IMPACT OF ANTHROPOGENIC ACTIVITIES ON BACTERIOLOGICAL WATER QUALITY OF NYANGORES RIVER, MARA BASIN-KENYA

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been submitted or presented for examination in any University

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RECOMMENDATION

This thesis has been submitted for examination with our approval as university supervisors

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DEDICATION

I dedicate this work to my beloved wife, children, and mum, to all friends of Mau catchment, Mara Basin and to all Environmentalists.

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ABSTRACT

The Mara River basin is the lifeline to Maasai Mara Game reserve in Kenya and the Serengeti National park in Tanzania. Its major perennial tributaries are Nyangores and Amala both originating from Mau Escarpment. Unprecedented evidence on change of land use for development purposes in the upper catchment has affected the water quantity, quantity and the environmental sanitation in general. In addition, the municipal town of Bomet situated close to Nyangores River lack adequate sanitation facilities, these might have greatly degraded the water quality through discharge of raw faecal matter into the river. Another notable area of concern is the nearby Tenwek Mission Hospital whose waste water lagoons are located close to the bank of the same river with their waste effluents being directly disposed to this river. Such waste disposal methods create point and non-point sources of pollution with different degrees of pollution. This study investigated the effect of human settlement and development on the microbial water quality of Nyangores River at various points along its river channel based on the intensities of human settlement and development. To establish the microbiological water quality, the study involved the use of Membrane Filtration Technique (MFT) to determine the densities of total coliforms, *Escherichia coli*, intestinal enterococci, *Clostridium perfringens* and *Salmonella spp.* followed by plating on selective differential media for the bacteria being sought. Pollution with easily biodegradable organic wastes was detected by Heterotrophic Plate Count (HPC) procedures and BOD_5 determination. Physico-chemical parameters; temperature, dissolved oxygen (DO), conductivity, turbidity, total dissolved solids and pH of the water at the sampling sites were also measured at the time of sampling using appropriate measuring meters. The collected data was analysed using Statistical Package for Social Sciences (SPSS) version 17 software with a confidence level of 95%. The results indicated spatial and temporal variation in the densities of faecal contamination indicators $P<0.05$. Indicators of contamination with easily degradable organic matter (BOD and HPC) also showed significant spatial and temporal variations, $P<0.05$. All the sites studied except site 1 at Kiptagich were found to be contaminated with *Salmonella spp.* Physicochemical parameters studied also showed significant spatial variation except DO, $P<0.05$. In conclusion, the presence of anthropogenic activities along Nyangores River have impacted negatively on quality of its water and therefore appropriate corrective mechanisms are necessary to help improve or restore its water quality so as to uphold its ecological integrity and be safe for domestic use.

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A LIST OF ABBREVIATIONS AND ACRONYMS

APHA…….American Public Health Association

BOD……Biological Oxygen Demand

BGLB……..Brilliant Green Lactose Bile

CFUs……...Colony Forming Units

CP……*Clostridium perfringens*

DPSIR……..Driving forces, Pressure, State, Impacts and Response

DO……...Dissolved Oxygen

EC…….*Escherichia coli*

UFSA……...European Food Safety Authority

EMB…….Eosin Methyl Blue

E.I.A…….Environmental Impact Assessment

EPHTI……..Ethiopian Public Health Training Initiative

HPC……..Heterotrophic Plate Count

IE……..Intestinal Enterococci

IWRM……..Integrated Water Resource Management

MDGS…….Millennium Development Goals

MRA…….Microbial Risk Assessment

NTU…….Nephelometric Turbidity Unit

OAEL……. Observed Adverse Effect level

TC……Total Coliform

TDS……..Total Dissolved Solutes

UNDP……..United Nations Development Programme

USEPA……United States Environmental Protection Agency

WBDs……..Waterborne Diseases

WRMA……Water Resource Management Authority

WRUA'S…… Water Resource User's Association

WHO……World Health Organization

CHAPTER ONE
INTRODUCTION

1.1 Background information

Mara basin is a trans-boundary water catchment tower comprising of various small rivers, streams and their tributaries that merge together to form the great River Mara. The River Mara is shared between Kenya and Tanzania and drains its water into Lake Victoria. It flows through open savannah grasslands and eventually into Maasai Mara Game Reserve and Serengeti National Park, an important tourist destination in Africa famous for the seven big animals and the spectacular wonders of the annual wildebeest migration. The main tributaries of Mara River are Amala and Nyangores Rivers which are both under serious threat as a result of change in land use to accommodate various purposes like human settlement and urbanization among others in the catchment. This has eventually led to drastic reduction in forest cover which has hampered the recharge of the river with faster surface runoff leading to the degradation of the water quality and quantity (Mati and Mutunga, 2005).

Human development which entails land clearing, urbanization and poor waste disposal measures along the tributaries of most rivers as in the case of the Mara River has significantly degraded the biological and chemical quality of its water. The consequence of this is to trigger the occurrence of point and non-point sources of pollution which have been found in other rivers too (Yillia *et al.*, 2009). Several other studies have also shown that increased intensity of human activities such as arable farming, livestock keeping, mining, industrial activities and urban settlement adjacent to river water bodies as in the case of Mara basin often impacting negatively on the quality and quantity of water (Mokaya *et al.*, 2004). River Nyangores originates from Mau forest and flows through an area with intensive anthropogenic activities (Mati and Mutunga, 2005) (Plate 1). Human activities such as settlement, urbanization and poor farming methods have not only been perceived to be the major cause of degradation to the quality of water in this river, but also to both River Mara and Lake Victoria where water from the tributary is emptied (Dadwell, 1993). Faecal pollution to water sources is a serious threat to the quality of water with a negative impact on the integrity of aquatic ecosystems and therefore is a risk to the health of the community consuming water from such sources. It is believed that 80% of all diseases in the world are caused by inadequate sanitation, polluted water or unavailability of water (WHO,

2002). Both direct contact and consumption of water contaminated with faeces of ill individuals can lead to human illness and even death (United State Environmental Protection agency (USEPA), 1995).

To test for the microbial quality of any water source, faecal contamination indicator organisms are preferred as the approach is fast and cheap (APHA, 2005). While a variety of pathogenic indicators have been proposed, the mostly commonly used estimator of faecal pathogenic bacteria presence is faecal coliforms and faecal streptococci abundance (Dadwel, 1993, Ford and Colwell, 1996). Traditionally, indicator micro-organisms have been used to suggest the possibility of presence of pathogens (Berg and Metcalf 1978). A direct epidemiological approach could be used as an alternative or adjunct to the use of index micro-organisms. However epidemiologic methods are generally too insensitive and miss the majority of waterborne disease transmissions (Frost *et al.* 1996). Other useful indicators include intestinal enterococci and *Clostridium perfrigens*. Organic matter loading from catchment activities results in vigorous consumption of oxygen attributable to large oxygen requirement by heterotrophic microbes in oxidative degradation processes. High Biological Oxygen Demand (BOD) is experienced in such systems and oxygen deficit is greatly increased often leading to destruction of other aquatic organisms. Thus BOD_5 is used as a measure of oxygen consumption and aerobic heterotrophic activities (Rheinheimer, 1991). Inorganic nutrients (PO_4 and NO_2) from agricultural activities also affect microbial flora of streams (Yuan *et al.*, 2001).

Plate 1: Anthropogenic activities along Nyangores River; (i) Farming activities and (ii) Settlement and human waste disposal

1.2 Statement of the problem

The Mara River basin is facing various challenges posed by ever increasing anthropogenic activities ranging from destruction of its riparian vegetation and catchment areas particularly the Mau forest to poor agricultural prectices, urbanization, municipal waste disposal, and dam construction. In addition the upper catchment has been encroached up to the river bank to pave way for human settlement and inhabitation. Sanitary facilities in households along the river are inadequate or ineffective while households still defaecates on land using bush as hide sites thus discharging raw sewage into the river during rains. Therefore there is increased faecal pollution into Nyangores River through surface run-offs and underground percolation and seepage. Rapidly growing towns like Bomet Municipality with unplanned developments adjacent to the river bank and other mashrooming urban centres along the river with no sewerage treatment plants also discharge raw sewage into the River. All these activities have resulted to the degradation of the microbial water quality of the river consequently exposing the local communities which heavily depend on its water for domestic use to incidences of waterborne and water related diseases. This adds more burdens on medical cost to already impoverished citizens. This study was therefore initiated to determine the influence of human settlement and development on the microbial quality of the water within Nyangores River through the use of faecal contamination indicator organisms. The results of this study will go a long way in future management of Mara River basin by relevant authorities like Water Resource Management Authority, Kenya Forest Services, Kenya Wildlife Services and other interested stakeholders like the Nyangores Water Resource User's Association among others

Plate 2: Anthropogenic activities affecting the bacteriological quality of Nyangores river; (i) Laundry work, (ii) sewage discharge from Tenwek hospital

1.3 Objectives

1.3.1 General objective

This study was to determine the effects of anthropogenic activities on the microbial water quality of Nyangores River.

1.3.2 Specific objectives

The specific objectives for this study were to;

1. Determine the density of faecal contamination indicators along eight selected points on Nyangores River.
2. Analyse for the temporal variation in the abundance of faecal contamination indicators along Nyangores River.
3. Detect the presence of *salmonella* spp in Nyangores river water
4. Determine pollution level by easily degradable organic matter in various sites within Nyangores river

1.4 Hypotheses

1. The densities of faecal contamination indicators have no significant variation along various points within Nyangores River.
2. The density of faecal contamination indicators in water of Nyangores River does not vary significantly with respect to time.
3. *Salmonella* spp. are not in detectable concentrations in water of Nyangores river
4. There is no significant temporal and spatial variation in pollution level by easily degradable organic matter within Nyangores River.

1.5 Justification of the study

This study established the influence of human settlement and development on microbial quality of the water in river Nyangores using cheap and reliable water quality monitoring method. The results of the study gave the microbial quality status of the water that can be attributed to human settlement and development at various points along the river channel. The data obtained under this herein will also form a directive framework towards sustainable development and proper management of the entire Mara river basin. The sustainability in development will consequently enhance proper long term catchment area based solutions to the problems of pollution arising from faecal contamination from this part of Lake Victoria basin. Sustainable development will also ensure good quality and adequate water supply from the Mara basin for sustainable

ecosystem functioning and basic human need. It is therefore paramount that the bacteriological water quality was ascertained so that appropriate intervention measures on water pollution are put in place. Good water quality provision can improve on the general human health and environmental sanitation and enhance the country's economically significant sectors such as tourism, fisheries and industries.

1.6 Definition of terms

Bacteria: Are microscopic single celled organisms which have neither a membrane-bounded nucleus nor other membrane-bounded organelles like mitochondria and chloroplasts.

Clostridium perfringens: *Clostridium* spp. is a Gram-positive, and endospore forming anaerobic, sulphite-reducing bacilli.

Ecosystem: Is a complete community including both biotic and abiotic factors functioning together as group.

Thermotolerant coliforms: They are a group of bacteria capable of fermenting lactose at higher temperatures of 44-45˚C

Escherichia coli: They are gram negative rod bacteria which form a subset of the total coliform group that can ferment lactose at higher temperatures and produce indole from tryptophan or produce an enzyme β-galactosidase and β-glucuronidase.

Index organism: They are bacteria that give the actual level of pathogenic infection.

Indicator organism: Are those bacteria that indicate the presence of pathogenic bacteria in water

Total Coliform bacteria: These groups of bacteria include a wide range of aerobic and facultative anaerobic which are Gram-negative, non-spore-forming bacilli capable of growing in the presence of relatively high concentrations of bile salts with the fermentation of lactose and production of acid or aldehyde within 24 hours at temperature of between 35–37˚ C.

CHAPTER TWO
LITERATURE REVIEW

2.1 Importance of water

Water is one of the most abundant and indispensable vital substances for the life of plants and animals. There could be no life without water. The human body has been determined to consist of 60% water (European Food Safety Authority (EFSA), 2010). Water is used mainly by man for domestic purposes like drinking, bathing, cooking and washing among others. Water is also used for watering domestic and wild animals. Other purposes of water are irrigations, transportation, sewage disposal and recreation. Potable water which should meet all the set standards by WHO or by individual states cannot be judged by ordinarily looking at the colour and smell. This is due to the presence of minute micro-organisms like bacteria, viruses and fungi which cannot be seen by naked eyes, However they are commonly found in water and are more dangerous to human health and to the general environment than physical turbidity since they cause most of the known diseases on earth and this pathogenic enteric bacteria enters the environment from human or animal excreta (Dadwel, 1993). Therefore water in that sense is a good medium that can accommodate various types of pathogenic and non-pathogenic bacteria, thus it requires adequate treatment before it can be rendered fit for domestic purposes or consumption. Where no treatment is given to drinking water then appropriate protection and management of the water source should be greatly take care of this to minimize pollution or contamination of water since currently, about 20% of the world's population lacks access to safe drinking water or adequate sanitation (Hunter *et al*., 2000).

In areas where water for domestic purposes is unreliable in quality the child mortality rate is high causing more than three million deaths from diseases caused by unsafe water (WaterDome, 2002). Efforts to improve health conditions, especially by providing safe water supplies and adequate waste disposal, obviously are frustrated by poverty. Unlike in developed countries, improvement in water sanitation are usually ranked below a number of other needs by the people living in developing countries like Kenya, Tanzania and many other African countries. Those given higher priorities are education, employment opportunities, better shelter, and health. Infectious diseases in advanced economies like Europe and North America have declined drastically as results of closeness to clean water supplies and hygienic toilets and in fact at any

given time almost half of the people in developing countries suffer from water related diseases (WaterDome, 2002).

It was a desire of Kenya Government that in the year 2000 every home should be close to a source of piped water or other safe sources like boreholes and wells and that the distance moved looking for these sources should be within 1 kilometer from every homestead (Kimani and Ngindu, 2007). Most of the homes along the Mara basin wholly depend on the raw water of Mara River and its tributaries for domestic purposes and any other uses hence posing a serious challenge to their health. In September 2000 the United Nations General Assembly adopted The Millennium Goals in which Goal no. 7 Target 21 called for the world community to halve by the year 2015 the proportion of people who are unable to reach or afford safe drinking water. In Ministerial statement from the water forum at the Hague-Netherland, called for efforts to guarantee "that every person has access to enough safe water at an affordable cost to lead a healthy and productive life and that the vulnerable are protected from the risks of water related health hazards. Additional actions or efforts should be taken to accelerate the rate at which access to safe water is provided considering that 2015 is drawing closer. There is need to carry out an integrated water resource management for the benefit of all stakeholders (Thomas and Durham, 2003).

2.2 Effects of anthropogenic activities on water quality

Anthropogenic activities such as access by people and livestock to surface water systems are common in developing countries, particularly in poor rural communities where most residents lack access to portable clean water. As a result, they usually obtain water for their daily needs from surface water systems that are often contaminated (Nevondo and Cloete, 1999; Venter *et al*., 2001 Obi *et al*., 2002). Visits are periodic and frequent, especially during dry periods when other sources of water such as piped water, rainwater or groundwater are irregular, lacking or inaccessible. During visits, livestock may be watered, people bath or swim, waste is disposed off, clothes and vehicles may be washed and water is usually abstracted for domestic needs (Nevondo and Cloete, 1999; Mathooko et al., 2001;Yillia *et al*., 2009;). Since these activities occur largely within and beside stream channels, they are collectively called in-stream activities and could constitute a major source of diffuse pollution, especially in shallow water systems. Specifically, in-stream activities may influence microbial water quality as faecal matter is

deposited of during visits and the surrounding area is usually littered with faeces (Fatoki and Madhabadha, 2001; Zamxaka *et al*., 2004).

