University of Natural Resources and Life Sciences, Vienna

UNESCO-IHE Institute for Water Education

EGERTON UNIVERSITY

EVALUATION OF FAECAL INDICATOR BACTERIA AND ANTIMICROBIAL RESISTANCE OF *Escherichia coli* ISOLATED FROM RIVER NJORO, NAKURU COUNTY, KENYA

Master of Science Thesis

by

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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 institution.

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SM19/13728/19

Recommendation

This thesis has been submitted with our approval as supervisors for examination according to Egerton University regulations.

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DEDICATION

I dedicate this work to my beloved parents and siblings for their love, support and encouragement.

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ABSTRACT

Agriculture, urbanization and industrial activities are a threat to surface water quality. The wastes generated from these activities are discharged into rivers directly or indirectly consequently altering the physical, chemical and biological quality of rivers. Human settlements and associated land use activities have compromised water quality of River Njoro, Nakuru County, Kenya causing environmental and public health concerns including diseases and antimicrobial resistance of microbial risks to medical drugs. The aim of this study was to investigate the effect of human activities on faecal pollution of River Njoro and to evaluate antimicrobial resistance of *Escherichia coli* isolated from the river water to a selected group of antibiotics. Water samples were collected at sites with varying land use. At each site, *in situ* physico-chemical variables; dissolved oxygen, temperature, pH, conductivity, total dissolved solids and turbidity were measured after collecting triplicate water samples from the river. Samples were stored in a cool box and taken to Limnology and Wetland Management laboratory at Egerton University for nutrients and bacteriological analyses. Antibiotic susceptibility of *E. coli* isolated from the river water samples was tested using Kirby-Bauer disc diffusion assay. The strain *E. coli* ATCC 25922 was used as antibiotic susceptibility reference standard. All data was subjected to normality and heterogeneity tests. All variable records were summarized as means and standard deviations. Spearman`s correlation coefficient was used to determine any significant relationships among variables measured in River Njoro. Non-parametric test, Kruskal-Wallis was used to assess significant differences between median concentrations of faecal indicator bacteria, physico-chemical variables and antimicrobial resistance of *E. coli* among the sites. Both physico-chemical and bacteriological variables varied significantly among the sampled sites except for total suspended solids ($P<0.05$). Significant correlations were observed between dissolved oxygen, temperature, ammonium and faecal indicator bacteria among others ($P<0.05$). A significant difference in antimicrobial resistance of *E. coli* isolated from River Njoro was observed between the sampling sites except for amikacin and amoxicillin ($P<0.05$). Antimicrobial susceptibility test revealed that *E. coli* isolated from water at different sections of the river were resistant to multiple antibiotics. A high resistance prevalence was recorded in streptomycin (95.83%), chloramphenicol (86.11%), ciprofloxacin (86.31%), amoxicillin (85.71%) and tetracycline (82.14%). Multiple antimicrobial resistance index were greater than the threshold of 0.2 in all the sites. Results from this study can be used in predicting potential microbial risks to human health and to provide mitigation measures towards protection of water resources against pollution.

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LIST OF ABBREVIATIONS AND ACRONYMS

APHA	American Public Health Association
AR	Antimicrobial Resistance
ARB	Antibiotic Resistant Bacteria
ARG	Antibiotic Resistant Genes
ATCC	American Type Culture Collection
CFUs	Colony Forming Units
CLSI	Clinical Laboratory Standard Institute
FIB	Faecal Indicator Bacteria
IMVIC	Indole Methyl Red Voges-Proskauer Citrate
LWM	Limnology and Wetland Management
MARI	Multiple Antibiotic Resistance Index
MPN	Most Probable Number
NEMA	National Environment Management Authority
NH_4-N	Ammonium-Nitrogen
NO_2-N	Nitrite-Nitrogen
NO_3-N	Nitrate-Nitrogen
NTU	Nephelometric Turbidity Unit
SDG	Sustainable Development Goal
SPSS	Statistical Package for the Social Sciences
SRP	Soluble Reactive Phosphorus
TDS	Total Dissolved Solids
TP	Total Phosphorus
TSC	Tryptose Sulphite Cycloserine
TSS	Total Suspended Solids
UK	United Kingdom
USA	United States of America
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
WHO	World Health Organisation
WWTP	Wastewater Treatment Plant

CHAPTER ONE
INTRODUCTION

1.1 Background information

Globally, surface water resources are vulnerable to pollution (Mul *et al*., 2015). Human activities such as agriculture, industries and urbanization have been linked to land use change in river basins (Kang *et al*., 2010; Ngoye & Machiwa, 2004) which in turn affects water quality (Tanaka *et al*., 2016; Tran *et al*., 2010). Water pollution by faecal bacteria can either be from point sources such as sewage effluents, or non-point sources like run-offs from grazed lands, and re-suspension of sediments in the channel (Collins *et al*., 2007; Dorner *et al*., 2006).

Faecal contamination in rivers readily occurs in disturbed catchments especially during storm events (Paruch *et al*., 2018). Removal of trees and vegetation cover for agriculture or urban development reduces water infiltration rates and increases surface runoff, consequently leading to deposition of bacteria laden influents into rivers (Garcia-Armisen & Servais, 2007). Surface run off carries animal and human wastes from surrounding areas into the river channel causing pollution (Kistemann *et al*., 2002). Vegetation ground cover plays a critical role in preventing and improving river water quality by reducing faecal bacteria contamination (Tong & Chen, 2002). Physico-chemical and biological factors affect the growth and survival of microbes in aquatic environments. In a review done by Ferguson *et al*. (2003) temperature, moisture, availability of nutrients, pH, exposure to sunlight influenced the fate and transport of microbes.

Human and animal faecal matter is regarded to be of great risk to human health. In most cases, it contains enteric pathogens (Scott *et al*., 2002). Bacterial pathogens such as *Salmonella spp.*, *Shigella spp.*, *E. coli* and *Vibrio cholerae* are capable of causing debilitating diseases to humans or even death (USEPA, 2001). Consumption of water containing these pathogens causes gastrointestinal infections and diarrhoeal diseases in susceptible humans such as infantile diarrhoea (Momba *et al*., 2006). They also increase resistance of humans to antimicrobial therapy from direct and horizontal bacterial or viral gene transfer (Da Silva & Mendonca, 2012). Antimicrobial use is of great importance in Njoro River surface waters as agriculture and livestock rearing is one of the major land uses in the catchment where animals are treated with antibiotics for control of diseases and as a means of growth enhancement. Medical use of antibiotics is also prevalent in human settlements in the area. The highest prevalence of antimicrobial resistant bacteria is found in areas where antibiotics are used at high rates for prevention and treatment of microbial infections in both humans and livestock

(Kümmerer, 2004). The prevalence of resistant bacteria is due to presence of antibiotic residues even at low concentrations (Stepanauskas *et al*., 2006). Such residues have been recorded in River Njoro (Itotia *et al*., 2018). Antimicrobial resistance can either cause prolonged illness or in severe cases, death (Cosgrove, 2006).

The faecal indicator bacteria (FIB) including; intestinal enterococci and *Escherichia coli* are commonly used as indicators of faecal contamination (Leclerc *et al*., 2001). These bacteria are found in gastrointestinal tract of both warm and cold-blooded animals and are shed in faeces together with pathogens (Byappanahalli *et al*., 2012; Harwood *et al*., 1999). Although total coliforms have been used as indicators of faecal pollution, it was discovered that some species in the coliform group can multiply and survive in the environment for extended periods of time and their detection is not necessarily an indication that they are of faecal origin. It is therefore necessary to determine presence of *E. coli* or faecal coliforms which indicate recent faecal pollution (Gauthier & Archibald, 2001). Thus, epidemiological studies by Fewtrell and Bartram (2001) showed that human health risks associated with water are better indicated by *E. coli* and intestinal enterococci than coliforms.

1.2 Statement of the problem

Population increase has necessitated intensive agriculture (crop and livestock farming), establishment of settlements and industries as well as urbanization in River Njoro catchment. These activities have led to loss of vegetation which helps to maintain and improve river water quality. Urbanization has led to migration of people from rural to peri-urban/urban settlements resulting to population increase and more effluents. Most people around River Njoro catchment use pit latrines for collection and disposal of wastes. The pit latrines can be inadequate or faulty hence others resort to open defecation accelerating pollution into the river. Treated discharges which may be of unacceptable qualities arising from settlements and industries also find their way into the river. Nevertheless, the river still serves the riparian communities with water for domestic purposes such as drinking as well as recreation. Fifty percent (50 %) of all illnesses reported at Njoro Health Centre have been linked to water contamination by bacterial pathogens. Therefore, water pollution in River Njoro poses a serious public health threat.

1.3 Objectives

1.3.1 General objective

To assess water quality of low order tropical stream passing through a human impacted catchment.

1.3.2 Specific objectives

(i) To determine physico-chemical variables along River Njoro including; total suspended solids, turbidity, dissolved oxygen, conductivity, total dissolved solids, pH, temperature, discharge, nitrates, nitrites, ammonium, total nitrogen, soluble reactive phosphorus and total phosphorus at different sampling sites along River Njoro.

(ii) To determine the concentrations of *E. coli*, intestinal enterococci and *Clostridium perfringens* at different sampling sites along River Njoro.

(iii) To determine antimicrobial resistance of *E. coli* isolates from different sampling points along River Njoro.

1.4 Hypotheses

(i) There is no significant difference in physico-chemical variables including; total suspended solids, turbidity, dissolved oxygen, conductivity, total dissolved solids, pH, temperature, discharge, nitrates, nitrites, ammonium, total nitrogen, soluble reactive phosphorus and total phosphorus between the sampling sites along River Njoro.

(ii) There is no significant difference in concentrations of *E. coli*, intestinal enterococci and *Clostridium perfringens* between sampling sites along River Njoro.

(iii) There is no significant difference in antimicrobial resistance of *E. coli* isolates between sampling points along River Njoro.

1.5 Justification

Rivers are subjected to climate change effects and are continuously affected by the anthropogenic influences from in-stream activities and also in the catchment area. As such, these dynamic ecosystems undergo continuous changes on local and global scales, meaning that water quality at any time is an expression of such phenomena. A good water quality monitoring programme is essential to constantly monitor health effects on our aquatic systems from time to time in order to strategize best management and suitable practices for the protection of water bodies and health of communities. Previous studies have attempted to assess the microbial water quality of the river but did not consider the influence of particular land uses in the Njoro catchment. There is need to beef-up such research efforts and come up with data that can help make concrete decisions in the protection of this resource. The frequent use of antibiotics for treatment of diseases or growth enhancement in humans and livestock can cause antibiotics to be ineffective. This study will build up on previous studies on the influence of land use on microbial populations in rivers. It will also be useful in understanding the health risks associated with faecal pollution due to human impacts and form a basis for evidence-based management strategies to reduce faecal contamination in River Njoro. Furthermore, the

UN 2030 agenda on Sustainable Development Goals (SDG) aims to have good health and wellbeing (SDG 3), clean water and sanitation for all (SDG 6). The management of faecal pollution is still a challenge in Kenya especially in rural areas since a substantial number of the population utilize surface waters directly for various domestic purposes. Any research efforts on issues of water and sanitation in individual countries to support achievement of these goals is a big boost to these agenda.

1.6 Definition of terms

Faecal coliforms	Gram negative bacteria that ferment lactose at a temperature of 35-37°C with production of gas, acid and aldehyde.
Escherichia coli	Thermophilic coliforms that produce indole from tryptophan. The bacterium lives in the intestines of warm blooded animals.
Faecal streptococci	Gram positive cocci bacteria, occurring in chains, non-spore forming and grows on selective media such as m-*Enterococcus* agar.
Enterococci	Subset of faecal streptococci that are capable of aerobic growth at 44±0.5°C.
Pathogens	Microorganisms that cause disease and in this study, waterborne illnesses.
Total coliforms	Gram negative, anaerobic or facultative anaerobic bacteria from the family Enterobacteriaceae that ferment lactose to produce acid and gas after 24-48 hours of incubation at 35 to 37°C. On solid medium they are expressed as CFU/100 mL.
Clostridium perfringens	Gram positive, spore forming, non-motile anaerobes, sulphite-reducing bacilli.
Antibiotic intermediate	A category defined by a breakpoint that include isolates with zone diameters within the intermediate range and for which response rates may be lower than for susceptible isolates.
Antibiotic susceptible	A category defined by a breakpoint that implies that isolate with zone diameters at or above the breakpoint are inhibited by achievable concentration of antimicrobial agent when the dosage recommended to treat the site of infection is used, resulting in likely drug efficacy.

Antibiotic resistant	A category defined by a breakpoint that implies that isolates with zone diameters at or below the resistant breakpoint are not inhibited by the usually achievable concentrations resulting in likely drug inefficacy.

CHAPTER TWO
LITERATURE REVIEW

2.1 Rivers as a source of livelihood

River water is an important source of livelihood for riparian communities. Many people from the rural and peri-urban depend directly on river water since they have no access to tap or borehole water particularly in developing countries (Mathooko *et al*., 2009). About 144 million people depend on surface water to meet their basic needs (WHO, 2017). Therefore, they make frequent visits to the river to obtain water for domestic purposes such as drinking and also to water their livestock. The demand for water is higher in dry seasons than in wet seasons and this has caused conflicts between communities. Approximately 2300 L of water is abstracted from a single point of a river per day (Mathooko *et al*., 2009).

2.2 Water quality and microbial risks from contaminated water

Contamination of water with bacterial pathogens is a public health concern. Exposure to waterborne bacterial pathogens occurs through ingestion or inhalation. Waterborne bacterial pathogens listed in Table 1 are capable of causing diseases even at low concentrations and can remain in water for a long period of time (Arnone *et al*., 2007). According to WHO (2011) there should be no bacteria in 100 mL sample potable water. This same reference also discusses other microbial risks emanating from consumption of contaminated water.

Table 1: List of waterborne diseases and their effects

Source: WHO (2011)

Pathogen	Disease	Effect
Bacteria		
Salmonella typhi	Typhoid fever	Typhoid fever, diarrhea, food poisoning, enteritis
Shigella spp.	Shigellosis	Bacillary dysentery
Campylobacter jejuni	Gastroenteritis	Acute diarrhoea
E. coli-pathogenic	Gastroenteritis	Diarrhoea, Urinary Tract Infections vomiting, meningitis, Crohn`s disease, Dysentery
Legionella spp.	Legionellosis	Acute respiratory disease
Leptospira interrogans	Leptospirosis	Fever, Jaundice
Vibrio cholera	Cholera	Heavy diarrhoea
Salmonella enterica	Salmonellosis	Diarrhoea

Yersinia enterocolitica	Yersiniosis	Diarrhoea
Protozoal		
Cryptosporidium parvum	Cryptosporidiosis	Diarrhoea
Giardia Lamblia	Giardiasis	Diarrhoea, Nausea
Entamoeba histolytica	Amebiasis	Diarrhoea
Viral		
Astroviruses	Gastroenteritis	Diarrhoea, Vomiting
Hepatitis A	Infectious hepatitis	Jaundice, Fever
Rotavirus	Gastroenteritis	Diarrhoea, Fever
Helminthic		
Dracunculus medinensis	Dracuncliasis	Fever, Vomiting
Schistosoma spp	Schistosomiasis	Diarrhoea

2.3 Indicators for determining bacteriological quality of river water

Transport of faecal pollutants to the environment comes from wastewater, sludge (human faecal origin), and/or slurry and manure (animal faecal origin). Through these media, high numbers and many types of bacteria enter water sources, soils and vegetation. It is not practically feasible to identify each pathogen that can be found in water due to the excessive costs, labour involved and very importantly that some pathogens cannot grow in culture media or are difficult to identify easily in culture media. Instead, one or more microorganisms are chosen to indicate the possible presence of pathogens in water (Ashbolt, 2015).

Many countries have been using faecal indicator bacteria, comprising total coliforms, faecal coliforms, *Escherichia coli*, intestinal enterococci and *Clostridium perfringens* as a monitoring tool to predict the probability of the presence of bacterial pathogens originating from faecal contamination and associated microbial risks (Haller *et al*., 2009; Savichtcheva & Okabe, 2006). According to Medema *et al*. (2003), microbial faecal indicators should be easy to isolate, identify, and enumerate, not multiply in the environment, inexpensive to test thereby permitting many samples to be taken, present in greater numbers than the pathogenic microorganisms, absent in unpolluted water and lastly should respond to natural environmental conditions and water treatment processes in a manner similar to the pathogens of concern.

Although some strains of *E. coli* are pathogenic (Anastasi *et al*., 2012), their occurrence in the environment does not necessarily result in the threat of disease. Nevertheless, their presence indicates faecal matter contamination (Haller *et al*., 2009). They have been found in higher concentrations in bed sediments and biofilms than in the water column (Kim & Carlson,

2007; Rehmann & Soupir, 2007). However, it is difficult to study bacteria attached on sediments since sediments are usually difficult to collect and analyze. Furthermore, land use has been linked to *E. coli* concentration in water column than in the sediment (Pandey *et al*., 2018).

Faecal indicator bacteria are typically used to demonstrate the potential presence or absence of groups of pathogens associated with wastewater and sewage sludge. *Escherichia coli* and intestinal enterococci are two representative organisms that have been used as indicators for faecal contamination (Kator & Rhodes, 2003). These bacteria have been used to investigate the spread of antibiotic resistance through waterborne transmission because they reside in gastrointestinal tract of warm-blooded animals. They are frequently exposed to antibiotics such as ampicillin, ciprofloxacin, gentamicin, and tetracycline which are used in treatment of both livestock and humans (Edge & Hill, 2005).

Escherichia coli belongs to the family Enterobacteriaceae described as facultative anaerobic, gram-negative bacteria and commonly found in the intestinal tract of livestock and humans (Sorum & Sunde, 2001). Epidemiological studies done in 1980s showed that concentration of *E. coli* in recreational waters was associated with sewage contamination correlated with prevalence of gastrointestinal illnesses. Thus, *E. coli* has been used widely as an indicator of faecal contamination (Edberg *et al*., 2000).

Intestinal enterococci are found in the gastrointestinal tract of healthy humans and livestock. They are Gram-positive facultative anaerobic bacteria, spherical, which occur singly, in pairs or short chains (Ciftci *et al*., 2009). *Enterococcus faecalis* represent 80-90 % of human clinical enterococcal infections, whereas 5-15% are caused by *E. faecium*. Common infections caused by intestinal enterococci include those of the urinary tract, bloodstream, endocardium and wounds (Shepard & Gilmore, 2002). Enterococci can survive under unusually wide ranges of temperatures, pH, and salinity, as well as resisting the bactericidal effects of detergents such as bile salts (Flahaut *et al*., 1996). Majority of clinical intestinal enterococci infections in humans are associated with *Enterococcus faecalis* and *Enterococcus faecium* (Mundy *et al*., 2000). They are of major significance in decision making about bacteriological quality of any source of water especially when *E. coli* detection is negative (Maraccini *et al*., 2016).

Whitlock *et al*. (2002) effectively used faecal coliforms as an indicator to identify the contaminant sources in an urban watershed, while Maul and Cooper (2000) used both enterococci and faecal coliform bacteria concentrations to assess variation in water quality in an agricultural field. Similarly, Islam *et al*. (2017) used both intestinal enterococci and faecal

coliform bacteria concentrations to assess variation in river water quality hence the selection of these organisms in this study.

Not all bacterial indicators currently used meet all ideal criteria established for water quality. Difficulties related to conventional faecal indicators could be partly avoided by using alternative biological and chemical faecal indicators including faecal anaerobes bacteriophages such as coliphages (Mcminn *et al*., 2017). Faecal anaerobes account for a significant portion of faecal bacteria (Matsuki *et al*., 2002) and are limited to warm-blooded animals (Franks *et al*., 1998). The disadvantage of faecal anaerobes as indicators is the short survival in non-host environments due to their low oxygen tolerance. The degree of their tolerance to atmospheric oxygen is a genus related characteristic (Avelar *et al*., 1998).

The need to maintain anoxic conditions for cultivation, isolation and biochemical identification limits the usage of anaerobic *Bacteroides* as faecal indicator. However, the increasing use of molecular methods overcomes this problem. Since certain *Bacteroides* are highly host-specific, it is possible to identify the source of faecal contamination by tracking host-specific *Bacteroides* (Simpson *et al*., 2004). Avelar *et al*. (1998) documented that *Bacteroides* could survive for up to 6 days under oxygen stress conditions. Human-specific *Bacteroides* marker could persist in freshwaters for up to 24 days at 4 and 12°C, and up to 8 days at 22°C, indicating high possibilities of being detected after a discharge event (Santiago-Rodriguez *et al*., 2013). A study done in Virginia tidal creeks by Kator and Rhodes (1999) showed that *Bifidobacterium spp* was no longer detectable after 5-9 days in water at 23 and 30°C. Their relatively short survival time is presently a problem in terms of their recovery. High background levels of predators and Gram-positive rods and cocci could prevent growth and/or detection of *Bifidobacterium spp* in the aquatic environment. They also concluded that environmental conditions that are not conducive for their persistence could limit their detection.

Clostridium perfringens produces spores, which are resistant to environmental stress and last longer than other indicator bacteria such as faecal coliforms and faecal streptococci (Horman *et al*., 2004). *C. perfringens* has the advantage of having an extended viability, a wide distribution in aquatic sediments, and the potential to be used when other indicators are unavailable hence suitable for detecting remote pollution. Spores of *C. perfringens* can be detected even in long distance from contamination sites, indicating remote or old faecal pollution (Desmarais *et al*., 2002). Furthermore, their concentrations vary among different animal species (Sorensen *et al.*, 1989). Just like other alternative faecal indicators, *C. perfringens* standards have not yet been evaluated based on epidemiological studies on the acceptable risk associated with faecal pollution. They have also not been adopted since their

distribution in the environment is limited or the methods of recovering them are very complex and have low tolerance to oxygen (Leclerc *et al*., 2001).

Ribonucleic acids (RNA) coliphages are found in livestock and human faeces hence they can be used to predict source of faecal pollution from domestic sewage (Scott *et al*., 2002).The presence of F-specific RNA bacteriophage in water used to be an indicator of sewage pollution (Osawa *et al*., 1981). As a matter of fact, presence of SARS-CoV-2 RNA has been proposed as a very effective tool for wastewater based epidemiological (WBE) studies since it is difficult to test all members of community for coronavirus disease of 2019 (COVID-19) (Prado *et al*., 2021). However, methods of concentration and recovery from water bodies are complex hence they are not frequently used.

Heterotrophic plate count is used as indicator of organic pollution especially from domestic effluents. The method estimates the concentrations of live heterotrophic bacteria in water. High concentrations of heterotrophic bacteria results to high biological oxygen demand and low dissolved oxygen concentration (APHA, 2005).

2.4 Methods for testing coliforms in water

2.4.1 Most Probable Number (MPN) method

This method is also called multiple fermentation tube test method. The details of the method are described in APHA (2005).The test is conducted in three steps, presumptive test, confirmed test and completed test. Positive results are indicated by gas production in inverted Durham tubes and acid production (yellow colour) using phenol red as pH indicator in lactose broth growth medium. The tubes showing acid and gas production in each dilution series are counted and recorded. The numbers estimated as MPN per 100 mL from McCrady's statistical tables. This method is suitable even for highly turbid samples as long as suitable dilutions are made. The disadvantages of this method are; it is not precise, requires a lot of time to perform since it requires 48 hours for presumptive results and it is tedious (Rompré *et al*., 2002).

2.4.2 Membrane filtration method

In this method, the volume of sample to be filtered depends on the source of water. The details of the method are found in APHA (2005). Between 0.1-100 mL of sample is filtered through a sterile filtration unit that consist of a sterile, gridded, filter paper. The filter membrane is then put on a petridish containing suitable culture medium followed by incubation at appropriate temperature. After incubation the colonies are counted and expressed as Colony Forming Units (CFU) per 100 mL. However, it is not suitable for very turbid samples due to high loads of particles that easily block the filter paper (APHA, 2005). The advantages of membrane filtration method over MPN include; more sensitive and reliable, enumeration of

coliforms is quantitative unlike MPN which is semi-quantitative and the method is simpler than MPN. The disadvantage of this method is that a confirmatory test is needed which requires additional 24 hours after the first incubation period (Rompré *et al*., 2002).

2.5 Antibiotic resistance characterization

Although culture-independent approaches have been used, determination of prevalence values and resistance patterns is more frequently based on culture-dependent methods (Czekalski *et al*., 2012). Culture based methods such as membrane filtration techniques are used for enumeration on *E. coli* and intestinal enterococci in water (Rompré *et al*., 2002). The technique is adapted to frequently isolate bacteria for further characterization of antibiotic resistance. Mostly for this reason, the microbiological indicators of water quality coliforms and intestinal enterococci are frequently the major targets of such analyses (APHA, 2005). After purification, isolates can be identified and typed for their antibiotic resistance patterns, allowing the calculation of resistance rates.

Although culture-based methods are laborious and time consuming (Ferreira da Silva *et al*., 2007) they produce good results. It involves use of selective culture media supplemented with antibiotics at concentrations similar to or above those reported as inhibitory for the target bacteria (Figueira *et al*., 2011). In this case, the percentage of resistance is estimated as the ratio between the number of bacteria growing in the presence and in the absence of antibiotic (Novo & Manaia, 2010). Watkinson *et al*. (2007) proposed this method to estimate the prevalence of resistance of *E. coli* to ampicillin, tetracycline, ciprofloxacin and sulfamethoxazole, and emphasized it`s great potential as a representative for assessment of antibiotic resistance in *E. coli* using many samples. Novo and Manaia (2010) adapted the same method to assess the resistance prevalence to amoxicillin, tetracycline and ciprofloxacin in heterotrophs, enterobacteria and intestinal enterococci, since it was a feasible approach to compare the resistance loads in the inflow and outflow of three wastewater treatment plants.

2.6 Microbial source tracking methods

Several studies have been published on various methods for distinguishing between human and non-human sources of faecal pollution in water. These methods include; use of bacteriophages (somatic coliphages and F-specific RNA bacteriophage) (Duran *et al*., 2003; Muniesa *et al*., 2012), faecal coliform to faecal streptococci ratios (Sinton *et al*., 1998) and use of genetic markers from faecal *Bacteroides* that are specific to faecal bacteria (Sauer *et al*., 2011). Patterns of antibiotic resistance are also used to identify source of bacteria from human or animal origin. This approach assumes that human faecal bacteria will have a higher resistance to specific antibiotics than animal faecal bacteria. Isolates of faecal streptococci or

E. coli are grown in different concentrations of antibiotics (Hager, 2001). It is still not clear on the number of isolates that are required to represent a catchment. However, sample level analysis is used when a sample is obtained from a single source even though it is not possible that a single sample source can represent the entire catchment. Nonetheless, isolate level analysis can be used when a sample is contaminated by many sources (Wiggins *et al*., 1999). Previously, *E. coli* and intestinal enterococci ratios have been used to tract source of water faecal pollution whereby ratios greater than 4 indicated human source while ratios less than 0.7 indicated faecal pollution by animals (Sinton *et al*., 1998). Gene markers are currently used as modern methods of tracking faecal contaminants (Donde *et al*. 2018)

2.7 Sources of antibiotics and antimicrobial resistance in natural ecosystems

Antibiotics have been extensively used in the environment to treat or prevent infections in both human and animals hence they circulate in the environment (Figure 1). Frequent use and exposure to antibiotics can result to selection pressure in that bacteria that are sensitive to antibiotics undergo mutational or genetic change making them to grow and survive as antibiotic resistant bacteria due to presence of antibiotic resistant genes (ARG) (Martínez, 2009). Genetic materials like plasmids and integrons that contain ARG, transform, conjugate or transduct through horizontal gene transfer (Martínez *et al*., 2015).

