BACTERIOLOGICAL AND PHYSICO-CHEMICAL WATER QUALITY OF OMUBHIRA STREAM IN KAKAMEGA COUNTY, KENYA

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A thesis submitted in partial fulfilment for the requirements of the award of Master of Science in Environmental Biology of Masinde Muliro University of Science and Technology

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DECLARATION

DECLARATION

This thesis is my original work prepared with no other than the indicated sources and support and has not been presented elsewhere for a degree or any other award.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a thesis entitled 'Bacteriological and Physico-chemical water quality of Omubhira stream, Kakamega County, Kenya'.

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DEDICATION

To my entire family that stood by me, persevered and provided me with much inspiration to go on.

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ABSTRACT

Omubhira stream drains a predominantly densely populated suburban catchment of Kakamega Town and is an important source of water for domestic use. In the recent past, health of the stream has been in question due to increased anthropogenic activities that are potential sources of bacterial contamination and pose health risk to the stream users. The general objective of the study was to investigate the physico-chemical and coliform gradients of the Omubhira stream and specifically to determine physico-chemical gradients; *Escherichia coli* (*E. coli*) and Total Coliforms (TC) counts of Omubhira stream and to identify members of enteric bacteria contaminating the stream. Stratified random sampling was applied and four strata with 15 selected sampling sites identified. Samples were collected in triplicate from the 15 selected sampling sites after every two weeks for six months between January to March and May to July in the year 2015. Physico-chemical water quality parameters were measured *insitu* except for TSS that was further analysed in the laboratory. Coliforms/*E. coli* levels were analyzed using 3M Petrifilm *E. coli*/coliforms count plate technique. Identification was based on biochemical test namely Triple Sugar Iron Agar (TSI) test, Motility Indole Lysine (MIL) test and Simmons Citrate Agar test. Data was analyzed using Ms Excel windows 2007 and Statistical Package for Social Sciences (SPSS) version 20. Results indicated mean pH value was 7.53 in dry season and 7.10 in the wet season. Mean Temperature in the dry season was 21.11°C while 12.45°C in the wet season. Electrical Conductivity (EC) in dry season had a mean of 156.62µS/cm while the wet season had 64.89 µS/cm. Dissolved Oxygen (DO) in dry season had a mean of 6.45 mg/l while wet season had mean of 6.74 mg/l. Total Dissolved Solids (TDS) mean in the dry season was 104.61 mg/l while wet season mean was 43.20 mg/l. Total Suspended Solids (TSS) had a mean of 0.033 mg/l in the dry season with 0.018 mg/l in the wet season. Bacteriological water quality revealed *E.coli* mean count values ranged from 20-1593 Cfu/100 ml during the dry season and 93-1229 Cfu/100 ml during the wet season. The total coliforms mean count values ranged between 300-4476 Cfu/100 ml during the dry season and 1160-6209 Cfu/100 ml during the wet season. A Pearson product-moment correlation showed that there was a positive correlation during the wet season between *E.coli* and two physico-chemical parameters that is EC (r = .349, n = 78, p< .01), TDS(r = .351, n = 80, p< .01) and TC (r = .241, n=80, p< .05) that was statistically significant. During the dry season, there was a negative correlation between *E.coli* and DO (r = .421, n = 80, p< .01) while positive correlation between *E. coli* and TDS(r = .302, n=80, p<.01); TC(r = .545, n= 80, p< .01) that were statistically significant. Enteric bacteria isolated from water samples in this study included *E. coli, Enterobacter* spp., *Citrobacter* spp., *Proteus* spp., *Serratia* spp., *Shigella* spp., *Providencia* spp., *Morganella* spp., *Salmonellae* spp. and *Klebsiella* spp. The presence of *Shigella* spp. and S*almonella* spp. raise serious public health concerns over the quality and safety of the water of the Omubhira stream. Generally, water samples analysed contained both total coliforms and *E. coli* except for the First Strata (1S) representing the source that had zero counts at some occasions. This indicates that water source could be safe and clean without bacterial contamination. Bacterial counts increased downstream in both seasons. This study therefore has generated relevant information that will be helpful in formulating an ecological watershed management plan for Omubhira stream and the larger River Isiukhu. Water Resource Users Association (WRUA) should be strengthened through Water Resources Management Authority (WRMA) to ensure that stream users do not pollute the stream ecosystem.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
APHA	American Public Health Association
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
Cfus	Colony Forming Units
DO	Dissolved Oxygen
EC	Electrical Conductivity
E. coli	*Escherichia coli*
EU	European Union
FC	Faecal coliform
MMUST	Masinde Muliro University of Science and Technology
MPN	Most Probable Number
NEMA	National Environment Management Authority
pH	Potential of Hydrogen ions concentration
TC	Total Coliform
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
US EPA	United States Environment Protection Agency

WHO	World Health Organization
WTP	Waste Water Treatment Plant
WRUA	Water Resources Users Association

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Water quality is an important factor influencing the distribution and abundance of stream fauna and flora (Lenat and Crawford, 1994). The quality of a stream or a river is normally affected by many physical, chemical and biological parameters that are usually introduced by either natural forces or human (anthropogenic) activities. More so, an environment that is healthy usually refers to one in which its water quality supports a rich and varied community of organisms and protects public health (Adekoyeni and Salako, 2012).

In the recent past, pollution of streams and rivers with deleterious microbes including bacteria has been on an increase with major source of microbes being faeces from humans and other mammals through point, non- point sources or both (Musyoki *et al*., 2013). Nutrient overload due to poor farming methods, partially treated effluents, organic waste dumping into water bodies and other related activities within stream catchment areas subsequently deteriorate the water quality (Shivoga *et al.,* 2005). Most Kenyan cities and towns are situated either at the source or on the main rivers/streams flowing into major water systems. Nairobi River flows through the residential and industrial areas of Nairobi City (Mbui *et al.,* 2016).

It receives and drains untreated and treated discharges of various types. People living downstream use water from this river for agriculture and domestic purposes. Following the inadequate handling of waste management, the river may be highly polluted with urban industrial waste alongside other domestic wastes (Mbui *et al*.,

2016). Ogendi *et al.,* (2015) in the study of River Nyanchwa- Riana found that the river is within Kisii town with urban settlement located upstream and along the river. Urbanization, agriculture and industrialization being the sources of organic and inorganic pollutants impacting negatively on the water quality.

Kakamega among the Kenyan towns with Omubhira stream having its origin within it. Urban settlements are located upstream and along the stream. Omubhira stream in Kakamega County of Western Kenya drains a small predominantly suburban catchment. The stream traverses through the main campus of Masinde Muliro University of Science and Technology and flows through middle and low-class human settlements. Omubhira stream drains its water into River Isiukhu that flows into River Nzoia that is one of the inlets feeding Lake Victoria. This makes it an interesting stream to investigate how human activities can influence water quality of small streams in emerging urban centres.

Accordingly, pollution extends its influence to the international environment, since the waste flows downstream and pollutes larger rivers at junctions, which may ultimately pour its contents into the larger water body, effectively dispensing the waste and thus degrading the aquatic environment. The study of Omubhira stream is therefore important since it empties its waters into River Isiukhu and to the larger River Nzoia and eventually into Lake Victoria.

According to Onyango (2012), anthropogenic activities including effluent discharge from a sewage treatment plant; increased agricultural activities; livestock grazing fields; watering points and increased settlements could have an impact on the water quality of the stream. These activities could also have an influence on the bacteriological quality of the stream by contributing organic loads that is source of

bacterial contamination. Additionally, with a growing population in the town, there has also been increased demand for its water to meet domestic chores. These activities are bound to influence the microbial profile and physico-chemical parameters (Shitu *et al.,* 2008). Furthermore, the microbial health risks associated with the Omubhira stream are not clearly understood. Even though measures have always been put in place to ensure industries treat their effluent before releasing into the sewer, this may not be the case, since effluents from domestic and unclassified source may still pollute waters recommended for drinking and domestic use (Milandri *et al.,* 2012).

Bacterial contamination of river and stream waters usually have public health implications to the users of the water because low doses of waterborne pathogenic microorganisms are infective and may lead to diarrheal and other enteric diseases (Shitu *et al.,* 2008). Since the range of pathogenic microorganisms is extensive, microbiological indicators of contamination in water was examined based on the assumption that if they are present then pathogen may also be present and if absent, then water is safe (Okafor *et al.,* 2012). These bacteria are usually not pathogenic themselves, but are of similar faecal origin (Fripp *et al.,* 2013).

Principal bacteria indicators of water quality are the coliforms namely the total coliforms, faecal coliforms and *E. coli.* Their presence in water is undesirable; nonetheless, they are not necessarily indicative of any specific health hazard, although some strains of *E. coli* may be pathogenic (Gichana *et al*., 2014). It is against the said background that this study therefore focuses on water quality of Omubhira stream for physico-chemical parameters and measures the levels of coliforms and *E.coli*. This study has therefore provided information on the status of

the physico-chemical and bacteriological water quality of the stream that was unknown.

1.2 Statement of the Problem

Omubhira stream drains a densely populated suburban catchment of Kakamega Town. The stream harbours a number of tropical biota and is an important source of water for domestic use, irrigation, fishing and recreational use.

In recent times, the quality of water in Omubhira stream has been of great concern due to a number of anthropogenic activities that are point and non-point sources of pollution and potential sources of bacterial contamination. Anthropogenic activities including effluent discharge from sewage treatment plant; increased agricultural activities; livestock grazing fields; watering points and increased settlements are contributing factors to the organic loads that may affect bacteriological water quality and physico-chemical water quality of the stream. Moreover, indiscriminate and uncontrolled discharges of wastes into streams and rivers influence negatively on the streams, ecosystems and human health directly or indirectly. In this effect, physicochemical and bacteriological pollution parameters are lacking for Omubhira stream and therefore it was necessary to investigate physico-chemical and bacteriological parameters of Omubhira stream to evaluate how it may influence infection and disease health implication.

According to WHO (2005) and National Environment Management Authority (NEMA Regulations, 2013) microbiological water quality guidelines, faecal coliforms (FC) should not exceed 10^3 per 100 ml with 0 per 100 ml for *E. coli* in water used for domestic, irrigation and recreational purposes. These regulations

therefore have been put into consideration with the findings of the study to ascertain the health and safety of the stream water to the beneficiaries.

1.3 Research objectives

1.3.1 General objective

To investigate the Physico-chemical and faecal coliforms gradients of the Omubhira stream in Kakamega town, Western Kenya.

1.3.2 Specific objectives

The study was guided by the following specific objectives:

i. To determine the physico-chemical gradients for water for Omubhira stream course in Kakamega town.
ii. To determine the *E. coli* and total coliforms, gradient for Omubhira Stream in Kakamega town.
iii. To identify members of enteric bacteria contaminating the Omubhira stream in Kakamega town.

1.4 Research hypothesis

The study was guided by the following specific null hypotheses:

1. The Omubhira stream is not contaminated with either total coliforms or *Escherichia coli.*
2. The Omubhira stream physico-chemical water quality is not within acceptable limits.

3. There is no relationship between the levels of Dissolved Oxygen, Electrical conductivity, pH, TDS, TSS and Water temperature in respect to either total coliforms or *E. coli* along the Omubhira stream.

1.5 Justification of the study

Currently, the most affected part of the environment is the water resources (Walakira 2011). On a global scale, environmental pollution has become a threat to plants and animals and may ultimately threaten the quality of human life (Olaniyi *et al*., 2012). Pollution usually extends its influence to the larger environment, since waste flows downstream and pollutes larger rivers (Mbui et al., 2016). Water quality of Omubhira stream is thus of great importance to ascertain that its waters are not polluted and prevent the extension of its pollutants to the river Isiukhu and further to the larger River Nzoia that drains into Lake Victoria.

The increased infrastructure developments within the Kakamega County and especially near the stream according to the gazette notice no. 14531, of the Environmental Management and Coordination act (No. 8 of 1999) were likely to affect Omubhira stream through pollution. Bacteriological water quality of Omubhira stream concerning Total Coliforms and *E. coli* levels had not been done on this stream. Study carried out on Omubhira stream so far only involved macro-invertebrates diversity in relation to water quality (Onyango 2012). All the parameters studied were within the recommended levels for water for domestic use.

The findings of this study forms useful and potential database information for further research if need be. Furthermore, the study is a source of information to the beneficiaries of the stream that include the domestic water users of the stream, Water Resource Management Authority and National Environment Management Authority, local communities and academia; and assist in developing ecologically- sound management plans and enhance the integrity of the stream.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

Water quality is defined by its physical, chemical, biological and aesthetic characteristics (Shalom *et al*., 2011). Water has no substitute and there is need for regular analysis to identify the physical, chemical and bacteriological characteristics of any water in order to ascertain its acceptability (Ngele and Opara, 2013).

2.2 Microbial water quality

Microbial water quality describes levels of microscopic organisms such as bacteria, protozoa and viruses in water. Two microorganisms commonly used for surface water quality testing are Total Coliforms (TC) and *E. coli*. Coliforms are gram-negative rods that produce acid and gas from lactose during metabolic fermentation. Coliform colonies growing on the Petrifilm EC plate produce acid causing the pH indicator to make the gel colour darker red. Gas trapped around red coliform colonies indicates confirmed coliforms. Testing of harmful pathogens directly among faecal coliforms in a water source is impractical since it requires lengthy, complex, and expensive testing procedures. Testing for indicator organisms is thus the norm as recommended by World Health Organisation (WHO 2005). Indicator organisms are bacteria found present when certain pathogens are present, and absent when those pathogens are absent (Fripp *et al*., 2013).