Unsanitary means of disposing human waste and faecal droppings from livestock are routes through which faecal matter may enter aquatic systems. Faecal matter degrades water quality due to the possible introduction of pathogens, nutrients and organic matter (Vinneras *et al*., 2003; Langergraber and Muellergger, 2005; Vikaskumar *et al*., 2007). Degraded water quality may result in increase in cost of drinking water treatment or loss of opportunities for recreation, aquaculture and fishing (Sinton *et al*., 1998; Parveen, *et al*., 2001; Ebdon *et al*., 2007; Edge and Hill 2007). Prominently, pollution with faecal matter may present significant health risk to the public (Sinton *et al*., 1998; Byamukama *et al*., 2005). The level of risk will depend considerably on the origin and level of contamination (Scott *et al*., 2002). In particular, contamination from human excreta is of greater risk to public health as it is more likely to contain human-specific enteric pathogens although reliable epidemiological evidence is lacking (Sinton *et al*., 1998). To minimize health risk, it is often required to undertake regular monitoring of indicator parameters in aquatic systems (Kong *et al*., 2002; Wheeler *et al*., 2002; McLellan and Salmore, 2003; Noble *et al*., 2003; Shah *et al*., 2007). Such assessment studies are useful not only for evaluating health risk, but also for determining the course of action that may be required to solve the problem (Parveen *et al*., 2001; Ahmed *et al*., 2007; Graves *et al*., 2007). Other out-stream anthropogenic activities include; industrial sources e.g. pulp and paper mills, chemical manufacturers, steel plants, metal process and product manufacturers, textile manufacturers and food processing plants, municipal sewage treatment plants, urbanization, agricultural activities e.g. crop production, pastures, rangeland, feedlots, other animal holding areas, silviculture, deforestation, construction, mining, channelization, dredging, dam construction and stream bank modification, (Amy *et al.*, 2003). All these pollution activities are occurring along Nyangores River and have might impacted the environment negatively hence further contributing to eutrophication of the receiving water body, Lake Victoria. This has eventually degraded the quality of the lake water and greatly led to the diminishing of the economically significant fisheries resources within the lake.

2.3 Waterborne diseases

Waterborne Diseases (WBDs) are those diseases which generally arise from contamination of water by human or animal faeces or urine infected by pathogenic viruses or bacteria and are

directly transmitted when unsafe water is drunk or used in food preparation (WHO 2002). Like other diseases, WBDs also have a negative effect on the socio-economic development (Wilconsin, 1993; Patricia *et al*., 2009).

Table 1: Waterborne and water related Diseases (WBDs)

Category of causative agent	**Diseases**	**Causative Organism**	**Common transmission Route**
Bacterial	Shigellosis	*Shigella species*	Man-feces-(flies) water and food – man
	Typhoid fever	*Salmonella typhi and paratyphi*	Man-feces-food and water – man
	Cholera	*Vibrio cholera*	Man-feces-water and food – man
	Acute Gastroenteritis	*E. coli*	Man-feces-water-Man
Viral	Infectious hepatitis	*Hepatitis A virus Hepatitis E virus*	Man-feces-water and food – man
	Poliomyelitis	Polio virus	Man-feces-water –Man
	Acute Gastroenteritis	Rota Virus	Man-feces-water-Man
Protozoan	Amoebiasis	*Entameoba hystolitica*	Man-feces-water and food – man
	Giardiasis	*Giardia lamblia*	Man-feces-water and food –man
	Cryptosporidiosis	*Cryptosporidium parvum*	Man-feces-water and food – man
Helminths	Dracunculiasis (Guinea Worm)	*Dracunculus medinesis*	Man-water-man

Source: Adopted and modified from EPHTI Report, 2003

2.4 Faecal contamination pathways of water sources

All waterborne pathogens also called 'pathogenic micro-organisms' are transmitted to humans through drinking or otherwise ingesting contaminated water, either directly or via food or poor hygiene. During heavy rain, many of these pathogenic microorganisms can be washed into waterways, increasing the risk of getting into water supplies. Therefore, the keys to breaking the process of transmission of these pathogenic microorganisms are to protect the water source by preventing contamination from occurring, protect the population from ingesting contaminated water by treating a contaminated supply and limiting its use and inform the community if something goes wrong (Ministry of Health New Zealand, 2007).

2.4.1 Transmission Routes for WBDs

Water-borne transmission occurs when pathogens in faeces from an infected person or from a healthy carrier are discharged into water and when a susceptible person consumes this water or contaminated food, infection occurs through faecal oral route. This may lead to cholera, typhoid, infectious hepatitis and dysentery among others (Rabie and Curtis 2006).

2.4.2 Water washed route

Water-washed disease is defined as one whose transmission will be reduced following an increase in volume of water used for hygienic purposes, irrespective of its quality. They depend on quantity of water used rather than its quality and this lead to many infections of intestinal tract which include diarrheal diseases. This also causes infection of eyes like trachoma and skin infections such as bacterial skin sepsis, scabies as well as fungal infection (Rabie and Curtis 2006).

2.4.3 Water based route

A water based disease is one in which the vector of pathogens spend a part of its cycle in a water snail or other animals. These diseases are due to infection by parasitic worms (helminthes) which spend part of life cycle on aquatic intermediate hosts to complete their life cycles (Rabie and Curtis 2006).

2.5 Historical development in the use of contamination indicator organisms

The use of bacteria as indicators of the sanitary quality of water dates back to 1880 when Von Fritsch described *Klebsiellapneumoniae* and *Krhinoscleromatis* as micro-organisms characteristically found in human faeces (Geldreich, 1978). In 1891, the Franklands came up with the concept that organisms characteristic of sewage must be identified to provide evidence of potentially dangerous pollution (Hutchinson and Ridgway, 1977). The concept of 'coliform' bacteria was in use in Britain in 1901.Sanitary significance of finding various coliforms along with streptococci and *C. perfringens* was recognized by bacteriologists by the start of the twentieth century (Hutchinson and Ridgway, 1977). Water sanitary engineers, however, require simple and rapid methods for the detection of faecal indicator bacteria and as such standard methods for bacterial water quality analysis have since been described for use in many parts of the world (APHA, 2005; WHO, 2002). One of the first generally accepted methods for coliforms was called the Multiple-Tube Fermentation Test.

2.6 Importance and characteristics of indicator organisms in water quality assessment

A complete epidemiological investigation is normally expensive and time consuming, public health authorities and water quality managers are inclined to follow the Observed Adverse Effect level (OAEL) approach, which is usually integrated into the routine water quality monitoring programme. OAEL requires testing the water for the presence of a preferential faecal indicator bacterium at the point of exposure, usually a recreational site or a water source. When the indicator bacterium is present in excess of referential water quality guidelines and the tolerable risk threshold is breached, a sanitary survey is executed to detect faecal sources (WHO, 2002).

The concept of using indicator organisms as signals of faecal pollution is a well-established practice in the assessment of drinking-water quality (Noble *et al*., 2003). The organisms used as indicators and index organisms must have the following qualities; need not to be pathogenic, should be universally present in faeces of humans and animals in large numbers, should not multiply in natural waters and should persist in water in a similar manner to faecal pathogens. They should also be present in higher numbers than faecal pathogens and respond to treatment processes in a similar fashion to faecal pathogens and be readily detected by simple inexpensive method (Jay, 1992).

The density of pathogenic micro-organisms is smaller in number compared to non-pathogenic organisms which interferes with detection of pathogenic micro-organisms. It is quite tedious to examine pathogenic micro-organisms and is of no use since it takes a lot of days before conclusive and meaningful results are got and by that time water would have already been consumed by clients which necessitated the use of non-pathogenic micro-organisms to determine the extent of the water pollution.

2.7 Microbial indicators of faecal pollution

The common bacteria indicators currently in use are total coliforms (TC), faecal coliforms (FC), faecal streptococci (FS), *Escherichia coli* (EC) and intestinal enterococci (IE). Most regulatory agencies are interested in EC and IE given that they correlate well with the rate of gastrointestinal illnesses in recreational waters (Cabelli *et al*., 1982; Cabelli *et al.*, 1983; Dufour, 1984; Fattal *et al*., 1986; Medema *et al*., 1997; Haile *et al*., 1999; Turbow *et al*., 2003; Wade *et al*., 2003; Dwight *et al*., 2004). This correlation has been useful for the development of microbial water quality guidelines. Nonetheless, the ideal is to validate appropriate index organisms by way of epidemiological studies. A good example is the emerging use of an enterococci guideline for recreational water quality (World Water Forum, 1998). Often epidemiologic studies fail to show any relationship to microbial indicators, due to poor design (Fleisher, 1990) and/or due to the widely fluctuating ratio of pathogen(s) to faecal indicators and the varying virulence of the pathogens. The validity of any indicator system is also affected by the relative rates of removal and destruction of the indicator versus the target hazard. The differences due to environmental resistance or even ability to multiply in the environment will therefore influence their usefulness. Similarly, viral, bacterial, parasitic protozoan and helminthes pathogens are unlikely to behave in the same way as a single indicator group, and certainly not in all situations. Moreover, viruses and other pathogens are not part of the normal faecal microbiota, but are only excreted by infected individuals and the higher the number of people contributing to sewage or faecal contamination, the more likely the presence of a range of pathogens. The occurrence of specific pathogens varies further according to their seasonal occurrence (Berg and Metcalf, 1978).

A guideline value stipulates a theoretical health safety limit that is often associated with the maximum acceptable health risk. It is usually the tolerable concentration of an indicator rather

than the detectable harmful dose of infectious pathogens (Steyn *et al.*, 2005). The choice of indicator over pathogen is largely due to methodological problems (Cabelli, 1983). A lot of time and resources may be needed to adequately detect any type of pathogen and because they are diverse and occur in low numbers in the environment, large errors and costs may be incurred in sampling and enumeration (Kong *et al.*, 2005). Also, many pathogenic bacteria could be described as viable but non-culturable and the densities of most pathogens in environmental waters are unpredictable (Cabelli *et al.*, 1983; Kong *et al*., 2005). As a result, bacteria indicators such as EC and IE are used during routine microbiological assessment. However, both indicators have been criticized for not being representative enough, especially, for viral and protozoan pathogens and may be present where bacteria indicators are shown to be absent (Barrell *et al*., 2000).

2.8 Microbial risk assessment methods in water quality monitoring

Exposure to contaminated water has been associated with illnesses such as gastroenteritis and infections of the skin, eyes, ears, nose and throat (Cabelli *et al*., 1982; Cabelli *et al.*, 1983; Dufour, 1984). The threat of microbial pollution-related illnesses is predictable with microbial risk assessment (MRA). In particular, MRA can function as a valuable tool for risk identification and management in situations where epidemiological investigations are lacking (Gibson *et al*., 2002; Westrell *et al*., 2004). With the observed adverse effect level (OAEL) approach, the level of faecal contamination is indicated by the presence of an indicator organism (Steyn *et al.*, 2004). A negative health effect can be expected if the indicator is present and the level of risk increases with increase in the indicator density (Wade *et al*., 2003).

2.9 *Salmonella typhi*

This is a gram-negative pathogenic enteric bacillus belonging to the family *Enterobacteriaceae*. It is a motile, facultative anaerobe that is susceptible to various antibiotics. It is responsible for enteric fever or typhoid disease in man and other animals. Diagnostic identification can be attained by growth on MacConkey and EMB agars, and the bacteria is strictly non-lactose fermenting. It also produces no gas when grown in TSI media, which is used to differentiate it from other *Enterobacteriaceae* (Kelly *et al.*, 1999). Infection of *S. typhi* leads to the development of typhoid, or enteric fever. The encounter of humans to *S. typhi* is made via fecal-oral route from infected individuals to healthy ones. Poor hygiene of patients shedding the organism can

lead to secondary infection, as well as consumption of water contaminated with the pathogen. The most common source of infection, however, is drinking water tainted by urine and feces of infected individuals. The entry of this bacterial species into the human body is most commonly achieved by ingestion (Al-Wasify *et al.,* 2011). Once ingested, the organisms multiply in the small intestine over the period of 1-3 weeks, breech the intestinal wall, and spread to other organ systems and tissues. Transmission of *S. typhi* has only been shown to occur by fecal-oral route (Nye *et al.,* 2002).

2.10 Commonly used methods for bacteriological water testing

2.10.1 Membrane filtration technique

This is a rapid and quantitative method for detection of indicator bacteria. It has been described in detail in (APHA 2005). A summary of method description is presented here. Thin membrane filters with a pore size that will retain bacteria but allow water or diluents to pass through are used. Following the collection of bacteria upon filtering a given volume, the membrane is placed on an agar plate or an absorbent pad saturated with culture medium of choice, and incubated appropriately and after growth, colonies are enumerated to give colony forming units (CFU, s) per 100ml. The method offers ability to work with flexible sample volume range enabling the use of large sample volume and therefore increased sensitivity. When confirmation is needed, isolation from well separated colonies on membrane is easy. On the other hand it causes some difficulties as quality of membranes varies, solid particles and chemicals adsorbed from sample to the membrane during filtration may interfere with the growth of the target organism, not applicable to turbid samples and scoring of typical colonies not always easy as well as the expensive cost of membrane and filtration units.

2.10.2 Most probable number (MPN)

The method consists of inoculating a series of tubes with appropriate decimal dilutions of the sample. Production of gas, acid formation or abundant growth in the test tube after a certain period of time incubation at 35^0C constitutes a positive presumptive reaction. Both lactose and LauryTryptose broths can be used as presumptive media. All tubes with positive presumptive reaction are subsequently subjected to a confirmation test. The formation of gas in a Brilliant Green Lactose Bile (BGLB) broth fermentation tube at any time within 48 hours at 35^0C constitutes a positive confirmation test. A test using an EC medium can be applied to determine

TC that is FC (APHA 2005): the production of gas after 24 hours of incubation at 44.5^{0}C in an EC broth medium is considered a positive result (Rompré *et al*., 2002).

2.10.3 Other methods for detection of faecal contamination

Faecal contamination organisms can also be detected by other methods. One of these methods is the addition of fluorogenic and chromogenic substrates to cultivation media (agar and liquid) to detect enzymatic activities of TC and *E. coli* (Deere *et al*., 2002). This method has increased the sensitivity of the classical methods for estimating the microbial contamination of drinking water. In addition, the flow cytometry which is a technology in which particles are made to flow one at a time through a light beam (laser beam) in a sensing region of a flow chamber can be used for detecting non-culturable cells. As particles flows through the beam, both light scattered by the particles and fluorescence light from the labeled particles are collected. This is done either by a photomultiplier or photodiode in combination with light splitters (dicroic mirrors) and filters (Vesey *et al.,* 1994). Molecular methods have also been developed to increase the rapidity of analysis. They are able to achieve high degree of sensitivity and specificity without the need for a complex cultivation and additional confirmation tests, (Rosina *et al.,* 2010; Rompre *et al.,* 2002).

