *et al*., 2000). Duckweed species have shown characteristics that make duckweed based wastewater treatment (DWWT) very attractive. They are used for wastewater treatment for nutrient removal. The reason for this is the rapid multiplication of duckweeds and high protein content of its biomass

(Hasar *et al*., 2000). Duckweed has the capability to purify wastewater in collaboration with both aerobic and anaerobic bacteria. The duckweed mat, which fully covers the water surface, results in two zones created. These are the aerobic zone and the anoxic zone (Cheng *et al*., 2002). In the aerobic zone, organic materials are oxidised by aerobic bacteria using atmospheric oxygen transferred by duckweed roots (Tchobanoglous and Burton, 1991). Nitrification and denitrification takes place in anoxic zones, where organic nitrogen is decomposed by anoxic bacteria into ammonium and ortho-phosphate, which are intermediate products used as nutrients by the duckweed (Ashby *et al*., 1949). Duckweed family (Lemnaceae) is composed of small floating or submerged plants whose populations expand nearly exclusively through asexual propagules (Landolt, 1986). The development of propagules occurs by the branching and subsequent fragmentation of the shoot into separate units called fronds (Lemon and Posluszny, 2000), and results in rapid population growth rates. *Lemna minor* has been reported to live for 4–5 weeks and produce between 4 and 12 daughter fronds (Ashby *et al*., 1949). Unfortunately, population growth in these plants is rarely expressed in terms of frond demography, obscuring aspects of development that regulate frond production.

2.5 Biology and Ecology of *Azolla pinnata*

The genus *Azolla* belongs to division Pteridophyta, class Polypodiopsida and order Salviniales. It belongs to the family Salviniaceae and has two subgenera and six living species (Lumpkin and Plucknett, 1980). The subgenus *Azolla*, characterized by three megaspore floats and septate glochidia, include four species: *A. filiculoides*, *A. caroliniana*, *A. microphylla*, and *A. Mexicana*. The subgenus *Rhizosperma*, characterized by nine megaspore floats, include two species: *A. pinnata* with simple glochidia, and *A. nilotica*, with no glochidia. *Azolla pinnata* is a free floating aquatic plant typically found in clusters or in large mats (Raja *et al*., 2012). Each plant is 1-2.5 cm in diameter with a feathered triangular shape; midsection is typically straight with pinnately arranged side branches that are longer towards their base (Saunders and Fowler 1992). Each leaf is 1-2 mm long and overlap in a two-ranked pattern (Pereira *et al*., 2011). *Azolla* is a genus of small water ferns with a world-wide distribution. The genus possesses intrinsic interest in that its members are capable of assimilating atmospheric nitrogen, the actual agent of fixation presumably being the blue-green alga that is almost invariably present in cavities in their leaves. *Azolla pinnata* generally grows in freshwater in tropical, subtropical, and warm-temperate regions throughout the world. *Azolla pinnata* is a highly productive plant. It doubles its biomass in 3–10 days, depending on conditions (Wagner *et al*., 1997) and reproduces both vegetatively and sexually. Vegetative fragments form when the main axis deteriorates and lateral branches break free. When reproducing sexually, round sporocarps (1-1.5 mm in diameter) form on the underside of the leaves. *Azolla pinnata* grows optimally between 29°C -33°C (Watanabe and Berja 1983). It tolerates salt concentrations up to 30 PSU, but can be preincubated in lower concentrations to increase salinity tolerance up to as high as 60 PSU (Raji et al., 2015). Three *Azolla* species that is *A. caroliniana*, *A. microphylla*, and *A. pinnata* are commonly found all over the African subcontinent (Lamarck, 1983). The macrophyte of *Azolla pinnata* is called frond which ranges from 1 cm to 2.5 cm in length in species such as *A. pinnata* and 15 cm or more in the largest species like *A. nilotica* (Raji *et al*., 2012). It has a main rhizome which branches into secondary rhizomes, all of which bear small leaves alternately arranged. Numerous unbranched, adventitious roots hang down into the water from nodes on the ventral surfaces of the rhizomes. The roots absorb nutrients directly from the water and in shallow water they may touch the sediment, deriving nutrients from it. Each leaf consists of two lobes: an aerial dorsal lobe, which is chlorophyllous, and a partially submerged ventral lobe, which is colourless and cup-shaped and provides buoyancy. Each dorsal lobe contains a leaf cavity which houses the symbiotic *Anabaena Azollae* (Vymazal, 2007).

2.6 *Lemna minor* and *Azolla pinnata* in wastewater treatment

Uptake of nutrients by macrophytes is one of the major mechanisms for the removal of nutrients and other pollutants from wastewater. The process, which is also known as plant assimilation, converts inorganic nitrogen and phosphorous into organic compounds, the building blocks for plant cells and tissues (Vymazal, 2007). Rooted macrophytes obtain nearly all nutrients from the sediment, whereas floating plants assimilate nutrients directly from the water column (Wetzel, 2012). *Lemna minor* and *Azolla pinnata* are capable of absorbing any form of soluble nitrogen and phosphorous (Bornette *et al*., 2008). However, their preferential uptake depends on the nitrogen forms available in the sediments and soils (Bornette and Puljalon, 2011). For example, most plants prefer NH_4 since it is easily assimilated into physiological processes, while NO^{3-} has to be converted further into NH_4 (Skillikorn *et al*., 1993).

Duckweeds are small green floating plants of the *Lemnaceae* family. The family of *Lemnaceae* consists of four genera: *Lemna, Spirodella, Wolffia and Wolffiella*. About forty species are identified worldwide (Tripathi and Upadhyay, 2003). However, duckweed has the great capacity in absorbing the nutrients and their high nutrient removal efficiency can be used to clean wastewater in an effective, cheap and simple way. Compared to most other plants, duckweed has low fiber content (about 5%), since it does not require structural tissue to support leaves and stems. The applications of duckweed in wastewater treatment is found to be very effective in the removal of nutrients, soluble salts, organic matter, heavy metals and in eliminating suspended solids, algal abundance and total and faecal coliform densities. Duckweed is found world-wide on the surface of nutrient rich fresh and brackish waters (Zimmo, 2003). The nutrients taken up by duckweed are assimilated into plant protein. Under ideal growth conditions more than 40% protein content on dry weight basis may be achieved (Skillikorn *et al*., 1993).

Duckweed has been used for tertiary treatment of municipal and industrial wastewater for many years (Skillicorn *et al*., 1993). Ammonia can be removed by duckweed uptake, nitrification-denitrification, ammonia stripping (at pH higher than eight) and algal and microbial assimilation (Iram *et al*., 2012). Phosphorus removal can also be attributed to duckweed uptake, microbial assimilation, precipitation with cations and adsorption on clay and organic matter (Priya *et al*., 2012).

The suitability of floating macrophytes on the purification of wastwater depends on the type of wastewater and the nature of pollutants in it (Ra *et al*., 2007). In general *Azolla pinnata* and *Lemna minor* have shown wide application in phytoremediation as these plants are small in size, free floating and growing at faster rate, having heavy rates of absorption and uptake of nutrients and different pollutants (Dixit *et al*., 2011).

Application of *Azolla pinnata* and *Lemna minor* is a very common practice in phytoremediation, because they have very good potential for hyper accumulation of different pollutants, minerals and heavy metals, thus restoring polluted aquatic resources (Muradov *et al*., 2014). They have the ability for altering water quality by regulating oxygen balance through photosynthetic and respiration activities and nutrient cycles (Sood *et al*., 2012).

Duckweed species are promising macrophytes for use in sustainable secondary wastewater treatment due to their rapid growth, ease of harvest, and feed potential as a protein source. Macrophyte-based wastewater treatment systems have several potential advantages like being relatively inexpensive to construct and operate, easy to maintain, provide effective, reliable and ecologically sound wastewater treatment and can tolerate both great and small volumes of water and varying contaminant levels (Wu *et al*., 2015). If well treated, Wetlands water can be reused for productive purposes and they are aesthetically pleasing and provide habitat for wildlife and human enjoyment compared with conventional treatment systems (Park and

Roesner, 2012). However, there are certain limitations like the screening of efficient duckweed species and sensitivity to potentially toxic high concentrations of Ammonia in wastewater. However, few reports are available on the ability of *Azolla pinnata* for removal of nutrients from wastewaters and its use in wetlands. *Azolla pinnata* the aquatic microphytic fern is advantageous because of its high productivity and free floating nature for easy harvest (Saeed and Sun, 2012). Its biomass has high protein content and can be used as animal feed and as biofertiliser. *Azolla pinnata* is known to remove metals and nutrients like nitrogen and Phosphates from wastewaters. A recent report has shown ability of *Azolla pinnata* fresh biomass to remove chemical dissolved oxygen and Polyphenols from Wastewater (Saeed and Sun, 2011). The capacity of aquatic macrophytes to assimilate and store nitrogen is dependent on their net productivities (growth rates), the concentration of nutrients in plant tissue, and the ultimate potential for biomass accumulation (that is maximum standing crop). Thus, desirable features of vegetation used to remove nutrient would include rapid growth, high concentration of nutrients in the plant tissue, and the capability to attain a high standing crop (Vymazal, 2007). However, at the end of growing season, aquatic plants may die back and the leaves and stalks eventually fall into wetland beds where they break down (Kadlec, 1999). If the bulk of nutrients have not been stored at the roots and rhizomes, phosphorus will eventually return back to the wetland systems (Reddy and Rodrigues, 1999; Vymazal, 1999). New growth of these macrophytes will require phosphorus uptake during early spring the next year so that a cycle can eventually develop where the uptake phosphorus in growing seasons will equal the phosphorus release due to dead plant decomposition. Thus, if the vegetation is not harvested, the macrophytes will bring about no net phosphorus removal (Verhoeven and Meuleman, 1999; Vymazal, 2007).

2.7 The use of *Lemna minor* and *Azolla pinnata* for biomonitoring

Bioassessment is used to determine if human activity has caused changes to the biological properties of an ecosystem, by comparison to natural reference conditions, whilst biomonitoring is the systematic use of biological responses in the target groups, to assess environmental change as a quality control measure. The assessment of biotic responses is seen to have advantages over physico-chemical monitoring by integrating long-term response to pollution. However, biotic responses do not give insight into the specific causes of pollution, and should be used in conjunction with insights gained from physico-chemical monitoring. There are several advantages of using aquatic macrophytes in wastewater monitoring, including their longevity, relative ease of sampling, identification, and their contribution to the physical habitat of other target groups (Kennedy, 2012)

Aquatic macrophytes have been used for monitoring contamination level by various pollutants in aquatic environments (Sawidis *et al*., 1995), including organic matter and nutrients such as phosphorous and nitrogen in wetland systems (Borges *et al*., 2008) and heavy metals, such as Zn (Wolff *et al*., 2009), Hg (Molisani *et al*., 2006) and others as these plants have the ability to accumulate metallic ions (Devi *et al*., 1996). Methods to assess a stressing agent, such as alterations in the growth rate and species composition in a community, have been using inadequate indicators. It is necessary to use biological indicators capable of detecting, predicting and quantifying a stressing agent before large-scale visible damage and loss occur. Bioassays are important tools to select better indicators. Several aquatic organisms at different trophic levels have been widely used in ecotoxicological assessments. Biological methods can provide a qualitative description of both the presence and toxicological potential of a certain pollutant. It is possible to expose an organism to different toxicity tests and then estimate the potential hazard of the pollutant (Feiler *et al*., 2006).

2.8 Legal and Policy Framework on Wetlands in Kenya

The global policy context concerning wetland management planning is defined by the processes around the Ramsar Convention and other relevant environmental conservation treaties and conventions, notably the Rio Declaration and Agenda 21, the United Nations Convention to Combat Desertification, and the Convention on Biological Diversity. The regional policy context on the other hand is defined by the integration arrangement between Kenya and its four neighbouring countries within the framework of the East African Community. Currently, there are two important draft policies relating to the management of wetlands: the draft Wetlands Conservation and Management Policy 2013 and the draft Environment Policy 2013. These are important policy discourses that have set the motion towards sustainable wetland management in Kenya. The national context is defined largely by the Constitution, Draft Environment and Wetland Policies 2013, the National Land Policy and legislation introduced to give effect thereto. Also of relevance are other sector specific policies and laws touching on wetland/water resources management.

2.9 National Environmental Policy (2013) on Water and Sanitation

Water supply and sanitation in Kenya is characterized by low levels of access, particularly in urban informal settlements and in rural areas, as well as poor service quality in the form of intermittent water supply. Dirty water and lack of basic sanitation continue to undermine efforts to reduce disease prevalence in the country. Seasonal and regional water scarcity exacerbates the difficulty to improve water supply. These challenges persist despite the water sector undergoing considerable reforms over the years. In addition, sewerage systems and wastewater treatment plants experience inadequate operation and maintenance and low connection rate to sewers. Mixing industrial effluent and domestic sewage in mixed sewer system often cause poor performance in pond treatment systems. Cases of pollution by wastewater emptying into storm sewers, soak-ways and cesspits designed for kitchen waste are a common occurrence. Access to clean drinking water and basic sanitation facilities could transform the lives of millions of citizens, prevent thousands of deaths and free up hours each day for women and children to go to work or school.

2.10 Environmental Management and Coordination (Wetlands, Riverbanks, Lakeshores and Seashores Management) Regulations 2009 (EMCA Wetland Regulations)

The objectives of the EMCA Wetland Regulations are to, among others, provide for the conservation and sustainable use of wetlands and their resources, ensure their protection as habitats for floral and faunal species, prevent and control their pollution and siltation and to provide a framework for public participation in their management. Regulation 5 also reiterates the need to have Environmental Impact Assessments and Environmental Audits as provided for by EMCA and discussed in the preceding section. Regulation 6 states that the Standards and Enforcement Review Committee established under EMCA shall advise NEMA on the wise use, management and conservation of wetlands.

The Minister responsible for the environment can, under Regulation 8, declare a wetland to be a protected area on account of its biological diversity, ecological importance, landscape, natural heritage, or aesthetic value. This declaration automatically triggers the prohibition of all activities in wetlands other than those touching on research, ecotourism, restoration or enhancement of the wetland or the activities identified in the management plan. Regulation 9 sets out an elaborate procedure that must be followed before a wetland is declared a protected area while Regulation 10 obligates NEMA to develop and maintain a national wetland inventory. Regulation 11 lists the permitted uses of wetlands and includes harvesting of papyrus, medicinal plants, trees and reeds on a subsistence scale; collection of water for domestic use and; fishing. By Regulation 14, owners, occupiers and users of land which is adjacent or contiguous to wetlands have a duty to prevent its degradation or destruction. The regulations are evidently comprehensive and their enforcement would help to address many of the issues that bedevil wetland management in the country.

2.11 Constitution of Kenya, 2010

Although the Constitution, which is Kenya's paramount law, does not expressly refer to wetlands, it enshrines a number of novel environmental provisions that can potentially improve wetland management. The preamble which is founded on the principle of respect for the environment affirms the determination and commitment of Kenyans to pursue a sustainable development path. Further, Article 42 entitles every person to a clean and healthy environment while Article 70 provides for redress in case of right infringement. According to Article 64, sustainable management of land resources as well as sound conservation and protection of ecologically sensitive areas are some of the principles that undergird water management.

2.12 Standards for Discharge of Wastewater

Below are the standards provided by the National Environment Management Authority that the wastewater treatment need to meet before being discharged into the environment.

Table 2.1: National Environment Management Authority (NEMA) Maximum Permissible Limits/Effluent discharge regulations

Parameter	**Limit**
Ammonia –NH_4	0.5 (mg/L)
Ammonia Nitrogen	10mg/L
Total Nitrogen	10 mg/1
Nitrate – NO_3	10 mg/1
Nitrite – NO_2	3.0 mg/1
Chemical Oxygen Demand	50 mg/1
Biochemical Oxygen Demand (5days at 20^{o}C)	30 mg/l
pH	6.5-8.5
Total Phosphate	10 mg/1
Soluble Phosphate	5.0 mg/1
Total Dissolved Solids	1200 mg/1
Temperature	20-35^{0}C
Total Suspended Solids	30 mg/1

Source: The National Environment (Standards for Discharge of Effluent into Water or on Land) Regulations, S.I. No 5/1999.

The following are the guidelines given by the World Health Organisation that need to be met during the wastewater treatment before being released to the environment.

Table 2.2: World Health Organization (WHO) Environmental, Health, and Safety (EHS) Guidelines/ Wastewater and Ambient Water Quality

Parameter	**Limit**
pH	6 – 9 pH
Biological Oxygen Demand	30 mg/l
Chemical Oxygen Demand	125 mg/l
Total nitrogen	10 mg/l
Total phosphorus	2 mg/l
Total suspended solids	50 mg/l

Source: World Bank Group; Environmental, Health, and Safety (EHS) Guidelines (2007).

