EFFICACY OF SEWAGE TREATMENT PLANT IN MOI UNIVERSITY, UASIN GISHU COUNTY, KENYA

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DECLARATION

I, Ali Kipchumba Ronoh declare that this project is my original work and has not been submitted for a degree in any other institution.

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DEDICATION

This thesis is dedicated to my dear wife Josephine Chepkorir, daughters Rehema Jemutai, Mariam Jerop and Aida Chebet, and son Abubakar Kipsang, who gave me moral support as I conducted the study. Finally I dedicate it to my young brother Stanley Kiptoo and my parents the late Mr. John Rono and Mrs. Gladys Rono for their encouragement and financial support.

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ABSTRACT

Sewage comprises of about 99% water, with the remainder being ions, suspended solids and harmful bacteria that must be removed before the water is released into the ecosystem. Moi University sewage treatment plant (STP), with a capacity of 3,200m^3/day, performs biological sewage treatment processes. The major environmental concern with the STP is the contamination of the effluent receiving Sambul River. In this study, efficacy of the STP was analyzed using key water quality parameters, and comparisons made with those of the National Environmental Management Authority (NEMA) and Moi University Effluent Discharge Monitoring Standards (MUEDMS). Purposive sampling design was used to select five sampling points; STP inlet where sewage is received, STP outlet where sewage has undergone bio-treatment, wetland where pollutants have been trapped from outlet sewage, downstream of Sambul River where bio-treated sewage has mixed with river Sambul waters, and upstream (control) of Sambul River where the river water does not mix with STP effluent. At each sampling point, triplicate water samples for analysis were collected bi-weekly in sterilized plastic bottles from May to August 2015. First, levels of water temperature, pH, and dissolved oxygen (DO) were determined *in situ* using meter probes, while biochemical oxygen demand (BOD_5), turbidity, total dissolved solids (TDS), total suspended solids (TSS), total phosphorus (TP) and total nitrogen (TN) were determined *ex situ* using standard laboratory methods. The concentration of *Escherichia coli* was measured using Idexx Quanti-Tray method. Finally, macroinvertebrates as bioindicators of water quality were collected using manual grab method. The impact of bio-treated effluent on the abundance of aquatic macroinvertebrates at river Sambul was evaluated using Shannon-Wiener diversity index. Results showed that levels of most physicochemical parameters were within the acceptable standards of NEMA and MUEDMS. Upstream parameters showed no significant differences with those of downstream (water temperature $F_{4,\ 115}$ = 8.45; P = 0.9813; pH $F_{4,\ 115}$ = 20.77 P = 0.9781; BOD_5 $F_{4,\ 115}$ = 38965.46; P = 0.9734; TDS $F_{4,\ 115}$ = 123.27; P = 0.9997; TSS $F_{4,\ 115}$ = 708.50; P= 0.9999; ammonia $F_{4,\ 115}$ = 50.78; P = 1.0000; nitrates $F_{4,\ 115}$ = 412.78; P = 0.1919; nitrites $F_{4,\ 115}$ = 943.53; p = 0.9986; phosphates $F_{4,115}$ = 1125.73; P = 0.9931; total phosphorus $F_{4,115}$ = 2107.17; P = 0.9972; total nitrogen $F_{4,115}$ = 81.12; P = 0.9354, indicating improved sewage quality after bio-treatment. Levels of turbidity and *E. coli* downstream were significantly higher ($F_{4,\ 115}$ = 872.0; P < 0.0001 and $F_{4,\ 115}$ = 935593; P < 0.0001, respectively) than those of upstream, outlet or wetland. Treated effluent had no significant effect on the aquatic macroinvertebrates' abundance at the receiving river, as demonstrated by Shannon-Wiener diversity index (H) values at upstream (H=2.504), wetland (H=2.4096) and downstream (H=2.371). High turbidity indicates presence of colloidal matter, which affect water acceptability to consumers while high concentration of *E. coli* indicates possible faecal contamination after bio-treatment, hence the risk of pathogens presence. Moi University sewage treatment plant was found to be relatively efficient based on the tested parameters.

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ACRONYMS

ANOVA	Analysis of Variance
APHA	American Public Health Association
BOD_5	Five day Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
E. coli	*Escherichia coli*
EMCA	Environmental Management and Co-ordination Act
GIS	Geographic Information System
mg/L	Milligrams per litre
MPN	Most Probable Number
MUEDMS	Moi University Effluent Discharge Monitoring Standards
NEMA	National Environment Management Authority
NTU	Nephelometric Turbidity Units
pH	Potential of Hydrogen ions
SEM	Standard Error of Means
STP	Sewage Treatment Plant
SPSS	Statistical package for social sciences
TDS	Total Dissolved Solids
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
UNICEF	United Nations Children's Fund
UNEP	United Nations Environmental Programme
WHO	World Health Organization

WRMA	Water Resources Management Authority
WWAP	World Water Assessment Programme

CHAPTER ONE

INTRODUCTION

1.1 Background information

Wastewater is any water degraded in quality by human factors. Sources of wastewater include surface runoff, domestic or sewage effluents, industrial discharges and agricultural activities. Increased urbanization and industrialization has lead production of wastes that eventually enter wastewater treatment plants. Wastewater must be treated effectively to prevent pollution of the environment and to safeguard public health. Numerous microbes, solids, and other contaminants exist in raw sewage hence treatment of sewage is thus vital before effluent is released to the ecosystem (Yapo *et al*, 2014). The physical, chemical and biological treatment methods have been developed to treat wastewater before effluent is discharged into the environment (Naidoo & Olaniran, 2013).

Wastewater treatment plants have different designs to produce an effluent of specific quality from wastewater of known composition. Consequently, the selection and design of treatment plants are based on factors that include the characteristic of wastewater to be treated and the environmental standard that must be met and maintained where the wastewater effluent is to be discharged. Consequently, each sewage treatment plant must obtain a permit with a list of allowed range of physicochemical and biological parameters that must be adhered to before the effluent is discharged into water bodies. In Kenya, wastewater discharge permits are issued by the Water Resources Management Authority (WRMA) and the National Environmental Management Authority (NEMA). NEMA coordinates and publishes regulations on all matters relating to the environment while WRMA regulates and protect water resources from adverse impacts in addition to monitoring and enforcement of conditions attached to water permits and water use.

Treatment plants have undergone expansion and upgrading to meet ever increasing stricter effluent discharge standards. Sewage treatment process involves multiple steps of sanitization. Secondary treatment phases have been added to primary treatment plants and tertiary phases are being added to the secondary treatment plants. The application of chitinous products in treatment of wastewater has gained prominence in recent years (Gregorio & Pierre-Marrie, 2008) as it coagulates organic materials and suspended solids in addition to removing toxic metallic ions from wastewater. Furthermore, biological, physical and chemical treatment methods have been developed to treat wastewater of specific composition.

In many developing countries poor sanitation condition is widespread. In 2012, only 30% of the population in sub-Saharan Africa had access to improved sanitation (World Health Organization [WHO] and the United Nations Children Fund [UNICEF], 2012). The main improved sanitation systems such as pit latrines and toilets dominated in many countries whereas systems that safeguarded the collection, transportation and proper treatment of wastewater remained very low. In kenya the available good quality water is presently projected at 650m^3 per year per capita and could drop to about 350m^3 per year per capita by the year 2020 (Kaluli, Githuku, Home & Mwangi, 201l) due to pollution and drought factors. Water scarcity in Kenya slows development hence the need for water saving and regulation policies.

The major environmental concern with the Moi University sewage treatment plant (STP) is the potential pollution of the effluent receiving Sambul River. The treatment plant has a design capacity of 3200m^3/day as per Moi University Estates Department records and sewage undergoes biological treatment in a series of stabilization ponds. The receiving Sambul River could be contaminated through nutrient loading which is likely to lead to eutrophication and

algal blooms. Algal blooms results in bad taste and odour as a consequent of organic decomposition. Furthermore possible pollution of river Sambul will reduce its water quality to harmful levels for aquatic life and human beings downstream. The physicochemical and biological characteristics of sewage effluent must be within the set environmental levels for discharge provided by NEMA for the effluent receiving environments and public health protection.

Microbial contamination of the river can occur if the received effluent is loaded with pathogenic microbes and can lead to outbreaks of waterborne diseases in the community. Pollution of the river may also influence the abundance and diversity of macroinvertebrates as bioindicators of water quality, resulting in bioaccumulations through food chains and more microbial contamination of water downstream. This study investigated changes in the quality of effluent released from Moi University sewage treatment plant by direct measurement of selected physicochemical parameters, determination of the concentration of *Escherichia coli* and the evaluation of aquatic macroinvertebrate abundance and diversity bioindicators of water quality.

1.2 Statement of the problem

Moi University programmes are offered in trimester basis currently implying that the volume of wastewater released to the STP has increased due to the rise in student. However, due to very high populations settled in one place, pollution of aquatic systems such as streams and rivers through sewage effluents in these places is possible. Moi University sewage treatment plant discharges its effluent into the receiving nearby Sambul River that serves the Sambul community. The major environmental concern with the Moi University STP is the potential pollution of the effluent receiving Sambul River through nutrient loading which poses

dangers of eutrophication and pathogenic microbes flourishing under anaerobic conditions, and negative effects on aquatic organisms. Efficacy of such treatment plants in pollution abatement of sewage discharges must be ascertained to enhance environmental protection and to cultivate good public relationship with the surrounding communities.

1.3 Study objectives

1.3.1 Overall objective

This study investigated the efficacy of sewage treatment plant in of Moi University by using key water quality parameters.

1.3.2 Specific objectives

1. To analyse the levels of selected physicochemical parameters at the sewage treatment plant and the effluent receiving Sambul River.
2. To determine the concentration of *Escherichia coli* at the sewage treatment plant and the effluent receiving Sambul River.
3. To evaluate the effect of treated effluent on aquatic macroinvertebrates composition at the effluent receiving Sambul River.

1.4 Hypotheses

1. The sewage treatment plant in Moi University is effective in treating effluent as shown by levels of the selected physicochemical parameters at the sewage treatment plant and the effluent receiving Sambul River.
2. The sewage treatment plant in Moi University is effective in treating effluent as shown by the concentrations of *E. coli* at the sewage treatment plant and the receiving Sambul River.

3. There are no significant changes in the composition of aquatic macroinvertebrates at the receiving Sambul River that may be attributed to the effect of discharged treated effluent.

1.5 Justification

There is no data on the effect of effluent discharged into Sambul River on aquatic macroinvertebrates abundance and diversity as bioindicators of water quality. Data from WRMA routine checks on the efficacy of Moi University treatment plant lacks macroinvertebrate status of river Sambul. In order to assure the surrounding community on the efficiency of sewage treatment plant in Moi University, it is important to examine its efficiency. The use of aquatic macroinvertebrates as bioindicators has the advantage that they are capable of integrating all the biological effects of the mix of contaminants in effluents. This could be difficult to predict by measuring physicochemical concentrations alone in the abiotic environment as shown by available data from Kenya's WRMA. The results of the study are expected to be useful to Moi University and the community by offering information on the effectiveness of sewage treatment plant in removing pollutants from its effluent.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter presents the historical background on wastewater treatment; characteristics of domestic wastewater; the physicochemical and biological parameters; effect of sewage treatment plant effluent on biodiversity; wastewater treatment processes and the National Environmental Management Authority (NEMA) and Moi University Effluent Discharge Monitoring Standards (MUEDMS).

2.2 Historical background on wastewater treatment

Growing civilizations, increased urbanization and the establishment of centralized wastewater collection has resulted in accumulations of wastewater. Apart from the domestic sources, centralized systems for wastewater collection have other sources of wastewater such as storm water and industrial wastewater. From an aesthetic perspective and because of its typical bad odour, and the fact that its main constituent is human waste, wastewater is usually looked upon as an undesirable resource. Wastewater treatment efforts have evolved from the fact that untreated wastewater discharged into aquatic ecosystems constitute a great hazard for the environment and a public health risk (Malik, Hsu, Johnson, & De Sherbinin, 2015). The solution to this problem is through treatment of the raw wastewater before discharge into the environment. More advanced treatment techniques were developed and designed for specific constituents in the wastewater (Britannica, 2012). At conventional treatment plants, tertiary treatment steps for removal of nitrogen and phosphorus which contribute to eutrophication have been widely introduced especially where the recipient aquatic body is vulnerable.

According to the [WHO/UNICEF (2012),] the population in sub-Saharan Africa that has access to enhanced sanitation is about 30%. A trend from 1990 to 2010 shows that increase in access to improved sanitation has been lowest in Sub-Saharan Africa at about 4%. The prevention of infectious diseases and hygiene as part of the main concerns of the sustainable development goals have necessitated the need to reduce pathogens released by humans into the ecosystem through wastewater treatment efforts (Tsuzuki, 2012). According to Mara (2003) at least 15% of the wastewater in most developing nations is treated before discharge into the ecosystem. The inadequate level of wastewater treatment coverage in developing countries is mainly attributed to lack of funds. In the Asian continent, most countries had 10-30% of the populations linked to public collection sewers as septic tanks are mainly used in urban areas while pit latrines dominate in rural areas.

The development of environmental policy and a legal framework in Kenya reflects government's commitment to ensure sustainability of natural resources by mitigating the impacts of socio-economic growth. Subsequent to the Brundtland Commission report entitled "*Our Common Future*", the application of environmental management models from industrialized countries, was adopted in the enactment of the Environmental Management and Co-ordination Act (EMCA) by Kenyan parliament (EMCA, 1999). EMCA is based on principles such as sustainability of the environment and natural resources and the precautionary approach to mitigate environmentally deleterious impacts of socio-economic activities. The legislation also led to the creation of NEMA, whose core functions include ensuring that the key players that include industrialists, government enterprises and establishments, entrepreneurs, private developers, and individuals comply with the laid down provisions of EMCA, including the proper treatment of wastewater in accordance to the environmental management requirements in the protection of human health.

In Kenya the main challenge today is how to maintain sustainable development without degrading the natural ecosystem on which people relies on. Development projects are expected to be economically viable and acceptable to the environment. Land degradation, loss of biodiversity, environmental pollution, and water resource degradation are the major environmental problems being experienced in many parts of Kenya today. This situation is intensified by lack of awareness and limited involvement of the local communities and key stakeholders on the consequences of environmental degradation as well as management of sewage treatment (Nzuki, 2008).

2.3 Characteristics of domestic wastewater

Domestic wastewater has physicochemical and biological factors which are substantial in the treatment performance of sewage treatment plants and the subsequent environmental impact. These components are divided into those causing an environmental threat and those causing hazard for public health. The environmental hazards are linked greatly to eutrophication, algal blooms and a subsequent decline of aquatic organisms. The decomposition of algae requires large quantities of oxygen and results in reduced levels of oxygen in the water body, causing aquatic life kills. Health hazards are caused by presence of pathogenic agents in wastewater effluents discharged into the environment (Kemira, 2003).