2.10.4 Source tracking for faecal contamination

Faecal contamination sources are numerous. Some of the possible human sources include effluents from sewage treatment plants and faulty on-site septic systems. Equal important sources are the non-human sources of pollution such as domestic animals which also contribute to the contamination of surface water. Surface runoff from animal grazing areas and manure-treated agricultural land for example can find its way into nearby bodies of water. Other domesticated animals including cats and dogs must also be considered possible sources. Finally, wildlife can contribute significant amounts of faecal contamination to otherwise pristine environments that are minimally impacted by humans (Weaver *et al.,* 2005). Several methods of source tracking of faecal contamination have been proposed, and the strengths and weaknesses of these methods have been reviewed (Scott *et al.,* 2002). These methods are grouped into; non molecular library independent methods, molecular library dependent methods, molecular library independent methods and chemical methods. Non molecular library independent methods include the use of faecal bacterial ratios and non-molecular host specific indicators. Molecular

library dependent methods are repetitive polymerase chain reaction, pulse field gel electrophoresis, ribotyping and randomly amplified polymorphic DNA. Molecular library independent methods are bacteriophage indicators and virus (human pathogen) indicators (Simpson *et al.,* 2002).

Faecal bacterial ratios is one of the techniques developed in the source tracking field and is mainly based on the ratios of faecal coliform (*E. coli*) to fecal streptococci (intestinal enterococci) (FC:FS). In this method CFUs (Colony Forming Units) of faecal coliforms and faecal streptococci are counted on plates and a ratio between the two is determined. A ratio of more than 4 is considered human contamination and a ratio of less than 0.7 suggests non-human sources. Die-off rates are monitored through time and the change in the FC:FS ratio is then used to further interpret possible sources. FC:FS ratios is still useful as a general indicator of human verses non-human faecal bacterial contamination (Weaver *et al.,* 2005).

2.11 Summary

This study has shown the importance of water and how its quality affect community health status. Various bacteriological water quality testing techniques have been explored on various domestic water sources. However, the quality of Nyangores river is one of the water sources whose bacteriological quality has not been adequately studied despite the impact it may have on the people, hence the basis for this study.

2.12 Conceptual framework

The state of water is determined by natural factors such as geology and climate and also by the pressures exerted by human activities (Fig 1). Many of the pressures and the underlying driving forces are common to all or a number of the issues. For example, agriculture, human settlement and industrialization are significant driving forces in terms of ecological quality, nutrient and organic pollution, hazardous substances and water quantity. The aim of managing water resources is to safeguard human health while sustainably managing aquatic and associated terrestrial ecosystems. It is, therefore, important to quantify and identify the current state of, and impacts on water environment and how these are changing with time. In water assessment at global, regional, national and by river basins level, is important to put into consideration the following generic levels; Driving forces which are the needs for human survival, Pressure on

water to meet human needs, State of the water quality, Impact on the water quality state due to change in physical, chemical and biological state of environment and Response by relevant parties due to undesired impacts (DPSIR). The DPSIR model can be used as an analytical framework for assessing water quality and quantity issues. This allows for a comprehensive assessment through examination of the relevant generic forces.

Independent Variables	**Intervening Variables**	**Dependent Variable**
Driving forces	Responses &pressure	Impacts & State

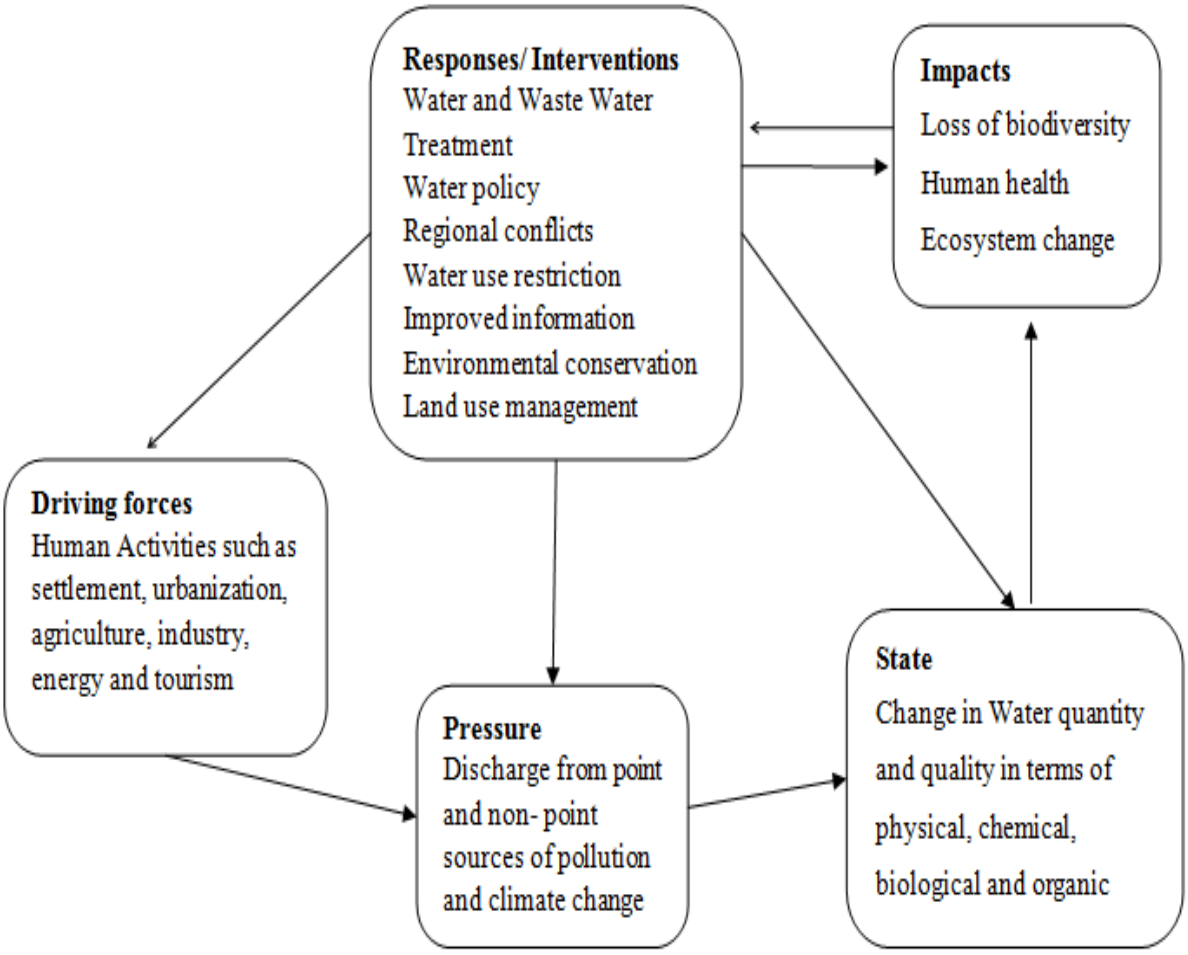

Figure 1: Conceptual framework on water quality management

Source: Adapted and modified from National Environmental Research Institute, Denmark Department of Policy Analysis-2004

CHAPTER THREE
MATERIALS AND METHODS

3.1 The study area

The Mara River is located in Mau escarpment with its source mainly in Kenya and flow through Tanzania and drains into Lake Victoria at Musoma bay. The Mara River covers a surface area of 13,504km^2 and distributed in the two countries in proportion of 60% Kenyan and 40% Tanzania. The basin is located between longitudes 33.88372^0 and 35.907682^0 West, latitude -0.331573^0 and -1.975056^0 South (Mati and Mutunga, 2005).The major catchment is Mau-Complex Forest which borders Nakuru, Kericho, Bomet, and Narok Counties in Kenya and flows for a distance of 395 Km. The basin is characterized by different land cover and land uses. Some of the land uses include urban settlement and villages, subsistence and large scale agriculture, forestry, livestock, fisheries, tourism, conservation areas and mining.

The main tributaries are Amala and Nyangores rivers which are both facing a serious threat as a result of change in land use for various purposes like settlement, urban development among others in the catchment. This has led to drastically reduced forest cover which has hampered the recharge of the river with faster surface runoff leading to water pollution. The river meanders through open savannah grasslands that are mostly governed by Maasai group ranches and eventually into Maasai Mara National Reserve and Serengeti National and finally drains into Lake Victoria in Tanzania (Mati and Mutunga, 2005).

This study mainly focused on Nyangores River from the source where pollution was not or least expected to the confluence of Amala River (Fig 2). Eight sampling stations were systematically established (Table 2), in which point and non- point sources of pollution were considered for sampling. Also included was non polluted sources and the intensity of anthropogenic activities giving eight sampling stations as follows; Site 1 at the source before forested portion near Kiptagich (S 00° 34′ 55.8″, E 035° 36′ 13.0″), Site 2 located at at Masese point (S 00° 42′ 25.7″, E 035° 25′ 24.5″) Site 3 located at the upstream of Tenwek Mission hospital (S 00° 44′ 15.9″ E 035° 21′ 45.2″), Site 4 located at the downstream of Tenwek Mission hospital (S 00° 44′ 53.2″ E 035° 21′ 52.8″), Site 5 located at the upstream of Bomet municipality at Raiya village (S 00° 46′ 30.6″, E035° 21′ 05.0″), Site 6 located at the downstream of Bomet Municipality near St.

Michael secondary school (S 00° 47′ 44.8″, E 035° 20′ 18.5″), Site 7 located at Olbutio trading centre at the bridge (S 00° 51′ 30.0″, E 035° 16′ 44.8″) and finally Site 8 located after the confluence of Amala and Nyangores river at Tuiyobei (S 00° 56′ 14.6″ , E 035° 14′ 30.4″).

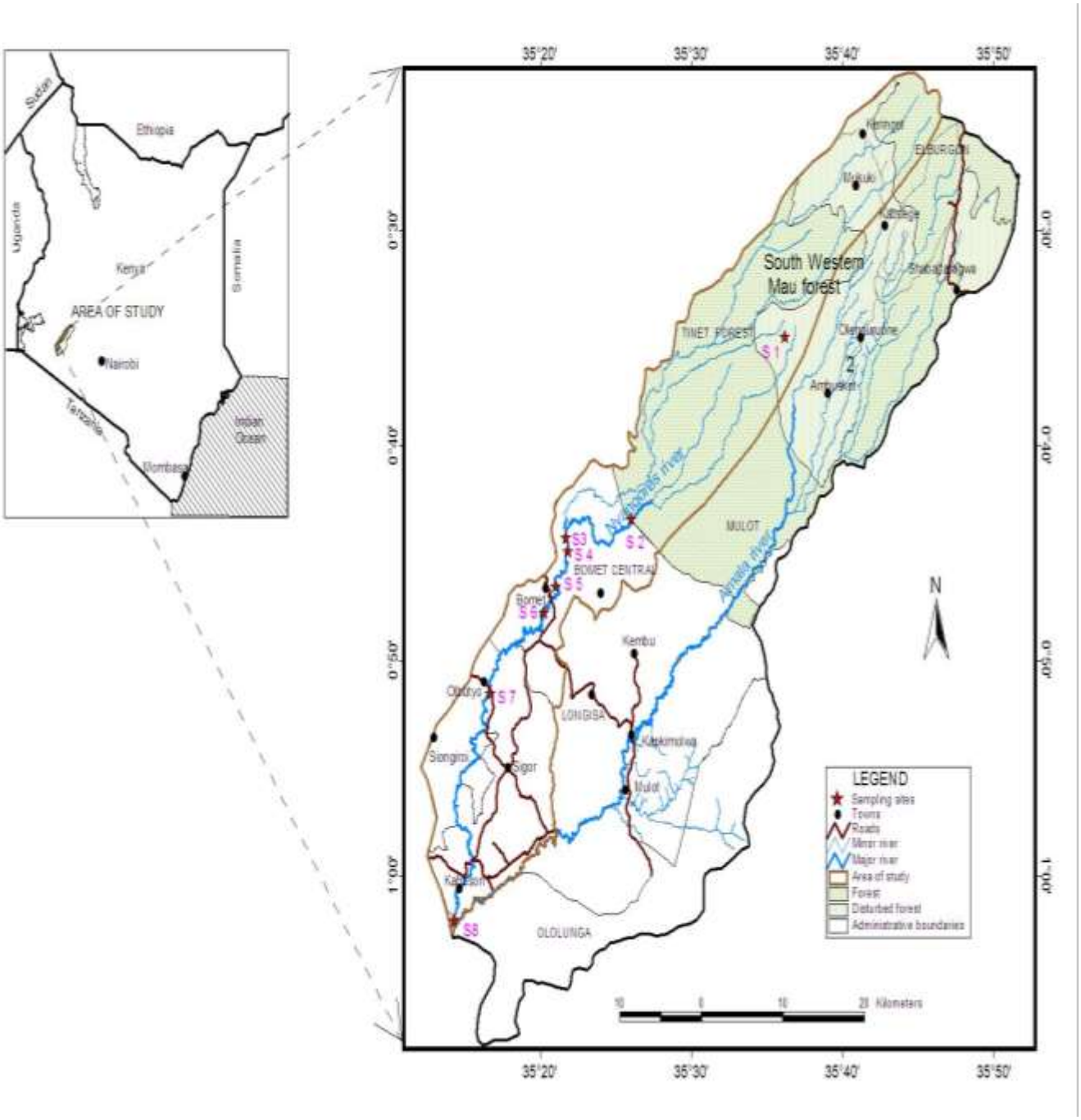

Figure 2: Map of the study area showing upper Mara basin (Constructed in the year 2011 by Geofrey Maina- Senior GIS Technologist at Environmental Science Department of Egerton University).