2.13 Conceptual framework

The conceptual framework (Figure 2.1) shows the independent variable with its indicators being the water physicochemical parameters (temperature, EC, PH, dissolved oxygen, biological oxygen demand and total suspended solids). The dependent variable is the efficiency whose indicators are nutrients concentration (nitrates, nitrites, ammonia, total nitrogen, soluble reactive phosphorous and total phosphorous) and biomass of *Lemna minor* and *Azolla pinnata.* The intervening variables are the ecological succession, pest interference, wastewater volume, seasonality and chemical composition. These variables show the link that exists between physicochemical water qualities and the floating macrophyte species.

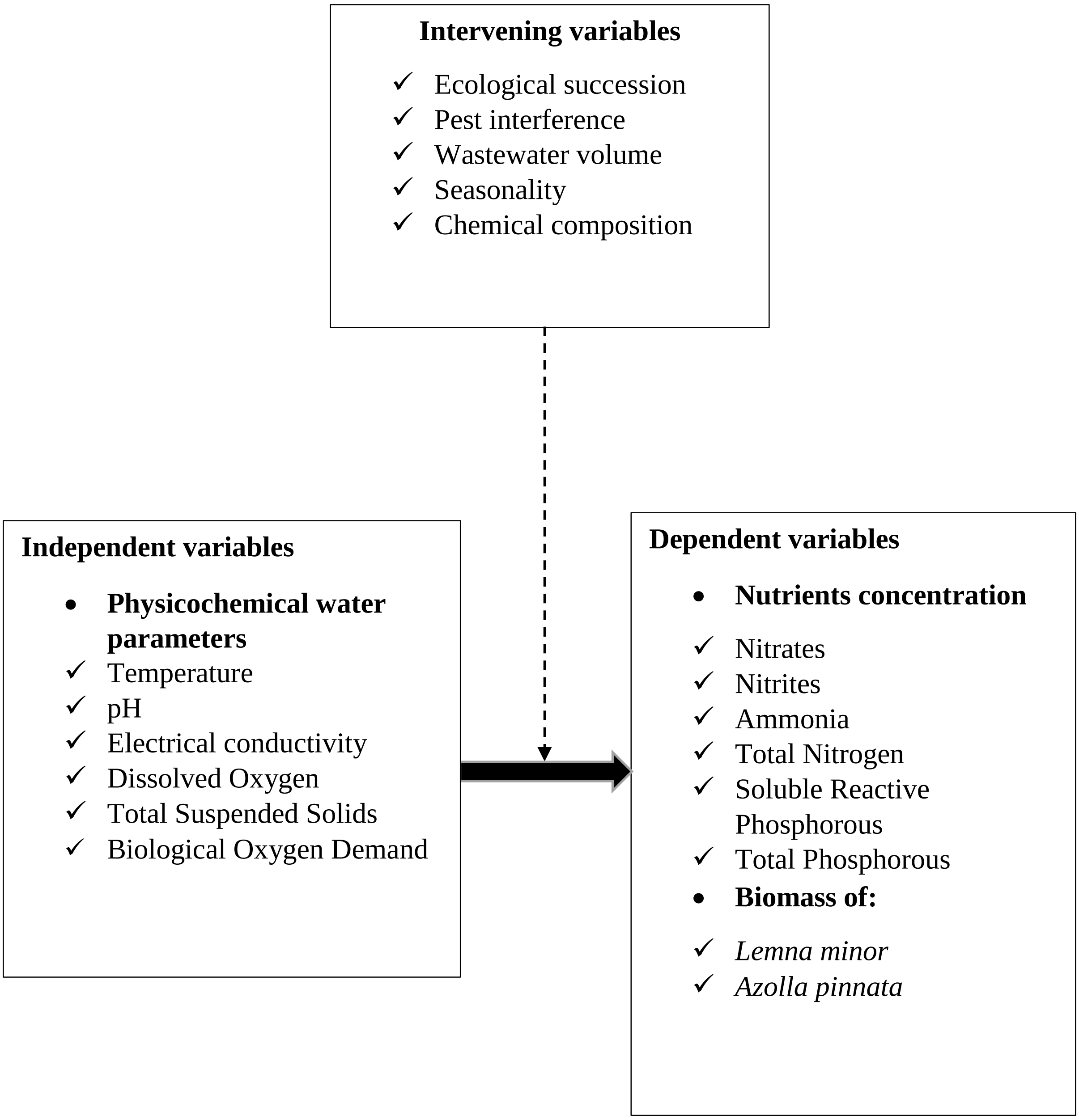


Figure 2.1: Interelationships between independent, intervening and dependent variables.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Study Area

This study was carried out in Egerton University in Kenya, located about 25 Km South-west of Nakuru town in Nakuru County within latitude 0 15′ and between longitudes 35° 50'and 35 05' E. (Figure 3.1). The institution is located on a land of 1580 hectares within the River Njoro watershed at an altitude of 1890 - 2190 metres above sea level. The institution lies in an agricultural area characterized by bimodal precipitation pattern ranging from 760 - 1270 mm per annum with the long rains falling between March and May while the short rains occur in September – November. It experiences a daily temperature range of 14.9 - 21.9°C (Odongo and Partners, 1989).

The University has a population of about 18,000 people and generates about 800 m^3 per day of wastewater which is treated in wastewater stabilization ponds (lagoons) and the constructed wetland within the University (Mwanyika *et al*., 2016). The constructed wetland is a free-water surface wetland that covers 0.25 hectares of land. It was constructed in 2007 to polish the pre-treated wastewater effluent from the wastewater stabilization ponds. The system consists of one vegetated sedimentation/gravel bed that has always been dominated by emergent macrophytes and some floating macrophytes that include *Pistia stratiotes*, *Cyperus alopecuroides* and *Scirpus lacustris*. This compartment is followed by a series of three connected, vegetated wetland cells. The dominant plant species in the first two cells has been *Eichhornia crassipes,* while the last cell is largely an open pond with few tufts of *Cyperus alopecuroides*.

The vegetation in the cells had however changed with the harvesting and removal of the emergent macrophytes and the introduction of *Lemna minor* and *Azolla pinnata* both of which are floating macrophytes that now dominate the cell. The system was designed to purify about $100m^3$ of water per day with an approximate retention time of 10 to 14 days before discharging into River Njoro.

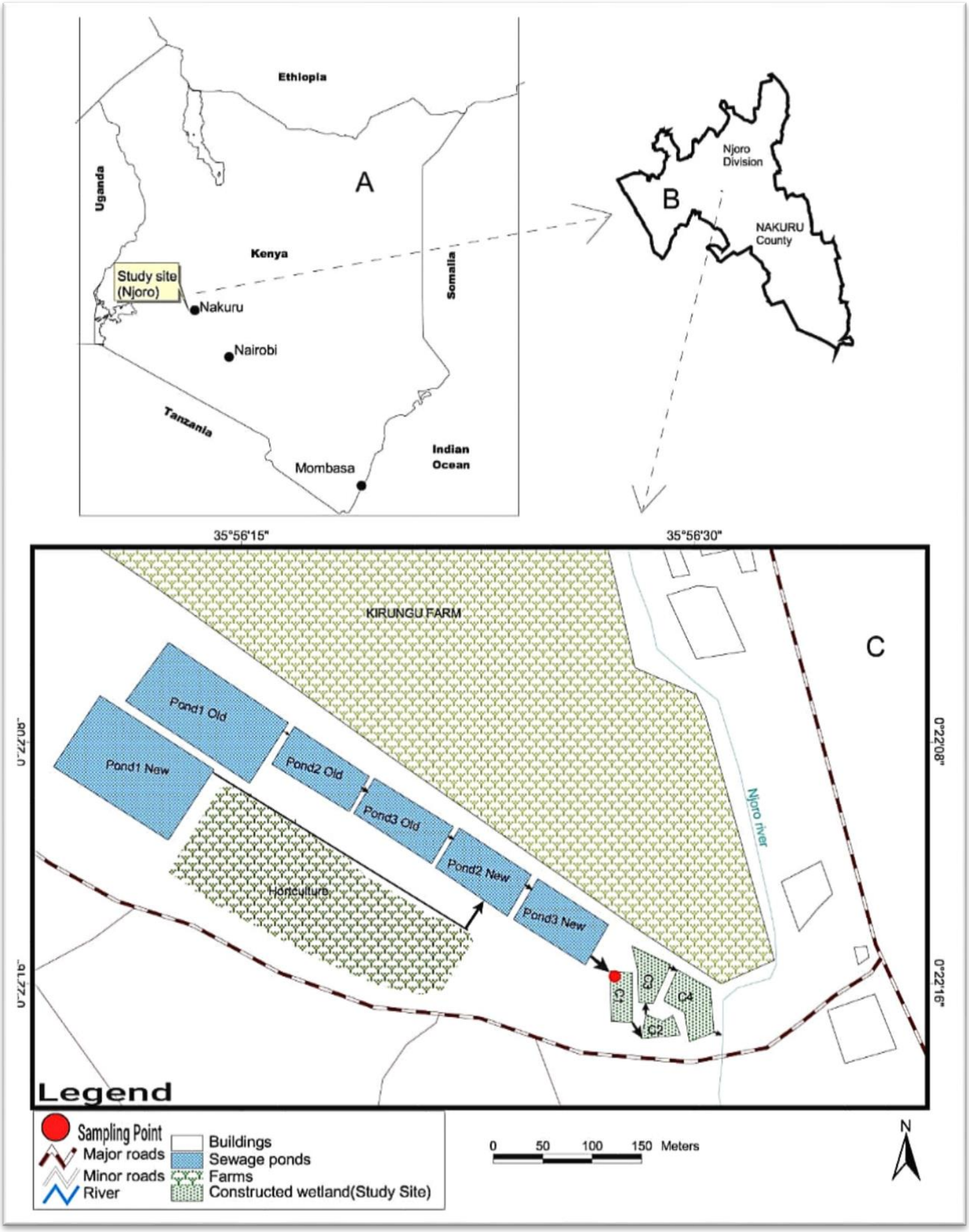


Figure 3.1: Location of the study site

3.2 Research design

This study employed completely randomized design as the experimental design. The research design was based on understanding the effectiveness of floating macrophytes (*Lemna minor* and *Azolla pinnata*) in removal of nutrients (nitrates and phosphates) when growing within the constructed wetland. The two plants were grown in buckets filled with wastewater collected from first cell of the constructed wetland.

The experiment was conducted with forty five buckets and on each sampling occasion wastewater from nine buckets was sampled, three having *Azolla pinnata*, three with *Lemna minor* and the other three from the control with no macrophytes. Before the introduction of the wastewater to the buckets, baseline sampling was done to establish the status of the wastewater (nutrients concentration and physicochemical parameters). The buckets were divided into three groups of nine where wastewater of 7.5 litres was put in each and the predetermined weight of 10g of the treatments (*Lemna minor* and *Azolla pinnata*) was introduced in six buckets separately (three buckets each treatment) and the rest three had no plants in them (the Controls). Sampling from the nine buckets was done after every 5 days (5th, 10th, 15th, 20th, and 25th). Physicochemical parameters in the wastewater were measured *in situ* while water samples for analysis of suspended solids, biological oxygen demand and nutrients concentration were collected from the nine buckets in which the macrophytes were growing and from the control buckets. Samples of *Lemna minor* and *Azolla pinnata* were harvested and their change in weight over time was determined in each sampling occasion. Nutrients concentrations (phosphates and nitrates) were also determined in the laboratory.

3.3 Sampling procedure and sample collection

Replicate samples were taken from each bucket after every five days up to the twenty fifth day. A total of twenty seven composite samples of wastewater were collected from the buckets on each sampling occasion since the samples were in triplicates. The floating macrophytes (*Lemna minor* and *Azolla pinnata*) in each of the six buckets were harvested for further biomass analysis in the laboratory. On each of the sampling occasion water quality parameters; pH, temperature, EC, dissolved oxygen, were measured *in situ* by use of an electronic multimeter (model Jenway 3405 electrochemical analyzer) fitted with the appropriate detection probes. Water samples for suspended solids and nutrients analyses were collected in rinsed plastic bottles from the wastewater buckets and poured in to sampling bottles. One sample was collected randomly from each sampling site (bucket). The samples were kept in a cooler box and transported to the laboratory for further analyses within six hours. All the samples were collected between 9:00 a.m. and 11:00 a.m. when there was minimal diurnal variations in the suspended solids and the nutrients (Ayres and Mara, 1996). All *Lemna minor* and *Azolla pinnata* contained in each sampling site were harvested by use of a hand sieve whose weight had been determined and recorded. All the collected plant samples of *Lemna minor* and *Azolla pinnata* were placed into a labeled plastic bag and drained of excess water while in the field. The samples were taken to the laboratory for biomass analysis. All the selected physicochemical parameters affecting water quality were determined following methods described in American Public Health Association (APHA 2005).

3.4 Laboratory analysis

3.4.1 Total Nitrogen

Determination of total nitrogen concentration in wastewater was done using a semi-micro Kjeldahl method according to APHA, (2005). Wastewater sample of 25ml was transferred to flasks, a mixture of sodium hydroxide, potassium peroxodisulphate and boric acid was added. The flasks were covered with a cotton plug and aluminium foil, mixed carefully and put in the autoclave for 1 hour at 110°C. A 1ml of concentrated sulphuric acid was added followed by 40ml of distilled water. The absorbance was measured at 220nm and 275nm against distilled water in spectrophotometer.

3.4.2 Nitrates and nitrites

Nitrates concentration was determined using the sodium-salycilate method (APHA, 2005). 0.5grams of sodium salycilate was dissolved in 100ml distilled water, 400grams sodium hydroxide was dissolved in 1litre distilled water, with potassium-sodium-tartrate for this solution. 20ml of filtered wastewater sample was placed in an evaporation bottle and to this as well as to the standard series, 1ml of sodium salycilate solution freshly prepared was added. The bottles were then put in an oven and the samples evaporated to dryness at a temperature of 95°C. The resulting residue was dissolved by adding 1ml of concentrated sulphuric acid and the bottles were swirled carefully while still warm. Next, 40ml of distilled water was added and mixed. Finally 7ml of potassium-sodium hydroxide-tartrate solution was added mixed and the absorbance of this solution was determined at a wavelength of 420nm using spectrophotometer.

The nitrite concentration analysis was carried out using the reaction between sulfanilamide and N-Naphthyl-(1)-ethylendiamin-dihydrochlorid. Sulfanilamide solution (25ml concentrated hydrochloric acid (37%) diluted to 150ml with distilled water. Then, 2.5grams of sulfanilamide added. The solution then diluted to 250ml with distilled water). To 25ml of the filtered wastewater sample, 1ml of sulfanilamid solution was added. After 2 – 8 minutes, 1ml of N-Naphthyl-(1)-ethylendiamin-dihydrochlorid solution was added to the mixture and gently mixed. The solution was left standing for 10 minutes after which its absorbance was read from the sphectrophotometer at a wavelength of 543nm.

3.4.3 Ammonia

Determination of ammonium concentration in the wastewater was done by semi-automated colorimetry method according to O'Dell, (1993). Sodium salicylate solution (130grams of sodium salicylate and 130grams of trisodium-citrate dehydrate was mixed in 800ml of distilled water. 0.97grams of sodium nitroprusside was then added to this solution. The solution was then filled up to 1000ml using distilled water). Hypochlorid solution (0.2grams of sodium dichloroisocynurate was added and mixed with 100ml of 32grams sodium hydroxide solution dissolved in 1000ml distilled water). To 25ml of the sample, 2.5ml of sodium salicylate solution was added followed immediately by the addition of 2.5mlof hypochorid solution. The sample was then placed in a water bath at a temperature of 25°C in the dark for 90 minutes. Absorbance was then determined at a wavelength of 665nm in a spectrophotometer after this incubation.

o3.4.4 Soluble Reactive Phosphorus

Determination of the soluble reactive phosphorous content in wastewater was done using the ascorbic acid method as described by APHA, (2005) on the filtered samples. The four reagents (15 grams of ammonium molybdate dissolved in 500ml distilled water , concentrated sulphuric acid 140ml diluted up to 1000ml with distilled water, 2.7 grams ascorbic acid dissolved in 50ml distilled water) were mixed in the respective ratios 2:5:2:1, where the order of mixing was very critical. The resulting solution was added to the sample in a ration of 1:10 (2.5ml reagent added to 25ml of the sample). The treated sample's absorbance was measured after 15 minutes in a spectrophotometer at a wavelength of 885nm with distilled water as a reference.