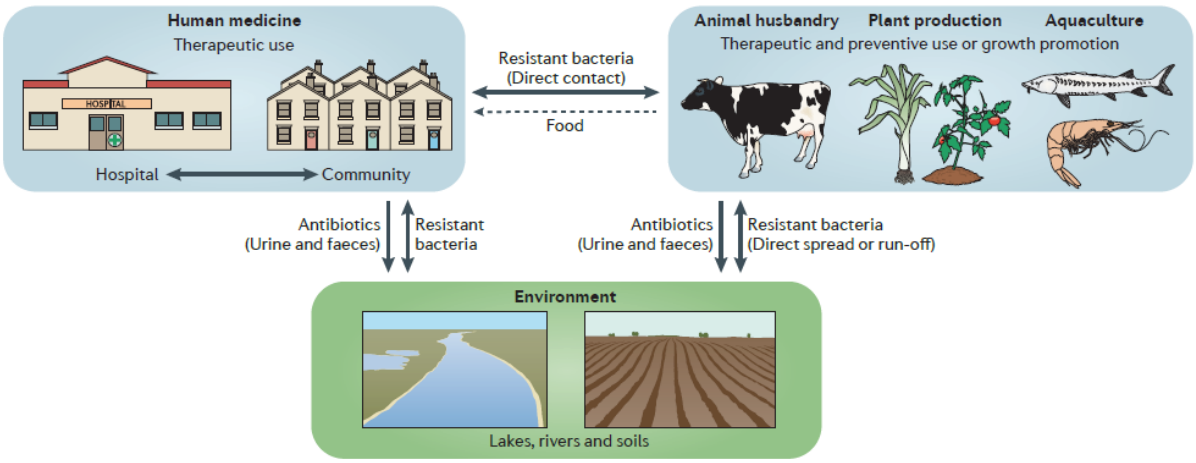


Figure 1: Sources of antibiotics, their use and how they are cycled in the environment
Source: Andersson and Hughes (2014)

Antibiotics are not fully metabolized and end up discharged with excreta, sometimes up to 90% of original antibiotic compound as shown in Table 2 (Dolliver & Gupta, 2008; Kumar *et al*., 2005). This could result to accumulation of antibiotics in the system, increasing the risk for development of antimicrobial resistance in the environment and subsequent transfer to human and zoonotic hosts. The natural process of degradation of antibiotics depends on pH,

temperature, moisture and microbiota. It involves, chemical degradation, photodegradation and biodegradation. The use of antibiotics selects resistant microorganisms (Livermore, 2005).

Table 2: Human prescription amounts and excretion rates of commonly used antibiotics and some used in the past

Source: Kumar *et al*. (2005).

Antibiotic compound	Daily dose (mg)	Excretion of original compound (%)
Amoxicillin	750-2250	80-90
Ampicillin	3000-6000	30-60
Penicillin V	2000	~ 40
Penicillin G	240-720	50-70
Sulfamethaxole	400-1600	~15
Trimethroprim	80-360	~ 60
Erythromycin	200-1000	> 60
Roxithromycin	150-300	> 60
Clarithromycin	125-250	> 60
Chloramphenicol	-	5-10
Chloratetracycline	-	> 70
Tetracycline	-	80-90
Minocycline	100-200	~ 60
Oxytetracycline	-	> 80
Doxycycline	100-200	> 70

Antimicrobials are used in agriculture to promote growth, increase productivity and for prophylaxis (Cogliani *et al*., 2011). In integrated agriculture where, aquaculture is sustained by livestock wastes, the risk of exposure to antibiotics of humans, animals and environment increases (Kim *et al*., 2013). During storms or irrigation, the antibiotics are washed away by surface run off and end up in aquatic systems (Kümmerer, 2004). Wastewater treatment plants are also sources of antibiotic resistant bacteria (Yamashita *et al*., 2017). Watkinson (2007) reported that sites close to wastewater treatment plants had higher concentration of antibiotic resistant *E. coli*. Seemingly, human wastes (urine and faeces) from hospitals contain unmetabolised antibiotics as well as resistant bacteria (Finley *et al*., 2013).

Gram negative bacteria have higher level of resistance to antibiotics than gram positive bacteria. They have an impermeable cell wall that inhibits easy penetration of antibiotics into

the cell (Ruppé, 2015; Sohlenkamp & Geiger 2016). It is worthwhile to note that *E. coli* has been used to determine antimicrobial resistance in the environment and their continued spread of antibiotic resistant genes is a public health risk (Nnadozie & Odume, 2019; Huijbers *et al*., 2020). It has been documented that resistance in enterobacteriaceae is more common in rivers than lakes (Nnadozie & Odume, 2019).

Antibiotic resistance genes are transferred from a resistant bacteria to a susceptible bacteria either through horizontal or vertical gene transfer. Vertical gene transfer is where resistant gene is transferred form the parent to the offspring (El-Demerdash *et al*., 2018; Dodd, 2012). Horizontal gene transfer occurs through transduction (transfer of resistant gene by a phage), conjugation (transfer of resistant gene through cell to cell contact) or transformation (uptake of resistant extracellular DNA and genes directly from the environment) (Vikesland *et al*., 2017).The fate of antibiotic resistant bacteria in the environment depends on oxygen, temperature, pH, organic content and concentration of the antibiotics in the environment (Gullberg *et al*., 2014).

Antimicrobials such as tetracycline, streptomycin, and β-lactams are commonly used for treatment of infections caused by *E. coli* (Theobald *et al*., 2019). Previous studies have reported *E. coli* resistance to streptomycin (88.25%), ampicillin (91.25%) and tetracyclines (95.25%), sulphamethaxole (100%), amoxicillin (59%) (Titilawo *et al*., 2015; Azad *et al*., 2019; Theobald *et al*., 2019). Another study by Itotia *et al*. (2018) found multidrug resistant *E. coli* and this was linked to surface run off from an agricultural land. The authors found *E. coli* isolates were resistant to tetracycline (71%), ampicillin (81%), streptomycin (33%) and chloramphenicol (43%).

Antimicrobial resistance leads to mortality, prolonged treatment due to ineffectiveness (Berendonk *et al*., 2015; Sanganyado & Gwenzi, 2019). Restrictions to use drugs used in treatment of infections caused by gram negative bacteria has shown to reduce the spread of antibiotic resistant bacteria (Chalmers *et al*., 2017). Use of bacteriophages instead of antibiotics has been shown to reduce antibiotic resistance (Ghosh *et al*., 2019).

2.8 Factors influencing the growth and survival of *E. coli* and other microorganisms

Faecal Indicator Bacteria (FIB) are sensitive to stress therefore, their survival rate in water depends on physico-chemical conditions. In some studies, TSS positively correlated with FIB (Huey & Meyer, 2010) while a study done in Neuse river estuary TSS and FIB had no significant correlation (Fries *et al*., 2006). This implies that the relationship between TSS and FIB varies with different characteristics of catchments.

Turbidity and Dissolved Oxygen influence the effect of UV on FIB. High turbidity reduces light penetration in water column (Bolton *et al*., 2010). Inactivation of microorganisms by UV is important since it reduces the cost of water treatment by lowering their survival rate. Dissolved Oxygen has a direct relationship with sunlight inactivation of FIB. An increase in Dissolved Oxygen could result to photo oxidative damage of the microorganism hence lowering their survival rate (Christensen & Linden, 2003).

E. coli are thermotolerant and can grow in temperatures ranging between 7 to 66 °C (Jones *et al*., 2004). A study by Byamukama *et al*. (2000) revealed that *E. coli* survived at a temperature range between 23 to 26 °C. This characteristic makes them most suitable faecal indicators since they can survive in diverse habitats. As temperature decreases in surface waters, the survival rate of FIB increases (Medema *et al*., 2003).

Nutrient availability influences bacterial morphology and cell size thereby increasing their vulnerability or resistance to predation. Excess nutrients can cause bacteria to form filaments that are inedible to grazers (Matz & Jurgens, 2003). On the other hand, nutrient limitation can cause bacteria to form small cells making them easily fed on by protozoan grazers. However, formation of filaments on the cells increases their resistance to grazing hence increasing their survival (Corno & Jurgens, 2006). Faecal coliforms and Enterococci significantly correlated with ammonium in a study that was done in Febros River in Portugal (Cabral & Marques, 2006). These correlations were attributed to breakdown of organic matter and ammonification by bacteria.

Escherichia coli interacts with other micro-organisms in all natural habitats. It can be predated by protozoa and lysed by phages. These two biological mechanisms have been reported to be responsible for up to 70 % of the faecal indicator bacteria removal in river water (Korajkic *et al*., 2014) and in biological processes of sewage treatment (Wu *et al*., 2019).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area

3.1.1 Location

The River Njoro catchment is located at 0° 30′ S, 35° 20′ E in the Rift Valley, Kenya (Shivoga *et al*., 2007). It is approximately 50 Km long, flowing from the eastern Mau escarpment. It lies at an altitude of 1759-3000 m above sea level (Aera *et al*., 2019) and drains into Lake Nakuru (Figure 2).

3.1.2 Climate and land use

The region experiences trimodal rainfall pattern. Long rains occur from April to May, with a small peak in August while short rains occur from November to December. The average rainfall per year is 939 mm with an atmospheric temperature ranging between 9 to 4°C (Baldyga *et al*., 2008). River Njoro buffer strip is approximately 5-20 m on each side of river channel. The predominant land use types in upper zones of the river are moorland and forest plantations (Mathooko & Kariuki, 2000). The middle and lower sections of the river are characterized by small, medium and large scale agriculture and settlements (Lelo *et al*., 2005).

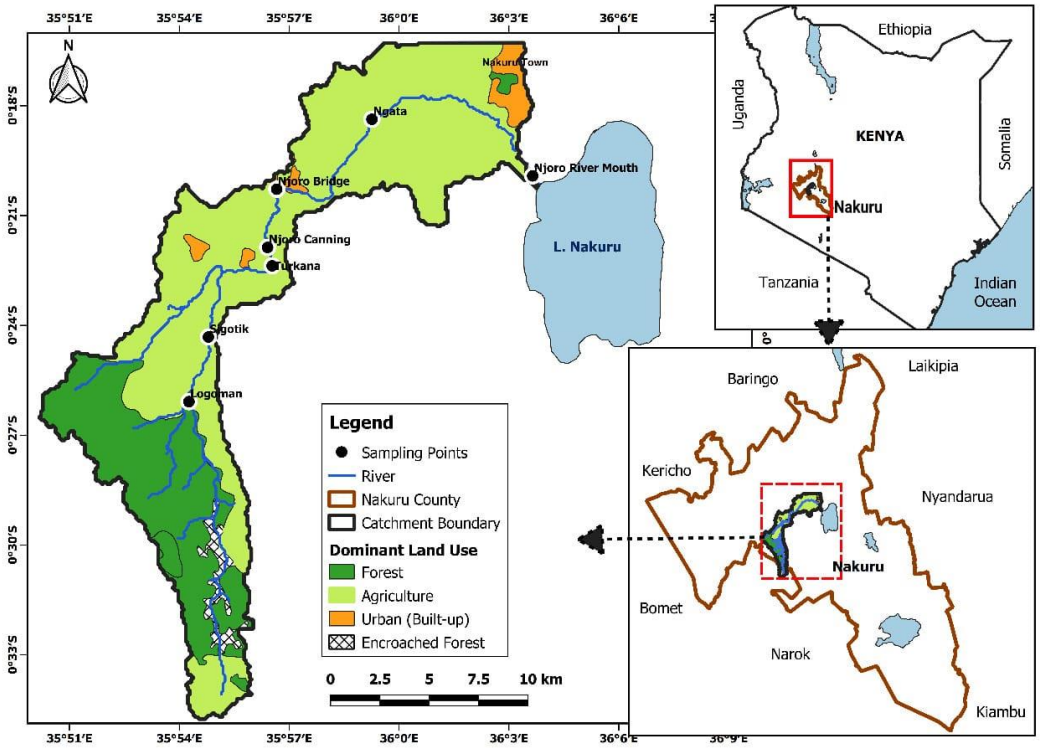


Figure 2: A map showing location of River Njoro catchment and sampling points
Source: Drawn using QGIS courtesy of hassaeed2@gmail.com

Selection and description of the sampling sites

Sampling sites were selected based on land use types and accessibility; forest, mixed (forest and agriculture), agriculture, industrial and urban as described below;

(i) Logoman

This was the most upstream site located at S 00° 26' 05.25" (longitude) and E 35°54' 15.8" (latitude), altitude of 2513 m above sea level. The site was a rehabilitated forest with pure stands of cedar. This site was minimally disturbed compared to other sites. Traces of cow pat were found implying that livestock accessed the river at this site (Plate 1).

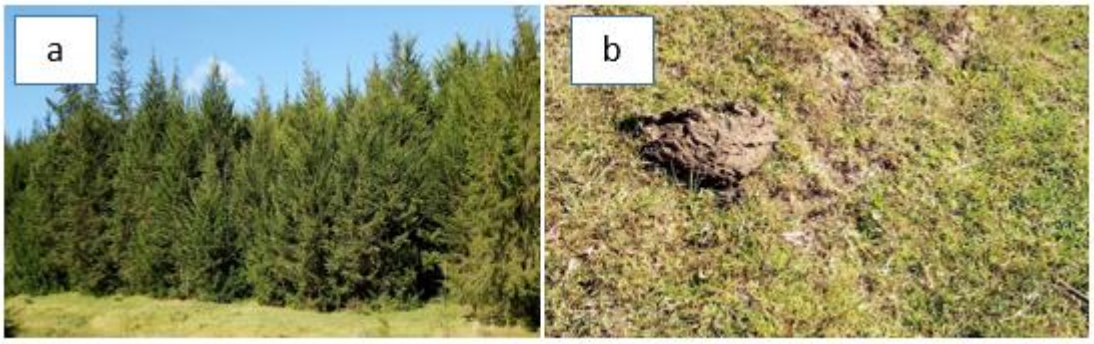


Plate 1: Photographs showing (a) Logoman forest (b) trace of cow pat at Logoman

(ii) Sigotik

This was the second sampling site located S 00° 24' 19.2" (longitude) and E 35° 54' 47.6" (latitude), altitude of 2413 m above sea level. This site was slightly disturbed than Logoman site. The site had mixed land use (forest and agriculture). There were grazing livestock and maize plantations on the riparian zones. Visits by people at this site to obtain water for domestic use was observed during sampling sessions (Plate 2).

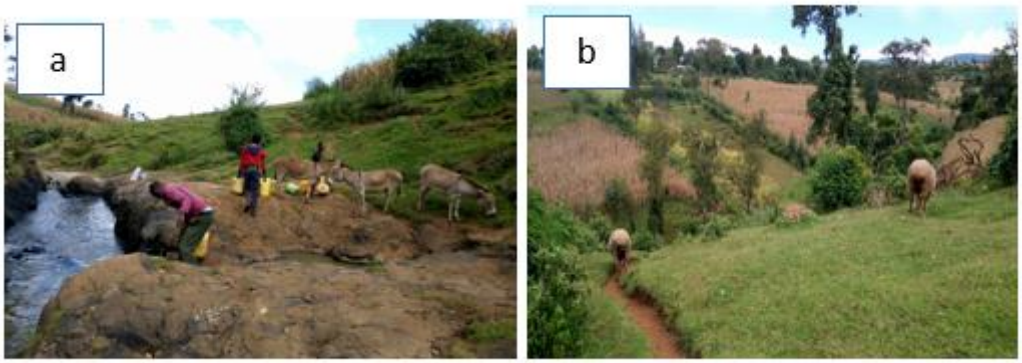


Plate 2: Photographs taken at Sigotik showing (a) water abstraction (b) sheep grazing

(iii) Turkana

This was the third sampling site located at S 00° 22' 22.5" (longitude) and E 35°56' 24.6" (latitude), altitude of 2220 m above sea level. The dominant land use at this site was agriculture. Visits by people to water their livestock directly in the river, to wash clothes and to obtain water for domestic use were observed during sampling sessions (Plate 3).

Plate 3: Photographs taken at Turkana showing (a) laundry (b) cattle watering and (c) water abstraction

(iv) Canning

This was the fourth sampling site located at S 00° 21' 52.3" (longitude) and E 35° 56' 24.6" (latitude), altitude of 2190 m above sea level. The riverbanks were fairly vegetated and there was a food manufacturing industry (Njoro Canning factory) on the riparian zone. This site was visited by people to water their livestock directly in the river consequently disturbing the sediments (Plate 4).

Plate 4: A photograph taken at Canning showing channel modification to create pools for watering livestock

(v) Njoro Bridge

This was the fifth sampling site located at S 00° 20' 16.9" (longitude) and E 35° 56' 39.6" (latitude), altitude of 2148 m above sea level. The dominant land use at this site was agriculture. Livestock watered directly in the river channel hence disturbing the sediments. Visits by people to obtain water for domestic use, to wash clothes and to clean motorcycles

was observed at this site. Pools at this site were used by children for recreation activities (swimming) (Plate 5).

Plate 5: Photographs taken at Njoro Bridge showing (a) cattle watering and (b) recreation

(vi) Ngata

This was the sixth sampling site located at S 00° 18' 22.2" (longitude) and 35° 59' 15.6" (latitude), altitude of 2048 m above sea level. The dominant land use at this site was agriculture. Livestock watered directly in the channel hence disturbing the sediments. Visits by people to obtain water for domestic use and to do laundry was observed at this site. (Plate 6).

Plate 6: Photographs taken at Ngata showing (a) cattle watering and (b) laundry

(vii) River Mouth

This was the last sampling site located at S 00° 19' 37.3" (longitude) and E 36° 03' 53.7" (latitude), altitude of 1789 m above sea level. The dominant land use at this site was urban. Nakuru old town sewage treatment plant was located approximately 100 m from this site. The sewage treatment plant treats effluents from Nakuru town before discharging to River Njoro. No livestock were found watering or grazing at this site during the sampling sessions. Solid wastes, organic matter and fish kills were found at this site (Plate 7).

Plate 7: Photographs taken at River Mouth showing (a) decomposed Nile tilapia and (b) solid wastes and organic matter

3.2 Study design

3.2.1 Sample collection

Sampling was done every Tuesday from end of November 2020 to mid - January 2021 between 8:00 a.m. and 2:00 p.m. Sampling began upstream at Logoman, which was the reference site and ended downstream at River Mouth draining into Lake Nakuru (Figure 3).

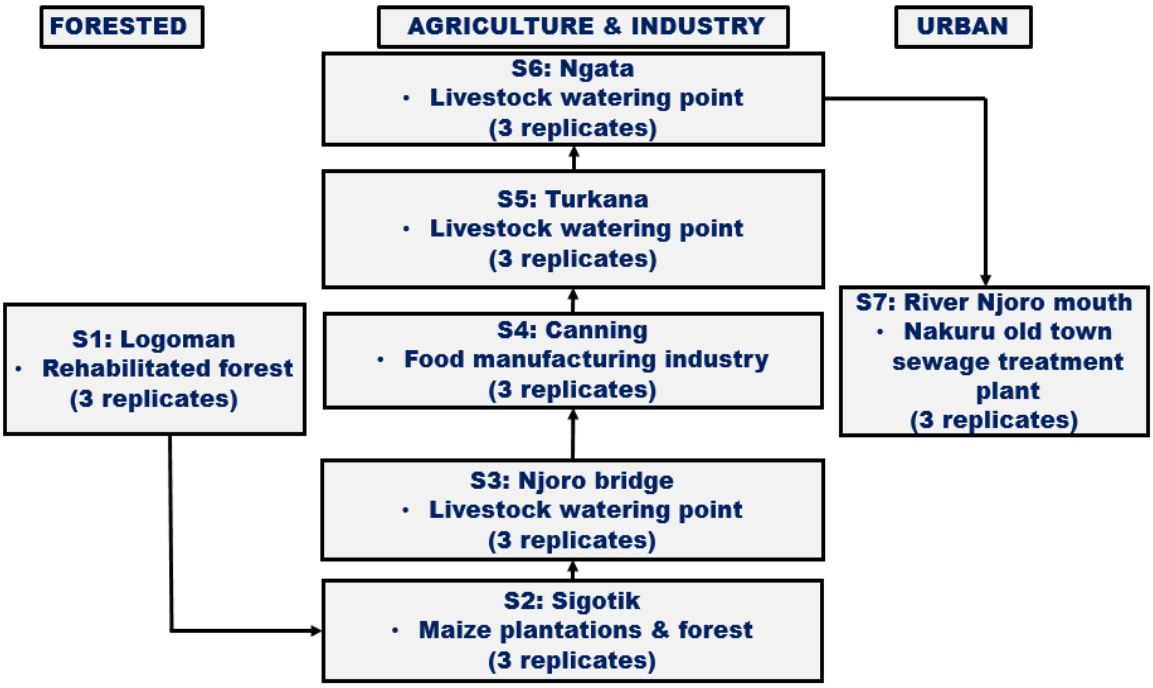


Figure 3: Sampling layout of River Njoro

A total of 168 river water samples (7 sites, 3 samples per site for 8 sessions) samples for nutrients and Total Suspended Solids (TSS) analysis were collected in the middle of the river channel at every site using 1 Litre acid washed bottles while, for bacteriological analysis were collected using 250 mL sterile nalgene bottles, at the surface to avoid disturbing the

stream bed. All the samples were stored in a cool box and transported to the LWM lab at Egerton University for analysis within 8 hours.

a) Determination of *in situ* physico-chemical variables

Electrical conductivity, pH and temperature were measured using a HACH HQ 40d meter, dissolved oxygen was measured using a HACH HQ 30d meter. Turbidity was measured using a HACH HQ 11d meter at every sampling site. The probes were rinsed with distilled water after use in each site. All measurements were recorded in triplicate.

b) Determination of river discharge

Velocity area method was used to determine river discharge at each sampling site. A portable automatic flow metre (Flo-Mate, model 2000, Marsh McBirney) was used to measure the average water velocity at 60% water depth across the river channel. Discharge was then calculated according to the formula by Wetzel (2001) as indicated in equation 1.

$$Q = \sum VA \quad (1)$$

Where;

Q = Discharge (m^3/s)

V = Mean current velocity (m/s)

A = Cross-sectional area of the river channel (m^2)

3.2.2 Nutrients and TSS analysis

a) Determination of nitrogen and phosphorus in water samples

Different forms of nitrogen were determined; Ammonium-Nitrogen (NH_4-N), Nitrate-Nitrogen (NO_3-N), Nitrite-Nitrogen (NO_2-N) and Total Nitrogen (TN). Ammonium Nitrogen (NH_4-N) was determined by adding 2.5 mL of sodium-salicylate solution and 2.5 mL of hypo chloride solution to 25 mL of filtered water samples. The samples were incubated in the dark for 90 minutes after which their absorbance ere read at a wavelength of 665nm using GENESYS ™ 10S UV-Vis spectrophotometer. The final concentration of NH_4-N was calculated from equation generated from standard calibration curve. NO_3-N was determined using sodium-salicylate method, where 1 mL of freshly prepared sodium salicylate solution was added to 20 mL of filtered water sample. The processed samples were placed in the oven and evaporated to complete dryness at 95°C. The resulting residue was dissolved using 1 mL H_2SO_4, followed by addition of 40 mL of distilled water and 7 mL potassium-sodium hydroxide-tartarate solution respectively and read at a wavelength of 420 nm. The concentration of NO_3-N was calculated from linear equation generated from NO_3-N standard calibration curve. Nitrite-nitrogen (NO_2-N) was determined using the sulphanilamide method. 1 mL of sulphanilamide solution was added to 20 mL of filtered water samples. The samples

were left for 8 minutes then 1 mL of N-Naphthyl-(1)-ethylendiamine-dihydrochloride solution was added and left to settle for 10 minutes. The absorbance was read at a wavelength of 540 nm. The concentration of NO_2-N was calculated from linear equation generated from NO_2-N standard calibration curve (APHA, 2005).

Total Nitrogen (TN) was determined through persulphate digestion where 1 mL of warm potassium persulphate was added to 25 mL of unfiltered water sample to convert the nitrogen forms into ammonium. The samples were autoclaved for 90 minutes at 120°C and 1.2 atm. After digestion, the total reduced forms into ammonium was analysed using sodium-salicylate method. The concentration of TN was calculated from the linear equation generated from TN standard calibration curves (APHA, 2005).

Soluble Reactive Phosphorus (SRP) was analysed using the ascorbic acid method. Water samples were filtered using Whatman GF/C, pore size 0.45µm. The following chemicals including; ammonium molybdate solution (A), sulphuric acid (B), ascorbic acid (C) and potassium antimony tartrate solution (D) were mixed in a ratio of A:B:C:D= 2:5:2:1. 2.5 mL of resulting solution was added to 25 mL of filtered water sample. The absorbance was read after 15 minutes at 885 nm wavelength using GENESYS ^{TM} 10S UV-Vis spectrophotometer. The concentration of SRP was calculated from linear equation generated from SRP standard calibration curve (APHA, 2005).

Total phosphorus (TP) was determined by persulphate digestion of unfiltered water to reduce the forms of phosphorus present into SRP. 1mL of warm potassium persulphate solution was added to 25 mL of water samples followed by autoclaving for 90 minutes at 120°C and 1.2 atm. After digestion, TP was analysed as SRP using ascorbic acid method. The concentration of TP was calculated from linear equation generated from TP standard calibration curves (APHA, 2005).

b) Determination of nutrients loading rates

Phosphorus and Nitrogen loading rates at every site was calculated using the formula by Kitaka (2000) as indicated in equation 2.

$$Nutrient\ loading/loss\ = Discharge\ x\ nutrient\ concentrations\ x\ 0.0864 \quad (2)$$

Where;

Nutrient loading or loss expressed as (Kg/day)

Discharge expressed as (L/s)

Nutrients concentration expressed as (mg/L)

0.0864 = concentration time conversion factor from mg/day to Kg/day

c) Determination of TSS

Total Suspended Solids (TSS) was estimated gravimetrically on glass-fibre filters (Whatman GF/C filters, pore size 0.45µm) (APHA, 2005). Between 300-500 mL of water sample was filtered using pre-weighed Whatman GF/C filter and then dried at 95°C to a constant weight. The total suspended solids was estimated according to the formula by Wetzel (2001) as indicated in equation 3.

$$TSS = ((Wc - Wf)x10\text{^}6))/V \quad (3)$$

Where;

TSS = Total Suspended Solids (mg/L)

Wf = Weight of pre-combusted filter (g)

Wc = Constant weight of filter + residue (g)

V= Volume of water sample (mL)

3.2.3 Determination of bacteriological indicators

a) Enumeration of coliform bacteria

The procedure described in APHA (2005) was used. A preliminary sampling and river water analysis was done to determine the volume of sample to be filtered for every site. 1 mL, 5 mL, 10 mL of river water sample from each site was filtered then filter papers were placed on chromocult coliform agar (Merck, Germany) and incubated at 37°C for 24 hours. After 24 hours, colonies in each petridish were counted. The chosen volume to be filtered for every site was based on the principle that the number of colonies counted in each petridish should not be less than 20 and should also not exceed 300 since they would be too numerous to count.

