SOME MICROBIAL AND HEAVY METAL POLLUTION OF RIVER KADUNA FROM KADUNA SOUTH WATERWORKS TO 100M DOWNSTREAM OF RIVERS ROMI-KADUNA CONFLUENCE

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AUGUST, 2018

DECLARATION

I declare that the work in this thesis titled ***SOME MICROBIAL AND HEAVY METAL POLLUTION OF RIVER KADUNA FROM KADUNA SOUTH WATERWORKS TO 100M DOWNSTREAM OF RIVERS ROMI-KADUNA CONFLUENCE*** was carried out by me in the Department of Microbiology, Faculty of Life Sciences, under the supervision of Prof. S.E. Yakubu, Prof. J.B. Ameh and Prof. C.A. Okuofu.

The information derived from the literature review has been duly acknowledged in the text and a list of the references provided. No part of this thesis has been presented in any previous application for another degree or diploma here or any other Institution.

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CERTIFICATION

This Thesis titled Some microbial and heavy metal pollution of River Kaduna from Kaduna South Waterworks to 100m downstream of Rivers Romi-Kaduna Confluence by Cordelia Ngozi OKAFO meets the regulations governing the award of the Doctor of Philosophy Degree of Ahmadu Bello University, Zaria, Nigeria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my dear mother whom the Lord called to Glory during the course of the study. May her gentle soul rest in peace. Amen.

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ABSTRACT

In Nigeria, waste discharges from domestic, municipal, trade, industrial and agricultural set-ups contain various pollutants including heavy metals and microbes. The river water is also contaminated with fecal samples from humans and animals introducing enteric microbes including *E. coli* O157:H7 and *Cryptosporidium* oocysts. These waste discharges are conveyed (mostly untreated) through drains, water-ways and soils into inland water-bodies. The water is used for irrigation of vegetable crops and, fish, being relatively situated at the top of the aquatic food chain, can accumulate heavy metals from water, food and sediments. The pollution of River Kaduna from Kaduna South Waterworks to 100 meter downstream of River Romi-Kaduna confluence was studied. The study investigated the levels of some heavy metals including chromium (Cr), cadmium (Cd), lead (Pb) and zinc (Zn) in the water and fish samples obtained from the river, and in cabbage, cucumber, jute, lettuce and amaranthus cultivated on the banks of the river. The microbial quality of the river water including *Escherichia coli* O157:H7 and *Cryptosporidium* was also studied. Two hundred and eight (208) water samples, 56 vegetable samples and 57 fish samples were used for the study. Determination of the heavy metals in the different samples was carried outby means of Atomic Absorption Spectrophotometer (Varian, model AA240FS) after digestion. *Cryptosporidium* oocysts were detected by the flocculation method and microscopy. *Escherichia coli* was isolated from water samples using the Tryptic Soya Broth (TSB) enrichment and high temperature (44.5°C) incubation, followed by plating for isolation on Eosine Methylene Blue (EMB) agar. Biochemically confirmed *E. coli* isolates were screened on Sorbitol MacConkey agar and presumptive *E. coli* O157:H7 further confirmed by testing for agglutination with *E. coli* O157:H7 antisera. The levels of the

heavy metals (Cr, Cd, Pb and Zn) in the river water were within the limits for discharge of effluents into surface water but Cd and Cr were above the 0.01mg/l and 0.1mg/l Food and Agriculture Organization (FAO) limit for irrigation water. All the fish samples tested contained the metals in varying concentrations. The levels of the metals in the fish species were however, within the permissible limits set by FAO and US FDA. Vegetable samples obtained from the banks of the river were also evaluated for the same elemental toxicants. The concentrations of Cd, Cr and Zn in the vegetables were within the FAO/WHO recommended safe limits of 0.2 mg/kg, 2.3 mg/kg and 20 mg/kg respectively. However, the mean level of Pb in cabbage (0.70 mg/kg), cucumber (0.77mg/kg) and jute (0.43mg/kg) exceeded the 0.3mg/kg permissible limits. *Cryptosporidium* oocysts were detected at some locations along the river. Similarly, *Escherichia coli* O157:H7 was found in some of the water samples. The *E. coli* O157:H7 isolated from the water samples exhibited high resistance to amoxicillin, sulphamethoxazole/trimethroprim and doxycycline. The presence of some antibiotic resistance genes (*TEM, SHV, CTX-M, dfrII, Sul* and *TET*) was established among some of the *E. coli* O157:H7 isolates. All the *E. coli* O157:H7 isolates expressed the virulence gene shiga toxin 1 while 66.7% of them expressed the gene shiga toxin 2. The study has established that the stretch of the River Kaduna studied was polluted. It is recommended that an integrated wastes treatment technology employing Best Management Practices (BMP) should be adopted by the industries within the river vicinity and there is need for epidemiological studies on the immediate communities that have direct or indirect contact with the river water in order to properly assess the health hazards of the pollutants.