3.4.5 Total Phosphorus

For total phosphorous concentration determination in wastewater, the nitric acid-sulphuric acid method was used (APHA, 2005). 12 grams Potassium persulphate was dissolved in 100ml of distilled water by sonification for about half an hour. To 25ml of the unfiltered sample, 1ml of the still warm Potassium persulphate solution was added. The bottles with 25ml of sample were weighed without the lids and their weight noted. The lids were put back but not closed tightly after which they were autoclaved for 90 minutes at about 120°C and 1.2 atm. After cooling the bottles were re-weighed and the evaporated water replaced by addition of distilled water. After digestion, samples analysed using procedure for soluble reactive phosphorous analysis.

3.4.6 Determination of nutrients uptake efficiency/capacity by the plants

$$\textbf{uptake capacity} = \frac{\textbf{IC} - \textbf{FC}}{\textbf{IC}} \textbf{X100}$$

Where; IC= Initial Concentration

FC= Final Concentration

3.4.7 Biological Oxygen Demand (BOD_5) determination

Determination of the BOD_5 in the wastewater was done by "Five Day BOD" test method according to Delzer and McKenzie, (2003)

The general equation used in the determination of a BOD_5 value was:

$$B0D5 = \frac{D1 - D2}{P}$$

Where; D_1= initial DO of the sample.

D_2= final DO of the sample after 5 days.

P= decimal volumetric fraction of sample used.

3.4.8 Total Suspended Solids (TSS)

Total suspended solids was determined by Gravimetric method according to APHA (2005). Glass-fibre filters (Whatman GFC filters) were pre-dried to constant weight at 95°C. Wastewater sample volume of 150ml was filtered using pre-weighed filter and dried at 95°C until a constant weight was achieved (APHA, 2005).

The suspended solids weighed was worked out using the formulae:

$$TSS = \frac{Wc - Wf}{v} X10^6$$

Where; TSS = Total suspended solids

Wf = Weight of pre-combusted filter in gram

V = Volume of water sample used in ml

3.4.9 Biomass of Lemna minor and Azolla pinnata

At the beginning of the experiment, an initial damp weight of 10 grams of *Azolla pinnata* and *Lemna minor* were introduced into their respective buckets. On each sampling occasion (5th, 10th, 15th, 20th, and 25th day) *Azolla pinnata* and *Lemna minor* were sieved from the buckets using a hand sieve and muslin cloth of known damp weight. They were dried up of excess water then taken to the lab. Then later *Azolla pinnata* and *Lemna minor* were kept for five hours to dry up of the remaining water and their weight determined by use of a balance machine and recorded. The damp weight of the muslin cloth and the hand sieve was subtracted from the total weight to compute the total damp weight of *Azolla pinnata* and *Lemna minor*. To determine the weight increase of *Azolla pinnata* and *Lemna minor*, the initial weight was subtracted from the final weight.

$Aw - Iw = \mathrm{Fw}$

Where: Aw = weight after

Iw = initial weight

Fw = final weight

3.5 Data analysis

All data on physico-chemical water quality and temporal nutrients concentration in wastewater was statistically analysed using descriptive and inferential statistics. The inferential statistics included Two-way ANOVA and Tukey Test to determine if there was significant difference in the: physicochemical water parameters, nutrients concentrations between the sampling occasions, significant difference in nutrients uptake between *Azolla pinnata* and *Lemna minor* and whether there was significant difference in weight increase of the two plants. During the study period. In all determinations, significance level was 0.05.

Table 3.1: Data analysis summary

Objective	**Research hypotheses**	**Variables**	**Statistical tools**
To determine the physico-chemical parameters of the wastewater in the experimental design	There was no significant variation in the physico-chemical water parameters in the wastewater in the experimental design.	• Dissolved oxygen • Electrical conductivity • Temperature • pH • Biological oxygen demand	Descriptive Statistics (mean ± SD) Inferential Statistics • ANOVA • Tukey's Test
To assess the temporal variation in nutrients concentration in the wastewater containing *Lemna minor*, *Azolla pinnata* and the control	There was no significant variation in the temporal concentration of nutrients in the wastewater experimental design containing *Lemna minor*, *Azolla pinnata* and the control.	• Nitrates • Nitrites • Ammonia • Total nitrogen • Soluble Reactive Phosphorous • Total Phosphorous	• Descriptive Statistics (mean ± SD) • Inferential Statistics ANOVA. Tukey's Test.
To assess temporal variation in the biomass of *Lemna minor* and *Azolla pinnata* grown in the wastewater in the experimental design.	There was no significant temporal variation in the biomass of *Lemna minor* and *Azolla pinnata* grown in the wastewater in the experimental unit.	Biomass of: • *Lemna minor* • *Azolla pinnata*	• Descriptive Statistics (mean ± SE) • Inferential Statistics. ANOVA Tukey's Test.
To compare the nutrients removal efficiencies between the two floating macrophytes (*Lemna minor* and *Azolla pinnata*) grown in the experimental design.	There was no significant variation in the removal of nutrients from the wastewater in the experimental unit between the two floating macrophytes (*Lemna minor* and *Azolla pinnata*).	Comparison of changes in nutrients uptake between the two plants (*Azolla pinnata* and *Lemna minor*)	• Descriptive Statistics (mean ± SE) • Inferential Statistics ANOVA Tukey's Test

To correlate the physicochemical parameters with the nutrients uptake by the floating macrophytes (*Lemna minor* and *Azolla pinnata*) and the biomass productivity by the macrophytes	There was no statistically significant effect of the physicochemical parameters on the nutrients uptake by the macrophytes (*Lemna minor* and *Azolla pinnata*) and on the macrophytes biomass productivity	Physicochemical parameters • Dissolved oxygen • Electrical conductivity • Temperature • pH • Biological oxygen demand Nutrients concentration • Nitrates • Nitrites • Ammonia • Total nitrogen • Soluble Reactive Phosphorous • Total Phosphorous Biomass of: • *Lemna minor* • *Azolla pinnata*	• Pearson's correlation

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Physicochemical parameters

The baseline/ zero day's sampling occasion recorded the highest temperature (26.80±0.10°C) which was uniform for all the treatments (*Azolla pinnata, Lemna minor* and control) while the twenty days sampling occasion recorded the lowest mean temperature for *Lemna minor* (17.77±0.41°C) and *Azolla pinnata* (18.30±0.51°C) and twenty fifth day recorded the lowest for the control (18.70±0.52°C) (Table 4.1). The mean temperature values recorded in the experiment varied slightly during the investigation period as well as the pH of the wastewater. The mean concentration of dissolved oxygen was lower during the first sampling occasion and kept on fluctuating during the other sampling occasions. The mean BOD_5 values obtained in the wastewater from consecutive sampling occasions were different in each sampling occasion (Table 4.1). There was a statistically significant temporal variation in physicochemical parameters except for TSS (Table 4.1).

Temperature in the baseline sampling occasion was slightly higher than the other sampling occasions. Temperature variations amongst sampling occasions would be attributable to the influence of the vegetation and the existing air temperature at the study site. The presence of macrophytes results in more stable temperature in the experimental set up (Sirage *et al*, 2017). The lowest temperature value was obtained in the control at the twentieth day of research work (17.77±0.41°C) by the end of March while the highest wastewater temperature was recorded during end of January which was uniform in all the treatments (26.80±1.00°C). Fluctuating trend in temperature was observed during the study period (Table 4.1) and there was a statistically significant difference amongst the sampling occasions (F=249.81, P= 0.00). Lepcha (2016) noted that, any temperature slightly above or below normal room temperature is ideal for the microorganism to carry out their metabolism which helps in degradation and assimilation of organic pollutants present in the wastewater. Yuan *et al*. (2013) showed that low temperatures had negative effects on the pollutants removal by aquatic plants. According to their study, removal efficiencies of the pollutants by macrophytes increased with the increasing temperature throughout their study period. Guo-feng *et al.* (2000) conducted a study where the water temperature ranged from 13°C to a maximum of 32°C and found that the macrophytes were effective in the treatment of wastewater at that temperature range.

Table 4.1: Physicochemical parameters (means ±SD) at different sampling occasions for all the treatments

Physicochemical parameters		Electrical Conductivity (µS/cm)	Wastewater Temperature (°C)	Wastewater pH	Wastewater Dissolved Oxygen (mg/L)	Total suspended solids (mg/L)	Biological oxygen demand (mg/L)
Sampling occasion	Plants/ control						
Baseline/Zero days	*Azolla pinnata*	839.67±2.30	26.80±1.00	8.55±0.06 - 9.01±0.006	5.65±0.32	48.33±12.58	50.37±0.63
	Lemna minor	839.67±2.30	26.80±1.00	8.55±0.06 - 9.01±0.006	5.65±0.32	48.33±12.58	50.37±0.63
	Control	839.67±2.30	26.80±1.00	8.55±0.06 - 9.01±0.006	5.65±0.32	48.33±11.58	50.37±0.63
Five days	*Azolla pinnata*	668.33±27.38	21.17±0.51	8.54±0.07 - 10.±0.079	17.00±0.49	50.00±30.00	175.78±1.35
	Lemna minor	655.00±0.00	22.40±0.57	9.32±0.00 -10±0.00	17.85±0.19	45.51±14.10	182.66±3.08
	Control	638.33±6.43	24.97±0.55	9.57±0.01 - 10±57	20.60±0.13	37.78±26.96	214.18±1.00
Ten days	*Azolla pinnata*	478.33±0.58	19.20±0.61	7.15±0.01 - 9.12±0.01	11.06±0.02	51.67±24.66	111.44±0.50
	Lemna minor	514.00±0.57	19.07±0.51	7.02±0.00 - 9.12±0.00	11.76±0.03	51.57±11.11	119.15±0.22
	Control	540.67±0.61	19.67±0.49	7.05±0.01 - 9.12±0.01	10.19±0.03	45.86±12.46	99.96±0.23
Fifteen days	*Azolla pinnata*	483.00±0.62	18.90±0.57	7.21±0.02 - 9.55±0.02	10.46±0.01	51.74±29.43	95.26±0.65
	Lemna minor	350.00±0.57	18.53±0.61	7.14±0.01 - 9.55±0.01	8.91±0.02	22.21±8.96	87.67±0.71
	Control	395.00±0.51	19.60±0.65	7.05±0.04 - 9.55±0.04	11.85±0.02	35.56±9.18	120.08±0.55
Twenty days	*Azolla pinnata*	603.33±0.53	18.30±0.51	9.55±0.03 - 10.06±0.03	12.42±0.11	44.44±7.70	124.63±0.05
	Lemna minor	631.00±0.63	17.77±0.41	10.0±0.01- 10.06±0.01	11.89±0.04	53.34±23.09	111.78±0.71
	Control.	665.33±0.57	20.00±0.57	10.6±0.03 - 10.06±0.03	14.60±0.11	28.89±10.18	148.74±0.23
Twenty five days	*Azolla pinnata*	613.67±0.54	18.63±0.51	6.90±0.03- 8.11±0.03	10.81±0.17	68.89±26.94	100.18±0.22
	Lemna minor	650.33±0.55	18.60±0.51	7.05±0.02 - 811±0.02	9.43±0.02	68.89±43.37	85.63±0.21
	Control	700.67±0.61	18.70±0.52	7.28±0.03 - 8.11±0.03	11.82±0.03	31.11±7.70	118.29±0.63
df= 5	F statistic	169.351	249.809	433.929	344.705	1.906	326.282
	P value	0.00	0.00	0.00	0.00	0.100	0.00

Shah *et al.* (2014) observed maximum performance of macrophytes at a temperature range of 15°C to 38°C which was favorable for treatment of wastewater by the macrophytes. Temperature influences plant production and distribution within and among habitats in both terrestrial and aquatic ecosystems. Continuous stimulation of growth of the plants with fluctuating temperature was observed across the entire study period and at the noted temperature range the macrophytes had the ability to accumulate high biomass and remove nutrients and therefore had high potential in biological nutrient removal process.

The highest pH value ranging from 10.6±0.03 to 10.06±0.03 was recorded during twenty days sampling occasion while the lowest pH value ranging from 6.90±0.03 to 8.11±0.03 was recorded at twenty five days sampling occasion (Table 4.1). The pH range during phytoremediation is an important factor to be considered because it is essential to maintain acidic conditions (low pH) or basic conditions (high pH)) for the growth of plants (macrophytes) for maximum uptake of the nutrients (Mesania Rizwana, 2014). The pH range for the maximum nutrients uptake by *Lemna minor* and *Azolla pinnata* is between 5.0 – 7.5 (Xu and Shen, 2011). Maintaining such pH range is because big fluctuations can alter the structure of enzymes and stop growth of the plants. This suggests that the macrophytes can function optimally when the pH is neutral because this is the time the uptake of nutrients was high in both macrophytes. Plants modify wetland environments by excreting protons, organic acids and carbon dioxide via their roots and this maintains the pH below 7 which is favorable for several biochemical transformations (Dipu Sukumaran, 2011). Lower pH in the presence of plants could also be related with the imbalances between nitrification and denitrification (Coleman *et al*., 2001). pH is one of the important parameters that influence the performance of wetland systems (Lepcha, 2016). During the study period, pH particularly for 15-20-25 days changed from 7 to 10 then to 7 respectively. This can be attributed to several biochemical and physical processes that occurred during the biological purification of the wastewater and the stability in the buffer capacity of the wastewater. Priya *et al.* (2012) did a similar study and noted that any increase in pH in the treatment system was due to the photosynthetic activities of the plants in the wastewater. An upturn in pH of the control indicates that there was algal growth whose photosynthetic activities resulted in the increase of pH in the wastewater made for the control purpose. Ammonia oxidation again contributed to the increase of pH from 7 to 10. Gustin and Marinsek-Logar, (2011) noted that dissolved ammonia raises the pH of wastewater to above 11 with a strong base and can pose inhibitory effects on a variety of microorganisms involved in different biological wastewater treatment process.

According to Buchauer, (1998), a high pH value will be harmful to the various biochemical processes in wastewater treatment. He also states that the upper limit for biological purification lies at pH 12. During this period, it is suspected that there were high rates of respiration by the selected plants quantitatively releasing carbon IV oxide into the wastewater leading to the decrease in pH from 10 to 7 because Carbon IV oxide is much more soluble in water than is oxygen.

The highest electrical conductivity value (839.67±2.30µS/cm) was recorded in zero days/baseline sampling occasion, while the lowest mean value (350.00±0.57µS/cm) was recorded during the fifteen days sampling occasion. Electrical conductivity gives a measure of the concentration of various types of ions present in experimental set up (Dipu Sukumaran, 2013). The decrease in electrical conductivity during phytoremediation indicates uptake of nutrients by macrophytes (Lepcha, 2016). There was reduction in electrical conductivity over the study period. High levels of conductivity would indicate high concentration of ions in the experimental set up that could be problematic during treatment (Dalu and Ndamba 2003). There is limited data correlating EC with duckweed uptake of nutrients. Iqbal *et al*. (2017) conducted experiments correlating EC with duckweed growth. They reported that after 25 days of retention time of duckweed, maximum removal of nutrients and growth was observed at 1,000 µS/cm EC. Wang *et al*. (2010) also conducted similar study and noted that growth rate and nutrient removal efficiency by duckweed and *Azolla pinnata* decrease with an increase in EC. Highest electrical conductivity (EC) was recorded in the absence of aquatic plants (during baseline sampling occasion) as compared to the presence of *Azolla pinnata* and *Lemna minor*. The results clearly revealed reduction in electrical conductivity in the presence of plants. The range of electrical conductivity mostly depends on the concentration of various types of soluble salts in wastewater (Dipu Sukumaran *et al*., 2013).

Dissolved oxygen concentration ranged from 5.65±0.32 mg/L to 20.60±0.13 mg/L (Table 4.1) during zero days and five days sampling occasions respectively. Photosynthetic activity by macrophytes increases the dissolved oxygen in water, thus creating aerobic conditions which favor the aerobic bacterial activity to reduce the BOD and COD (Mesania Rizwana, 2014). The lowest dissolved oxygen concentration was recorded during the initial sampling occasion then after introduction of the macrophytes the dissolved oxygen rose significantly (table 4.1), for the other sampling occasions the dissolved oxygen value remained above 10 mg/L (Table 4.1). The difference in dissolved oxygen concentration during the study period was statistically significant (F= 344.71, P= 0.00). Sirage *et al.* (2017) noted that supply of oxygen through the plant roots is much higher than atmospheric diffusion. This means that though there could have been atmospheric diffusion of oxygen in the wastewater, it was minimal as compared to the supply of oxygen via the roots of the macrophytes.