Coliforms are enterobacteriaceae (enteric) that are able to ferment lactose. They are gram- negative rod- shaped bacteria that inhabit intestinal tract of humans and other warm-blooded animals. Coliforms are considered non- pathogenic in intestinal tract

(e. g *E. coli*) (Rompre *et al.,* 2002). However, presence of faecal coliforms and *E.coli* indicates contamination of surface water from human or animal waste. Indicator organism provides information on the health of a water body through organism's presence, condition or numbers.

There have been links between different land uses and amount of TC and *E.coli* found in nearby surface waters. High faecal coliforms counts have been positively related to urban development, agriculture and amount of erodible soils. High TC counts are also dependent on amount of precipitation that the stream receives in previous days. Factors affecting microbiological quality of surface waters are discharges of sewage works and run-off from informal settlements (Raju & Renuga, 2012).

According to Cohen and Hillel (1972), Coliforms group of bacteria- faecal coliforms (FC) have been widely used as bacterial indicators of fecal pollution and are present in large numbers among the intestinal flora of humans and other warm-blooded animals and are thus found in faecal wastes (Rompre *et al*., 2002). Total coliforms (TC) comprise bacterial species of fecal origin as well as other bacterial groups and they indicate severe water pollution but it does not have to be directly correlated to an anthropogenic source of pollution. *E.coli* does not occur naturally in water but in warm-blooded animals and thus is a good indicator of water quality. It is one of the current bacterial pathogens of concern found in human and animal faecal material (SEAWA Watershed Report, 2009).

The presence of faecal coliforms in drinking water must at least be considered as a possible threat or indicative of microbiological water quality deterioration (Rompre *et al.*, 2002). High faecal coliforms (FC) and Total coliforms (TC) in water are usually manifested in the form of diarrhea and sometimes by fever and other secondary complications. Bathing and swimming is common among children and adults in the local community and the probability of ingesting infective dose of disease causing microorganism is high considering the fact that waterborne pathogens generally have low infective dose (Raju & Renuga, 2012). In many developing countries, availability of water has become a critical and urgent problem and it is a matter of great concern to families and communities depending on non-public water supply (Eze & Madumere, 2012).

The analysis of the total and faecal coliform counts for Nyanchwa-Riana River indicated the presence of pathogenic and non-pathogenic bacteria in the water at the source of Nyanchwa stream. This source is used for drawing water for domestic use hence, indicating that a large number of springs within the Kisii Municipality, which provide drinking water for the majority of the residents, could be contaminated by pathogenic coliform counts. The concentrations of the coliform counts showed a strong increase with distance from the source to the lower reaches of the river. This showed that there is an input of raw sewage or animal waste at certain points along the stream transect. It was observed that people defecate near bushes along the stream and majority of the municipality residents use pit latrines that are dug along the stream and when it rains, they overflow thus leaking into the stream that in turn become major sources of pathogenic bacteria inputs and later infections (Ogendi *et al*., 2015).

2.3 Physico-chemical water quality

Physico-chemical parameters are used to characterise and define water quality. Chemical parameters include pH, total solids, nitrates, sulphates, dissolved oxygen, alkalinity, acidity, residual chlorine, fluorides, phosphates, nitrites, hardness, and heavy metals generally as well as some other elements. Physical quality involves such parameters as odour, colour, taste, temperature, turbidity. Parameters studied included temperature, electrical conductivity, total dissolved solids, total suspended solids, dissolved oxygen and pH.

Temperature is one of the most important parameters with an impact on acceptability of various inorganic constituents and chemical contaminants affecting taste. Water temperature is one of the critical parameters used to assess rivers and streams for aquatic habitats health. Water temperature does not change as fast as air temperature. However, smaller increases in water temperature can have negative impact on water quality and ecosystems. Water temperature has thus become an important indicator in light of the climate changes that affect our ecosystems (Nathanson, 2003).

High temperature enhances growth of microorganisms and may increase problems related to taste, odour, colour and corrosion. High temperature observed during the rainy season in Ruguti River were attributed to insulating effect of increased nutrient loading from surface runoff and coffee factory discharge (Ombaka and Gichumbi, 2012). Ogendi *et al.,* (2015) reported that the washing site of vehicles along the Nyanchwa -Riana River could be responsible for the relatively high conductivity measured (53.70 –257.00 μScm^{-2}) as a result of discharge of lead-laden and soap effluents from the washing of the cars which therefore affects the water quality.

pH level is a measurement of acidity or alkalinity of water and can indicate chemical changes in water and the availability of nutrients in water. A safe level of pH of water ranges between 6.5 and 8.5 units. Effluent discharges may have higher or lower pH levels that in turn change the pH of stream water. High acidity or alkalinity deteriorates water quality for both aquatic and recreational purposes and may cause irritation or damage to skin or eyes (Nathanson, 2003).

In the study of River Nyanchwa- Riana, selected physical and chemical parameters were done in situ at each sampling site using respective meters. The water quality standards of Nyanchwa-Riana River showed that five out of the six parameter levels exceeded the National Environment Management Authority (NEMA 2013) quality standards. The total dissolved solids (TDS) did not exceed the NEMA Standards except for the upper range. This implied external pollutants input into the stream. This implied that there was material input into the stream at different times of the thirteenth month sampling period. The dissolved oxygen concentration had the lower limit (3.29 mg/l) of the range approach concentrations in anoxic waters. Concentrations below 5 mg/l are stressful to fish growth, while fish kills are observed at concentrations below 3 mg/l. The pH of pure water is approximately neutral (nearly 7), however, the lower pH range (5.12-9.00) measured in the River Nyanchwa- Riana indicated the discharge of organic or acidic effluents being discharged into the stream. This concurred with the observation of raw sewage discharged into the stream at three different sites (NY3, R1 and R2) and also the organic effluent runoff from the waste dumpsites and the Daraja Mbili agricultural products market straddling the stream downstream (Ogendi *et al.,* 2015).

According to Mbui *et al.,* 2016, a pH value obtained in the study of Nairobi River ranged from 6.89 to 7.77 and was within the acceptable World Health Organization (WHO, 2005) limit for natural water (6.0-8.5). High pH values recorded at Kirichwa Ndogo (KN) (7.64 ± 0.19) may be attributed to fertilizers from the potted plants near the sampling point and effluents containing acid from car wash and garage may also result in lower pH values at Kileleshwa Kirichwa Kubwa (KKK) (6.89) during the dry season. The electrical conductivity values were above the WHO 2005 limits of 600 µS /cm for natural water at Lenana School (LS) and Riara (RR) during both the dry and the wet seasons, and Kileleshwa Kirichwa Kubwa (KKK) during the wet season. This was attributed to domestic effluent discharges into the river, which increased the concentration of the ions. The concentration of total suspended solids (TSS) was observed to be significantly higher during the wet season as compared to the dry season. This variation was attributed to runoff, which carries particles into the river during the wet season.

Dissolved oxygen is a measure of amount of oxygen dissolved in the water (percent or milligrams of oxygen per litre of water). The dissolved oxygen (DO) concentrations were found to be higher during the wet season (17.23-24.29 mg/L), as compared to the dry season. This is probably due to increased volume of water during the wet season hence high aeration due to turbulence brought about by storm water. During the dry season, the water volume was less leading to minimal aeration and less dissolved oxygen in the water. The study concluded that pH, TDS, DO and temperature values of Nairobi River are acceptable for a pristine river.

2.4 Economic/ Health Importance of Enteric Bacteria

Worldwide, the most common bacterial diseases transmitted through water are caused by *Shigella, Salmonella*, Enterotoxigenic *Escherichia coli* and *Vibrio cholera* (Chetna *et al.*, 2006).

2.4.1 *Escherichia coli*

E. coli are common inhabitants of the human digestive tract. However, some cause diarrheal diseases in humans (Fenwick, 1995). These pathogenic *E. coli* fall under five groups: enterotoxigenic (ETEC), enterohemorrhagic (EHEC), enterroinvasive (EIEC), enteropathogenic (EPEC) and the newly recognized group called enteroadherent-aggregative *E. coli* (EAAggEC) for its aggregate or "stacked-brick" – like adherence to cultured mammalian cells (Vial *et al*., 1998). Although pathogenic *E. coli* has most often been implicated in food-borne illness, several major waterborne outbreaks have been reported (Fenwick, 1995). Outbreaks have involved both water supplies and recreational waters.

2.4.2 Opportunistic pathogens

Opportunistic pathogens are naturally present in the environment and are not formally regarded as pathogens. They are able to cause diseases in people with impaired local or general defense mechanisms such as the elderly or the very young, patients with burns or extensive wounds, those undergoing immunosuppressive therapy, or those with acquired immunodeficiency syndrome (AIDS) (Nelson *et al.*, 2004). Water used by such patients for drinking or bathing, if it contains large numbers of these organisms, can produce various infections of the skin and mucous membranes of the eye, ear, nose and throat. Examples of such agents are

Pseudomonas aeruginosa and species of *Klebsiella, Serratia, Aeromonas* and certain "slow growing" mycobacteria.

2.4.3 Pathogenic bacteria

Salmonella

Salmonella is a bacterium that is most commonly associated with eating undercooked meat, poultry, and eggs, but can affect water supplies. It can be acquired directly from animals. *Salmonella* causes an illness, known as enteric fever, which is usually uncomfortable but not dangerous, except for young children, the elderly, and the immuno-compromised, who may become dangerously dehydrated (CDC, 2004). Symptoms of enteric fever include diarrhea, fever, and abdominal cramps.

Shigella

Shigella is a genus of bacteria that cause illnesses very similar to *Salmonella* and *Campylobacter*, but usually spreads differently, through the fecal-oral route. In the developing world, *Shigella dysenteriae* type 1 causes deadly epidemics whose symptoms include diarrhea, bloody diarrhea, cramping, and fever. As with several other bacterial infections, *Shigella* may necessitate hospitalization of the very young or elderly because of profound diarrhea (CDC, 2005). Some infected individuals show no symptoms but easily pass the illness on to others. About 3% of victims of *Shigella flexineri* suffer a serious, long-term complication known as Reiter's syndrome, which causes painful joints, irritation of the eyes, painful urination, and in some cases long-term arthritis. The syndrome may last for months or years. It is still an important public health problem, especially in developing countries, where there is substandard hygiene and unsafe water supplies (Niyogi, 2005). *Shigella* still

accounts for a significant proportion of bacillary dysentery in many tropical and subtropical countries (Zafar *et al.,* 2005).

2.5 Related Studies

Petrifilm *E. coli*/ Coliform count plates previously validated for enumerating *E. coli* in food were tested for monitoring E. coli in environment and counts were significantly correlated with commonly used methods. (Vail *et al*., 2003). Liesl *et al*., (2008) investigated fecal indicator bacteria level during dry weather in a reference stream in Southern California and results indicated that bacteria levels fluctuated seasonally. Temperature at all sites, explained about one- half the variation in total coliforms density suggesting that stream temperature regulated bacterial populations. Meador *et al*., (2012) in an attempt to quantify aerobic bacteria in irrigation water used petrifilm technology as an onsite monitoring technique.

Musyoki *et al*., (2013) assessed the quality of surface water in Nairobi River and the adjacent River Athi to ascertain whether it meets local and international microbiological standards for safe human consumption. Standard bacteriological techniques were used to describe bacteria content from water samples collected from the two confluent sources. The waters were highly contaminated with human pathogenic bacteria and the most dominant bacteria in combined waters of the two rivers was *Escherichia coli* while the least was *Shigella flexneri*. Other bacteria were *Klebsiella aerogenes*, *Enterococcus fecalis, Salmonella typhi, Pseudomonas aeruginosa, Salmonella paratyphi and Vibrio cholera*.

According to Kiruki *et al.,* (2011), study on Bacteriological quality and diarrhoeagenic pathogens on River Njoro and Nakuru Municipal Water revealed that Bacteria indicator numbers (arithmetic mean MPN/ml) varied from 24.4 (source) to

>2700.0 (midstream) for total coliforms and 3.6 (source) to 1880.0 (midstream) for faecal coliforms in River Njoro. The sampling sites at River Njoro were selected to encompass potential sources of contamination included: pristine watersheds, farming areas, sewage polluted and industrial discharge points.

Coliforms were estimated using a three-tube most probable number method (MPN) according to American Public Health Association (APHA) Standard Methods (2008). There was a consistent increase in bacteria loading as the river flowed from the source (Nessuit) to downstream sites. The biochemical oxygen demand (BOD) ranged from 2.0 mg/l at the source of the river to 44.0 mg/l at Njoro Bridge. The current study involved the use of *E .coli*/Coliform petrifilm count plates that are specifically manufactured using a plate technology to ensure there is less contamination during preparation of media and less time is consumed in the process of sample analysis.

Gichana *et al*., (2014) conducted a study to assess the effects of human activities on the microbial water quality along Nyangores stream, Mara River Basin. Seven sampling stations were selected to correspond to different human activities along the stream. Significant variation ($p<0.05$) of physical chemical parameters were observed downstream. The same trend was observed with TC and *E. coli*. Higher densities of bacteria were recorded during the wet season. The study concluded that human activities influenced the quality of water in Nyangores stream.

Gerhard *et al*., (2002) assessed the microbial water quality of the river Danube (km 2581-km 15): longitudinal variation of pollution and the results indicated highest pollution in the tributaries. Highest levels of faecal pollution were found in the middle part of Danube, particularly downstream of major cities (Budapest, Beograd).

The main sources affecting microbiological water quality were raw discharges, discharges from wastewater treatment plants, impaired tributaries and impact by diffuse sources. In a remarkable number of sampling sites, microbiological parameters alone indicated anthropogenic impacts.

Bacterial analysis carried out on Ananthanar channel water of Kanyakumari district, Tamil Nadu, India by Raju & Renuga (2012) reported that water samples were contaminated with high amount of bacterial population than Indian acceptable limit. Results indicated fecal coliform counts varied from 12 to 180 MPN/100 ml while *E. coli* counts ranged from 6 to 161 MPN/ 100 ml for all the sampled sites. This was above the desirable limit of coliform in water of 10 MPN/100 ml according to Indian Standards.