TABLE OF CONTENT

Cover Page i

Fly Leaf ii

Title Page iii

Declaration iv

Certification v

Dedication vi

Acknowledgement vii

Abstract viii

Table of Content x

List of Tables xv

List of Figures xvii

List of Plates xviii

Appendices xix

List of Abbreviations xx

1.0 INTRODUCTION 1

1.1 Background to the study 1

1.2 Statement of Research problem 3

1.3 Justification for the study 4

1.4 Aim of the study 5

1.5 Objectives of the study..... 5

2.0 LITERATURE REVIEW 6

2.1 Introduction to SurfaceWater Quality Degradation 6

2.2 Sources of Water Pollution in Nigeria..... 8

2.2.1 Domestic Based Water Pollution and Urban Run-off..... 8

2.2.2 Industrial discharges 9

2.2.3 Agricultural wastes 12

2.3 Indicators of water pollution..... 13

2.3.1 Bacteriological Indicators 13

2.3.2 Physico-Chemical Indicators 14

2.4 Heavy Metals in the Environment 16

2.5 *Escherichia coli*..... 21

2.5.1 Adaptation and Importance of *E. coli*..... 21

2.5.2 *Escherichia coli* infections..... 22

2.6 *E.coli* O157:H7 Infections 28

2.6.1 Virulence factors of *E.coli* O157:H7 28

2.6.2 Modes of Transmission of *E.coli* O157:H7 30

2.6.3 Emergence of *E.coli* O157:H7 in Africa..... 32

2.6.4 Survival of *E.coli* O157:H7 in the environment 33

2.7 *Cryptosporidium* 34

2.7.1 Cryptosporidiosis 34

2.7.2 *Cryptosporidium* Oocysts in Water Supplies 36

3.0 MATERIALS AND METHODS 40

3.1 Materials 40

3.2 The Study Area 40

3.3 Collection of Samples 45

3.3.1 Preparation of water samples for heavy metals analyses 46

3.3.3 Preparation of vegetable samples for heavy metals analyses 47

3.4 Identification and detection of *Cryptosporidium* oocysts 48

3.4.1 Concentration of Cryptosporidium from water: Chemical Flocculation 48

3.4.2 Preparation of Samples for coccidia detection using floatation method 49

3.4.3 Sample Staining by the Modified Ziehl-Neelsen (mZN) Method 50

3.5 Isolation and Biochemical Identification of *Escherichia coli* and *Escherichia coli* O157:H7 50

3.5.1 Antibiotic Susceptibility Testing 51

3.5.2 Determination of Multiple Antibiotic Resistance (MAR) Index 52

3.5.3 Molecular Analysis 52

3.6 Analysis of data 56

4.0 RESULTS 59

4.1 Physicochemical and faecal coliforms analysis 59

4.2 Heavy metals 59

4.2.1 Heavy Metal Contamination of the Water Samples 59

4.2.2 Contamination of Vegetable Samples 64

4.2.3 Contamination levels of heavy metals in fish samples 67

4.2.4 Constellation Plot of the sampling points 70

4.3 Prevalence of *Cryptosporidium* oocysts in water samples from Kaduna River 70

4.4 *Escherichia coli* and *Eschrichia coli* O157:H7 78

4.4.1 Isolation and Biochemical Identification of *E. coli* and *E.coli* O157:H7 78

4.4.2 Antibiotic Susceptibility Profile of the *E.coli* O157:H7Isolates 78

4.4.3 Multiple Antibiotic Resistance Index (MARI) and Resistance Pattern of *E.coli* O157:H7……...……78

4.4.4 Molecular Characterization of Antibiotics Resistance Genes in *E. coli* O157:H7 Isolated…….…….……...83

4.4.4.1 Genomic DNA extraction and characterization of extended spectrum beta lactamase genes……..83

4.4.4.2 Molecular Characterization of Trimethoprim Resistance Genes in *E. coli* O157:H7……83

4.4.4.3 Molecular Characterization of Sulfamethoxazole Resistance Genes in *E. coli* O157:H7……83

4.4.4.4 Molecular Characterization of Tetracycline Resistance Genes in *E. coli* O157:H7 83

4.4.5 Molecular Characteristic of Virulence genes in the *E.coli* O157:H7 Isolates 94

4.4.6 Sequencing of *E. coli* O157:H7 isolates from water sources 94

4.4.6.1 BLAST Analysis of Sequenced *E. coli* O157:H7 isolates from River Kaduna water samples……..…….94

4.4.6.2 Phylogenetic Study of the Sequenced *E. coli* O157:H7 isolates 94

5.0 DISCUSSION 99

5.1 Heavy Metal concentration in water samples..... 99

5.2 Heavy Metals in Vegetable Samples..... 105

5.3 Heavy Metals in Fish Samples 108

5.4 Prevalence of *Cryptosporidium* oocysts in River Kaduna..... 112

5.5 Identification of *Escherichia coli* and *Escherichia coli* O157:H7 in River Kaduna ……..……114

5.5.1 Antibiotic Susceptibility 116

5.5.2 Molecular characterization of Antibiotic Resistance genes in *E.coli* O157:H7 ... 120

5.5.3 Molecular charaterization of shiga toxin genes in *E. coli* O157:H7..... 122

5.5.4 Sequencing of the isolates..... 124

6.0 CONCLUSION AND RECOMMENDATIONS 128

6.1 Conclusion 128

6.2 Recommendations..... 129

REFERENCES 131

APPENDICES..... 171

LIST OF TABLES

Table	Title	Page
3.1	Coordinates of the sampling points	44
3.2	Primers for antibiotic resistance genes	57
3.3	Primers for Virulence Genes	58
4.1	Mean heavy metal concentration in water samples at the different sampling points	61
4.2	Mean heavy metal concentration in water samples at the locations (pooled results)	62
4.3	Seasonal variation of heavy metals concentration of the sampling locations of the river	63
4.4	Comparison of mean heavy metal concentration in some vegetables cultivated along the banks of River Kaduna	65
4.5	Spatial variation of heavy metal concentration in vegetable samples	66
4.6	Seasonal variation of heavy metal concentration in vegetable samples from River Kaduna	68
4.7	Heavy metal concentration of fish samples caught in River Kaduna	69
4.8	Spatial variation in the mean heavy metal concentration of the fish samples	72
4.9	Seasonal variation of mean heavy metal concentrations in fish samples from River Kaduna	73
4.10	Incidence of *Cryptosporidium* oocysts in water samples obtained from the different sampling locations	76
4.11	Prevalence of *E. coli* O157:H7 isolated from Kaduna River	80
4.12	Percentage of *E.coli* O157:H7 resistant to antibiotics used (n=15)	81
4.13	Multiple Antibiotic Resistance Index (MARI) and Resistance Pattern of *E.coli* O157:H7 Isolates	82

4.14 Distribution of Some Antibiotic Resistance and Virulence Genes among the *E. coli* O157:H7 isolates 88

LIST OF FIGURES

Figure **Title** **Page**

3.1 Map of the study area……...……...43

4.1 Constellation Plot of the sampling points……74

4.2 Incidence of coccidia and *Cryptosporidium* oocysts in water samples obtained from the different sampling locations……75

4.3 Seasonal occurrence of the identified oocysts……77

4.4 Incidence of *E.coli* O157:H7 among confirmed *E.coli* isolates……..79

4.5 Neighbour-joining Phylogenetic Tree for *E. coli* O157:H7 isolates from the water samples sequences……98

LIST OF PLATES

Plates	Title	Page
I	Genomic DNA extracted from the *E. coli* O157:H7 isolates	84
II	Agarose Gel Photo of Amplicons of *TEM* gene from *E. coli* O157:H7 isolates	85
III	Agarose Gel Photo of Amplicons of *SHV* gene from *E. coli* O157:H7 isolates	86
IV	Agarose Gel Photo of Amplicons of *CTX-M$_{15}$* gene from *E. coli* O157:H7 isolates	87
V	Agarose Gel Photo of Amplicons of *DfrXII* gene from *E. coli* O157:H7 isolates	89
VI	Agarose Gel Photo of Amplicons of *Sul1* gene from *E. coli* O157:H7 isolates	90
VII	Agarose Gel Photo showing no *Sul2* gene amplified	91
VIII	Agarose Gel Photo of Amplicons of *TetA* gene from *E. coli* O157:H7 isolates	92
IX	Agarose Gel Photo showing no *TetB* gene amplified	93
X	Agarose Gel Photo of Amplicons of *Stx1* gene from *E. coli* O157:H7 isolates	95
XI	Agarose Gel Photo of Amplicons of *Stx2*gene from *E. coli* O157:H7 isolates	96
XII	Agarose Gel Photo of Amplicons of 16S rRNA gene from *E. coli* O157:H7 isolates	97

APPENDICES

Appendix	Title	Page
I	Materials used in the study	171
IIa	Results of Physiocochemical analysis of the river water samples	173
IIb	Dissolve Oxygen (DO) and Biochemical Oxygen Demand (BOD) of the water samples	174
III	Feacal coliform counts (MPN/100ml) at the different sites along River Kaduna	175
IV	Monthly concentration of heavy metals in water samples	176
IV	contd	177
IV	contd	178
IV	contd	179
V	Distribution of enterobacteriaceae among the isolates	180
VI	Blasting of the *E.coli* O157:H7 isolates from Kaduna River	181
VII	*Cryptosporidium* oocyst (arrowed) as seen under the microscope	182
VIII	Colored water from Kudenda drain emptying into River Kaduna	183