2.4 Physicochemical parameters of water quality

2.4.1 Temperature

Many of the biological, physical and chemical characteristics of wastewater are directly affected by temperature. Temperature is affected by depth of the water, season, time of the day, cloudiness of the sky and the air temperature in lotic systems. Wastewater discharges can also affect temperature. Generally, wastewater temperature is higher than the local water

sources due to high content of warm water from households or industries. Variations in temperature alter dissolved oxygen, thus higher temperatures mean the water holds less dissolved oxygen. The distribution and number of aquatic macroinvertebrate changes as temperature varies in aquatic environments. High water temperature is unsuitable for sensitive macroinvertebrate species. The optimum temperature for biological treatment is in the range of 25 to 35°C. Microbial reactions are reduced at lower temperatures while nitrification stops at very high temperatures. Natural water bodies that receive effluent water with higher temperatures have had their aquatic life decline significantly (Metcalf & Eddy, 2004).

2.4.2 Dissolved oxygen (DO)

Dissolved oxygen (DO) refers to microscopic bubbles of gaseous oxygen (O) that are mixed in water and available to aquatic organisms for respiration. The amount of dissolved oxygen in the aquatic environment (i.e., river) is dependent on factors which include water temperature and the amount of oxygen taken out of the aquatic ecosystem by respiring and decaying organisms. The amount of oxygen reverted back into the system by river flow, aeration and photosynthesizing plants also affect levels of dissolved oxygen in aquatic systems. The atmospheric pressure and the concentration of impurities such as salts and suspended solids have also been attributed to the fluctuation of dissolved oxygen in aquatic environments.

In aerobic biological wastewater treatment, dissolved oxygen is essential for bacterial respiration. The assessment of DO levels before and after wastewater treatment is of great significance since it is indicative of the rate of biological activity within the treatment system. Discharge of organic wastes alters oxygen balance of the receiving aquatic bodies because their breakdown utilizes oxygen. The temperature of effluent receiving water bodies affects

the amount of dissolved oxygen present; less oxygen dissolves in warm water than cold water. For this reason, there is cause for concern for rivers with warm water. A concentration of 4-6 mg/L DO in natural water bodies is essential for supporting aquatic life (Omoto, 2006).

2.4.3 Water pH

pH is the measure of the acidity or alkalinity of the water on a scale from 1–14 (1 is very acidic, 7 neutral and 14 very alkaline). The pH of water affects the solubility of many toxic and nutritive chemicals consequently affecting the availability of these substances to aquatic organisms in the effluent receiving aquatic ecosystems. The optimum pH levels for microbial activities are from 6-9. Outside of optimum ranges, metabolic activities become impaired and can lead to declines in organisms. If the pH of water is too high or too low, the aquatic organisms living within it will die hence the hydrogen-ion concentration is a key factor in biological treatment (Shu, Wait, Bliss, Fane, & Jegathessan, 2005).

2.4.4 Turbidity

Turbidity is the cloudiness of a fluid caused by large numbers of individual particles that are generally invisible to the naked eye. Wastewater effluent with high turbidity can negatively impact the effluent receiving aquatic bodies such as rivers and lakes. Turbidity is caused by particles suspended or dissolved in water that scatter light making the water appear cloudy. This includes suspended matter such as clay, silts, finely divided organic and inorganic matter, plankton debris and microorganisms.

Turbidity affects the physiological processes in aquatic plants found in water bodies such as rivers and lakes. High turbidity levels blocks light from reaching submerged plants hence hindering photosynthesis. When photosynthesis stops levels of plant productivity and

dissolved oxygen concentration drops in the water body. Reduced levels of dissolved oxygen in water will impair metabolic reactions in aquatic organism and leads to increased levels of organic materials in water. Decomposition of the additional organic waste requires more oxygen further decreasing the limited dissolved oxygen in water. Microbial contamination of water will increase as microbes will increase in number. Water quality will deteriorate and the entire consumers will be affected. In a lake or river, turbidity may also reduce visibility of underwater structures such as logs or large boulders, negatively affecting a water body's recreational use (Shittu, Olaitan, & Amusa, 2008).

2.4.5 Total suspended solids (TSS)

Total suspended solids are solids in water that can be trapped by a filter including decaying plants, silt, animal matter, industrial wastes, and sewage. In general, raw wastewater comprises of solids of different types and sizes. The presence of excess suspended solids is harmful to the health of a water body consequently affecting aquatic life. TSS values are used to monitor and assess efficacy of wastewater treatment plants to guarantee the health of effluent receiving water bodies.

Discharge of settleable solids to water a body increases sedimentation rates and often destroy and alter habitats for aquatic organisms. Macroinvertebrates and fish become buried and die and their decomposition will deplete dissolved oxygen subsequently lowering the quality of water. Mineralization can reduce egg and embryo survival by reducing oxygen supply and crusting over the egg, preventing the embryo from escaping. As sediment build-up increases, the shallower body of water means an increased risk of flooding.

Release of sewage effluents with excess suspended solids to rivers and other aquatic systems can increase levels of pathogens and contaminants including bacteria, protozoa, nitrates and phosphorus, pesticides, mercury, lead and other metals. Pathogens can attach to suspended materials and increase the risk of disease outbreaks. This is why an increase in TSS can indicate potential pollution, not just a decrease in water quality. High levels of TSS prevents penetration of light to the submerged plants leading to reduced photosynthesis which results to less oxygen levels in water as a by-product (Giller & Malmqvist, 1998). This inability of plants to photosynthesise will eventually lead to death and the decaying process that demands more oxygen use from water. High TSS can also cause increased temperatures of water as the suspended particles absorb heat from sunlight.

2.4.6 Total dissolved solids (TDS)

Total dissolved solids (TDS) comprise inorganic salts (mainly calcium, phosphates, chlorides, nitrates, sodium, magnesium, potassium, bicarbonates, and sulfates) and some small amounts of organic matter that are dissolved in water. Other sources come from fertilizers and pesticides used on farms.

The effluent from wastewater treatment plants can increase dissolved solids in aquatic systems. However, a constant level of minerals in the water is necessary for aquatic life. And any variations in the amounts of dissolved solids can be harmful to organisms as the process of osmosis can be impaired. In addition, concentration of total dissolved solids that are too high or too low may limit the growth and may lead to the death of many aquatic organisms (Vijay, Sardar, Dhange, Kelkar, & Gupta, 2010). The presence of excess salt concentration act to dehydrate the animals and causes unpleasant taste of water in rivers and lakes.

2.4.7 Organic matter in wastewater

Organic matter comprises of carbon-based chemicals that are the building blocks of most living organisms. Organic compounds contain carbon, hydrogen, oxygen, nitrogen, and other non-metallic elements. Many organics are proteins, carbohydrates, or fats and are biodegradable. However, even biodegradable materials can cause pollution if discharged into aquatic environments (Wiesmann, Choi & Evamaria, 2007). Large amounts of biodegradable materials are hazardous to aquatic bodies because microorganisms will initiate biochemical reactions by utilising dissolved oxygen in the water to breakdown the wastes. Those biochemical reactions is measured in the laboratory as the biochemical oxygen demand (BOD).This can deplete the supply of oxygen in the water needed by aquatic life, resulting in fish and macroinvertebrate kills, odours, and overall dilapidation of water quality (Jagai, Li Wang, Messier, Wade, & Hilborn, 2015). The discharge of oxidizable chemicals from wastewater treatment plants into a natural water body will initiate chemical reactions that are measured as chemical oxygen demand (COD).

The amount of oxygen organisms need to breakdown wastes in wastewater is referred to as the biochemical oxygen demand (BOD). The BOD test measures the biodegradable fraction of the wastewater by monitoring the assimilation of organic material by aerobic microorganisms and therefore a suitable indicator of treatment efficiency. If effluent with high levels of BOD is discharged into a stream or river, it will accelerate bacterial growth in the river and consume the oxygen levels in the river (Ramesh, Bhadrinarayana, Meera & Anantharaman, 2007).

2.4.8 Nutrients in wastewater

Nutrients in wastewater comprises of both organic and inorganic matter. The discharge of nutrients such as nitrogen and phosphorus into aquatic bodies causes eutrophication (Gücker, Brauns & Pusch, 2006). Excessive presence of nutrients in a river or lake causes algal blooms and too much growth of aquatic plants and destroys habitats for the aquatic animals. The decomposition of dead plant debris would utilise and eventually deplete oxygen from water and results in aquatic animals' kills. Water quality in such an environment deteriorates due to the addition of microbes and other organic materials into a river system as more aquatic life perish.

Wastewater contains nitrogen in the forms of ammonia, nitrite, nitrate and organic nitrogen.. The discharge of these nitrogen compounds into the ecosystem may lead to several environmental and health risks. Nitrogen compounds, therefore, need to be removed from the wastewater before the effluent is discharged into a water body. Phosphorus occurs naturally in low concentrations and is very essential to plants and animals. In wastewater treatment plants organic matter microbial decomposition leads to phosphorus accumulation. In aquatic environments phosphorus levels may increase as a result of discharge of untreated sewage, surface runoff containing fertilizers and organic matter. High phosphorus concentrations leads to problems such as algal blooms, foul smelling, excessive weed growth and the loss of biodiversity in rivers and lakes (Waiser, Tumber, & Holm 2011.)

2.4.9 Total nitrogen (TN)

Nitrogen forms that are significant in wastewater include organic nitrogen, nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), and nitrogen gas (N_2). Organic nitrogen is nitrogen bound to carbon which is the key nitrogen component in faeces. Organic nitrogen also includes urea

(H_2NCONH_2) which is the principal constituent in urine. Organic nitrogen needs bacterial conversion to nitrate before it is available for plants. Total nitrogen is the sum of the inorganic and organic compounds of nitrogen. Wastewater contains organic nitrogen in bonded form and inorganic forms such as nitrate or nitrite. Nitrite is rarely observed in water sources because it is readily converted to nitrate by microbial processes (McHale & McChesney, 2007). Ammonia exists in water as either the ammonia gas (NH_3^-) or ammonium ion (NH_4^-) depending on the pH of the water. At pH levels above 9.3, ammonia gas is the predominant form while the ammonium ion is the predominant form at pH levels below 9.3. Ammonia is usually present in surface water and is due to the chemical transformation of urea and anaerobic processes. Ammonium binds to soil which is negatively charged.

In aquatic environments, nitrates are readily available to aquatic life as it is the most oxidized form of nitrogen. The reduction of nitrogen in wastewater effluent discharges is therefore required to reduce the influx in water bodies. Nitrate is used as water quality indicator as it results from eutrophication in aquatic bodies, and in high concentrations it can be detrimental to aquatic animals (Wolfgang *et al*, 2002). Nitrate result from nitrification process where ammonia (NH_4^- N) is oxidized to nitrite (NO_2^- N) before nitrite is oxidized to nitrate (NO_3^- N), both steps under the presence of oxygen and *Nitrosomonas* and *Nitrobacter* bacteria respectively. Denitrification of nitrates leads to more nitrogen compounds removal in wastewater treatment ponds. This occurs when nitrate is reduced to nitric oxide, nitrous oxide and finally nitrogen gas with the aid of *Pseudomonas* bacteria.

2.4.10 Phosphorus and phosphates

Phosphorus exists in wastewater in form of soluble orthophosphate ion (PO_4^{-3}), organically-bound phosphate, and other phosphorus- oxygen compounds. Inorganic phosphates are

present in organic molecules such as DNA, RNA and nucleotides while organically-bound phosphate in wastewater is from human wastes. A number of cleaning agents contain phosphate. Phosphorus is usually the limiting nutrient in water and is the main cause of eutrophication in surface water bodies such as lakes and rivers (Burks and Minnis, 1994). As a result of precipitation reactions occurring simultaneously with high alkaline conditions in stabilization ponds, about 50% of phosphorus removal can be expected (Dates, 1994). In wastewater treatment organic phosphate is accumulated as algal biomass while phosphorus remains attached to sediments. Consequently phosphorus removal in wastewater treatment plants can be enhanced by increasing the number of primary and secondary facultative ponds (Omoto, 2006). In wastewater treatment plants phosphorus in organic materials is finally oxidized to phosphates. Effluent with excess phosphates discharged into an aquatic system lead to eutrophication of lakes and rivers consequently affecting aquatic life.

2.5 Biological parameters of wastewater

Sewage comprises of a diverse range of organisms originating not only from faeces but also from soil and water. Pathogenic microorganisms such as viruses, bacteria, fungi, rotifers, protozoa, and worms occur chiefly in human excreta and urine. Presence of pathogens in water can cause fatal infectious water borne and water related diseases such as cholera, giardiasis, typhoid, amoebic dysentery, yellow fever, skin infections or malaria. These pathogens usually originate from animals and human beings that are infected. High concentrations of pathogens exist in wastewater from domestic facilities. Consequently, in sewage treatment plants these pathogens are removed through natural die off as a result of exposure to ultra violet light and disinfection of effluent using before it is discharged into receiving water bodies (Wakelin, Colloff & Kookana, 2008).

In developing countries, analysis of pathogenic organisms remains a challenge due to limited laboratory equipment and materials. Therefore evaluation of wastewater treatment systems is accomplished by use of indicator organisms such as faecal coliforms and *Escherichia coli (E. coli)* as they can be detected by simple methods and do not grow in natural waters (Sanders, Yuan, & Pitchford, 2013). Fecal coliforms and *E. coli* exist in large numbers in the digestive tracts of humans and animals and when present in water samples it indicates contamination of water. Effluent receiving rivers with a positive test for *E. coli* shows possible contamination and a risk for waterborne disease.

2.6 Effect of treated effluent on macroinvertebrates

Anthropogenic factors for instance discharge of untreated sewage effluents into the ecosystems are the main causes of environmental degradation (Dixon, Simon, & Burkitt, 2003). Environmental regulations on wastewater effluent discharge to the receiving aquatic bodies such as rivers are essential to safeguard water quality and to protect aquatic life. The deterioration of water quality is harmful to both human beings and animals. Macroinvertebrates are animals without backbones, bottom dwelling in freshwater bodies, visible without a microscope and can be easily identified in the field. The taxonomic composition, abundance and diversity of macroinvertebrates have been used to monitor water quality in effluent receiving aquatic systems such as rivers and lakes. They are used to determine water quality in streams and rivers as they are high sensitive to pollution including changes in pH, DO, temperature and turbidity. Macroinvertebrate species can be found around vegetation and in sediments at the bottom of rivers and streams. Some macroinvertebrates exists as larval stages of crustaceans such as snails and leeches while others are insects such as caddis flies, mosquitoes and dragonflies. Benthic organisms exist in aquatic ecosystems living on or inside deposits of the bottom substrate and are sedentary with

reduced or no mobility. They are significant component in food chain and energy flow, source of food for other higher organisms such as fish and play an important role in the circulation of nutrients (Oben, Oben, Ugwumba, Okorie, & Pleysier, 2003).