Eze & Madumere (2012) conducted physico-chemical and microbiological analysis of water bodies in Uturu, Abia state - Nigeria involving two streams, a spring and a borehole. Physiochemical and chemical analysis included the determination of pH, total solid present, total dissolved solids (TDS), biological oxygen demand (BOD), chemical oxygen demand (COD), and total hardness using standard methods. Microbiological analysis was done by assessing the bacterial counts (MPN/100 ml) coliforms. The total viable counts of all water samples were generally high exceeding WHO (2006) standard that is 1.0 x 10 Cfu/100 ml for potable water. It was recommended that there was need for water to be treated before it is fit for drinking. The pH range of 6.5-8.1 was considered as being within the acceptable range for natural water.

Kolarevic *et al*., (2011) investigated microbiological quality of River Tisa in Serbia. Microbial analyses were conducted on purposively selected sampling sites. Samples were monitored using membrane filtration method and identification of isolated coliform bacteria was tested using API 20e identification kit. Standard procedures for an assessment of sanitary quality and organic load were applied and 16 parameters analysed. The results of analyses indicated that water quality was unsatisfactory at all sampling sites since fecal coliform numbers ranged from 10^3 to 1.7 x 10^4 Cfu/100 ml.

Muhibbu *et al.,* (2011) through their study on investigating effluents impacts on physicochemical parameters of stream waters in Obafemi, Nigeria reported that there was an adverse impact on physicochemical characteristics of receiving stream due to discharge of inadequately treated effluents from the wastewater treatment facility. Muhibbu assessed physicochemical qualities of effluent impacted stream over duration of seven months by measuring using standard methods and unacceptable high levels of assayed parameters outside compliance levels of the World Health Organization (WHO) was realized. This posed a health risk to several rural communities who rely on the receiving water bodies primarily as their source of domestic water. The study recommended that there was need for intervention of appropriate regulatory agencies to ensure production of high quality treated final effluents by wastewater treatment facilities in rural communities.

Ngele and Opara (2013) conducted a study on streams used for drinking water in Ebonyi State Nigeria. Streams were selected randomly using table of random numbers. Microbial and biochemical assessments indicated that the parameter values conformed to the WHO (2005) standard for drinking water while iron contents were above recommended contents. Otokunefor and Obiukwu (2005) investigated the

physicochemical qualities of a refinery effluent, water and sediment of an effluent receiving water body and results indicated high concentrations of phenol, ammonia, COD and TDS. High concentrations were observed in water at the point of effluent impact.

Lekwot *et al*., (2012) examined public health effects of effluent discharge into River Romi for 2 years where samples were collected in two seasons and a range of physicochemical and microbial parameters were determined. Results revealed that all parameters measured were far above WHO (2005) maximum permissible limits. This study also identified potential health effects of the presence of these parameters in water. The significance of water to the health and well-being of human populations was increasingly apparent. Microbial analysis revealed that microbial water quality of River Romi is poor, total and fecal coliform pollution was wide spread and entire course of the river sampled is not suitable for domestic consumption without treatment.

Sections of Asa River along its course were investigated for twelve months to determine effects of detergent effluent on aspect of water quality of river. Samples were screened for microbiological analysis using pour plate method using nutrient agar and results indicated that level of pollution varied depending on season and that water acceptable at upstream became impaired at some points downstream indicating significant pollution. Analysis of microbiological characteristics of water samples from the three sampling points revealed high microbial and fecal contamination (Adewoye, 2010).

Nafarrnda *et al*., (2012) investigated bacteriological characteristics of Abattoir effluents, water source and receiving water bodies in Abiya, Nigeria using the multiple- tube fermentation technique. Total coliform bacteria count for effluents exceeded recommended limits for discharge into surface water. No significant difference was observed between bacterial counts of effluents and receiving water bodies 100 meters downstream: an indication of contamination of receiving water bodies by effluent and possible public and environmental health hazards.

According to Srivastava & Srivastava (2011) in the assessment of physico-chemical properties and sewage pollution indicator bacteria in surface water of River Gomti in Uttar Pradesh, water conductivity showed significant positive correlation with total coliform and fecal coliform. Correlation analysis revealed a significant negative correlation of pH with total coli and fecal coliform. Temperature also showed significant negative correlation with fecal coliform, total coliform and biological oxygen demand.

Salama *et al.*, (2013) evaluated fecal coliform levels in the discharges from the city of El Jadida, Morocco. Faecal coliforms had a positive and highly significant correlation with temperature, pH and total suspended solids. In addition, a poorly significant and negative correlation was observed with electrical conductivity. Among the physicochemical parameters studied, electrical conductivity appeared as the parameter to be monitored during treatment of wastewater to reduce load of fecal coliforms.

Omubhira stream is one of the streams that has received less attention in the recent past despite the fact that its waters in the end empty into Lake Victoria via River Nzoia. A study had been conducted on Omubhira stream concerning macro-

invertebrates diversity in relation to physico-chemical gradients and relating to Lurambi stream, which meet with Omubhira stream at a confluence before emptying into River Isiukhu. Macro-invertebrates were used as bio-indicators of water quality in this study. Results indicated that macro-invertebrates populations correlated with physicochemical parameters with Omubhira stream recording higher values for electrical conductivity measured (Onyango 2012). Investigating the bacteriological and selected physico-chemical water quality using total coliforms and *Escherichia co*li as indicators of pollution reveals the occurrence of the various enteric bacteria that might have economic and public health implication to the stream water users.

2.6 Water Quality Regulations

Table 2.1 : Threshold levels of some parameters according to Inland water quality classification (Nurcihan & Basaran, 2009).

Parameters	**Water Quality Categories**			
	High quality waters	**Moderate quality waters**	**Polluted waters**	**Highly polluted waters**
Temperature ºC	25	25	30	>30
Dissolved oxygen(mg/ L)	8	6-8	3-6	<3
BOD_5 (mg/L)	4	4- 8	8-20	>20
pH	6.5- 8.5	6.5-8.5	6.0-9.0	6.0- 9.0
Electrical conductivity (µS/cm)	250	250-750	750-2250	2250-5000
Total coliform(MPN/100 ml)	100	100-20,000	20×10^3-10×10^4	$>10 \times 10^4$
Fecal coliform (MPN/100 ml)	10	10-200	200-2000	> 2000

Table 2.2: NEMA Water Quality Regulations guideline value- first schedule

Parameter	**Guide value**
pH	6.5- 8.5
BOD (5 days at 50ºC)	30 (mg/l) max
Suspended solids	30 mg/l max
Total dissolved solids	1200 (mg/l)
Escherichia coli	Nil/100 ml
Total coliforms	1000/100 ml

CHAPTER THREE

MATERIALS AND METHODS

3.1 Overview

This chapter presents area of study, research design, detailed description of the sampling sites, sampling protocol, sample collection procedure, sample analysis and data analysis.

3.2 Area of study

Omubhira stream is a first order stream that flows in an Easterly direction with its origin situated in Milimani estate in Kakamega town within Kakamega County. Kakamega lies at latitude: 0°17′03″N and longitude: 34°45′08″E. Its elevation above sea level is 1563 m = 5127 ft. Kakamega County is a county in the former Western Province of Kenya. Its capital and largest town is Kakamega. The stream is approximately 0.98KM long and approximately 100 cm wide. Temperature ranges from a minimum of 10.3ºC to a maximum of 30.8ºC with an average of 20.5ºC. The rainfall ranges between 1250- 1750 per annum (KARI Kakamega Annual report 2011). The residents along the stream are majorly engaged in small-scale crop farming, livestock rearing and aquaculture. Omubhira stream joins Lurambi stream at a confluence and both drain into River Isiukhu and eventually into River Nzoia; one of the major rivers draining into Lake Victoria. Important features along the stream include a narrow strip of natural wetland on either side that comprises majorly of the sedges, settlement schemes, fishponds, wastewater treatment plant, farmlands and Main campus of Masinde Muliro University of Science and Technology. Generally, a high population density characterizes its catchment area.

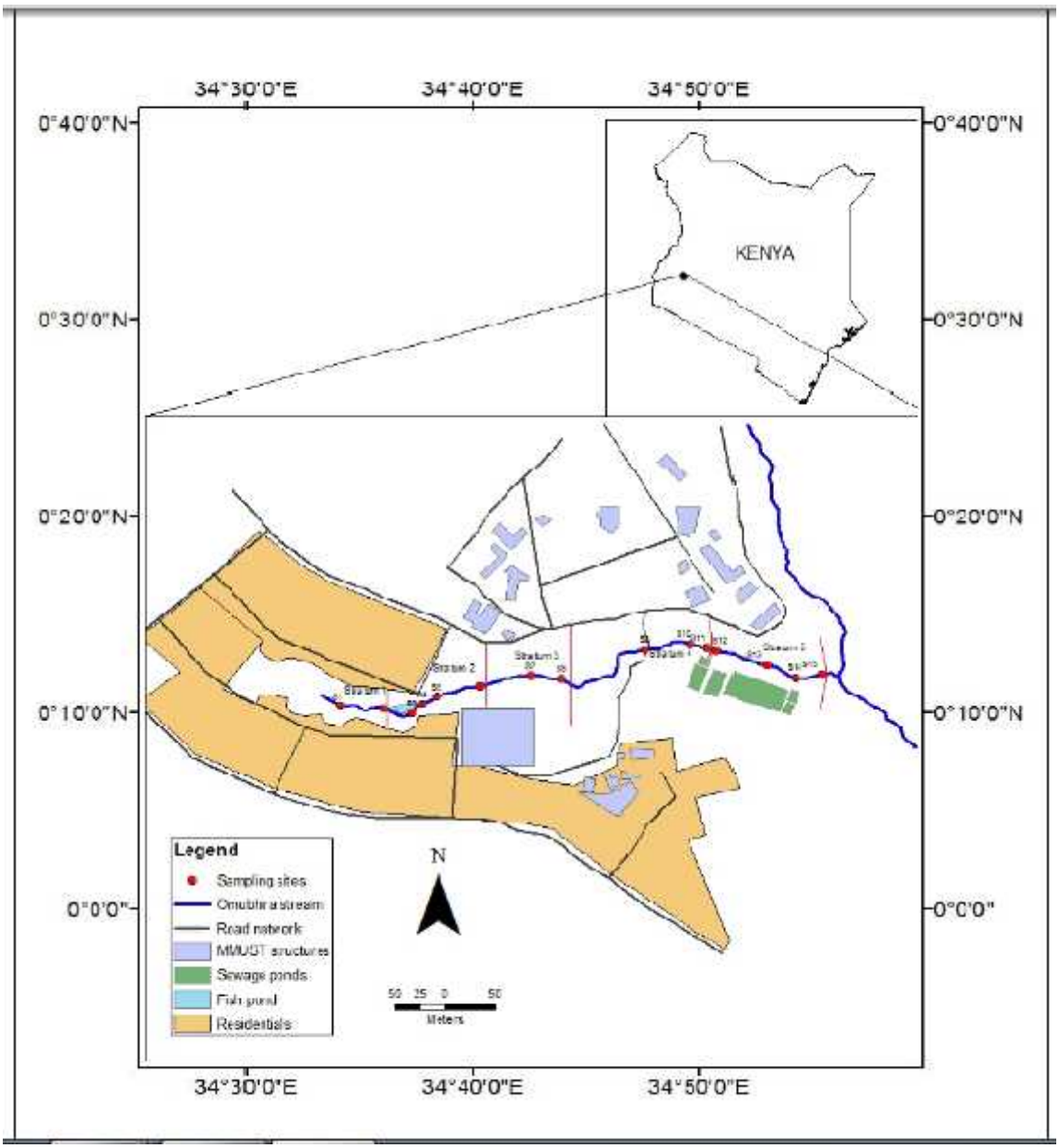

Figure 3.1: Map of Omubhira stream showing the study area

Source: Modified from GoK (2012)

3.3 Research design

Stratified random sampling design was used in this study. The stream was divided into four strata in relation to the purported sources of bacterial contamination. Fifteen sampling sites were identified depending on the watering points where water was being collected for various domestic use; livestock watering points and numerous point or non- point sources of potential faecal pollution. Coordinates of sampling sites were taken by GPS ("Garmin Etrex") and plotted using the ArcGIS software. At each sampling site, three samples were collected twice in a month randomly between 9 am – 12 noon during the wet season (May-2015 to July-2015) and dry season (January- 2015 to March - 2015). Five hundred and forty samples were collected within the period of six months.

3.4 Description of sampling sites

Sampling sites were selected to represent different ecological and environmental variations in the stream and to understand influence of natural and human induced stress.

Table 3.1: Description of strata and sampling sites

Strata	**Strata description**	**Sampling site and GPS Coordinates**
Stratum 1(1S)	Situated upstream on a valley. It is the source of the stream. It is influenced by farmland activities and water run offs	Sampling site 0 Site Lat Long 0.0.285284 34.760049
Stratum 2(2S)	Situated up stream and upper mid upstream. It is also influenced by farmland activities, storm water, and fishpond outlet. Also borders area for grazing livestock and watering point for the livestock	Sampling site 1,2 and 3 Site Lat Long 1. 0.285498 34.760281 2. 0.285545 34.761081 3. 0.285580 34.761377
Stratum 3(3S)	Situated midstream. There is less human use with a stretch of wetland vegetation. It is affected by water from a valley. A Dairy farm situated on the upper riparian area of the stream also influences it.	Sampling site 4, 5, 6, 7, 8 & 9 Site Lat Long 4. 0.285649 34.761566 5. 0.285774 34.761722 6. 0.285954 34.762444 7. 0.286166 34.763273 8. 0.286156 34.763850 9. 0.286269 34.765794
Stratum 4(4S)	Situated on the lower midstream and downstream and is influenced by effluents from the wastewater treatment plant and watering point for livestock, washing and bathing area	Sampling site 10, 11, 12, 13, 14 & 15. Site Lat Long 10. 0.286752 34.765778 11. 0.286720 34.766074 12. 0.286721 34.766288 13. 0.286400 34.767033 14. 0.286363 34.767479 15. 0.286397 34.767772

3.5 Sampling protocol

Sampling was carried out between 9.00 am – 12.00 noon during each sampling trip. Samples were taken from the downstream site first and continued up to the upstream site. This ensured that downstream water quality was not altered due to disturbances when sampling. Samples were taken from the stream by holding the 250 ml sampling bottle near its base in the hand and plunging it, neck downward, below the surface. Then turning the bottle until neck points slightly upward and mouth directed toward the current. The sampling bottle was filled up to the brim and 20 mm to 30 mm space was left for effective shaking of the bottle.