10 mL (forest and mixed land use), 5mL (agriculture, industrial and urban land use) of water sample was put aseptically into a sterile stainless-steel stainless multichannel apparatus (Plate 8) containing sterile gridded membrane filter of 0.45 µm pore size and 47 mm (whatman) diameter in a funnel.

Plate 8 : Photographs showing (a) vaccum pump and (b) multichannel filtration unit

The filters were removed carefully from the funnel immediately after filtering using a pair of sterile forceps then placed on a petridish with chromocult coliform agar (Merck, Germany), a

selective and differential medium for coliforms. The plates were incubated at 37°C for 24 hours. Dark blue colonies were identified as *Escherichia coli* while pink colonies were identified as non-faecal coliforms according to manufacturer`s instructions. Colonies were counted using a FISHER ACCU- LITE COLONY COUNTER MODEL 133-8002A. Total coliforms (TC) were obtained by adding pink colonies and dark blue colonies. Final results were calculated using equation 4.

$$CFUs\ per\ 100\ mL = ((No.of\ colonies\ counted)/(volume\ filtered))x100 \quad (4)$$

Where;

100 is the standard volume for reporting colony counts (mL)

CFUs are the colony forming units

Presumptive *E. coli* were isolated from chromocult coliform agar media using sterile wire loop and stored in nutrient agar slants (Plate 9) for indole, methyl red, Voges-Proskauer and Citrate (IMVIC) biochemical tests.

Plate 9: A photograph showing nutrient agar slants for growing and preserving cultures

Biochemical test for confirmation of *E. coli*

The IMVIC biochemical test described by Cheesbrough (2000) was used. Each letter of IMViC stands for an individual test: I - Indole production test; M - Methyl red test; V - Voges-Proskauer test and C - Citrate utilization as described below;

Indole test: A wire loop was used to inoculate overnight growth cultures in a test tube containing 5 mL of peptone water. The inoculation was incubated at 37°C for 24 hours then 5 drops of Kovac`s indole reagent was added and shaken gently. Development of a red layer showed a positive test.

Methyl Red-Voges-Proskauer: *E. coli* isolates were grown in Methyl Red-Voges-Proskauer broth and incubated at 35°C for 48 hours. 1 mL of the broth was transferred into a test tube then 2 drops of methyl red was added. Formation of yellow colour indicated negative test while a formation of red colour indicated positive test. 15 drops of 15% alpha-napthol was added to

the remaining broth. 5 drops of 40 % potassium hydroxide was added then shaken gently. The cap of the test tube was loosened and the tube was observed after one hour. No colour change indicated a negative test while development of a red colour indicated positive test.

Citrate test: The isolates were inoculated on simmon`s citrate agar in a bijou bottle followed by incubation for 48 hours. Formation of a deep blue colour indicated a positive reaction.

b) Enumeration of intestinal enterococci

The procedure described in APHA (2005) was used. A preliminary sampling and river water analysis was done to determine the volume of sample to be filtered for every site. 1 mL, 5 mL, 10 mL of river water sample from each site was filtered, filter papers were placed on m-enterococcus agar (Difco, USA) and incubated at 37°C for 24 hours. After 24 hours, colonies in each petridish was counted. The chosen volume to be filtered was based on the principle that the number of colonies counted in each petridish should not be less than 20 and should not exceed 300 since they would be too numerous to count.

An aliquot of 10 mL (forest and mixed land use), 5mL (agriculture, industrial and urban land use) of sample was put aseptically into a sterile stainless-steel filtration multichannel apparatus (Plate 8) containing sterile gridded membrane filter of 0.45 μm pore size and 47 mm diameter (Whatman) in a funnel. The filters were removed immediately from the funnel after filtering using a pair of sterile forceps and placed on a petri dish with m-*Enterococcus* agar (Difco, USA), a selective and differential medium, and incubated at 37°C for 24 hours. Red/Maroon colonies were identified as intestinal enterococci. The colonies were counted using a FISHER ACCU-LITE COLONY COUNTER MODEL 133-8002A. Final results were calculated using equation 5.

$$CFUs\ per\ 100\ mL = ((No.\ of\ colonies\ counted)/(volume\ filtered))x100 \quad (5)$$

Where;

100 is the standard volume for reporting colony counts (mL)

CFUs are the colony forming units.

c) Enumeration of *Clostridium perfringens*

The procedure described in APHA (2005) was used. A preliminary sampling and river water analysis was done to determine the volume of sample to be filtered for every site. 1mL, 5 mL, 10 mL of river water samples from each site was filtered then filter papers were placed on Tryptose Sulphite Cycloserine agar (Merck, Germany) and incubated at 44°C for 24 hours. After 24 hours, colonies in each petridish was counted. The chosen volume to be filtered for each site was based on the principle that the number of colonies counted in each petridish

should not be less than 20 and should not exceed 300 since they would be too numerous to count.

10 mL of sample from each site was filtered through a membrane filter of 0.45 µm pore size and 47 mm diameter (Whatman) on a sterile stainless-steel filtration multichannel apparatus (Plate 1) then filter papers were placed on Tryptose Sulphite Cycloserine (TSC) agar (Merck, Germany) plates. The plates containing filters were put in an anaerobic jar with anaerocult strips (Merck, Germany) and incubated at 44°C for 18-24 hours. Black fluorescent counts of *Clostridium perfringens* was done under 360 nm UV light using UVP UVGL-25 MINERALIGHT LAMP. Final results were calculated using equation 6.

$$CFU\ per\ 100\ mL = ((No.\ of\ colonies\ counted)/(volume\ filtered))x100 \quad (6)$$

Where;

100 is the standard volume for reporting colony counts (mL)

3.2.4 Determination of antimicrobial resistance of *E. coli*

Selection of antibiotics to be tested was based on availability and frequency of use in humans and animals for treatment of diseases caused by gram negative bacteria. A total of nine antibiotics were selected. These include; streptomycin (10 µg) (Oxoid, UK), ciprofloxacin (5 µg) (Oxoid, UK), chloramphenicol (30 µg) (Oxoid, UK), gentamicin (5 µg) (Oxoid, UK), amoxicillin (Oxoid, UK) (20 µg), tetracycline (30 µg) (Himedia, India), amikacin (30 µg) (Himedia, India), levofloxacin (5 µg) (Oxoid, UK) and ampicillin (10 µg) (Oxoid, UK). Antibiotic susceptibility test was done according to Kirby-Bauer disc diffusion method (Bauer *et al*., 1966). Where, an overnight culture (16 hours), equivalent to 0.5 McFarland standard solution (cell density equivalent to 10^8 cells /mL, prepared by reacting 0.5 mL of 0.048 M $BaCl_2$ and to 99.5 mL of 0.18 M H_2SO_4) was shaken and visually compared to freshly prepared 0.5 McFarland turbidity standard (Plate 10).

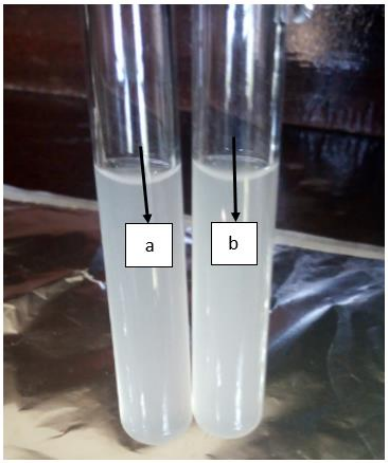


Plate 10: A photograph showing visual turbidity comparison

(a) 0.5 McFarland standard and (b) overnight nutrient broth with *E. coli* isolated from Canning sampling site resulting in confluent growth

A sterile cotton swab was used to spread evenly bacterial suspension from nutrient broth (Difco, USA) for each site on separate Mueller-Hinton (Himedia, India) agar plates within a period of 10 minutes. Antibiotic discs impregnated with various concentrations of antibiotics were placed on inoculated surface within 10 minutes of streaking the bacterial suspensions followed by incubation at 37°C for 24 hours. After incubation, the diameter of growth inhibition zone of each drug was measured horizontally, vertically and diagonally to the nearest mm (Plate 11). The diameter of the zone was related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. Every zone of inhibition of bacterial growth was interpreted as either susceptible, intermediate or resistant according to CLSI (2020). A resistant breakpoint meant there was a high probability of drug inefficacy hence the risk of pollution increased. A susceptible breakpoint meant there was a high probability of drug efficacy while an intermediate breakpoint meant that the drug efficacy was lower than susceptible breakpoint hence the therapeutic effect is uncertain (Table 3). *E. coli* ATCC 25922 was used as antibiotic susceptibility reference standard it`s inhibition zone was interpreted as indicated in Table 4.

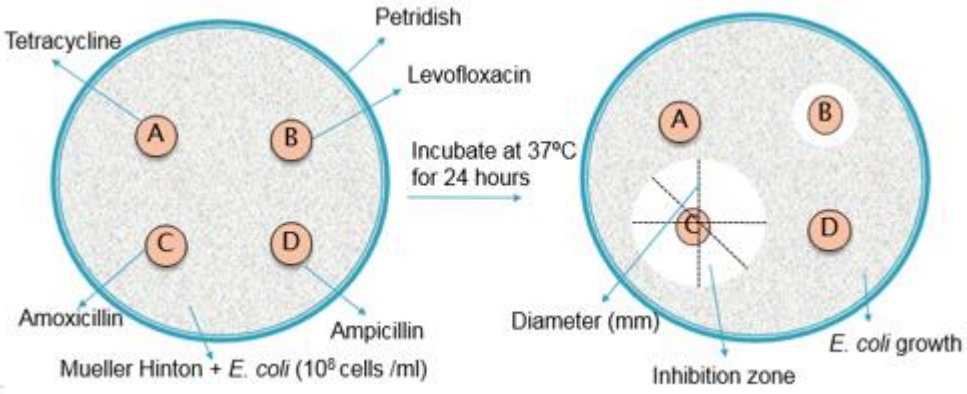


Plate 11: An illustration showing the Kirby-Bauer test

Multiple Antibiotic Resistance Index (MARI) was estimated using equation 7 as developed by Krumperman (1983).

$$MARI = a/b \quad (7)$$

Where;

MARI = Multiple Antibiotic Resistance Index

a = number of antibiotics to which the isolate was resistant

b = number of antibiotics to which the isolate was exposed

Table 3: Zone diameter and minimum inhibitory concentration for *Escherichia coli*

Source: CLSI (2020)

Antimicrobial agent	Disc Content (µg)	Interpretive categories and zone diameter breakpoints (mm)		
		Susceptible	Intermediate	Resistant
Tetracycline	30	≥ 15	12-14	≤ 11
Ciprofloxacin	5	≥ 26	22-25	≤ 21
Gentamicin	10	≥ 15	13-14	≤ 12
Levofloxacin	5	≥ 21	17-20	≤ 16
Amoxicillin	20/10	≥ 18	14-17	≤ 13
Amikacin	30	≥ 17	15-16	≤ 14
Ampicillin	10	≥ 17	14-16	≤ 13
Streptomycin	10	≥ 15	12-14	≤ 11
Chloramphenicol	30	≥ 18	13-17	≤ 12

Table 4: Minimum inhibitory concentrations of quality control strain *Escherichia coli* ATCC 25922

Source: CLSI (2020)

Antimicrobial agent	Disc content (µg)	Disc diffusion ranges (mm)
Amikacin	30	19-26
Ampicillin	10	15-22
Amoxicillin	20/10	18-24
Chloramphenicol	30	21-27
Ciprofloxacin	5	29-37
Gentamicin	5	19-26
Levofloxacin	5	29-27
Streptomycin	10	12-20
Tetracycline	30	18-25

3.3 Data analysis

Physico-chemical variables (pH, temperature, dissolved oxygen, turbidity, total dissolved solids, conductivity, TN, NH_4-N, NO_3-N, NO_2-N,SRP,TP and TSS) and bacteriological variables (*E. coli*, *Clostridium perfringens*, total coliforms, intestinal enterococci) were summarized as means and standard deviations. To decide whether parametric or non-parametric test were to be used, all data from measured variables were

subjected to normality test using Shapiro-Wilk test (hypothesis test) and quantile-quantile plots (graphical test) (Appendix A). Non-parametric tests, Kruskal-Wallis followed by post-hoc pairwise Mann-Whitney tests with Bonferonni correction were used due to skewness of all data even after log transformation. Bonferonni correction was done to avoid type 1 error. Kruskal-Wallis test was done to determine if there were significant differences between sample medians from different sampling sites with varying land use along River Njoro. Spearman`s correlation coefficient was used to determine if there were significant relationships of various physico-chemical variables (pH, temperature, dissolved oxygen, turbidity, total dissolved solids, TN, NH_4-N, NO_3-N, NO_2-N,SRP,TP) and bacteriological variables (*E. coli*, *Clostridium perfringens*, total coliforms, intestinal enterococci) of River Njoro. Antimicrobial resistance of *E. coli* isolates for each antibiotic at every site was classified according to CLSI (2020). Kruskal-Wallis test was done to determine whether the antimicrobial resistance of *E. coli* among the sampling points were statistically different. All the tests were done at significance level of 0.05 ($P < 0.05$). Data was stored in Microsoft Excel (version 2016) and analysed using R software version 3.3.1 (R Core Team, 2018) and SPSS software (version 21).

CHAPTER FOUR

RESULTS

4.1 Longitudinal variation of physico-chemical variables along River Njoro

A summary of physico-chemical parameters are presented in Table 5. The highest mean concentration of dissolved oxygen (7.86±0.70 mg/L) was recorded at Logoman while the lowest concentration was recorded at the River Mouth (3.53±1.87 mg/L). Dissolved oxygen decreased from upstream sites to downstream sites significantly (Kruskal-Wallis, H= 72.823, df =6, $P<0.05$). As expected, a significant variation in dissolved oxygen was observed between the River Mouth and all the sites (pairwise Mann–Whitney U-test, $P<0.05$) (Appendix B2).

The highest temperature (20.56±0.38°C) was recorded at the River Mouth while the lowest temperature (12.59±0.91°C) was recorded at Logoman. Although there was a decrease in temperature between Turkana and Canning, the decrease was not significant (Table 5). Mean water temperatures differed significantly among the sites (Kruskal-Wallis, H=148.681, df=6, $P<0.05$). There was a significant difference in temperature between Logoman and all the sites except Sigotik; Canning and Ngata; Canning and River Mouth; Njoro Bridge and Ngata; Njoro Bridge and River Mouth; Turkana and River Mouth (pairwise Mann–Whitney U-test, $P<0.05$) (Appendix B3). Dissolved oxygen showed a significant negative relationship with temperature (Spearman's, r= -0.149). This means that as the water temperature increased, the oxygen concentration decreased.

The river water had a neutral to slightly alkaline pH in all the sites. The highest pH ranged from 7.50 to 8.20 at Canning while the lowest pH ranged from 6.80 to 7. 28 at Logoman (Table 5). There was a significant difference in pH along the river (Kruskal-Wallis, H=48.594, df=6, $P<0.05$). Significant variation in pH was observed between River Mouth and Canning; River Mouth and Turkana; River Mouth and Ngata; Sigotik and Canning; Sigotik and Turkana; Sigotik and Ngata; Njoro Bridge and Canning; Njoro Bridge and Turkana; Njoro Bridge and Ngata; Logoman and Ngata (pairwise Mann–Whitney U-test, $P<0.05$) (Appendix B4).

Total Dissolved Solids (TDS) concentration increased from upstream to downstream. Logoman had the lowest TDS concentration (41.42±2.17 mg/L) while the highest TDS concentration was recorded at the River Mouth (117.79±24.00 mg/L) (Table 5). A significant increase in TDS was observed from Logoman to River Mouth (Kruskal-Wallis, H=112.406, df=6, $P<0.05$). Significant differences were observed between Logoman and all the sites except Sigotik (pairwise Mann–Whitney U-test, $P<0.05$) (Appendix B5).There was a positive correlation between pH and conductivity although it was not significant (Spearman`s, r=0.2908, $P>0.05$), pH and total dissolved solids (Spearman`s, r=0.304, $P<0.05$), TDS and

conductivity (Spearman`s, r=0.994, P<0.05), Total suspended solids and turbidity showed a significant positive correlation (Spearman`s, r= 0.664, P<0.05).

Turbidity varied significantly among the sites during the sampling period (Kruskal-Wallis, H=39.921, df=6, P<0.05). The highest turbidity (41.91±19.56 NTU) was recorded at Njoro Bridge while the lowest (22.13±8.37 NTU) was recorded at Sigotik (Table 5). Significant differences in turbidity were observed between Sigotik and all the sites except Logoman; Logoman and Ngata (pairwise Mann–Whitney U-test, P<0.05) (Appendix B6).

Logoman had the lowest conductivity of 87.55±4.70 µS/cm while the River Mouth had the highest conductivity (245.92±47.15 µS/cm) followed by Ngata (211.40±34.08 µS/cm) (Table 3).There was a significant difference in conductivity among the sites (Kruskal-Wallis, H=112.161, df=6, P<0.05). Significant differences in conductivity were observed between Logoman and all the sites except Sigotik (Appendix B7).

A summary of mean nutrients concentration are presented in Table 5. A significant difference in mean nutrients concentration from upstream to downstream was observed. The highest average NH_4-N concentration was recorded at the River Mouth (114.46±63.07 µg/L) while the lowest was recorded at Sigotik (25.67±13.43 µg/L). Significant difference was observed in NH_4-N concentration among the sites (Kruskal-Wallis, H=68.192, df=6, P<0.05). The differences were between Sigotik and Njoro Bridge; Sigotik and River Mouth; Logoman and River Mouth; Turkana and River Mouth; Canning and River Mouth; Ngata and River Mouth; Njoro Bridge and River Mouth (pairwise Mann–Whitney U-test, P<0.05) (Appendix B8).

Nitrate levels (NO_3-N) were highest at Canning (2.90±0.56 µg/L) and lowest at Logoman (0.28±0.46 µg/L) (Table 5). Significant differences were observed in nitrate concentration among the sites (Kruskal-Wallis, H=123.117, df=6, P<0.05).The differences were between Logoman and all the sites except River Mouth and Sigotik; Sigotik and all the sites except Logoman; River Mouth and Ngata; River Mouth and Njoro Bridge; River Mouth and Turkana (pairwise Mann–Whitney U-test, P<0.05) (Appendix B9).

Table 5: Mean values and standard deviation of physico-chemical and nutrients along River Njoro (n=168)

	Sites and their respective land use						
Variables	Logoman Forest	Sigotik Mixed	Turkana Agriculture	Canning Industrial	Njoro Bridge Agriculture	Ngata Agriculture	River Mouth Urban
Temperature (°C)	12.59±0.91	14.10±0.68	15.95±0.61	15.69±0.66	15.83±0.91	17.67±0.55	20.56±0.38
Dissolved oxygen (mg/L)	7.86±0.70	7.65±0.66	7.42±0.56	7.47± 0.54	7.41± 0.58	7.36± 0.54	3.53±1.87
% saturation	97.87±7.37	98.49±8.24	97.52±7.80	97.20±7.64	96.06±8.12	97.93±7.89	48.65±25.68
pH range	6.80-7.28	7.15-7.99	7.45-8.25	7.50-8.20	7.43-7.93	7.58-8.34	7.35-7.85
Conductivity (µS/cm)	87.55±4.70	102.56±11.59	200.44±31.40	202.49±29.98	208.79±33.15	211.40±34.08	245.92±47.15
Turbidity (NTU)	26.45±9.51	22.13±8.37	31.45±5.89	33.21±8.70	41.91±19.56	41.09±17.00	38.63±28.11
TDS (mg/L)	41.42±2.17	51.00±5.38	95.04±15.00	96.65±14.65	99.58±15.72	96.92±14.39	117.79±24.00
TP (µg/L)	37.14±8.57	39.29±12.82	71.76±42.84	67.02±14.14	80.42±22.87	89.82±16.27	159.64±48.19
SRP (µg/L)	7.62±5.32	8.27±6.65	20±12.31	22.62±10.48	28.69±9.62	36.78±11.52	56.72±28.00
NO_2-N(µg/L)	7.44±9.16	14.61±16.04	20.33±12.31	19.44±23.86	23.69±17.24	32.89±14.57	40.41±23.22
NH_4-N(µg/L)	36.13±23.37	25.67±13.43	35.75±12.43	39.13±15.87	40.54±12.23	35.83±13.28	114.46±63.07
NO_3-N(mg/L)	0.28±0.46	0.68±0.14	2.90±0.56	2.89±0.64	2.73±0.49	2.61±0.47	1.01±0.82
TN (mg/L)	22.22±15.25	25.24±15.79	32.92±11.92	34.70±12.19	34.83±11.95	33.97±10.50	28.09±12.47
TN loadings (Kg/day)	0.68±0.46	0.42±0.26	0.57±0.21	0.81±0.28	0.93±0.37	0.13±0.39	0.84±0.37
TP loadings (Kg/day)	1.14±0.26	0.65±0.21	1.26±0.75	1.56±0.32	2.14±0.60	3.35±0.60	4.79±1.45
TSS (mg/L)	17.31±18.52	8.81±4.33	19.43±21.22	12.25±6.27	16.84±12.28	17.17±15.75	17.72±14.03

The highest NO_2-N concentration was recorded at River Mouth (40.41±23.22 µg/L) while the lowest concentration was recorded at Logoman (7.44±9.16 µg/L) (Table 5). There was a significant difference in NO_2-N concentration among the sites (Kruskal-Wallis, H=115.124, df=6, P<0.05). The differences were between Logoman and all the sites except Sigotik; Sigotik and all the sites except Logoman and Canning; Canning and River Mouth; Turkana and River Mouth; Njoro Bridge and River Mouth; Ngata and River Mouth (pairwise Mann–Whitney U-test, P<0.05) (Appendix B10).

The highest TN concentration was recorded at Njoro Bridge (34.83±11.95 mg/L) while the lowest concentration was recorded at Logoman (22.22±15.25 mg/L) (Table 5). The mean Total Nitrogen concentration varied significantly among the sites (Kruskal-Wallis, H=55.711, df=6, P<0.05). The significant differences were between Logoman and all the sites except Sigotik (pairwise Mann–Whitney U-test, P<0.05) (Appendix B11). Generally, TN loading rate was highest at Njoro Bridge (0.93±0.37 Kg/day) and lowest at Ngata (0.13±0.39 Kg/day) (Table 5).

Total Phosphorus varied significantly among the sites (Kruskal-Wallis, H= 127.373, df=6, P<0.05). The lowest concentration of TP was recorded Logoman (37.14±8.57 µg/L) and increased slightly at Sigotik (39.29±12.82 µg/L) while the highest concentration was recorded at River Mouth (159.64±48.19 µg/L) followed by Ngata (89.82±16.27 µg/L) (Table 5). Significant differences were between Logoman and all the sites except Sigotik; Sigotik and all the sites except Turkana and Logoman; Turkana and Ngata; Turkana and River Mouth; Canning and River Mouth; Njoro Bridge and River Mouth (pairwise Mann–Whitney U-test, P<0.05) (Appendix B12).Generally, the highest TP loading rate was recorded at River Mouth (4.79±1.45 Kg/day) and lowest was recorded at Sigotik (0.65±0.21 Kg/day) (Table 5).

A similar trend to TP was observed for SRP. The lowest concentration was recorded at Logoman (7.62±5.32 µg/L) while the highest concentration was recorded at the River Mouth (56.72±28.00 µg/L). A significant difference in SRP concentration among the sites was recorded (Kruskal-Wallis, H= 96.448, df=6, P<0.05). The differences were between Sigotik and all the sites except Logoman; Logoman and all the sites except Turkana and Sigotik, Turkana and Ngata, Turkana and River Mouth, Canning and River Mouth (pairwise Mann–Whitney U-test, P<0.05) (Appendix B13).

The highest TSS was recorded at Turkana (19.43±21.22 mg/L) while the lowest was recorded at Sigotik (8.81±4.33mg/L) (Table 5). However, Total suspended solids did not vary significantly among the sites (Kruskal-Wallis, H= 10.957, df=6, P>0.05).

4.2 Longitudinal variation of faecal indicator bacteria along River Njoro

Escherichia coli concentration ranged from median of 5.96-8.77 $\log_e$ CFU/100 mL (Figure 4) while total coliforms ranged from median of 6.80-9.13 $\log_e$ CFU/100 mL (Figure 5). The highest concentration of *E. coli* was recorded at the River Mouth while the lowest concentration was recorded at upstream Logoman site. The concentrations of *E. coli* (Plate 12) varied significantly among the sites (Kruskal-Wallis, H=107.502, df=6, P<0.05). Significant differences in *E. coli* concentration was observed between Logoman and all the sites except Sigotik; Sigotik and Ngata; Sigotik and Njoro Bridge; Sigotik and River Mouth; Turkana and River Mouth; Canning and River Mouth; Ngata and River Mouth; Njoro Bridge and River Mouth (pairwise Mann–Whitney U-test, P<0.05) (Appendix B14).

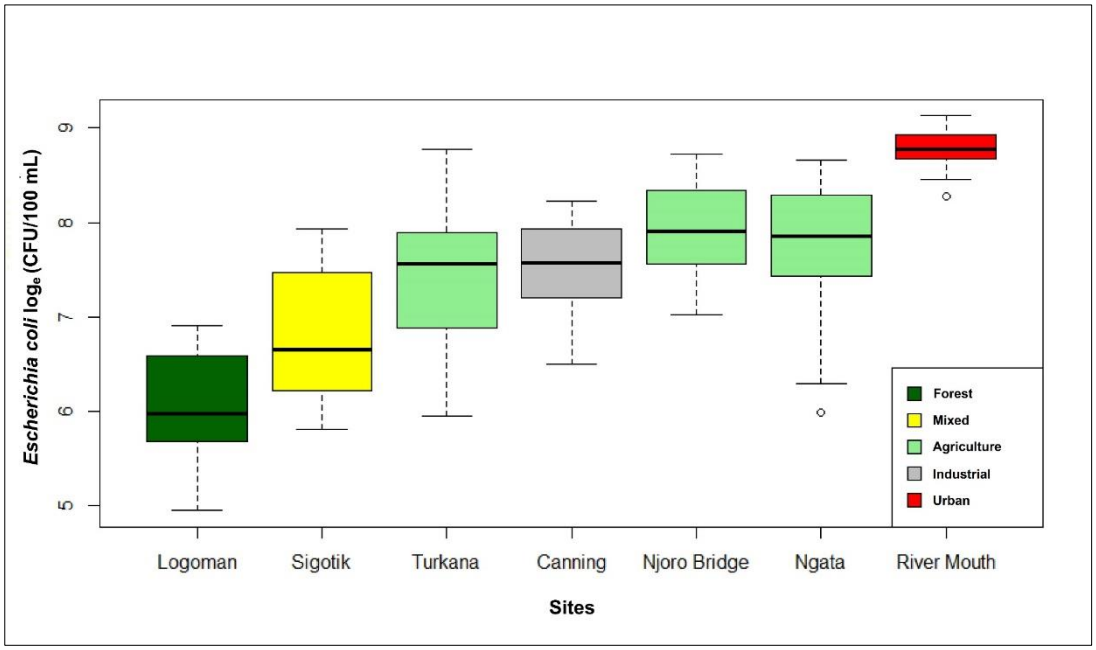


Figure 4: Box-whisker plots showing concentrations of *Escherichia coli* at each site
The circles represent outliers, horizontal line within each box represents the median, the whiskers above and below each box indicate the maximum and minimum respectively.