Total Suspended Solids (TSS) are particles in water that can be trapped by a filter. The highest total suspended solids value (68.89±43.37 mg/L) was recorded on the 25th day of the study period while the 15th day of experiment recorded the lowest value (22.21±8.96 mg/L) (table 4.1). Total suspended solids values varied throughout the sampling occasions. The removal of TSS in the wastewater is mainly attributed to the sedimentation. Other factors such as the hydraulic behavior of the system and microbiological breakdown of organic matter may contribute to TSS reduction (Ugya and Imam, 2015). The mean TSS value of the effluent was significantly low this is due to the stabilization ponds present before the wastewater is allowed to flow into the constructed wetland where the effluent for this study was obtained. There was no significant difference in TSS removal amongst the treatments (*Lemna minor* and *Azolla pinnata*) and the control (p>0.05). The removal of TSS though mostly a physical process than microbiological, is affected by the retention time (Saraiva *et al*., 2018). Further, TSS removal mechanism is also influenced by the properties of substrate media used (Lepcha, 2016).

Biological oxygen demand (BOD_5) is a measure of the oxygen consumption of microorganisms in the oxidation of organic matter present in a given water sample at certain temperature over a specific time period. Attached and suspended microbial growth is responsible for the removal of BOD_5 (Sirage *et al*., 2017). Sampling at the start (zero days) recorded the lowest BOD_5 value (50.37±0.63 mg/L) while five days sampling occasion recorded the highest (214.18±1.00 mg/L) (table 4.1). The lowest value of BOD_5 was observed in the buckets containing *Azolla pinnata*. This indicates that the BOD_5 value can be reduced a lot by treating wastewater with *Azolla pinnata*. The BOD removal efficiency by the two macrophytes was better than the control and there was a statistically significant difference between the plants' BOD_5 and the control (F= 326.282, P= 0.00). The substantial reduction in BOD_5 in the buckets containing the macrophytes was due to the presence of both aerobic and anaerobic microorganisms associated with the macrophytes' surfaces and suspended in the water column (Priya *et al*., 2012). It may be also due to the direct uptake of small hydrocarbon by duckweeds. Again, the high BOD_5 removal can be attributed to the high oxygen supply by diffusion from the air because the buckets were not covered. Furthermore, it can be concluded that *Azolla pinnata* remove organic wastes from the wastewater due to the action of microorganisms present on the root surface.

4.2 Temporal Variation in nutrients concentrations during the study period

There was uptake of nutrients by the macrophytes (*Lemna minor* and *Azolla pinnata*) since the concentration of the nutrients was reducing over the study period (Figure 4.1, 4.2, 4.3, 4.4). The uptake of the nutrients by the macrophytes was statistically significant since there was significant reduction in the nutrients concentration during the study period. (NH_4-N: F= 195.572, P= 0.00, TP: F= 56.500, P= 0.00, SRP: 37.11, P= 0.00, TN: F= 104.025, P= 0.00). The decreasing nutrients were likely taken up by the plants grown in the buckets. Similar results were obtained by Shah *et al*. (2015) who carried out a similar study and found out a greater nutrients reduction over time due to uptake by water hyacinth and water lettuce. Macrophytes take up nutrients to build up their biomass overtime. Ammonia, total phosphorous, soluble reactive phosphorous and total nitrogen concentrations decreased throughout the study period. The reduction was principally due to the nutrients uptake by the plants grown in the buckets. But it was noted that there was reduced nutrients concentration reduction towards the end of the study which could be explained by the reduced plants' growth in the wastewater. Similar results were obtained by Sooknah and Wilkie, (2004) who found out that there was reduced SRP uptake due to reduced growth of water hyacinth in wastewater as compared to the initial days of the study.

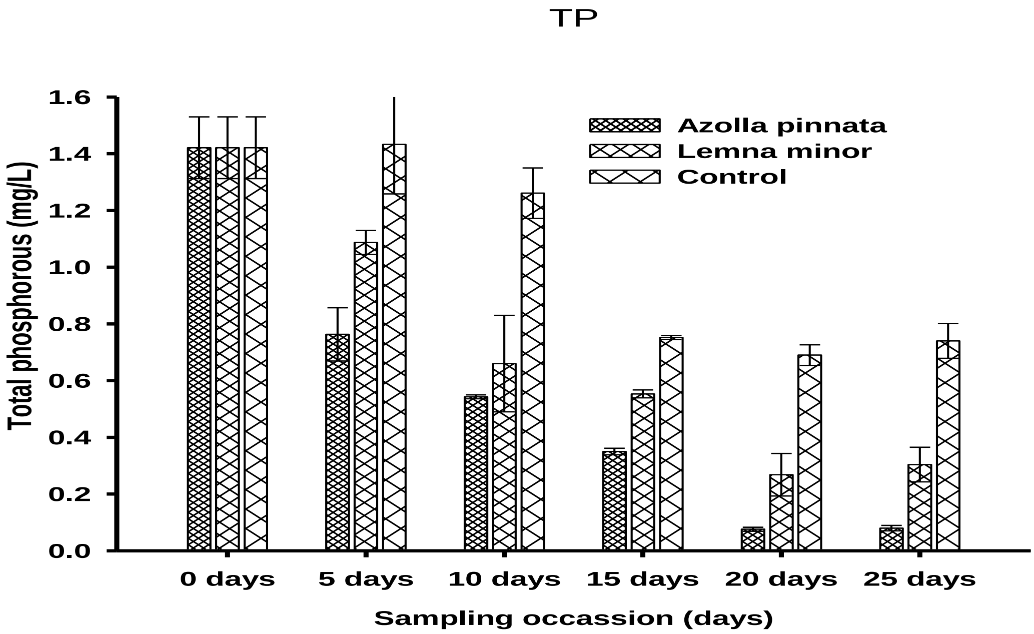


Figure 4.1: Total phosphorous concentration at different sampling occasions (Mean ± SD)

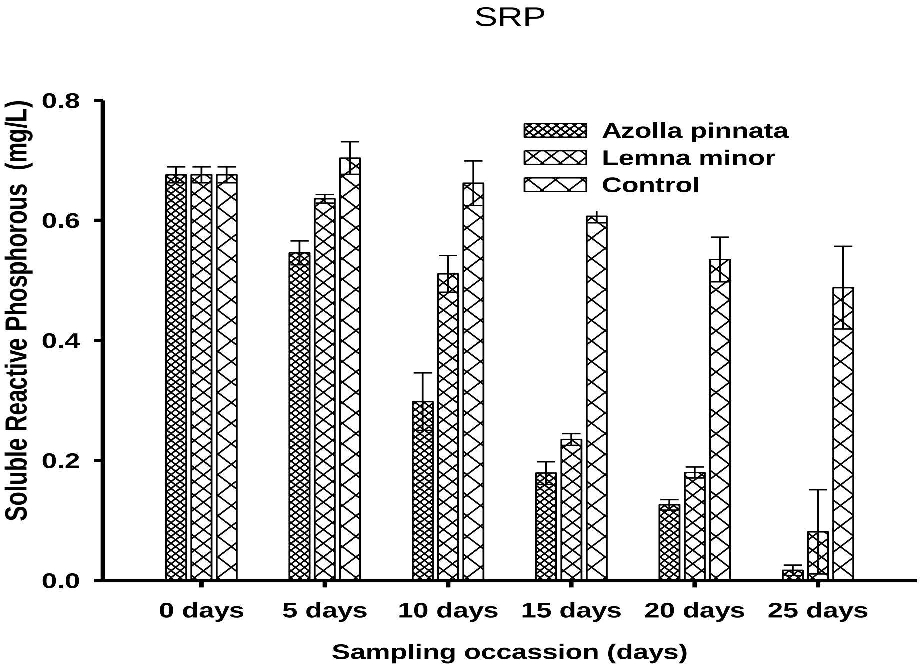


Figure 4.2: Soluble Reactive Phosphorous concentration at different sampling occasions (Mean ±SD)

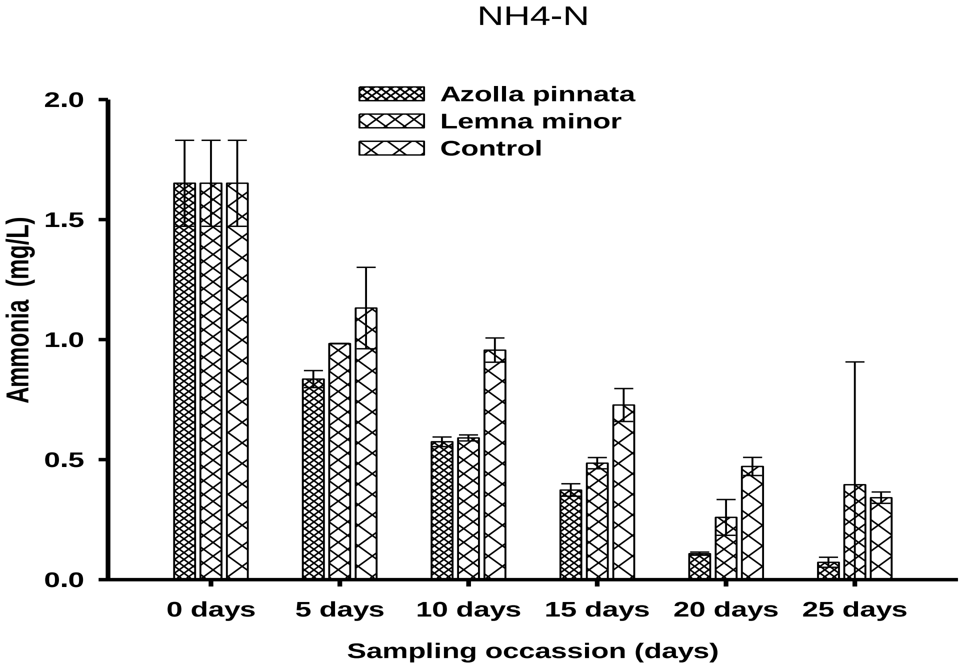


Figure 4.3: Ammonia concentration at different sampling occasions (Mean ±SD)

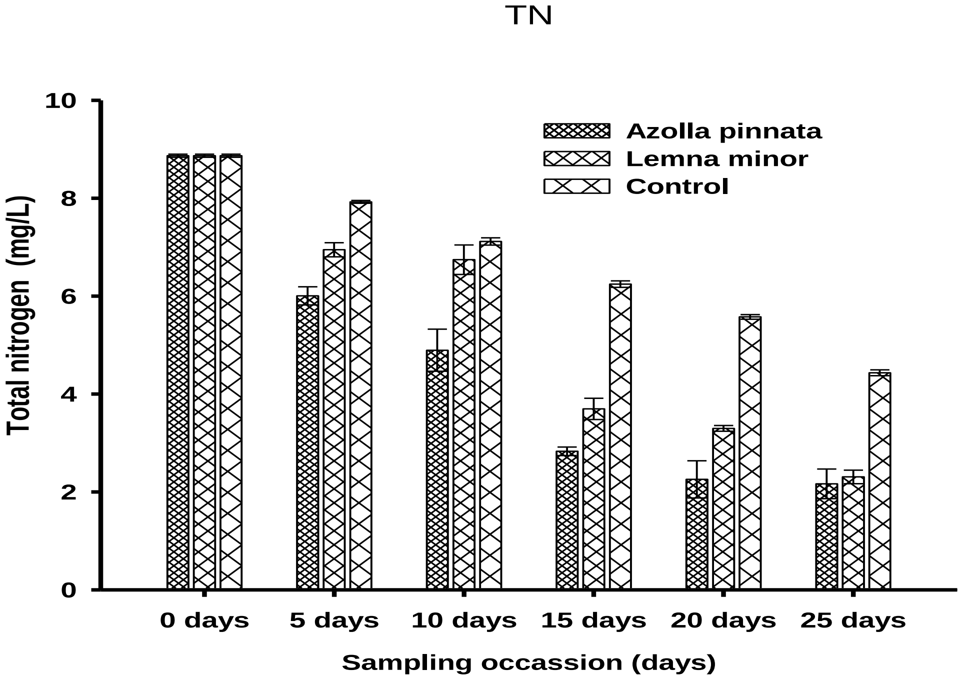
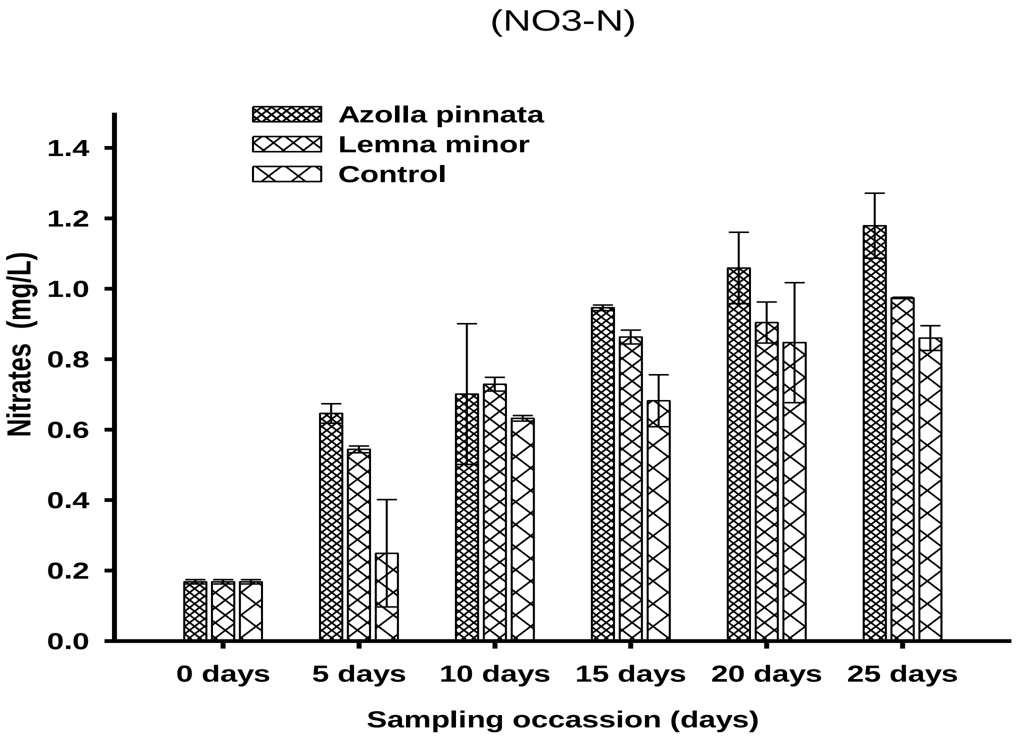
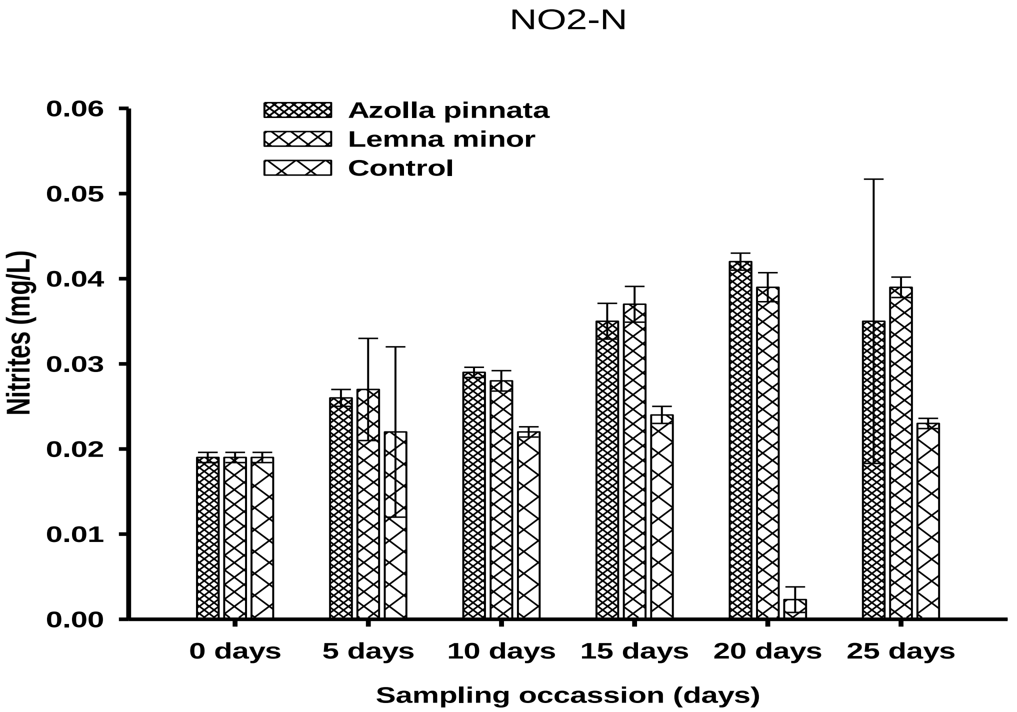


Figure 4.4: Total nitrogen concentration at different sampling occasions (Mean ±SD)



(NO3-N)

Figure 4.5: Nitrate concentration at different sampling occasions (Mean ±SD)



NO2-N

Figure 4.6: Nitrite concentration at different sampling occasions (Mean ±SD)