The samples were transported in ice packed cooler boxes. All samples were processed and analysed in the Department of Biological Sciences - Microbiology laboratory at Masinde Muliro University of Science and Technology within 8 hours from the time of sampling to avoid changes of bacteria count due to growth or die off. Analysis followed guidelines outlined in APHA (2005). Aseptic technique was observed in all the analysis procedures.

3.6 Field Sample collection procedure

Grab sampling procedure was as described in APHA (2005). Physico-chemical parameters namely: water temperature, Electrical Conductivity, pH, Dissolved Oxygen, TDS and TSS were measured *in situ* at the time of sampling using electrical probes. All field meters and equipment were checked and calibrated according to the manufacturer's instructions before fieldwork. Electrical conductivity (EC), total dissolved solids (TDS) and temperature were analyzed using Cond/TDS/Salt/Temp meter CTS-406K. The conductivity probe was rinsed with distilled water, immersed

into the sample and the reading recorded in a table. pH was measured using HI 2211 pH/ORP meter Hannia instrument.

The meter was calibrated by inserting its probe in a standard solution at pH 7.0 then rinsed with distilled water and then at pH 4.0. Then rinsed with distilled water and inserted in water. The pH was read off above the temperature level displayed on the screen. Dissolved oxygen was measured using digital oxygen meter of M.R.C model. For the determination of total suspended solids (TSS), filter paper was weighed using an electronic digital balance and the initial reading noted. 100 ml of the sample was filtered through and the filter paper oven dried at 50ºC for 1 hour. The filter paper was then re-weighed and the final weight of the filter paper gave the value of TSS in grams.

3.7 Enumeration of faecal coliform levels

The levels of total coliforms and *E.coli* was determined using the 3M *E.coli*/Coliform petrifilm count plates as described by the manufacturer. Petrifilm is a plating technology with a ready-made dehydrated culture medium with nutrients and tetrazolium salts that fluoresces living bacteria colonies on a flat card with a laminate cover slip. Petrifilm plate was placed on level surface with top film lifted and using a pipette, 1 ml of sample pipetted onto the center of bottom film. The top film was then rolled down to avoid entrapping air bubbles making sure the top film does not drop. With flat side down, spreader was placed on top film over innoculum and pressure gently applied on spreader to distribute innoculum over circular area before gel was formed. Spreader was lifted and a minimum of one minute given for gel to solidify.

Plates were placed in the incubator with clear side up in stacks of no more than 20 and incubated for 24h ± 2h at 37°C. The incubator was humidified to minimize

moisture loss. Petrifilm plates were then counted on a standard colony counter. Typical coliform colonies appeared pink/red whereas the *E. coli* appeared dark blue. The colonies were enumerated, characterized and recorded as Cfu/100 ml. The results were expressed as the number of *E.coli* or TC in 100 ml of water.

3.8 Isolation and characterization of enteric bacteria present along the Omubhira stream

Bacteriological characterization of isolates was confirmed and determined by conventional biochemical tests (Cheesbrough, 2002). Selected *E. coli* and total coliform colonies that had formed gas from the petrifilm plate were inoculated onto MacConkey Agar, nutrient agar and Eosin Methylene blue ((Hymenia Lab. Pvt. Mumbai, India). All the plates were incubated aerobically at 37ºC for 24 to 48 hours. Subsequently, isolates were then characterized and identified based on cultural and cell morphology; gram reaction; motility and biochemical tests including Triple Sugar Iron agar (TSI Agar test), Motility Indole Lysine (MIL) test and Simmons Citrate Agar test all from HIMedia Lab. Pvt. Mumbai, India as described by Cheesbrough,(2002). All enterobacteriaceae isolates were later preserved in - 80°C under refrigeration in Tryptic Soy Broth (HIMedia Lab. Pvt. Mumbai, India) plus 15% glycerol after identification.

3.9 Quality control

All field meters and equipment were checked and calibrated according to the manufactures instructions. All reagents used were of analytical grade and contaminations were checked by running blanks of all determinations. For microbial media *E. coli* (ATCC 25922) was used as a control. Distilled water was used all through and all glass apparatus used were first soaked in hot soap solution, later in

H_2SO_4/HNO_3 acid (1:1) mixture and then rinsed with copious amount of hot water followed by several portions of distilled water. All plastic containers were equally given same treatment. Samples were run in triplicate and shaken vigorously to reduce bacterial clumping. Reagents were measured using calibrated weighing balance Sartirius AG Gottingen CP2245 ISO 9001 certified.

3.10 Statistical analysis

Collected data was subjected to statistical analysis using MS Excel and Statistical Package for Social Sciences (SPPS) version 20. Microsoft Excel package was used to derive the mean and standard errors and standard deviations. Data was also subjected to analysis by using Correlation and Analysis of Variance (ANOVA). Pearson product moment correlation procedure was used to perform correlation analysis and determine whether there were significant relationships between different physicochemical parameters and coliform levels. To check whether there was any significant difference between the values of physico-chemical parameters at different stations, ANOVA was performed.

CHAPTER FOUR

RESULTS

4.0 General Overview

Assessment of Omubhira stream environment during study revealed that sampling locations were mostly frequented by the nearby residents and livestock as watering points and especially strata four (4S) that represents downstream. Observations also revealed that some people living in the stream basin and downstream use stream to water their crops with a number of fishponds constructed along the stream.

4.1 Physico-Chemical water quality gradients along the Omubhira stream course

Results obtained from all samples were calculated in triplicates and the average calculated for results. These were presented in tables.

4.1.1. Water Temperature (ºC)

The overall mean water temperature for Omubhira stream was 21.11 º C during the dry season and 12.45 º C during the wet season (Table 4.1). During dry season, water temperatures varied widely among the study strata with a range of 20.1 to 21.7 ° C. The lowest mean temperature (20.7 ° C) was recorded in the first stratum while highest mean temperature (21.2 ° C) was recorded in the third stratum. Using the one-way ANOVA test, it was established that there was no significant difference in water temperature during sampling occasions for the dry season ($p \leq 0.719$, DF = 4), (Appendix 4). During wet season, water temperatures varied widely among the study strata between 11.7 to 15.0° C. The lowest mean temperature (12.17 ° C) was

recorded in the third stratum(3S) while the highest mean temperature (14.6 °C) was recorded in the first stratum (Table 4.1).Using the one way ANOVA test, it was established that there was no significant difference in water temperature during sampling occasions for the wet season ($p \leq 0.103$, DF = 4), (Appendix 3).

Table 4.1: Water Temperature levels (°C) of Omubhira stream during the dry and wet season

Water Temperature (° C)					
	Strata	**Mean**	**Std. Error**	**Min**	**Max**
Dry season	1S	20.7	0.245	20.1	21.2
	2S	21.09	0.191	20.8	21.7
	3S	21.2	0.139	20.8	21.5
	4S	21.15	0.129	20.9	21.5
	Overall	21.12	0.349		
Wet season	1S	14.6	0.447	13	15
	2S	12.67	0.204	12.33	13.33
	3S	12.17	0.228	11.67	12.83
	4S	12.27	0.516	11.83	12.67
	Overall	12.45	0.176		

4.1.2. Electrical Conductivity (µS/cm)

The overall mean for electrical conductivity was 156.6 µS/cm for dry season and 64.89 µS/cm for wet season. During dry season, electrical conductivity levels ranged between 118 µS/cm and 183.6 µS/cm. The lowest mean EC of 126.3 µS/cm was recorded at the third stratum while the highest mean of 178 µS/cm was recorded at the fourth stratum (Table 4.2). Using a one-way ANOVA test, it was noted that there was no significant difference in mean EC of water during the sampling occasions for the dry season ($p \leq 0.968$, DF=4), (Appendix 4). During the wet season, electrical conductivity (EC) of stream water ranged from 26.83µS/cm and 165.1783µS/cm. The highest mean EC value was 98 µS/cm in the fourth stratum while the lowest mean was 41.2 µS/cm in the second stratum (Table 4.2). Using a one-way ANOVA test, it was noted that there was no significant difference in mean EC of water during the sampling occasions for the wet season ($p \leq 0.236$, DF=4), (Appendix 3).

Table 4.2: Electrical conductivity levels (µS/cm) of Omubhira stream during the dry and wet season

Electrical Conductivity(µS/cm)					
	Strata	**Mean**	**Std. Error**	**Min**	**Max**
Dry season	1S	173.4	3.77	161	180
	2S	168.40	9.45	126.3	183.3
	3S	126.33	2.41	118	129.3
	4S	178.83	4.85	161.5	183.7
	Overall	156.63	5.12		
Wet season	1S	53.8	6.80	30	63
	2S	41.2	3.54	34	51.33
	3S	45	10.36	26.833	78.5
	4S	98.83	19.27	71.33	165.17
	Overall	64.90	9.9		

4.1.3. pH

During dry season, the average mean pH of water recorded was 7.54 (Table 4.3). The pH values varied from 7.43 to 7.66. The lowest mean pH of 7.47 was recorded in the third stratum while the highest mean pH (7.65) was recorded in the first stratum. Using the one-way ANOVA test, it was established that there was no significant difference in water pH during the sampling occasions for the dry season ($p \leq 0.585$, DF = 4), (Appendix 4). During wet season, the average mean pH of water was 7.12 (Table 4.3) .The pH values varied widely among the study strata from 6.53 to 7.52. The lowest mean pH (6.88) was recorded in the first stratum while highest mean pH

(7.21) was recorded in the fourth stratum. Using the one-way ANOVA test, it was established that there was significant difference in water pH during the sampling occasions for the wet season ($p \leq 0.0005$, DF = 4), (Appendix 4).

Table 4.3: pH levels of Omubhira stream during the dry and wet season

pH					
	Strata	**Mean**	**Std. Error**	**Min**	**Max**
Dry season	1S	7.65	0.007	7.65	7.66
	2S	7.62	0.01	7.596	7.65
	3S	7.47	0.03	7.43	7.56
	4S	7.53	0.019	7.53	7.58
	Overall	7.54	0.016		
Wet season	1S	6.88	0.207	6.53	7.42
	2S	7.10	0.184	6.58	7.52
	3S	7.07	0.103	6.78	7.28
	4S	7.21	0.068	7.02	7.33
	Overall	7.12	0.14		

4.1.4. Dissolved Oxygen (DO) (mg/l)

During dry season, the overall average mean value of dissolved oxygen was 6.45 mg /l (Table 4.4). Dissolved oxygen values in water from the sampling stratum varied from 5.80 to 9.3 mg /l. The lowest mean DO value of 5.80 was recorded in the first stratum (1S) while the highest mean DO values of 9.3 mg/ l in the fourth stratum (4S). Using a one-way ANOVA test, it was observed that the difference in DO among the sampling occasions during the dry season was not significant ($p \leq 1$, DF = 4), (Appendix 4).

During the wet season, the overall average mean of Dissolved oxygen was 6.74 mg /l (Table 4.4). DO levels varied widely among the study strata from 5.4 to 9.3 mg /l. The lowest mean DO value was recorded in fourth stratum (5.99 mg/ l) while first stratum recorded the highest mean DO value of 9.2 mg/ l. (Table 4.4). Using a one-way ANOVA test, it was observed that the difference in DO of stream water during the sampling occasions for the wet season was significant ($p \leq 0.009$, DF = 4). (Appendix 3).

Table 4.4: Dissolved Oxygen levels (mg/l) of Omubhira stream during the dry and wet season

Dissolved Oxygen (mg/l)					
	Strata	**Mean**	**Std. Error**	**Min**	**Max**
Dry season	S1	9.2	0.071	9.0	9.3
	S2	6.71	0.081	6.53	6.87
	S3	6.39	0.005	6.38	6.4
	S4	5.99	0.054	5.8	6.067
	Overall	6.45	0.053		
Wet season	1S	8.2	0.489	6.8	9.3
	2S	7.16	0.397	5.9	7.8
	3S	6.743	0.333	5.8	7.52
	4S	6.19	0.317	5.4	6.92
	Overall	6.74	0.3840		

4.1.5. Total Dissolved Solids (mg/l)

The overall average mean value for the TDS during dry season was 104.61 mg/l (Table 4.5) with the lowest mean of 84.37 mg/l recorded in the third stratum and the highest mean of 119.3 mg/l recorded in the fourth stratum. During Wet season, TDS recorded an average mean of 43.2051 mg/l (Table 4.5). The lowest mean of 27.4 mg/l was recorded at the second stratum and the highest mean of 65.73 mg/l in the fourth stratum (Table 4.5). Using a One-Way ANOVA test, it was observed that the difference in TDS was not significant during the sampling occasions for the wet season ($p \leq 0.252$, DF = 4) (Appendix 3) and during the dry season ($p \leq 0.960$, DF = 4) (Appendix 4).