Similarly, the highest concentration of total coliforms was recorded at the River Mouth while the lowest concentration was recorded at Logoman. The median values total coliforms are shown in Figure 6. Total coliforms concentration varied significantly among the sites (Kruskal-Wallis, H=115.244, df=6, P<0.05).Significant difference was observed between Logoman and all the sites except Sigotik; Sigotik and Ngata; Sigotik and Njoro Bridge; Sigotik and River Mouth; Turkana and River Mouth; Canning and River Mouth; Ngata and River Mouth (pairwise Mann–Whitney U-test, P<0.05) (Appendix B15).

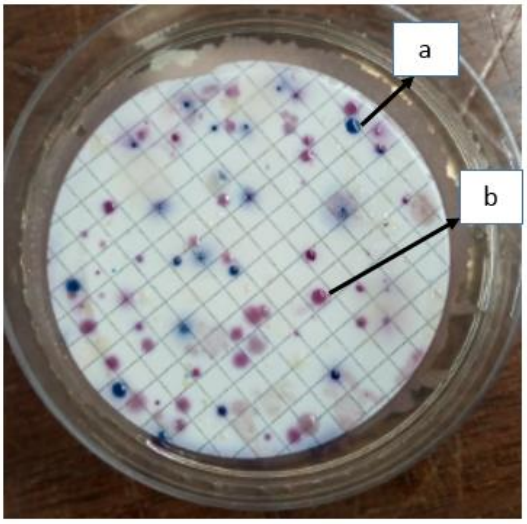


Plate 12: A photograph showing faecal and non-faecal coliforms on chromocult agar (Merck) (a) dark-blue colonies are *Escherichia coli* and (b) pink colonies are non-faecal coliforms. An example at Sigotik site.

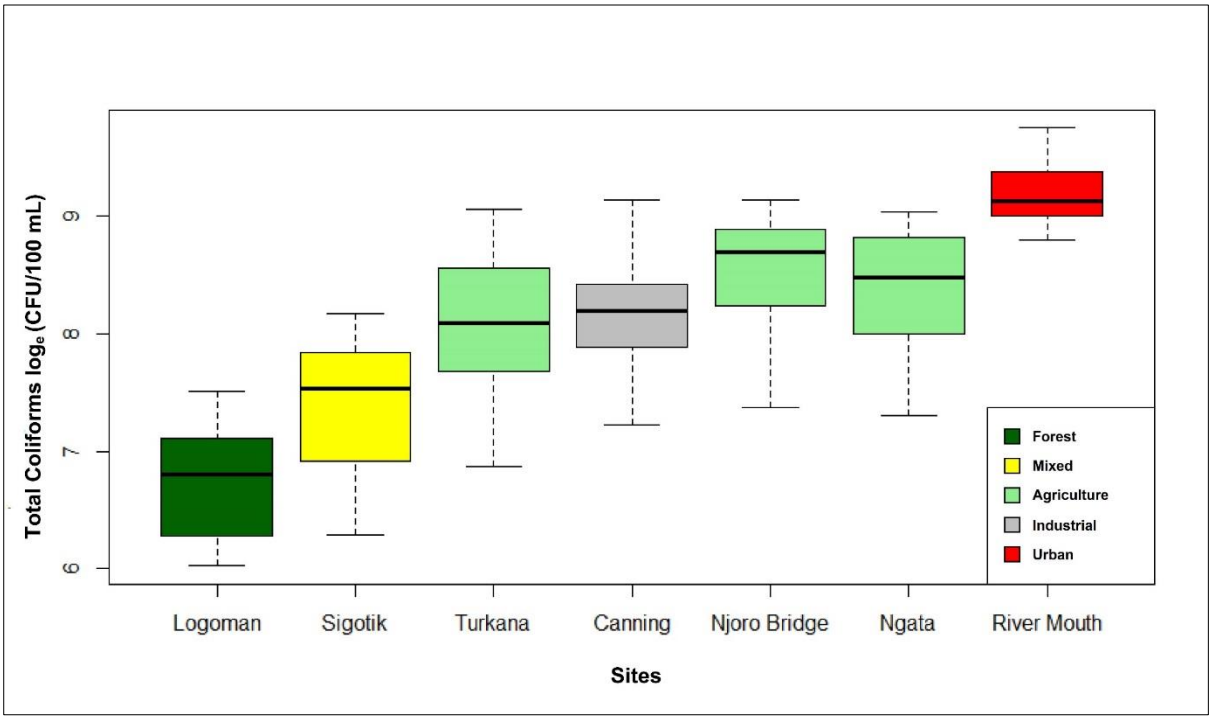


Figure 5: Box-whisker plots showing concentrations of total coliforms at each site
The horizontal line within each box represents the median, the whiskers above and below each box indicate the maximum and minimum respectively

For intestinal enterococci (Plate 13), the concentrations ranged from median of 5.84-8.26 log$_e$ CFU/100 mL. The highest concentration was recorded at the River Mouth while the lowest concentration was recorded at Logoman (Figure 7). There was a significant difference in intestinal enterococci concentration among the sites (Kruskal-Walllis, df=6, H=107.666,

P<0.05). Significant difference was observed between Logoman and all the sites except Sigotik; Canning and River Mouth (pairwise Mann–Whitney U-test, P<0.05) (Appendix B16).

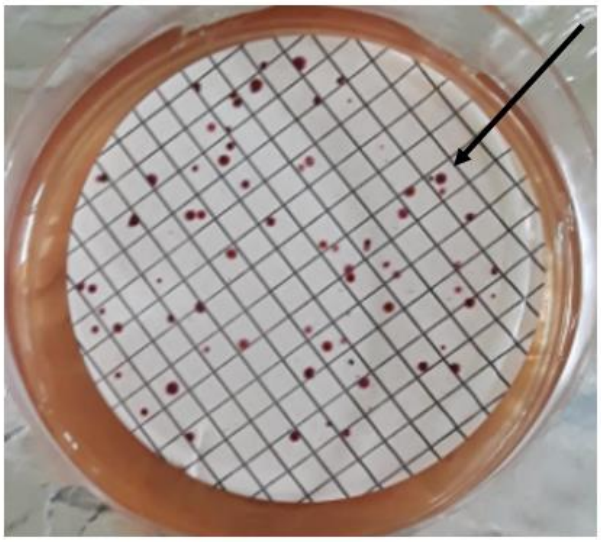


Plate 13: A photograph showing intestinal enterococci on m-enterococcus agar (Difco) Appearing red in colour.

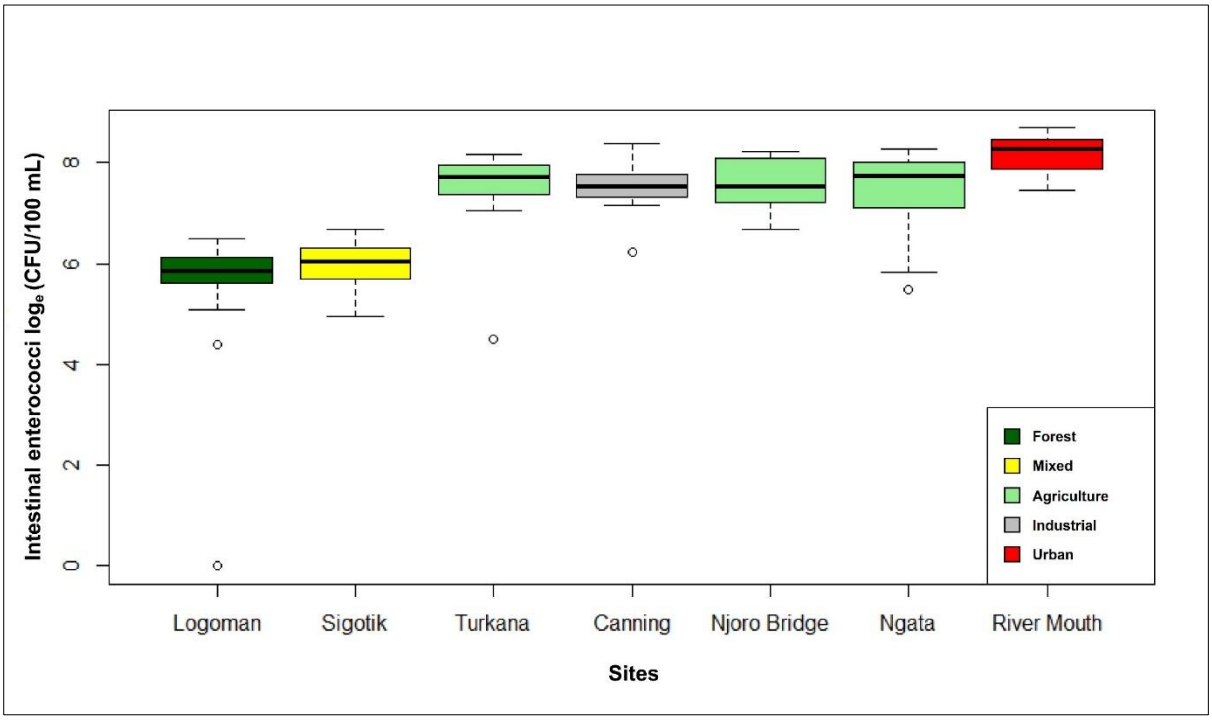


Figure 6: Box-whisker plots showing concentrations of intestinal enterococci at each site The circles represent outliers, horizontal line within each box represents the median, the whiskers above and below each box indicate the maximum and minimum respectively.

The ratio of faecal coliform to faecal streptococci was lowest at Turkana (1.02) and highest at Sigotik (2.60) (Table 6) implying that during the sampling period wildlife could have played a major role in impacting faecal pollution of the river.

Table 6: The ratio *of Escherichia coli* (EC) to Intestinal enterococci (IE)

Sites	Land use	EC:IE ratio	EC:IE ratio (Sinton *et al*., 1998)
Logoman	Forest	1.42	
Sigotik	Mixed	2.60	
Turkana	Agriculture	1.02	> 4 pollution from human source
Canning	Industrial	1.05	< 0.7 pollution from animal source
Njoro Bridge	Agriculture	1.46	
Ngata	Agriculture	1.31	
River Mouth	Urban	1.74	

The concentrations of *Clostridium perfringens* (Plate 14) were lower than *Escherichia coli* and intestinal enterococci in all the sites. The concentrations ranged between median of 5.00-6.51 $\log_e$ CFU/100 mL (Figure 8). Just like *Escherichia coli* and intestinal enterococci, the highest concentration was recorded at the River Mouth while the lowest concentration was recorded at Logoman. A significant difference in concentration of *Clostridium perfringens* among the sampled sites was observed (Kruskal-Wallis, $H=76.669$, $df=6$, $P<0.05$). Significant differences were between Logoman and all the sites except Sigotik; Sigotik and Ngata; Sigotik and Njoro Bridge; Sigotik and River Mouth; Turkana and River Mouth, Canning and River Mouth (pairwise Mann–Whitney U-test, $P<0.05$) (Appendix B17).

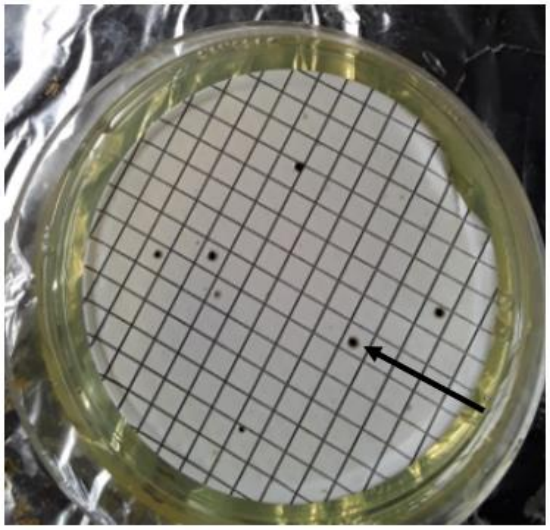


Plate 14: A photograph showing *Clostridium perfringens* on TSC (Merck) indicated by an arrow

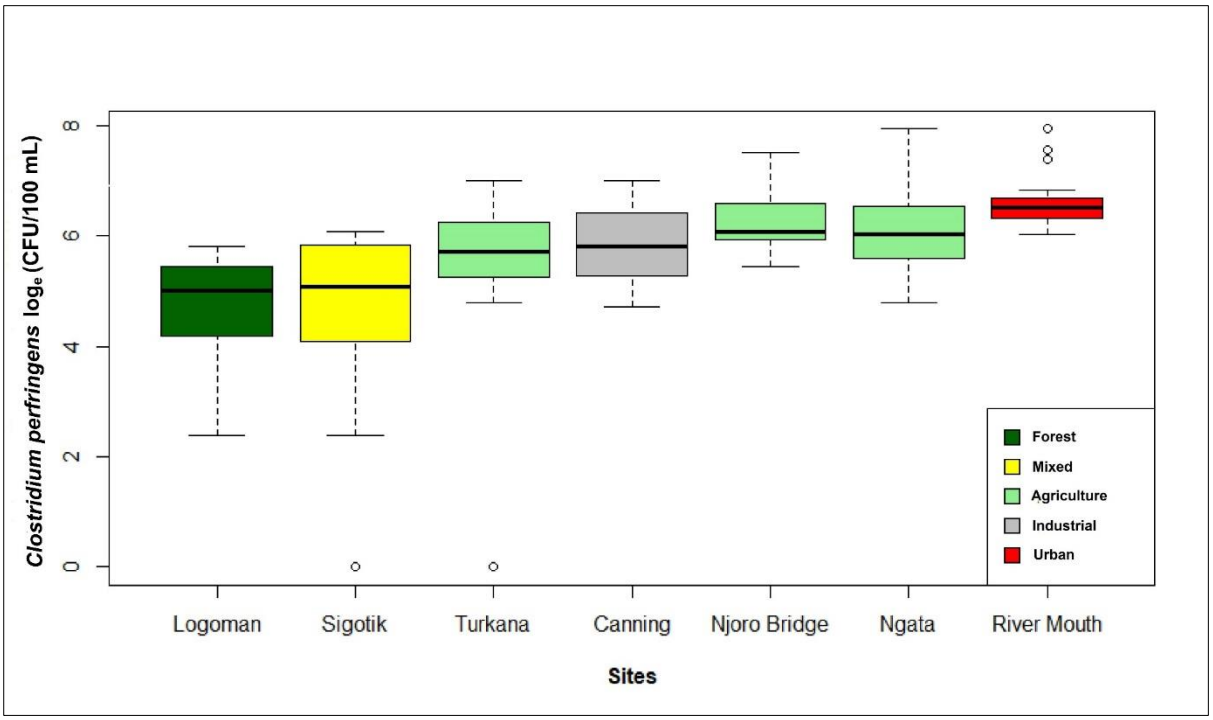


Figure 7: Box-whisker plots showing concentrations of *Clostridium perfringens* at each site

The circles represent outliers, horizontal line within each box represents the median, the whiskers above and below each box indicate the maximum and minimum respectively

The correlations between physico-chemical and bacteriological variables are presented in Table 7 and Table 8. There was significant positive correlations between all the faecal indicator bacteria and conductivity, nitrites, ammonium and total phosphorus. Positive correlations were observed between *E. coli* and intestinal enterococci (Spearman`s, r=0.820, P>0.05), temperature and *E. coli* (Spearman`s, r=0.705, P<0.05), temperature and total

coliforms (Spearman`s, r= 0.734, P<0.05) temperature and intestinal enterococci (Spearman`s, r=0.675, temperature and *Clostridium perfringens* (Spearman`s, r=0.57, P>0.05). All the faecal indicator bacteria also positively correlated with nitrates, ammonium, total phosphorus, total nitrogen nitrites and total nitrogen. Significant negative correlation was observed between all the FIB and dissolved oxygen. Negative correlations were also noted between all the FIB and TSS although the correlations were weak and not significant. Turbidity correlated positively with *Escherichia coli*, total coliforms, enterococci but negatively with *Clostridium perfringens* (Table 7).

Table 7: Spearman`s correlation between physico-chemical and bacteriological variables

	E.coli	TC	IE	*C. perf*	DO	Temp	pH	EC	TDS	NTU	TSS
E. coli	1										
TC	0.829*	1									
IE	0.820*	0.665*	1								
C. perf	0.751*	0.544*	0.671*	1							
DO	-0.759*	-0.499*	-0.654*	-0.738*	1						
Temp	0.705*	0.734*	0.675*	0.571	-0.149*	1					
pH	-0.066	0.01	0.157	-0.176	0.148	-0.016	1				
EC	0.764*	0.657*	0.860*	0.635*	-0.614*	0.620*	0.2908	1			
TDS	0.766*	0.651*	0.857*	0.645*	-0.630*	0.621*	0.304*	0.994*	1		
NTU	-0.025	0.158	0.048	-0.133	-0.297*	0.430*	0.002	0.01	0.002	1	
TSS	-0.128	0.094	-0.122	-0.180	0.272	0.292*	-0.044	-0.158	-0.164	0.664*	1

*correlation is significant at 0.05 level (2-tailed). TC-total coliforms IE-intestinal enterococci, *C. perf-Clostridium perfringens,* DO-dissolved oxygen Temp- temperature, TDS-total dissolved solids, NTU-turbidity, EC-electrical conductivity, and TSS- Total Suspended Solids.

Table 8: Spearman`s correlation between nutrients and bacteriological variables

	E. coli	TC	IE	*C. perf*	NO_2-N	NO_3-N	NH_4-N	SRP	TP	TN
E. coli	1									
TC	0.829*	1								
IE	0.820 *	0.665*	1							
C. perf	0.751*	0.544*	0.671*	1						
NO_2-N	0.632*	0.659*	0.737*	0.449*	1					
NO_3-N	0.268	0.251	0.447*	0.248	0.332*	1				
NH_4-N	0.471 *	0.411 *	0.459 *	0.472 *	0.425 *	-0.015	1			
SRP	0.537 *	0.560*	0.646*	0.388*	0.845*	0.330*	0.349 *	1		
TP	0.635*	0.643*	0.706 *	0.494 *	0.867*	0.242	0.471 *	0.825*	1	
TN	0.304*	0.348*	0.328 *	0.223	0.317*	0.491*	0.038	0.231	0.375*	1

*correlation is significant at 0.05 level (2-tailed). TC-total coliforms, IE-intestinal enterococci, *C. perf-Clostridium perfringens.*

4.3 Antimicrobial susceptibility of *E. coli* isolated from river Njoro

The *E. coli* control strain, ATCC 25922 was susceptible to all the tested antibiotics (Plate 15).

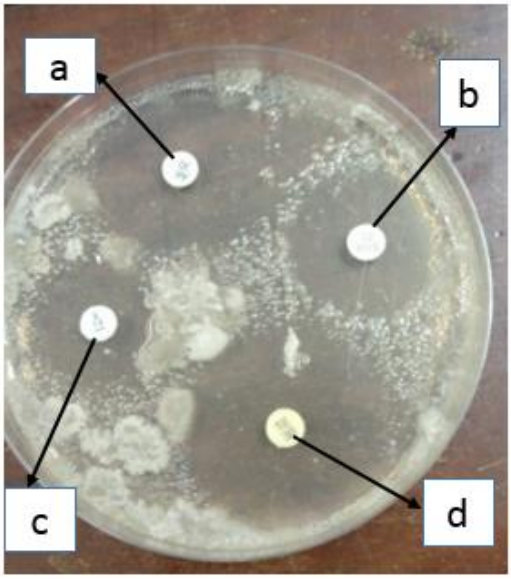
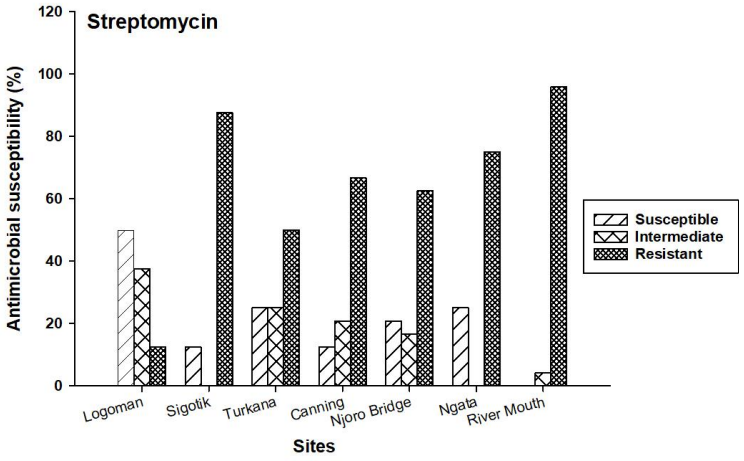
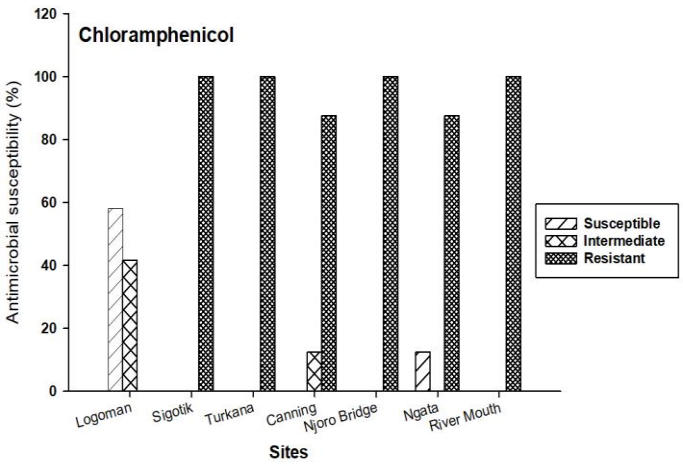


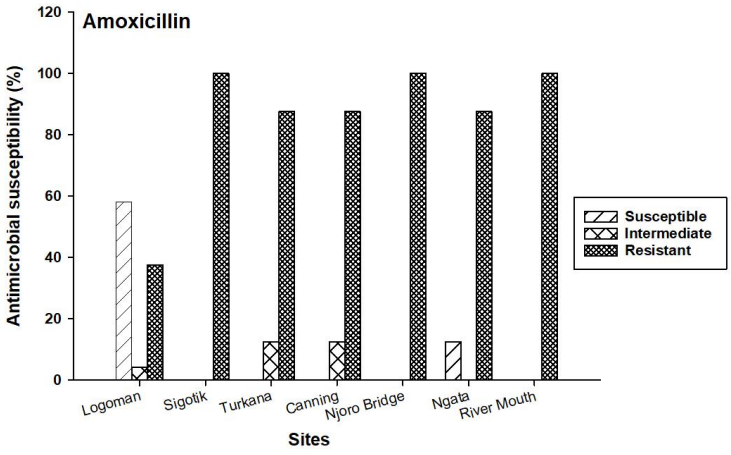
Plate 15: A photograph showing susceptibility of control strain (ATCC 25922)

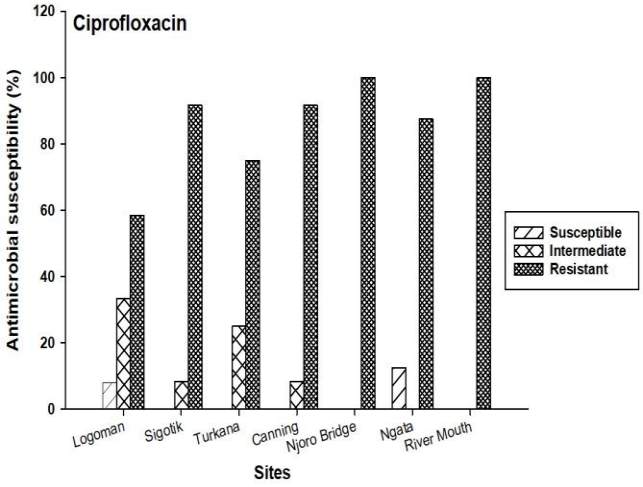
The control strain was susceptible to (a) amikacin (b) amoxicillin (c) streptomycin and (d) tetracycline as indicated by the clear zones of inhibition

The tested antibiotics showed variations in their susceptibilities at different sites as illustrated in Figure 9 and 10. All the sites had isolates resistant to one or all the tested antibiotics. Isolates from the River Mouth, Turkana, Canning, Njoro Bridge and Ngata were resistant to all antibiotics.









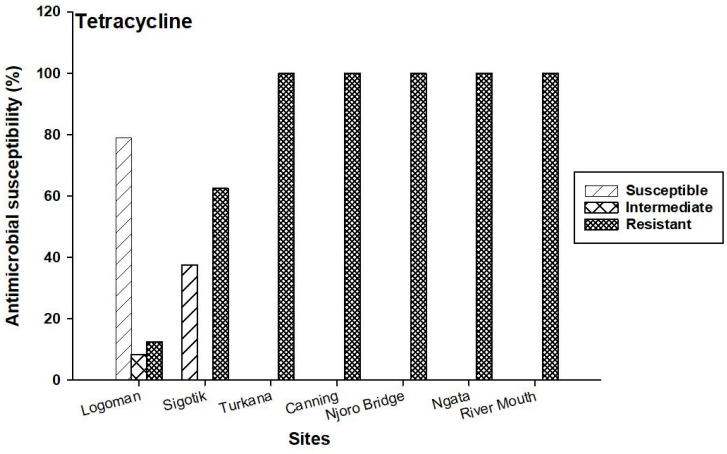


Figure 8: Clustered bar graphs showing high percentage antimicrobial resistance of *E. coli* along River Njoro

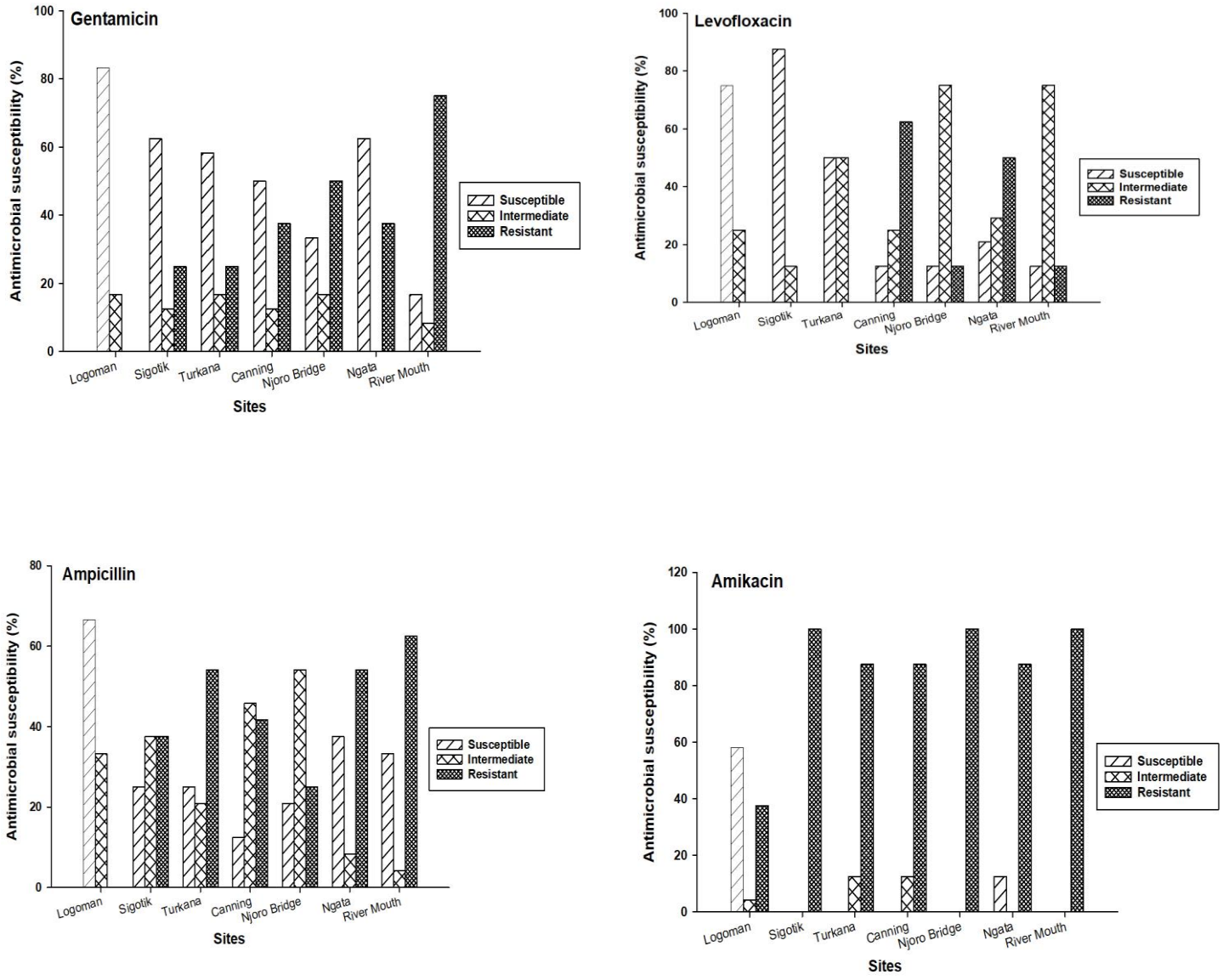


Figure 9: Clustered bar graphs showing low antimicrobial resistance of *E. coli* along River Njoro

Some isolates were both susceptible and intermediate to tetracycline, ciprofloxacin, gentamicin, levofloxacin, amoxicillin, amikacin, ampicillin and streptomycin and chloramphenicol while others were susceptible, intermediate and resistant to ciprofloxacin and tetracycline. No susceptible or intermediate bacteria to streptomycin, chloramphenicol and tetracycline was isolated from Turkana (Figure 9). *E. coli* isolated from Logoman was susceptible to (a) gentamicin (b) resistant to tetracycline (c) intermediate to amikacin and (d) resistant to amoxicillin as indicated by the size of clear zone of inhibition (Plate 16).