Bioassessment of macroinvertebrates are useful in evaluating pollution in freshwater bodies due to their tolerance to contaminants. If exposed to environmental stressors, the intolerant species such as Mayflies (Ephemeroptera), Stoneflies (Plecoptera*)* and Caddisflies (Trichoptera) may die while tolerant species such as leeches (Hirudinea), Tubifex worms (*Tubifex sp.*), and Pouch Snails (Gastropoda) will occupy the space left by the intolerant group, consequently creating a totally different population of macroinvertebrates (Carey & Migliaccio, 2009).

Evaluation of macroinvertebrates is a procedure that uses inexpensive equipment and is scientifically valid if done properly. Bioassessment of macroinvertebrates can be used to define rehabilitation goals and to monitor trends in rivers. Macroinvertebrate data analysis for streams and rivers is accomplished by using the multimetric approach including taxa richness, proportional abundance measures and various diversity indices. Measures of diversity and abundance on macroinvertebrates have been significantly applied in determining habitat quality of freshwater ecosystems (Merrit & Cummins, 1996).

2.7 Wastewater treatment processes

Wastewater treatment plant is a combination of different treatment processes tailored to produce an effluent of a definite quality from wastewater influent of known composition. Most treatment plants have primary, secondary and tertiary phases (Dixon *et al*., 2003). Primary treatment comprises of physical processes involving mechanical screening, grit

removal, and sedimentation which is aimed at removal of oil and fatty acids, settleable, suspended and floating solids such as plastics simultaneously. The main objective of this treatment step is to remove a large fraction (50-70%) of the suspended solids in the wastewater. Since suspended solids also contribute to the content of BOD in the wastewater, it is expected that 25-40% of the total BOD is removed in the process (Metcalf & Eddy, 2004).

In general secondary treatment mainly converts biodegradable organic matter (thereby reducing BOD) and organic nitrogen to carbon dioxide, water, and nitrates by means of aerobic and/ or anaerobic microbial processes. In secondary treatment phase biological removal of dissolved solids is accomplished and can be removed from the wastewater stream as sludge (Tilley, 2011). At optimized performance, reduction of pathogenic bacteria up to 2 log units can also be achieved in secondary treatment systems, depending on the suspended solids concentration (World Health Organization [WHO], 2006). Therefore secondary treatment removes readily biodegradable BOD and suspended solids that have escaped the primary treatment.

Other treatment plants have a tertiary treatment option which provides a final treatment stage to raise the effluent quality before it is discharged to the receiving aquatic environment such as the sea, river or lake. Basically, tertiary treatment phase in wastewater treatment systems is aimed at controlling eutrophication in sensitive effluent receiving surface waters and some reuse schemes. It is designed to remove nutrients, total nitrogen and total phosphorus from secondary effluent. Additional BOD reduction and total suspended solids removal and is accomplished by tertiary processes (Davis, 2011).

2.8 Moi University sewage treatment plant

Moi University sewage treatment plant (STP) (Appendix 1) provides biological treatment of wastewater. The waste stabilization ponds constructed are simple to operate and maintain. Routine tasks comprises of cutting the embankment grass, removing scum, and any other floating vegetation from pond surface, keeping the inlet and outlet channels clear and repairing any damage to the embankments. Wastewater quality is evaluated every three months by the Moi University Estates department together with Water Resources and Management Authority (WRMA).

The STP at Moi University receives and treats wastewater from the entire premises. The mean inflow of influent into the treatment plant is 3,200 m^3/day. STP Inlet (Appendix 1) is the point where wastewater is received at the treatment plant. Screening of wastewater occurs at this point. Wastewater flows through a coarse screen (size opening 50 mm), where all the floatable solids are trapped for removal. A fine screen (size opening 25 mm) is also fitted downstream of the coarse screen to trap materials that passes through the coarse screen. If the coarse screen in the channel is blocked due to excessive solids in the wastewater, the level of wastewater rises in the channel and wastewater starts flowing through the emergency bypass channel, which has another coarse screen (size opening 75 mm).

From the inlet channel, wastewater flow into stabilization ponds which are six in number. There are two anaerobic ponds, primary and secondary facultative ponds, and two maturation ponds (Appendix 1). An anaerobic pond (width 51 m, length 51 m and depth 3 m) allows for sewage settling, some BOD removal and anaerobic digestion of solids. Anaerobic breakdown occurs when solids settle at the bottom and are eliminated as bacteria decompose organic matter. In addition, organic nitrogen is hydrolysed to ammonia in anaerobic ponds. From the

anaerobic pond, wastewater flows into the primary facultative pond (width 80 m, length 220 m, and depth 1.7 m). Phosphorus and nitrogen removal occur in facultative ponds. The primary facultative pond provides some anaerobic digestion in the benthic zone, and aerobic respiration at the water surface. The primary pond discharges wastewater into the secondary facultative pond (width 70 m, length 220 m, and depth 1.7 m) where wastewater is aerobically treated.

Maturation ponds (each; width 70 m, length 70 m, and depth 0.5 m) receive effluent from the secondary facultative pond to polish the wastewater. In this ponds pathogens die as the levels of suspended solids is low and pathogens are exposed to ultra violet radiations and perish and remaining BOD is removed by heterotrophic bacteria. Maturation ponds discharge wastewater into the outlet channel where effluent is disinfected by the charcoal and gravel deposits along the channel. From the outlet channel, wastewater effluent is discharged into the wetland. The effluent released into the ecosystem has undergone biological treatment in the stabilization ponds within the sewage treatment plant. At the wetland more nutrients are absorbed by the plants through biofiltration thus reducing the nutrient load. From the wetland wastewater effluent is then discharged to Sambul River. The quality of effluent discharged into river Sambul must be within the standards outlined by environmental agencies such as NEMA and WRMA.

2.9 NEMA and MUEDMS Standards

A number of the characteristics of sewage effluent are likely to cause problems with regards to treatment in a conventional water works. The high content of non-biodegradable organics, total solids, and ammonia and nitrate nitrogen are likely to degrade the environment. The high content levels of viral and bacteriological impurities would be a source of concerns to water

treatment authorities. Third and fifth schedules of the Environmental Management and coordination Act give Standards for effluent discharge into the environment and public sewers respectively (Appendix 2). Consequently, Moi University has established standards from NEMA for monitoring its wastewater before and after treatment (Appendix 3).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter outlines the geographical location and climatic conditions of the study area. Moi University sewage treatment plant inlet and outlet works and the description of the five sampled points are presented in the chapter. Research design, sampling techniques and procedures for the study is highlighted. The procedure for the determination of each study objective is described; the levels of the selected physicochemical parameters, the presence of *Escherichia coli* and collection and evaluation of aquatic macroinvertebrate abundance are elaborated in this chapter. In addition, data analysis methods are also covered in this chapter.

3.2. Study area

3.2.1 Description of study area

The study was conducted at Moi University main campus, situated in Uasin Gishu County, Kenya. The study area is described by latitude 0^{o} 06´N to 0^{o} 08´N and longitude 5^{o} 08´E to 35^{o} 10´E at an elevation above 2000 m above sea level (Figure 3.1).

3.2.2 Climatic conditions

Uasin Gishu County experiences two wet seasons: the short rains from October to November and the long rains from March to June. The rains support the moisture in the ground for much of the year and this favours farming activities in the area. Data from Moi University weather station indicates that on average, Moi University receives between 1,200 and 2,000 mm of rainfall per annum, and daily temperatures range between 12 and 25°C.

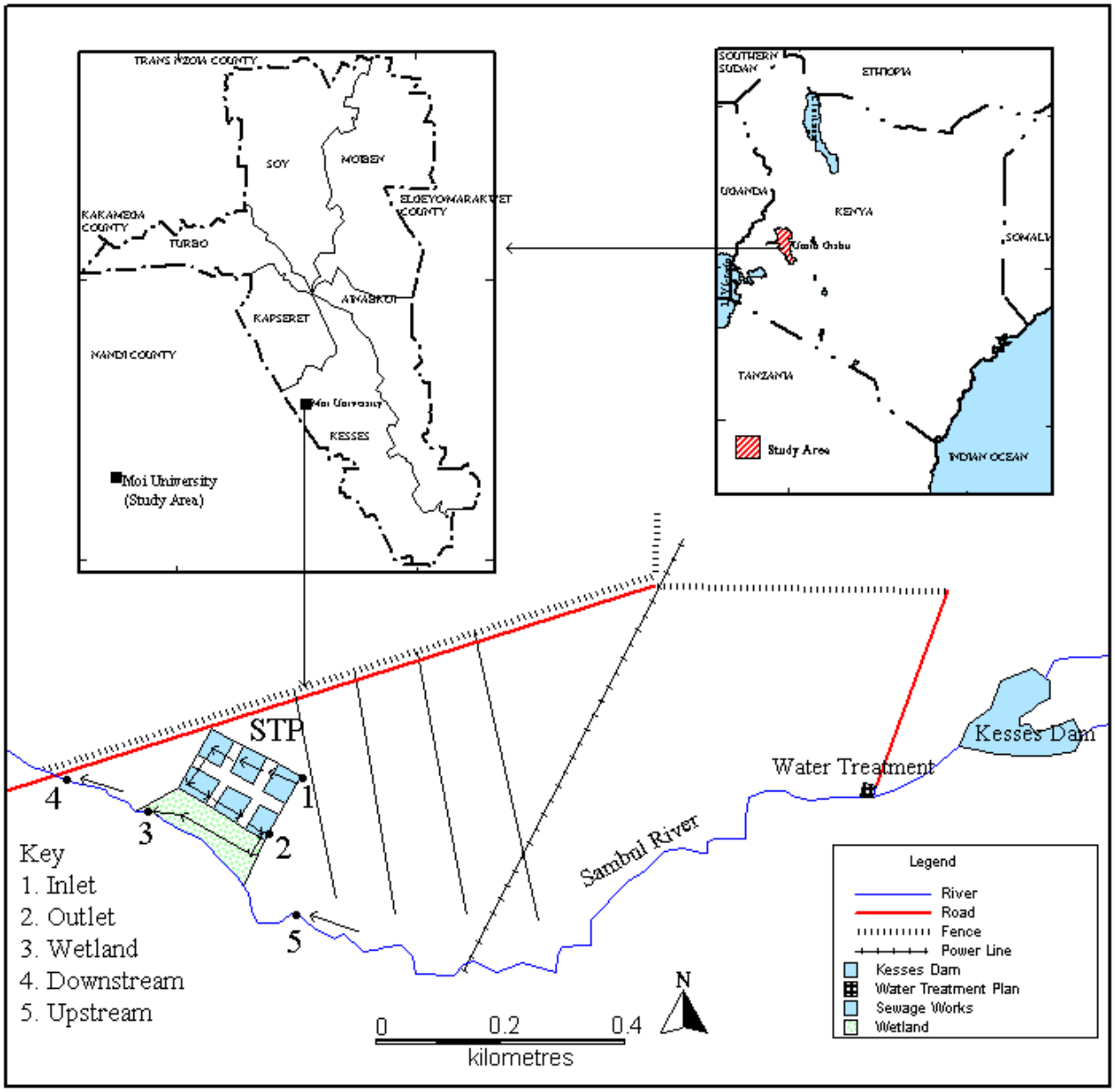


Figure 3.1: Map showing sampling points at Moi University sewage treatment plant and Sambul River (by Kanda, Geographic information systems laboratory, Moi University, 2015).

3.3 Experimental design

3.3.1 Selection of sampling points

Sampling points at Moi University sewage treatment plant were selected using a purposive design. This generated wastewater samples that were used to evaluate the effectiveness of the plant in treating sewage, and further determine the possible impacts of the treated effluent on physicochemical and biological integrity of the receiving Sambul River. Five sampling points (Figure 3.1) were selected as follows; sampling point 1 (SP1), which is the inlet where influent is discharged into the sewage treatment plant to undergo biological treatment. Sampling point 2 (SP2), which is the outlet where effluent is released from the sewage treatment plant into the wetland and finally to Sambul River. At this point, effluent has undergone biological treatment. SP2 was 100 m from SP1. Sampling point 3 (SP3), which is the point at the wetland where the effluent joins the Sambul River. SP3 was 100 m from SP2. Sampling point 4 (SP4), which is at downstream of Sambul River where biologically treated effluent is mixed with waters from the Sambul River. SP4 was 100 m from SP3. The last point was sampling point 5 (SP5), which is upstream of Sambul River a point before the Sambul river water mixes with effluent from the sewage treatment plant. This point served as a control, reflecting the most naturally preserved conditions of river ecosystem without the influence of the sewage effluent. SP5 was 100 m from SP3.

3.4 Sampling techniques and procedures

Manual- grab sampling technique was used to collect wastewater samples at each of the five (SP1- SP5) sampling points. Samples were collected bi- weekly in 2015 between 0900 and 1500 hours from May to June, (wet season) and from July to August 2015 (dry season). For physicochemical analyses, wastewater samples were collected in 1 L inert plastic bottles. Prior to usage, the bottles were acid washed to remove phosphate ions (PO_4).

During sampling, sample bottles were first rinsed with sample water three times before collecting the water samples. At each sampling point, a capped sampling bottle was inserted into the water vertically. Once at a depth of 15 cm below the water surface, the bottle was opened to allow the sample water to flow in. Care was taken to ensure that no floating films or large organic material were collected. The bottle was then capped before withdrawing it from the water. Samples were collected in triplicate. The samples were then labelled and transferred into a cooling box and then transported to the laboratory (Prof L. Huisman, Moi University) for analysis. They were then stored in a refrigerator at 4.0 ± 1.0° C with the aim of arresting physical, chemical and biochemical reactions that may take place in the sample bottles, leading to changes in the intrinsic quality of the samples. Each sample was implemented as a composite sample out of the triplicate samples. The amounts are correlated to the actual minimum amount needed to carry out the appurtenant analyses. For microbial analyses, 250 ml glass bottles were used to collect water samples while 1,000 ml glass jars were used to preserve samples for macroinvertebrate analyses. Measurements for dissolved oxygen (DO), temperature and pH were taken on site because concentrations of these parameters can be significantly changed during transport and storage.