LIST OF ABBREVIATIONS

APHA	American Public Health Association
ATSDR	Agency for Toxic Substances and Disease Registry
CDC	Centers for Disease Control and Prevention
DEHP	Department of Environment and Heritage Protection
DHMH	Department of Health and Mental Hygiene
EPA	United States Environmental Protection Agency
FAO	Food and Agriculture Organization
FEPA	Federal Environmental Protection Agency (Nigeria)
FGN	Federal Government of Nigeria
FMEnv	Federal Ministry of Environment
GBDDD	Global Burden of Disease Diarrhoeal Diseases
IAEA	International Atomic Energy Agency
IARC	International Agency for Research on Cancer
IPCS	International Programme on Chemical Safety
US FDA	United States Food and Drug Administration
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Increasing industrialization and urbanization have led to a wide-scale contamination of Nigeria's surface waters from industrial effluents, domestic sewage discharges and excessive usage of fertilizers and pesticides (Ajayi and Osibanjo, 1981; Moussa and Kawo, 2005). With increasing economic growth and urbanization in Nigeria, the nature and quantity of wastes generated changed significantly. Most domestic wastes in the country now contain substances that pose additional risk to public health (Nweke and Sanders, 2009). The volume of industrial wastes is growing at an alarming rate, and the country still lacks adequate technology, resources and manpower required to effectively manage these wastes in an environmentally safe manner (Adeyemo, 2003).

Many factories in Nigeria are located on river banks and use the rivers as open sewers for their effluents. The World Bank (1995) observed that there was a gross contamination of major rivers across the nation due to the discharge of industrial effluents, sewage and agricultural wastes into them. Incidentally, a significant proportion of the Nigerian population still relies on surface water for drinking, washing, fishing and swimming (Emere and Dibal, 2013).

The Federal Environmental Protection Agency, FEPA, was set up by Decree 58 in 1988 with the statutory responsibility for overall protection of the environment (FGN, 1988a). Similarly, the Hazardous Waste Decree was promulgated with the intention of discouraging reckless and illegal dumping of hazardous and harmful wastes on land and into water

courses (FGN, 1988b). One year later, the National Policy on the Environment was launched (FEPA, 1989). Part of the implementation of the policy and the environmental pollution abatement strategy contained therein was the introduction of guidelines and standards. In addition, uniform effluent limits based on the assimilative capacity of the receiving water have been drawn up for all categories of industrial effluents in Nigeria (FEPA, 1991a). Unfortunately, Ekiye and Zejiao (2010) noted that the different regulations put in place to protect the marine environment in Nigeria have not been effective in controlling the indiscriminate dumping of effluents into open water bodies.

Kaduna is one of the few highly industrialized cities in Nigeria with over ninety-two industries (Yusuf, 2004) and most of the industries are located downstream of the Kaduna South Waterworks which is the main area where this project is domiciled. River Kaduna is the main source of water supply to Kaduna town and environs and its numerous industries. It is important to the socio-economic growth of the State and its environs. Besides these, the river is used for fishing and for recreational purposes. Apart from being a source of water supply, the river also serves as a terminal drain for industrial and domestic wastes. The river receives over 500,000 m^3/day of untreated effluent from various industries in Kaduna through 53 tributaries (Ali *et al*., 2005). Human settlements and physical developments are fast emerging in the floodplains; these have encouraged neighbouring communities on the bank to farm. During heavy rains, the farmlands are eroded, resulting in the washing of plant residues, pesticides and fertilizers into the water body. River Kaduna is therefore subjected to effluent discharge and in fact, serves as a sink for all industrial effluents in Kaduna city, making it one of the most polluted inland waters in Nigeria (Beetseh, 1985).

Industrial effluents mostly contain heavy metals, acids, hydrocarbons and atmospheric deposition (Alam *et al*., 2007). Agricultural run-off is another major water pollutant as it contains nitrogenous compounds and phosphorus from fertilizers, pesticides, salts, poultry wastes and washdown from abattoirs (Omole and Longe, 2008). Untreated domestic sewage discharged into the aquatic environment (Adeyemo, 2003) and severe flooding events (Wolf-Rainer, 2011) are major sources of human pathogens. All of these can have a negative effect on the aquatic ecosystem as well as pose potential health risk to humans.

1.2 Statement of Research problem

River Kaduna is the major source of water supply to Kaduna City. The river basin is a booming crop farming area in both dry and rainy seasons: the banks of the river are predominantly used for peasant vegetable crop farming of lettuce, cabbage, jute, tomatoes, and dry season fresh corns. Fertilizers, herbicides and insecticides are used on these crops – and are eventually washed into the river via leaching and surface run off. All the major industries in Kaduna town (Textile factories, Petroleum Refinery, Peugeot Automobile Assembly Plants, etc) are located near the river and discharge their wastes mostly untreated directly into it. Trade wastes (from auto-mechanics, metal fabrication/finishing, abattoirs, etc) are also directly or indirectly discharged into the river. Domestic sewage and refuse also find their way into the river from many settlements along the river banks via leaching, direct discharge and surface run off. Thus, there is every possibility of contamination of the water and sediments of the river by heavy metals and harmful microorganisms. The health of the public is in danger as most of the vegetables produced are eaten raw or not sufficiently cooked. People spend a lot of time in the river taking their bath or washing or even fetching water for domestic use. Farmers and fishermen are in constant contact with

the river. Moreover, fish is caught in the river and sold to the public. The fish caught may accumulate heavy metals and release same in the food chain. In addition, people come in direct and constant contact with the river water for various purposes and could be exposed to water-related diseases. Not only can disease occur from ingestion of contaminated water, significant associations have been found between waterborne disease occurrence and the use of a river for bathing, laundry, washing cooking utensils, and from teeth brushing (Hamner *et al*., 2006).

1.3 Justification for the study

The River Kaduna is the major source of water for irrigation, fishing, and industrial uses for the inhabitants of Kaduna town, and is the major source for the Kaduna South Waterworks. Numerous activities take place in the segment of the river where the study was carried out. These activities may lead to the release of industrial as well as municipal wastes into River Kaduna. As aresult of industrial activities and other anthropogenic sources of contamination, the river Kaduna may be grossly polluted and may contain high concentrations of heavy metals and microbes. Since these heavy metals are not biodegradable they are bound tobioaccumulate in fishes or plants which eventually can be consumed by humans leading to severe diseases. The presence of heavy metals in water can be detrimental to human health and the aquatic ecosystem. Outbreaks of *Escherichia coli* O157:H7 related to consumption and use of contaminated water and food have been reported and is the leading cause of bloody diarrhea, hemolytic colitis and the more life-threatening hemolytic ureamic syndrome. *Cryptosporidium* transmission may occur by contamination of water used for recreation or swimming pools, drinking or food, and is responsible for profuse diarrhea which can be fatal. The result of this study may help in

formulating remedial measures, if necessary, to safeguard the environment and the health of people especially those who make contact with the river water or those who consume aquatic resources or vegetables which had contact with the water. It will also help in stricter enforcement of the law for the industries to comply appropriately, and will also serve as a data base on the microbiological quality of the river water.