Table 2: Description of study sites

SITES	LOCATION/ELEVATION	DESCRIPTION
1	S 00° 34′ 55.8″, E 035° 36′ 13.0″ and 2441 m	Kiptagich at eye of the spring source. The vegetation cover is bamboo and still very intact. Canopy cover is good. The surrounding community has preserved this source and water is tapped directly from the spring eye. This area is ecological zone 2 characterized by bamboo vegetation. Major farming activities are tea plantation and dairy keeping.
2	S 00° 42′ 25.7″, E 035° 25′ 24.5″ and 2070m	Masese at about1 km inside the forest. There is little disturbance of the forest by adjacent community. The canopy cover is good and composes of large indigenous tree spp. This site is zone 2. Plantation of tea and maize mixed with dairy keeping.
3	S 00° 44′ 15.9″ E 035° 21′ 45.2″ and 1953m	Tenwek Hospital upstream. The canopy cover is poor as community surrounding has cleared the vegetation to pave way for agricultural activities like maize. Car washing, bathing and clothe washing are being exercised inside the river channel. This is zone 3.
4	S 00° 44′ 53.2″ E 035° 21′ 52.8″ and 1929m	Tenwek Hospital downstream. The Hospital located waste water treatment lagoons at the bank of the river plate 2 (ii). Also close to the river is an open dumping site. At this site water is dammed to generate electricity to serve the hospital. This is zone 3. Maize plantation continues dominating in this study area.
5	S 00° 46′ 30.6″, E035° 21′ 05.0″ and 1940m	Bomet Town upstream at Raiya area. This is densely populated slum site. Methods of human waste disposal are pit latrines and bush which leaves the human faeces scattered all over. Riparian vegetation has been cleared up to the river bank. Bathing and washing takes place too here. This is ecological zone 3.
6	S 00° 47′ 44.8″, E 035° 20′ 18.5″ and 1916m	Bomet Town downstream at St. Michael's secondary. There is an open dumpsite close to the river used by Bomet municipal as solid waste dump site. Open waste water and storm water channels from the town drain to the river at this site. Zone 3
7	S 00° 51′ 30.0″, E 035° 16′ 44.8″ and 1855m	Olbutio shopping centre at the bridge. It is sparsely settled area and mainly livestock farming is being practice. The area is moderately dry with no canopy cover and is transition zone between 3 and 4.
8	S 00° 56′ 14.6″ , E 035° 14′ 30.4″ and 1805m	Confluence of Amala and Nyangores at Tuiyobei. This is the site where the two rivers join to form Mara river. There is mixing of Waters from the two rivers. This area is dry and main activities are livestock rearing. This is zone 4 characterized by cactus and acacia spp

3.2 Sampling

Water samples were collected in triplicates from the eight selected sites within the river as indicated in the study area. Sampling was conducted two times a month from each station for six months (February to June) covering both wet and dry months (Appendix 2) during the year 2012. The dry season was on the months of February and March while wet season was on the months of April, May and June. Sterilized glass sample bottles were used to collect samples from the river 30 centimetres below the surface and at the middle of the river channel. The following physico-chemical parameters were measured on site; Temperature, pH, Electrical Conductivity (EC), Total Dissolved Solutes (TDS) by use of H1 991301 portable pH/EC/TDS/Temp meter. Dissolved Oxygen (DO) was measured by the use of H1 9143 microprocessor and also Turbidity was measured using 2100 isoTurbidimeter. These were all measured *in- situ* at the time of sampling from each site. All samples were marked appropriately, placed in a cool box packed with ice and transported to Ministry of Water and Irrigation regional laboratory in Nakuru town, Kenya for analysis.

Plate 3: Sampling sites along Nyangores River; i & ii Site 1, iii Site 2 and iv Site 8.

3.3 Sample analysis

Analysis of microbiological parameters was conducted according to guidelines outlined in APHA (2005) and Scott *et al.*, (2002). This was done within 6-24 hours after sampling to avoid changes of the bacteria count due to growth or die off. To avoid contamination during sampling and analysis aseptic technique involving complete sterilization of the working area and equipment was strictly observed at all the stages. Methods of analysis involved the use of Heterotrophic Plate Count (HPC) procedure to estimate the number of live heterotrophic bacteria. Membrane Filtration Technique (MFT) was also used in the analysis of samples for the presence of indicator organisms. The nutrients and selective media were prepared in advance for each procedure as per the manufacturer's instruction and kept in a refrigerator at 4˚C apart from

HPC which was prepared afresh. Serial dilutions of samples were also made as appropriate for each test depending on the water source (APHA, 2005).

3.3.1 Heterotrophic plate count (HPC) procedure

One ml of each sample or dilutions of it was placed onto 80mm diameter plates and mixed with molten plate count agar and incubated at 37˚C for 48 hours in triplicates. Counting was made on the dilution with plates containing 30 to 300 colonies. The numbers of HPC bacteria per ml of water sample were estimated by multiplying mean numbers of CFU's recorded with the reciprocal of the dilution.

3.3.2 Membrane filtration technique

Aseptic filtration was done separately for each dilution. Sterile funnel was detached, a sterile filter membrane placed on sterile filtration device using a pair of forceps, flamed for a short while and the funnel reattached. A known volume of water and or its dilutions where necessary were filtered starting with the highest to the lowest dilution. After sucking off the whole samples, the tap or pump was turned off and the funnel detached. The membrane filter was taken off using a pair of sterilized forceps and placed on the surface of the corresponding culture media. For total coliforms and *E. coli* counts, filters were placed onto chromocult agar plates and incubated at 37°C for 18-24 hours. Typical colonies appearing pink and dark blue were counted as total coliforms. For *E. coli* only blue colonies were counted on the same plate as for total coliforms. For all colonies forming units (CFU) counted, total numbers per 100ml was expressed as; No/100ml = (CFU's × Dilution/Volume filtered) × 100 (APHA 2005).For intestinal enterococci counts, filters were placed onto enterococci agar (Merck) plates and incubated at 44˚C for 24-48 hours. Typical colonies appearing pink were counted as intestinal enterococci. For all colonies forming units (CFU) counted, total numbers per 100ml were expressed as; No/100ml = (CFU's × Dilution/Volume filtered) × 100 (APHA, 2005).

For *C. perfringens* counts, filters were placed onto Tryptose Sulphite Cyclocerine (TSC) agar plates. The plates were then placed in an anaerobic jar with an anaerocult strip and incubated at 44˚C for 18-24 hours. Black fluorescent counts of *C. perfringens* were then observed under 360 nm UV light. For all colonies forming units (CFU) counted, total numbers per 100ml were expressed as; No/100ml = (CFU's × Dilution/Volume filtered) × 100 as stipulated in (APHA, 2005).

For *Salmonella typhi* filters were placed on HiCrome ™ Salmonella agar improved plates and incubated at 37°C for 24 hours. Typical colonies appearing light pink were identified and recorded (Al-Wasify *et al.*, 2011; APHA, 2005).

3.4 Measurement of Biochemical Oxygen Demand

Samples were collected in 250ml aluminium foil-coated BOD bottles and transported to the laboratory for BOD analysis using BOD OxiTop® meter (Yuan *et al.*, 2001). 450 mls of sample was put into dark BOD bottles with magnetic stirrer. Two pellets of sodium hydroxide were placed in the bottles and tightly corked. They were then put into BOD meter and incubated at 20°C for 5 days. After which the BOD_5 results were obtained directly from the metre reading.

3.5 Tracking of faecal contamination sources

Faecal contamination source identification was achieved through *E. coli*: intestinal streptococci ratio determination based on their CFU values. It was based on the knowledge that a ratio of above 4 was to indicate high levels of human faecal contamination while below 0.7 was to indicate contamination by faeces from non-human sources (Scott *et al.*, 2002).

3.6 Rainfall data

Daily rainfall data was obtained from Metrological weather station based at Bomet where raingauge was used in measuring the rainfall values in milliliters.

3.7 Data analysis

Data analysis was done using Statistical Package for Social Sciences (SPSS) version 17 software. In all the analysis, 95% level of significance was used as the critical point ($P = <0.05$). The collected data on the density of indicator organisms and HPC from the water sources were statistically analysed. Data on physicochemical parameters (pH, Temperature, DO, TDS, Turbidity, BOD, and Conductivity) were also computed for all the water sources to calculate their mean values. One way Analysis of variance (ANOVA) was used to compare means recorded at different sampling sites and in different months for various variables. The means were separated using Least Significance difference (LSD) as the *post hoc* test.

CHAPTER FOUR

RESULTS

4.1 Physical and chemical parameters

Physicochemical parameter values recorded in this study are represented in Table 3 and Fig. 3. Temperature values had a range of between 12.4 °C at site 4 to 23.3 °C at site 7. Sites 1 and 2 furthermost upstream had lower values as compared to the other sites (Sites 3 to 8). Temperature values showed significant spatial variation, F= 21.239, P=0.001 and df= 7,239. Dissolved Oxygen (DO) values ranged between 4.6 to 9.2 mg/l with Site 7 recording the highest mean value but there was no significant variation between sites, F=1.752 at P=0.098 and df= 7,239. The values recorded for pH ranged between 5.0 to 7.9 and site 1 recording the lowest mean value. These values for pH showed significant variation with respect to sites, F= 90.769 at P=0.001 and df= 7,239. Total dissolved solids (TDS) had values ranging between 0.0 to 400.0 ppm, Sites 1 recorded zero reading for the mean value while Sites 4, 5 and 6 recorded the highest mean values. TDS also showed significant spatial variation, F=30.560 at P=0.001 and df= 7,239. Turbidity had values ranging between 5.7 to 147.7 NTUs, Site 1 recorded the lowest mean value while Sites 5, 6 and 7 recorded the highest mean values, with significant spatial variations, F= 6.893 at P= 0.001 and df = 7, 239. Electrical Conductivity values ranged between 100 to 900 µs/cm with Sites 3, 4, 5 and 6 recording the highest mean values.

Table 3: Mean values for physicochemical parameters recorded at different study sites on Nyangores River.

	TEMPERATURE (°C)		DO (mg/l)		pH	TDS (ppm)		TURBIDITY (NTU)		CONDUCTIVITY (µs/cm)	
SITES	Mean±SE	Range	Mean±SE	Range	Mean±SE	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range
1	16.7±0.1	15.8-17.8	6.3±0.2	4.7-9.2	5.4±0.0	0.0±0.0	0.0-0.0	6.8±0.1	5.7-7.9	153.0±13.3	100.0-305.0
2	14.9±0.2	12.6-16.1	7.2±0.2	6.0-9.0	6.8±0.1	39.5±7.2	0.0-100.0	41.1±4.8	7.5-7.9	284.4±20.8	109.0-500.0
3	20.2±0.4	15.8-22.8	6.9±0.2	4.6-8.6	7.1±0.1	210.3±20.6	100.0-400.0	71.1±7.2	19.0-130.4	517.2±36.3	270.0-880.0
4	18.7±0.5	17.9-21.7	6.7±0.2	4.6-8.9	7.0±0.1	268.434.8	90.0-100.0	92.0±6.1	21.2-134.1	538.4±42.3	240.0-804.0
5	18.4±0.7	12.4-21.2	6.5±0.2	5.3-8.0	6.8±0.0	245.7±18.9	100.0-400.0	110.5±8.3	26.6-187.5	715.9±190.0	230.0-616.0
6	18.9±0.4	14.9-21.2	6.8±0.2	5.0-8.6	6.5±0.1	256.7±19.1	100.0-400.0	125.6±16.7	12.3-369.0	554.0±34.6	280.0-800.0
7	19.6±0.4	15.5-23.3	7.3±2.0	6.0-9.2	7.1±0.1	232.3±21.9	100.0-400.0	147.7±44.3	22.8-1414.7	541.7±46.7	240.0-900.0
8	20.2±0.2	17.7-21.6	7.6±0.1	6.7-8.7	7.3±0.1	107.0±3.8	80.0-160.0	112.6±8.3	21.5-180.7	305.5±19.3	204.0-515.0

DO (dissolved Oxygen), TDS (Total Dissolved Solids) and n=30.

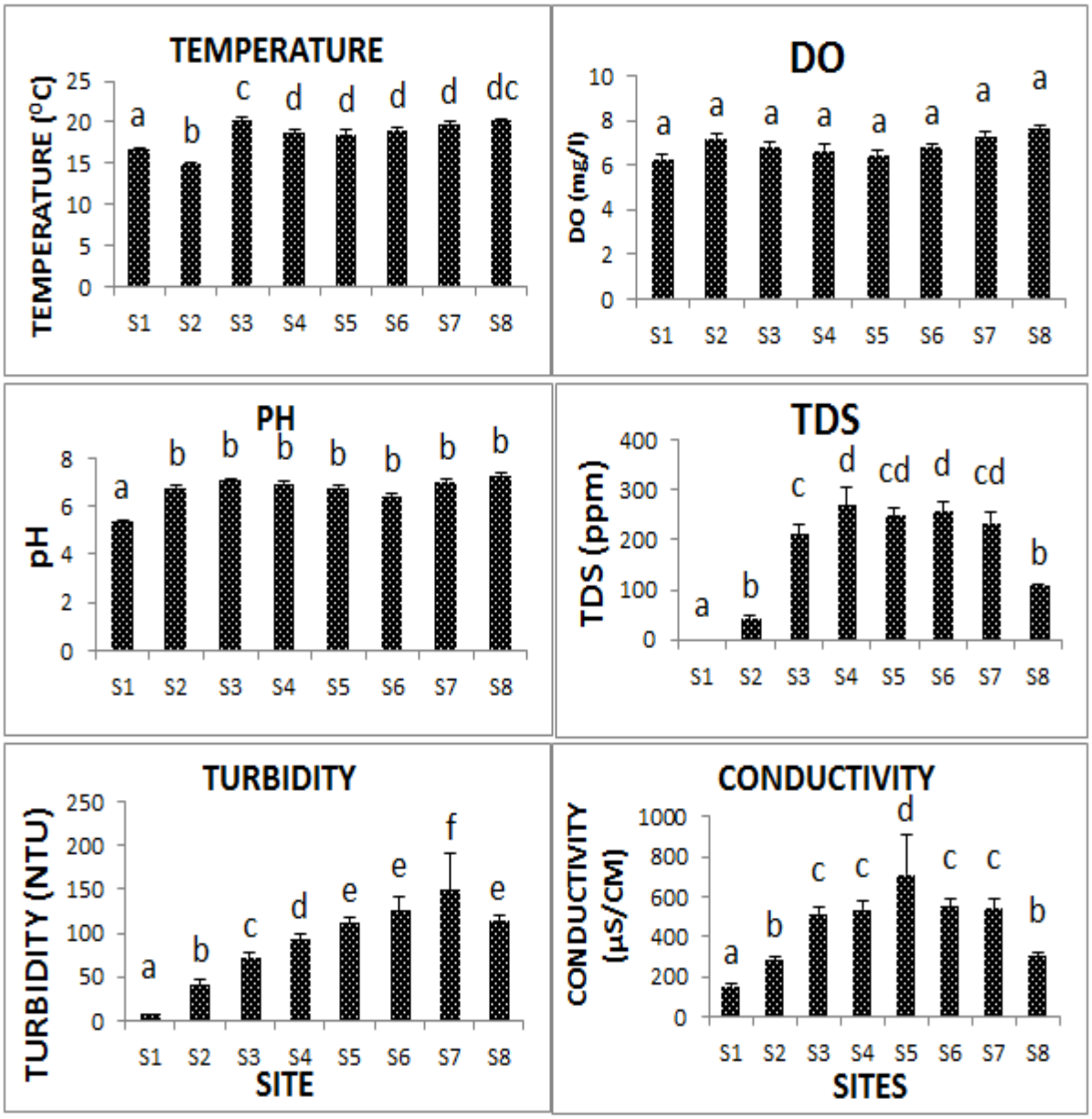

Figure 3: Graphs of mean values of physicochemical parameters from all the sites.

(Vertical bars indicate standard error of mean, sites with significant mean differences are shown by different letters at $P<0.05$ and $n= 30$)

4.2 Spatial variation in faecal contamination indicator densities

The results on mean densities of microbiological indicators of pollution are shown in Table 4 and Fig.4. Total Coliforms (TC) had values ranging between0-3 log units of Colony Forming Units per 100ml (CFUs/100ml), with Site 1 which was the furthest upstream recording mean density value of zero while Site 6 giving the highest mean density value in 3 powers of magnitude. TC showed significant variation with respect to sites, F= 31.972, P= 0.001 and df= 7, 239. *E. coli* had values ranging between 0.0 to 6900.0 cfu/100ml, with Site 1 recording a mean value of zero while Site 6 recording the highest mean value. *E. coli* also showed significant spatial variation, F= 19.253, P=0.001 and df= 7, 239. Intestinal enterococci had values ranging between 0.0 to 1400.0 cfu/100ml. Site 1 had a mean value of zero while Site 6 recorded the highest mean value. With respect to IE, there was significant spatial variation, F= 46.244, P= 0.001 and df= 7, 239.*C. perfringens* had values ranging between 0.0 to 1830.0 cfu/100ml with Site 6 recording the highest mean value. *C. perfringens* also indicated significant variation with respect to sampling sites, F= 38.461, P=0.001 and df= 7, 239.