3.4.1 Determination of levels of selected physicochemical parameters.

3.4.1.1 Wastewater temperature

Wastewater temperature for each sampling point was measured using a DO meter (YSI 550A Hanna instruments, USA) simultaneously with the DO-readings. The sensor was washed with distilled water after every measurement. On each sampling date, triplicate readings were taken and recorded. Mean values were later calculated (American Public Health Association

[APHA], 1998). Calibration of DO meter was done every week by filling the membrane with electrolyte and connecting the probe to the meter and wait till the percentage tag reading is stable.

3.4.1.2 pH of wastewater

Wastewater pH readings were taken using a handheld pH-meter (pH 1000 H, phenomenal labtech, India). Calibration of the meter was done using standard pH buffers of pH 4, 7 and 9 which were commercially obtained. 100 mL of wastewater sample was dispensed in a 100mL beaker and the pH meter electrode dipped until the pH-value was stabilized within a one decimal point range. Readings were taken directly from the pH meter and mean values of the triplicate samples were recorded (APHA, 1998).

3.4.1.3 Dissolved oxygen (DO)

Readings of the dissolved oxygen (mg/L) in the wastewater samples were taken using a handheld -YSI 550A dissolved oxygen meter. Three replicate samples were measured and their mean values recorded (APHA, 1998). The sensor was cleaned between every measurement using distilled water. Distilled water was used to calibrate the DO meter to a zero reading before samples were measured in situ.

3.4.1.4 Biochemical oxygen demand (BOD_5)

To determine the wastewater BOD_5 (mg/L) dilution water was first prepared, where 1 ml each of phosphate buffer, magnesium sulphate, and calcium chloride and ferric chloride solution was added to 1,000 mL of de-ionized water in a volumetric flask. 1 mL of wastewater sample was added to a 500 mL beaker, and then filled up to 300 mL with dilution water. The pH was adjusted to a value within the range of 6.8-7.5 by adding acid or alkali. 300 mL of dilution

water was also dispensed in another 500 mL beaker and served as a control. Both the prepared and control samples were then dispensed into 300 mL BOD bottles. The DO for each sample was measured using a DO meter, before they were incubated in a BOD incubator for 5 days at 20 ± 1°C. The DO values were again measured 5 days after incubation. For wastewater samples from each sampling point, three replicate samples were measured and their mean values recorded. Calculation for BOD_5 (mg/L) was determined by the following formula:

$$BOD\ (mg/L) = \frac{D_1 . D_2}{P} \quad \text{..... Equation (3.1)}$$

Where,

D_1 = DO (mg/L) value in initial sample

D_2 = DO (mg/L) value in final sample

P = decimal volumetric fraction of sample used (ml of sample/300 ml).

3.4.1.5 Turbidity

A turbidity meter (LaMotte Turbidity, model 2008, code1790; USA) was used to measure turbidity (NTU) of wastewater samples. Calibration of the turbidity meter was done using a cuvette with distilled water (zero NTU). 10 mL of each wastewater sample was then dispensed into the cuvette and readings recorded after a stable value was achieved on the turbidity meter. All samples were measured and three readings recorded and their mean values calculated (APHA, 1998).

3.4.1.6 Total dissolved solids (TDS)

A TDS meter (TDS 3, HM Digital, PAT Design NO. ZL 2004 3 0048169.1; USA) was used to measure total dissolved solids (mg/L) of wastewater samples. Calibration of the TDS meter

was done using sodium chloride. 10 mL of each wastewater sample was then dispensed into the beaker and TDS meter was then immersed into the sample up to the maximum immersion level (2") and readings recorded after a stable value was achieved on the TDS meter. After usage, the meter was wiped with a tissue before the next sample reading was taken. All samples were measured in triplicates and their mean values recorded (APHA, 1998).

3.4.1.7 Total suspended solids (TSS)

Well-mixed wastewater sample (100 mL) from each sampling point was measured and filtered through the glass-fibre filter paper (pore size 0.7 μm) under slight suction and the residue washed three times with 5 mL distilled water, allowing it to drain free from water after each wash. The filter paper was then removed and placed on a watch glass, dried in an oven at 105°C for 1 hour, cooled in a desiccator and filter paper weighed plus solids until constant weight was achieved upon cooling to room temperature. Measurements were taken in triplicate per wastewater sample and the average weight obtained and recorded (APHA, 1998). The following equation was then used to get TSS (mg/L) in a sample.

$$\text{TSS (mg/L)} = \frac{W1-W2}{V} \quad \text{Equation (3.2)}$$

Where,

W1 = mass of dried residue and filter paper in grams.

W2 = initial weight of filter paper

V = volume of sample water taken

3.4.1.8 Ammonia

Ammonia content in the wastewater samples was determined using the Wagtech Ammonia Test (Andrew, Lenore & Arnold, 1995). The method is based on the principal that a green-blue indophenols complex is formed when ammonia reacts with alkaline salicylate in the presence of chlorine. The intensity of the colour produced is directly proportional to the ammonia concentration in the sample. 10 mL of wastewater sample was filled into a test tube. One Ammonia No. 1 tablet and one Ammonia No. 2 tablet were then added and mixed into the sample. The sample was then left standing for 10 min fo20r colour to develop, before a photometer reading was performed on wavelength 640 nm in a spectrophotometer (Model SP75UV/VIS SANYO, U.K) for all the samples in triplicates. A blank sample was prepared for zero adjustment of photometer using distilled water while the calibration curve was prepared by making a standard series with five concentrations in a proper range (0 – 100 mg/L) on ammonia concentrations expected in the samples. The amount of ammonia in the wastewater sample was then determined by reading the transmittance percentage from the photometer and extrapolating the value from an ammonia calibration curve to get the ammonia concentration. Three replicate samples were measured and mean values recorded (APHA, 1998).

3.4.1.9 Nitrates

The amount of nitrate in the water samples was determined with the aid of the Wagtech Nitratest (Andrew *et al.,* 1995). In this test, nitrate was first reduced to nitrite, and the resulting nitrite was determined by a diazonium reaction to form a reddish dye. By using a zinc-based Nitratest powder and a Nitratest tablet, the reduction stage was carried out. 1 mL of wastewater sample was pipetted into a Nitratest tube. The Nitratest tube was then filled up to 20 mL with de-ionized water. One Nitricol tablet was added and dissolved in the solution.

A blank sample was prepared for zero adjustment of photometer using distilled water. The cuvette was then filled and the absorbance of the solution was measured in the photometer at 540 nm and all samples measured in triplicates. The calibration curve was then prepared by making a standard series with five concentrations in a proper range (0 – 100 mg/L) on nitrate concentrations expected in the samples. The colour intensity was read on a Wagtech Photometer at wavelength 540 nm. Three replicate samples were measured and mean values recorded (APHA, 1998). The transmission percentage given from the photometer was then extrapolated from the standards curve. The given value was then multiplied by 20 to get the nitrate concentration in the original sample in mg/L.

3.4.1.10 Nitrites

Nitrites concentration was determined using sulfanil acid method (APHA, 1995), where 50 mL of water sample was dispensed into Erlenmeyer flask and 1 ml of Sulphanilamide solution added. After a thorough mixing for 5 minutes, 1 mL of NEDD (n- [1- naphthyl] ethylene diamine dihydrochloride solution) solution was added and mixed again. The solution was then left to react for 10 minutes. A blank sample was prepared for zero adjustment of photometer using distilled water. The cuvette was then filled and the absorbance of the solution was measured in the photometer at 540 nm. Three replicate samples were measured and mean values recorded (APHA, 1998). The calibration curve was prepared by making a standard series with five concentrations in a proper range (0 –100 mg/L) on nitrite concentrations expected in the samples. The nitrite was found out from the plotted graph values.

3.4.1.11 Phosphates

Phosphate in wastewater samples was determined by Ammonium molybdate method (Andrew *et al.,* 1995). Erlenmeyer flask (100 ml) was filled with 50 mL of filtered water-samples and

1.5 mL of the mixed reagent (sulfuric acid, Ammonium molybdate solution and potassium antimony tartrate) was added. The solution was then mixed and 0.75 mL of ascorbic acid solution was added and left to react for 5 minutes. A blank sample was prepared using distilled water for zero adjustment of photometer. A cuvette was then filled and absorbance measured in the photometer at a wavelength of 890 nm. All samples were measured in triplicates and the sample reading derived from the standard curves. Three replicate samples were measured and their mean values recorded. The calibration curve was drawn from a series with five concentrations in a range of 0.00 – 0.100 mg/L derived from the phosphate stock solution.

3.4.1.12 Total Phosphorus (TP)

Wastewater total phosphorus analysis was determined by potassium persulphate digestion followed by ascorbic acid procedure (Andrew *et al.*, 1995). Hach COD tubes were filled with 50 mL of unfiltered samples in triplicate. Then 1 mL of still warm potassium persulphate was added and the tubes were weighed without lids. The weights were recorded and the lids were put into the tubes. The tubes were then autoclaved for 50 minutes at 121°C (15 psi). After cooling the tubes were weighed again and the evaporated water was replaced by addition of distilled water. Then 100 mL Erlenmeyer flask was filled with 50 mL of filtered water-samples and 1.5 mL of the mixed reagent (sulfuric acid, Ammonium molybdate solution and potassium antimony tartrate) was added. The solution was mixed and 0.75 mL of ascorbic acid solution added and left to react for 5 minutes. A blank sample was prepared using distilled water for zero adjustment of the photometer. A cuvette was then filled and absorbance measured in the photometer at a wavelength of 890 nm. All samples were measured in triplicates and the sample reading derived from the standard curves. Three replicate samples were measured and mean values recorded (APHA, 1998). The calibration

curve was drawn from a series with five concentrations in a range of 0.000 – 0.100 mg/L derived from the phosphate stock solution.

3.4.1.13 Total nitrogen (TN)

Total nitrogen in wastewater samples was determined using Kjeldahl method (Andrew *et al.*, 1995). 20 mL of unfiltered samples was placed in a 50 mL Erlenmeyer flask and 0.5 mL of sulfuric acid was then added and mixed. The solution was placed in an oven at 130°C overnight. On the next day 100 µl of hydrogen peroxide was added to the samples and placed in an oven at 230°C for 40 min. The samples were taken out of the oven, cooled and the flasks weighed again and 30 mL of distilled water and 3 mL of 5M NaOH was added. The samples were then neutralized by titrating with 1M NaOH to pH 5.6. The samples were then filled up to 40 mL and 4 mL of Na- salicylate and 4 mL of hypochlorite solutions was added, mixed and the samples were placed in the dark for 1 hr. The samples were then measured in the photometer at 650 nm in triplicates. Three replicate samples were measured and mean values recorded. Blank samples were prepared for zero adjustment of the photometer using distilled water. The calibration curve was drawn from a series of five concentrations in a range of 0 – 100 mg/L derived from the stock solution.

3.4.2 Determination of the concentration of *Escherichia coli*

The concentration of *E. coli* in every sample was quantified using the Idexx Quanti-Tray method in triplicates. Dilution series of the raw wastewater samples was prepared by taking 10 mL of sample and diluting in 90 mL of distilled water. When the desired dilution was obtained, 10 mL of the selected dilution was pipetted into an Erlenmeyer flask containing 90 mL of distilled water. The solution was then suspended in one snap pack of Colisure reagent and shaken thoroughly and rested for some time until no large particles were visible. The

reagent was then poured into a Quanti-Tray and sealed using the Quanti-Tray sealer. The sealed plates were then incubated at 35°C for 24 hours. After the incubation, enumeration of *E. coli* was performed. *E. coli* was determined by keeping the tray under a 6 watt, 365 nm UV light and counting the number of red/magenta or fluorescent wells appearing. The Most Probable Number (MPN) table was used to quantify the level of *E. coli* per 100 mL of sample. The enumeration was completed by implementing the number of positive wells in the MPN table (Appendix 4), getting the quantity of bacteria per 100 mL of sample.

3.4.3 Evaluation of aquatic macroinvertebrate composition and abundance

Macroinvertebrate samples were collected from the upstream, wetland and downstream sampled points. The aquatic macro-invertebrate samples were collected by placing a D-frame (Merrit & Cummins, 1996) aquatic net (500 μm) and scooping mud with a core-sampler from a 0.25 m^2 transect placed immediately upstream of the net. The mud samples were scooped from up to a depth of 15 cm and placed in a plastic container. Any dislodged organisms trapped in the D-frame net were emptied into plastic containers and immediately killed using 2% formalin. Different life stages (larvae/nymph, pupae and adult) of aquatic macroinvertebrates were collected depending on the taxa encountered. All debris was removed from the samples after picking all attached organisms. The macroinvertebrate samples were then sieved through a 500 μm mesh sieve in the field to separate the substrate and the benthic fauna (Sutherland, 1997). The sieve retained some organisms which were then preserved in 4% formalin and identified up to the family level using a standard identification key (Macan, 1977; APHA, 1998) in the laboratory.

3.5 Data analyses

Statistical analyses of wastewater quality parameters, *E. coli* and aquatic macro-invertebrate data was performed using both parametric and nonparametric tests. Statistical Package for Social Science (SPSS version 22) was used to perform the analyses. Mean values data for temperature, pH, DO, TSS, TDS, turbidity, BOD, ammonia, nitrate, nitrite, TN, TP, Phosphate and *E. coli* were pre-checked to ensure conformity using one way Analysis of Variance (ANOVA) and means separated by Tukey test. The diversity indices of aquatic macroinvertebrate community samples were analysed as indicators of water quality. Community composition and relative abundance of families also were analysed. The Shannon-Weiner diversity index was used in the form described by the equation as follows

$$H = -\sum \text{Pi}\ (\ln \text{Pi})$$

……. *Equation (3.3)*

Where,

H = Shannon-Weiner diversity index.

ln = Natural logarithm

Pi = Proportional abundance of a given family (i).

The proportional abundance (Pi) was calculated as follows:

$$\text{Pi} = \frac{\text{ni}}{\text{N}}$$ …… *Equation (3.4)*

Where:

ni is the number of individuals of a given family.

N is the total number of individuals of all families in the sample.

CHAPTER FOUR

RESULTS

4.1 Introduction

This chapter presents results on the levels of selected physicochemical parameters, concentration of *Escherichia coli* and effect of effluent on Aquatic macroinvertebrate abundance and composition of the macroinvertebrate families at the five sampled points. The results for the three study objectives were tabulated for each of the study objective and significant differences shown for physicochemical and *E. coli* objectives. Shannon -Weiner index (H) of macroinvertebrate diversity and abundance are also highlighted. The results of the study are outlined in the following subsections.