Concentration of nitrate and nitrite increased during the study period (Figure 4.5, 4.6) and the increase was statistically significant (NO_2-N: F= 24.780, P= 0.00, NO_3-N: F= 198.261, P= 0.00). The increase could be attributed to mineralization of ammonia and nitrogen and reaction of nitrogen with dissolved oxygen in the wastewater (Lee *et al*., 2009). Similar observations were made in the control pointing to the role of algae growth in the control wastewater thus producing oxygen that could actively transform organically bound nitrogen to nitrite and nitrate over the sampling period. Similar observation was meant by Srivastava *et al.* (2008) who conducted a similar study and attributed the nutrients reduction in the control to the uptake by algae and the growth of microbes and other biological activities taking place that utilize nutrients during their growth. Since ammonia is known to be volatile, the portion that was not taken up by the macrophytes most likely ended up in the sediment or was released to the atmosphere by coupled nitrification-denitrification (Tang *et al*., 2017). Quan (2014) noted that along the growing season of macrophytes, nitrates concentrations increased similar to nitrites concentrations. This increase may result from a combination of reduced plant uptake, nutrient leaching from senescing plants and reduced denitrification rates. Nitrogen is an essential plant nutrient and is removed through plant uptake of ammonium or nitrate, and stored in organic form in wetland vegetation (Ozengin and Elmaci, 2007). The optimum pH for nitrification is in the range 7.5 – 9.0. Below pH 7.0 and above pH 9.8 nitrification rate is less than 50% of the optimum (Ozengin and Elmaci, 2007). The wastewater showed pH ranging from 7.07±0.06 to 9.14±0.47 (table 4.1) which is within the required range for the macrophytes to function well. Other studies where increase of NO3-N and NO_2-N concentration was observed due to nitrification include those of Belmont and Metcalfe, (2003). But this phenomenon did not result in extreme oxygen depletion in the wastewater as was the case in Sirage *et al.* (2017). It is known that in wastewater excessive nitrogen is bound organically and nitrate is normally released through biological transformation. Therefore, the high rate of organic nitrogen transformation through mineralization and nitrification was the key factor that explains the increase in nitrate and nitrite concentration in the wastewater (Sirage *et al*., 2017). Zhang, (2014) also attests that duckweed preferentially absorbs ammonia rather than nitrate and nitrite because nitrogen in ammonia form is transformed directly to plant protein, rather than being assimilated and subsequently reduced, as in the case of nitrate (El-Shafai *et al*., 2007). Changes in conditions throughout the day could explain variations, as conditions like temperature affect nitrification and denitrification processes. Nitrification was the other pathway for NH_4-N removal, which resulted in the increase of NO_3-N and NO_2-N concentration, like reported by

Xu and Shen, (2011) and Zhao *et al*. (2014). But as observed during the study period, all the other nutrients were decreasing suggesting that there was enhanced uptake by the floating macrophytes and also the phytoplankton growing in the wastewater. Based on the current study results, uptake of nutrients by the macrophytes (*Lemna minor* and *Azolla pinnata*) led to significant reductions in the studied nutrients during the study period.

4.3 Temporal variation in macrophytes biomass (*Lemna minor and Azolla pinnata*)

The biomass production by the plants revealed a lag phase for the first five days followed by an exponential growth until fifteenth day, beyond which changes in growth were negligible (Figure 4.7). Similar results were found by Yin *et al*. (2015) obtaining the maximum biomass production at day 12. The ability of duckweeds to assimilate nutrients from culture medium has been reported by different authors as comparable (Xu and Shen, 2011; Zhao *et al*., 2014). The study on the growth aspects of macrophytes clearly indicated that the wastewater had no detrimental effects on the plants because none of the plant introduced in wastewater died, and despite the nutrients uptake difference, the biomass for both plants increased over the experimental period. There was a significant increase in biomass in the two macrophytes over the study period from one sampling occasion to the next over the study period (*Azolla pinnata*: F= 621.713, P= 0.00; *Lemna minor*: F= 786.494, P= 0.00). This suggests that there was great uptake of nutrients from the wastewater since the macrophytes use the nutrients to build their biomass.

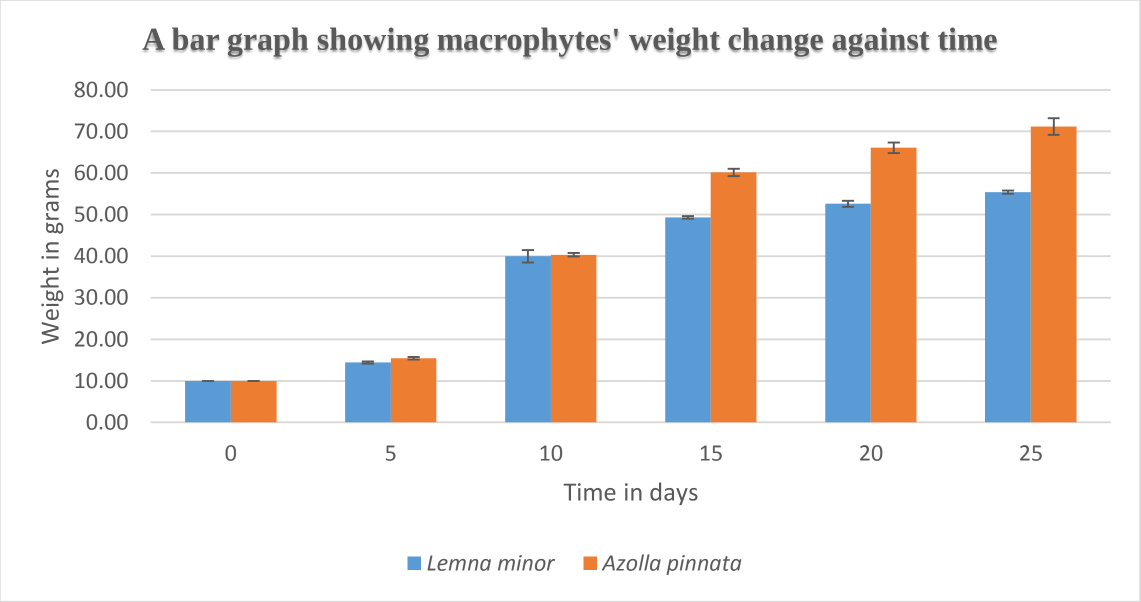


Figure 4.7: Macrophytes (*Lemna minor* and *Azolla pinnata*) biomass increase at different sampling ocassions (mean+SE, df= 5)

Uptake of nutrients by macrophytes is essential for their growth and reproduction (Sooknah and Wilkie, 2004). The rapid growth of the biomass was also measured within the same retention time as the nutrients thus the biomass increase indicating the uptake of the nutrients by the macrophytes. The high productivity of macrophytes enables substantial amounts of nutrients to be stored in plant biomass. Since the aquatic macrophytes' uptake of nutrients is depended on their biomass production and thus on their photosynthesis, the nutrients uptake would happen optimally only in the growing period of the macrophytes (Crispim *et al*., 2009). The growth of the macrophytes or biomass increase reduced significantly during the last days of the study period as compared to the initial days of the experiment where their growth was fast (Figure 4.7). Solano *et al*. (2004) suggested that for more nutrients removal, regular harvesting of the macrophytes is necessary. Biomass yields of small-leaf floating macrophytes are quite lower than for large-leaf floating aquatic macrophyte such as *Eichhornia crassipes* or *Pistia stratiotes* (Pena *et al.*, 2017). Macrophytes have a key function in relation to wastewater purification by provisioning a surface area for attached microorganisms (Xu and Shen, 2011), pollutant uptake, enhancing filtration, and releasing oxygen (Zhang *et al*., 2009). The removal efficiency of the plants may be due to a combination of mechanisms favoured by the plants and adsorption of certain nutrients (Zhao, 2014). However, other researchers suggest that 25 days is sufficient time for duckweed to polish wastewater effluent. Nasr *et al*. (2009) operated duckweed ponds as post-treatment at 10 days and 15 days. They noted that a 15-day hydraulic retention time (HRT) gave the best results and the macrophytes removed 73.4% of nitrogen and 65% of phosphorus. El-Shafai *et al*. (2007) stated that while a floating macrophytes treatment system is not strongly temperature dependent at high HRT, it may be affected by temperature at low HRT. This study was contacted in buckets with stagnant water as opposed to a constructed wetland where there is always flow of the wastewater as it is treated by the plants. In a constructed wetland, macrophytes are always affected by water flow through direct effects (stretching, uprooting, breakage) and indirect effects (changes in uprooting, gas exchange, bed material distribution, sediment suspension (Han *et al*., 2018). During this study, there was no wastewater flow hence there was no effect of flow rate on the reduction of the nutrients using the selected plants. Water flow inhibits the growth of the macrophytes and also alters the vertical distribution of water velocity. Levi *et al*. (2015) noted that flow turbulence could inhibit plant growth, induce oxidant stress and photosynthetic efficiency and reduce the carbon content in the tissue of the macrophytes. But the case in this study was different since there was no flow of the wastewater from the start to the end of the study.

Primary productivity of macrophytes is higher than that of terrestrial plants and agricultural crops because they do not suffer from a shortage of water. Macrophytes have a high tolerance for the fluctuations in environmental conditions and show high photosynthetic efficiencies (Sand- Jensen *et al*., 2007). The high productivity of macrophytes enables substantial amounts of nutrients to be stored in plant biomass. Some plants must be harvested for the most effective removal of pollutants which means large amount of biomass would be available for different uses. By regularly harvesting these plants, nutrients may be removed from the system. The aquatic biomass can then be used in various bio-based applications, for instance, as a bio-fertilizer or as fodder for livestock (Hauck, 1978; Biswas and Sarkar, 2013). The optimum period for the macrophytes growth for harvesting is by day 15. Shah et al. (2014) was of the opinion that macrophytes could be harvested weekly or biweekly due to their high growth rates. After the 15th day of this study, macrophytes growth was slow as well as the nutrients uptake, may be because they had reached maximum growth period (Figure 4.7). Tang *et al*. (2017) found out that there was major rise in plants biomass in first ten days during their study however, in remaining days it was much less as compared to initial growth. His experimental run consisted of a thirty-day period. At the optimum point, the growth rate of the plant is lowest. *Azolla pinnata* had more biomass increase than *Lemna minor* which had a low biomass build up while both were exposed to similar conditions. The biomass produced by *Azolla pinnata* can as well be used for inoculating paddy fields or for other applications and wastewaters can be reused for irrigation purposes (Arora and Saxena, 2005). This is also supported by Zhang *et al*. (2018) who found out that *Azolla pinnata* has distinct advantages as it has high biomass productivity coupled with high rate of nitrogen fixation, ability to grow in varied environments and multiple applications in biomonitoring, animal feed, biofilter, biofertilizer, and its ability to concentrate nutrients from wastewaters.

4.4 Nutrients uptake capacity/efficiency of *Lemna minor* and *Azolla pinnata*

The aim of this study was to experimentally test the magnitude of nutrient uptake by the two floating macrophytes from the effluent generated by Egerton University. The results show that under test conditions, *Azolla pinnata* has a greater capacity to remove nutrients as compared with *Lemna minor* (Table 4.2). The average removal efficiency of each nutrient by a particular macrophyte (*Lemna minor* and *Azolla pinnata*) is demonstrated in the table 4.2 below.

Table 4.2: Nutrients uptake capacity by the macrophytes in percentage (%)

Nutrient	**Macrophyte' nutrient uptake capacity/efficiency (%)**	
	Azolla pinnata	***Lemna minor***
Nitrite (NO_2	-84.21	-105.26
Nitrate (NO_3)	-601.79	-479.76
Ammonia (NH_4)	95.64	76.08
Total Phosphorous (TP)	94.37	78.61
Soluble Reactive Phosphorous (SRP)	97.49	88.02
Total Nitrogen (TN)	75.59	73.99

The study showed that both *Lemna minor* and *Azolla pinnata* can be successfully engaged to enhance dissolved nutrient uptake in wastewater treatment systems (table 4.2). Though both plants have shown to be effective in removing nutrients from wastewater, *Azolla pinnata* showed the highest capacity of nutrients uptake than *Lemna minor.* Nutrients removal efficiency was different for all the nutrients by each plant and this may mainly be due to the fact that nutrient components are removed by different processes in phytoremediation (Shah, 2014). For example, ammonia, nitrite and organic nitrogen are initially oxidized to nitrate by rhizoremediation (Tang *et al*., 2017). Nitrate is then absorbed and extracted by plants. Therefore, the oxidation of the former may be completed in shorter retention times, while the extraction of the latter may take longer (Ghosh and Gopal, 2010). The results of this study indicate that both macrophytes (*Lemna minor* and *Azolla pinnata*) play a very important part in the soluble reactive phosphorous (SRP) removal from the wastewater. Both micro-organisms and Plants utilize SRP as a crucial nutrient and their tissues have phosphorous (Shah *et al*., 2015). Total phosphorous removal during the study in the macrophytes treatment was 94.37% for *Azolla pinnata*, and 78.61% for *Lemna minor*. Vermaat and Hanif, (1998) had different findings after performing several batch growth of macrophyte plants that lasted for 12 days using domestic wastewater. Their results showed that *Lemna minor* and *Azolla pinnata* were responsible for around 56% and 18% uptake of total phosphorus, respectively. Their outcome demonstrated that under experimental conditions, *Lemna minor* has a higher capability to remove nutrients which is contrary to the results of the current study. Again Srivastava *et al*. (2008) performed similar study on *Lemna minor* uptake of phosphorous and nitrogen from wastewater and demonstrated that *lemna minor* achieved 74% and 77% removal of nitrogen and phosphorus, respectively. The current study results are concurring with Azarpira *et al*. (2013) results who found out that *Azolla pinnata* had high growth rate and productivity and was very promising in improving pre-treated wastewater quality. *Azolla pinnata* removed nutrients more efficiently than *Lemna minor* hence the nutrients content in the wastewater was significantly lowered in presence of *Azolla pinnata* than in the presence of *Lemna minor*. Again, Forni *et al*. (2001) encouraged interest in using *Azolla pinnata* for the purpose of decontamination of wastewater in low cost wastewater treatment systems and also documented *Azolla pinnata* as the macrophyte with the ability to purify wastewater by removing nitrogen and phosphorous nutrients which are the elements responsible for eutrophication. The nutrient removal efficiencies for two floating macrophytes are shown in table 4.2. *Lemna minor* and *Azolla pinnata* macrophytes have demonstrated potential for removing nutrients in wastewater but some nitrification was detected during the experiment in all treatments which was because there was production of a lot of oxygen in the wastewater. Basically nitrites and nitrates increased over the study period which is implicated by the negative uptake capacity of the two nutrients. It is known that in wastewater excessive nitrogen is bound organically and nitrate is normally released through biological transformation. Therefore, the high rate of organic nitrogen transformation through mineralization and nitrification was the key factor that explains the increase in nitrate and nitrite concentration in the wastewater (Sirage *et al*., 2017). Zhang, (2013) also attests that *Lemna minor* preferentially takes up more ammonia than nitrite and nitrate. This is because nitrogen in form of ammonia is converted directly in to plant protein, rather than being subsequently reduced, as is the case with nitrate once assimilated (El-Shafai *et al*., 2007). Nitrification process may explain the increase in nitrate and nitrite concentration. Findings by Alexia Mackey, (2017) support this hypothesis. In his study, as ammonia concentrations decreased, nitrite and nitrate concentrations increased, indicating that nitrification occurred. Changes in conditions throughout the day could explain variations, as conditions like temperature affect nitrification and denitrification processes. NH_4-N removal was also done by means of nitrification pathway which gave rise to the upturn of NO^{3-}N and NO^{2-}N concentrations as has been reported by Xu and Shen, (2011) and Zhao *et al*., 2014). But nitrate was likely taken up by the plants as levels of ammonia decreased significantly in the wastewater. Since the uptake of nutrients by aquatic macrophytes depends on their biomass production and thus on macrophyte photosynthesis, nutrients uptake would only function optimally during the growing season of the macrophytes (Crispim *et al*., 2009). Aging of the macrophytes may have contributed to low nutrients uptake hence the uptake capacity reducing significantly towards the end of the experiment. This was also observed on the macrophytes biomass increase and this should be considered in future designs. Therefore, a periodical removal of the aging macrophytes to avoid decomposition and a consequential nutrient feedback may enhance nutrient uptake in wastewater. Moreover, longer hydraulic retention time increased the action of the selected floating macrophytes on the wastewater. Removal of the nutrients by the selected plants was strongly correlated to retention time. Thus, the efficiency of the tested macrophytes could be improved by adjusting the technical methods and increasing the hydraulic retention time (Merino-Solís *et al.*, 2015). Sehar *et al.* (2013) found out that After 20 days' retention time, the treated wastewater was free of almost all nutrients and microbial pollutants. Hence, increasing hydraulic retention time was found to ameliorate the operational competence of a wastewater treatment system.