Table 4.5: Total Dissolved Solids levels (mg/l) of Omubhira stream during the dry and wet season

Total Dissolved Solids (mg/l)					
	Strata	**Mean**	**Std. Error**	**Min**	**Max**
Dry season	1S	116.2	2.924	106	121
	2S	111.9	6.346	85.67	121.67
	3S	84.37	1.693	78.33	86.17
	4S	119.3	3.419	107.2	123.7
	Overall	104.6	3.60		
Wet season	1S	36.8	4.45	21	45
	2S	27.4	2.247	22.67	32.7
	3S	29.83	6.47	17.17	49.3
	4S	65.73	13.02	47	110.7
	Overall	43.21	6.55		

4.1.6. Total Suspended Solids (mg/l)

During the dry season, TSS recorded an overall average mean of 0.033 mg/l (Table 4.6) with the lowest mean value of 0.031 mg/l recorded in the first stratum and the highest mean of 0.034 recorded in the fourth stratum. During Wet season, the TSS recorded an overall average mean of 0.018 mg/l (Table 4.6). The lowest mean value of 0.0015 mg/l was recorded in the first stratum while the highest mean value of 0.0099 mg/l recorded in the fourth strata. TSS was significant during sampling occasions for the wet season ($p \leq 0.002$, DF = 4) (Appendix 3) while not significant ($p \leq 1$, DF = 4) (Appendix 4) during the dry season.

Table 4.6: Total Suspended Solids levels (mg/l) of Omubhira stream during the dry and wet season

Total Suspended Solids(mg/l)					
	Strata	**Mean**	**Std. Error**	**Min**	**Max**
Dry season	1S	0.0308	0	0.031	0.0308
	2S	0.0312	0.00001	0.0312	0.0312
	3S	0.0335	0.0003	0.033	0.0339
	4S	0.0345	0.0002	0.034	0.0349
	Overall	0.033	0.0001		
Wet season	1S	0.01	0.006	0.0015	0.0074
	2S	0.014	0.006	0.0022	0.0093
	3S	0.015	0.006	0.0038	0.0099
	4S	0.019	0.005	0.0090	0.0194
	Overall	0.018	0.006		

4.2. Bacteriological water quality gradients along the Omubhira stream course in Kakamega town

4.2.1. Total Coliforms (Cfu/100 ml)

The Total Coliform levels during the dry and wet season are as presented in Table 4.7. During dry season, TC counts in Omubhira stream recorded an overall average mean value of 3691 Cfu/100 ml (Table 4.7). The lowest mean of TC counts of 300 Cfu/100 ml was recorded in the first stratum (1S) while the fourth stratum (4S) recorded the highest mean value of 4477 Cfu/100 ml. During wet season, TC overall average mean counts in Omubhira stream was 4,780 Cfu/100 ml. The lowest mean of 1160 Cfu /100 ml were recorded in the first stratum (1S) while the fourth stratum (4S) recorded the highest mean count of 6210 Cfu /100 ml (Table 4.7). Mean comparison using a one-way ANOVA test revealed that the difference in TC counts in Omubhira stream water during the wet season was significant ($F = 17.76$, $p \leq 0.0005$), (Appendix 3). Mean comparison using a one way ANOVA test revealed that the difference in TC counts in Omubhira stream water during the dry season was not significant ($F = 1.687$, $p \leq 0.162$), (Appendix 4).

Table 4.7: Total Coliforms levels (Cfu/100 ml) of Omubhira stream during the dry and wet season

Total Coliforms counts (Cfu/100 ml)					
	Strata	**Mean**	**Std. Error**	**Min**	**Max**
Dry season	S1	300	61	100	400
	S2	3673	270	3300	4333
	S3	3480	332	3150	4600
	S4	4477	382	3650	5533
	Overall	3691	261		
Wet season	1S	1160	653	400	3400
	2S	4280	268	3700	5066
	3S	4203	567	3500	6200
	4S	6210	1480	4716	11500
	Overall	4780	742		

4.2.2. *Escherichia coli* (*E. coli*) (Cfu/100 ml)

During dry season, *E. coli* counts in Omubhira stream recorded an overall average mean value of 1096 Cfu /100 ml (Table 4.8). The least mean value of 20 Cfu /100 ml was recorded in the first stratum (1S) while the fourth stratum (4S) recorded the highest mean value of 1593 Cfu /100 ml. One-way ANOVA test revealed that the difference in *E. coli* counts during sampling occasions of Omubhira stream in dry season was not significant ($F = 2.538$, $p \leq 0.047$) (Appendix 4).

During wet season, *E. coli* counts in Omubhira stream recorded an overall average mean value of 651 Cfu/100 ml (Table 4.8) with the first stratum (1S) recording the least mean value count of 93 Cfu/100 ml while the fourth (4S) stratum recorded the highest mean value of 1230 Cfu /100 ml. As in the case of TC, mean comparison using a one way ANOVA test revealed that the difference in *E.coli* counts during the sampling occasions was significant during the wet season ($F=16.60$, $p \leq 0.05$),(Appendix 3).

Table 4.8: The E. coli levels (Cfu/100 ml) of Omubhira stream during the dry and wet season.

***E. coli* counts (Cfu/100 ml)**					
	Strata	**Mean**	**Std. Error**	**Min**	**Max**
Dry season	S1	20	22	0	100
	S2	880	247	333	1533
	S3	886	243	366	1450
	S4	1593	232	766	1850
	Overall	1096	186		
Wet season	1S	93	79	0	366
	2S	294	54	133	433
	3S	356	71	283	550
	4S	1230	164	933	1733
	Overall	651	92.50		

4.2.3. Relationship between Escherichia coli, Total Coliform and Physico-chemical Parameters

During the wet season, Pearson product-moment correlation as presented in Appendix 5 showed that there was a relationship between *E. coli* and EC, TDS and TC. There was a positive correlation between *E.coli* and electrical conductivity (EC) which was statistically significant (r = .349, n = 80, $p \leq 0$ .05), TDS (r = .351, n = 80, $p \leq$ .05) and TC (r= .241, n = 80, $p \leq$ .05). During the dry season, Pearson product-moment correlation showed that there was a positive relationship between *E. coli* and TDS and EC which was statistically significant ; TDS (r = .302, n = 80, $p \leq$ .0005) and TC (r= .545, n = 80, $p \leq$ .0005) dissolved oxygen showed a negative correlation with *E. coli* ;DO (r = .421, n = 80, $p \leq$ .0005), (Appendix 6).

Table 4.9: Pearson correlation coefficients between Escherichia coli and the other studied parameters of Omubhira stream during the dry and wet season

Parameter	Temp	pH	EC	TDS	DO	TSS	TC
E.coli (Dry season)	-.128	-.135	.307	.302*	-.421*	.159	.545*
E.coli (Wet season)	-.081	-.002	.349*	.351*	-.198	.072	.241*

4.3. Isolation and Characterization of Enteric Bacteria from Omubhira stream

The study recovered ten genera namely *E. coli, Enterobacter* spp., *Citrobacter* spp., *Salmonellae* spp., *Providencia* spp., *Proteus* spp., *Klebsiella* spp., *Shigella* spp., *Morganella* spp. and *Serratia* spp. as presented in figure 4.1 and 4.2 below. *E. coli* accounted for 55% of the isolates recovered during the dry season whereas it only accounted for 48% during the wet season. *Citrobacter* spp. accounted for 21 % of isolates recovered in the wet season while 14% for dry season. *Citrobacter freundii* accounted for 3% in the wet season while it was not recovered in the dry season.

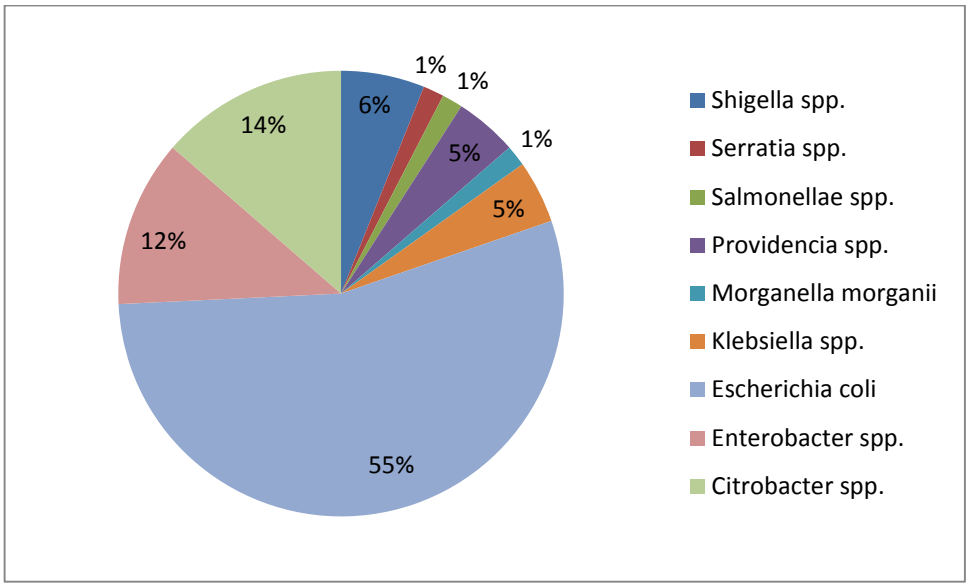

Figure 4.1: Bacterial isolates recovered from Omubhira stream during the dry season

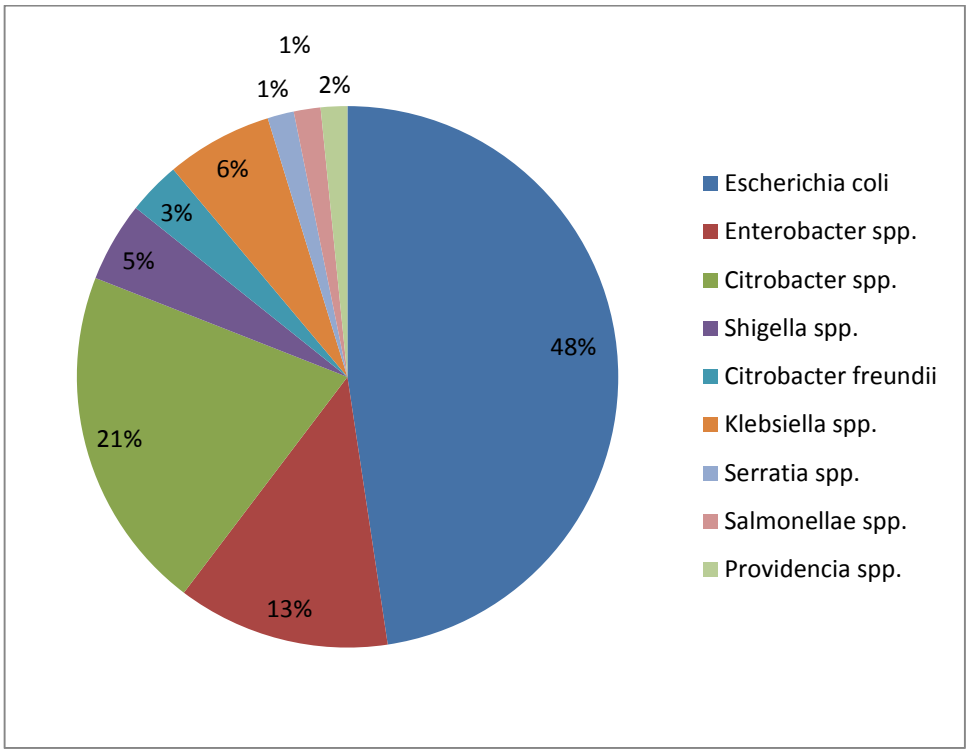

Figure 4.2: Bacterial isolates recovered from Omubhira stream during the wet season

CHAPTER FIVE

DISCUSSION

5.1 Physico-Chemical water quality gradients along the Omubhira stream course in Kakamega town

The physical chemical parameters of water in Omubhira stream exhibited significant variations in the dry and wet seasons. Furthermore, there were spatial variations of the physico- chemical parameters from upstream to downstream. Data in tables 4.1-4.6 shows that all the physical chemical parameters studied were within the permissible limit as per the WHO standards (WHO 2005) and NEMA (2013) regulations for natural waters. However, stratum four (4S) that is situated at the lower reaches of the stream had significantly high mean levels of electrical conductivity and TDS than in the other three sampling strata.

High levels of electrical conductivity and total dissolved solids could have been due to the high organic load, which in this study was from the wastewater effluent discharge and livestock watering points. This corresponds to a study on Nyangores stream, Mara river basin whereby the levels of electrical conductivity, total suspended solids and total dissolved solids increased downstream with the low temperature, conductivity, TSS and BOD levels recorded in upstream station increasing downstream due to the increased run-off from agricultural activities and sewage effluents (Gichana *et al.,* 2014). On the other hand, results indicated that EC, TDS and TSS had higher values in the dry season than in wet season. More so, similar observation was also made for Nairobi River whereby section at Lenana

School had highest electrical conductivity due to the effluent discharge from laboratory, which has high levels of ionic species.

The trend for Total Dissolved Solids (TDS) values was similar to that observed for electrical conductivity. Electrical conductivity levels increased downstream as the total dissolved solids levels also increased in both seasons. This is expected, since most dissolved solids in water are ionic species, which tend to increase electrical conductivity. Therefore, TDS values predictably increased with increase in electrical conductivity. TDS values were below the acceptable National Environment Management Authority (NEMA, 2013) limits of 1200 mg/l for natural water with similar observation also made for Nairobi River (Mbui *et al.,* 2016).

Water temperature variations at the various sampling sites were attributed to the differences in the amount of water present at the site and presence of vegetation shielding water source from direct insulation. The water temperature also depends on the time of sampling, season, and the temperature of effluent, which are discharged into the river. High temperatures during the dry season could have been influenced by factors that majorly include solar radiation and turbidity. Bacteriological and physico-chemical studies of the rural catchments of the Lake Victoria showed high temperature levels during the dry season (Ouma *et al.,* 2016). In general, the entire Omubhira stream system surface water samples had temperature means that were within the permissible limit in both seasons and averagely suitable for survival of most tropical aquatic organisms.