4.2 Levels of selected physicochemical parameters.

Mean water temperature at the sampled points ranged from 20.25 ± 0.13 to 21.75 ± 0.28°C (Table 4.1). The mean water temperature value for upstream (control) was not significantly different from that of downstream ($F_{4, 115} = 8.45$; $p = 0.8680$), outlet ($F_{4, 115} = 8.45$; $p = 0.2731$) or inlet ($F_{4, 115} = 8.45$; $p = 0.07534$) but was significantly higher than that of the wetland ($F_{4, 115} = 8.45$; $p = 0.0044$) (Table 4.1). Water temperature at inlet was significantly higher than that of outlet ($F_{4, 115} = 8.45$; $p = 0.0152$) and wetland ($F_{4, 115} = 8.45$; $p < 0.0001$). Water temperature at the outlet was significantly lower than that of downstream ($F_{4, 115} = 8.45$; $p = 0.0293$) but not significantly different from that of wetland ($F_{4, 115} = 8.45$; $p = 0.5075$). Water temperature values for downstream was significantly different from that of wetland ($F_{4, 115} = 8.45$; $p = 0.0001$). There were no other significant differences ($p > 0.05$).

Mean pH for the sampled points ranged from 7.01 ± 0.05 to 7.72 ± 0.11 on pH Scale (Table 4.1). The mean pH values for the upstream (control) was not significantly different from that

of downstream ($F_{4, 115} = 20.77$; $p = 0.9781$), wetland ($F_{4, 115} = 20.77$; $p = 0.9255$) or inlet ($F_{4, 115} = 20.77$; $p = 0.4550$) but was significantly lower from that of outlet ($F_{4, 115} = 20.77$; $p < 0.0001$) (Table 4.2). Water pH at outlet was significantly higher than those of inlet ($F_{4, 115} = 20.77$; $p < 0.0001$), wetland ($F_{4, 115} = 20.77$; $p < 0.0001$) and downstream ($F_{4, 115} = 20.77$; $p < 0.0001$). There were no other significant differences ($p > 0.05$).

Mean DO for the sampled points ranged from 0.02 ± 0.00 to 5.73 ± 0.02 mg/L (Table 4.1). Mean DO for upstream (control) was not significantly different from that of wetland ($F_{4, 115} = 5156.61$; $p = 0.8394$), but was significantly higher than that of downstream ($F_{4, 115} = 5156.61$; $p = 0.0040$), outlet ($F_{4, 115} = 5156.61$; $p < 0.0001$) and inlet ($F_{4, 115} = 5156.61$; $p < 0.0001$). Mean DO from the wetland was not significantly different from that of downstream ($F_{4, 115} = 5156.61$; $p = 0.0802$), but was significantly higher than that from the outlet ($F_{4, 115} = 5156.61$; $p < 0.0001$) and inlet ($F_{4, 115} = 5156.61$; $p < 0.0001$) (Table 4.1). Mean DO of the inlet was significantly lower than that from all other sampled points ($p > 0.05$).

The mean BOD for the sampled points ranged from 1.06 ± 0.04 to 668.1 ± 13.15 mg/L (Table 4.1). The mean BOD for upstream (control) was not significantly different from that of downstream ($F_{4, 115} = 38965.46$; $p = 0.9734$) and wetland ($F_{4, 115} = 38965.46$; $p = 0.9277$), but was significantly lower than that of outlet ($F_{4, 115} = 38965.46$; $p < 0.0001$) and inlet ($F_{4, 115} = 38965.46$; $p < 0.0001$) (Table 4.1). Mean BOD for inlet was significantly higher than that of outlet ($F_{4, 115} = 38965.46$; $p < 0.0001$), wetland ($F_{4, 115} = 38965.46$; $p < 0.0001$) and downstream ($F_{4, 115} = 38965.46$; $p < 0.0001$). At the STP, BOD removal efficiency was 93.13% between the inlet and outlet sampled points, and 99.59% at wetland point before the effluent joined river Sambul (table 4.2).

Mean turbidity for the different sampled points ranged from 2.67 ± 0.03 to 5.16 ± 0.03 NTU (Table 4.1). The mean turbidity for upstream (control) was significantly lower than that of downstream ($F_{4, 115}$ = 871.98; $p < 0.0001$) and inlet ($F_{4, 115}$ = 871.98; $p < 0.0001$), but was significantly higher than that of wetland ($F_{4, 115}$ = 871.98; $p < 0.0001$) and outlet ($F_{4, 115}$ = 871.98; $p < 0.0001$) (Table 4.1). Turbidity reduction levels were 48.26% and 38.57% (Table 4.2) at outlet and wetland sites respectively.

Total dissolved solids mean values for the different sampled points ranged from 129.83 ± 1.43 to 584.77 ± 15.33 mg/L (Table 4.1). The Mean total dissolved solids for upstream (control) was significantly lower than that of outlet ($F_{4, 115}$ = 123.27; $p < 0.0001$) and inlet ($F_{4, 115}$ = 123.27; $p < 0.0001$), but showed no significant differences from that of downstream ($F_{4, 115}$ =123.27; $p = 0.9997$) or wetland ($F_{4, 115}$ = 123.27; $p = 0.9978$) (Table 4.1). There were no other significant differences ($p > 0.05$) observed. The percentage removal of TDS at the outlet was 7.19% and 77.80% at the wetland point (Table 4.2).

Total suspended solids values for the different sampled points ranged from 27.69 ± 0.86 to 672.94 ± 22.88 mg/L (Table 4.1). The mean total suspended solids for upstream (control) was significantly lower than that of wetland ($F_{4, 115}$ = 708.50; $p < 0.0001$) and inlet ($F_{4, 115}$ = 708.50; $p < 0.0001$), but was not significantly different from that of downstream ($F_{4, 115}$ = 708.50; $p = 0.9999$) or outlet ($F_{4, 115}$ = 708.50; $p = 0.5068$). Mean TSS for inlet was significantly higher ($P < 0.05$) among all sampled points (Table 4.1). There was 92.13% (Table 4.2) TSS removal at outlet sampled point and was higher than at wetland site which was 75.14%.

Ammonia values for the different sampled points ranged from 0.42 ± 0.04 to 5.42 ± 0.66 mg/L (Table 4.1). The mean ammonia level for upstream (control) was significantly lower than that of inlet ($F_{4, 115}$ = 50.78; $p < 0.0001$), but showed no significant differences from that of downstream ($F_{4, 115}$ = 50.78; p = 1.0000), wetland ($F_{4, 115}$ = 50.78; p = 0.9617) or outlet ($F_{4, 115}$ = 50.78; p = 0.5797) (Table 4.1). There were no other significant ($p > 0.05$) differences observed. The inlet ammonia value was reduced by 75.65% and 92.25% at outlet and wetland studied sites respectively (Table 4.2).

Nitrate levels for the different sampled points ranged from 0.12 ± 0.02 to 16.06 ± 0.10 mg/L (Table 4.1). Mean nitrates for upstream (control) was significantly lower than that of outlet ($F_{4, 115}$ = 412.78; $p < 0.0001$), but was significantly higher than that of ($F_{4, 115}$ = 412.78; p = 0.0005) However, the mean nitrates for upstream was not significantly different from that of downstream ($F_{4, 115}$ = 412.78; p = 0.1919) or inlet ($F_{4, 115}$ = 412.78; p = 0.8011). Mean nitrates for downstream showed no significant differences ($F_{4, 115}$ = 412.78; p = 0.2577) from that of wetland (Table 4.1). At the outlet point, there was an increase of 92.95% of nitrates from that of inlet value (Table 4.2) while a decreased level of 92.31% was observed at the wetland.

Nitrite values for the different sampled points ranged from 0.04 ± 0.01 to 0.99 ± 0.02 mg/L (Table 4.1). Mean nitrites for upstream (control) was significantly lower than that of outlet ($F_{4, 115}$ = 412.78; $p < 0.0001$), but showed not significant differences from that of downstream ($F_{4, 115}$ = 943.53; p = 0.9986), wetland ($F_{4, 115}$ = 943.53; p = 1.0000) or inlet ($F_{4, 115}$ = 943.53; p = 0.3207) (Table 4.1). There were no other significant differences ($p > 0.05$) observed. A significant increased level of nitrites at outlet point (1137.5%) and a reduction of 50% at wetland were observed (Table 4.2).

Phosphate values for the different sampled points ranged from 0.05 ± 0.01 to 5.50 ± 0.15 mg/L (Table 4.1). Mean phosphate level for upstream (control) was significantly lower than that of outlet ($F_{4, 115}$ = 1125.73; $p < 0.0001$) and inlet ($F_{4, 115}$ = 1125.73; $p < 0.0001$), but showed no significant differences from that of downstream ($F_{4, 115}$ = 1125.73; $p = 0.9931$) and wetland ($F_{4, 115}$ = 1125.73; $p = 1.0000$) (Table 4.1). Also, mean phosphate levels for inlet and outlet were not significantly different ($F_{4, 115}$ = 1125.73; $p = 0.7250$). There were no other significant differences ($p > 0.05$) observed. The outlet samples showed an additional 3% from the inlet samples while the wetland samples had a reduction of 99.06% (Table 4.2).

Total Phosphorus values for the different sampled points ranged from 0.04 ± 0.01 to 2.88 ± 0.05 mg/L (Table 4.1). Mean total phosphorus level for upstream (control) was significantly lower than that of outlet ($F_{4, 115}$ = 2107.17; $p < 0.0001$) and inlet $F_{4, 115}$ = 2107.17; $p < 0.0001$), but showed no significant differences from that of downstream ($F_{4, 115}$ = 2107.17; $p = 0.9972$) and wetland ($F_{4, 115}$ = 2107.17; $p = 0.2285$) (Table 4.1). Mean TP for inlet was significantly higher ($F_{4, 115}$ = 2107.17; $p < 0.0001$) than that of outlet. TP removal efficiency was 29.86% and 95.83% (Table 4.2) at the outlet and wetland respectively.

Total nitrogen values for the different sampled points ranged from 1.06 ± 0.02 to 5.63 ± 0.35 mg/L (Table 4.1). Mean total nitrogen level for upstream (control) was significantly lower than that of wetland ($F_{4, 115}$ = 81.12; $p < 0.0001$) and inlet ($F_{4, 115}$ = 81.12; $p < 0.0001$), but showed no significant differences from that of downstream ($F_{4, 115}$ = 81.12; $p = 0.9354$) or outlet ($F_{4, 115}$ = 81.12; $p = 0.0753$) (Table 4.1). Mean total nitrogen level for inlet was significantly higher ($F_{4, 115}$ = 81.12; $p < 0.0001$) than that of the wetland. There were no

other significant differences ($p > 0.05$) observed. At the outlet, 81.17% of TN was removed and 45.47% (Table 4.2) reduction was observed at the wetland.

Table 4.1 Levels of selected physicochemical parameters and NEMA and MUEDS (n = 24)

Parameter	Inlet	Outlet	Wetland	Downstream	Upstream	NEMA/MUDMS Acceptance values
Temp °C	21.75 ± 0.28^{a}	20.75 ±0.12^{bc}	20.25 ±0.13^{c}	21.68 ± 0.29^{a}	21.38 ± 0.22$^{a b}$	25-35
pH	7.01 ± 0.05^{b}	7.72 ± 0.11^{a}	7.09 ± 0.05^{b}	7.12 ± 0.04^{b}	7.16 ± 0.04^{b}	6-9
DO	0.02 ± 0.00^{d}	3.59 ± 0.02^{c}	5.68 ± 0.07^{ab}	5.56 ± 0.01^{b}	5.73 ± 0.02^{a}	*
BOD	668.10 ± 13.15^{a}	41.99 ± 1.02^{b}	2.76 ± 0.33^{c}	2.34 ± 0.07^{c}	1.06 ± 0.04^{c}	30/20
Turbidity	5.16 ± 0.03^{a}	2.67 ± 0.03^{e}	3.17 ± 0.03^{d}	4.09 ± 0.05^{b}	3.76 ± 0.02^{c}	*
TDS	584.77 ± 15.33^{a}	542.71 ±44.79^{a}	129.83 ± 1.43^{b}	133.40 ± 1.09^{b}	139.33 ± 3.15^{b}	1200
TSS	672.94 ± 22.88^{a}	52.94 ± 1.98^{c}	167.26 ± 2.91^{b}	27.69 ± 0.86^{c}	29.63 ± 0.95^{c}	30
Ammonia	5.42 ± 0.66^{a}	1.32 ± 0.05^{b}	0.42 ± 0.04^{b}	0.66 ± 0.03^{b}	0.70 ± 0.01^{b}	100/1.5
Nitrate	1.56 ± 0.05^{b}	16.06 ± 0.10^{a}	0.12 ± 0.02^{c}	1.06 ± 0.03$^{b c}$	2.08 ± 0.73^{b}	100/15
Nitrite	0.08 ± 0.01^{b}	0.99 ± 0.02^{a}	0.04 ± 0.01^{b}	0.05 ± 0.01^{b}	0.04 ± 0.01^{b}	100
Phosphate	5.34 ± 0.12^{a}	5.50 ± 0.15^{a}	0.05 ± 0.01^{b}	0.11 ± 0.01^{b}	0.06 ± 0.01^{b}	*
TP	2.88 ± 0.05^{a}	2.02 ± 0.04^{b}	0.12 ± 0.01^{c}	0.05 ± 0.01^{c}	0.04 ± 0.01^{c}	2 Guideline value
TN	5.63 ± 0.35^{a}	1.06 ± 0.02^{c}	3.07 ± 0.22^{b}	1.56 ± 0.07^{c}	1.78 ± 0.11^{c}	2 Guideline value

Mean (±SEM) in the same row followed by the same alphabet letter are not significantly different at p = 0.05.

Table 4.2 STP pollutants removal efficiency in sewage effluents (n = 24).

Parameter	Inlet (I)	Outlet (O)	Wetland (W)	% Decrease (I/O)	% Decrease (I/W)
BOD	668.10 ± 13.15	41.99 ± 1.02	2.76 ± 0.33	93.72	99.59
TDS	584.77 ± 15.33	542.71 ±44.79	129.83 ± 1.43	7.19	77.80
TSS	672.94 ± 22.88	52.94 ± 1.98	167.26 ± 2.91	92.13	75.14
Turbidity	5.16 ± 0.03	2.67 ± 0.03	3.17 ± 0.03	48.26	38.57
Ammonia	5.42 ± 0.66	1.32 ± 0.05	0.42 ± 0.04	75.65	92.25
Nitrate	1.56 ± 0.05	16.06 ± 0.10	0.12 ± 0.02	+92.95	92.31
Nitrite	0.08 ± 0.01	0.99 ± 0.02	0.04 ± 0.01	+1137.5	50.00
Phosphate	5.34 ± 0.12	5.50 ± 0.15	0.05 ± 0.01	+3.00	99.06
TP	2.88 ± 0.05	2.02 ± 0.04	0.12 ± 0.01	29.86	95.83
TN	5.63 ± 0.35	1.06 ± 0.02	3.07 ± 0.22	81.17	45.47
E.coli	817.83 ± 0.35	5.83 ± 0.35	24.83 ± 0.35	99.29	96.96

4.3 Concentration of *Escherichia coli*.

Escherichia coli mean values for the different sampled points ranged from 5.83 ± 0.35 to 817.83 ± 0.35 MPN/100 ml (Table 4.3). Mean concentration of *E. coli* for upstream (control) was significantly lower than that for downstream ($F_{4, 115}$ = 935593.30; $p < 0.0001$) and inlet ($F_{4, 115}$ = 935593.30; $p < 0.0001$), but was significantly higher than that for wetland ($F_{4, 115}$ = 935593.30; $p < 0.0001$) and outlet ($F_{4, 115}$ = 935593.30; $p < 0.0001$) (Table 4.3). Mean concentration of *E. coli* for inlet was significantly higher ($p < 0.05$) than that of the other sampled points, while the concentration for outlet was significantly lower ($p < 0.05$) than that of all other sampled points. There were no other significant differences ($p > 0.05$) observed. The removal efficiency of *E. coli* at the outlet and wetland sampled points was 99.29% and 96.96% respectively (Table 4.2).