4.5 Nutrients uptake capacity between the two plants during the study period

Nutrient removal efficiency was statistically significant between the control and the two macrophytes for all the nutrients ($P<0.05$) except for the uptake of nitrate (NO_3-N) and ammonia (NH_4-N) by *Lemna minor* where the difference was not statistically significant (F= 5.563; P= 0.162, F= 4.95, P= 0.079 respectively). The uptake of the nutrients between the two floating macrophytes (*Azolla pinnata* and *Lemna minor*) was statistically significant for the soluble reactive phosphorus only (F= 35.183, P= 0.044) (Table 4.3). Table 4.3 below shows the statistical difference between the two plants (*Azolla pinnata* and *Lemna minor*) in the uptake of the nutrients during the study period. Control was used as a standard with the assumption that the nutrients content in the control wastewater remained constant from the beginning of the study to the end since it had no plant in it.

Table 4.3: Comparison of nutrients uptake between the two plants (*Lemna minor* and *Azolla pinnata*) (ANOVA, $P<0.05$)

Nutrients	**F statistic**	**P value**
Nitrite (NO_2)	22.229	0.325
Nitrate (NO_3)	5.563	0.295
Ammonia (NH_4)	4.952	0.652
Soluble Reactive Phosphorous (SRP)	35.183	0.044
Total Phosphorous (TP)	23.183	0.183
Total Nitrogen (TN)	15.949	0.207

During the study period, it was noticed that there was also reduction of nutrients in the control which can be attributed to the role of phytoplankton growing in the control wastewater. Though from the results it is evident that *Azolla pinnata* is better in nutrients uptake, *Lemna minor* might serve dual benefits as an effective environmental reservoir for nutrient removal and producing a protein rich plant with significant fast growth rate that can double its mass within 16 to 48 hours only and can be suitable for use as a supplement for fish and animal feed since its rich in protein (Nassar *et al*., 2015). The higher variability of floating macrophytes in nutrients removal can be due to the fast growth rate and short life span and could result in high turnover rate of biomass and enhanced die backs which ultimately may cause remobilization of nutrients (Tang *et al*., 2017). During peak growth, floating macrophytes can achieve high efficacy whereas during die back nutrients uptake will virtually be low and associated conducive conditions for microbial activities could be impacted (Sooknah and Wilkie, 2004). This condition makes these systems unpredictable and hardly possible to get the steady state conditions (Sirage *et al*., 2017). The main advantage of using floating macrophytes instead of emergent macrophytes is, however, that they can be harvested multiple times a year and that they take up nutrients from both the water layer and the sediment (Sooknah and Wilkie, 2004). Plant growth rate and hydraulic retention time can influence the reduction of contaminants. But the removal efficiency of the plant may vary with the type of plant selected, temperature, treatment method and the properties of the contaminants present in the wastewater. Therefore, an available knowledge and techniques for removal of water contaminants and advances in wastewater treatment can be integrated to assess and control water pollution. According to Greenway, (2003) the rate of nutrients uptake and the assimilation into the plant is key in determining the potential of macrophyte species to be utilized for phytoremediation. Macrophytes species with potential nutrient removal should be chosen. The highest removal of nutrients was observed in *Azolla pinnata* than in *Lemna minor*. Phosphorus is an essential nutrient for all life forms, and is the eleventh-most abundant mineral in the earth’s crust (Shah *et al*., 2015). It is needed for plant growth and is required for many metabolic reactions in plants. According to Shah, (2014), maximum removal of phosphorous by *Lemna minor* showed 15.25% average reduction within 30 days experimental period. In this study, *Lemna minor* showed an uptake capacity of 94.37% of phosphorous in 25 days (Table 4.2). Total phosphorus uptake is mainly dependent upon sorptive physicochemical processes taking place on the substrate (Van de Moortel *et al.*, 2010). Large quantities of phosphate present in wastewater is one of the main causes of eutrophication that negatively affects many natural water bodies, both fresh water and marine (Khanijo, 2002). It is desirable that wastewater treatment facilities remove phosphorus from the wastewater before they are returned to the environment. Total removal or at least a significant reduction of the nutrient is obligatory, if not always fulfilled, in wastewater treatment. This is because mostly eutrophic problems are highly associated with phosphorous.

4.6 Pearson's correlation between various wastewater parameters

To find out how the various wastewater parameters were correlating, Pearson's correlation analysis was carried out. Table 4.4 shows Pearson's Correlation coefficients between various water parameters studied.

Temperature had a statistically significant strong negative correlation with nitrates and nitrites. This is because both nitrates and nitrites were increasing during the study period. Again, temperature had a statistically significant strong positive correlation with ammonia, total phosphorous, soluble reactive phosphorous and total nitrogen nutrients over the experimental period (Table 4.4). This is because these nutrients were decreasing during the study period due to uptake by the macrophytes which were grown in the wastewater. Temperature is one of the limiting factors in the aquatic environment (Lawson, 2011; Murhekar and Rathod, 2011). Macrophytes are sensitive to changes in water temperature and require a certain temperature range to survive and thrive. If water temperature is outside the range for a long time, macrophytes can be stressed and die. Dissolved oxygen had a weak correlation with all the nutrients which was only significant in ammonia and nitrates. Oxygen concentration in the wastewater was increasing due to the effect of photosynthetic activities carried out by the macrophytes and higher solubility of oxygen in the wastewater due the atmospheric diffusion. Temperature also affects the amount of oxygen water can hold. Cold water holds more oxygen than warm water, and all aquatic organisms need oxygen to survive. pH had a weak negative correlation with nitrate and nitrite though the correlation was significant with nitrate (Table 4.4). This suggests that the pH was decreasing as the nitrates and nitrites concentrations were increasing. The correlation was positive with all other nutrients but not statistically significant. Variations in pH can be attributed to microorganisms in the water which break down organic materials to simpler products like CO_2. The CO_2 dissolves in water to produce carbonic acid (H_2CO_3), which lowers the pH. The lowering of pH of water also comes from decaying vegetation and organic matter. Photosynthesis, and respiration are also responsible for variations of pH in water (Lawson, 2011); Limoli *et al*., 2016). During the study period, it was found that pH was within the favourable range which was in favour of the functioning of the macrophytes in the uptake of the nutrients. For the electrical conductivity, the correlation with all the nutrients was statistically significant though weak in nitrites and soluble reactive phosphorous (Table 4.4). For all the other nutrients the correlation was strong and statistically significance. The correlation between the total suspended solids and all the nutrients was weak and not statistically significant. The correlation between biological oxygen demand and all the nutrients was weak. But it was only significant in nitrates, ammonia and soluble reactive phosphorous. Evaporation rates, and nutrient status are the major factors that influence conductivity in wastewater (Kinnear and Garnett, 1999). This is because the level of conductivity in wastewater gives a good indication of the amount of charged ions dissolved in it (Li *et al*., 2008).

The correlation between macrophytes' productivity of biomass and all the nutrients was statistically significant and positive between nitrites and nitrates (Table 4.4). This is a clear indication that as nitrite and nitrate nutrients concentration in the wastewater were increasing there was also high biomass yield by the macrophytes as a consequence of nutrient uptake. All the other nutrients were negatively correlated with macrophytes' biomass productivity meaning that as the biomass increased, the nutrients were decreasing due to their uptake by the plants. The removal of pollutants by macrophytes may occur through a number of processes not limited to plant uptake/removal efficiency and adsorption (Shah *et al*., 2015) hence the nutrients reduction. At the same time that pollutant removal from wastewater occurred, great quantities of biomass of the macrophytes were produced due to their nutrient absorbing capacity. Primary productivity and biomass increase are the important parameters in macrophytes uptake of nutrients (Shah *et al*., 2014). Macrophytes have high tolerance for the fluctuations in environment conditions and show high photosynthetic efficiencies. Uptake of nutrients by macrophytes is an essential for their growth and reproduction. The high productivity of macrophytes enables substantial amounts of nutrients to be stored in plant biomass (Shah *et al*., 2015). All the physicochemical parameters were negatively correlated with biomass except total suspended solids. Suggesting that as the biomass was increasing, all the physicochemical parameters were decreasing or static except total suspended solids that were increasing. This is a clear indication that the physicochemical parameters values were suitable for the growth of the macrophytes and had made the wastewater environment suitable for the growth of the macrophytes hence increase in biomass.

Table 4.4: Pearson's correlation between the various wastewater physico-chemical variables

	TEMP	**D.O**	**pH**	**EC**	**TSS**	**BOD$_5$**	**NO$_2$**	**NO$_3$**	**NH$_4$**	**TP**	**SRP**	**TN**
D.O	-0.191	1										
P value	0.015											
pH	0.215	0.421	1									
P value	0.006	0.000										
EC	0.656	-0.289	0.318	1								
P value	0.000	0.000	0.000									
TSS	0.011	-0.185	-0.074	0.155	1							
P value	0.907	0.055	0.449	0.108								
BOD$_5$	-0.185	0.997	0.418	-0.295	-0.203	1						
P value	0.018	0.000	0.000	0.000	0.035							
NO$_2$	-0.667	0.021	-0.077	-0.458	0.056	0.012	1					
P value	0.000	0.788	0.331	0.000	0.564	0.882						
NO$_3$	-0.888	0.194	-0.155	-0.549	0.037	0.182	0.752	1				
P value	0.000	0.013	0.049	0.000	0.706	0.021	0.000					
NH$_4$	0.874	-0.195	0.085	0.507	-0.008	-0.184	-0.731	-0.933	1			
P value	0.000	0.013	0.281	0.000	0.933	0.019	0.000	0.000				
TP	0.808	-0.063	0.091	0.520	-0.090	-0.052	-0.795	-0.887	0.889	1		
P value	0.000	0.426	0.247	0.000	0.357	0.509	0.000	0.000	0.000			
SRP	0.681	0.143	0.121	0.354	-0.156	0.159	-0.811	-0.809	0.811	0.887	1	
P value	0.000	0.070	0.126	0.000	0.107	0.043	0.000	0.000	0.000	0.000		
TN	0.856	-0.072	0.117	0.528	-0.063	-0.058	-0.823	-0.923	0.926	0.937	0.912	1
P value	0.000	0.361	0.138	0.000	0.518	0.460	0.000	0.000	0.000	0.000	0.000	
BIOMASS	-0.676	-0.089	-0.255	-0.446	0.231	-0.132	0.819	0.778	-0.751	-0.862	-0.944	-0.874
P value	0.000	0.522	0.062	0.000	0.093	0.340	0.000	0.000	0.000	0.000	0.000	0.000

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

i). Physicochemical parameters were stable and within the optimum range for growth of the floating macrophytes

ii). There was temporal change in nutrients concentration in the wastewater after macrophytes phtoremediation

iii). There was temporal increase in the macrophytes' biomass (*Azolla pinnata* and L*emna minor*) which was attributed to nutrients uptake from the wastewater.

iv). Based on this study *Azolla pinnata* proved to be better than *Lemna minor* in the nutrients removal from the wastewater.

5.2 Recommendations

i). The effluent should be monitored regularly to ensure that the physicochemical parameters meet the given threshold before releasing it to river Njoro to prevent their detrimental effects on the receiving water bodies.

ii). Nutrients concentrations in the effluent should be monitored regularly to save the receiving water body from the risk of eutrophication which is detrimental to aquatic life.

iii). There should be a periodical removal of dead macrophytes in the effluent treatment system to avoid decomposition and a consequential nutrient release to the wastewater to enhance nutrient uptake by the macrophytes.

iv). This study recommends increasing the population of *Azolla pinnata* in the constructed wetlands meant for wastewater treatment especially within the tropics.

5.3. Suggestion for further research

This study recommends mixed plants nutrients uptake verses single plants as an area of further study.

REFERENCES

Admasu, T. (2007). Assessment of biological integrity using physico-chemical parameters and macroinvertebrate community index along Sebeta River, Ethiopia (Doctoral dissertation, Addis Ababa University).

Akpor, O. B. (2011). Wastewater effluent discharge: Effects and treatment processes. In *Int. Conf. Chem. Biol. Environ. Eng* (Vol. 20, pp. 85-91).

Akpor, O. B., Momba, M. N. B., and Okonkwo, J. (2008). Effect of nutrient/carbon supplements on biological phosphate and nitrate uptake by protozoan isolates. *Journal of applied sciences*, *8*(3), 489-495.

Alexia Mackey, (2017). The use of *Lemna minor* duckweed to remove nitrogen and phosphorous in wastewater effluent from a decentralized treatment system (DEWATS). California State Polytechnic University, Pomona Department of Civil Engineering (Thesis).

American Public Health Association (APHA), (2005). Standard methods for the examination of water and waste water, 21st edition, American public health association Washington D.C. USA

Arora, A.; Saxena, S., (2005). Cultivation of *Azolla microphylla* biomass on secondary-treated Delhi municipal effluents. *biomass bioenergy*, *29*(1), 60-64.

Ashby, E., Wangermann, E., and Winter, E. J. (1949). Studies in the Morphogenesis of Leaves: III. Preliminary Observations on Vegetative Growth in *Lemna minor*. *New phytologist*, *48*(3), 374-381.

Ayres, R. M., and Mara, D. D. (1996). Analysis of wastewater for use in agriculture. A laboratory manual of parasitological and bacteriological techniques [on line].

Azarpira, H.; Behdarvand, P.; Dhumal, K.; Pondhe, G., (2013). Phytoremediation of municipal wastewater by using aquatic plants. *Advances in environmental biology*, 21(2), 4649-4655.

Bani, R. (2011). Wastewater Management. *Wastewater: evaluation and management*, 379.

Bastviken, S. (2006).Nitrogen removal in treatment wetlands –Factors influencing spatial and temporal variations. PhD *Dissertation No 1041*. Linköping University, Sweden

Belmont, M. A., and Metcalfe, C. D. (2003). Feasibility of using ornamental plants (*Zantedeschia aethiopica*) in subsurface flow treatment wetlands to remove nitrogen, chemical oxygen demand and nonylphenol ethoxylate surfactants—a laboratory-scale study. *Ecological engineering*, *21*(4-5), 233-247.

Bick, A., Gillerman, L., Manor, Y., and Oron, G. (2012). Economic assessment of an integrated membrane system for secondary effluent polishing for unrestricted reuse. *Water, 4*(1), 219-236.

Biswas, S., and Sarkar, S. (2013). Azolla cultivation: A supplementary cattle feed production through natural resource management. *Agriculture update, 8*(4), 670-672.

Borges, A. K. P., Tauk-Tornisielo, S. M., Domingos, R. N., and Angelis, D. D. F. D. (2008). Performance of the constructed wetland system for the treatment of water from the Corumbataí River. *Brazilian archives of biology and technology*, *51*(6), 1279-1286.

Bornette, G., and Puijalon, S. (2011). Response of aquatic plants to abiotic factors: a review. *Aquatic Sciences,* 73(1), 1-14.

Bornette, G., Tabacchi, E., Hupp, C., Puijalon, S., and Rostan, J. C. (2008). A model of plant strategies in fluvial hydrosystems. *Freshwater biology*, *53*(8), 1692-1705.

Brix, H. (1994). Use of constructed wetlands in water pollution control: historical development, present status, and future perspectives. *Water science and technology*, *30*(8), 209-223.

Brix, H. (1997). Do macrophytes play a role in constructed treatment wetlands? *Water science and technology, 35*(5), 11-17.

Brix, H., Arias, C. A., and Del Bubba, M. (2001). Media selection for sustainable phosphorus removal in subsurface flow constructed wetlands. *Water science and technology, 44*(11-12), 47-54.

Buchauer, K. (1998). A comparison of two simple titration procedures to determine volatile fatty acids in influents to waste-water and sludge treatment processes. *Water SA-Pretoria-, 24*, 49-56.

Bunting, S. W. (2013). *Principles of sustainable aquaculture: promoting social, economic and environmental resilience*. Routledge.

Chapman, D. V. (Ed.). (1996). Water quality assessments: a guide to the use of biota, sediments and water in environmental monitoring. CRC Press.

Cheng, J., Landesman, L., Bergmann, B. A., Classen, J. J., Howard, J. W., and Yamamoto, Y. T. (2002). Nutrient removal from swine lagoon liquid by Lemna minor 8627. *Transactions of the ASAE, 45*(4), 1003.

Coleman J, Hench K, Garbutt AS, Bissonnette G, Skousen J (2001) Treatment of Domestic Wastewater by Three Plant Species in Constructed Wetlands. Water, Air and Soil Pollution 128: 283-295.

Corcoran, E. (Ed.). (2010). Sick water: the central role of wastewater management in sustainable development: *a rapid response assessment*. UNEP/Earthprint.