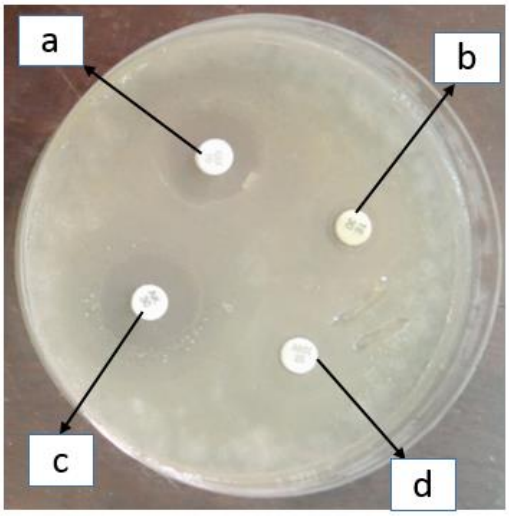


Plate 16: A photograph showing zones of inhibition of (a) gentamicin (b) tetracycline (c) amikacin and (d) amoxicillin

A significant difference in antimicrobial resistance of *E. coli* isolated from different sites was observed except for amoxicillin (Kruskal-Wallis, df=6, H=11.292, P>0.05) and amikacin (Kruskal-Wallis, df=5, H= 3.1187, P>0.05). For tetracycline (Kruskal-Wallis, df=6, H= 19.432, P<0.05), significantly different sites in tetracycline resistance was between Ngata and Njoro Bridge (pairwise Mann–Whitney U-test, P<0.05) (Appendix B18). For ciprofloxacin (Kruskal-Wallis, df=6, H=42.33, P<0.05), significantly different sites in ciprofloxacin resistance was between Ngata and Canning; Ngata and Logoman; Turkana and Canning; Njoro Bridge and Canning; Njoro Bridge and Logoman (pairwise Mann–Whitney U-test, P<0.05) (Appendix B19). For gentamicin (Kruskal-Wallis, df=5, H=39.388, P<0.05), significantly different sites in gentamicin resistance was between Sigotik and River Mouth; Sigotik and Canning; Sigotik and Ngata; Njoro Bridge and River Mouth; Turkana and River Mouth, Turkana and Njoro Bridge; Turkana and Ngata (pairwise Mann–Whitney U-test, P<0.05) (Appendix B20). For levofloxacin (Kruskal-Wallis, df=3, H=9.3377, P<0.05), significantly different sites in levofloxacin resistance was between Njoro Bridge and Canning; Njoro Bridge and Ngata; River Mouth and Canning; River Mouth and Ngata (pairwise Mann–Whitney U-test, P<0.05) (Appendix B21). For ampicillin (Kruskal-Wallis, df=6, H=21.547, P<0.05), significantly different sites in ampicillin resistance was between Njoro Bridge and Sigotik (pairwise Mann–Whitney U-test, P<0.05) (Appendix B22). For streptomycin (Kruskal-Wallis, df=5, H=35.137, P<0.05), significantly different sites was between Ngata and Turkana; Ngata and River Mouth; Njoro Bridge and River Mouth (pairwise Mann–Whitney U-test, P<0.05) (Appendix B23). For chloramphenicol (Kruskal-Wallis, df=5, H=17.505), significantly

different sites in chloramphenicol resistance was between Njoro Bridge and Turkana; Ngata and Turkana (pairwise Mann–Whitney U-test, $P<0.05$) (Appendix B24).

Although isolates from Logoman showed resistance to four out of the nine antibiotics, all isolates from this site were susceptible to chloramphenicol. At Sigotik, isolates were resistant to all antibiotics except for Levofloxacin. Gentamicin, amikacin and chloramphenicol. At midstream and downstream sites, all isolates were resistant to tetracycline. Most bacteria showed highest resistance to streptomycin and lowest resistance to Levofloxacin. Despite the fact that isolates of *E. coli* showed resistance to each tested antibiotic, Gentamicin, Levofloxacin and Amikacin showed resistance below average (Table 9).

Table 9: Overall resistance of the tested antibiotics in percentage

Antibiotic	Resistance (%)
Tetracycline	82.14
Ciprofloxacin	86.31
Gentamicin	41.67
Levofloxacin	34.38
Amoxicillin	85.71
Amikacin	45.84
Ampicillin	64.29
Streptomycin	95.83
Chloramphenicol	86.11

Generally, prevalence of resistant bacteria increased from upstream to downstream. Highest average percentage resistance was recorded at the River Mouth and lowest at Logoman. Canning and Ngata had similar mean percentage resistance. MARI (Multiple Antibiotic Resistance Index) ranged from 0.44-1.00. Logoman had the least index while Canning, Njoro Bridge, Ngata and River Mouth had the highest MARI (Table 10). Resistance index greater than 0.2 indicates high pollution risk.

Table 10: Average percentage antimicrobial resistance and MARI at each site

Sites	% resistance	Resistance index
Logoman	30.21	0.44
Sigotik	67.71	0.89
Turkana	73.96	0.89

Canning	75.00	1.00
Njoro Bridge	72.22	1.00
Ngata	75.00	1.00
River Mouth	81.02	1.00

CHAPTER FIVE

DISCUSSION

5.1 Physico-chemical variables and nutrients concentrations

From this study, the extent of pollution at different sections of the river varied with anthropogenic activities. Thus, anthropogenic activities in the River Njoro catchment coupled with decrease in proportion of forested area have impacted the water quality, a fact noted by other researchers (Aera *et al*., 2019). Presence of vegetation on riparian land has been reported to improve river water quality by absorption and filtration of pollutants (Tanaka *et al*., 2016).

Lower temperatures were recorded at Logoman, forested site, which was the most upstream station studied than other sites. This could be due to presence of a denser vegetation canopy while higher temperatures in other sites could be due to lack of (or reduced) vegetation canopy hence direct penetration of sunlight. Riparian vegetation protects the river water from heating due to solar radiation (Wondzell, 2019).The variation in temperature could also be due to differences in altitude (Ashley Steel *et al*., 2016). Logoman (forested) and Sigotik (mixed land use), situated at higher elevations had lower temperatures than downstream sites. Optimum temperature for growth and survival of microorganisms ranges between 16 to 25°C (Wang *et al*., 2017). Therefore, lower temperatures recorded at upstream sites were sufficient to eliminate contaminating bacteria.

Significant negative correlation between dissolved oxygen and temperature showed that an increase in temperature caused a decrease in dissolved oxygen. These results are similar to previous studies (He *et al*., 2011; Martinez-Travera *et al*., 2017). The concentration of dissolved oxygen in water depends on other factors such as altitude and water temperature which in turn influence metabolic processes (Shah *et al*., 2017). High temperatures at downstream sites reduced the solubility of gases and this could be the reason why dissolved oxygen was higher at forested site as compared to other sites (Goldman, 1983). Such findings would mean that microbial activities increase towards downstream sites in River Njoro. There is need to increase vegetation cover to filter overland inputs of nutrients and reduce biological pollution (Chen *et al*., 2019). This will also provide shading to attenuate direct heating and consequently improve on oxygen dissolution in the river.

It was also noted that dissolved oxygen decreased as turbidity, TDS and TSS increased. Solid wastes discharged into the river increased downstream hence reducing river flow at the River Mouth. This provided a suitable environment for bacterial attachment and growth and consequently oxygen consumption by in-stream microbial activities. Kebede *et al*. (2020) also reported an increase in FIB downstream of Awash River and the authors attributed

this to microbial attachment on solid wastes. Decomposition of organic matter could have caused a decrease in oxygen at the River Mouth. Lower levels of dissolved oxygen recorded at River Mouth (urban) compared to other sites could be the reason why fish kills were found at this site during sampling sessions. Fish such require oxygen for respiration hence low dissolved oxygen is lethal to them.

Nutrient concentrations influenced the concentration of FIB. Both nutrients and FIB could have been from the same origin. Significant positive correlations was observed between, nitrates, nitrites, ammonia and bacterial concentrations. Ammonia could have originated from inorganic fertilizers used in agriculture and also from animal excreta explaining the positive correlations between bacterial concentrations and ammonia. It would also mean that the source of some faecal indicators was of animal excreta origin (Álvarez, 2017).These results are similar to a study by where *E. coli* positively correlated with nutrients significantly (Gotkowska-Płachta *et al*., 2016). High ammonia concentrations at agricultural, industrial and urban land uses could be due microbial degradation of organic matter that resulted to ammonium production (Li *et al*., 2016).

Turbidity is caused by suspended and colloidal particles (non-settlable solids) in water (Lee *et al*., 2016). Turbidity negatively correlated with *Clostridium perfringens* and *E. coli* but positively with intestinal enterococci and total coliforms although the correlations were weak. Findings from this study are different from Liang *et al*. (2015) where turbidity correlated positively with *E. coli*. This is perhaps because colloidal particles could be from different sources other than animal excreta (Lee *et al*., 2016). Furthermore, there is an assumption that *E. coli* presence in water was of recent origin and the colloidal particles (non settlable solids) especially at River Mouth (urban) could have remained in suspension for a longer time due to reduced river flow at the site. Surface runoff from surrounding catchment eroded river banks contribute to turbidity in the rivers (Nkwonta & Ochieng, 2009) therefore turbidity varies depending on location of the sampling site. Livestock disturbing sediments during watering and eroded river banks could have caused resuspension and higher turbidity at downstream sites compared to upstream, forested site (Hartwig *et al*., 2016). Negative correlation between turbidity and dissolved oxygen showed FIB were adsorbed to resuspended particles and consumed oxygen during decomposition of organic matter from effluents discharged in the river and consequently increased their counts.

Total suspended solids could be due to soil erosion from farmlands along the river indicating an effect of land use on water quality in a stream. Although low concentration of TSS was recorded at the site with mixed land use (Sigotik), there was no significant difference

in TSS among the sites. This could be due to minimal entry of sediments into the river at the site from overland transport due to rainfall events that could have occurred frequently on higher grounds and not in downstream stretches of the river a common phenomenon in the area. Vegetation at forested site could have played a key role in retention of suspended matter from the catchment. The role of vegetation in removal of pollutants is well known (Olilo *et al*., 2016). Significant positive correlation between TSS and temperature would mean that clearing vegetation led to deposition of suspended particles in water and consequently increasing water temperature. In addition, it is possible that particles suspended in water absorbed heat especially in an open system increasing water temperature.

Concentrations of dissolved ions in water influence the conductivity hence this variable is used to measure the purity of water (Oyem *et al*., 2014; Yilmaz & Koç, 2014). TDS was lowest at forested site compared to other sites and these results are similar to studies by Muchukuri *et al.* (2014) who found out that TDS varied longitudinally in Subukia rivers mainly influenced by human activities within the catchment. Total dissolved solids (TDS) e.g. from detergents influence the taste and odour of water hence reducing the palatability. Furthermore, they can consist of disease causing organisms such as *E. coli* which can be harmful to humans (Shah *et al*., 2017). High TDS recorded at agricultural, industrial and urban land uses could be due to human activities within the channel; washing of motorcycles, laundry, runoff from agricultural fields, discharge of organic effluents from industrial wastewater and institutions adjacent to the riparian land as well as treated effluents from WWTP.

The concentration of dissolved ions in a solution determines it`s ability to conduct electric current (Shah *et al*., 2017). The positive correlation obtained in this study between TDS and conductivity could be due to dissolved ions (Sibanda *et al*., 2014). Furthermore, evaporation of water due to direct penetration of sunlight could have increased the concentration of TDS, consequently resulting to increase in conductivity.

It is notable that pH was generally neutral and it varied significantly along the river. pH was expected to be slightly acidic especially at sites with agricultural, industrial and urban land uses of the river due to release of carbon dioxide as a result of decomposition of organic effluents. Von Schiller *et al*. (2017) reported that carbon dioxide is a product of microbial decomposition of organic matter. The neutral pH at the River Mouth (urban) could be due to backflow of water from Lake Nakuru, an alkaline lake. According to Wu *et al*. (2021) lake water influences downstream part of the river water through exchange of solutes.

Lower nutrient levels at Logoman (Table 5) could be due to forested catchment which acted as filter to pollutants coupled with minimal anthropogenic activities especially livestock

rearing. Studies by Vettorazzi and Valente (2016) found out that in agricultural catchments with reduced forest cover the nutrients concentration increased along the rivers. Higher nitrate levels at downstream sites could be due to oxidation of excreta from grazed fields and discharge of organic effluents and excessive use of fertilizers. Nitrates is the highest level of oxidized form of Nitrogen and it`s easily leached. It is important to note that excess nitrates have a significant effect on other biota. For example, it lowers the respiration rate of aquatic organisms such as fish. In addition, nitrates play a key role in eutrophication rates surface waters such as River Njoro. Nitrate leaching is common in agricultural catchments where chemical fertilizers are applied in farmlands (Fowler *et al*., 2013). However, leaching is lower in forested sections since little or low rates of fertilizers are applied on farmlands (Di & Cameron, 2002). It is well documented that clearing vegetation results to high amounts of nitrates leached or washed off through surface run-off (Cameron *et al*., 2013).

The rate of nitrogen denitrification depends on oxygen concentration, temperature, concentration of nitrates and presence of microbes (Kröger *et al*., 2014). Lower oxygen at downstream sites accelerated activities of denitrifiers and this explains why higher ammonium concentrations were recorded at downstream sites compared to upstream forested site. Similar results have been obtained in other studies (Glińska-Lewczuk, 2016; Lin *et al*., 2020).

Total phosphorus and soluble reactive phosphorus increased from upstream to downstream (Table 5). At downstream sites, instream activities such as laundry and bathing was observed during sampling sessions. Detergents used for laundry and bathing could have been the potential source of phosphorus in the river. Phosphorus could also be from human and livestock excreta as well as inorganic fertilizers used in agriculture. This study is similar to other studies that reported higher concentrations of SRP and TP at sections of the river impacted by human activities (Aera *et al*., 2019; Gotkowska-Plachta *et al*., 2016). McCartney (2010) also found high SRP levels along the Mara River and this was attributed to the livestock wastes within the area.

Nutrient loadings are a product of amount of water passing through a particular point at a given time (discharge) and constituent nutrient concentrations. Lower TP and TN loadings at forested site compared to other sites shows that small amounts of nutrients from point or non-point sources were exported into the river at thise site. Morales-Marín *et al*. (2015) modelled nutrient loadings in Lake Diefenbaker using SPARROW models and higher nutrient loadings were recorded at upstream sites of Lake Diefenbaker while low nutrient levels were observed at downstream sites. High nutrient loadings downstream sites could be due to high organic matter exported from the surrounding into the river, accelerated by highly disturbed

riparian zones or high conversion of riparian zones to agricultural areas. During storms, nutrient loads increase due to increase in volume of water and nutrient loads input from the catchment (Brezonik & Stadelmann, 2002). Increased nutrient loads in rivers has been reported to be the cause of eutrophication resulting to algal blooms (Li *et al*., 2014).

5.2 Faecal indicator bacteria

Concentrations of faecal indicator bacteria in River Njoro varied among the sites due to different anthropogenic activities (Figure 5, 7 and 8). Around Njoro catchment there are informal settlements close to the river, agricultural activities including livestock keeping and cultivation of farmlands and discharge of effluents from households, industries and wastewater treatment plant. The concentrations of FIB increased downstream but were lowest at upstream Logoman, a forested site compared to other sites. In all cases the levels were above nil CFU/100 ml, amounts recommended by WHO and NEMA. High levels of faecal indicator bacteria at River Mouth station (urban) could be coming from the Nakuru old town sewage treatment plant, located approximately 100 metres away from the site or from contaminated water from upstream sites while bacterial contaminants at agricultural sites could have come from livestock that watered directly into the river and open defecation since human faeces were spotted at these sites during sampling sessions. These results are similar to other studies conducted in Awash River in Ethiopia that reported concentrations of FIB increased from downstream due to human activities (Kebede *et al*., 2020). Xue *et al*. (2018) also found high concentrations of *E. coli* and total coliforms in a river that drained through an agricultural catchment. Studies by Gichana *et al*. (2014) also found out that faecal indicator bacteria varied longitudinally in the Mara River in Kenya.

Escherichia coli and intestinal enterococci are recommended as faecal indicator bacteria since they exclusively live in the intestines of warm blooded animals hence their presence in the environment indicates recent pollution (Cloutier *et al*., 2017). Generally, concentrations of *Escherichia coli* were higher than of enterococci in all the sampling sites (Figure 5 and 7).The results obtained in this study are similar to a study by Kebede *et al*. (2020) who reported higher concentrations of *Escherichia coli* than intestinal enterococci in a mixed land use river catchment. Intestinal enterococci are less sensitive FIB than *E. coli* especially for detecting human pollution sources (Thoe *et a*l., 2018). This is because *E. coli* can grow survive longer in surface water than enterococci (Jang *et al*, 2017; Korajkic *et al*., 2019) most likely indicating *E. coli* was of human faecal pollution.

Clostridium perfingens is a more specific indicator of faecal pollution especially in tropical environments since it does not multiply in the environment (Byappanahalli *et al*.,

2012). Presence of *C. perfringens* in surface waters has been associated mostly with sewage contamination (Fujioka *et al*., 2015). In this study, *Clostridium perfringens* found in the river most likely was from effluents from households, industries and sewage treatment plant. A study by Abia *et al*. (2015) also recorded *Clostridium perfringens* in Apies river at a site near wastewater treatment facilities. The authors recommended use of *Clostridum perfringens* to detect pollution at sites next to WWTP. The concentration of *Clostridium perfringens* was also noted to be lower than that of *E. coli* and intestinal enterococci since *Clostridium perfringens* are anaerobic bacteria. *Clostridium perfringens* have low oxygen tolerance and they survive for only a short time in non-host environments (Avelar *et al*., 1998). It should be noted that their presence in the river is an indicator that faecal pollution in River Njoro has occurred over a period of time but their numbers were always much lower to suspect remote pollution in any of the sampling sites. Just like other alternative faecal indicators, *C. perfringens* standards have not yet been evaluated based on epidemiological studies on the acceptable risk associated with faecal pollution. They have also not been adopted since their distribution in the environment is limited or the methods of recovering them are very complex and have no tolerance to oxygen (Leclerc *et al*., 2001).

Findings from this study showed that faecal pollution was likely from animal origin since the ratio of *E. coli* to intestinal enterococci was less than 4. This could be true because livestock (cows, goats, donkeys) are reared in the catchment and it was very common to find many types of wild animals, birds, monkeys, snakes near the riparian areas of the river during sampling sessions. It is important to note that even at River Mouth (urban), located few metres from Nakuru old town sewage treatment plant, the *E. coli* to intestinal enterococci ratio was less than 4 hence did not indicate human origin as expected. Faecal pollution at the River Mouth could be from wild animals from the Lake Nakuru National park since no livestock were spotted at this site during sampling. Although faecal coliform to faecal streptococci ratio is easy and fast it can be inaccurate since the survival rates of faecal coliforms and faecal streptococci vary with physico-chemical factors. Furthermore, faecal coliforms and faecal streptococci have different die off rates (Sinton *et al*., 1993, 1998). Other methods of source tracking such as *Bacteroides* to track faecal pollution uses human-specific gene markers hence could be the most appropriate to track pollution from human origin.

It has been documented that some strains of bacteria can cause diseases in humans such as diarrhoea. However, this study did not conduct tests to find out if the antibiotic resistant *E. coli* isolated from the river were either pathogenic or non-pathogenic. Presence of enteric bacteria in River Njoro is an indication of possible presence of other pathogens therefore

increasing human microbial risks to waterborne illnesses such as typhoid and cholera. At agricultural sites, there were recreational instream activities such as swimming and this has been documented in another study (Yillia *et al*., 2008). This is a public health risk since the contaminated water maybe ingested involuntarily during swimming or through direct contact with contaminated water during washing or water abstraction for domestic use. A study by Ashbolt (2015) found out that exposure to contaminated water during recreation caused gastro intestinal illnesses.

Turbidity and total suspended solids correlated positively with FIB from this study and this correlations have been reported in other studies (Paule-Mercado *et al*., 2016). Suspended solids promote the growth of total coliforms by protecting the bacteria from UV and providing both organic and inorganic nutrients. Although it was expected that TSS would positively correlate with FIB, the correlations were negative and very weak leading to the speculation that a good proportion of TSS was inorganic clay particles constituting non-filtrable solids that could have persisted in the river water for longer time especially in downstream sites. Significant positive correlations between FIB (particularly total coliforms) and temperature implied that water temperature favoured their growth and survival. Previous studies have also reported positive correlations between FIB and temperature (Abia *et al*, 2015; Islam *et al*., 2017). Inactivation rates of FIB is dependent on temperature. Warmer temperatures increases the rate of metabolism hence promoting growth of FIB. As temperature increases beyond optimum range, inactivation rates of FIB also increases (Blaustein *et al*., 2013; Pachepsky *et al*., 2014).Total Phosphorus, soluble reactive Phosphorus and total nitrogen positively correlated with FIB indicating presence of manure from farmlands and deposition of animal excreta into the river. Similar correlations were observed by Paule-Mercado *et al*. (2016). The authors attributed these correlations to deposition of animal wastes into the river and use of fertilizers within the catchment.

5.3 Antimicrobial resistance of *E. coli* isolated from River Njoro

It was observed that at every site, *E. coli* was resistant to more than one antibiotic (Figure 9 and 10). These results are similar to a study by Martinez (2012) who also found *E. coli* resistant to multiple antibiotics including tetracycline. Tadesse (2012) also reported high resistance to tetracyclines due to their frequent use. In this study, high level of resistance at agricultural, industrial and urban sites could be from effluents that have ARB, direct defecation in the river by livestock or open defecation by humans. Continued spread of antibiotic resistance in the environment could result to risk of death, prolonged illness or higher cost of treatment especially from various pathovars of *E. coli*.

Antibiotics are not easily degraded in the body hence they are sometimes excreted in human or livestock excreta in their original form (DeWitte *et al*., 2008; Luo *et al*., 2014; Verlicchi & Zambello, 2016). Antibiotic Resistant genes are found in genetic elements such as plasmids and they spread through transduction, conjugation or transformation (Andersson & Hughes, 2010). These genes are excreted together with faeces and this could result to accumulation of antibiotics in the system. The potential health risk is development of antimicrobial resistance in the environment and subsequent transfer to human and zoonotic hosts. The low resistance observed in gentamicin, levofloxacin and amikacin from this study implies that resistant genes and bacteria containing this antibiotics were limited in the environment. It could also be due to their limited use in River Njoro catchment. In a study done by Goñi-Urriza *et al*. (2000), the authors found out that streptomycin are frequently used in treatment of tuberculosis and this could be the reason why it had higher resistance compared to other aminoglycosides such as amikacin and gentamicin.

Multiple antibiotic resistance index observed in all the sites implied that the environment has been exposed with high amounts of antibiotics indicating high usage within the catchment for treatment or as growth promoters. Drug overuse cause the targeted bacteria to develop resistance. Generally, resistance of *E. coli* to the tested antibiotics was as follows; streptomycin>ciprofloxacin>chloramphenicol>amoxicillin>tetracycline>ampicillin>amikacin >gentamicin>levofloxacin (Table 9). These results are similar to a study by Itotia *et al*. (2018) who also reported high resistance of *E. coli* to streptomycin and chloramphenicol. This could be attributed to indiscriminate use of these antimicrobials especially for chloramphenicol in the past as it is currently not prescribed. Alhaj *et al*. (2007) also found *E. coli* resistant to gentamicin, tetracycline, chloramphenicol. The authors attributed this to contamination of the river by agricultural and domestic wastes. Other studies also found *E. coli* resistant to tetracycline since this antibiotic was frequently used in livestock and humans for treatment or growth enhancement thus accelerated the spread of tetracycline resistant bacteria (Kümmerer, 2004; Ribeiro *et al*., 2012).

Both ciprofloxacin and levofloxacin belong to fluoroquinolones antibiotic group and they are used to treat bacterial infections. In this study, ciprofloxacin showed higher percentage resistance than levofloxacin (Table 9) and this indicates overuse of ciprofloxacin in Njoro catchment since it is listed as an essential drug in Kenya (MOH, 2019). A study by Wu *et al*. (2016) found higher resistance of *E. coli* to levofloxacin compared to ciprofloxacin but after controlling the use of levofloxacin, the incidence of *Escherichia coli* resistance to levofloxacin

reduced. Levofloxacin could be the most suitable antibiotic to treat *E. coli* infections in case of waterborne disease outbreak in Njoro catchment due to low resistance.

All the sites had MARI indices greater than the threshold of 0.2 (Table 10) suggested by Krumperman (1983) to distinguish between high risk and low risk contamination sites although the author acknowledged that the value was arbitrary. Values greater than 0.2 indicate high health risks to people who directly consume the river water. Despite upstream forested site having exceeded the threshold value of 0.2, the overall percentage resistance at this site was lower compared to other sites. Development of resistance of *E. coli* to multiple antibiotics especially downstream sites could have been caused by anthropogenic activities such as livestock watering, open defecation and discharge of effluents. These findings suggest that *E. coli* isolates could have been overexposed to antibiotics due to overuse of antibiotics in River Njoro catchment. Appropriate measures are needed to be put in place to reduce the spread of multidrug resistant *E. coli*.