Table 4: Mean densities of microbiological indicators of faecal pollution.

	TC		***E. coli***		**IE**		***C. perfringens***	
SITES	**Mean±SE**	**Range**	**Mean±SE**	**Range**	**Mean±SE**	**Range**	**Mean±SE**	**Range**
1	0.0±0.0	0.0-0.0	0.0±0.0	0.0-0.0	0.0±0	0.0-0.0	0.0±0.0	0.0-0.0
2	1085.3±101.2	300.0-1900.0	284.2±23.6	100.0-560.0	182.8±10.2	102.0-300.0	506.8±49.8	120.0-940.0
3	1821.8±209.1	450.0-3606.0	1144.4±148.3	150.0-2480.0	370.2±36.5	132.0-680.0	565.6±61.7	200.0-1480.0
4	3366.3±302.9	1300.0-6400.0	2075.6±282.0	280.0-4980.0	571.6±41.3	270.0-915.0	782.3±68.7	340.0-1480.0
5	3867.0±369.5	1500.0-7300.0	2731.0±376.3	300.0-6900.0	605.1±44.4	244.0-1200.0	1008.7±75.2	400.0-1780.0
6	4728.8±378.8	1760.0-8300.0	3369.3±452.3	412.0-7420.0	739.7±50.8	349.0-1400.0	1144.9±79.6	600.0-1830.0
7	3301.3±350.7	620.0-6300.0	2356.3±344.8	225.0-5700.0	505.0±37.0	150.0-827.0	721.7±49.2	300.0-1430.0
8	2025.6±244.1	330.0-4900.0	1555.8±213.9	158.0-3480.0	353.0±30.0	128.0-630.0	721.7±38.1	2.4-820.0

Total coliforms (TC), *E. coli*, Intestinal enterococci (IE) and *C. perfringens* from different sites on Nyangores River, (n=30).

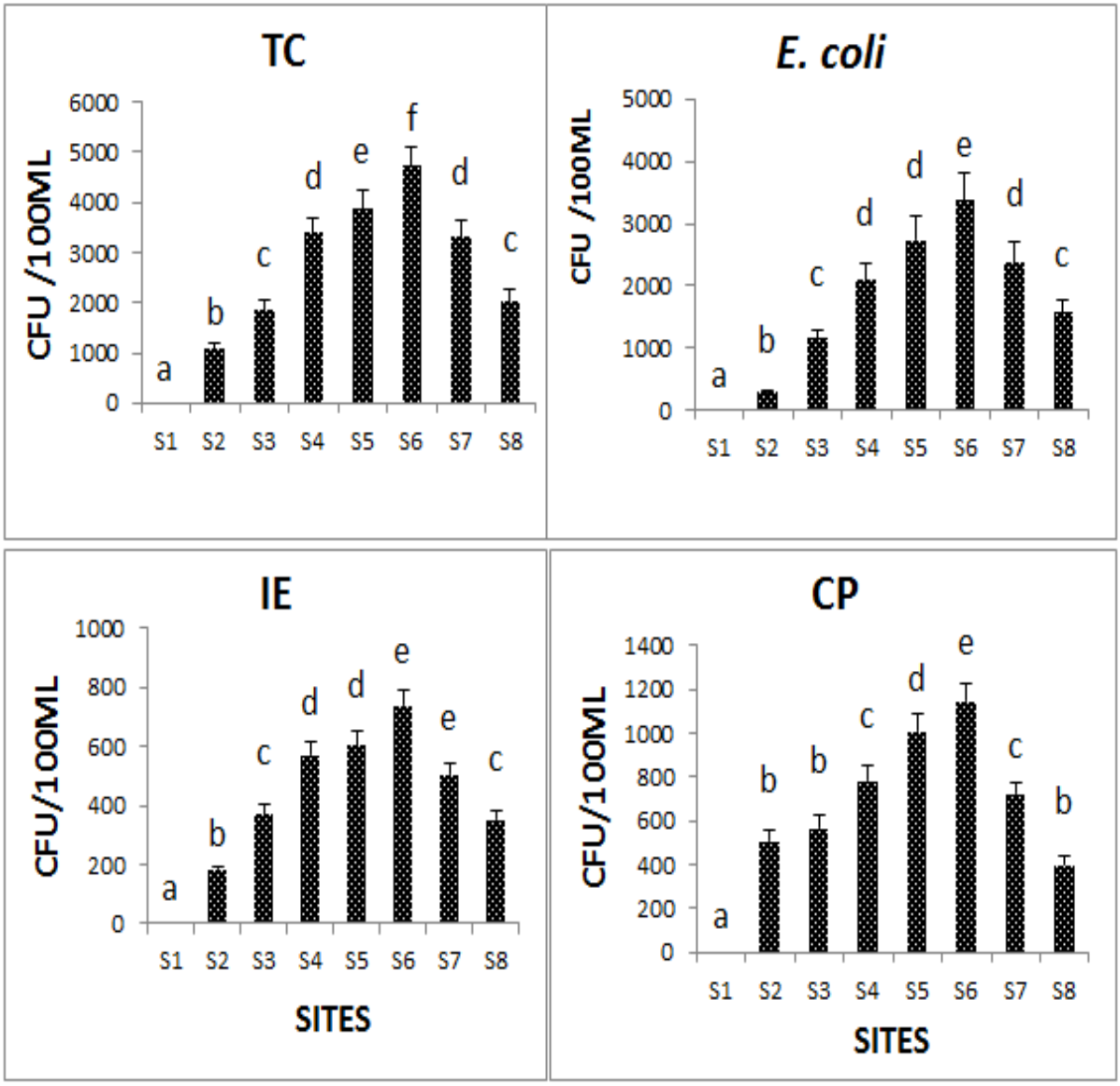

Figure 4: Graphs of mean values of microbiological contamination indicator parameters from all the sites.

(Vertical bars indicate standard error of mean and sites with significant mean differences are shown by different letters at $P<0.05$. n= 30)

4.3 Temporal variation of faecal contamination indicators

Monthly variations in the values of *E. coli* and total coliforms are shown in Figure 5. For all the parameters, there was a clear indication in the values sampled in different months based on rainfall intensity. *E. coli* showed statistical variation with reference to sampling months (February, March, April, May and June 2012), F= 40.35, P= 0.000 and df= 4 and 239. Total coliforms also showed significant variation with respect to month of sampling, F= 26.464, P= 0.000 and df= 4 and 239.

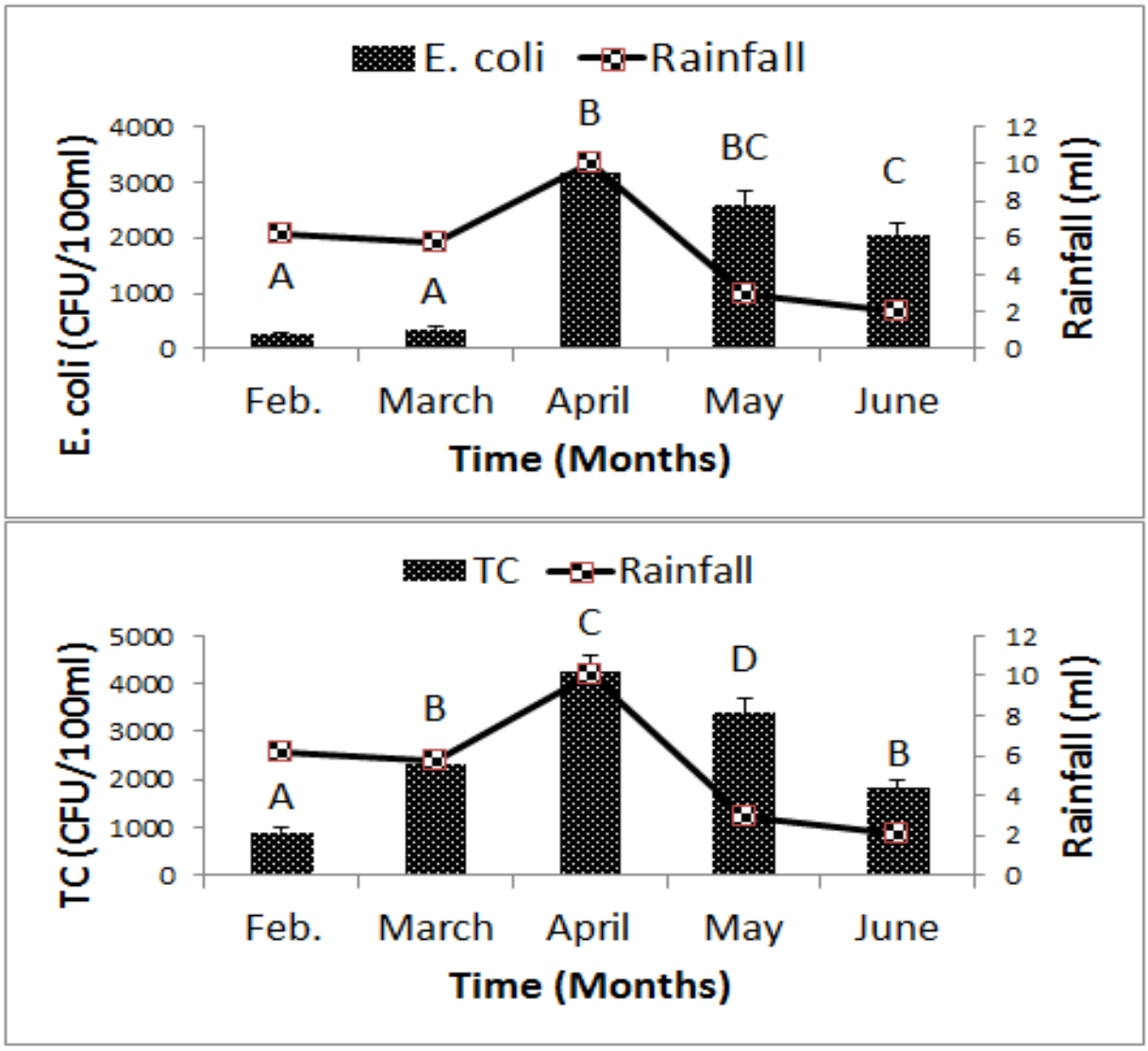

Figure 5: Graphs showing variability of *E. coli* and TC based on time.

(Vertical bars indicate standard error of mean and sites with significant mean differences are shown by different letters at P<0.05).

Monthly variations in the values of *C. pefringens* and intestinal enterococci are shown in Figure 6. For all the parameters, there was a clear indication in the values sampled in different months based on rainfall intensity. *C. pefringens* showed significant variation with respect to month of sampling, F= 25.96, P= 0.000 and df= 4 and 239. Intestinal enterococci also showed significant statistical variation with respect to sampling months (February, March, April, May and June 2012), F= 17.49, P= 0.000 and df= 4 and 239.

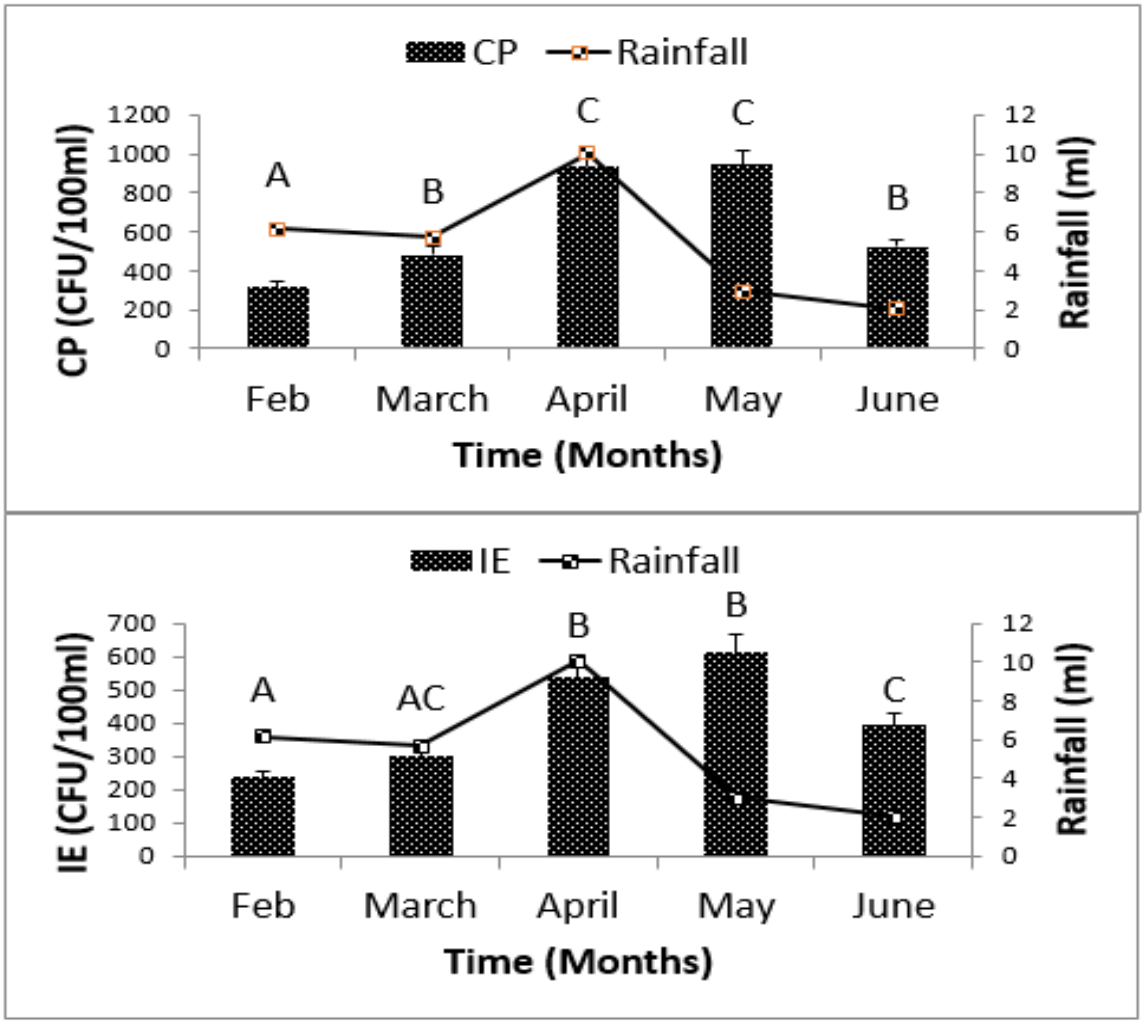

Figure 6: Graphs showing variability in CP and IE based on sampling time.

(Vertical bars indicate standard error of mean and sites with significant mean differences are shown by different letters at P<0.05)

4.4 Detection of *salmonella* spp

The results of *Salmonella* spp. detection are as shown in Table 5. All the samples from Site 1 were free from *Salmonella* spp. while all the remaining other samples indicated presence of *salmonella* spp. at particular sampling dates.