Crispim, M. C., Vieira, A. C. B., Coelho, S. F. M., and Medeiros, A. M. A. (2009). Nutrient uptake efficiency by macrophyte and biofilm: practical strategies for small-scale fish farming. *Acta limnologica brasiliensia*, *21*(4), 387-391.

Culley, D. D., Rejmánková, E., Květ, J., and Frye, J. B. (1981). Production, chemical quality and use of duckweeds (Lemnaceae) in aquaculture, waste management, and animal feeds. *Journal of the world mariculture society, 12*(2), 27-49.

Dalu, J. M. and Ndamba, J. 2003. Duckweed based wastewater stabilization ponds for wastewater treatment (a low cost technology for small urban areas in Zimbabwe). Physics and Chemistry of the Earth, Parts A/B/C, 28(20), 1147-1160.

Daud, M. K., Ali, S., Abbas, Z., Zaheer, I. E., Riaz, M. A., Malik, A. and Zhu, S. J. (2018). Potential of Duckweed (L*emna minor*) for the Phytoremediation of Landfill Leachate. *Journal of chemistry, 2018*. (2018).

Delzer, G. C., and McKenzie, S. W. (2003). Five-day biochemical oxygen demand: US Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A7, section 7.0.

Devi, M., Thomas, D. A., Barber, J. T., and Fingerman, M. (1996). Accumulation and physiological and biochemical effects of cadmium in a simple aquatic food chain. *Toxicology and environmental safety*, *33*(1), 38-43.

Dhote, S., and Dixit, S. (2009). Water quality improvement through macrophytes – a review. *Environmental monitoring and assessment*, *152*(1-4), 149-153.

Dipu Sukumaran. (2013). "Phytoremediation of Radionuclide Polluted Industrial Effluent by Constructed Wetland Technology", *Advances in agriculture, sciences and engineering research*, Volume 3 (4) pp: 768 - 774

Dipu, S., Kumar, A. A., and Thanga, V. S. G. (2011). Phytoremediation of dairy effluent by constructed wetland technology. *The environmentalist*, *31*(3), 263-278.

Dixit, A. R. C. H. A. N. A., Dixit, S. A. V. I. T. A., and Goswami, C. S. (2011). Process and plants for wastewater remediation: a review. *Scientific reviews and chemical communications*, *11*, 71-7.

Droste, R. L., and Gehr, R. L. (2018). *Theory and practice of water and wastewater treatment*. John Wiley and Sons.

El-Shafai, S. A., El-Gohary, F. A., Nasr, F. A., Van Der Steen, N. P., and Gijzen, H. J. (2007). Nutrient recovery from domestic wastewater using a UASB-duckweed ponds system. *Bioresource technology*, 98(4), 798-807.

Environmental, F. (2014). Conductivity, salinity and total dissolved solids. *Fundamentals of environmental measurements. Fondriest environmental, USA.*

Environmental, I. F. C. (2007). Health, and Safety (EHS) Guidelines. *Environmental energy conservation.*

Fabris, L. (2013). The influence of vegetation distribution on wetland efficiency.

Feiler, U., Krebs, F., and Heininger, P. (2006). Aquatic plant bioassays used in the assessment of water quality in German rivers. In *Macrophytes in Aquatic ecosystems: From biology to management* (pp. 67-71). Springer Netherlands.

Forni, C., and Nicolai, M. A. (2001). Potential of the Small Aquatic Plans Azolla and Lemna for Nitrogenous Compounds Removal from Wastewater. *WIT transactions on ecology and the environment, 49.*

Forni, C.; Chen, J.; Tancioni, L.; Caiola, M. G., (2001). Evaluation of the fern Azolla for growth, nitrogen and phosphorus removal from wastewater. *Water Res., 35*(6), 1592-1598.

Fraser, L. H., Carty, S. M., and Steer, D. (2004). A test of four plant species to reduce total nitrogen and total phosphorus from soil leachate in subsurface wetland microcosms. *Bioresource technology, 94*(2), 185-192.

Ghosh, D., and Gopal, B. (2010). Effect of hydraulic retention time on the treatment of secondary effluent in a subsurface flow constructed wetland. *Ecological engineering, 36*(8), 1044- 1051.

Gibson, A., and Maniocha, M. (2004). The use of magnesium hydroxide slurry for biological treatment of municipal and industrial wastewater. *Martin marietta magnesia specialties, LLC, white paper, 7.*

Greenway, M. (2003). Suitability of macrophytes for nutrient removal from surface flow constructed wetlands receiving secondary treated sewage effluent in Queensland, Australia. *Water science and technology, 48*(2), 121-128.

Guo-feng, L., Cheng-xin, F., Shi-qun, H., Jun, H., and Paerl, H. W. (2000). The Response of Macrophytes to Nutrients and Implications for the Control of Phytoplankton Blooms in East Taihu Lake, China. *Journal of pollution effects and control, 51*(5), 1-5.

Gustin, S.; Marinsek-Logar, R., (2011). Effect of pH, temperature and air flow rate on the continuous ammonia stripping of the anaerobic digestion effluent. Process saf. Environ. Prot. *89*(1), 61-66.

Han, B.; Zhang, S.; Wang, P.; Wang, C., (2018). Effects of water flow on submerged macrophyte-biofilm systems in constructed wetlands. *Scientific reports*, *8*(1), 2650.

Hanna, S. M. (2008). Examples of radiation wastewater treatment implemented in various countries. Twelfth International Water Technology Conference, Alexandria, 1-7

Hasar, H., and Cuci, Y. (2000). Removal of Cr (VI), Cd (II), and Cu (II) by activated carbon prepared from almond husk. *Environmental technology*, *21*(12), 1337-1342.

Hauck, F. W. (1978). China: recycling of organic wastes in agriculture. *Food and agricultural organization of the United Nations, Rome*.

Hey, D., Kostel, J., and Sullivan, G. (2007). Nutrient Farming: A Means to Finance Large-Scale Wetland Restoration. *Best practices in conservation and restoration*, *12*(6), 13.

Ijaz, A., Shabir, G., Khan, Q. M., and Afzal, M. (2015). Enhanced remediation of sewage effluent by endophyte-assisted floating treatment wetlands. *Ecological engineering*, *84*, 58-66.

Iqbal, J., Saleem, M., and Javed, A. (2017). Effect of electrical conductivity (EC) on growth performance of duckweed at dumpsite leachate. *International journal of science, environment and technology, 6, 1989-1999.*

Iram, S., Ahmad, I., Riaz, Y., and Zahra, A. (2012). Treatment of wastewater by *Lemna minor* Shazia Iram, iftikhar Ahmad, Yousaf Riaz and Ayesha Zahra. *PAK. J. BOT*, *44*(2), 553-557.

Kadlec, R. H. (1999). Chemical, physical and biological cycles in treatment wetlands. *Water science and technology*, *40*(3), 37-44.

Kadlec, R. H., and Li, X. M. (1990). Peatland ice/water quality. *Wetlands*, *10*(1), 93-106.

Kalff, J. (2002). Limnology*: inland water ecosystems* (No. 504.45 KAL).

Karczmarczyk, A., and Renman, G. (2011). Phosphorus Accumulation pattern in a subsurface constructed wetland treating residential wastewater. *Water*, *3*(1), 146-156.

Kassa, Y., and Mengistou, S. (2014). Nutrient uptake efficiency and growth of two aquatic macrophyte species under constructed wetlands, Ethiopia. *SINET: Ethiopian journal of science*, *37*(2), 95-104.

Kennedy, M. P., Soulsby, C. H. R. I. S., Gibbins, C. H. R. I. S., Murphy, K. J., Tapia Grimaldo, J., and Varandas Martins, S. (2012). Development of a macrophyte-based biomonitoring protocol for Zambian rivers. In Proceedings of 9th International Symposium Ecohydraulics, Vienna, *http://www. Ise2012 boku. Ac. at/papers/15451* (Vol. 2).

Khanijo, I. (2002). Nutrient Removal from Wastewater by Wetland System. *Wetlands*, *10*.

Kiepper, B. H. (2013). Microalgae utilization in wastewater treatment. *Cooperative extension bulletin, 1419, 1-5*

Kinnear, A., and Garnett, P. (1999). Water chemistry of the wetlands of the Yellagonga Regional Park, Western Australia. *Journal of the royal society of western Australia*, *82,* 79-85.

Körner, S., Das, S. K., Veenstra, S., and Vermaat, J. E. (2001). The effect of pH variation at the ammonium/ammonia equilibrium in wastewater and its toxicity to *Lemna gibba*. *Aquatic botany*, *71*(1), 71-78.

Lamarck, J. D. (1783). Encyclopedia methods. *Botanique*, *1*(1), 1-344.

Landolt, E. (1986). The family Lemnaceaea monographic study, Vol 1. Veroff Geoff Inst ETH stiftung. *Rubel. Zurich*.

Lawson, E. O. (2011). Physico-chemical parameters and heavy metal contents of water from the Mangrove Swamps of Lagos Lagoon, Lagos, Nigeria. *Advances in biological research*, *5*(1), 8-21.

Lee, C. G., Fletcher, T. D., and Sun, G. (2009). Nitrogen removal in constructed wetland systems. *Engineering in life sciences*, 9(1), 11-22.

Lemon, G. D., and Posluszny, U. (2000). Comparative shoot development and evolution in the Lemnaceae. *International journal of plant sciences*, *161*(5), 733-748.

Leong, L. Y., Kuo, J., and Tang, C. C. (2008). Disinfection of wastewater effluent: Comparison of alternative technologies. Alexandria, Va.: *Water environment research foundation*.

Lepcha, O. T. (2016). Wastewater Treatment Using Aquatic Plants.

Levi, P. S.; Riis, T.; Alnøe, A. B.; Peipoch, M.; Maetzke, K.; Bruus, C.; Baattrup-Pedersen, A., (2015). Macrophyte complexity controls nutrient uptake in lowland streams. *Ecosyst. 18*(5), 914-931.

Levlin, E. (2010). Conductivity measurements for controlling municipal waste-water treatment. In *Proceedings of a polish-Swedish-Ukrainian seminar* (pp. 51-62).

Li, H., Jin, Y., Mahar, R., Wang, Z., and Nie, Y. (2008). Effects and model of alkaline waste activated sludge treatment. *Bioresource technology*, *99*(11), 5140-5144.

Limoli, A.; Langone, M.; Andreottola, G., (2016). Ammonia removal from raw manure digestate by means of a turbulent mixing stripping process. *J. Environ. Manage*. *176*, 1-10.

Liu, Y. (2007). Wastewater purification: aerobic granulation in sequencing batch reactors. *CRC Press*.

Luklema, L. (1969). Factors affecting pH change in alkaline waste water treatment—I. *Water Research*, *3*(12), 913-930.

Lumpkin, T. A. and Plucknett, D. L. (1980). Azolla: botany, physiology, and use as a green manure. *Economic botany*, *34*(2), 111-153.

Maine, M. A., Sune, N., Hadad, H., Sánchez, G., and Bonetto, C. (2007). Removal efficiency of a constructed wetland for wastewater treatment according to vegetation dominance. *Chemosphere*, *68*(6), 1105-1113.

Maruthi, Y. A., and Rao, S. R. (2001). Effect of Sugar mill Effluent on Organic Reserves of Fish. *Pollution research*, *20*(2), 167-171.

Mayes, W. M., Batty, L. C., Younger, P. L., Jarvis, A. P., Kõiv, M., Vohla, C., and Mander, U. (2009). Wetland treatment at extremes of pH: a review. *Science of the total environment, 407*(13), 3944-3957.

Merino-Solís, M.; Villegas, E.; de Anda, J.; and López-López, A. (2015). The effect of the hydraulic retention time on the performance of an ecological wastewater treatment system: An anaerobic filter with a constructed wetland. *Water*, *7*(3), 1149-1163.

Meyer, D., Chazarenc, F., Claveau-Mallet, D., Dittmer, U., Forquet, N., Molle, P., and Campà, R. S. (2015). Modelling constructed wetlands: scopes and aims–a comparative review. *Ecological engineering*, *80*, 205-213.

Mitsch, W. J., Day, J. W., Gilliam, J. W., Groffman, P. M., Hey, D. L., Randall, G. W., and Wang, N. (2001). Reducing Nitrogen Loading to the Gulf of Mexico from the Mississippi River Basin: Strategies to Counter a Persistent Ecological Problem Ecotechnology—the use of natural ecosystems to solve environmental problems—should be a part of efforts to shrink the zone of hypoxia in the Gulf of Mexico. *Bioscience*, *51*(5), 373-388.

Molisani, M. M., Rocha, R., Machado, W., Barreto, R. C., and Lacerda, L. D. (2006). Mercury contents in aquatic macrophytes from two reservoirs in the Paraíba do Sul: Guandú River

Momba, M. N. B. (2010). Wastewater protozoan-driven environmental processes for the protection of water sources. *Biomass*, pp183.

Muradov, N., Taha, M., Miranda, A. F., Kadali, K., Gujar, A., Rochfort, S., and Mouradov, A. (2014). Dual application of duckweed and *azolla pinnata* for wastewater treatment and renewable fuels and petrochemicals production. *Biotechnology for biofuels*, *7*(1), 30.

Murhekar Gopalkrushna, H., and Rathod, R. G. (2011). Determination of physico-chemical parameters of surface water samples in and around Akot City. *International journal of research in chemistry and environment*, *1*(2), 183-187.

Mwanyika F. T., Ogendi G. M. and Kipkemboi J. K. (2016). Removal of heavy metals from wastewater by a constructed wetland system at Egerton University, Kenya, *Journal of environmental science, toxicology and food technology (2319-2402), p- ISSN: 2319-2399.Volume 10.*

Nair, C. S., and Kani, K. M. (2016). Phytoremediation of dairy effluent using aquatic macrophytes. *International journal of scientific and engineering research*, *7*(4), 253-259.

Nasr, F., Doma, H., and Nassar, H. 2009. Treatment of domestic wastewater using an anaerobic baffled reactor followed by a duckweed pond for agricultural purposes. *The environmentalist*, 29(3), 270-279.

Nassar, H. F., Shaban, A. M., Bassem, S. M., and Abdel-Gawad, F. K. (2015) Utilization of duckweed (DW) in nutrient removal from agricultural waste water and producing alternative economic animal fodder.

O’Dell, J. W. (1993). Determination of nitrate–nitrite nitrogen by automated colorimetry. *Methods for the determination of inorganic substances in environmental samples. US Environmental Protection Agency, Washington, DC.*

Odongo, O. and Partners consulting Engineers. (1989). Egerton University Water Supply and Sewerage Systems Rehabilitation and Expansion Programme, Egerton university, Kenya.

Ozengin, N., and Elmaci, A. (2007). Performance of Duckweed (*Lemna minor*) on different types of wastewater treatment. *Journal of environmental biology*, *28*(2), 307-314.

Park, D., and Roesner, L. A. (2012). Evaluation of pollutant loads from stormwater BMPs to receiving water using load frequency curves with uncertainty analysis. *Water research*, *46*(20), 6881-6890.

Pena, L., Oliveira, M., Fragoso, R., & Duarte, E. (2017). Potential of duckweed for swine wastewater nutrient removal and biomass valorisation through anaerobic co-digestion. *Journal of sustainable development of energy, water and environment systems*, *5*(2), 127-138.

Pereira, P. H. F., Voorwald, H. C. J., Cioffi, M. O. H., Mullinari, D. R., Da Luz, S. M., and Da Silva, M. L. C. P. (2011). Sugarcane bagasse pulping and bleaching: Thermal and chemical characterization. *Bio. Resources*, *6*(3), 2471-2482.

Priya, A., Avishek, K., and Pathak, G. (2012). Assessing the potentials of *Lemna minor* in the treatment of domestic wastewater at pilot scale. *Environmental monitoring and assessment*, *184*(7), 4301-4307.

Quan, J., Tie, X., Zhang, Q., Liu, Q., Li, X., Gao, Y., and Zhao, D. (2014). Characteristics of heavy aerosol pollution during the 2012–2013 winter in Beijing, China. *Atmospheric environment*, *88*, 83-89.

Ra, J. S., Kim, H. K., Chang, N. I., and Kim, S. D. (2007). Whole effluent toxicity (WET) tests on wastewater treatment plants with *Daphnia magna* and *Selenastrum capricornutum*. *Environmental monitoring and assessment*, *129*(1-3), 107-113.

Raji, M. I. O., Ibrahim, Y. K. E., Tytler, B. A., and Ehinmidu, J. O. (2015). Physicochemical Characteristics of Water Samples Collected from River Sokoto, Northwestern Nigeria. *Atmospheric and climate sciences*, *5*(03), 194.

Reddy, G. V., and Rodrigues, V. (1999). A glial cell arises from an additional division within the mechanosensory lineage during development of the microchaete on the Drosophila notum. *Development*, *126*(20), 4617-4622.