Table 4.3: Mean (± SEM) *Escherichia coli* of wastewater at the sampled points.

Sampled point	n	*Escherichia coli* (MPN/ 100mL)
Inlet	24	817.83 ± 0.35 [a]
Outlet	24	5.83 ± 0.35[e]
Wetland	24	24.83 ± 0.35[d]
Downstream	24	444.83 ± 0.35[b]
Upstream (control)	24	418.83 ± 0.35[c]

Mean (±SEM) in the same column followed by the same alphabet letter are not significantly different at p = 0.05.

4.4 Effect of effluent on aquatic macroinvertebrate diversity and abundance

The aquatic macroinvertebrate composition and abundance in the sampling points (upstream, wetland and downstream) are summarized in Tables 4.4, 4.5 and 4.6 respectively. At the sampling points, a total of 12 orders and 14families in were identified from a total of 5,365 macroinvertebrate individuals collected. A total of 1,928 individual macroinvertebrates was collected at the upstream (control) point while 1,721 and 1,716 were collected from wetland and downstream sampled points, respectively. The derived Shannon- Weiner diversity index (H) value for upstream (control), wetland and downstream indicate that in terms of aquatic macroinvertebrate populations, upstream had the highest value (H=2.504) followed by downstream (H=2.409) while wetland had the lowest (H=2.371) (Tables 4.4, 4.5 and 4.6). The highest H value was identified at the upstream (control) implying that macroinvertebrate diversity at upstream was higher than that of wetland and downstream. However, downstream sample point showed higher macroinvertebrate diversity than wetland point. In addition,

upstream (control) had a total of 14 macroinvertebrate families which was higher than that of wetland and downstream with 13 and 11 respectively (Tables 4.4, 4.5 and 4.6).

The relative abundance of EPT (Ephemeroptera, Plecoptera and Tricoptera) at upstream (control) sampled point were 5.91%, 8.77% and 7.05% respectively. Downstream showed a relative abundance of 5.35%, 8.54% and 6.68% and wetland had 3.96%, 6.18% and 10.90% respectively for EPT. Families of Coleoptera, Diptera, Gastropoda, Chilopoda and Hirudinea were equally represented in the sampled points (Table 4.7). The family Gammaridae showed a relative abundance of 5.08% at the upstream (control) sampled point while it was absent at wetland and downstream. Similarly family Lumbricidae had a relative abundance of 0.07% and 1.34% at the upstream (control) and downstream sampled points, but was absent at wetland sampled point Hygrobatidae family was absent at wetland sampled point but had a relative abundance of 1.35% and 0.01% at the upstream (control) and downstream sampled points (Table 4.7).

Table 4.4: Shannon -Weiner diversity index of macroinvertebrate families at the upstream study site of Sambul River

Phylum	Class	Order	Family (S)	Number of individuals (n)	n/N	pi	ln pi	pi ln pi
Arthropoda	Insecta	Ephimeroptera	Baetidae	114	114/1928	0.059	−2.830	−0.167
		Plecoptera	Nemouridae	169	169/1928	0.088	−2.430	−0.214
		Trichoptera	Hydropsychidae	136	136/1928	0.071	−2.645	−0.188
		Odonata	Gomphidae	189	189/1928	0.098	−2.323	−0.228
		Coleoptera	Helodidae	267	267/1928	0.138	−1.981	−0.273
		Diptera	Chironomidae	110	110/1928	0.057	−2.865	−0.163
			Tibulidae	126	126/1928	0.065	−2.733	−0.178
			Simulidae	213	213/1928	0.110	−2.207	−0.243
	Crustacea	Amphipoda	Gammaridae	98	98/1928	0.051	−2.976	−0.152
	Malacostraca	Decapoda	Hygrobatidae	26	26/1928	0.013	−2.674	−0.056
Mollusca	Gastropoda	Basommatophora	Limnaeidae	133	133/1928	0.069	−4.343	−0.185
Annelida	Oligochaeta	Enchytraeida	Lumbricidae	13	13/1928	0.007	−4.962	−0.035
	Clitellata	Hirudinea	Erpobdellidae	189	189/1928	0.098	−2.323	−0.228
Platyhelminthes	Turbellaria	Planaria	Planariidae	145	145/1928	0.075	−2.590	−0.194

S (number of families) = 14, N (total number of individuals) = 1928, Σ (sum) of - Σpi ln pi= - 2.504, H= 2.504

Table 4.5: Shannon - Weiner diversity index of macroinvertebrate families at the wetland study site of Sambul River

Phylum	Class	Order	Family (S)	Number of individuals (n)	n/N	pi	ln pi	pi ln pi
Arthropoda	Insecta	Ephimeroptera	Baetidae	92	92/1721	0.0535	-2.9281	-0.1567
		Plecoptera	Nemouridae	147	147/1721	0.0854	-2.4604	-0.2101
		Trichoptera	Hydropsychidae	115	115/1721	0.0668	-2.7061	-0.1808
		Odonata	Gomphidae	167	167/1721	0.0970	-2.3330	-0.2263
		Coleoptera	Helodidae	155	155/1721	0.0901	-2.4068	-0.2169
		Diptera	Chironomidae	216	216/1721	0.1255	-2.0754	-0.2605
			Tibulidae	134	134/1721	0.0779	-2.5523	-0.1988
			Simulidae	221	221/1721	0.1284	-2.0526	-0.2636
Mollusca	Gastropoda	Basommatophora	Limnaeidae	145	145/1721	0.0843	-2.4734	-0.2085
Annelida	Clitellata	Hirudinea	Erpobdellidae	173	173/1721	0.1005	-2.2976	-0.2309
Platyhelminthes	Turbellaria	Planaria	Planariidae	156	156/1721	0.0906	-2.4013	-0.2176

S (number of families) = 11, N (total number of individuals) = 1721, Σ (sum) of - Σ pi ln pi= -2.371, H= 2.371

Table 4.6: Shannon - Weiner diversity index of macroinvertebrate families at the downstream study site of Sambul River

Phylum	Class	Order	Family (S)	Number of individuals (n)	n/N	pi	ln pi	pi ln pi
Arthropoda	Insecta	Ephimeroptera	Baetidae	68	68/1716	0.0396	-3.2289	-0.1279
		Plecoptera	Nemouridae	106	106/1716	0.0618	-2.7839	-0.1720
		Trichoptera	Hydropsychidae	187	187/1716	0.1090	-2.2164	-0.2416
		Odonata	Gomphidae	155	155/1716	0.0903	-2.4046	-0.2171
		Coleoptera	Helodidae	137	137/1716	0.0798	-2.5282	-0.2018
		Diptera	Chironomidae	197	197/1716	0.1148	-2.1646	-0.2485
			Tibulidae	160	160/1716	0.0932	-2.3730	-0.2212
			Simulidae	186	186/1716	0.1084	-2.2219	-0.2409
	Malacostraca	Decapoda	Hygrobatidae	9	9/1716	0.0052	-5.2591	-0.0273
Mollusca	Gastropoda	Basommatophora	Limnaeidae	129	129/1716	0.0752	-2.5876	-0.1946
Annelida	Oligochaeta	Enchytraeida	Lumbricidae	23	23/1716	0.0134	-4.3125	-0.0578
	Clitellata	Hirudinea	Erpobdellidae	243	243/1716	0.1416	-1.9547	-0.2768
Platyhelminthes	Turbellaria	Planaria	Planariidae	116	116/1716	0.0676	-2.6941	-0.1821

S (number of families) = 13, N (total number of individuals) = 1716, Σ (sum) of - Σ pi ln pi= -2.4096, H = 2.409

Table 4.7: Composition and relative abundance (%) of the macroinvertebrate families in the upstream, wetland and downstream study sites of Sambul River.

Order	Family	Upstream (control)	Relative abundance (%)	Wetland	Relative abundance (%)	Downstream	Relative abundance (%)
Ephimeroptera	Baetidae	114	5.91	92	5.35	68	3.96
Plecoptera	Nemouridae	169	8.77	147	8.54	106	6.18
Trichoptera	Hydropsychidae	136	7.05	115	6.68	187	10.90
Odonata	Gomphidae	189	9.80	167	9.70	155	9.03
Coleoptera	Helodidae	267	13.85	155	9.01	137	7.98
Diptera	Chironomidae	110	5.71	216	12.55	197	11.48
	Tipulidae	126	6.54	134	7.79	160	9.32
	Simulidae	213	11.05	221	12.84	186	10.84
Amphipoda	Gammaridae	98	5.08	-	-	-	-
Basommatophora	Limnaeidae	133	6.90	145	8.43	129	7.52
Oligochaeta	Lumbricidae	13	0.07	-	-	23	1.34
Hirudinea	Erpobdellidae	189	9.80	173	10.05	243	14.16
Planaria	Planariidae	145	7.52	156	9.06	116	6.76
Decapoda	Hygrobatidae	26	1.35	-	-	9	0.01
	Total (N)	**1928**		**1721**		**1716**	

n = Number of individuals in a family, N = Total number of individuals in all families.

CHAPTER FIVE

DISCUSSION

This chapter provides a discussion of the findings for the three study objectives. The dynamics of the physicochemical parameters are discussed by outlining the significance of maintaining the allowable levels of the selected parameters based on the NEMA and MUEDMS environmental standards; the significance of *E. coli* in aquatic environment and its subsequent impacts and macroinvertebrate composition in terms of abundance and diversity for the sampled points.

The significant changes found in effluent quality from the five sampled points of the study area are an indication of the presence and removal of pollutants in the wastewater effluent. This could be attributed to considerable biological treatment of the wastewater in the sewage stabilization ponds. Sewage originates from Moi University premises that include hostels, laboratories, administrative units and kitchens.

Wastewater temperature mean value at the inlet was significantly higher than at the outlet and downstream. This could be attributed to a high content of warm water from received from University premises (Metcalf & Eddy, 2004). The mean water temperature was within the allowable limits 25-35°C by NEMA (Appendix 2). This mean water temperature range is favourable in biotreatment of wastewater as microbial reactions slow down at low temperatures, while nitrification and aerobic digestion stops at very high temperatures.

Outlet pH values were higher from that of inlet. This is attributed to the influence of biotreatment of wastewater at the treatment plant. The mean pH values were within the Moi

University effluent discharge monitoring standard (MUEDMS) range of 6 to 9 (Appendix 2 & 3). Droste (1997) noted that the pH of wastewater needs to be between 6 and 9 to protect beneficial microbial organisms. Influent water with exceptionally high or low pH-values can be difficult to treat by biological means and effluent discharges may affect the pH of the natural waters in the recipient.

There was a significantly higher level of DO in sewage effluent at the outlet channel (3.59 mg/L) than the inlet (0.02 mg/L). The study findings are similar to the findings made by Omoto in 2006 on a sewage treatment plant, which also gave a range of 4-6 mg/L for DO values. The rise in DO at the outlet could be a result of the wastewater being exposed to free oxygen at the facultative and maturation ponds through the air currents. The increase in DO level could be attributed to degradation of organic matter in the stabilization ponds thereby decreasing the BOD_5 and raising the DO. The means of DO at the inlet and outlet was inversely proportional to the means of BOD_5. There were high levels of BOD_5 at the inlet (668.10 mg/L) than outlet (41.99 mg/L). The BOD_5 values were not within the NEMA standard of 500mg/l and 30 mg/L for inlet and outlet respectively. However, the removal efficiency of BOD_5 at the outlet point was 93.72% implying that the treatment plant is efficient. The reduced levels of BOD_5 at outlet also explains the functioning of anaerobic zones of the stabilization ponds where anaerobic bacteria converts organic carbon into methane subsequently removing upto 60% of the BOD_5 (Navaraj, 2005). The significantly high levels of BOD_5 at the inlet point could be attributed to high organic matter content in raw sewage that flows to the treatment plant and that requires high levels of oxygen to be decomposed. The high level of BOD_5 indicates the pollution strength of the wastewaters and low oxygen availability for living organisms in the wastewater. If sewage effluent with high

BOD_5 levels is discharged into a river, it will accelerate bacterial growth in the river and consequently deplete the oxygen levels in the river (Ramesh *et al*., 2007).

The mean turbidity value at the inlet (5.16 mg/L) was significantly higher from that of outlet (2.67 mg/L). The reduced turbidity levels at outlet is attributed to sedimentation of suspended matter such as clay, silts, plankton debris and degradation of microorganisms by ultra violet rays at the stabilization ponds. The high level of turbidity downstream (4.09 mg/L) river Sambul is attributed to the frequent surface runoff from farms and murram road near the sampled point. The effluent turbidity levels out of the sewage treatment plant and downstream river Sambul were less than 10 NTU. This is the maximum limit of which physiological processes such as photosynthesis in aquatic plants could be lowered if exceeded according to the findings of by Shittu, Olaitan, & Amusa in 2008. High turbidity levels can block light from reaching aquatic plants and causes an increase in contaminants and pathogens loads in effluent receiving water bodies.

The study findings showed that mean value for TDS at the inlet (584.77 mg/L) was significantly higher from that of outlet (542.71 mg/L). These levels were within the NEMA standards of 2000mg/L and 1200mg/L for inlet and outlet respectively (Appendices 2). The observed reduction of 7.19% at the outlet could be attributed to the sedimentation of inorganic and organic salts in the stabilization ponds. The pronounced TDS reduction of 77.8% observed at wetland could be attributed to biofiltration of excess solids bringing the levels down before the effluent was discharged into Sambul River. The reduced levels on TDS concentrations is vital in sewage effluents as high levels inhibit growth of many aquatic species, and death may occur as excess salts may dehydrate aquatic organisms and hinder physiological processes like osmosis (Giller & Malmqvist, 1998) resulting in poor water

quality. The presences of algae in the maturation pond imply that salts such as calcium, phosphates and magnesium are utilized for the various metabolic reactions within the algal cells, thus contributing to lower levels of TDS at the outlet channel.