The spread of ARB in the environment has also been attributed to WWTP by introduction of antibiotics in the environment creating direct contact of the bacteria which enhances gene transfer. Similarly, this could explain high resistance observed at the River Mouth (urban) (Table 10) which is situated below Nakuru old town sewage treatment plant that treats effluents from Nakuru town. WWTP and septic tanks have been documented as sources of antibiotic resistant bacteria (Kümmerer, 2004; Yamashita *et al*., 2017). This agrees with Watkinson (2007) results that sites close to WWTP had higher concentration of antibiotic resistant *E. coli*. Although WWTP are designed to reduce enteric microbes, they can still promote the spread of antibiotic resistant genes (Marti *et al*., 2014). Furthermore, biological treatment of wastewater does guarantees that antibiotic resistant genes will not be spread in the environment (Da Costa *et al*., 2006).

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

There were several conclusions to be derived from this study. From the first (1) specific objective, this study concluded that forested site had lower levels of physico-chemical variables; total suspended solids, turbidity, dissolved oxygen, pH, temperature, total dissolved solids, conductivity and nutrients (nitrates, nitrites, ammonium, total nitrogen, soluble reactive phosphorus and total phosphorus) as compared to agricultural, industrial and urban sites. Thus, the null hypothesis that there is no significant difference in physico-chemical variables between the sampling sites along River Njoro is rejected.

From specific objective 2, this study concluded that there was significant difference in faecal indicator bacteria between the sites with varying land use. Therefore, null hypothesis that there is no significant difference in concentrations of *Escherichia coli,* intestinal enterococci and *Clostridium perfringens* between sampling sites along River Njoro is rejected. River Njoro water should not be used for domestic chores without being subjected to some kind of treatment process.

From specific objective 3, this study concluded that there was significant difference in antimicrobial resistance of *E. coli* isolates between the sampling points along River Njoro, with Logoman having the least resistance and River Mouth the highest resistance. *E. coli* was highly resistant to streptomycin, chloramphenicol, amoxicillin and tetracycline. Discharge of effluents and direct defecation in the river has led to spread of antibiotic resistant bacteria therefore limiting treatment options for residents during infections. Based on these results, the null hypothesis that there is no significant difference in antimicrobial resistance between sampling points along River Njoro is rejected except for amikacin and amoxicillin.

6.2 Recommendations

Farmers around River Njoro catchment should be sensitized on proper agricultural practices. These include; timely application of fertilizers and use of alternative methods to water their livestock to minimize pollution of River Njoro. Inhabitants of River Njoro catchment should also be involved in riparian management activities such as planting of indigenous trees to increase vegetation cover which is important for improving and maintaining river water quality.

Inhabitants of River Njoro catchment should be sensitized on water treatment technologies before using water for domestic chores such as UV disinfection, water boiling, and chlorination. This will help to kill or remove enteric bacteria and associated pathogens.

Microbial source tracking using molecular techniques should also be done to determine the exact source of faecal bacteria of River Njoro.

Policies should also be put in place to control the unregulated use of antimicrobials to minimize the spread of resistant bacterial strains. Antimicrobials should only be sold upon prescription by a physician or a veterinary.

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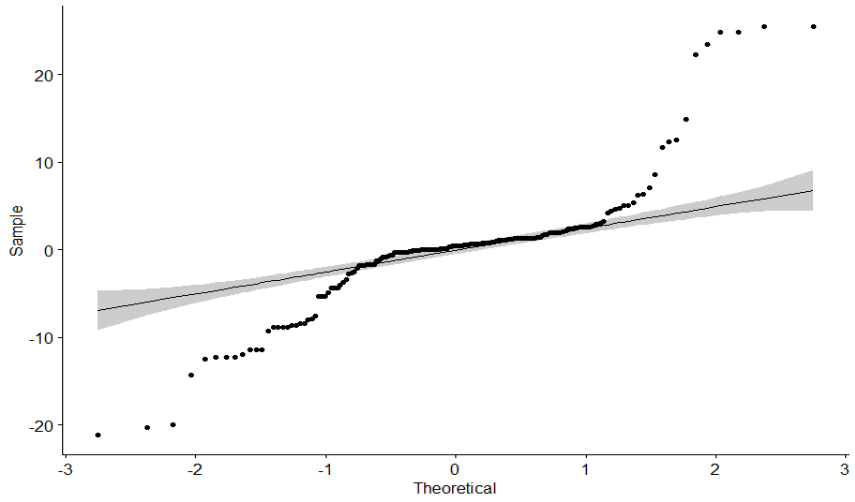
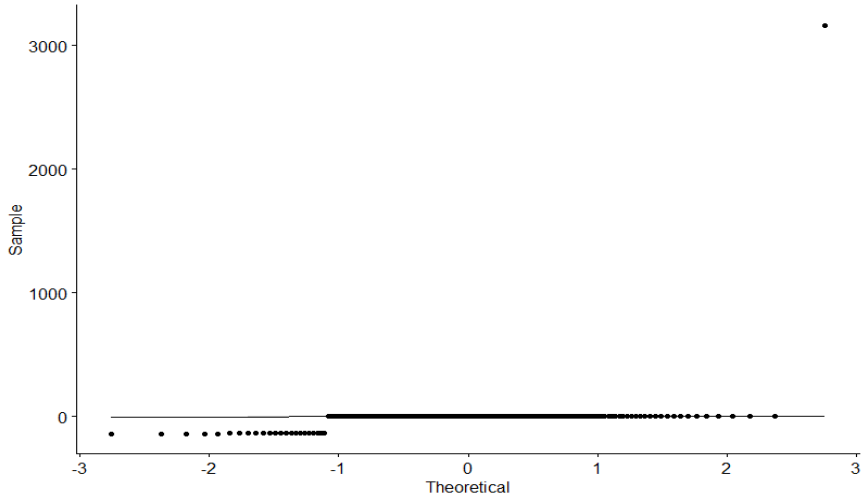
APPENDICES

Appendix A: Quantile-Quantile (Q-Q) plots showing skewness of the variables

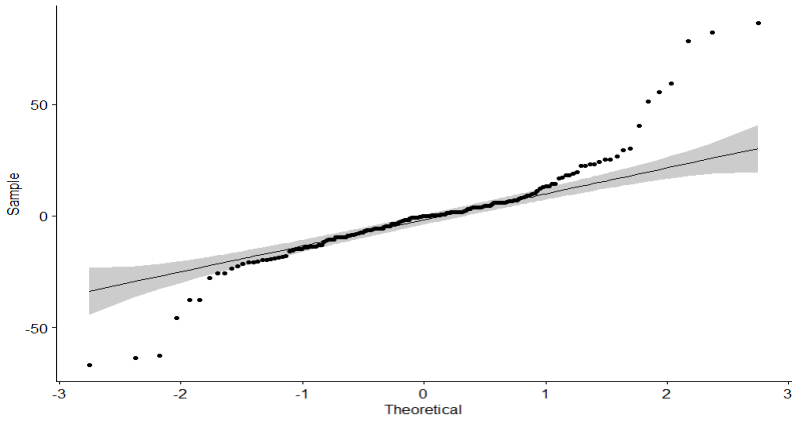
Nitrites



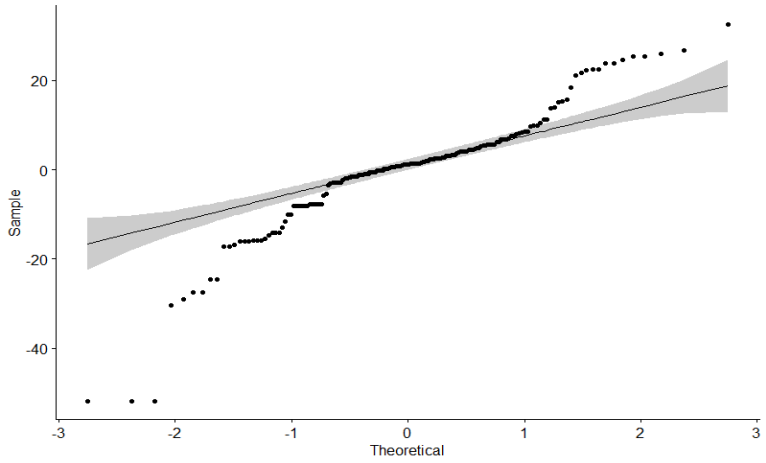
Nitrates



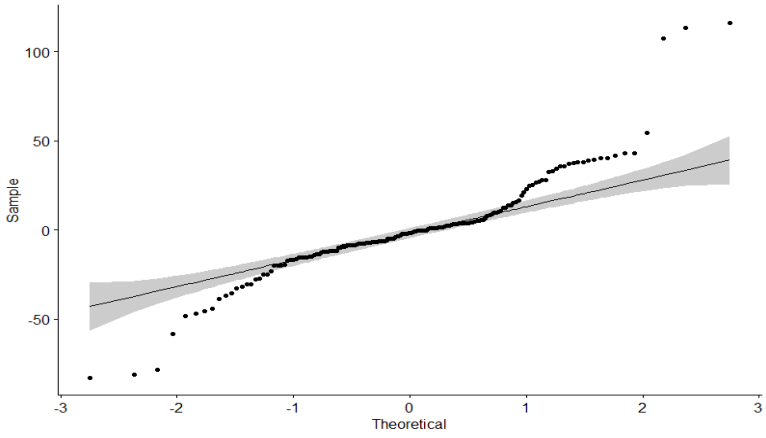
Ammonium



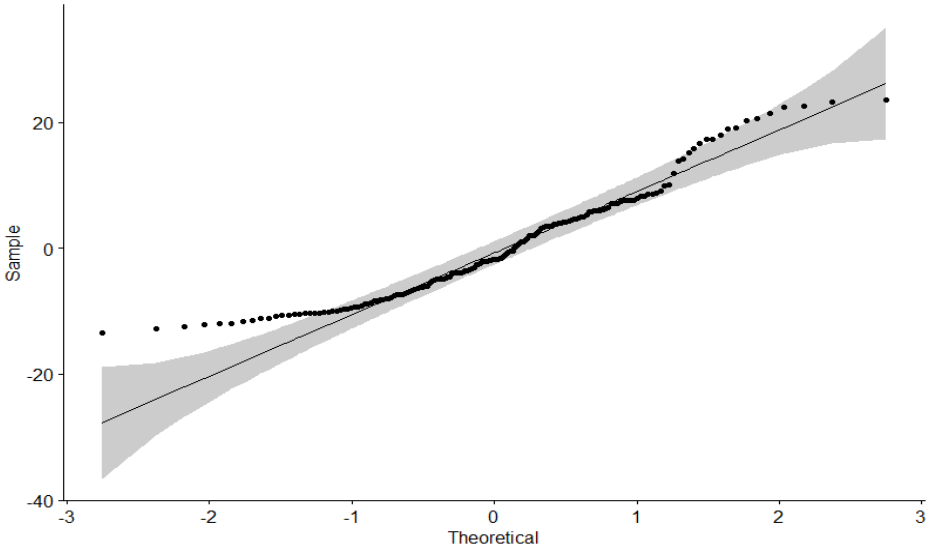
Soluble reactive phosphorus



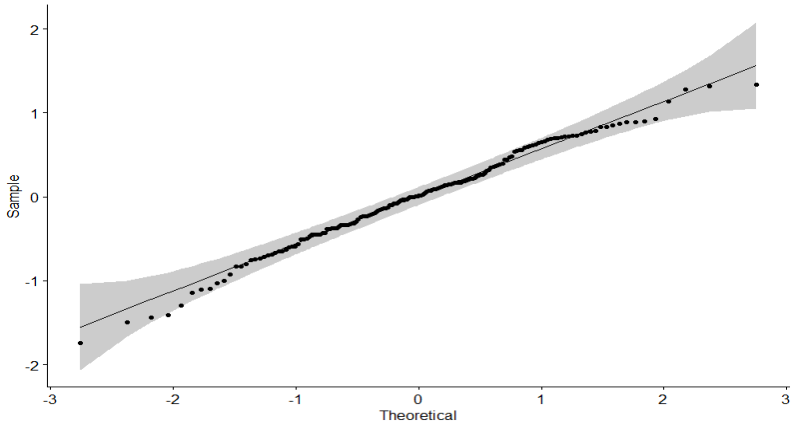
Total phosphorus



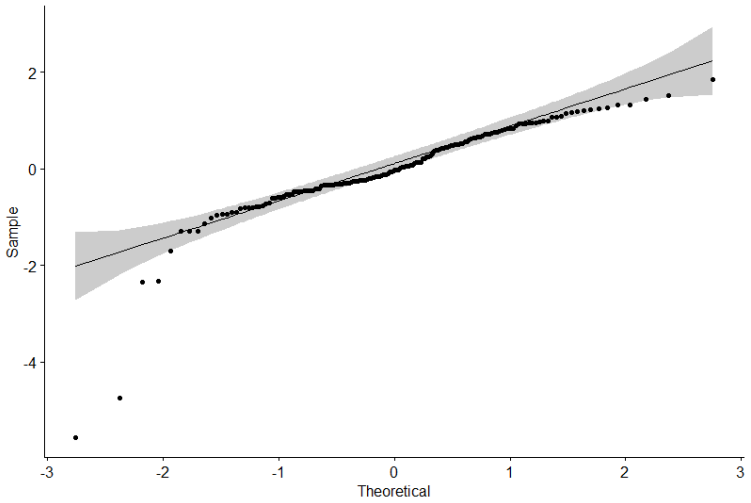
Total nitrogen



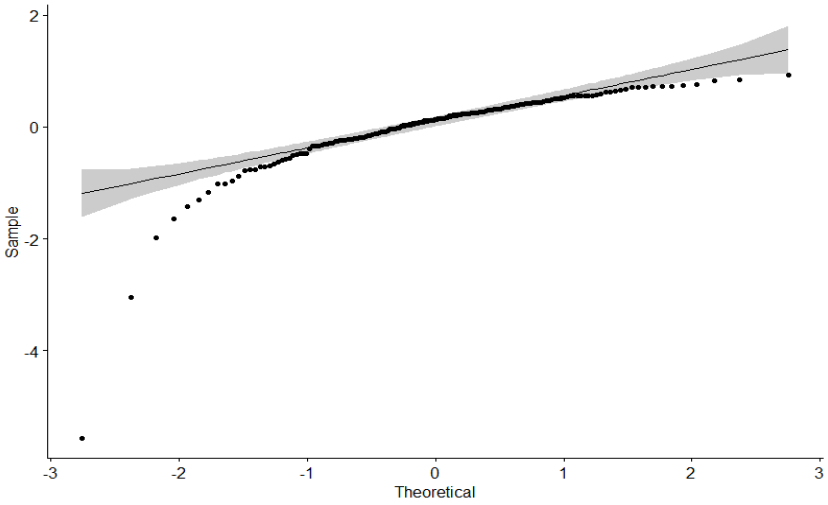
Escherichia coli



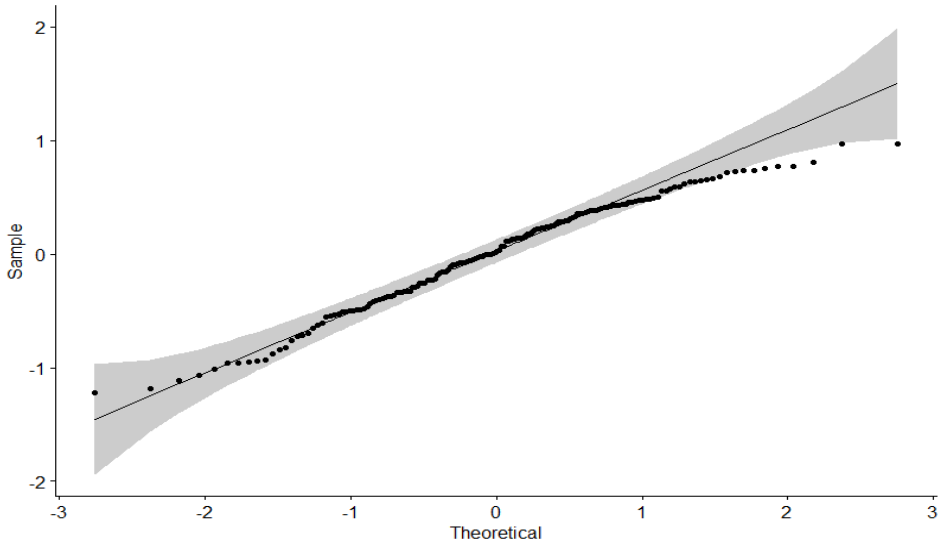
Clostridium perfringens



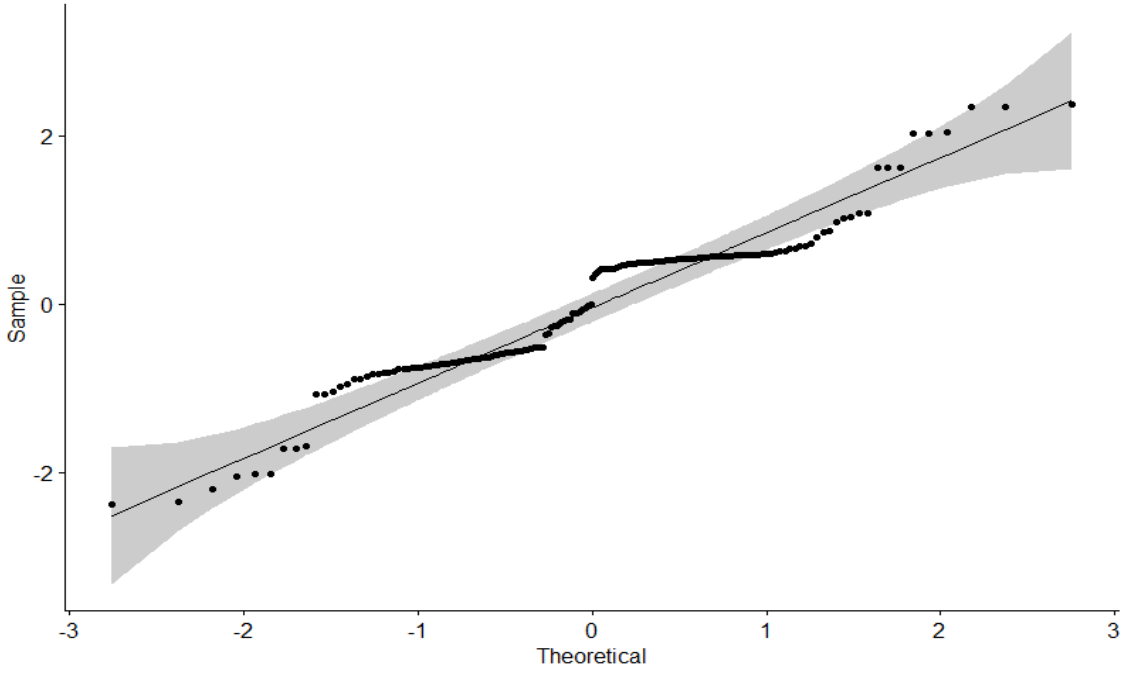
Intestinal enterococci



Total coliforms



Dissolved Oxygen



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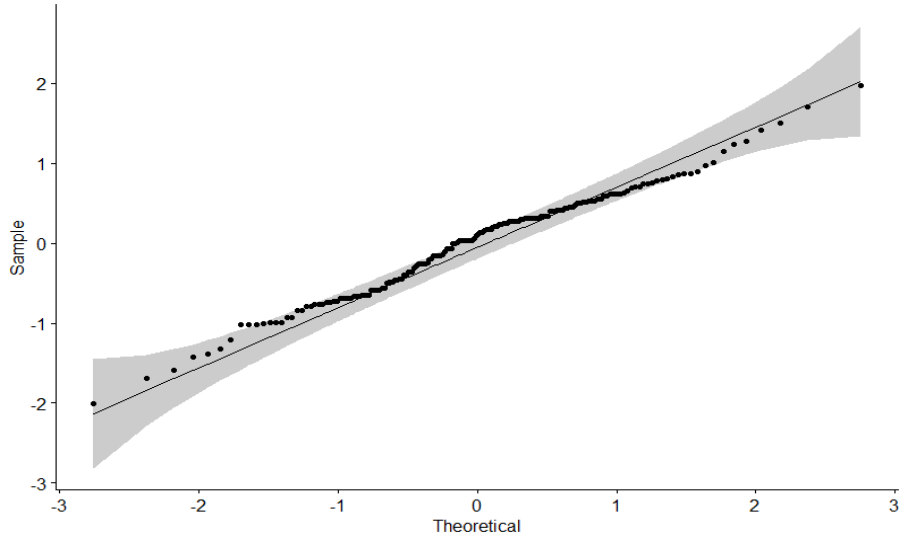

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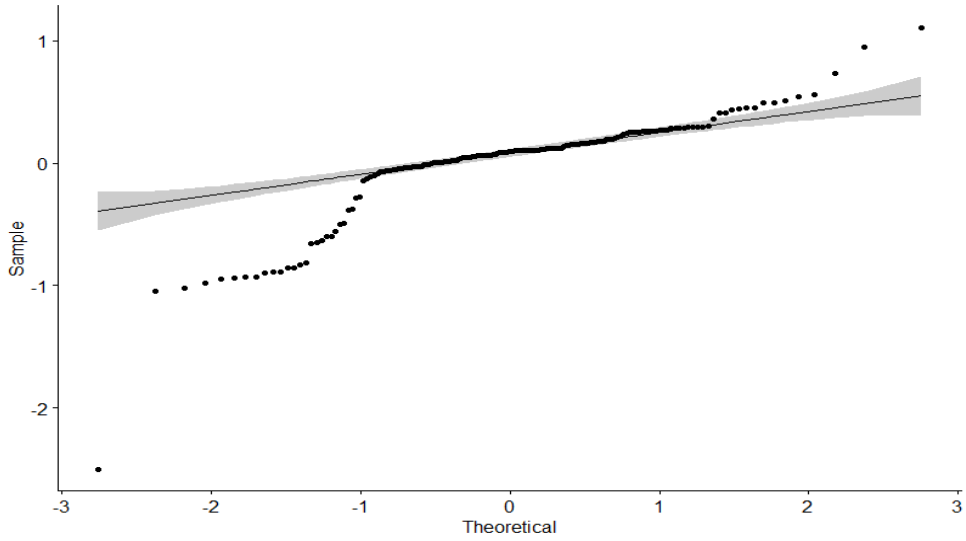
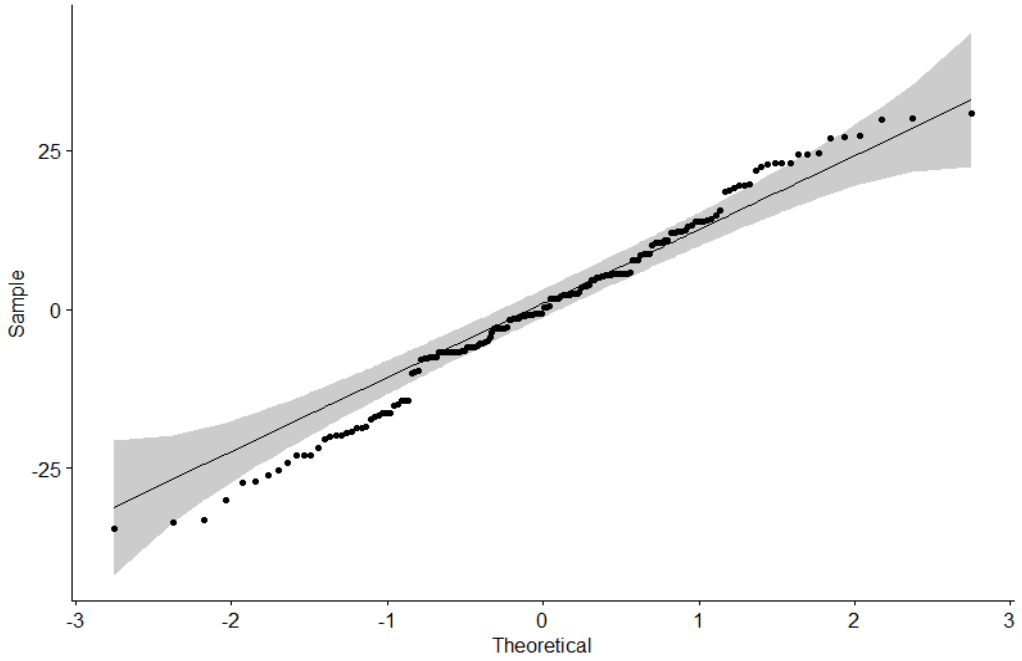
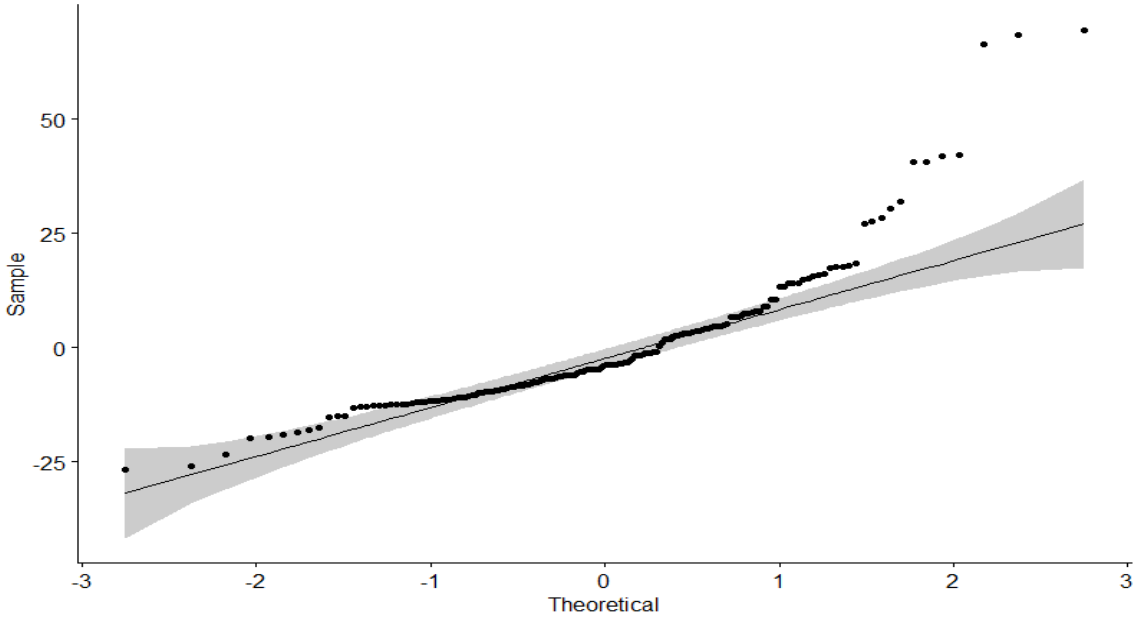