Rizwana, M., Darshan, M., and Nilesh, D. (2014). Phytoremediation of textile waste water using potential wetland plant: eco sustainable approach. *International journal of Interdisciplinary and multidisciplinary studies (IJIMS)*, *1*(4), 130-138.

Saeed, T., and Sun, G. (2012). A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: dependency on environmental parameters, operating conditions and supporting media. *Journal of environmental management*, *112*, 429-448.

Sahu, O. P., and Chaudhari, P. K. (2015). The Characteristics, Effects, and Treatment of Wastewater in Sugarcane Industry. *Water Quality, Exposure and Health*, *7*(3), 435-444.

Samuel, S. (2011). Physico-chemical analysis of sugar mill effluent, contaminated soil and its effect on seed germination of paddy (Oryza sativa L.). *International journal of pharmaceutical and biological archive*, *2*(5).

Sand- Jensen, K., Binzer, T., and Middelboe, A. L. (2007). Scaling of photosynthetic production of aquatic macrophytes–a review. *Oikos*, *116*(2), 280-294.

Saraiva, C. B., Matos, A. T., Matos, M. P., and Miranda, S. T. (2018). Influence of Substrate and Species Arrangement of Cultivated Grasses on the Efficiency of Horizontal Subsurface Flow Constructed Wetlands. *Engenharia agricola*, 38(3), 417- 425.

Saunders, R. M., and Fowler, K. (1992). A morphological taxonomic revision of *Azolla pinnata* LAM. Section Rhizosperma (MEY.) METT. (Azollaceae). *Botanical journal of the linnean society, 109*(3), 329-357.

Sawidis, T., Chettri, M. K., Zachariadis, G. A., and Stratis, J. A. (1995). Heavy metals in aquatic Plants and sediments from water systems in Macedonia, Greece. *Ecotoxicology and environmental safety*, *32*(1), 73-80.

Saxena, C., and Madan, S. (2012). Evaluation of adsorbents efficacy for the removal of pollutants from sugar mill effluent. *ARPN J. Agric. Biol. Sci*, *7*(5), 325-329.

Sehar, S., Aamir, R., Naz, I., Ali, N., and Ahmed, S. (2013). Reduction of contaminants (physical, chemical, and microbial) in domestic wastewater through hybrid constructed wetland. *ISRN microbiology*, *20 (13) 101.*

Shah, M., Hashmi, H. N., Ali, A., and Ghumman, A. R. (2014). Performance assessment of aquatic macrophytes for treatment of municipal wastewater. *Journal of environmental health science and engineering*, *12*(1), 106.

Shah, M., Hashmi, H. N., Ghumman, A. R., and Zeeshan, M. (2015). Performance assessment of aquatic macrophytes for treatment of municipal wastewater. *Journal of the South African institution of civil engineering*, *57*(3), 18-25.

Shelef, O., Gross, A., and Rachmilevitch, S. (2013). Role of plants in a constructed wetland: current and new perspectives. *Water*, *5*(2), 405-419.

Siddiqui, W. A., and Waseem, M. (2012). A comparative study of sugar mill treated and untreated effluent-a case study. *Orient J. Chem*, *28*(4), 1899-1904.

Sipaúba-Tavares, L. H., Favero, E. G. P., & BRAGA, F. D. S. (2002). Utilization of macrophyte biofilter in effluent from aquaculture: I. Floating plant. *Brazilian journal of biology*, *62*(4A), 713-723.

Sirage Ali A, Piet Lens PN, Hans Van Bruggen JJA (2017) Purifying Municipal Wastewater Using Floating Treatment Wetlands: Free Floating and Emergent Macrophytes. *Adv. Recycling Waste Manag* 2: 138. doi:10.4172/2475-7675.1000138

Sivasanthi, K., and Pandian, R. S. (2012). Effect of sugar mill effluent on seed germination of peanut (*Arachis hypogaea*) and green gram (*Vigna radiata*). *International journal of pharmaceutical and chemical sciences*, *1*, 804-806.

Skillicorn, P., Spira, W., and Journey, W. (1993). A New Aquatic Farming System for Developing Countries. *The world bank group*.

Smith, V. H., Tilman, G. D., and Nekola, J. C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental pollution*, *100*(1), 179-196.

Solano, M. L.; Soriano, P.; Ciria, M. P., (2004). Constructed wetlands as a sustainable solution for wastewater treatment in small villages. *Biosystems engineering*. *87*(1), 109-118.

Sood, A., Uniyal, P. L., Prasanna, R., and Ahluwalia, A. S. (2012). Phytoremediation potential of aquatic macrophyte, *Azolla pinnata*. *Ambio*, *41*(2), 122-137.

Sooknah, R. D., and Wilkie, A. C. (2004). Nutrient removal by floating aquatic macrophytes cultured in anaerobically digested flushed dairy manure wastewater. *Ecological engineering*, *22*(1), 27-42.

Spinosa, L. (Ed.). (2011). Wastewater Sludge. *Iwa publishing*.

Srivastava, J.; Gupta, A.; Chandra, H., (2008). Managing water quality with aquatic macrophytes. *Rev. Environ. Sci. Biotechnol.*, *7*(3), 255-266.

Stottmeister, U., Wießner, A., Kuschk, P., Kappelmeyer, U., Kästner, M., Bederski, O. and Moormann, H. (2003). Effects of plants and microorganisms in constructed wetlands for wastewater treatment. *Biotechnology advances*, *22*(1-2), 93-117.

Tang, Y., Harpenslager, S. F., van Kempen, M. M., Verbaarschot, E. J., Loeffen, L. M., Roelofs, J. G. and Lamers, L. P. (2017). Aquatic macrophytes can be used for wastewater polishing but not for purification in constructed wetlands. *Biogeosciences*, *14*(4), 755-766.

Tchobanoglous, G., and Burton, F. L. (1991). Wastewater engineering. *Management*, *7*, 1-4.

Tripathi, B. D., and Upadhyay, A. R. (2003). Dairy effluent polishing by aquatic macrophytes. *Water, air, and soil pollution*, *143*(1-4), 377-385.

U.S.EPA, (1998). Total Suspended Solids Laboratory Method 160.2, http://www.epa.gov/region09/lab/sop

Ugya, A. Y., and Imam, T. S. (2015). The efficiency of *Eicchornia crassipes* in the phytoremediation of waste water from Kaduna Refinery and petrochemical company. *Journal of environmental science and toxicology*, 43-47.

Uwidia, I. E., and Ukulu, H. S. (2013). Studies on electrical conductivity and total dissolved solids concentration in raw domestic wastewater obtained from an estate in Warri, Nigeria. *Greener journal of physical sciences*, *3*(3), 110-114.

Van de Moortel A, Meers E, De Pauw N, Tack F (2010) Effects of Vegetation, Season and Temperature on the Removal of Pollutants in Experimental Floating Treatment Wetlands. *Water, air and soil pollution*, 212: 281-297.

Verdegem, M. C. (2013). Nutrient discharge from aquaculture operations in function of system design and production environment. *Reviews in aquaculture*, *5*(3), 158-171.

Verhoeven, J. T., and Meuleman, A. F. (1999). Wetlands for wastewater treatment: opportunities and limitations. *Ecological engineering,* 12(1), 5-12.

Vermaat, J. E., and Hanif, M. K. (1998). Performance of common duckweed species (Lemnaceae) and the waterfern *Azolla filiculoides* on different types of waste water. *Water research*, *32*(9), 2569-2576.

Vymazal, J. (1999). Removal of BOD5 in constructed wetlands with horizontal sub-surface flow: Czech experience. *Water science and technology, 40*(3), 113-138.

Vymazal, J. (2007). Removal of nutrients in various types of constructed wetlands. *Science of the total environment*, *380*(1), 48-65.

Vymazal, J. (2010). Constructed wetlands for wastewater treatment. *Water*, *2*(3), 530-549.

Wagner, P., Nock, S., Spudich, J. A., Volkmuth, W. D., Chu, S., Cicero, R. L. and Chidsey, C. E. (1997). Bioreactive self-assembled monolayers on hydrogen-passivated Si (111) as a new class of atomically flat substrates for biological scanning probe microscopy. *Journal of structural biology*, *119*(2), 189-201.

Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y, and Ruan, R. (2010). Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant. *Applied biochemistry and biotechnology*, 162(4), 1174-1186.

Watanabe, I., and Berja, N. S. (1983). The growth of four species of Azolla as affected by temperature. *Aquatic botany*, *15*(2), 175-185.

Wetzel, R. G. (Ed.). (2012). Periphyton of Freshwater Ecosystems: Proceedings of the First International Workshop on Periphyton of Freshwater Ecosystems Held in Växjö, Sweden, 14–17 September 1982 (Vol. 17). *Springer science and business MEDIA.*

WHO, (1993) *Guidelines for Drinking-Water Quality. Volume 1. Recommendations.* Second edition, World Health Organization, Geneva, 188 pp.

Wilén, B. M. (2010). Variation in dissolved oxygen concentration and its effect on the activated sludge properties studied at a full scale wastewater treatment plant. In *In the proceedings of IWA World water congress and exhibition, 19-24 September 2010 in Montreal*.

Wolff, G., Assis, L. R., Pereira, G. C., Carvalho, J. G., and Castro, E. M. (2009). Efeitos da toxicidade do zinco em folhas de Salvinia auriculata cultivadas em solução nutritiva. *Planta daninha*, *27*(1), 133-137.

Wu, H., Zhang, J., Ngo, H. H., Guo, W., Hu, Z., Liang, S., and Liu, H. (2015). A review on the sustainability of constructed wetlands for wastewater treatment: design and operation. *Bioresource technology, 175*, 594-601.

Xu, J., and Shen, G. (2011). Growing duckweed in swine wastewater for nutrient recovery and biomass production. *Bioresource technology*, *102*(2), 848-853.

Yadav, S. B., Jadhav, A. S., Chonde, S. G., and Raut, P. D. (2011). Performance Evaluation of Surface Flow Constructed Wetland System by Using *Eichhornia crassipes* for Wastewater Treatment in an Institutional Complex. *Universal journal of environmental research and technology*, *1*(4), 32-34.

Yao, N., Wang, J., and Zhou, Y. (2014). Rapid determination of the Chemical Oxygen Demand of water using a thermal biosensor. *Sensors*, *14*(6), 9949-9960.

Yin, Y., Yu, C., Yu, L., Zhao, J., Sun, C., Ma, Y., and Zhou, G. (2015). The influence of light intensity and photoperiod on duckweed biomass and starch accumulation for bioethanol production. *Bioresource technology*, *187*(6), 84-90.

Yuan, H., Nie, J., Zhu, N., Miao, C., and Lu, N. (2013). Effect of temperature on the wastewater treatment of a novel anti-clogging soil infiltration system. *Ecological engineering*, *57*, 375-379.

Zhang, D., Gersberg, R. M., and Keat, T. S. (2009). Constructed wetlands in China. *Ecological engineering*, *35*(10), 1367-1378.

Zhang, D.; Gersberg, R. M.; Keat, T. S., (2009). Constructed wetlands in China. *Ecol. Eng.*, *35*(10), 1367-1378**.**

Zhang, K., Chen, Y. P., Zhang, T. T., Zhao, Y., Shen, Y., Huang, L., and Guo, J. S. (2014). The logistic growth of duckweed (Lemna minor) and kinetics of ammonium uptake. *Environmental technology*, *35*(5), 562-567.

Zhang, P., Grutters, B., Van Leeuwen, C. H., Xu, J., Petruzzella, A., van den Berg, R. F., and Bakker, E. S. (2018). Effects of rising temperature on the growth, stoichiometry and palatability of aquatic plants. *Frontiers in plant science*, *9*, 1947.

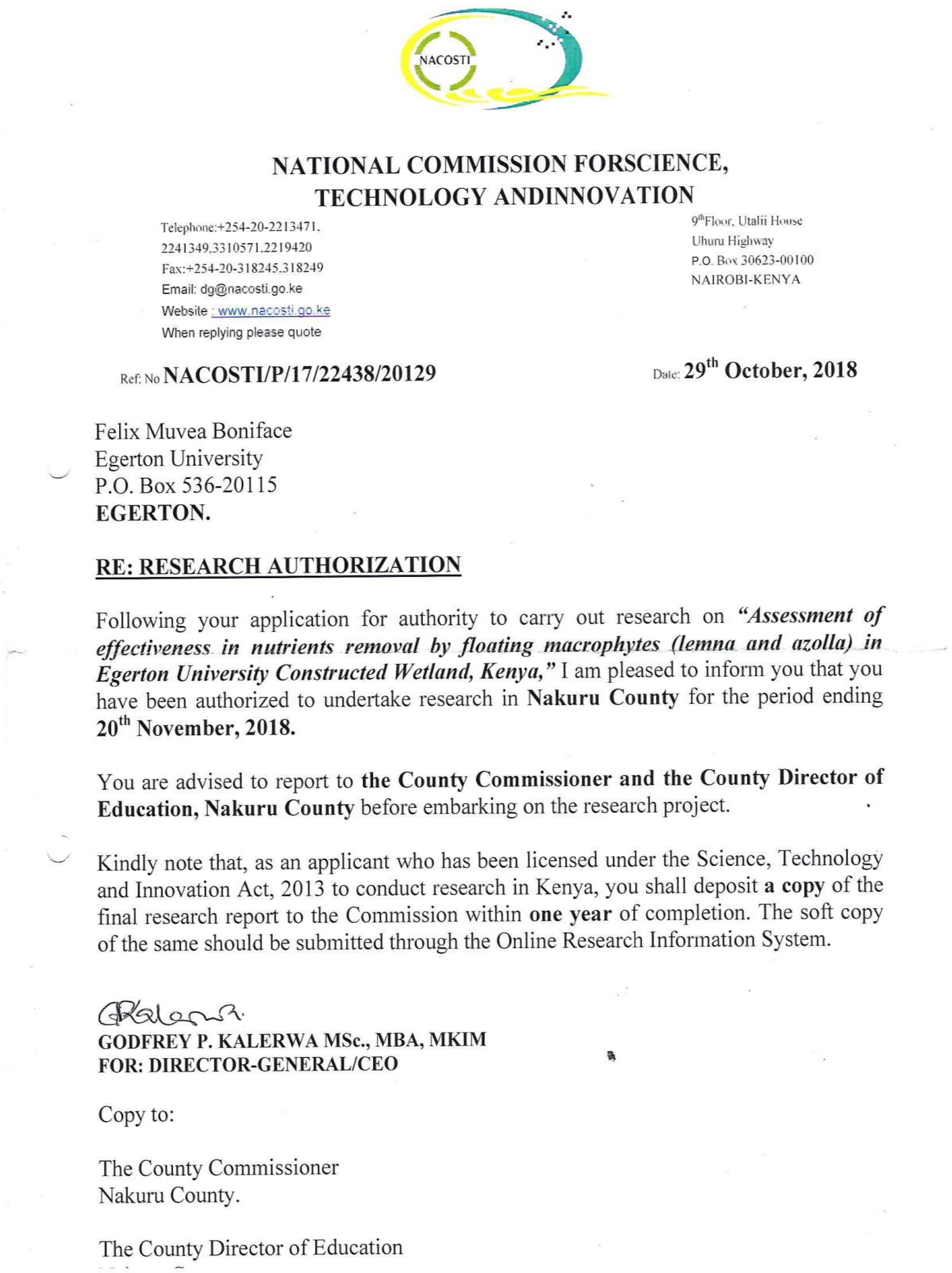
Zhang, Y. (2012). Design of a Constructed Wetland for Wastewater Treatment and Reuse in Mount pleasant, Utah.

Zhao, Z., Shi, H., Liu, Y., Zhao, H., Su, H., Wang, M., and Zhao, Y. (2014). The influence of duckweed species diversity on biomass productivity and nutrient removal efficiency in swine wastewater. *Bioresource technology*, *167*, 383-389.

Zimmo, O. (2003). Nitrogen transformations and removal mechanisms in algal and duckweed waste stabilization ponds. CR

APPENDICES

Appendix I: Research Permit



NATIONAL COMMISSION FORSCIENCE,
TECHNOLOGY ANDINNOVATION

Telephone:+254-20-2213471.
2241349.3310571.2219420
Fax:+254-20-318245.318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No **NACOSTI/P/17/22438/20129**

Date: **29th October, 2018**

Felix Muvea Boniface
Egerton University
P.O. Box 536-20115
EGERTON.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Assessment of effectiveness in nutrients removal by floating macrophytes (lemna and azolla) in Egerton University Constructed Wetland, Kenya,"*** I am pleased to inform you that you have been authorized to undertake research in **Nakuru County** for the period ending **20th November, 2018.**

You are advised to report to **the County Commissioner and the County Director of Education, Nakuru County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nakuru County.

The County Director of Education

Appendix II: Experimental layout