1.4 Aim of the study

The aim of this study was to carry out pollution study of Kaduna River from Kaduna South waterworks to one hundred meters (100m) downstream of Romi-Kaduna river confluence.

1.5 Objectives of the study

The specific objectives of the study were to:

1. Determine the concentrations of some heavy metals in the river water, irrigated crops and in fish caught in the river.
2. Detect the presence of *Cryptosporidium* oocysts in the water samples
3. Isolate and characterize *E.coli and E.coli* O157:H7 from the water samples
4. Determine the antibiotic susceptibility profile and Multiple Antibiotic Resistance Index (MARI) of the *E. coli* O157:H7 isolates.
5. Determine the presence of some antibiotic resistance genes in the *E. coli* O157:H7 isolates.
6. Identify *Shiga* toxin genes (*Stx1* and *Stx 2*) in the *E. coli* O157:H7 using PCR.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction to Surface Water Quality Degradation

Freshwater resources all over the world are threatened by over exploitation and poor management as well as ecological degradation. In additon, water as a universal solvent has the ability to dissolve many substances be it organic or inorganic compounds. With this outstanding property, nevertheless it is almost impossible to have water in its pure form since it cannot be held up in a vacuum. Human and industrial activities result in the discharge of various pollutants into the aquatic environment thus, threatening the health of the population and damaging the quality of the environment by rendering water bodies unsuitable (Abowei and Sikoki, 2005). Surface water quality is controlled by anthropogenic factors such as urbanization, industrial and agricultural practices, and also by natural processes like erosion and the regional climatic conditions (Rahman *et al*., 2014).

Fresh water is fundamental for sustenance of lives of humans, animals and plants. Water is typically referred to as polluted when it is impaired by anthropogenic contaminants and so, either does not support human use (like serving as drinking water), and/or if it undergoes a marked shift in its ability to support its constituent biotic communities, such as fish. According to Ncube, (2015) aquatic pollution is the introduction of chemical, physical or biological material into fresh or marine water that degrade the quality of water and affects organisms living in it. Globally, natural phenomena such as volcanoes, algal blooms, storms, and earthquakes also cause major changes in water quality and the ecological status of water (Farid, 2002).

The various water bodies in the country are used for various purposes such as fishing, transportation, sand mining, irrigation, recreation, abstraction for industrial and domestic purposes as well as electricity generation. Many factories in Nigeria are located on river banks and use the rivers as open sewers for their effluents discharge. The major industries responsible for water pollution in Nigeria include petroleum, mining (for gold, tin and coal) wood and pulp, pharmaceuticals, textiles, plastics, iron and steel, brewing, distillery fermentation, paint and food. From time to time accidental oil spillages occur which endanger local sources of water supply and freshwater living resources, especially in the rural areas (FEPA, 1991a). Degradation of water quality is most severe in the five states that contain 80 percent of the nations industries namely Lagos, Rivers, Oyo, Kano and Kaduna States. Coloured, hot and heavy metal laden effluents especially from the textile, tannery and paint industries are discharged directly into open drains and water channels, constituting direct danger to water users and organisms. This has continued to pose a threat to health and economic development in Nigeria (Adekunle *et al*., 2007; Adeyemi *et al*., 2008; Mustapha, 2008; Adewolu *et al*., 2009; Makinde, 2013). According to the Federal Ministry of Environment (2017):

> Industries were the major sources of pollutants in Nigeria in 1980s and 1990s when well over 5,000 industrial facilities and 10,000 small scale industries were in operation on the Nigerian landscape. Although many of the industries have closed down, pollution has by no means abated as those functioning still rely entirely on diesel-powered generators. Besides, the country has witnessed a tremendous upsurge in the number of automobiles and commercial motorcycles on our roads and streets as well as electric generators in homes and offices. These contraptions have become the new and widespread sources of pollution in Nigeria. Used crankcase oil from mechanic workshops, industries, power stations and commercial houses are dumped directly into drains and ground surfaces in urban centres…..Main challenges of environmental pollution in Nigeria include: (i) poor identification of sources of specific pollutants due to the use of inappropriate

technologies; and (ii) weak legal framework, institutions and human capacity to bring the pollutants to acceptable levels that conform to international standards.

2.2 Sources of Water Pollution in Nigeria

2.2.1 Domestic Based Water Pollution and Urban Run-off

Domestic based water pollution has been identified as a serious problem of developing countries (Fakayode, (2005). This type of pollution arises as a result of inappropriate management of enormous wastes generated by human activities and its unsafe disposal into the surrounding environment including water bodies, especially freshwater resources. This unsafe practice has regularly rendered water unsuitable for both primary and/or secondary usage (Fakayode, 2005).

Contrary to urban building regulations, provision of sanitation and drainage facilities usually come much later, if at all, after the settlements have been completed and inhabited. This results in the discharge of human and animal wastes into water courses. Household refuse are profusely dumped into water ways as a way of getting rid of them. Untreated domestic sewage discharged into the aquatic environment is a major source of human pathogens (Adeyemo, 2003). In addition, rainfall runoff carries all sorts of pollutants from houses, industries, farmland and dumping sites into water bodies. Surface water, such as rivers, lakes and ponds can provide a reservoir for pathogens (Oliveira *et al*., 2011). Among the pathogens occurring in water sources, enteric pathogens are the ones most frequently encountered.

Exposure to contaminated water through drinking, recreation or irrigation is a significant mode of transmission of gastrointestinal infections. *Cryptosporidium* species are protozoan

parasites that cause widespread gastrointestinal illnesses in humans and can potentially shorten the life spans of immunocompromised individuals (Carmena *et al*., 2007). These microorganisms are transmitted via the faecal–oral route, with the consumption of contaminated water and contact with recreational waters significant avenues for acquisition of infection (Slifko *et al.*, 2000). *Cryptosporidium* oocysts can survive for months or years in surface water and in soil (Robertson *et al*., 1992; Kato *et al*., 2004). They are resistant to conventional disinfectants at the concentrations and exposure times commonly used, and their infectious dose in humans has been estimated to be as low as 30 oocysts (DuPont *et al*., 1995). Surface water becomes contaminated through the discharge of untreated and partially treated sewage and run-off of manure (Hunter and Thompson, 2005); urban and farm runoffs, discharges from sewage treatment facilities, failing septic systems, wildlife, farm animals and direct faecal contamination by humans and animals (Payment and Riley, 2002; Okafo *et al*., 2003).