Table 5: Results of the *Salmonella* spp. detection from different sites at different sampling dates

SAMPLING DATE	SITES							
	1	2	3	4	5	6	7	8
04/02/2012	ND	ND	ND	ND	ND	ND	ND	ND
25/02/2012	ND	ND	ND	ND	ND	ND	ND	ND
10/03/2012	ND	ND	ND	ND	ND	ND	ND	ND
24/03/2012	ND	D	D	D	D	D	D	ND
07/04/2012	ND	D	D	D	D	D	D	D
21/04/2012	ND	D	D	D	D	D	D	D
05/05/2012	ND	D	D	D	D	D	D	D
19/05/2012	ND	D	D	D	D	D	D	D
02/06/2012	ND	D	D	D	D	D	D	D
16/06/2012	ND	D	D	D	D	D	D	D

ND= Not Detected and D= Detected.

From all the sites *Salmonella* spp were detected in 58 out of a total 80 samples. This gave 72.7% pollution level by *Salmonella* spp., while only 27.5% of the samples were free of this disease causing pathogen (Fig.7).

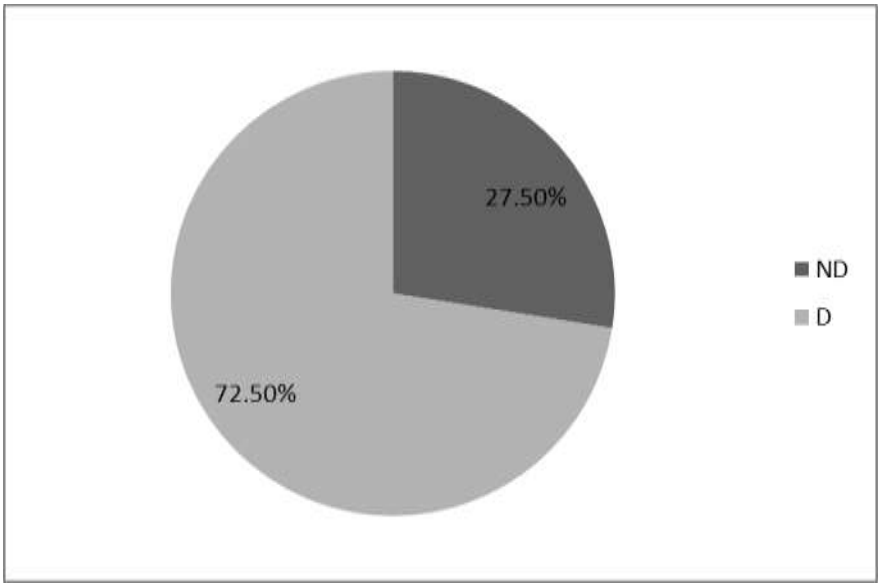

Figure 7: Percentage of samples polluted by *salmonella* spp.

ND= Not detected/not polluted, D= Detected/polluted.

4.5 Pollution level by easily degradable organic matter

Values of indicators for easily degradable organic matter are shown in Table 5 and Figure 7. Biological Oxygen Demand (BOD_5) had values ranging between 0.0 to 4.0 mg/l. BOD showed significant variation with respect to sampling sites, F= 215.695 at P=0.001 and df= 7, 239. Site 1 recorded the lowest mean values than the other remaining six sites. Heterotrophic Plate Count (HPC) had values ranging between 2-5 log units cfu/1ml. Sites 1 recorded the lowest mean value while Site 5 recorded the highest values. There was significant spatial variation in HPC values, F= 44.996, P= 0.001 and df= 7, 239.

Table 6: Mean values, standard error of mean (SE) with respective range for indicators of organic pollution from different sites.

SITES	**BOD (mg/l)**		**HPC (cfu/ml)**	
	Mean±SE	**Range**	**Mean±SE**	**Range**
1	1.3±0.1	0.0-2.0	756.2±93.5	120-1620
2	1.8±0.05	1.3-2.2	2112.4±382.1	408-6900
3	2.9±0.04	2.3-3.4	28450.0±4526.0	1620-72000
4	3.0±0.0	2.1-3.4	96905.3±12791.6	20400-260000
5	3.4±0.05	2.6-3.9	88033.3±6815.6	12000-150000
6	3.8±0.0	3.3-4.4	71030.0±4084.0	40000-130000
7	3.2±0.08	2.3-4.0	32596.7±3695.5	3210-72800
8	2.7±0.06	2.1-3.3	12613.3±986.4	5000-24000

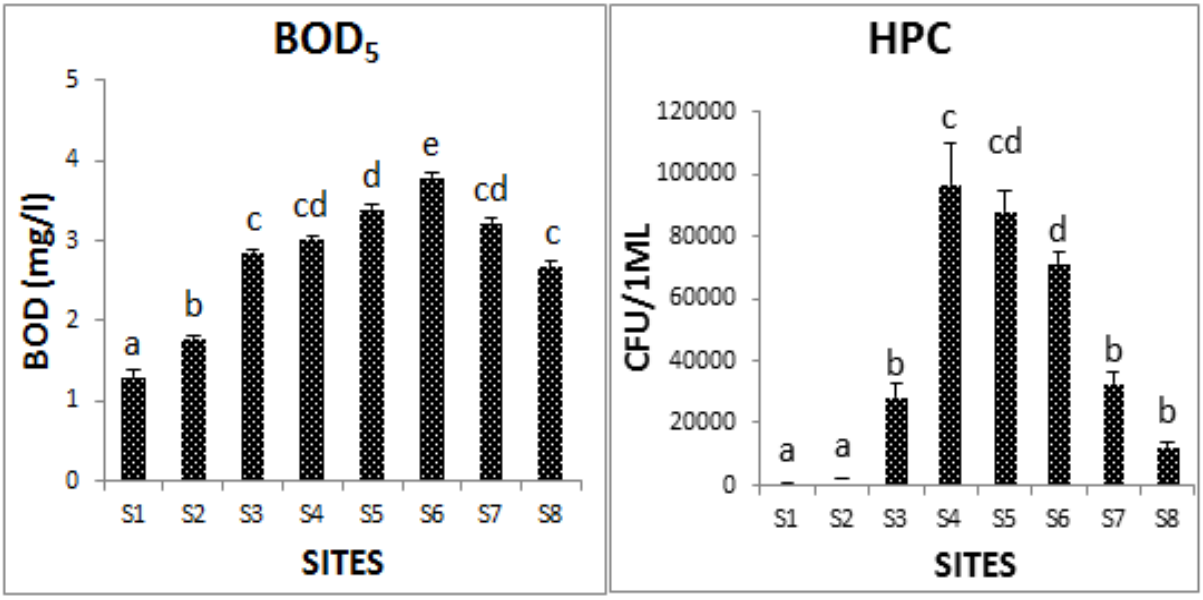

Figure 8: Graphs showing the mean values of organic pollution indicator parameters from all the sites.

(Vertical bars indicate standard error of mean, sites with significant mean differences are shown by different letters at $P<0.05$ and n= 30)

4.6 Temporal variation in indicators of organic pollution

Monthly variations in the values of HPC and BOD are shown in Figure 9. For all the parameters, there was a clear indication in the difference in values sampled in different months based on rainfall intensity. HPC showed significant variation with respect to month of sampling (February, March, April, May and June 2012), F= 7.496, P= 0.000 and df= 4 and 239. BOD also showed significant statistical variation with respect to sampling months, F= 84.861, P= 0.000 and df= 4 and 239.

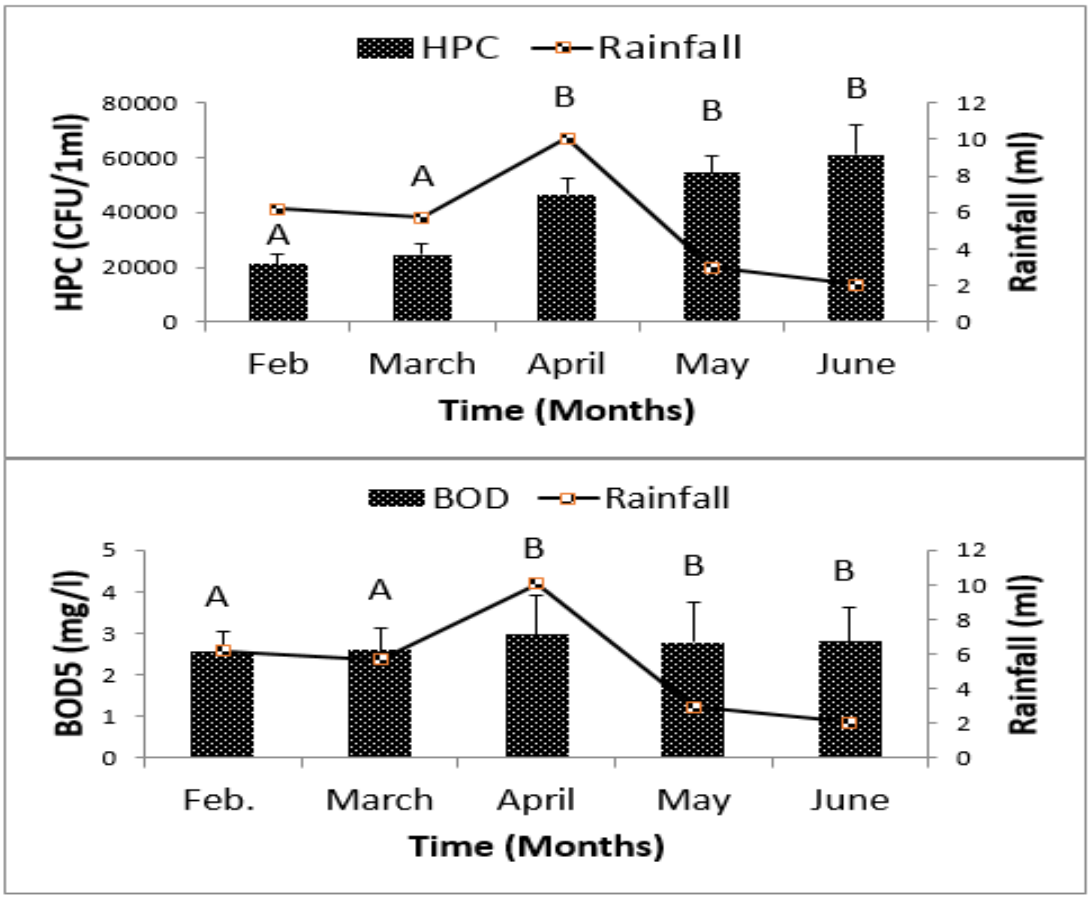

Figure 9: Graphs showing variability in HPC and BOD based on sampling time.

(Vertical bars indicate standard error of mean and sites with significant mean differences are shown by different letters at $P<0.05$)

4.7 Correlation between microbiological and physicochemical parameters

The relationship between various physicochemical parameters and microbiological parameters were as in Table 7. Most of the parameters showed significant positive correlation. There was significant correlation within microbial parameters as well as between microbial parameters and physicochemical parameters. *E. coli*, total coliforms, intestinal enterococci and *C. pefringens* showed positive significant correlation between themselves. All these microbiological parameters also showed positive significant correlation with physicochemical parameters like temperature, TDS, turbidity, and BOD. There was also significant correlation between some of the physicochemical parameters as well as between physicochemical parameters and microbiological parameters. Indicators of pollution by easily degradable organic matter (BOD and HPC) also showed significant correlation.

Table 7: Correlation between physical, chemical and microbiological parameters

	EC	TC	IE	CP	TEMP	DO	pH	COND.	HPC	TURBIBITY	TDS	BOD
EC	1	.818**	0.809**	0.796**	0.113	0.140*	0.012	-0.094	0.595**	0.415**	0.028	0.673**
TC	0.818**	1	0.879**	0.885**	0.207**	0.129*	0.160*	0.105	0.571**	0.410**	0.337**	0.737**
IE	0.809**	0.879**	1	0.894**	0.214**	0.045	0.201**	0.091	0.613**	0.352**	0.335**	0.781**
CP	0.796**	0.885**	0.894**	1	0.156*	0.061	0.135*	0.098	0.586**	0.355**	0.296**	0.720**
TEMP	0.113	0.207**	0.214**	0.156*	1	0.079	0.392**	0.271**	-0.009	0.085	0.414**	0.365**
DO	0.140*	0.129*	0.045	0.061	0.079	1	0.053	-0.01	-0.003	0.803**	-0.021	0.060
pH	0.012	0.160*	0.201**	0.135*	0.392**	0.053	1	.291**	0.005	0.131*	0.494**	0.392**
COND.	-0.094	0.105	0.091	0.098	0.271**	-0.01	0.291**	1	0.052	0.07	0.497**	0.278**
HPC	0.595**	0.571**	0.613**	0.586**	-0.009	-0	0.005	0.052	1	0.284**	0.186**	0.582**
TURBIDITY	0.415**	0.410**	0.352**	0.355**	0.085	0.803**	0.131*	0.07	0.284**	1	0.093	0.389**
TDS	0.028	0.337**	0.335**	0.296**	0.414**	-0.02	0.494**	0.497**	0.186**	0.093	1	0.533**
BOD	0.673**	0.737**	0.781**	0.720**	0.365**	0.060	0.392**	0.278**	0.582**	0.389**	0.533**	1

****** Correlation is significant at 0.01 level (two tailed)

*****Correlation is significant at 0.05 level (two tailed)

4.7 Faecal contamination source tracking

Source tracking for faecal contamination was done through *E. coli* and intestinal enterococci ratio determination. Results are shown in Table 8. The values from all the sites except sites 1 and 2 were closer to a ratio of 4.0 than 0.7.

Table 8: Mean values of *E. coli* and intestinal enterococci and ratios of *E. coli* to intestinal enterococci from all the sites

Sites	1	2	3	4	5	6	7	8
E. coli	0	284.2	1144.4	2075.6	2731.0	3369.3	2356.3	1555.8
IE	0	182.8	370.2	571.6	605.1	739.7	505.0	353.0
Ratio	-	1.6	3.1	3.6	4.5	4.6	4.7	4.4

CHAPTER FIVE
DISCUSSION

5.1 Physical and chemical parameters

In stream and out stream activities are considered to be the major factors controlling the physical chemical as well as the bacteriological water quality of a river. This is because an integrated approach gives a complete picture of the present state of the water ecosystem. Therefore the physical and chemical parameters of a river must be equally observed. Apart from point sources of pollution, diffused sources of pollution such as agricultural pollution and various in stream anthropogenic activities such as cattle watering, bathing, open defeacation and cloth washing pose an additional contribution to the deterioration of river water quality (Yilia *et al.,* 2009; Donde *et al.,* 2013). The results from this study to a greater degree concur with such findings and reasoning. Temperature exhibited spatial variation with the upper-most/upstream site at Kiptagich giving the lowest values. This could be attributed to the density and canopy coverage of riparian vegetation that determined the shading effect on the stream at Site 1.The lowest temperature values was noted to be at the area with the highest riparian vegetation cover which kept the water underneath cool for a long time. The amount of shading at various sites contributed to differences in stream temperature (Sherri, 2004). In general the mean temperature values increased downstream with an exception of Site 3 which was not significantly different from Site 8(Furthest downstream). This was because both site 3 and 8 lacked canopy cover resulting to direct heating of water within the channel at those particular sites by the heat from the sun's radiation.