The total suspended solids (TSS) for outlet (52.94 mg/L) significantly lower than that of inlet (672.94 mg/L). The reduced TSS levels at the outlet could be attributed to the sewage treatment plant TSS removal efficiency through the microbial decomposition of organic matter and sedimentation of settleable solids. The significantly higher inlet level is attributed to the raw sewage with considerably large amounts of TSS components that include decaying plants, silt, animal matter, industrial wastes, and human excreta. The outlet value was not within the NEMA and MUEDMS standards of 30mg/L (Appendices 2 and 3). However TSS removal of 92.13% was observed in the study implying that the treatment plant is relatively efficient. Discharge of effluents with high levels of suspended solids into aquatic bodies lowers water quality and depletes dissolved oxygen available for aquatic life.

The study findings showed Total phosphorus (TP) mean value at the inlet (2.88mg/L) was higher than that of the outlet (0.12mg/L). Phosphorus exists in sewage water in form of organically-bound phosphate, soluble orthophosphate ion (PO_4^{-3}), and other phosphorus-oxygen compounds. This implies that at inlet where sewage is received, total phosphorus is expected to be significantly high as shown by the study results. The lower level of phosphorus at the outlet indicates that Moi university sewage treatment plant is efficient in the removal of nutrients. This lower level is attributed to oxidation of phosphorus to phosphates and adsorption at the ponds. The sedimentation processes at the ponds enables phosphorus molecules to bind to the solids that settles at the bottom of treatment ponds and will eventually be removed during desludging (Soares *et al.,* 1996). However, phosphorus must be

removed before effluent is released to the environment as high concentrations leads to eutrophication, algal blooms, foul smelling, excessive weed growth and the loss of species diversity in surface water bodies such as lakes and rivers. At the wetland sampled point the effluent showed a phosphate concentration of 0.05mg/L which represents a reduction of 99.06% compared to the inlet (2.88mg/L). The projected reduction of phosphates at the wetland is attributed to the biofiltration of nutrients by macrophytes present at the sampled point.

The study showed that mean values of total nitrogen (TN) were significantly lower at outlet (1.06mg/L) than that of inlet (5.63mg/L). The high levels of TN at inlet could be attributed to raw sewage which contains high levels nitrogen in forms of ammonia, nitrates, nitrite, urea, proteins and amino acids. The discharge of these nitrogen compounds into the receiving ecosystem would lead to environmental degradation and health risks. Removal of nitrogen compounds in sewage effluents is thus significant as exhibited by the removal percentage of 99% at outlet (1.06 mg/L). The observed high reduction of TN at outlet is attributed to the removal of nitrogen compounds through ammonification, nitrification and denitrification at the sewage treatment stabilization ponds (Mary, 2005). In nitrification, ammonia (NH_4-N) is oxidized to nitrite (NO_2-N) before nitrite is oxidized to nitrate (NO_3-N), both steps under the presence of oxygen and Nitrosomonas and Nitrobacter bacteria respectively. Denitrification of nitrates leads to more nitrogen compounds removal in the treatment ponds. This occurs when nitrate reduction to nitric oxide, nitrous oxide and finally nitrogen gas with the aid of Pseudomonas bacteria (Metcalf & Eddy 2004). At the outlet, ammonia concentration was reduced by 75.65% while upstream (control) sampled point showed a higher ammonia value of 0.7mg/L than that of wetland (0.42mg/L) point implying that Sambul River could be receiving ammonia compounds from other sources. Nitrates and nitrites showed pronounced

values of 16.06mg/L and 0.99mg/L respectively at the outlet and is also attributed to nitrification and denitrification processes at the treatment ponds. However, nitrates and nitrites levels significantly reduced to 0.12mg/l and 0.04mg/L respectively at wetland sampled point, and are attributed to the biofiltration by various plants. The wetland nitrate value of 0.12mg/L conformed to the MUEDMS (Appendix 3) of 15mg/L allowable for discharge into the environment. The present study agreed with Wolfgang, Michael, Erich, & Karl (2002) that reduction of TN and its forms in sewage effluents discharged into aquatic environments will aquatic protect the water quality and safeguard biodiversity and public health.

Mean *E. coli* value for inlet (817.83MPN/100ml) was significantly higher from that of the outlet (5.83MPN/100ml) sampled point that was slightly above the NEMA standard of Nil/100mL. The high levels of *E.coli* at the inlet channel are attributed to the untreated raw sewage flowing into the treatment plant. *E.coli* occurs predominantly in infected human excreta and urine that flows to the treatment ponds and is used as indicator of water quality. The present study agrees with that of Sanders, *et al.,* (2013) that *E. coli* has been widely used to evaluate the efficiency of wastewater treatment plants and is found in the faeces of warm blooded animals in large numbers and therefore used as an indicator of faecal content in wastewater samples. The low levels of *E.coli* at the outlet channel is attributed to the reduction of *E.coli* through natural die off at the stabilization ponds as a result of exposure to ultra violet rays in addition to disinfection by filtration process the effluent is subjected to before it is discharged into the environment. At the outlet BOD_5 removal level is 99.59% implying that bacterial growth has been greatly inhibited and *E.coli* concentrations declined. The observed 99.29% reduction of *E.coli* at the outlet channel implies that the sewage treatment plant is efficient in the removal of pathogens from sewage effluents. Similarly, *E.*

coli levels were high at upstream (418 MPN/100ml).The high levels of *E. coli* at upstream of Sambul River indicates that the river is polluted by other sources. The study finding is similar to that of 2011 by Masters *et al*, that discharge of untreated or improperly treated sewage effluent into aquatic bodies can cause increase pathogenic loads consequently resulting in infectious water borne and water related disease epidemics.

The selected water quality parameters of the receiving Sambul River at upstream and downstream were not significantly different. This implies that the effluent discharged from the Moi University Sewage Treatment Plant had no observable consequences on water quality parameters of the receiving Sambul River. Consequently, STP had no observable consequences on the aquatic macroinvertebrate community at the river. The aquatic macroinvertebrate composition and abundance at the upstream (control site), wetland and downstream sampled points showed no significant differences. The derived Shannon- Weiner diversity index (H) value for upstream (2.504), wetland (2.371) and downstream (2.409), indicate that in terms of aquatic macroinvertebrate diversity, upstream was the most diverse followed by downstream while wetland was the least diverse.

The abundance of EPT (Ephemeroptera, Plecoptera and Tricoptera), that are highly sensitive to pollution (intolerant), were not significantly different at upstream, downstream and wetland sampled points. The study finding is similar to the study by Barbour, Gerritsen, Snyder, & Stribling (1999) which showed that the families of Coleoptera, Diptera, Gastropoda and Chilopoda and Hirudinea that are tolerant to pollution were equally represented in the sampled points and that the presences of this taxa could be used to evaluate the health of effluent receiving aquatic environments.

The present study established no decrease of taxa richness and dominance by tolerant taxa downstream that would translate into low community diversity. This agrees with the study hypothesis that there are no significant changes in the abundance of aquatic macroinvertebrate at the receiving Sambul River that may be attributed to the effect of discharged treated effluent. Downstream, however, slight recovery in taxa richness was found and this could be attributed to effect of river dilution and self-cleansing. Lack of induced nutrient enrichment downstream from the effluent explains the fact that there was no concomitantly increase in productivity of the Sambul River waters downstream. Several studies maintain that numerically macroinvertebrates increases under moderate nutrient enrichment and decrease under high nutrient inputs (Landman, Van Den Heuvel & Ling, 2005). Upstream point of river Sambul was considered undisturbed while downstream and wetland sampled points were considered to be disturbed.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Based on the tested parameters, the following conclusions are drawn: the study confirms that Moi University sewage treatment plant is relatively efficient in the treatment of sewage as shown by the levels of measured physicochemical parameters and pollutants removal efficiencies. Most parameters were within the wastewater effluent standards of NEMA and MUEDMS and this implies that the STP operates within the environmental regulations and has no adverse impacts on the environment. Wetland sampled point plays a significant role in wastewater treatment process where wetland plants filters excess nutrients from the outlet channel of Moi University treatment plant.

The *E. coli* concentrations at outlet point of Moi University sewage treatment plant were negligible while upstream (control) and downstream had higher *E. coli* concentrations. This implies that Sambul River is contaminated at upstream (control) rather than by the Moi University sewage effluent. This implies that the Moi University sewage treatment plant is effective in treating its wastewater.

The effluent discharged into Sambul River had no effect on macroinvertebrate abundance and diversity as shown by the Shannon-Weiner indices at the upstream, wetland and downstream points. The macroinvertebrate families encountered during the study were represented in at the sampled points. The presence of the intolerant species to pollution at all the sampled sites implying that the effluent discharged into river Sambul had no significant effect on the macroinvertebrates. This category of organisms is used as bioindicators in freshwater bodies that receive effluents from wastewater treatment plants. Therefore the evaluated parameters

on macroinvertebrates at river Sambul confirm the relative efficacy of Moi University sewage treatment plant.

The study recommends further research to be undertaken in the months and times of the year not covered in this study for comparison of the efficiency of the treatment plant and assessment of the macroinvertebrates at the effluent receiving river Sambul. Parameters such as heavy metals, COD, oil and grease and laboratory chemicals should also be investigated at the sewage treatment plant in Moi University.

Moi University wetland is prone to external anthropogenic interference. There is need for the protection of this site to enhance biofiltration of excess nutrients by wetland plants. This will further prevent livestock grazing observed during the entire study period.

The upstream of Sambul River showed high levels of *E. coli* contaminations. There is need for sensitization of Sambul River riparian community on the environmental protection. This will avert possible outbreaks of water borne diseases such as cholera and typhoid within the community as water from Sambul River is utilized for domestic activities by the riparian community.

REFERENCES

Andrew, D.E., Lenore, S.C., & Arnold, E.G. (1995). Standard Methods for the Examination of Water and Wastewater. 19th Edition.

American Public Health Association (APHA), (1998). Standard Methods for the Examination of Water and Wastewater. 20th Edition. American Water Works Association/ Water Environmental Federation, Washington DC. PP:1287.

Barbour, M.T., Gerritsen, J., Snyder, B.D., & Stribling, J.B. (1999). Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish. 2nd Edition. Technical Report No. EPA 841-B-99-002. Office of Water, U.S. environmental Protection Agency.

Burks, B., & Minnis, M. (1994). Onsite Wastewater Treatment Systems. *Madison, WI: Hogarth House, Limited*: 25-34.

Britannica, (2012). Encyclopaedia Britannica checked/topic/666611/wastewater treatment/72337/Historical- background.

Carey, R.O., & Migliaccio, K.W. (2009). Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems: a review. *Environmental Management, 44*: 205–217.

Dates, G. (1994). Monitoring for phosphorus. *Volunteer Monitor,* 6: 39.

Davis, M. L. (2011). Water and Wastewater Engineering, Design Principles and Practice. New York: McGraw-Hill.

Dixon, A., Simon, M., & Burkitt, T. (2003). Assessing the environmental impact of two options for small-scale wastewater treatment: comparing a reed bed and an aerated biological filter using a life cycle approach. *Ecological Engineering*, *20*: 297–308.

Droste, R. (1997). Theory and Practice of Wastewater Treatment. *Technology and Engineering:* PP: 678-681.

Environmental Management and Co-ordination (EMCA) Act No 8 of 1999, Laws of Kenya, *Government press*.

Giller, P.S., & Malmqvist, B. (1998). *The Biology of Streams and Rivers*. *Biology of Habitats Series.* Oxford University Press, 1: 231-235.

Gregorio, C., & Pierre-Marrie, M. (2008). Application of chitosan, a natural amino polysaccharide, for dye removal from aqueous solutions by adsorption processes using batch studies. *Progress in Polymer Science, 33*: 4

Gücker, B., Brauns, M., & Pusch, M.T. (2006). Effects of wastewater treatment plant discharge on ecosystem structure and function of lowland streams. Journal North American. Benthol Society, 25:313–329

Jagai, J.S., Li Wang, Q.S., Messier, K.P., Wade, T.J., & Hilborn, E.D. (2015). *Environmental Health Perspectives, 110* (4): A174.

Kaluli, W., Githuku, C., Home, P., & Mwangi, M. (2011). Towards a National Policy on Wastewater Reuse in Kenya, *Journal of Agriculture, Science and Technology, 13* (1): 116-125.

Kemira, N. (2003). About Water Treatment. Helsingborg: Kemira Kemwater, PP: 220

Landman, M.J., Van Den Heuvel, M.R., & Ling, N. (2005). Relative sensitivities of common freshwater fish and invertebrates to acute hypoxia. New Zealand Journal of Marine and Freshwater Research, 39: 1061–1067.

Macan, T.T. (1977). A key to British Fresh and Brakish water Gastropods. Freshwater Biologicak Association, PP: 100.

Malik, O.A., Hsu, A., Johnson, L.A., & De Sherbinin, A. (2015). A global indicator of wastewater treatment to inform the Sustainable Development Goals (SDGs). Environmental Science Policy. *Elsevier Limited, 48*:172–85.

Masters, N., Wiegand, A., Ahmed, W., & Katouli, M. (2011). *Escherichia coli* virulence genes profile of surface waters as an indicator of water quality. *Water Research,* PP: 6321-6333.

McHale, R., & McChesney, D. (2007). Phosphorus concentrations in stream-water and reference samples. An assessment of laboratory comparability. *U.S. Geological Survey Open-File Report 1267*: 25.

Merrit, R. W., & Cummins, K. W. (1996). *An introduction to the aquatic insects of North America*, 3rd edition. Dubuque: Kendall-Hunt, IOWA.

Metcalf, W., & Eddy, I. (2004). Wastewater Engineering: Treatment and Reuse. 4th edition New York: McGraw-Hill, PP: 1819.

Naidoo, S., & Olaniran, A. (2013). Treated wastewater effluent as a source of microbial pollution of surface water resources. *International Journal of Environmental Res ources and Public Health, 11* (1): 249-270.

Navaraj, P.S., (2005). Anaerobic Waste Stabilization ponds: A low cost contribution to a sustainable Reuse Cycle, Asian development Bank.

Nzuki, S. (2008). Initial Environmental Audit Report for Kipevu/ west mainland Sewage Treatment works and Sewer System: PP 170-180.

Oben, B.O., Oben, P.M., Ugwumba, A.O, Okorie, T.G., & Pleysier, J. (2003). Trace metal dynamics in Water, Sediments and Shellfish of a Constructed Freshwater Wetland.