2.2.2 Industrial discharges

In Nigeria, domestic waste, industrial pollution, and agricultural run-off have been identified as principal causes of water pollution (FEPA, 1991). Also, Ekiye and Zejiao (2010) stated that studies carried out in most cities in Nigeria showed that industrial effluent is one of the main sources of surface water pollution in Nigeria. Dada (1997) indicated that less than 10 % of industries in Nigeria treat their effluents before being discharged into the rivers. In Jebba and Kaduna, for instance, partially treated discharges are dumped into the rivers Niger and Kaduna, respectively. In Lagos, the discharges are dumped into the open drains from which they flow into the streams and the ocean (Ojo, 1995). Industrial

effluents, when discharged directly into rivers without prior treatment have capacity of increasing water quality parameters.

The indiscriminate discharge of chemical toxins especially lead (Pb), cadmium (Cd), chromium (Cr), dobolt (Co), and mercury (Hg) into the environment could lead to their transfer into plants, animals and man. High concentrations of heavy metals in irrigation waters could destroy crops, interfere with the uptake of essential nutrients, form objectionable deposits on fruits and render the edible portions of plants toxic to human and grazing animals (Bichi, 1999). Nwaedozie *et al*. (2011) found high concentrations of heavy metals in soils along the River Kaduna bank and called for caution in the use of the river and its banks for cultivating Fadama crops because the soil could be highly polluted. Vegetables can absorb metals from soil as well as from deposits on the parts of the vegetables exposed to the air from polluted environments (Haiyan and Stuanes, 2003). These elements, at concentrations exceeding the physiological demand of vegetables, could administer toxic effects in them and also could enter food chains, get biomagnified and pose a potential threat to human health (Sharma *et al*., 2008a, b).

Heavy metals may enter the aquatic environment from both natural and anthropogenic sources. Natural sources include weathering of minerals and soils (Merian, 1991). Anthropogenic inputs are mainly from industrial and domestic effluents, rural and urban storm water runoff and spoil heaps (Agbozu and Ekweozor, 2001). Heavy metals are transported as dissolved substances in water or as an integral part of suspended sediments. The metals could be ingested directly through drinking or indirectly by consumption of plants cultivated along the bank of the water body and through the consumption of aquatic

animals such as fishes and crabs (Mathias and Kevern, 1995). Two factors contribute to the deleterious effects of heavy metals as environmental pollutants. Firstly, they cannot be eliminated through biological degradation as in the case of most organic pollutants. Secondly, they are easily assimilated and can be bioaccumulated in the protoplasm of aquatic organisms (Egborge, 1994). Potential toxicity of trace metals results from the fact that they are transitional elements able to form stable coordinated compounds with a range of both organic and inorganic ligands (Fergusson, 1990). The metals are not toxic as the condensed free elements but are dangerous in the form of cations and when bonded to short chains of carbon atoms (Ward, 1995).

Fish is considered as an important source of animal proteins and other essential minerals, vitamins and unsaturated fatty acids (Medeiros *et al*., 2012). However, the contents of toxic heavy metals in fish can counteract their beneficial health effects in humans (Castro-Gonzalez and Méndez-Armenta, 2008). Different fish samples from River Kaduna were analyzed for toxic elemental contaminants such as Hg, Cd, Pb, V, Zn and Fe and were found to contain these elements in appreciable amounts in all the fish samples studied (Nwaedozie, 1998). Lead is a dangerous element; it is harmful even in small amounts. Exposure to lead is cumulative over time. High concentrations of lead in the body can cause death or permanent damage to the central nervous system, the brain, and kidneys (Jennings *et al*., 1996). Cadmium has been known to have contributed to pulmonary disease, reduced glucose tolerance, severe kidney and liver damage and death in human beings (Voogt dept van *et al.,* 1985). Zinc causes slow growth in children, reduced fertility, dry mouth, headache and nausea (Castro-Gonzalez and Méndez-Armenta, 2008).

2.2.3 Agricultural wastes

Agricultural run-off containing pesticides, plant and animal wastes is also a major contributing source of organic pollution to water bodies in Nigeria. Many farmers use agrochemicals to increase their yields and in vector control campaigns. According to Galadima *et al.* (2011) globally agriculture is a single largest user of freshwater. It is also a major cause of degradation of surface and groundwater resources via erosion and chemical runoff; this has global implications on water quality. The primary agricultural pollutants are nutrients (nitrogen and phosphorus), sediment, animal wastes, pesticides, and salts. The pollutants from these sources get into the surface water through direct surface runoff or through seepage to ground water that discharges to a surface water outlet. The dominant nutrients in surface water are chemical fertilizers and manure from animal facilities. These excess nutrients lead to eutrophication (excessive growth of algae); thus diminish oxygen content (called oxygen debt) suppressing the growth of other water plants and animals, disrupting the food chain. This source of pollution is considered as non-point source as the pollution is generated over the entire agricultural field.

Over the years, acute toxicity of pesticides to aquatic organisms has been manifested by alterations in the behavioural patterns and a large kill of fish caused by the accidental release of organochlorine pesticides (OCPs), such as DDT, toxaphene, dieldrin, aldrin, and heptachlor into the aquatic environment (Yameogo *et al.*, 1993; Singh *et al*., 2018). Being lipophilic, these pesticides can be concentrated to harmful levels in the aquatic environment through bioaccumulation (Müller and Lloyd, 1994). Water pollution through surface run-off, has been reported in literature causing nutrient enrichment, water quality impairment,

marine lives spawning ground destruction and fish kill (Martin *et al*., 1998; Izonfuo and Bariweni, 2001; Idu, 2015).

2.3 Indicators of water pollution

2.3.1 Bacteriological Indicators

A wide range of pathogens may be found in drinking water including bacteria, viruses, protozoa and helminthes. In many cases routine analytical methods for pathogens do not exist or are expensive and time consuming. As a result, current approaches to water quality control are usually based on the absence of bacteria that indicate the presence of faecal contaminants in samples taken from the water supply. Absence of such indicator bacteria in samples is often taken to describe the water supply as 'safe' (Lawrence *et al*., 2001). Generally, indicator microorganisms are not themselves human pathogens (Verhille, 2013). The most commonly used indicators for surface waters are the fecal coliforms and E. coli, although another bacterial group, genus Enterococcus (made up of the three synonyms: Fecal streptococci, enterococci and intestinal enterococci) comprise different species of sanitary significance (Saxena *et al*, 2015). Alternate indicators for surface waters have been suggested, including Clostridium perfringens (WHO, 2008), Bifidobacterium spp. (Mushi *et al*., 2010), Bacteroides fragilis (Zheng *et al.,* 2013) and Bacteriophages (Mendez *et al*., 2004). However, in all cases faecal coliform counts and *Escherichia coli* detection in particular, remain the major and most reliable tool in the assessment of the health risks posed by pathogens in water (Byamukama *et al*., 2000; Odonkor and Ampofo, 2013). According to Vinten *et al*. (2004), faecal contamination increases the risk of enteric pathogenic microorganisms being endemic.