The low DO value in Site 1 could be linked to the volume and source of water. Site 1 had minimum aeration with low water volume, this volume increased as it flows downstream due to the joining in of other tributaries. The colder spring water from underground that is dominant at site 1 also contributed to low DO (Nicholes *et al.,* 2013). The high volume of water at the downstream sites was able to trigger high flows which resulted to aeration as a result of waterfalls hence increased the amount of DO downstream. An additional point to note in this case is the nature of the stream gradient at different points. High gradient triggers fast flow of water within the channel and high flow may result to aeration hence high DO values (Harrelson *et al.,* 1994). Increase in deforestation downstream also contributed to warming up of water as it flows downstream (Sherri, 2004). Microbial respiration could have also resulted to low DO

(Kerr, 2008). The absence of spatial variation in DO values could be attributed to the balancing effects of the water temperature as well as flow regime as a factor of the channel topography. This factor could have also been contributed by the presence of cataracts and waterfalls along the channel at the downstream sites which resulted to unexpected increase in DO values downstream. However significant variations may be evident on prolonged or increased frequency of sampling (Craig and Brian, 1994).

Site 1 showed the lowest pH values than all the other sites, probably because the water at that particular site was noted to be very clean with minimum anthropogenic activities. This trend was also noted for TDS, turbidity and conductivity values. The study was able to show the existence of "self-purification" of a river. From physicochemical parameters point of view, it was noted that the water from site1 was of good quality but as it moves down stream through Sites 2 to 3upto 6, this quality deteriorated. But as the flow continues further downstream beyond Site 6 through Site 7 and 8 the quality again starts to improve, this was due to self-purification as one of the functions of rivers and stream in water quality maintenance (Maddock, 1999).

5.2 Spatial variation in faecal contamination indicators

All the other Sites except Site 1 had values of faecal contamination indicators far much above the values by WHO for drinking water guidelines (WHO, 2002). Higher TC values recorded in most sites are probably contributed by proliferation in the environment. The significant variation in total coliforms between different sampling sites was an indication of the existence of different degree of the anthropogenic activities which also impacted differently onto the quality of Nyangores River. The same trend was also observed in *E. coli* values, Site 6 recorded highest values for both TC and *E coli* counts. A study result by CRCFWE (2001) which involved the monitoring of faecal coliform levels in a stream also found higher values similar to what was achieved in some sites in this study.

A study on bacteriological water quality status of River Yamuna in Delhi had indicated increased bacterial total count from upstream to downstream stretch of river Yamuna. It showed that bacterial count at any location was a factor of hydrological condition and anthropogenic activities prevalent on the location (Chetna *et al.,* 2006). Down-stream increase in feacal pollution in River Awach in Nyanza region of Kenya was also attributed to increase in

settlements in middle reaches of the River (Akoko *et al.,* 2012). Within Nyangores River, the high pollution levels recorded were attributed to contamination by sewage discharge or storm water discharges from Bomet Municipality at site 6. Additional similar study by Stoimir and other researchers on the assessment of the microbiological quality of the River Tisa in Serbia at selected sites was also attributed mainly to a large amount of raw or improperly treated urban wastewater, organic pollutants loads and increased agricultural activities in that area. The origin of organic pollution in an ecosystem can be attributed to organic manure, fertilizers, high stocking density, feed waste, faecal matter, algal bloom and human interference. The microbiological pollution detected at selected sites was mainly due to a large amount of raw or improperly treated urban wastewater. Increased agricultural activity in this area during the sampling period probably contributed to the detection of heavy loads of organic and inorganic pollution. For that study, it was therefore recommended that stringent law regulations on effluent discharge from aquaculture facilities be put in place to give incentive to the industries to reduce effluent volumes and organic and inorganic loads. This was a measure which would result in acceptable river water quality and compliance with national and international quality standards and directives as well as more profound quality standards of microbial pollution in aquatic environments (Stomir *et al.*, 2011).

There are a number of environmental conditions that point to 'in-stream re-growth of *E. coli*' as the source of high faecal coliform levels. In-stream re-growth derived *E .coli* is not an indicator of faecal pollution. However, Byamkhama *et al.,* (2005) had also showed that *E. coli* is still a dependable indicator of faecal pollution in tropical environments. On the other hand, other studies have highlighted that surface waters are always poor in water quality due to their openness. This exposes them to various forms of pollution. A study within Khamis Mushait Governorate in South Africa showed that water derived from traditional sources (shallow wells) showed high values for most of the investigated bacteriological parameters, followed by surface water as compared to bottled or desalinated water. This may be highly attributed to the fact that shallow wells and surface water of Khamis Mushait Governorate being open and exposed to numerous pollution sources are highly at risk of contamination as indicated by the higher levels of bacteriological parameters (Eed, 2009). Contrary to this argument, is a study on microbial quality, diversity and antibiotic susceptibility profiles of bacterial isolates from borehole water used by schools in Greater Giyani Municipality of Mopani District in South Africa. According to

the results obtained from that study, it was concluded that the borehole water used by school children at Khomisani primary school, Nyanisi high school, Holapondo high school, Maswanganyi Primary School, Hlaniki Primary School and Macema High School was of poor microbial quality despite the fact that the water sources were closed and protected from pollution. That study recommended that a possible follow up would be to identify the actual sources of contamination to the borehole water; it also recommended a short term solution that could be to disinfect the water in the storage reservoir tank before distribution through the school taps (Samie *et al.,* 2011).

Faecal pollution of rivers used as source of drinking water pose health risk to humans not only through direct infection by pathogens, but such water if used for irrigation can transmit pollutants through vegetables/fruits consumed by man. A study by Labani *et al.,* (2005) had indicated that river water was of the poorest quality with high densities of faecal coliforms. That finding was the same as that of another study by Marijke (2010), who concluded that the rivers he had investigated contained high levels of faecal contamination. The conclusion here was based on the fact that some of the pathogens he isolated from the river and irrigation water and the irrigated produce, suggested a carry-over of microbial contamination from the river water to the irrigated produce. His study was, however, only done using the traditional international methods and he recommended the confirmation of the presence of specific pathogens in future by means of molecular techniques. But according to the results on an assessment of the microbial quality of river water sources in rural Venda communities in South Africa, where modern membrane filtration technique was used, it was also concluded that the microbial quality of the water sources was poor and unacceptable for human consumption due to faecal pollution. This indicated the potential risk of infection to consumers and calls for prompt intervention to mitigate the socio-economic and health impact of water-borne diseases in these rural communities (Obi *et al.,* 2002).

Based on the densities of total coliforms and *E. coli*, the Nyangores river water was found to be unfit for human consumption without proper purification. This finding was also in agreement with the values of total coliforms and *E. coli* in drinking water sources used by the Cree community of Mistissini area in Canada. In that study, microbiological analysis of raw drinking water using colourimetric and membrane filtration methods revealed that selected lake or river

water sources in the area were too polluted to be directly used for drinking. This was because positive results for all feacal contamination indicators were obtained at a high frequency and with varying distribution patterns. Interestingly, the scientific data obtained in this study also supported the traditional community perception that a limited number of environmental sites constituted safer water sources. In this regard even if water harvesting practices mitigate risk, boiling water therefore remains the best and reliable simple method to inactivate pathogens before consumption (Jean-Luc *et al.*, 2009).

Values obtained for intestinal enterococci from different sites within Nyangores River showed that Site 1 had good water quality than other sites. The significant spatial variation for intestinal enterococci indicates variation in contaminant levels at different stages of the river course. This variation could have been caused by anthropogenic activities like direct discharge of sewage into the river, open defeacation, laundry work in the rivers as well as bathing at different points within the river channel. Values of *C. perfringens* also had a similar trend and the above reasons could have also been the cause. This makes *C. perfringens* to be considered as a backup indicator for faecal contamination alongside other parameters. For instance, a study by Medema *et al.*, (1997) showed that the rapid die-off of *E. coli* and faecal enterococci makes them less suitable as indicators of oocyst presence in water. *C. perfringens* being able to survive longer than oocysts in untreated river water, it may prove useful as indicator for the presence of *C. parvum*. This was also similar to the conclusion by Florence *et al.,* (2012) who had studied Malaysian tropical rivers and found out *C. perfringen*s as being a potential indicator of the influence of high human population along the bank of these Malaysian Selangor River to its water quality (Florence *et al.,* 2012).

From this study, Nyangores River was found to be of the same quality status like other highly polluted water bodies. The rivers of the Scheldt basin had also been found to be poor as far as their microbiological water quality was concerned. This was a product of several monitoring studies within Scheldt basin. The Zenne River within the Scheldt basin was particularly contaminated downstream from Brussels due to the release impact of the treated wastewaters of the city. At the sale of the basin, the point sources (wastewaters) of faecal bacteria were largely predominant. Batch experiments also showed that decay of intestina enterococci was lower than that of *E. coli*. The final objective of this work also developed a mathematical model which

described the dynamic of *E. coli* and intestinal enterocci in the rivers of the whole Scheldt drainage network. This was then used to evaluate the impact of wastewater management on microbiological water quality (Ouattara *et al.,* 2009). Other studies similar to current study on Nyangores River do lead to the understanding of the microbial contamination. They are therefore important for both industrialized and developing countries as in the case of Kenya, where the low microbiological water quality is responsible for numerous waterborne diseases.

5.3 Temporal variation of faecal contamination indicators

All the feacal contamination indicator organisms had high mean values in samples obtained during the months of February and March, the month May and June had higher values while the month of April had the highest values. The high values during the months of February and March was due to dilution effect brought about by rainwater falling direct into the river basin. The highest values in April were due to increase in rainfall intensity which resulted to overland flow sweeping feacal wastes from the bushes and flooded urban centers into the river. In the month of May and June, there was reduction in rainfall intensity, this consequently resulted to high mean values of feacal contamination indicator organisms due to increase in their concentration caused by the drop in the volume of water. These were based on the knowledge that rain water have the potential to dilute river water and lower the densities of microbiological parameters. It was found that the presence of surface run offs as a result of rain do lower the densities of these faecal contamination indicators if rains are prolonged.

Studies have revealed that the city sewage discharge agriculture and urban run-off were affecting the water quality of Shatt Al Hilla River in Iraq (Fikrat *et. al.*, 2007). However, a study on River Brada had the opposite finding where there was existence of many forms of bacteria especially during rainy season. This could have probably been caused by sampling immediately after rainfall event. It had also appeared that in-stream *E.coli* re-growth is a seasonal pattern and therefore could be contributing to its temporal variation (CRCFWE, 2001). Another study on the effects of time and watershed characteristics on the concentration of *Cryptosporidium* Oocysts in River Water had actually confirmed time as one of the factors influencing the density variation of microbes. In that study, the results and the discussion led to several conclusions. One of the conclusion was that *Cryptosporidium* oocysts were present in river water of both inhabited and uninhabited areas at concentrations above the detection ability of the study method used i.e.,

about 0.05 to 0.15 oocysts per liter. It also concluded that Oocyst concentrations in watersheds of appreciable size were continuous as opposed to intermittent seasonal factors, including runoff of land drainage. These factors may affect oocyst concentrations by 10-fold. The character and intensity of both human and domestic animal activities in a watershed was also found to affect oocyst concentrations in the surface water by as much as 10- to 15-fold. Final conclusion was that public water supply watershed management practices of limiting human activity may reduce oocyst concentrations by as much as fivefold (Jahn *et al.,* 1991).

Contrary to the findings on the quality of Nyangores River, the precipitation intensity has also shown a positive contribution to microbial load. This had been showed in a study on microbial load of drinking water reservoir tributaries during extreme rainfall and runoff. This study showed that for every situation at a watercourse an individual analysis had to be carried out, taking into account geo-ecological conditions in catchment areas as well as variability in precipitation and runoff. It was important to evaluate systematically the environmental conditions of the catchment areas and their roles in microbial contamination of surface water, in addition to routine analytical monitoring of chemical and microbial parameters of water samples (Kistemann *et al.,* 2002). However, it is probable that a clear influence of rainfall effects could have been noted if the frequency of sampling was increased during rainy days.

5.4 Detection of *salmonella* spp

Salmonella species are pathogenic microorganisms with potential of causing salmonellosis in human being (Nye *et al.,* 2002). This microorganism was found to be present in majority of samples obtained from different sites. This result was an indication of how risky it would be to use the water from this river for human consumption without proper purification. In addition, it also points out that there is high contamination rate of the water in this river by faeces from ill individuals or healthy carriers as evidenced by the presence of raw sewage discharge from the hospital and other settlements. Nyangores River may therefore stand a chance of inhabiting other disease causing microorganisms which were not considered under this study. The absence of *Salmonella* spp in samples obtained during wet period could be due to their low densities; this could not be detected in 100 mls samples. Based on the presence of *Salmonella* Spp together with faecal contamination indicator organisms (*E. coli, C. perfringens* and intestinal enterococci)

it shows that these indicators are adequate tools in studying the safety of river water for human consumption.

The microbial quality of Alcantara estuarine waters was strongly influenced by the contributions of domestic and agricultural waste, discharge of effluents from wastewater plants, and several feacal sources. The presence of bacterial pathogens in the Alcantara estuarine waters not only showed a major health concern, but also might have prevented the utilization of these waters for important economic resources, such as fishing, aquaculture and mussel farming (Concetta *et al.,* 2009). The same result had also been found by Marita and Okemo (2008) who showed that the correlation of faecal coliforms with *Salmonella* sp. and *Vibro cholerae* was 85% and 2% respectively. For the faecal streptococci, correlation with *Salmonella* sp. and *V. cholerae* was 78% and 12% respectively. This indicated that faecal streptococci should be included as indicator organisms of the potential health hazards of polluted water. Most international drinking water quality guidelines and standards include bacterial indicators as a measure of microbial water quality, and for compliance reporting. The results from the study of the bacteriological quality of Nyangores River support the idea of using both the faecal streptococci and coliforms as indicators of faecal pollution.

5.5 Pollution level by easily degradable organic matter

There was high pollution by easily degradable organic matter as depicted by high values of HPC and BOD. The pattern of the values of these parameters from Site 1 to the last site 6 and significant spatial variation also shows that the intensity of organic loading into Nyangores river increases from upstream to downstream upto site 6 and reduces thereafter. This trend is linked to the trend in the density of riparian vegetation which was noted to reduce from upstream to downstream. However, sudden drop from Site 6 to Site 8 may have resulted from dilution effect by other emerging small tributaries. This could also be due to the fact that the organic material in these sites not being easily degradable. In addition, other in stream activities like nitrification could have also led to reduced oxygen concentration, which had an ultimate influence on the values of HPC and BOD. This was comparable to a study by Etleva *et al.,* (2012) on Vjosa River, Masedonia and whose preliminary results, as it was expected, had high load of heterotrophs in sample stations near urban areas.