Omoto, E. (2006). A study of Nairobi wastewater treatment efficiency and effluent quality for safe discharge and possible beneficial uses. *MSc thesis, University of Nairobi:* PP: 5-50.

Ramesh, B., Bhadrinarayana, N.S., Meera, S.B., & Anantharaman, N. (2007). Treatment of tannery wastewater by electro-coagulation. *Chemical Technology and Metallurgy, 42* (2): 201-206.

Sanders, E.C., Yuan, Y., & Pitchford, A. (2013). Fecal coliform and *E. coli* concentrations in effluent-dominated streams of the upper Santa Cruz watershed. Water (Switzerland), 5 (1): 243–61.

Shittu, O.B., Olaitan, J.O. & Amusa, T.S. (2008). Physico-Chemical and Bacteriological Analyses of Water Used for Drinking and Swimming Purposes in Abeokuta, Nigeria. *African Journal of Biomedical Research, Vol. 11* (2008): 285 -290

Shu, L., Wait, T.D., Bliss, P.J., Fane, A., & Jegathessan, V. (2005). Nano filtration for the Possible Reuse of Water and Recovery of Sodium Chloride Salt from Textile Effluent, Desalination, PP: 235-243.

Soares, J., Silver A., Olivveira, R., Arujo, C., Mara, D., & Pearson, W. (1996). Ammonia removal in a pilot-scale WSP complex in Northeast Brazil. *Water Science Technology, 33* (7): 165-171.

Sutherland, W.J. (1997). Ecological Census Techniques (A hand book), Cambridge University Press, PP: 336.

Tilley, D. F. (2011). Aerobic Wastewater Treatment Processes. History and Development. IWA Publishing.

Tsuzuki, Y. (2012). Linking sanitation and wastewater treatment: From evaluation on the basis of effluent pollutant concentrations to evaluation on the basis of pollutant removal efficiencies. *Water Science Technology, 65* (2): 368-79.

Vijay, R., Sardar, K., Dhage, S., Kelkar, S., & Gupta, A. (2010). Hydrodynamic assessment of sewage impact on water quality of Malad Creek, Mumbai. *India. Environmental Monitoring Assessment, 165*: 559-571.

Waiser, M.J., Tumber, V., & Holm, J. (2011). Effluent-dominated streams. Part 1: presence and effects of excess nitrogen and phosphorus in Wascana Creek, Saskatchewan, Canada. Environ. Toxicol. Chem. 30:496–507 [PubMed]

Wakelin, S.A, Colloff, M.J., & Kookana, R.S. (2008). Effect of wastewater treatment plant effluent on microbial function and community structure in the sediment of a freshwater stream with variable seasonal flow. Application of Environmental Microbiology, 74:2659–2668 [PMC free article] [PubMed]

Wiesmann, U., Choi, I.S., & Evamaria, D. (2007). Fundamentals of Biological Wastewater Treatment. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Romania.

World Health Organization (WHO) and United Nations Children Fund (UNICEF) (2012). Progress on Drinking Water and Sanitation: 2012 Update.

World Health Organization (WHO) (2006). Guidelines for the safe use of wastewater, excreta and greywater, v. 2. Wastewater use in agriculture, PP: 196.

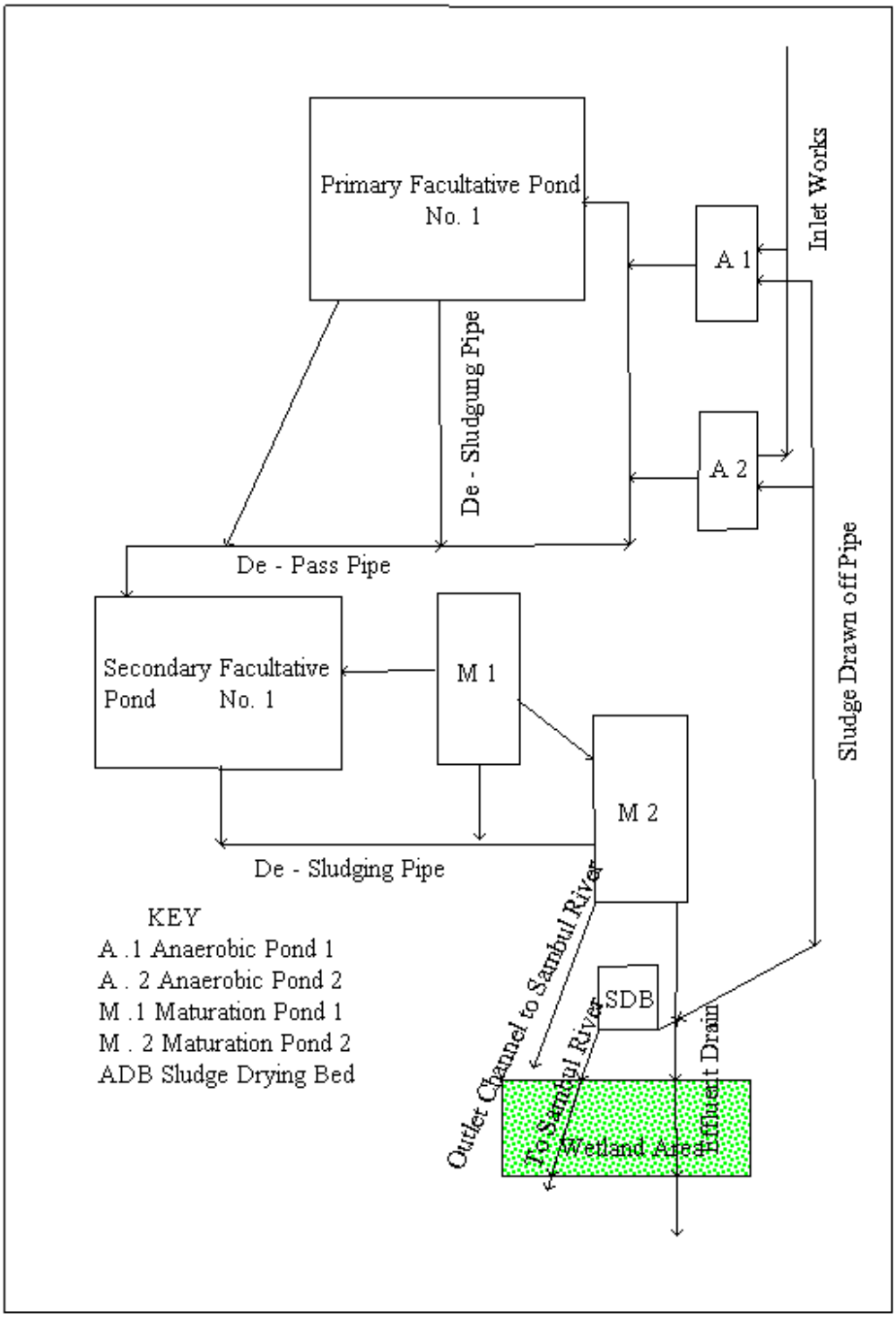
Wolfgang, L., Michael, T., Erich, S., & Karl, W. (2002). Nitrates and Nitrites. *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH: 265-280.

World Water Assessment Programme (WWAP) (2006). Case Study, Kenya. Kenya National Water Development Report. Prepared for the 2nd UN World Water Development Report *'Water: A shared responsibility'* UNESCO *World Water Assessment Programme* Secretariat.

Yapo, R.I., Koné, B., Bonfoh, B., Cissé, G., Zinsstag, J., & Nguyen-Viet, H. (2014). Quantitative microbial risk assessment related to urban wastewater and lagoon water reuse in Abidjan, Côte d'Ivoire. *Water Health*, 12 (2):301.

APPENDICES

Appendix 1: Moi University sewage treatment plan schematic flow diagram (by Kanda, Geographic Information Systems laboratory, Moi University, 2015).



Appendix 2: NEMA effluent standards for wastewater discharge into the environment

Parameter	Maximum Allowable Limits
Biological Oxygen Demand (BOD 5 days at 20°C, mg/L)	30
Total Suspended Solids (mg/L)	30
Total Dissolved Solids (mg/L)	1,200
pH	6.0-9.0
Oil and Grease	Nil
Chemical Oxygen Demand (COD, mg/L)	50
Total Phosphorus (mg/L)	2 Guideline value
Total Nitrogen (mg/L)	2 Guideline value
Nitrate, nitrite, Ammonia and ammonium compounds (mg/L)	100
Chromium VI (mg/L)	0.05
Lead (mg/L)	0.01
Cadmium (mg/L)	0.01
Zinc (mg/L)	0.5

Source: Environmental Management and Co-ordination Act (Water Quality) Regulations, 2006.

Appendix 3: Moi University Effluent Discharge Monitoring Standards

A) Effluent standard for acceptance into sewerage system

Parameters	**Acceptance Values**
B.O.D (5 days at 20^oC)	Not to exceed 450 mg/L
pH	To be in the Range of 6 to 9
Temperature	Not to exceed 35^oC
Suspended Solids (mg/L)	Not to exceed 300 mg/L

B) Effluent standard for direct discharge to natural water course

C.O.D	Not to exceed 50 mg/L
Total Nitrogen exclusive NO_3	1 mg/L
NH_3	1.5 mg/L
B.O.D (5 days at 20^oC)	Not to exceed 20 mg/L
pH	To be in the Range of 6 to 9
Temperature	Not to exceed 25^oC
Suspended Solids (mg/L)	Not to exceed 30 mg/L
Nitrate as NO_3	Not to exceed 15 mg/L

Source: Environmental Management and Co-ordination Act (Water Quality) Regulations, 2006.

Appendix 4: MPN table for quantifying *E. coli* levels in a wastewater sample

TABLE G.1

Most Probable Number of Coliforms per 100 mL of Sample

NUMBER OF POSITIVE TUBES				NUMBER OF POSITIVE TUBES				NUMBER OF POSITIVE TUBES				NUMBER OF POSITIVE TUBES			
10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN
0	0	0		1	0	0	2.0	2	0	0	4.5	3	0	0	7.8	4	0	0	13	5	0	0	23
0	0	1	1.8	1	0	1	4.0	2	0	1	6.8	3	0	1	11	4	0	1	17	5	0	1	31
0	0	2	3.6	1	0	2	6.0	2	0	2	9.1	3	0	2	13	4	0	2	21	5	0	2	43
0	0	3	5.4	1	0	3	8.0	2	0	3	12	3	0	3	16	4	0	3	25	5	0	3	58
0	0	4	7.2	1	0	4	10	2	0	4	14	3	0	4	20	4	0	4	30	5	0	4	76
0	0	5	9.0	1	0	5	12	2	0	5	16	3	0	5	23	4	0	5	36	5	0	5	95
0	1	0	1.8	1	1	0	4.0	2	1	0	6.8	3	1	0	11	4	1	0	17	5	1	0	33
0	1	1	3.6	1	1	1	6.1	2	1	1	9.2	3	1	1	14	4	1	1	21	5	1	1	46
0	1	2	5.5	1	1	2	8.1	2	1	2	12	3	1	2	17	4	1	2	26	5	1	2	64
0	1	3	7.3	1	1	3	10	2	1	3	14	3	1	3	20	4	1	3	31	5	1	3	84
0	1	4	9.1	1	1	4	12	2	1	4	17	3	1	4	23	4	1	4	36	5	1	4	110
0	1	5	11	1	1	5	14	2	1	5	19	3	1	5	27	4	1	5	42	5	1	5	130
0	2	0	3.7	1	2	0	6.1	2	2	0	9.3	3	2	0	14	4	2	0	22	5	2	0	49
0	2	1	5.5	1	2	1	8.2	2	2	1	12	3	2	1	17	4	2	1	26	5	2	1	70
0	2	2	7.4	1	2	2	10	2	2	2	14	3	2	2	20	4	2	2	32	5	2	2	95
0	2	3	9.2	1	2	3	12	2	2	3	17	3	2	3	24	4	2	3	38	5	2	3	120
0	2	4	11	1	2	4	15	2	2	4	19	3	2	4	27	4	2	4	44	5	2	4	150
0	2	5	13	1	2	5	17	2	2	5	22	3	2	5	31	4	2	5	50	5	2	5	180

APPENDIX 4 contd'

TABLE G.1 *(Cont.)*

NUMBER OF POSITIVE TUBES				NUMBER OF POSITIVE TUBES				NUMBER OF POSITIVE TUBES				NUMBER OF POSITIVE TUBES				NUMBER OF POSITIVE TUBES			
10 ML	1 ML	0.1 ML	MPN	10 ML	1 ML	0.1 ML	MPN	10 ML	1 ML	0.1 ML	MPN	10 ML	1 ML	0.1 ML	MPN	10 ML	1 ML	0.1 ML	MPN
0	3	0	5.6	1	3	0	8.3	2	3	0	12	3	3	0	17	4	3	0	27	5	3	0	79
0	3	1	7.4	1	3	1	10	2	3	1	14	3	3	1	21	4	3	1	33	5	3	1	110
0	3	2	9.3	1	3	2	13	2	3	2	17	3	3	2	24	4	3	2	39	5	3	2	140
0	3	3	11	1	3	3	15	2	3	3	20	3	3	3	28	4	3	3	45	5	3	3	180
0	3	4	13	1	3	4	17	2	3	4	22	3	3	4	31	4	3	4	52	5	3	4	210
0	3	5	15	1	3	5	19	2	3	5	25	3	3	5	35	4	3	5	59	5	3	5	250
0	4	0	7.5	1	4	0	11	2	4	0	15	3	4	0	21	4	4	0	34	5	4	0	130
0	4	1	9.4	1	4	1	13	2	4	1	17	3	4	1	24	4	4	1	40	5	4	1	170
0	4	2	11	1	4	2	15	2	4	2	20	3	4	2	28	4	4	2	47	5	4	2	220
0	4	3	13	1	4	3	17	2	4	3	23	3	4	3	32	4	4	3	54	5	4	3	280
0	4	4	15	1	4	4	19	2	4	4	25	3	4	4	36	4	4	4	62	5	4	4	350
0	4	5	17	1	4	5	22	3	4	5	28	3	4	5	40	4	4	5	69	5	4	5	430
0	5	0	9.4	1	5	0	13	2	5	0	17	3	5	0	25	4	5	0	41	5	5	0	240
0	5	1	11	1	5	1	15	2	5	1	20	3	5	1	29	4	5	1	48	5	5	1	350
0	5	2	13	1	5	2	17	2	5	2	23	3	5	2	32	4	5	2	56	5	5	2	540
0	5	3	15	1	5	3	19	2	5	3	26	3	5	3	37	4	5	3	64	5	5	3	920
0	5	4	17	1	5	4	22	2	5	4	29	3	5	4	41	4	5	4	72	5	5	4	1600
0	5	5	19	1	5	5	24	2	5	5	32	3	5	5	45	4	5	5	81				