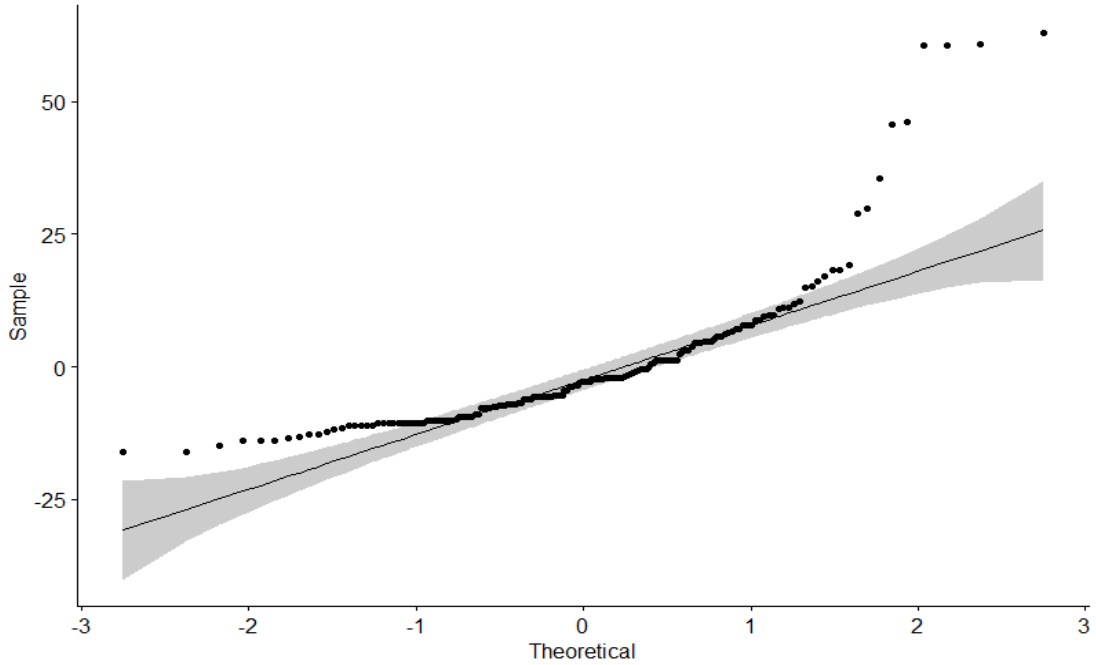
Total Dissolved Solids



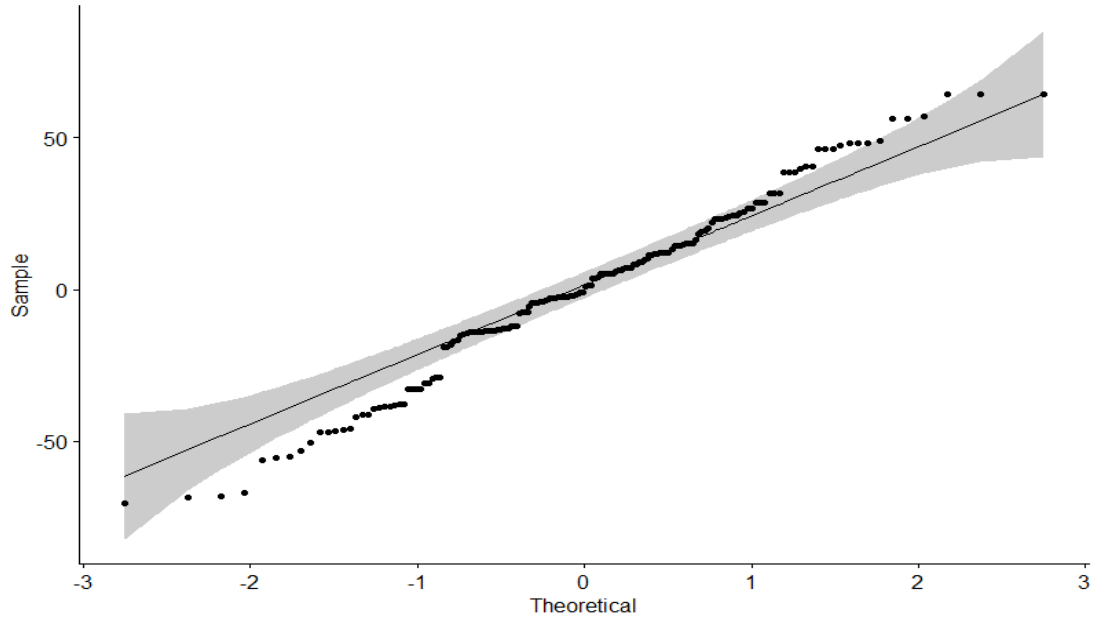
Turbidity



Total suspended solids



Conductivity



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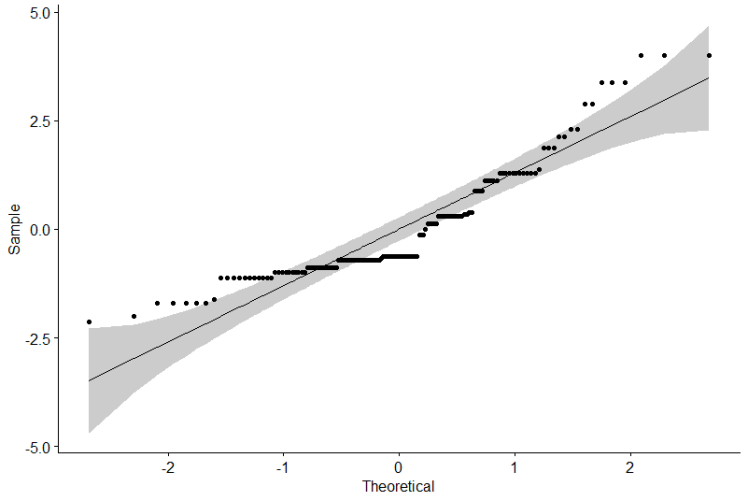

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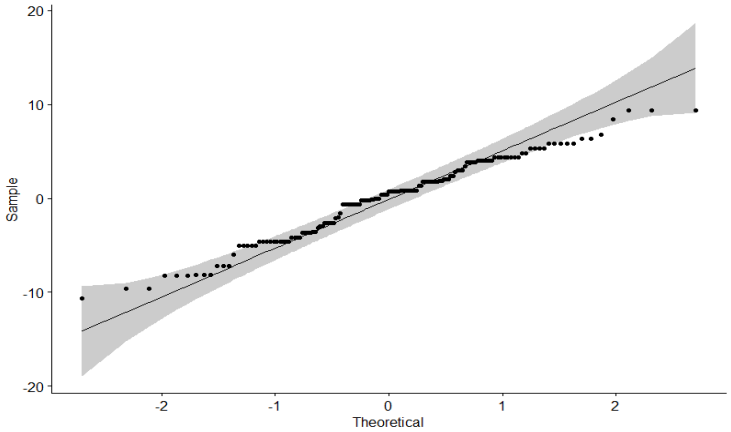

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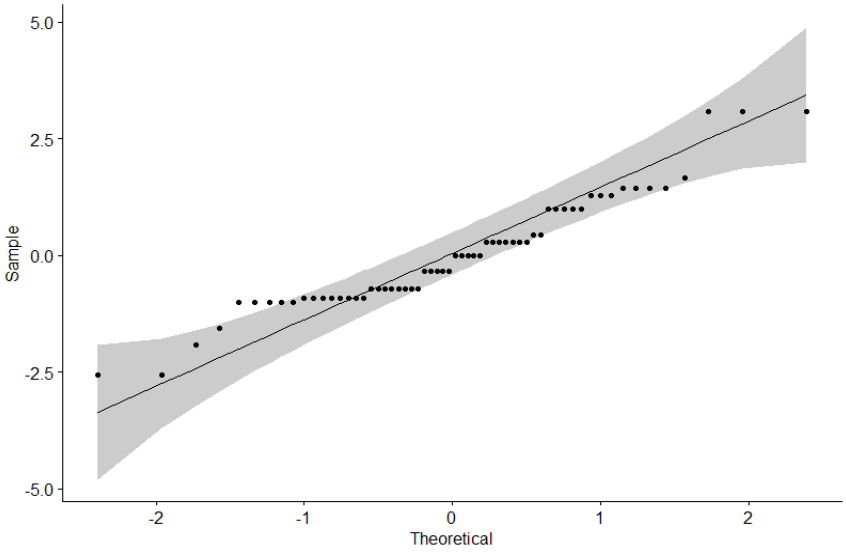

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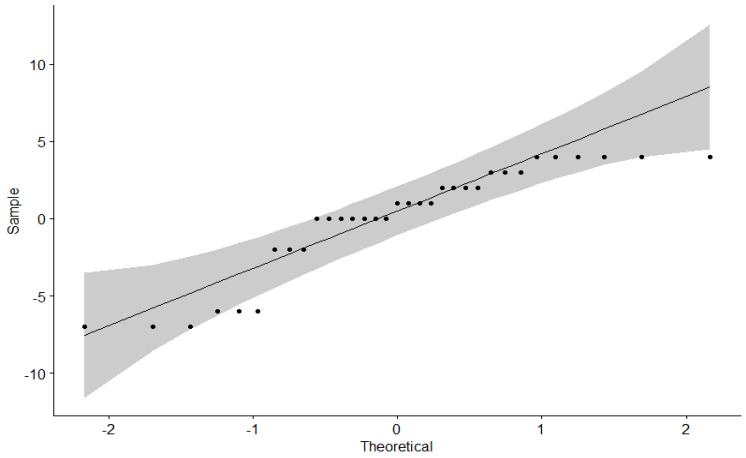

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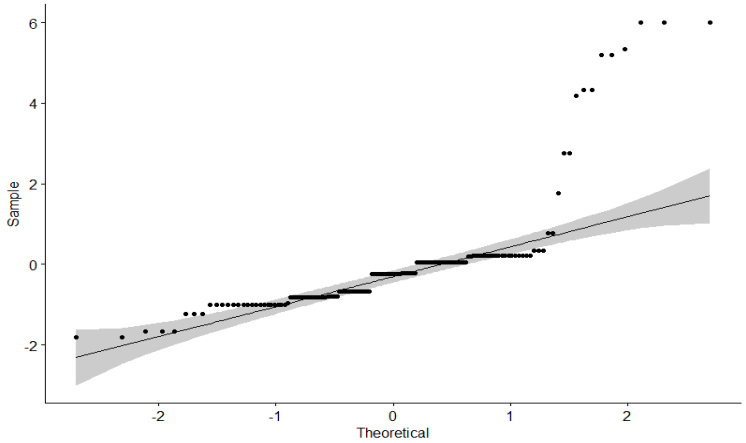

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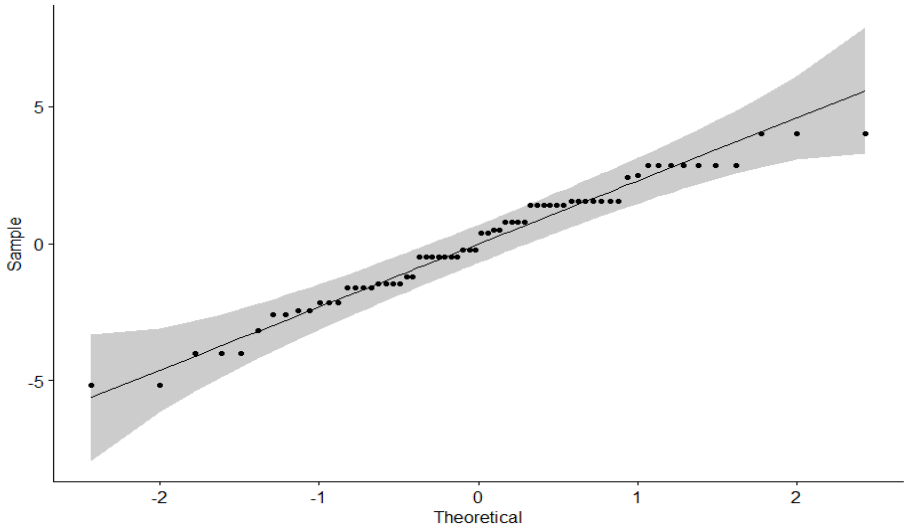

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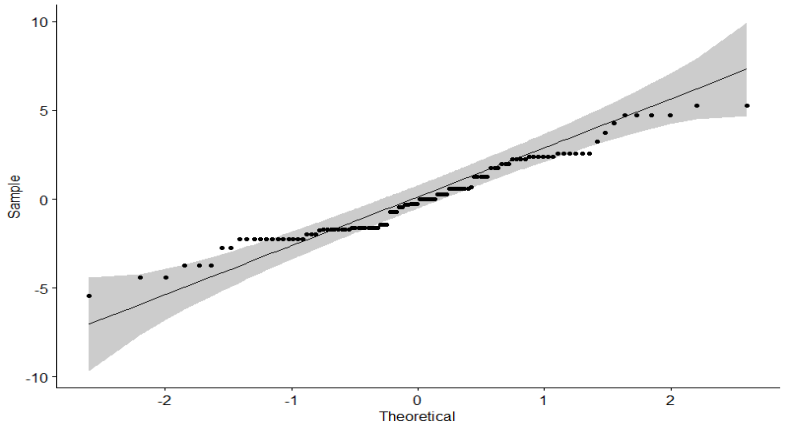
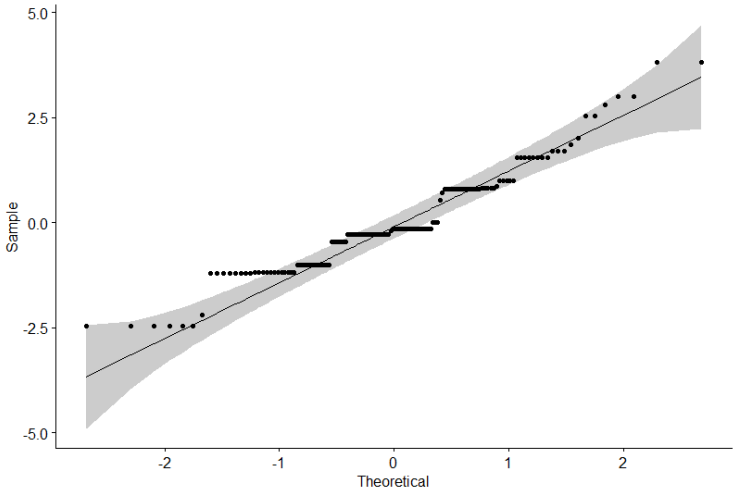


Streptomycin



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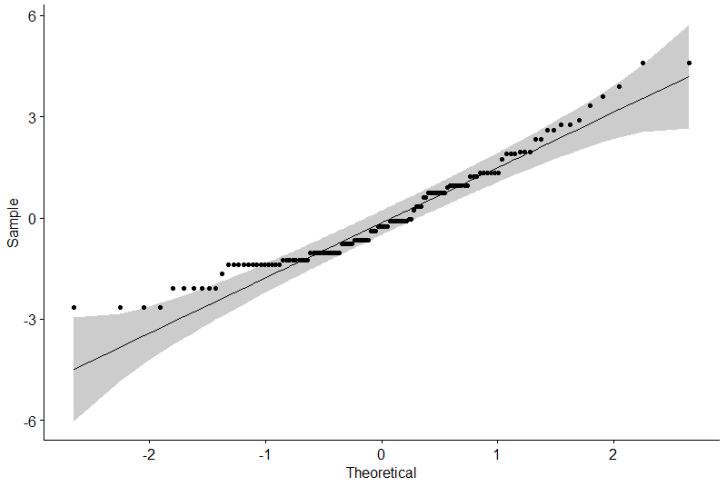

Appendix B: Additional results

Appendix B1: Table showing mean values, standard deviations (CFU/100 mL) of faecal indicator bacteria recorded during the study

	Sites and their respective land use						
	Logoman	Sigotik	Turkana	Canning	Njoro Bridge	Ngata	Mouth
Variables	Forest	Mixed	Agriculture	Industrial	Agriculture	Agriculture	Urban
E. coli	$4.89\times10^{2}\pm2.61\times10^{2}$	$1.11\times10^{3}\pm7.36\times10^{2}$	$2.22\times10^{3}\pm1.76\times10^{3}$	$2.07\times10^{3}\pm9.7\times10^{2}$	$3.16\times10^{3}\pm1.6\times10^{3}$	$2.85\times10^{3}\pm2.65\times10^{3}$	$6.57\times10^{3}\pm1.23\times10^{3}$
Total coliforms	$9.34\times10^{2}\pm4.23\times10^{2}$	$1.87\times10^{3}\pm8.88\times10^{2}$	$3.81\times10^{3}\pm2.13\times10^{3}$	$4.01\times10^{3}\pm2.04\times10^{3}$	$5.65\times10^{3}\pm2.17\times10^{3}$	$4.89\times10^{3}\pm2.26\times10^{3}$	$1.02\times10^{4}\pm2.88\times10^{3}$
Intestinal enterococci	$3.45\times10^{2}\pm1.51\times10^{2}$	$4.27\times10^{2}\pm1.87\times10^{2}$	$2.19\times10^{3}\pm8.15\times10^{2}$	$1.97\times10^{3}\pm7.16\times10^{2}$	$2.16\times10^{3}\pm1.00\times10^{3}$	$2.18\times10^{3}\pm1.15\times10^{3}$	$3.78\times10^{3}\pm1.30\times10^{3}$
Clostridium perfringens	$1.5\times10^{2}\pm9.6\times10^{1}$	$2\times10^{2}\pm1.53\times10^{2}$	$3.67\times10^{2}\pm2.37\times10^{2}$	$4.2\times10^{2}\pm2.63\times10^{2}$	$6.02\times10^{2}\pm3.81\times10^{2}$	$6.08\times10^{2}\pm6.23\times10^{2}$	$8.33\times10^{2}\pm5.39\times10^{2}$

Appendix B2: Pairwise comparison of dissolved oxygen concentration among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Mouth-Ngata	68.167	14.041	4.855	.000	.000
Mouth-Turkana	75.417	14.041	5.371	.000	.000
Mouth-Njoro Bridge	75.667	14.041	5.389	.000	.000
Mouth-Canning	80.250	14.041	5.716	.000	.000
Mouth-Sigotik	96.958	14.041	6.906	.000	.000
Mouth-Logoman	107.52	14.041	7.659	.000	.000
Ngata-Turkana	7.250	14.041	.516	.606	1.000
Ngata-Njoro Bridge	7.500	14.041	.534	.593	1.000
Ngata-Canning	12.083	14.041	.861	.389	1.000
Ngata-Sigotik	28.972	14.041	2.051	.040	.846
Ngata-Logoman	39.375	14.041	2.804	.005	.106
Turkana-Njoro Bridge	-.250	14.041	-.018	.986	1.000
Turkana-Canning	-4.833	14.041	-.344	.731	1.000
Turkana-Sigotik	21.542	14.041	1.534	.125	1.000
Turkana-Logoman	32.125	14.041	2.288	.022	.465
Njoro Bridge-Canning	4.583	14.041	.326	.744	1.000
Njoro Bridge-Sigotik	21.292	14.041	1.516	.129	1.000
Njoro Bridge-Logoman	31.875	14.041	2.270	.023	.487
Canning-Sigotik	16.708	14.041	1.190	.234	1.000
Canning-Logoman	27.292	14.041	1.944	.052	1.000
Sigotik-Logoman	10.583	14.041	.754	.451	1.000

Appendix B3: Pairwise comparison of temperature among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Logoman-Sigotik	-21.354	14.038	-1.521	0.128	1.000
Logoman-Canning	-65.167	14.038	-4.642	.000	.000
Logoman-Njoro Bridge	-68.729	14.038	-4.896	.000	.000
Logoman-Turkana	-74.792	14.038	-5.328	.000	.000

Logoman-Ngata	-116.708	14.038	-8.314	.000	.000
Logoman-Mouth	-141.792	14.038	-10.101	.000	.000
Sigotik-Canning	-43.812	14.038	-3.121	.002	.038
Sigotik-Njoro Bridge	-47.375	14.038	-3.375	.001	.016
Sigotik-Turkana	-53.438	14.038	-3.807	.000	.003
Sigotik-Ngata	-95.354	14.038	-6.793	.000	.000
Sigotik-Mouth	-120.438	14.038	-8.579	.000	.000
Canning-Njoro Bridge	-3.562	14.038	-2.54	.800	1.000
Canning-Turkana	9.625	14.038	.686	.493	1.000
Canning-Ngata	-51.542	14.038	-3.672	.000	.005
Canning-Mouth	-76.625	14.038	-5.458	.000	.000
Njoro Bridge-Turkana	6.062	14.038	.432	.666	1.000
Njoro Bridge-Ngata	-47.979	14.038	-3.418	.001	.013
Njoro Bridge-Mouth	-73.062	14.038	-5.205	.000	.000
Turkana-Ngata	-41.917	14.038	-2.986	.003	.059
Turkana-Mouth	-67.000	14.038	-4.733	.000	.000
Ngata-Mouth	-25.083	14.038	-1.787	.074	1.000

Appendix B4: Pairwise comparison of pH among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Mouth-Sigotik	12.229	14.039	.871	.384	1.000
Mouth-Njoro Bridge	13.375	14.039	.953	.341	1.000
Mouth-Logoman	23.833	14.039	1.698	.090	1.000
Mouth-Canning	57.500	14.039	4.096	.000	.001
Mouth-Turkana	59.708	14.039	4.353	.000	.000
Mouth-Ngata	71.646	14.039	5.103	.000	.000
Sigotik-Njoro Bridge	-1.146	14.039	-0.082	.935	1.000
Sigotik-Logoman	11.604	14.039	.827	.408	1.000
Sigotik-Canning	-45.271	14.039	-3.225	.001	.026
Sigotik-Turkana	-47.479	14.039	-3.382	.000	.000
Sigotik-Ngata	-59.417	14.039	-4.232	.000	.000
Njoro Bridge-Logoman	10.458	14.039	.745	.456	1.000
Njoro Bridge-Canning	44.125	14.039	3.143	.002	.035

Njoro Bridge-Turkana	46.333	14.039	3.300	.001	.020
Njoro Bridge-Ngata	-58.271	14.039	-4.150	.000	.001
Logoman-Canning	33.667	14.039	-2.398	.016	.346
Logoman-Turkana	-35.875	14.039	-2.555	.011	.223
Logoman-Ngata	-47.812	14.039	-3.406	.001	.014
Canning-Turkana	2.208	14.039	.157	.875	1.000
Canning-Ngata	-14.416	14.039	-1.008	.314	1.000
Turkana-Ngata	-11.938	14.039	-.850	.395	1.000

Appendix B5: Pairwise comparison of Total Dissolved Solids among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Logoman-Sigotik	-21.750	14.041	-1.549	.121	1.000
Logoman-Turkana	-84.688	14.041	-6.031	.000	.000
Logoman-Ngata	-87.646	14.041	-6.242	.000	.000
Logoman-Canning	-88.583	14.041	-6.309	.000	.000
Logoman-Njoro Bridge	-94.750	14.041	-6.748	.000	.000
Logoman-Mouth	-118.708	14.041	-8.54	.000	.000
Sigotik-Turkana	-62.938	14.041	-4.482	.000	.000
Sigotik-Ngata	-65.986	14.041	-4.693	.000	.000
Sigotik-Canning	-66.833	14.041	-4.760	.000	.000
Sigotik-Njoro Bridge	-73.000	14.041	-5.199	.000	.000
Sigotik-Mouth	-96.958	14.041	-6.905	.000	.000
Turkana-Ngata	-2.958	14.041	.211	.833	1.000
Turkana-Canning	-3.896	14.041	-.277	.781	1.000
Turkana-Njoro Bridge	-10.062	14.041	.717	.474	1.000
Turkana-Mouth	-34.021	14.041	-2.423	.015	.323
Ngata-Canning	.938	14.041	.067	.947	1.000
Ngata-Njoro Bridge	7.104	14.041	.506	.613	1.000
Ngata-Mouth	-31.062	14.041	-2.212	.027	.566
Canning-Njoro Bridge	-6.617	14.041	-.439	.661	1.000
Canning-Mouth	-30.125	14.041	-2.146	.032	.670
Njoro Bridge-Mouth	-23.958	14.041	-1.706	.088	1.000

Appendix B6: Pairwise comparison of Turbidity among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Sigotik-Logoman	23.500	14.041	1.674	.094	1.000
Sigotik-Mouth	-44.312	14.041	-3.156	.002	.034
Sigotik-Canning	-53.062	14.041	-3.779	.000	.003
Sigotik-Turkana	-54.562	14.041	-3.886	.000	.002
Sigotik-Njoro Bridge	-65.958	14.041	-4.698	.000	.000
Sigotik-Ngata	-74.188	14.041	-5.824	.000	.000
Logoman-Mouth	-20.182	14.041	-1.482	.138	1.000
Logoman-Canning	-29.562	14.041	-2.105	.035	.740
Logoman-Turkana	-31.062	14.041	-2.212	.027	.566
Logoman-Njoro Bridge	-42.458	14.041	-3.024	.002	.052
Logoman-Ngata	-50.688	14.041	-3.610	.000	.006
Mouth-Canning	8.750	14.041	.623	.533	1.000
Mouth-Turkana	10.250	14.041	.730	.465	1.000
Mouth-Njoro Bridge	21.646	14.041	1.542	.123	1.000
Mouth-Ngata	29.875	14.041	2.128	.033	.701
Canning-Turkana	1.500	14.041	.107	.915	1.000
Canning-Njoro Bridge	-12.896	14.041	-.918	.358	1.000
Canning-Ngata	-21.125	14.041	-1.505	.132	1.000
Turkana-Njoro Bridge	-11.396	14.041	-.812	.417	1.000
Turkana-Ngata	-19.625	14.041	-1.398	.162	1.000
Njoro Bridge-Ngata	-8.229	14.041	-.586	.558	1.000

Appendix B7: Pairwise comparison of conductivity among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Logoman-Sigotik	-19.125	14.040	-1.362	.173	1.000
Logoman-Turkana	-84.604	14.040	-6.026	.000	.000
Logoman-Ngata	-84.958	14.040	-6.051	.000	.000
Logoman-Canning	-86.250	14.040	-6.143	.000	.000

Logoman-Njoro Bridge	-94.292	14.040	-6.716	.000	.000
Logoman-Mouth	-117.708	14.040	-8.384	.000	.000
Sigotik-Turkana	-65.479	14.040	-4.664	.000	.000
Sigotik-Ngata	-65.833	14.040	-4.689	.000	.000
Sigotik-Canning	-67.125	14.040	-4.781	.000	.000
Sigotik-Njoro Bridge	-75.167	14.040	-5.354	.000	.000
Sigotik-Mouth	-98.583	14.040	-7.002	.000	.000
Turkana-Ngata	-.354	14.040	-.025	.980	1.000
Turkana-Canning	-1.646	14.040	-.117	.907	1.000
Turkana-Njoro Bridge	-9.688	14.040	-.690	.490	1.000
Turkana-Mouth	-33.104	14.040	-2.358	.018	.386
Ngata-Canning	1.292	14.040	.092	.927	1.000
Ngata-Njoro Bridge	9.333	14.040	.665	.506	1.000
Ngata-Mouth	-32.759	14.040	-2.333	.020	.413
Canning-Njoro Bridge	-8.042	14.040	-.573	.567	1.000
Canning-Mouth	-31.458	14.040	-2.241	.025	.526
Njoro Bridge-Mouth	-23.417	14.040	-1.668	.095	1.000