Indicator bacteria are of limited use in predicting the presence of protozoa and viruses both of which may be significantly more resistant to disinfection and may survive longer in the environment. In the case of protozoa, sanitary completion measures usually provide sufficient security for groundwater supplies. It is important that the analysis of indicator bacteria should be supported by risk assessment and source protection. An absence of indicators does not mean a total absence of pathogens. However, the absence of faecal indicators can be taken as an indication that water is relatively low risk (Lawrence *et al*., 2001). Routine monitoring of water supplies also involves screening for the presence of the waterborne parasite *Cryptosporidium parvum* (Fricker, 2000; Gale, 2001).

2.3.2 Physico-Chemical Indicators

These include dissolved oxygen, pH, temperature, salinity and nutrients (nitrogen and phosphorus). They also include measures of toxicants such as insecticides, herbicides and metals. Physico-chemical indicators provide information on what is impacting on the system. Although physico-chemical indicators can identify the cause of the problem, they only provide limited information on the extent that pollutants are actually impacting on fauna and flora (DEHP, 2016). This is related to chronic as opposed to acute effects. Temperature is one of the most important characteristics of an aquatic system. Most of the biological and chemical processes occurring in natural water bodies are greatly dependent on temperature.

All aquatic animals need dissolved oxygen, DO, to breathe. Low levels of oxygen (hypoxia) or no oxygen levels (anoxia) can occur when excess organic materials, such as large algal blooms, are decomposed by microorganisms. During this decomposition process, DO in the

water is consumed. As DO levels drop, some sensitive animals may move away, decline in health or even die (EPA, 2016). Dissolved oxygen is considered an important measure of water quality as it is a direct indicator of an aquatic resource's ability to support aquatic life.

Salinity refers to the total amount of inorganic mineral compositions contained in water. Regular consumption of low-mineral water will destroy the balance of alkali metals and alkaline earth metal ions in human body, resulting in disease. Regular consumption of high-mineral water will lead to stone disease. Salinity is an important indicator to determine the chemical compositions of water and is used in the evaluation of irrigation water applicability as a major indicator by evaluating the total water salinity. Salinity is commonly used in the analysis of natural water quality and is mainly expresses by the sum of the mass of major ions tested.

Nitrogen and phosphorus are essential elements for all living organisms: they are used in DNA and chemical processes in the body. Nitrogen and phosphorus are applied to crops in the form of artificial fertilizers to produce high yields. Phosphates originate from many sources, including sewage and manure, and are also found in many artificial fertilizers. Nitrogen and phosphorus support the growth of algae and aquatic plants, which provide food and habitat for fish, shellfish and smaller organisms that live in water (EPA, 2017). However, excess nitrate and phosphate in watercourses causes a nutrient boost which often gives rise to excessive algae growth. Some algal species produce toxins harmful to other organisms. Submerged vegetation can choke off a waterway, preventing boat access or swimming. When all this vegetation dies, it sinks to the bottom and decomposes. The

decomposition process depletes the dissolved oxygen in the water making it unfit for aquatic life and leading to the death of macroinvertebrates and fish. This degradation is known as eutrophication. Major sources of nitrate and phosphate are fertilizer, sewage and runoff from animal farms.

2.4 Heavy Metals in the Environment

Heavy metals are defined as metallic elements that have a relatively high density compared to water (Gabrielyan *et al*., 2018). Reported sources of heavy metals in the environment include geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources (He *et al*., 2005), mining and industrial wastes; vehicle emissions; lead-acid batteries; fertilisers; paints; treated woods; aging water supply infrastructure (Harvey *et al*., 2015); microplastics in the aquatic environments (Horton *et al*., 2017); and volcanic eruptions (Wendel, 2017). Heavy metals are an important class of pollutants which can produce considerable harm to the environment when they are above certain concentrations (Sierra *et al*., 2017; Zhang *et al*., 2016). These elements can be leached into the surface water or groundwater, taken up by plants, and can bond semi permanently with soil components such as clay or organic matter, which later affect human health (Gómez-Alvarez *et al*., 2009). After heavy metals enter into a water body, they can harm aquatic organisms, and, through the processes of chemical adsorption and physical precipitation, heavy metals can accumulate in the sediments of the water environment (Gao *et al*., 2014). Heavy metals are stable inorganic contaminants since they cannot be destroyed or biodegraded as most organic pollutants. They tend to bio accumulate in the environment especially in bottom sediment of rivers and lakes, associated with inorganic and organic chemical combinations. Multiple heavy metals may simultaneously enter the

human body through air, water or food. Once inside the body, heavy metals are not easily metabolized or excreted, they build up quickly and bioaccumulate leading to an increase in their concentration (Cooper *et al*., 2017). Some studies have shown the potential risk to human health due to the accumulation of heavy metals in plants (Balkhair and Ashraf, 2016; Alghobar and Suresha, 2017).

Metals such as cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) are essential nutrients that are required for various biochemical and physiological functions (WHO/FAO/IAEA, 1996). They are important constituents of several key enzymes and play important roles in various oxidation-reduction reactions (WHO/FAO/IAEA, 1996). Inadequate supply of these micro-nutrients results in a variety of deficiency diseases or syndromes (WHO/FAO/IAEA, 1996). On the other hand, an excess amount of such metals produces cellular and tissue damage leading to a variety of adverse effects and human diseases. Other metals such as aluminium (Al), antinomy (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), gallium (Ga), germanium (Ge), gold (Au), indium (In), lead (Pb), lithium (Li), mercury (Hg), platinum (Pt), silver (Ag), strontium (Sr), tellurium (Te), thallium (Tl), tin (Sn), titanium (Ti), vanadium (V) and uranium (U) have no established biological functions and are considered as non-essential metals (Chang *et al*., 1996). Due to their high degree of toxicity, the five elements namely arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of great public health significance. They are all systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure (Tchounwou *et al.*, 2012).

The major routes of heavy metal uptake by man are food, drinking water and air. Unlike other pollutants like petroleum hydrocarbons and litter which may visibly build up in the environment, trace metals may accumulate unnoticed to toxic level.

Lead is a naturally occurring bluish-gray metal present in small amounts in the earth's crust. It is present in nature only in small amounts but human activities have increased its concentration levels in many locations worldwide (Gupta *et al*., 2013). Although lead occurs naturally in the environment, anthropogenic activities such as fossil fuels burning, mining, and manufacturing contribute to the release of high concentrations. Lead has many different industrial, agricultural and domestic applications. Exposure to lead occurs mainly via inhalation of lead-contaminated dust particles or aerosols, and ingestion of lead-contaminated food, water and paints (ATSDR, 1992, 1999; CDC, 2016). Lead is the most systemic toxicant that affects several organs in the body including the kidneys, liver, central nervous system, hematopoetic system, endocrine system, and reproductive system (ATSDR, 1999). In 2013, the World Health Organization estimated that lead poisoning resulted in 143,000 deaths, and contributes to 600,000 new cases of children with intellectual disabilities, each year (WHO, 2013).