Increased turbidity increases water temperature. Suspended particles in water usually absorb heat from water thereby contributing to the heat absorbed by water temperature. This was observed during the dry season whereby defecation by

livestock directly into the water during feeding and watering increased the amount of suspended solids that absorbed heat from solar radiation and thus increasing temperature (Ogendi *et al.*, 2015).

In this study, it was observed that compared to other types of water sources, this water source was mainly located in high altitude areas where they are on most occasions sheltered by trees. Such an environment is likely to experience cool air temperature that influences water temperature. This study showed negative correlation between temperature and faecal coliforms and therefore is in agreement to the assessment of physico-chemical properties and sewage pollution indicator bacteria in surface water of River Gomti (India) whereby temperature showed significant negative correlation with faecal coliforms, total coliforms and biological oxygen demand (Srivastava & Srivastava, 2011).

Electrical conductivity values were higher in the dry season (118-183.6 µS/cm) when compared to the rainy/wet season (26.83- 165.1783µS/cm). This is consistent with the findings in River Moiben, Kenya whereby electrical conductivity values were higher in dry season (Masese *et al.,* 2009). This was due to the high solute concentrations in dry season because of evapo-transpiration losses from the channel. Moreover, run-off is supplied from ground water reservoirs, where water has a long residence time and solute release is prompted. In contrast, during the rainy season, run-off is generally translated much more rapidly to the stream channel with less opportunity for solute pick up and therefore has lower dissolved solids content.

The values of electrical conductivity observed during sampling periods are however within the range (WHO, 2005). The correlation coefficient for the electrical conductivity and dissolved solid values is 0.99, which implies that the presence of

the total dissolved solids is a major contributing factor to the electrical conductivity of water. However, relatively high conductivity of water observed at some sampling sites could have been due to the effect of released effluent from sewage treatment plant (Gichana *et al.,* 2014).

This study showed significant correlation between conductivity and coliforms and therefore is in agreement to a study of physico-chemical properties and sewage pollution indicator bacteria in surface water of River Gomti (India) whereby results revealed that EC showed significant positive correlation with faecal coliforms, total coliforms and biological oxygen demand (Srivastava & Srivastava, 2011).

Oxygen availability in an aquatic ecosystem is an indication of the systems health and general well-being and dissolved oxygen usually reflects the physical and biological processes prevailing in the water (Cohen and Hillel, 1972). Dissolved oxygen values for the fourth stratum (4S) of 5.99 and 6.12 mg/l in dry and wet season respectively indicated that there was slight pollution occurrence at this sampling location. The decreased dissolved oxygen levels downstream could have been attributed to the high organic load and partly due to increased water temperature that decreases solubility of oxygen in water (Gichana *et al.,* 2014). DO values of 9.2 mg/l and 8.2 mg/l for the dry and wet season respectively recorded at the first stratum (1S) which is the source of the stream indicated that the waters at this sampling location were high quality water. Dissolved oxygen concentrations above 5 mg/l in all the studied strata implies that the water is not stressful to fish growth since fish kills are usually observed at below 3 mg/l concentrations and thus suitable for fish farming.

Significant difference of dissolved oxygen levels during the wet season in the different sampling occasions could be due to the rapid water movement and turbulence that was as a result run-offs after precipitation the previous night to the sampling day that ensured trapping of air into the water thereby increasing amount of dissolved oxygen. Unlike when there had not been precipitation previous days to sampling, there was no rapid run-off into the stream except for the groundwater supplies that flowed calmly with less movement and turbulence mixing of water with atmospheric air while ensuring sufficient utilization of already dissolved oxygen by the aquatic organism.

TDS values in this study were within the prescribed limit given by WHO (2005) and the NEMA (2013). This could not interfere with the osmo-regulation of fresh water organisms in the stream. Excess sediment can harm the water quality. High level of solids in water increases water density, affects osmo-regulation of fresh water organisms, and reduces the solubility of gases such as oxygen (Nurcihan & Basaran 2009). The TSS levels were high for the dry season than for the wet season. This could have been due to the stampede of the stream basin by the livestock that are directly watering and feeding just by the stream banks during the dry season. However, low values of TSS during the wet season could account for the reason why the entire appearance of the water samples was clear, not turbid and having no odour. This result is similar to that obtained by Medema *et al*., (2001). Significant difference of TSS levels during the wet season could have been because of eroded soils, siltation and loose decomposed organic matter that was washed by rain during precipitation thereby increasing the levels of TSS.TSS levels were high on certain

sampling occasions especially when precipitation had occurred the previous night to the sampling day.

The Omubhira stream had a measured pH ranging from 6.88 to 7.654. The pH of water samples indicated that it was within the range set by WHO(2005). pH influences the survival of aquatic organisms in the water bodies since their metabolic activities are pH dependent and drastic changes in pH can have detrimental effects on stream health (Ouma *et al.*, 2016). This study showed negative correlation between pH and fecal coliform, which agrees to a study by Srivastava& Srivastava (2011) in the assessment of physico-chemical properties and sewage pollution indicator bacteria in surface water of River Gomti in Uttar Pradesh. Results revealed that pH showed significant negative correlation with fecal coliform, total coliform and biological oxygen demand.

5.2 Bacteriological water quality gradients along the Omubhira stream course in Kakamega town

The presence of total coliforms and *E. coli* counts in the stream water indicated contamination by raw sewage or defecations in the bush in the catchment or rather defecation by livestock during watering. This was similar to the findings in the study of Nyanchwa- Riana River (South West Kenya) (Ogendi *et al.*, 2015). The average mean for total coliforms and *E. coli* (faecal coliforms) counts were far above the NEMA (2013) regulations and WHO (2005) recommended standards for bacteria that stipulates that there should be nil Cfu/100 ml of *E.coli* counts and not more than 100 Cfu/100 ml of total coliforms in water for domestic and recreational use.

Moreover, the four strata representation of the stream also recorded TC and E.coli counts that were above the recommended NEMA and WHO (2011) standards for

drinking water. Generally, higher mean counts for TC were recorded in the wet season than in the dry season while *E. coli* mean counts were higher in the dry season than in the wet season.

High flows in streams tend to increase bacterial counts due to run-offs during the wet season (Muhibbu *et al.,* 2011) while the high levels of *E. coli* could have been due to increased turbidity from suspended particles during stampede by livestock while watering and feeding at the stream banks. Suspended particles could have facilitated the survival and growth of coliforms bacteria as they are shielded from ultra-violet radiation and attack by bacteriophage (Medema *et al.,* 2003). Moreover, the high counts in the dry season compared to the wet season of *E. coli* could be associated with rainfall occurrences that diluted and weakened the effects of point source pollution. While also increasing the contribution of non- point sources or diffuse pollution through land run-off from agricultural fields and leaches from refuse dumps (Muhibbu *et al.,* 2011).

The high mean E. coli counts during the dry season could also be associated with non- human warm-blooded animals' origin since domestic animals especially cows were a common precincts of sampling locations considered as watering and feeding points. This is in agreement with findings in the study of patterns and sources of faecal pollution in the heavily impaired River Njoro watershed (Kenya) (Jenkins, 2008). Moreover, *E. coli* counts were associated with humans because of the open defecation evident along the River Njoro and sewage treatment plants discharge point (Jenkins, 2008).

Concentrations of coliforms counts studied shows increase from the upstream (source) (1S) to downstream (4S). This indicates that there was input of raw sewage or animal waste at certain points along the stream transect. Zero *E. coli* counts at the source of the stream (1S) during various sampling occasions may be an indication that stream source is clean, safe, and not contaminated. Generally, the findings of the total coliforms (TC) and *E. coli* counts revealed that the human activities on most occasions increased the bacterial load of the water. Although total coliforms organisms may not always be directly related to the presence of faecal contamination or pathogens in the drinking water, this study found that almost all water samples from strata 2S, 3S, and 4S contained both TC and *E. coli*. except for a number of samples from 1S that recorded nil or rather, zero counts for *E. coli.*

In addition, the Omubhira stream is not well protected from direct access by animals, because most of the animals move along the streambed in search of water and pasture. In the process, they deposit many organic wastes inform of faecal matter directly into the stream or rather on the streambed floor. When it rains, the seasonal floods wash off bacteria and organic waste into the Omubhira stream hence contaminating them (Musyoki *et al.,* 2013) However, presence of a significant difference in TC and *E.coli* counts in Omubhira stream samples during the different sampling occasions in the wet season indicates impact of runoffs especially after precipitation the previous day that brought in high effluent from point and non-point sources of pollution that were contaminated with raw sewage and faecal matter.

According to Kavka *et al.,* (2002), although some community members put measures to protect their streams from direct faecal contamination by livestock, the high population of livestock and wildlife that visit the streambeds at different times

exposes the stream to some contamination. The downstream of Omubhira stream had the highest loads of TC and *E. coli*, while source had the least and in some occasions zero counts. Microbiological studies of river Danube showed similar characteristic with high levels of faecal pollution being particularly downstream. Main sources of pollution being raw discharges, discharges from wastewater treatment plants, impaired tributaries and impact by diffuse sources (Gerhard *et al.,* 2002) .It is therefore clear that should the water be qualified as potable, Omubhira stream must be fully protected from pollutants accordingly from its source in the first stratum to downstream at the fourth stratum.

5.3. Isolation and Characterization of Bacteria from Omubhira stream

Various bacterial isolates of public health concern were also identified from stream water samples in this study. *E. coli* were the most predominant enterobacterial isolate during both wet and dry seasons and across all the study sites. It is evident that the occurrence of pathogenic organism in stream water indicates the contamination of stream water with human or animal wastes and thus of public health significance (Shitu *et al.,* 2008). Though these bacteria are naturally found in the intestinal tract of warm-blooded animals, in soil and water, they can cause primary and opportunistic infections in humans and animals (Cheesbrough, 2002). Most are faecal-oral route transmitted and cause number of diseases from diarrhoeal, urinary tract infections, inflammation, and ulceration of intestinal tract, enteric fever to chest infections. However, *Serratia* spp., though found mostly in soil and water, has been reported to cause pulmonary and urinary infection. This is a unique micro- organism from all that were identified in the study since it produced a red pigment in nutrient agar at room temperature.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the findings of this study, the following conclusions can be derived.

i) The studied physico-chemical water quality of Omubhira stream was within the acceptable limits as per NEMA 2013 and WHO 2005 standards.
ii) The *E. coli* and TC levels were above the recommended standard levels.
iii) Enteric bacteria of public health concern were identified from the stream water. This provides new knowledge in the study of Omubhira stream.

Although the physico-chemical water quality of Omubhira stream was within the acceptable limits as per NEMA and WHO (2011) standards, the bacteriological water quality was above the recommended standards levels. This implies that the application of physico-chemical water quality analysis of water alone cannot be used to determine the safety of water as potable and suitable for other purposes. Identification of enteric bacteria that are of public health significance pose a health risk to stream water users which rely on the receiving water body primarily as their source of domestic water. Therefore, control of human activities to prevent faecal matter from entering water body is the key to avoiding bacterial contamination. Though the bacterial levels render stream water unfit for human consumption before treatment, the water can be used for other purposes depending on the particular use.

6.2 Recommendations

The following recommendations are derived.

i) Sustainable use and conservation of fresh water resources, water pollution prevention, institutional capacity building and creating awareness should be promoted. There is also need for intervention of appropriate regulatory agencies to ensure production of high quality treated final effluents by wastewater treatment facilities.

ii) Stream water users that majorly use the stream for watering livestock should be enlightened on the best method of watering by ensuring livestock do not directly drink from stream since it is during these occasions that they defecate in the same water leading to contamination of the stream by the faecal matter.

iii) Programmes must be organized to educate general population living around the stream on proper disposal of refuse and need to purify water to make it fit for drinking because the associable organisms are of public health significance and are implicated in one form of infection or the other.

iv) Programme for restoration, protection and conservation of Omubhira stream should be developed by Masinde Muliro University of Science and Technology, which is an institution of higher learning in Science and Technology and in close proximity to the stream.