The rise in the inflow of waste is clearly always due to the rapid growth of residential and commercial activities in an area. Due to the discharge of sewage, domestic wastes and human activities the phosphate and other nutrients load in river water exceeds the permissible limit of WHO drinking water standards. This permits the bloom of algae, fungi and other microbes, resulting to modification of BOD, DO and HPC qualities and quantities. Under such scenario, even though the sewage mixes with the river system, the river water could be used for irrigation but it remains unsuitable for drinking purposes due to the presence of faecal coliforms, *E. coli*, other bacterial population and higher concentration of phosphate, BOD and COD (Usharani *et al.,* 2010). The bacteriological counts in the river water make the water unfit for human consumption. In regard to that study, the groundwater around the Noyyal River was found to be suitable for irrigation purposes but the presence of phosphate, BOD and COD levels clearly indicated that the river water and ground water are getting contaminated with salt/nutrients that may affect soil and water conditioning. In such situation, suitable measures had to be taken to minimize the load of salts/nutrients in the soil and water, so that the fertility could be maintained and better yield may be obtained. That study concluded that the river was polluted as it was used as a sewer disposal site, but was also undergoing self-purification and had potential for significant improvement in water quality if discharges are ameliorated. Regular monitoring of river and taking suitable remedial measures like collection of domestic sewage and setting up the common treatment plant; before discharge of sewage into river system, was proposed. This was to control pollution and prevent the depletion of the quality of river waters (Usharani *et al.,* 2010).

5.6 Temporal variation in indicators of organic pollution

The presence of temporal variation in the densities of organic pollution indicators where dry season had higher density values for indicators of organic pollution than wet season was an indication that seasonality has a role in the amount of organic pollution. Much of the organic pollutant is getting swept from the allochthonous sources and brought into the river by surface run off but the dilution effect lowers the density of these parameters. A seasonal change had also been observed by Etleva *et al.,* (2012), in their study of Vjosa River. This study showed that for every situation at a watercourse an individual analysis has to be carried out, taking into account geoecological conditions in catchment areas as well as variability in precipitation and runoff. It is

important to evaluate systematically the environmental conditions of the catchment areas and their roles in microbial contamination of surface water, in addition to routine analytical monitoring of chemical and microbial parameters of water samples.

The study on the bacterial indicators of faecal contamination of the Gangetic river system right at its source had factored in the effect of pollutant variability on the level of pollution indicators. This involved studies on microbial ecology in the runoff of the glacier in relation to pollution levels. It clearly revealed that there was significant presence of bacterial indicators of faecal pollution in middle and lower stretch. That situation of Gangotri glacier was not very serious but alarming. Presence of bacterial indicators of faecal contamination in different altitudes of runoff of Gangotri glacier clearly revealed the bacteriological status of the water at various sites in various times (Vinay *et al.*, 2005).

5.7 Correlation between physicochemical and microbiological parameters

The significant correlation between *E. coli*, total coliforms, intestinal enterococci and *C. perfringens* was good evidence that all these parameters can be used as perfect indicators of organic pollution. It also showed that at the time of this study, there existed both the recent and the persistent faecal pollution into Nyangores River. Significant correlation between faecal contamination indicators (*E. coli*, total coliforms, intestinal enterococci and *C. perfringens*) and organic pollution indicators (HPC and BOD) also showed that human development activities along Nyangores River do contribute to both faecal and organic pollution into the river. The result is also comparable to studies by Desmarais *et al.*, (2002); Kei *et al.*, (2004) and Olga and Satoshi, (2006). In all those studies there was strong relationship between *E. coli*, total coliforms, intestinal enterococci and *C. perfringens*) and organic pollution indicators. However, a study by Sugumar *et al.*, (2008) on the occurrence of seasonal variation of bacterial indicators of faecal pollution along Thoothukudi Coast, Tamil Nadu had a different finding. The finding showed that there was no discernible relationship between faecal contamination indicators, organic pollution indicators and physical parameters.

Other studies have also quantified the relationship between microbiological parameters and the easily degradable organic and inorganic matter. A study of base-flow conditions had indicated that the turbidity in the Stock Creek watershed correlates most strongly with *E. coli* load rates

and not density. Moreover, there was a spatial dependency of the *E. coli* load rate on turbidity and precipitation. Due to the drainage–storage characteristics associated with individual sub-basins at base flow, the *E. coli* load rate showed difference responses. The first response was that it correlated best with antecedent precipitation in headwater sub-basins. The second one was that it also correlated better with turbidity in the higher order streams at the tail water end of the basin. The slow shedding of storm water in the tail water end of the watershed may have also resulted in higher sediment and *E. coli* mass loadings. That suggested a strong tie to *E. coli* persistence in the slow-draining sub-basins due to possible sediment attachment. However, this hypothesis needed to be tested in a more elaborated study. The persistence of *E. coli* in faster draining sub-basins may be tied more directly to the antecedent storm events and elevated base-flow response (Randall *et al.,* 2006). On a different perspective, wastewater effluents discharge has also been found to pose an influence not only to the microbiological parameters but also to the physico-chemical properties of water and vice versa (Jakhrani *et al.,* 2009).

5.8 Faecal contamination source tracking

The ratios of *E. coli* to intestinal enterococci at different sites within Nyangores River were closer to a ratio of 4.0 than 0.7. This showed that the major source of faecal pollution into the river was probably of human origin (Weaver *et al.,* 2005). This result was the exact opposite of what was reported for a river in North Area of Wigry National Park where it was found that source of faecal contamination was majorly coming from flows arising from other arable-forestry-pasture-meadow catchments as expressed by a larger number of streptococci than *E. coli*, (Niewolak, 1999). Generally, the source of microbial pollution into Nyangores River could be different and hence need to be determined. Indeed, all current methods and those in progress require additional investigation. However, all have merit and are important constituents of the constantly expanding microbial source tracking toolbox. Agencies like The Environmental Protection Agency are considering the inclusion of modern faecal contamination source tracking technologies to increase the accuracy in microbial source tracking. These methods are certain to play a pivotal role in identifying point and nonpoint sources of fecal pollution in the nation's impaired water systems as in the case of Nyangores River (Troy *et al.,* 2002).

CHAPTER SIX
CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Based on the results of this study, the following conclusions can be drawn;

- The densities of faecal contamination indicators vary significantly between various points within Nyangores River. The river water is of good quality as it comes from the source i.e. Site 1. This site at Kiptagich is one of the sources of the river and water spring out to the surface with no contamination. The quality deteriorates as the water flows to downstream; pointing out that there is significant influence of human activities on the quality of this Nyangores River as the water flows downstream.
- The densities of faecal contamination indicators in water within Nyangores River also had significant variation with respect to time/month of sampling. The influence of dilution effect as a result of rainfall events played a greater role in this case.
- In addition to faecal contamination indicators, the water within Nyangores River exhibited the presence of pathogenic *Salmonella* spp. This was present in all the sites along the river channel except site 1.
- There was also present of organic pollution indicators. The levels of these indicators varied significantly with respect to the sites (spatial) and time of sampling (temporal).

6.2 Recommendations

The following recommendations are necessary for the better management of Nyangores River to maintain the quality of its water as well as its ecological integrity and for the safety of the community who depend on this river as sources of their domestic water:

- Site 1 to be used as the reference point in managing the quality of water in Nyangores River. Conservation effort should be put in place to have the water quality in the entire river to be the same or close to Site 1.
- Proper sewerage treatment facilities should be put up in the existing towns and hospitals and also in any of such development to come. Each household should also have a proper way of waste disposal.
- All the development activities should put into consideration the environmental consequences and therefore development and environment management should go hand

in hand so as to achieve sustainable development and to meet the Millennium Development Goals (MDG).

- Water from Nyangores River is not safe for human consumption, unless adequate treatment procedure is done to it.
- The ecological integrity of Nyangores River should be maintained to enable the river carry out its natural processes like water quality monitoring through self-purification.
- The community using the water from Nyangores River to be sensitized on the quality of water and its potential danger to their health. They should also be made knowledgeable of the cheap and efficient methods of post harvesting water quality improvement measures is necessary to the local communities.
- This study also recommends that proper water quality management and continuous monitoring of microbes are the key factors to reduce the bacterial load and ultimately reduce the likelihood of disease outbreak.
- Further study that in-cooperate other techniques in tracking the sources of faecal and organic matter pollution in Nyangores River is also recommended.

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APPENDICES

Appendix 1: Plates showing colony forming units of microbiological parameters

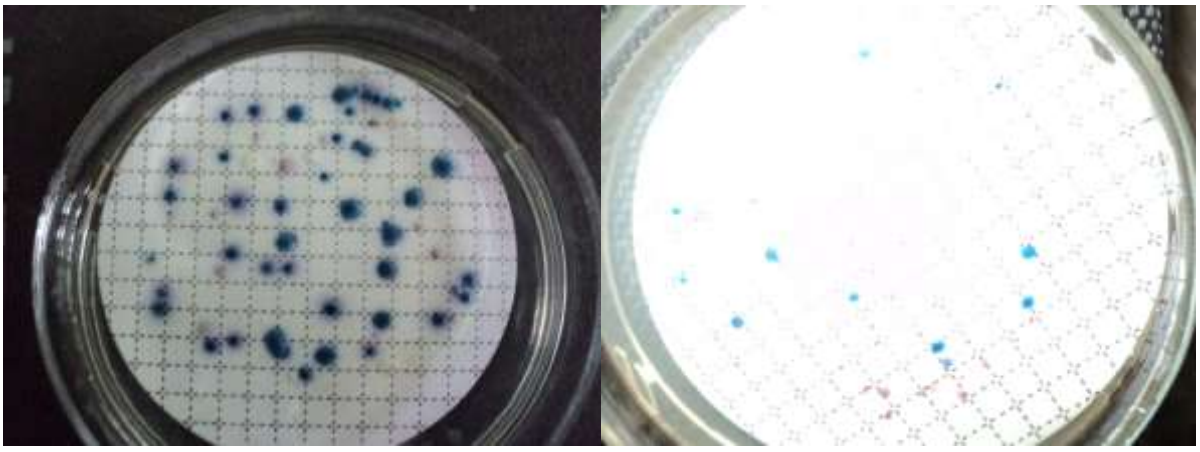

Plate 4: Photograph showing CFUs (*E. coli* - blue colonies) and (pink for other- coliforms)

Plate 5: Photograph showing CFUs (Salomonella- pink) (*E. coli*-light blue colonies)

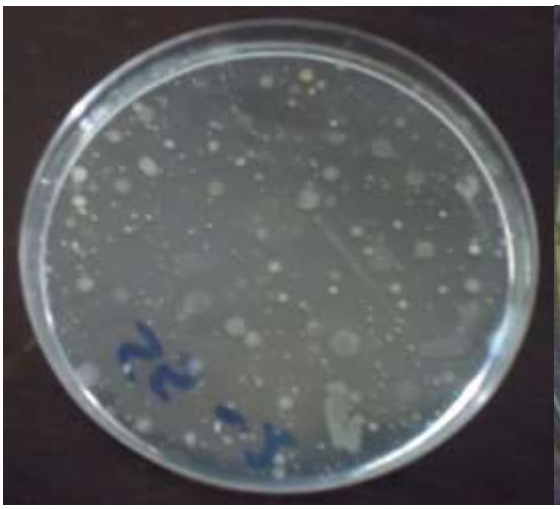

Plate 6: Photograph showing CFUs HPC

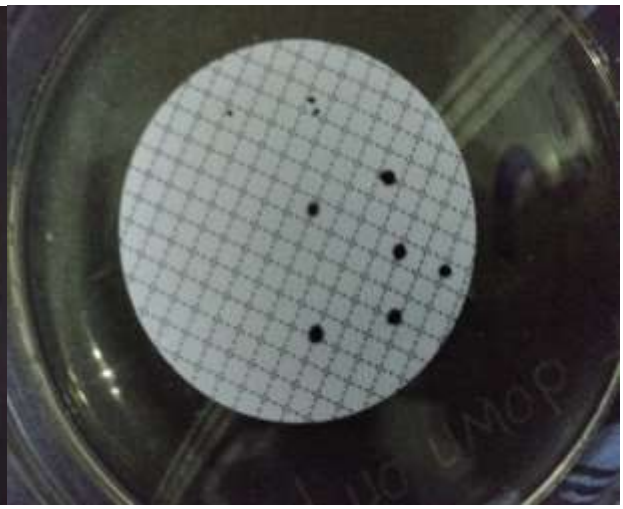

Plate 7: Photograph showing CFUs (*C. perfringens*–black frorescence colonies)

Appendix 2: Rainfall data (ml) for Bomet District during the study time (February to August 2012) Source: Water Resource Management Authority, Lake Victoria South Catchment Area.

Date/Time	February	March	April	May	June	July	August
01/02/2012 09:00	0.0	8.8	0.0	0.0	0.0	0.0	0.0
02/02/2012 09:00	0.0	9.9	7.7	2.0	4.9	0.0	4.2
03/02/2012 09:00	0.0	48.0	0.0	6.5	10.6	2.0	2.4
04/02/2012 09:00	0.0	17.1	18.3	0.0	19.5	0.0	0.0
05/02/2012 09:00	0.0	54.2	0.0	0.0	0.0	5.7	0.0
06/02/2012 09:00	0.0	18.3	6.5	0.0	0.0	0.0	0.0
07/02/2012 09:00	0.0	0.0	11.4	0.0	0.0	0.0	0.0
08/02/2012 09:00	0.0	0.0	0.0	6.9	0.0	0.0	2.0
09/02/2012 09:00	0.0	0.0	0.0	3.7	2.4	0.0	13.8
10/02/2012 09:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11/02/2012 09:00	0.0	0.0	19.1	0.0	0.0	0.0	0.0
12/02/2012 09:00	0.0	0.0	9.8	0.0	0.0	0.0	0.0
13/02/2012 09:00	0.0	0.0	0.0	0.0	2.0	0.0	15.5

14/02/2012 09:00	0.0	0.0	0.0	34.2	0.0	0.0	0.0
15/02/2012 09:00	0.0	0.0	0.0	5.1	0.0	0.0	0.0
16/03/2012 09:00	0.0	0.0	8.3	0.0	0.0	0.0	0.0
17/03/2012 09:00	0.0	0.0	10.3	0.0	0.0	0.0	0.0
18/03/2012 09:00	15.9	0.0	8.3	9.8	10.2	0.0	0.0
19/03/2012 09:00	0.0	18.4	17.6	8.3	0.0	0.0	0.0
20/03/2012 09:00	4.7	2.0	3.2	3.9	0.0	0.0	0.0
21/03/2012 09:00	6.7	0.0	0.0	0.0	0.0	0.0	0.0
22/03/2012 09:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23/03/2012 09:00	0.0	0.0	13.4	10.0	0.0	2.9	0.0
24/03/2012 09:00	5.6	0.0	0.0	0.0	4.9	0.0	97.7
25/03/2012 09:00	8.2	0.0	10.8	0.0	3.3	0.0	0.0
26/03/2012 09:00	18.7	0.0	0.0	0.0	3.7	0.0	0.0
27/03/2012 09:00	26.5	0.0	20.0	0.0	0.0	0.0	0.0
28/03/2012 09:00	36.2	0.0	34.2	0.0	0.0	0.0	0.0
29/03/2012 09:00	56.4	0.0	50.1	0.0	0.0	0.0	0.0
30/03/2012 09:00	-	0.0	52.9	1.8	0.0	0.0	0.0
31/03/2012 09:00	-	0.0	-	0.0	-	0.0	-
Mean	**6.2**	**5.7**	**10.1**	**3.0**	**2.0**	**0.3**	**4.5**