Appendix B8: Pairwise comparison of Ammonium concentration among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Sigotik-Logoman	24.625	14.036	1.754	.079	1.000
Sigotik-Turkana	-37.688	14.036	-2.685	.007	.152
Sigotik-Canning	-38.833	14.036	-2.767	.006	.119
Sigotik-Ngata	-41.167	14.036	-2.933	.003	.071
Sigotik-Njoro Bridge	-57.583	14.036	-4.103	.000	.001
Sigotik-Mouth	-108.396	14.036	-7.723	.000	.000
Logoman-Turkana	-13.062	14.036	-.931	.352	1.000
Logoman-Canning	-14.208	14.036	-1.012	.311	1.000
Logoman-Ngata	-16.542	14.036	-1.179	.239	1.000
Logoman-Njoro Bridge	-32.958	14.036	-2.348	.019	.396
Logoman-Mouth	-83.771	14.036	-5.968	.000	.000
Turkana-Canning	-1.146	14.036	-.082	.935	1.000

Turkana-Ngata	-3.479	14.036	-.248	.804	1.000
Turkana-Njoro Bridge	-19.896	14.036	-1.417	.156	1.000
Turkana-Mouth	-70.708	14.036	-5.038	.000	.000
Canning-Ngata	-2.333	14.036	-.166	.868	1.000
Canning-Njoro Bridge	-18.750	14.036	-1.336	.182	1.000
Canning-Mouth	-69.562	14.036	-4.956	.000	.000
Ngata-Njoro Bridge	16.417	14.036	1.170	.242	1.000
Ngata-Mouth	-67.229	14.036	-4.790	.000	.000
Njoro Bridge-Mouth	-50.812	14.036	-3.620	.000	.006

Appendix B9: Pairwise comparison of Nitrates concentration among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Logoman-Sigotik	-24.583	14.041	-1.751	.080	1.000
Logoman-Mouth	-25.562	14.041	-1.821	.069	1.000
Logoman-Ngata	-85.021	14.041	-6.055	.000	.000
Logoman-Njoro Bridge	-101.542	14.041	-7.232	.000	.000
Logoman-Turkana	-103.188	14.041	-7.349	.000	.000
Logoman-Canning	-104.312	14.041	-7.429	.000	.000
Sigotik-Mouth	-.979	14.041	-0.070	.944	1.000
Sigotik-Ngata	-60.438	14.041	-4.304	.000	.000
Sigotik-Njoro Bridge	-76.958	14.041	-5.481	.000	.000
Sigotik-Turkana	-78.604	14.041	-5.598	.000	.000
Sigotik-Canning	-79.729	14.041	-5.678	.000	.000
Mouth-Ngata	59.458	14.041	4.234	.000	.000
Mouth-Njoro Bridge	75.979	14.041	5.411	.000	.000

Appendix B10: Pairwise comparison of Nitrites concentration among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Logoman-Sigotik	-10.750	14.037	-.766	.444	1.000
Logoman-Canning	-52.458	14.037	-3.737	.000	.004

Logoman-Turkana	-57.188	14.037	-4.074	.000	.001
Logoman-Njoro Bridge	-75.250	14.037	-5.361	.000	.000
Logoman-Ngata	-78.854	14.037	-5.618	.000	.000
Logoman-Mouth	-128.583	14.037	-9.161	.000	.000
Sigotik-Canning	-41.708	14.037	-2.971	.003	.062
Sigotik-Turkana	-46.438	14.037	-3.308	.001	.020
Sigotik-Njoro Bridge	-64.500	14.037	-4.595	.000	.000
Sigotik-Ngata	-68.104	14.037	-4.852	.000	.000
Sigotik-Mouth	-117.833	14.037	-8.395	.000	.000
Canning-Turkana	4.729	14.037	.337	.736	1.000
Canning-Njoro Bridge	-22.792	14.037	-1.624	.104	1.000
Canning-Ngata	-26.396	14.037	-1.881	.060	1.000
Canning-Mouth	-76.125	14.037	-5.423	.000	.000
Turkana-Njoro Bridge	-18.062	14.037	-1.287	.198	1.000
Turkana-Ngata	-21.667	14.037	-1.544	.123	1.000
Turkana-Mouth	-71.396	14.037	-5.806	.000	.000
Njoro Bridge-Ngata	-3.604	14.037	-.257	.797	1.000
Njoro Bridge-Mouth	-53.333	14.037	-3.800	.000	.003
Ngata-Mouth	-49.729	14.037	-3.543	.000	.008

Appendix B11: Pairwise comparison of Total Nitrogen concentration among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Logoman-Sigotik	-23.021	14.042	-1.639	.101	1.000
Logoman-Mouth	-40.146	14.042	-2.859	.004	.089
Logoman-Turkana	-70.729	14.042	-5.037	.000	.000
Logoman-Njoro Bridge	-72.500	14.042	-5.163	.000	.000
Logoman-Ngata	-74.667	14.042	-5.318	.000	.000
Logoman-Canning	-75.062	14.042	-5.346	.000	.000
Sigotik-Mouth	-17.125	14.042	-1.220	.223	1.000
Sigotik-Turkana	-47.708	14.042	-3.398	.001	.014
Sigotik-Njoro Bridge	-49.479	14.042	-3.524	.000	.009

Sigotik-Ngata	-51.646	14.042	-3.678	.000	.005
Sigotik-Canning	-52.042	14.042	-3.706	.000	.004
Mouth-Turkana	30.583	14.042	2.178	.029	.617
Mouth-Njoro Bridge	32.354	14.042	2.304	.021	.445
Mouth-Ngata	34.521	14.042	2.458	.014	.293
Mouth-Canning	34.917	14.042	2.487	.013	.271
Turkana-Njoro Bridge	-1.771	14.042	-.126	.900	1.000
Turkana-Ngata	-3.938	14.042	-.280	.779	1.000
Turkana-Canning	-4.333	14.042	-.309	.758	1.000
Njoro Bridge-Ngata	-2.167	14.042	-.154	.877	1.000
Njoro Bridge-Canning	2.562	14.042	.182	.855	1.000
Ngata-Canning	.396	14.042	.028	.978	1.000

Appendix B12: Pairwise comparison of Total Phosphorus concentration among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Logoman-Sigotik	-6.792	14.040	-.484	.629	1.000
Logoman-Turkana	-49.396	14.040	-3.518	.000	.009
Logoman-Canning	-57.438	14.040	-4.091	.000	.001
Logoman-Njoro Bridge	-78.750	14.040	-5.609	.000	.000
Logoman-Ngata	-97.375	14.040	-6.396	.000	.000
Logoman-Mouth	-125.292	14.040	-8.294	.000	.000
Sigotik-Turkana	-42.604	14.040	-3.305	.002	.051
Sigotik-Canning	-50.646	14.040	-3.607	.000	.006
Sigotik-Njoro Bridge	-71.598	14.040	-5.125	.000	.000
Sigotik-Ngata	-90.583	14.040	-6.452	.000	.000
Sigotik-Mouth	-118.500	14.040	-8.440	.000	.000
Turkana-Canning	-8.042	14.040	-.573	.567	1.000
Turkana-Njoro Bridge	-29.354	14.040	-2.091	.037	.767
Turkana-Ngata	-47.979	14.040	-3.417	.001	.013
Turkana-Mouth	-75.896	14.040	-5.406	.000	.000
Canning-Njoro Bridge	-21.312	14.040	-1.518	.129	1.000
Canning-Ngata	-39.938	14.040	-2.845	.004	.093

Canning-Mouth	-67.854	14.040	-4.833	.000	.000
Njoro Bridge-Ngata	-18.625	14.040	-1.327	.185	1.000
Njoro Bridge-Mouth	-46.542	14.040	-3.315	.001	.019
Ngata-Mouth	-27.917	14.040	-1.988	.047	.982

Appendix B13: Pairwise comparison of Soluble Reactive Phosphorus concentration among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Sigotik-Logoman	3.083	14.034	.220	.826	1.000
Sigotik-Turkana	-38.229	14.034	-2.724	.006	.135
Sigotik-Canning	-51.583	14.034	-3.676	.000	.005
Sigotik-Njoro Bridge	-70.250	14.034	-5.006	.000	.000
Sigotik-Ngata	-93.271	14.034	-6.464	.000	.000
Sigotik-Mouth	-98.542	14.034	-7.022	.000	.000
Logoman-Turkana	-35.416	14.034	-2.504	.012	.258
Logoman-Canning	-48.500	14.034	-3.456	.001	.012
Logoman-Njoro Bridge	-67.167	14.034	-4.786	.000	.000
Logoman-Ngata	-90.188	14.034	-6.426	.000	.000
Logoman-Mouth	-95.458	14.034	-6.802	.000	.000
Turkana-Canning	-13.354	14.034	-.952	.341	1.000
Turkana-Njoro Bridge	-32.021	14.034	-2.282	.023	.473
Turkana-Ngata	-55.042	14.034	-3.922	.000	.002
Turkana-Mouth	-60.312	14.034	-4.298	.000	.000
Canning-Njoro Bridge	-18.667	14.034	-1.330	.183	1.000
Canning-Ngata	-41.688	14.034	-2.970	.003	.062
Canning-Mouth	-46.958	14.034	-3.346	.001	.017
Njoro Bridge-Ngata	-23.021	14.034	-1.640	.101	1.000
Njoro Bridge-Mouth	-28.292	14.034	-2.016	.044	.920
Ngata-Mouth	-5.271		-.376	.707	1.000

Appendix B14: Pairwise comparison of *E. coli* concentration among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Logoman-Sigotik	-28.250	14.041	-2.012	.044	.929
Logoman-Turkana	-59.542	14.041	-4.240	.000	.000
Logoman-Canning	-62.875	14.041	-4.478	.000	.000
Logoman-Ngata	-75.479	14.041	-5.376	.000	.000
Logoman-Njoro Bridge	-85.396	14.041	-6.082	.000	.000
Logoman-Mouth	-131.938	14.041	-9.396	.000	.000
Sigotik-Turkana	-31.292	14.041	-2.229	.026	.543
Sigotik-Canning	-34.625	14.041	-2.466	.014	.287
Sigotik-Ngata	-47.229	14.041	-3.364	.001	.016
Sigotik-Njoro Bridge	-57.146	14.041	-.4.070	.000	.001
Sigotik-Mouth	-103.688	14.041	-7.384	.000	.000
Turkana-Mouth	-72.396	14.041	-5.516	.000	.000
Canning-Ngata	-12.604	14.041	-8.98	.369	1.000
Canning-Njoro Bridge	-22.521	14.041	-1.604	.109	1.000
Canning-Mouth	-69.062	14.041	-4.919	.000	.000
Ngata-Njoro Bridge	9.917	14.041	.706	.480	1.000
Ngata-Mouth	-56.458	14.041	-4.021	.000	.001
Njoro Bridge-Mouth	-46.542	14.041	-3.315	.001	,019

Appendix B15: Pairwise comparison of total coliforms concentration among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Logoman-Sigotik	-25.917	14.041	-1.846	.065	1.000
Logoman-Turkana	-62.708	14.041	-4.466	.000	.000
Logoman-Canning	-68.354	14.041	-4.868	.000	.000
Logoman-Ngata	-79.667	14.041	-5.674	.000	.000
Logoman-Njoro Bridge	-91.271	14.041	-6.500	.000	.000
Logoman-Mouth	-133.500	14.041	-9.508	.000	.000
Sigotik-Turkana	-36.792	14.041	-2.620	.009	.185
Sigotik-Canning	-42.438	14.041	-3.022	.003	.053
Sigotik-Ngata	-53.750	14.041	-3.828	.000	.003

Sigotik-Njoro Bridge	-65.354	14.041	-4.654	.000	.000
Sigotik-Mouth	-107.583	14.041	-7.662	.000	.000
Turkana-Canning	-5.646	14.041	-.402	.688	1.000
Turkana-Ngata	-16.598	14.041	-1.208	.227	1.000
Turkana-Njoro Bridge	-28.562	14.041	-2.304	.042	.881
Turkana-Mouth	-70.792	14.041	-5.042	.000	.000
Canning-Ngata	-11.312	14.041	-8.06	.420	1.000
Canning-Njoro Bridge	-22.917	14.041	-1.632	.103	1.000
Canning-Mouth	-65.146	14.041	-4.640	.000	.000
Ngata-Njoro Bridge	11.604	14.041	.826	.409	1.000
Ngata-Mouth	-53.833	14.041	-3.834	.000	.003
Njoro Bridge-Mouth	-42.229	14.041	-3.007	.003	.055

Appendix B16: Pairwise comparison of intestinal enterococci concentration among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Logoman-Sigotik	-6.198	14.041	-.441	.659	1.000
Logoman-Canning	-68.646	14.041	-4.889	.000	.000
Logoman-Njoro Bridge	-75.579	14.041	-5.376	.000	.000
Logoman-Ngata	-76.312	14.041	-5.435	.000	.000
Logoman-Turkana	-76.750	14.041	-5.466	.000	.000
Logoman-Mouth	-117.062	14.041	-8.337	.000	.000
Sigotik-Canning	-62.458	14.041	-4.448	.000	.000
Sigotik-Njoro Bridge	-69.292	14.041	-4.935	.000	.000
Sigotik-Ngata	-70.125	14.041	-4.994	.000	.000
Sigotik-Turkana	-70.562	14.041	-5.026	.000	.000
Sigotik-Mouth	-110.875	14.041	-7.897	.000	.000
Canning-Njoro Bridge	-6.833	14.041	.487	.626	.000
Canning-Ngata	-7.667	14.041	-.546	.585	1.000
Canning-Turkana	8.104	14.041	.577	.564	1.000
Canning-Mouth	-48.417	14.041	-3.448	.001	.012
Njoro Bridge-Ngata	-.833	14.041	-.059	.953	1.000
Njoro Bridge-Turkana	1.271	14.041	.091	.928	1.000

Njoro Bridge-Mouth	-41.583	14.041	-2.962	.003	.064
Ngata-Turkana	.438	14.041	.031	.975	1.000
Ngata-Mouth	-40.750	14.041	-2.902	.004	.078
Turkana-Mouth	-40.312	14.041	-2.871	.004	.086

Appendix B17: Pairwise comparison of *Clostridium perfringens* concentration among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Logoman-Sigotik	-13.333	14.039	-.950	.342	1.000
Logoman-Turkana	-43.812	14.039	-3.121	.002	.038
Logoman-Canning	-52.583	14.039	-3.745	.000	.004
Logoman-Ngata	-63.479	14.039	-4.522	.000	.000
Logoman-Njoro Bridge	-77.729	14.039	-5.537	.000	.000
Logoman-Mouth	-102.125	14.039	-7.274	.000	.000
Sigotik-Turkana	-30.479	14.039	-2.171	.030	.629
Sigotik-Canning	-39.250	14.039	-2.796	.005	.109
Sigotik-Ngata	-50.146	14.039	-3.572	.000	.007
Sigotik-Njoro Bridge	-64.396	14.039	-4.587	.000	.000
Sigotik-Mouth	-88.792	14.039	-6.325	.000	.000
Turkana-Canning	-8.771	14.039	-.625	.532	1.000
Turkana-Ngata	-19.667	14.039	-1.401	.161	1.000
Turkana-Njoro Bridge	-33.917	14.039	-2.416	.016	.330
Turkana-Mouth	-58.312	14.039	-4.154	.000	.001
Canning-Ngata	-10.896	14.039	-.776	.438	1.000
Canning-Njoro Bridge	-25.146	14.039	-1.791	.073	1.000
Canning-Mouth	-49.452	14.039	-3.529	.000	.009
Ngata-Njoro Bridge	14.250	14.039	1.015	.310	1.000
Ngata-Mouth	-38.646	14.039	2.753	.006	.124
Njoro Bridge-Mouth	-24.396	14.039	-1.738	.082	1.000

Appendix B18: Pairwise comparison of tetracycline resistance among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Ngata-Sigotik	3.5554	12.211	.291	.771	1.000
Ngata-Mouth	-6.604	10.710	-.617	.537	1.000
Ngata-Canning	12.958	10.710	1.210	.226	1.000
Ngata-Bridge	16.458	10.710	1.537	.124	1.000
Ngata-Turkana	36.668	10.710	3.426	.001	.013
Ngata-Logoman	60.854	22.719	2.679	.007	.155
Sigotik-Mouth	-3.050	12.211	-.250	.803	1.000
Sigotik-Canning	-9.404	12.211	-.770	.441	1.000
Sigotik-Bridge	-12.904	12.211	-1.057	.291	1.000
Sigotik-Turkana	-33.133	12.211	-2.713	.007	.140
Sigotik-Logoman	57.330	23.464	2.442	.015	.307
Mouth-Canning	6.354	10.710	.593	.553	1.000
Mouth-Bridge	9.854	10.710	.920	.358	1.000
Mouth-Turkana	30.083	10.710	2.809	.005	.104
Mouth-Logoman	54.250	22.719	2.388	.017	.356
Canning-Bridge	-3.500	10.710	-.327	.744	1.000
Canning-Turkana	23.729	10.710	2.216	.027	.561
Canning-Logoman	47.896	22.719	2.108	.035	.735
Bridge-Turkana	20.229	10.710	1.889	.059	1.000
Bridge-Logoman	44.396	22.719	1.954	.051	1.000
Turkana-Logoman	24.167	22.719	1.064	.287	1.000

Appendix B19: Pairwise comparison of ciprofloxacin resistance among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Ngata-Turkana	2.052	13.421	.153	.879	1.000
Ngata-Bridge	2.190	12.485	.175	.861	1.000
Ngata-Mouth	-30.420	12.485	-2.436	.015	.312
Ngata-Sigotik	30.509	12.748	2.393	.017	.351
Ngata-Canning	42.577	12.748	.3.340	.001	.018

Ngata-Logoman	71.976	14.417	4.992	.000	.000
Turkana-Bridge	-.139	13.029	-.011	.991	1.000
Turkana-Mouth	-28.368	13.029	-2.177	.029	.619
Turkana-Sigotik	28.457	13.280	2.143	.032	.675
Turkana-Canning	-40.525	13.280	-3.052	.002	.048
Turkana-Logoman	69.925	14.890	4.696	.000	.000
Bridge-Mouth	-28.229	12.062	-2.340	.019	.405
Bridge-Sigotik	28.318	12.333	2.296	.022	.455
Bridge-Canning	40.386	12.333	3.275	.001	.022
Bridge-Logoman	69.86	14.052	4.966	.000	.000
Mouth-Sigotik	.089	12.333	.007	.994	1.000
Mouth-Logoman	41.557	14.052	2.957	.003	.065
Sigotik-Canning	-12.068	12.598	-.958	.338	1.000
Sigotik-Logoman	41.468	14.285	2.903	.004	.078
Canning-Logoman	29.399	14.285	2.058	.040	.831

Appendix B20: Pairwise comparison of gentamicin resistance among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Sigotik-Bridge	-2.833	8.529	-.332	.740	1.000
Sigotik-Turkana	-3.667	9.849	-3.372	.710	1.000
Sigotik-Mouth	-29.083	8.041	-3.617	.000	.004
Sigotik-Canning	30.667	8.990	-3.411	.001	.010
Sigotik-Ngata	-32.722	8.990	-3.460	.000	.004
Bridge-Turkana	.833	8.529	.098	.922	1.000
Bridge-Mouth	-26.250	6.357	-4.129	.000	.001
Bridge-Canning	27.833	7.522	3.700	.000	.003
Bridge-Ngata	-29.889	7.522	-3.974	.000	.001
Turkana-Mouth	-25.417	8.041	-3.161	.002	.024
Turkana-Canning	-27.000	8.990	-3.003	.003	.040
Turkana-Ngata	-29.056	8.990	-3.232	.001	.018
Mouth-Canning	1.583	6.964	.227	.820	1.000
Mouth-Ngata	3.639	6.964	.523	.601	1.000

Canning-Ngata	-2.056	8.041	-.256	.798	1.000

Appendix B21: Pairwise comparison of levofloxacin resistance among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Bridge-Mouth	.000	7.606	.000	1.000	1.000
Bridge-Canning	12.600	5.892	2.139	.032	.195
Bridge-Ngata	-13.125	6.013	-2.183	.029	.174
Mouth Canning	12.600	5.892	2.139	.032	.195
Mouth-Ngata	13.125	6.013	2.183	.029	.174
Canning-Ngata	-.525	3.608	-.146	.884	1.000

Appendix B22: Pairwise comparison of ampicillin resistance among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Bridge-Ngata	-4.389	10.679	-4.11	.681	1.000
Bridge-Turkana	8.125	11.831	.687	.492	1.000
Bridge-Mouth	-8.565	10.138	-.845	.398	1.000
Bridge-Canning	27.875	10.979	2.539	.011	.233
Bridge-Sigotik	34.119	10.327	3.304	.001	.020
Bridge-Logoman	46.000	19.320	2.381	.017	.363
Ngata-Turkana	3.736	11.384	.328	.743	1.000
Ngata-Mouth	-4.176	9.613	-.434	.664	1.000
Ngata-Canning	23.486	10.496	2.238	.025	.530
Ngata-Sigotik	29.730	9.812	3.030	.002	.051
Ngata-Logoman	41.611	19.049	2.184	.029	.608
Turkana-Mouth	-.440	10.878	-.040	.968	1.000
Turkana-Canning	-19.750	11.665	-1.693	.090	1.000
Turkana-Sigotik	25.994	11.054	2.352	.019	.393
Turkana-Logoman	37.875	19.718	1.921	.055	1.000
Mouth-Canning	19.310	9.944	1.942	.052	1.000
Mouth-Sigotik	25.554	9.220	2.772	.006	.117

Mouth-Logoman	37.435	18.751	1.996	.046	.964
Canning-Sigotik	6.224	10.137	.616	.538	1.000
Canning-Logoman	18.125	19.219	.943	.346	1.000
Sigotik-Logoman	11.881	18.854	.630	.529	1.000

Appendix B23: Pairwise comparison of streptomycin resistance among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Ngata-Bridge	4.625	10.693	.433	.665	1.000
Ngata-Sigotik	24.167	10.693	2.260	.024	.357
Ngata-Canning	25.357	11.043	2.296	.022	.325
Ngata-Turkana	32.729	10.693	3.061	.002	.003
Ngata-Mouth	-54.292	10.693	-5.077	.000	.000
Bridge-Sigotik	19.542	10.330	1.892	.059	.878
Bridge-Canning	20.732	10.693	1.939	.053	.788
Bridge-Turkana	28.104	10.330	2.721	.007	.098
Bridge-Mouth	-49.667	10.330	-4.808	.000	.000
Sigotik-Canning	-1.190	10.693	-.111	.911	1.000
Sigotik-Turkana	-8.562	10.330	-8.29	.407	1.000
Sigotik-Mouth	-30.125	10.330	-2.916	.004	.053
Canning-Turkana	7.372	10.693	.689	.491	1.000
Canning-Mouth	-28.935	10.693	-2.706	.007	.102
Turkana-Mouth	-21.562	10.330	-2.807	.037	.553

Appendix B24: Pairwise comparison of chloramphenicol resistance among the sampled sites along River Njoro

Sites	Test statistic	Std. Error	Std. test statistic	Sig.	Adj.sig.[a]
Sigotik-Bridge	-6.236	13.440	-.464	.643	1.000
Sigotik-Ngata	-7.343	13.519	-.543	.587	1.000
Sigotik-Canning	-10.924	13.440	-.813	.416	1.000
Sigotik-Mouth	-26.203	13.802	-1.899	.058	.864
Sigotik-Turkana	-38.069	13.440	-2.833	.005	.069

Bridge-Ngata	-1.107	10.033	-.110	.912	1.000
Bridge-Canning	4.688	9.926	.472	.637	1.000
Bridge-Mouth	-19.967	10.411	-1.918	.055	.827
Bridge-Turkana	31.833	9.926	3.207	.001	.020
Ngata-Canning	3.581	10.033	.357	.721	1.000
Ngata-Mouth	-18.860	10.513	-1.794	.073	1.000
Ngata-Turkana	30.726	10.033	3.062	.002	.033
Canning-Mouth	-15.279	10.411	-1.468	.142	1.000
Canning-Turkana	27.146	9.926	2.735	.006	.094
Mouth-Turkana	11.867	10.411	1.140	.254	1.000

Appendix C: Positive confirmation of *E. coli* using IMVIC biochemical test

Indole test	Methyl-Red test	Voges-proskauer test	Citrate utilization test
+	+	-	-

Appendix D: Additional results on percentage antimicrobial susceptibility of the tested antibiotics

Site	Diameter	Antibiotics								
		Tetracycline	Ciprofloxacin	Gentamicin	Levofloxacin	Amoxicillin	Amikacin	Ampicillin	Streptomycin	Chloramphenicol
Logoman	Susceptible	79.17	8.33	83.33	75	58.33	66.67	50	58.33	100
	Intermediate	8.33	33.33	16.67	25	4.17	33.33	37.5	41.67	-
	Resistant	12.5	58.33	-	-	37.5	-	12.5	-	-
Sigotik	Susceptible	37.5	-	62.5	87.5	-	25	12.5	-	8.33
	Intermediate	-	8.33	12.5	12.5	-	37.5	-	-	54.17
	Resistant	62.5	91.67	25		100	37.5	87.5	100	37.5
Turkana	Susceptible	-	-	58.33	50.0	-	25	25	-	-
	Intermediate		25	16.67	50.0	12.5	20.83	25	-	-
	Resistant	100	75	25	-	87.5	54.17	50	100	100
Canning	Susceptible	-	-	50	12.5	-	12.5	12.5	-	-
	Intermediate	-	8.33	12.5	25	12.5	45.83	20.83	12.5	-
	Resistant	100	91.67	37.5	62.5	87.5	41.67	66.67	87.5	100
Njoro Bridge	Susceptible	-	-	33.33	12.5	-	20.83	20.83	-	-
	Intermediate	-	-	16.67	75	-	54.17	16.67	-	-
	Resistant	100	100	50	12.5	100	25	62.5	100	100
Ngata	Susceptible	-	12.5	62.5	20.83	12.5	37.5	25	12.5	-
	Intermediate	-	-	-	29.17	-	8.33	-	-	4.17
	Resistant	100	87.5	37.5	50	87.5	54.17	75	87.5	95.83
Mouth	Susceptible	-	-	16.67	12.5	-	33.33	-	-	-
	Intermediate	-	-	8.33	75	-	4.17	4.17	-	16.67
	Resistant	100	100	75	12.5	100	62.5	95.83	100	83.33

Appendix E: Summary of drinking water guidelines by different authorities

PARAMETERS	UNITS	AUTHORITY WHO	NEMA-KENYA	US-EPA	EU-FRAMEWORK
PHYSICO-CHEMICAL					
Odour and taste			Not offensive to consumers		
Suspended matter				Nil	
pH	pH units	6.5-8.5		6.5-8.5	≤ 6.5≥9.5
Conductivity	µs/cm at 20^0C				2500
TDS	mg/l		1500	500	
NO_3-N (mg/L)		10			
NH_4-N (mg/L)		0.2			
MICROBIAL PARAMETERS					
Total viable counts at 37 ^{o}C/ml	CFU		100	500	20/mL
Total coliforms	CFU	not detected/100 mL	shall be absent	<1/100 mL	0/100 mL
E. coli	CFU	not detected/100 mL	shall be absent	<1/100 mL	0/100 mL
Enterococci	CFU	not detected/100 mL	shall be absent		0/100 mL
Sulphite reducing anaerobes	CFU	not detected/100 mL	shall be absent		0/100 mL

Appendix F: Research permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **206873**

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RESEARCH LICENSE

This is to Certify that Ms., Lorine Auma Omondi of Egerton University, has been licensed to conduct research in Nakuru on the topic: Effect of land use on faecal pollution in river njoro for the period ending : 25/March/2022.

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