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APPENDICES

Appendix I: Descriptive analysis of Physicochemical and Bacteriologic properties of Omubhira stream during the Dry Season

		Mean	Std. Error	Variance	Minimum	Maximum
Temperature	4S	21.15	0.1515	0.0918	20.85	21.5
	3S	21.2	0.2281	0.2082	20.816	21.4667
	2S	21.09	0.2041	0.1667	20.80	21.7
	1S	20.7	0.4473	0.8	20.1	21.2
Electrical Conductivity	4S	178.8332	4.8480	94.013	178.8332	183.667
	3S	126.33314	2.4073	23.180	126.333	129.333
	2S	168.39988	9.4535	357.4698	168.39988	183.333
	1S	173.4	3.7683	56.8	172	180
TDS	4S	119.3	3.419	46.75	119.3	123.667
	3S	84.37	1.693	11.46517	84.36668	86.1667
	2S	111.9	6.346	161.0751	111.9334	121.667
	1S	116.2	2.924	34.2	116.2	121
PH	4S	7.527	0.019	0.001	7.525	7.575
	3S	7.474	0.03	0.003568	7.425	7.563
	2S	7.62	0.01	0.000426	7.596	7.653
	1S	7.654	0.007	0.00018	7.654	7.66
TSS	4S	0.0345	0.000164	0.00000010	0.0343	0.0349
	3S	0.0335	0.00029	0.00000	0.03286	0.03391
	2S	0.0312	0.0000010	0.000000	0.03116	0.0312
	1S	0.0308	0	0	0.0308	0.0308
DO	4S	5.99	0.054	0.012	5.8	6.0667
	3S	6.393	0.005	0.000008	6.383	6.4
	2S	6.706	0.081	0.026297	6.533	6.866
	1S	9.2	0.071	0.02	9.1	9.3
TC	4S	4477	382	0.00000005	3650	5533.333
	3S	3480	332.3	441583.2	3150	4600
	2S	3673	270.9	293543.9	3300	4333.3
	1S	300	61.24	15000	100	400
E. coli	4S	1593	232.2	0.0000005	766.667	1850
	3S	886.7	243	236166.5	366.667	1450
	2S	880	247.9	245888.6	333.333	1533.33
	1S	20	22.36	2000	0	100

Appendix II: Descriptive analysis of Physicochemical and Bacteriological properties of Omubhira stream during the Wet season

		Mean	Std. Error	Variance	Minimum	Maximum
Temperature	4S	12.2667	0.129	0.067	11.833	12.667
	3S	12.1666	0.139	0.077441	11.667	12.833
	2S	12.666	0.191	0.146337	12.333	13.333
	1S	14.6	0.245	0.24	13.0	15.0
Electrical Conductivity	4S	98.83	19.27	1485	71.333	165.166
	3S	45	10.36	429.6444	26.833	78.5
	2S	41.2	3.536	50.01657	34	51.33
	1S	53.8	6.804	185.2	30	63
TDS	4S	65.73	13.02	678	47	110.667
	3S	29.83	6.47	167.4591	17.166	49.333
	2S	27.4	2.247	20.18991	22.667	32.667
	1S	36.8	4.45	79.2	21	42
pH	4S	7.206	0.068	0.019	7.015	7.33
	3S	7.067	0.103	0.042748	6.773	7.283
	2S	7.103	0.184	0.135839	6.583	7.52
	1S	6.88	0.207	0.1708	6.53	7.42
TSS	4S	0.019	0.005	0.000000005	0.00895	0.03433
	3S	0.015	0.006	0.000132	0.003783	0.03392
	2S	0.014	0.006	0.000127	0.0022	0.03116
	1S	0.01	0.006	0.000152	0.0015	0.0308
DO	4S	6.19	0.317	0.401	5.85	6.916
	3S	6.743	0.333	0.44371	6.383	7.5167
	2S	7.16	0.397	0.631891	6.866	7.8
	1S	8.2	0.489	0.955	7.8	9.3
TC	4S	6210	1481	8773280	4716.667	11500
	3S	4203	567.8	1289501	3500	6200
	2S	4280	268.4	288111.8	3700	5066.667
	1S	1160	653.5	1708000	400	3400
E. coli	4S	1230	164.2	107832.56	933.333	1733.33
	3S	356.7	71.83	20638.99	283.33	550
	2S	294.7	54.59	11919.79	133.333	433.33
	1S	93.33	79.41	25222.27	0	366.667

Appendix III: Determination of Significant difference between values of Physicochemical and Bacteriological parameters at different sampling occasions using one-way ANOVA for the Wet season

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Temperature	Between Groups	2.836	4	.709	2.005	.103
	Within Groups	25.805	73	.353		
	Total	28.641	77			
conductivity	Between Groups	34083.528	4	8520.882	1.420	.236
	Within Groups	437987.652	73	5999.831		
	Total	472071.179	77			
TDS	Between Groups	14809.914	4	3702.479	1.373	.252
	Within Groups	196824.804	73	2696.230		
	Total	211634.718	77			
PH	Between Groups	2.472	4	.618	9.586	.000
	Within Groups	4.706	73	.064		
	Total	7.178	77			
TSS	Between Groups	.009	4	.002	4.620	.002
	Within Groups	.036	73	.000		
	Total	.045	77			
DO	Between Groups	22.637	4	5.659	3.668	.009
	Within Groups	112.635	73	1.543		
	Total	135.272	77			
TC	Between Groups	636185297.494	4	159046324.373	17.760	.000
	Within Groups	653753553.545	73	8955528.131		
	Total	1289938851.038	77			
E. coli	Between Groups	1818176.465	4	454544.116	16.60	.000
	Within Groups	59437910.71	73	814217.955		
	Total	61256087.17	77			

Appendix IV: Determination of Significant difference between the values of Bacteriological and Physicochemical parameters at different sampling occasions using one way ANOVA for the Dry season

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Temperature	Between Groups	2.925	4	.731	.523	.719
	Within Groups	104.875	73	1.398		
	Total	107.800	77			
conductivity	Between Groups	5367.375	4	1341.844	.137	.968
	Within Groups	735431.375	73	9805.752		
	Total	740798.750	77			
TDS	Between Groups	2734.675	4	683.669	.155	.960
	Within Groups	329974.313	73	4399.658		
	Total	332708.988	77			
PH	Between Groups	.050	4	.012	.714	.585
	Within Groups	1.305	73	.017		
	Total	1.354	77			
TSS	Between Groups	.000	4	.000	.003	1.000
	Within Groups	.004	73	.000		
	Total	.004	77			
DO	Between Groups	.058	4	.015	.003	1.000
	Within Groups	320.681	73	4.276		
	Total	320.739	77			
TC	Between Groups	31589070.000	4	7897267.500	1.687	.162
	Within Groups	351067250.000	73	4680896.667		
	Total	382656320.000	77			
E.coli	Between Groups	10689500.000	4	2672375.000	2.538	.047
	Within Groups	78979375.000	73	1053058.333		
	Total	89668875.000	77			

Appendix V: Correlation between *E. coli* and Physico-Chemical Properties of Omubhira stream during **wet season**

		E.coli	Temperature	Conductivity	TDS	PH	TSS	DO	TC
	Pearson Correlation	1	-.081	.349**	.351**	-.002	.072	-.198	.241*
E.coli	Sig. (2-tailed)		.483	.002	.002	.989	.531	.083	.034
	N	78	78	78	78	78	78	78	78
	Pearson Correlation	-.081	1	.054	.051	.014	-.024	-.122	.188
Temperature	Sig. (2-tailed)	.483		.636	.659	.905	.836	.289	.099
	N	78	78	78	78	78	78	78	78
	Pearson Correlation	.349**	.054	1	.999**	.012	-.041	-.447**	.453**
Conductivity	Sig. (2-tailed)	.002	.636		.000	.920	.719	.000	.000
	N	78	78	78	78	78	78	78	78
	Pearson Correlation	.351**	.051	.999**	1	.016	-.047	-.443**	.449**
TDS	Sig. (2-tailed)	.002	.659	.000		.892	.685	.000	.000
	N	78	78	78	78	78	78	78	78
	Pearson Correlation	-.002	.014	.012	.016	1	.031	.142	-.107
PH	Sig. (2-tailed)	.989	.905	.920	.892		.787	.215	.352
	N	78	78	78	78	78	78	78	78
	Pearson Correlation	.072	-.024	-.041	-.047	.031	1	.031	-.114
TSS	Sig. (2-tailed)	.531	.836	.719	.685	.787		.790	.319
	N	78	78	78	78	78	78	78	78
	Pearson Correlation	-.198	-.122	-.447**	-.443**	.142	.031	1	-.481**
DO	Sig. (2-tailed)	.083	.289	.000	.000	.215	.790		.000
	N	78	78	78	78	78	78	78	78
	Pearson Correlation	.241*	.188	.453**	.449**	-.107	-.114	-.481**	1
TC	Sig. (2-tailed)	.034	.099	.000	.000	.352	.319	.000	
	N	78	78	78	78	78	78	78	78

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (1-tailed).

Appendix VI: Correlation between *E. coli* and all the other studied Properties of Omubhira stream during dry season

Correlations

		EC	Temperature	conductivity	TDS	PH	TSS	DO	TC
E. coli	Pearson Correlation	1	-.128	.307**	.302**	-.135	.159	-.421**	.545**
	Sig. (2-tailed)		.259	.006	.006	.234	.160	.000	.000
	N	80	80	80	80	80	80	80	80
Temperature	Pearson Correlation	-.128	1	.201	.199	.174	.017	.085	-.139
	Sig. (2-tailed)	.259		.074	.077	.122	.883	.455	.220
	N	80	80	80	80	80	80	80	80
Electrical conductivity	Pearson Correlation	.307**	.201	1	1.000**	-.154	-.127	-.478**	-.081
	Sig. (2-tailed)	.006	.074		.000	.171	.262	.000	.478
	N	80	80	80	80	80	80	80	80
TDS	Pearson Correlation	.302**	.199	1.000**	1	-.152	-.130	-.475**	-.081
	Sig. (2-tailed)	.006	.077	.000		.177	.250	.000	.475
	N	80	80	80	80	80	80	80	80
PH	Pearson Correlation	-.135	.174	-.154	-.152	1	-.226*	.117	-.103
	Sig. (2-tailed)	.234	.122	.171	.177		.044	.300	.365
	N	80	80	80	80	80	80	80	80
TSS	Pearson Correlation	.159	.017	-.127	-.130	-.226*	1	.194	.468**
	Sig. (2-tailed)	.160	.883	.262	.250	.044		.085	.000
	N	80	80	80	80	80	80	80	80
DO	Pearson Correlation	-.421**	.085	-.478**	-.475**	.117	.194	1	.025
	Sig. (2-tailed)	.000	.455	.000	.000	.300	.085		.828
	N	80	80	80	80	80	80	80	80
TC	Pearson Correlation	.545**	-.139	-.081	-.081	-.103	.468**	.025	1
	Sig. (2-tailed)	.000	.220	.478	.475	.365	.000	.828	
	N	80	80	80	80	80	80	80	80

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix VII: Determination of significant difference between the values of physico-chemical parameters using One-Way ANOVA for both Wet and Dry season

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Temperature	Between Groups	2968.169	1	2968.169	3393.656	.000
	Within Groups	136.441	156	.875		
	Total	3104.610	157			
conductivity	Between Groups	332297.615	1	332297.615	42.740	.000
	Within Groups	1212869.929	156	7774.807		
	Total	1545167.544	157			
TDS	Between Groups	148925.314	1	148925.314	42.680	.000
	Within Groups	544343.705	156	3489.383		
	Total	693269.019	157			
PH	Between Groups	7.312	1	7.312	133.686	.000
	Within Groups	8.533	156	.055		
	Total	15.845	157			
TSS	Between Groups	.009	1	.009	30.360	.000
	Within Groups	.048	156	.000		
	Total	.058	157			
DO	Between Groups	3.318	1	3.318	1.135	.288
	Within Groups	456.011	156	2.923		
	Total	459.328	157			
TC	Between Groups	17390419.221	1	17390419.221	1.622	.205
	Within Groups	1672595171.038	156	10721763.917		
	Total	1689985590.259	157			
E.coli	Between Groups	6435979.593	1	6435979.593	6.652	.011
	Within Groups	150924962.179	156	967467.706		
	Total	157360941.772	157			

Appendix VIII: Preparation of culturing media

Mac Conkey agar powder weighing 10.306 was dispensed into 200 ml of sterilized distilled water in a conical flask. After mixing the solution, it was heated gently to dissolve and then autoclaved at 121ºC for 15 minutes. The agar was allowed to cool to about 45ºC then be poured into sterile disposable petri-dishes.

EMB Agar (Levine- Eosin- Y, Methylene Blue) Agar ((HIMedia Lab. Pvt. Mumbai, India) weighing 9.375 g was weighed and dispensed into 250 ml sterilized distilled water and heat to boiling to dissolve the medium completely and autoclaved at 1 bar (121ºC) for 15 minutes. The medium was cooled to about 50ºC and shaken in order to oxidize the methylene blue (i.e. to restore its blue colour) and to suspend the precipitate that is an essential part of the medium. The agar was poured into sterile disposable petri- dishes.

The Eosin Y and Methylene Blue dyes in Levine EMB Agar render the medium slightly selective in that they inhibit growth of gram-positive bacteria to a limited degree. These dyes also play a role in differentiating between lactose fermenters and lactose non-fermenters due to the presence or absence of dye uptake in the bacterial colonies. Coliforms, as lactose fermenting organisms, are visualized as blue-black colonies whereas colonies of *Salmonella* and *Shigella*, as lactose non-fermenters, appear colourless, transparent or amber in colour.

Some gram-positive bacteria, such as fecal streptococci, staphylococci and yeasts, will grow on this medium and usually form pinpoint colonies. A number of non-pathogenic, lactose-non-fermenting gram-negative bacteria will grow on this medium and must be distinguished from the pathogenic strains by additional biochemical tests.

Tryptic Soy Broth weighing 3 grams was dispensed in 15% glycerol and heat to boiling to dissolve the medium completely. 15% glycerol was realized by measuring 15 ml glycerol with 85 ml of distilled sterilized water.

Prepared 15% glycerol and Tryptic Soy Broth was poured into the storing tubes and the lids loosely closed before autoclaving at 1 bar (121ºC) for 15 minutes. On cooling, isolates were then dispensed in the tubes and lids fitted tightly for refrigeration at - 80°C.

Appendix IX: Biochemical tests

Triple Sugar Iron (TSI) Agar Test

During preparation, tubes containing molten agar were angled and allowed to solidify. Using a straight inoculating needle, an isolated colony cultured on nutrient agar plates was picked. Inoculation was done on the TSI slant by first stabbing the butt down to the bottom, withdrawing the needle, and then streaking the surface of the slant. A loosely fitting closure was then used to permit access of air. Results were read after incubation at 37°C for 18 to 24 h.