Chromium (Cr) is a naturally occurring element present in the earth's crust. Chromium enters into various environmental matrices (air, water, and soil) from a wide variety of natural and anthropogenic sources with the largest release coming from industrial establishments (Tchounwou *et al.*, 2012). The industries with the largest contribution to chromium release include metal processing, tannery facilities, chromate production, stainless steel welding, and ferrochrome and chrome pigment production. Chromium

released into the environment from anthropogenic activity occurs mainly in the hexavalent form [Cr(VI)] (ATSDR, 2012). Hexavalent chromium [Cr(VI)] is a toxic industrial pollutant that is classified as human carcinogen by several regulatory and non-regulatory agencies (IARC, 1990; US EPA, 1992; ATSDR, 2012). Chromium is widely used in numerous industrial processes and as a result, is a contaminant of many environmental systems (Cohen *et al*., 1993). Occupational and environmental exposure to Cr(VI)-containing compounds is known to cause multiorgan toxicity such as renal damage, allergy and asthma, and cancer of the respiratory tract in humans (WHO/IPCS, 1988; Goyer, 2001).

Cadmium has been classified by the International Agency for Research on Cancer (IARC) as being carcinogenic in humans (group 1), especially lung cancer but with limited evidence for kidney, liver and prostate cancers. Cadmium is not an essential element in biological processes. Instead it is toxic for humans, animals and plants (Palma *et al*., 2013) and poisoning occurs through inhalation and ingestion. This heavy metal enters the environment mainly from industrial processes and phosphate fertilizers.

Plants have a natural ability to take up metals. Plants and vegetables take up elements by absorbing them from contaminated soils and waste water used for irrigating them; as well as from deposits of the metals on different parts of the plants exposed to the air from polluted environment (Funtua *et al*., 2008). Vegetables are known to naturally accumulate heavy metals from either wastewater or dumpsite (Arora *et al*., 2008). Factors influencing the bioavailability of metals and their occurrence in crops were found as soil pH, cation exchange capacity, organic matter content, soil texture, and interaction among the target elements. It is concluded that total metal concentrations in soils are the main controls on

their contents in plants (Jung, 2008). However, Farook *et al.* (2008) posited that agricultural soils have generally low levels of heavy metals and that contamination of the soils are through fertilizer application and irrigation with partially treated or untreated sewage.

Fishes are major sources of protein. They have been recognized as good bioaccumulators of organic and inorganic pollutants (King and Jonathan, 2003). Fish have the ability to accumulate heavy metals in their tissues byabsorption along gill surface, kidney, and liver and gut tract wall to higher levels than environmental concentration (Annabi *et al*., 2013). Accumulation of heavy metals by organisms may be passive or selective; and differences in accumulation of heavy metals by organisms could be as a result of differences in assimilation, egestion or both (Egila and Daniel, 2011). Research has shown that the extent of accumulation of heavy metals in fish is dependent on the metal types, fish species, and the tissues respectively (Gorur *et al*., 2012; Petrovic *et al*., 2013). In addition, many factors such as sex, age, size, reproductive cycle, swimming patterns, feeding behavior and living environment (i.e., geographical location) can influence metal uptake (Zhao *et al*., 2012). The rate of bioaccumulation of heavy metals in aquatic organisms depends on the ability of organisms to digest the metals and the concentration of such metals in the river. It also has to do with the concentration of the heavy metal in the surrounding soil as well as the feeding habits of the fish species (Eneji *et al*., 2011). The metals are finally transferred to other animals including humans through the food chain (Palma *et al*., 2013).

2.5 *Escherichia coli*

Escherichia coli (*E. coli*) is a Gram-negative, facultative anaerobe, non-sporulating, rod and belongs to the family Enterobacteriaceae of gamma-proteobacteria (Willey *et al.,* 2008). They are commonly found in the lower intestine of warm-blooded animals (endotherms), which makes their easy passage into faeces, urine, sewage and contaminated water possible (APEC, 2011). Using molecular assay through genomic sequencing and ecological study, some strains of *E. coli* have been found to survive naturally outside a warm blooded host (Kostas, 2014). Most strains of *E. coli* are harmless; others can cause illness (CDC, 2018).

2.5.1 Adaptation and Importance of *E. coli*

Genetic adaptations possessed by *E. coli* include the ability to respond to environmental signals such as chemicals, pH, temperature and osmolarity in a number of very remarkable ways. Some toxins dangerous to humans e.g. *Shigela* toxin may help *E. coli* fend off aquatic predators, enabling strains of *E. coli* that produce the toxin to survive longer in lake water than their counterparts (Mauro *et al., 2013*).

Some *E. coli* have been found to develop resistance to multiple antibiotics they were formally susceptible to (Poole, 2012). This *E. coli* adaptation has been found to raise the cost of medical treatment compared to treating normal infections. For example, *E. coli* with extended spectrum betalactamase (ESBLs) genes are one of the world's most pressing scourges in the health care industry. If they are pathogenic, they adapt quickly to external factors (mutation) and transfer genetic elements through horizontal gene transfer, etc, to become resistant to antibiotics, thereby creating a diminishing return in the efficacy of antibiotics aimed at targeting specific sites in the microbes.

However, as long as these bacteria do not acquire genetic elements encoding for virulent factors, they remain commensal, but in the presence of virulent factors they develop ability to produce lethal toxins, extended-spectrum β-lactamase genes or modification of genetic constituent induced by the influence of some intercalating agents like ethidium bromite, acridine dye etc, which cause mutation and resistance to antibiotics (Igwe *et al.,* 2013).

The harmless strains are part of the normal flora of the gut. They benefit their hosts by producing vitamin K_2 and also prevent the establishment of pathogenic bacteria within the intestine (Bentley and Meganathan, 1982). Non-pathogenic *Escherichia coli* strain Nissle 1917, also known as mutaflor, is used as a probiotic agent in medicine, mainly for the treatment of various gastroenterological diseases, like inflammatory bowel disease (Grozdanov *et al.,* 2004).

2.5.2 *Escherichia coli* infections

Escherichia coli is the predominant nonpathogenic facultative flora of the human intestine. The organism typically colonizes the infant gastrointestinal tract within a few hours after birth (Hooper and Gordon, 2001; Fanaro *et al*., 2003), and, thereafter, *E. coli* and the host derive mutual benefits for decades (Kaper *et al*., 2004). *E. coli* usually remains harmlessly confined to the intestinal lumen. However, in the debilitated or immunosuppressed host; or when gastrointestinal barriers are violated, even normal "non-pathogenic" strains of *E. coli* can cause infection. Infections due to pathogenic *E. coli* may be limited to the mucosal surfaces or can disseminate throughout the body. Three general clinical syndromes result from infection with inherently pathogenic *E. coli* strains: (i) urinary tract infection, (ii) sepsis/meningitis, and (iii) enteric/diarrheal disease.