TSI contains three carbohydrates: glucose (0.1%), sucrose (1%), and lactose (1%).Besides the carbohydrates mentioned, the medium also contains beef extract, yeast extract, and peptones, which are the sources of nitrogen, vitamins and minerals. Phenol red is the pH indicator, and agar is used to solidify the medium. The slant of the medium is aerobic, while the deep (or butt) is anaerobic. Based on carbohydrate utilization and hydrogen sulphide production, a TSI slant can be interpreted in several ways: A yellow butt (acid production) and red- pink slope indicate the fermentation of glucose only. The slope is pink – red due to a reversion of the acid reaction under aerobic conditions. This reaction is seen with Salmonella and Shigella species and other enteric pathogens. Cracks and bubbles in the medium indicate gas production from glucose fermentation. *S. paratyphi* and some faecal commensals. A yellow slope produce gas and a yellow butt indicate the fermentation of lactose and possibly glucose. This occurs with *E. coli* and other enterobacteria. A red- pink slope and butt indicate no fermentation of glucose or lactose. This is seen with most strains of *P. aeruginosa.* Blackening along the stab line or throughout the medium indicates

hydrogen sulphide (H_2S) production, for example, *S. typhi* produces a small amount of blackening whereas *S. typhimurium* causes extensive blackening.

Citrate utilization test

This test is based on the ability of an organism to use citrate as its only source of carbon. Using Simmon's citrate agar, slopes of the medium were prepared in bijou bottles as recommended and stored at 2-8°C. Using a sterile straight wire, the slope was first streak with a saline suspension of the test organism and then the butt was stubbed. Incubation was at 35°C for 48 hours. A bright blue colour in the medium indicates positive citrate test while no change in medium colour indicates a negative citrate test.

Lysine Decarboxylase (LDC) test

This test is important in identification of enterobacteria. This was conducted by use of a combined Motility Indole Lysine Agar. Some strains of enterobacteria break down lysine in decarboxylase reaction as a source of carbon. Blue/ violet colour indicates a positive LDC test while a yellow colour indicates a negative LDC test.

Motility test

Knowing whether an organism is motile or non- motile can often assist in its identification, for instance most species of *Salmonella* are motile whereas *Shigella* species are non- motile. This test was conducted by use of a combined Motility Indole Lysine Agar medium. Prepared media was dispensed in binjou bottles and allowed to cool down. Isolates was then picked using a sterile needle and innoculated through media in a straight line and needle removed carefully in the same manner to

avoid it spreading throughout the media. Results were read after incubation at 37°C for 18 to 24 h.

Indole test

Testing for indole production is important in the identification of enterobacteria. Most strains of enterobacteria break down the amino acid tryptophan with the release of indole. The test organism was cultured in a medium that contained tryptophan. Indole production was detected by Kovac's reagent. This reacts with the indole to produce a red coloured compound. Red surface layer indicates positive indole test while yellow surface layer indicates negative indole test.

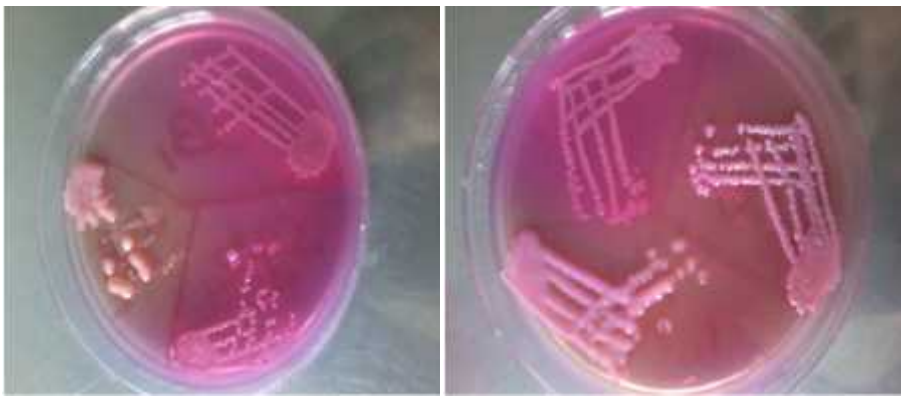
Appendix X: Growth of coliforms on petrifilm plates

Petrifilm plates showing colony growths of water samples from different sampling sites. Blue colonies indicate *E.coli* while Red colonies indicate total coliforms

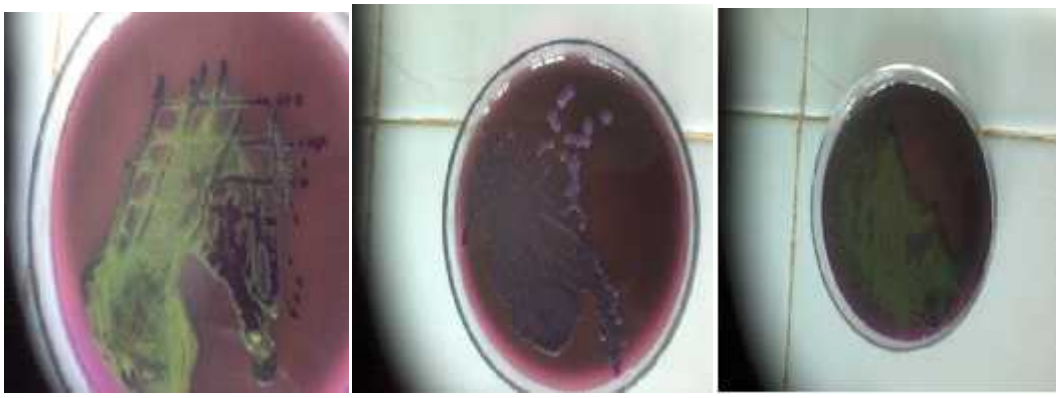
Growth of colonies from sampling site (S6), (S11), (S14) and (S3) respectively

Appendix XI: Growth of various coliforms on differential media

Selected blue and red colonies were isolated from petrifilm plates and cultured onto Mac Conkey and Eosin Methylene Blue agar plates. Pink colonies on Mac Conkey indicate lactose fermenters, light pink colonies indicate late lactose fermenters while colourless colonies indicate non- lactose fermenters.



Plates 1: Growth of colonies on Mac Conkey agar



Plates 2: Growth of *Escherichia coli* on EMB agar with a green metallic sheen

Appendix XII: Bacterial types isolated from Omubhira stream

DURING WET SEASON					MIL Medium			TSI Medium			
Site	Species	VR Medium	Mac Conkey agar	Cit	Mot	Ind	LDC	Slope	Butt	H_2S	Gas
SO	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Proteus spp.	R2	NL	+	-	-	-	R	Y	+	+
	Citrobacter spp.	R3	NL	+	+	-	-	R	Y	-	+
	Enterobacter spp.	R4	L	+	+	+	+	R	Y	-	+
S1	Serratia spp.	R2	L	+	+	-	+	Y	Y	-	+
	Enterobacter spp.	R3	L	+	+	-	+	Y	Y	-	+
	Shigella spp.	R4	L	-	-	+	+	Y	Y	-	+
	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B2	L	-	+	-	-	Y	Y	-	+
	Citrobacter spp.	R5	L	+	+	-	-	R	Y	-	+
S2	Escherichia coli	R1	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Klebsiella spp.	R2	L	+	-	-	+	Y	Y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B3	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B4	L	-	+	+	+	Y	Y	-	+
	Shigella spp.	R3	L	-	-	+	+	R	Y	-	+
S3	Proteus spp.	R1	NL	+	+	-	+	Y	Y	+	+
	Citrobacter spp.	R2	LL	+	+	-	-	Y	Y	-	+
	Citrobacter freundii	R3	LL	+	+	-	-	Y	Y	+	+
	Shigella spp.	R4	L	-	-	+	+	Y	Y	-	+
	Citrobacter spp.	R5	L	+	+	+	+	Y	Y	+	+
	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
S4	Enterobacter spp.	R1	L	+	+	-	+	Y	Y	-	+
	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Citrobacter spp.	R2	NL	+	+	-	-	R	Y	+	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+
S5	Citrobacter spp.	R1	L	+	+	-	-	Y	Y	-	+
	Escherichia coli	B1	L	-	+	-	+	Y	Y	-	+
	Escherichia coli	B2	L	+	-	-	-	Y	Y	-	+
	Enterobacter spp.	R2	LL	+	+	-	-	Y	Y	-	+
S6	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+

	Enterobacter spp.	R1	LL	+	+	-	-	Y	Y	-	+
	Enterobacter spp.	R2	L	+	+	-	-	R	Y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+
	Citrobacter spp.	R3	LL	+	+	-	-	Y	Y	-	+
	Enterobacter spp.	R4	L	+	+	-	-	R	Y	-	+
S7	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Enterobacter spp.	R1	L	+	+	-	-	R	Y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	R2	L	-	+	+	+	Y	Y	-	+
	Citrobacter spp.	R3	LL	+	+	-	-	Y	Y	-	+
	Escherichia coli	B3	L	-	+	+	+	Y	Y	-	+
S8	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+
	Klebsiella spp.	R1	L	+	-	-	+	Y	Y	-	+
	Klebsiella spp.	R2	L	+	-	-	+	R	Y	-	+
S9	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Klebsiella spp.	R1	L	+	-	-	+	Y	Y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+
	Citrobacter spp.	R2	LL	+	+	-	+	Y	Y	-	+
S10	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Klebsiella spp.	R1	L	+	-	+	+	Y	Y	-	+
	Escherichia coli	R2	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+
S11	Enterobacter spp.	R1	LL	+	-	+	-	Y	Y	-	+
	Providencia spp.	R2	NL	+	+	+	-	Y	Y	-	+
	Proteus spp.	R3	NL	+	+	-	-	Y	Y	-	+
	Citrobacter spp.	R4	LL	+	+	-	-	Y	Y	-	+
	Citrobacter spp.	R4	LL	+	+	-	-	Y	Y	-	+
	Proteus spp.	B2	NL	+	-	-	-	Y	Y	-	+
S12	Citrobacter freundii	R1	L	+	-	-	-	Y	Y	+	+
	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	R2	L	-	+	+	+	Y	Y	-	+
S13	Citrobacter freundii	R1	L	-	+	+	+	Y	Y	+	+
	Escherichia coli	R2	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Citrobacter spp.	R3	LL	+	+	-	-	Y	Y	-	+

	Citrobacter spp.	R4	LL	+	+	-	-	Y	Y	+	+
S14	Salmonellae spp.	R1	NL	-	+	-	+	Y	Y	-	+
	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	R2	L	-	+	+	+	Y	Y	-	+
S15	Citrobacter spp.	R1	LL	+	+	-	-	Y	Y	-	+
	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Citrobacter spp.	R2	LL	+	+	-	-	R	y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+

DURING DRY SEASON					MLI Medium			TSI Medium			
Site	Species	VR Medium	Mac Conkey medium	Cit	Mot	Ind	LDC	Slope	Butt	H_2S	Gas
SO	Providencia spp.	R1	NL	+	+	+	-	R	Y	-	-
	Salmonellae spp.	R2	NL	-	+	-	-	Y	Y	-	+
S1	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+
	Shigella spp.	R2	L	-	-	+	-	Y	Y	-	+
	Citrobacter spp.	R1	LL	+	+	-	-	R	Y	-	+
	Escherichia coli	R2	LL	-	+	+	+	Y	Y	-	+
	Klebsiella spp.	R3	L	+	-	-	+	Y	Y	-	+
S3	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+
	Providencia spp.	R2	NL	+	+	+	-	R	Y	-	-
	Klebsiella spp.	R4	L	+	-	+	+	Y	Y	-	+
S4	Escherichia coli	B1	LL	-	+	+	+	Y	Y	-	+
	Escherichia coli	B2	LL	-	+	+	+	Y	Y	-	+
	Citrobacter spp.	R3	LL	+	+	+	-	Y	Y	+	+
	Escherichia coli	B3	LL	-	+	+	+	Y	Y	-	+
S5	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Providencia spp.	R1	NL	+	+	+	-	Y	Y	-	+
	Serratia spp.	R2	L	+	+	-	+	Y	Y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+

S6	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Enterobacter spp.	R1	L	+	+	-	+	Y	Y	-	-
S7	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	R2	L	-	+	+	+	Y	Y	-	+
S8	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B3	L	-	+	+	+	Y	Y	-	+
	Citrobacter spp.	R3	LL	+	+	-	-	Y	Y	-	+
S9	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Shigella spp.	R1	NL	-	-	+	-	Y	Y	-	+
	Morganella morganii	R4	NL	-	+	+	-	Y	Y	-	+
S10	Escherichia coli	B1	L	-	-	+	+	Y	Y	-	+
	Escherichia coli	B2	L	-	-	+	+	Y	Y	-	+
	Citrobacter spp.	R1	LL	+	+	-	-	Y	Y	-	+
	Citrobacter spp.	R2	LL	+	+	-	-	Y	Y	-	+
	Escherichia coli	R3	LL	-	+	+	+	Y	Y	-	+
S11	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B3	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B4	L	-	+	+	+	Y	Y	-	+
	Citrobacter spp.	R4	LL	+	+	-	-	Y	Y	-	+
S12	Escherichia coli	B1	L	-	-	+	+	Y	Y	-	+
	Escherichia coli	B2	LL	-	+	+	+	Y	Y	-	+
	Citrobacter spp.	R2	LL	+	+	-	-	R	Y	-	+
	Escherichia coli	B3	LL	-	+	+	+	Y	Y	-	+

S13	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Klebsiella spp.	R1	L	+	-	-	+	Y	Y	-	+
	Klebsiella spp.	R2	L	+	-	-	+	Y	Y	-	+
	Shigella spp.	R3	NL	-	-	-	+	Y	Y	-	+
S14	Citrobacter spp.	R1	L	+	+	+	-	Y	Y	-	+
	Citrobacter spp.	R2	LL	+	+	-	+	Y	Y	+	+
S15	Escherichia coli	B1	L	-	+	+	+	Y	Y	-	+
	Escherichia coli	B2	L	-	+	+	+	Y	Y	-	+
	Shigella spp.	R1	NL	-	-	+	-	R	Y	-	+