Under normal conditions, the capacity of the gastrointestinal tract to absorb fluid and electrolytes is tremendous and from the 8-9 liters of fluids presented to the intestine daily, only 100-200 ml are excreted in the stool (Hodges and Ravinder, 2010; Fluid and electrolyte, 2017). Enteric pathogens, however, can alter this balance towards net secretion leading to diarrheal disease (Hodges and Ravinder, 2010). Diarrheagenic *E. coli* (DEC) strains are among the most common etiologic agents of diarrhea. They can be passed in the feces of humans and other animals. Transmission occurs through the fecal-oral route, primarily via contaminated food or water and also through person-to-person contact and contact with animals or their environment. The intestinal tracts of animals, especially cattle and other ruminants, are the primary reservoirs of STEC. This pathogenic *E. coli* are categorized into pathotypes on the basis of their virulence genes (O'Reilly *et al*., 2017) viz: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), Vero toxin-producing/Shiga toxin-producing *E. coli* (VTEC/STEC) which include its well-known subgroup including enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC).

Enteropathogenic *E. coli* (EPEC)

The distinctive histopathology induced by this group of *E. coli* is termed attaching and effacing (A/E) lesions and is caused by the intimate attachment of bacteria to the intestinal epithelial cells and effacement of enterocyte microvilli (Chen and Frankel, 2005). EPEC continues to be a significant cause of infantile diarrhea in developing nations, contributing to high morbidity and mortality. Prolonged and persistent episodes of diarrhoeal disease (diarrhoea > 14 days) constitute a significant portion of the global burden of diseases in children (Ochoa and Contreas, 2011). A systematic review of the illnesses in developing

countries has shown that EPEC, ETEC and EAEC are the main pathogens associated with this complication; and are responsible for 30-40% of all persistent diarrheal episodes in children (Abba *et al*., 2009). In developing countries, healthy carriage of enteric pathogens is common and has been attributed to the interplay of many factors including host susceptibility (related to the child's age, breastfeeding, nutritional and immunological status), bacterial virulence factors (different virulence genes), and environmental factors (poor hygiene, and high fecal contamination) (Ochoa and Contreas, 2011).

EPEC forms small microcolonies on the surface of infected epithelial cells, followed by intimate contact and localized degeneration of the epithelial brush border microvilli, cumulating in an attaching and effacing (A/E) lesion. The A/E lesion (or pedestal) is associated with the assembly of highly organized cytoskeletal structures in epithelial cells immediately beneath the adherent bacteria that include the cytoskeletal components actin, α-actinin, myosin light chain, ezrin, and talin (Albert *et al*., 1991; Arriaga *et al*., 1995).

Enterotoxigenic *Escherichia coli* (ETEC)

ETEC is the most important but least-recognized bacterial cause of diarrhea or cholera-like disease in all age groups in areas with poor sanitation and inadequate clean water (Jafari *et al*., 2012). Infection with ETEC is the leading cause of travelers' diarrhea and a major cause of diarrheal disease in lower-income countries, especially among children. ETEC is transmitted by food or water contaminated with animal or human feces.

ETEC strains adhere to intestinal epithelial cells via a heterogenous group of proteinacious surface structures called colonization factors (CFs), which can be fimbrial, non-fimbrial or fibrillar (Croxen and Finlay, 2010). Following the initial adhesion and colonization, ETEC

strains cause diarrhea by producing heat-labile (LT) and/or heat-stable (ST) enterotoxins, which are plasmid-encoded (Johnson and Nolan, 2009). ETEC strains are associated with two major clinical syndromes: weanling diarrhea among children in the developing world, and traveler's diarrhea. The infection in developing countries is usually frequent in infants younger than 2 years of age, with a decrease after 5 years of age. A report indicated that ETEC infection is the first cause of diarrhea illness experienced by infants from endemic areas of developing countries during their first year of life (Walker and Black, 2010). ETEC infections in areas of endemic infection tend to be clustered in warm, wet months, when multiplication of ETEC in food and water is most efficient (Levine *et al*., 1993). Infection can be prevented by avoiding or safely preparing foods and beverages that could be contaminated with the bacteria, as well as washing hands with soap frequently.

Shiga toxin-producing/ Enterohaemorrhagic *E. coli* (STEC/EHEC)

Entero-hemorrhagic *E. coli* (EHEC), also known by the acronyms Shiga toxin-producing *E. coli* (STEC), or verocytotoxin-producing *E. coli* (VTEC)] is one of the most common of the serogroups associated with gastroenteric illnesses and foodborne outbreaks (Islam *et al*., 2014). Among DEC, STEC includes the most virulent strains (Tozzolli and Scheutz, 2014). The Shiga toxins produced by this group of E. coli cause an array of complications in humans, ranging from uncomplicated diarrhea to life-threatening conditions such as hemorrhagic colitis (HC), which can progress into hemolytic uremic syndrome (HUS) and severe acute renal failure. The STEC/VTEC encompass hundreds of serotypes capable of causing severe illnesses in humans, among which O157, O26, O111, O103 and O145 (the so-called top five) are the most frequently reported serogroups associated with human

disease (De Rauw *et al*., 2014). The O157 serogroup is the most commonly reported and has been the main cause of serious outbreaks, especially of foodborne infections (Islam *et al*., 2014). As such, *E. coli* O157 has emerged as a particular public health concern. A key property of these strains is the production of "Attaching and Effacing" (AE) lesions.

Ruminants, particularly cattle, are the natural reservoir of *E. coli* O157, which inhabit the intestinal tract of healthy animals but are shed in their faeces. STEC are considered zoonotic microorganisms that can be transferred, through fecal contamination, from its wild and farm animal reservoir to meat and other products from animal origin, as well as to water, and further to fresh produce (Croxen *et al*., 2013, Feng, 2014). Infection of humans is typically acquired through contaminated food and drink (i.e. undercooked ground meat, raw milk, dairy products, raw vegetables, contaminated fruits) or through direct contact with animals (Islam *et al*., 2014; Ahmed and Abouzeed, 2014).

The major virulence factor, and a defining characteristic of EHEC, is a phage-encoded cytotoxin, *Stx*. This potent cytotoxin is the factor that leads to death and many other symptoms in patients infected with EHEC. *Stx1*, from EHEC, is identical to Shiga toxin from *Shigella dysenteriae I*. The high virulence of STEC strains such as O157:H7 is not only dependent on the virulence factors but partially also on the pathogen's ability to survive environmental stress conditions, such as low pH levels found in the gastrointestinal tract. These contribute to its very low infectious dose of <100 cells, which also facilitates STEC infections (Viazis and Diez-Gonzalez, 2011; Croxen *et al*., 2013).

The term enterohaemorrhagic *E. coli* (EHEC) is applied to those STEC serotypes that have the same clinical, epidemiological and pathogenetic features associated with the prototype

strain *E. coli* O157:H7. In addition to *E. coli* O157, many other serogroups of STEC may also cause disease. These are called "non-O157 STECs.

Enteroinvasive *E. coli* (EIEC)

The ability of these strains which before now were called enteroinvasive *E. coli* (EIEC) to cause diarrhoea was demonstrated in volunteer studies in 1971 (Nataro and Kaper, 1998). It has been shown that EIEC strains and *Shigella* species are biochemically, genetically, and pathogenetically very closely related so much so that it has been proposed that they should be classified as one species in genus *Escherichia* (Lan *et al*., 2004). However, molecular methods have been developed to distinquish EIEC from *Shigella* and other diarrhoeal pathogens (Kauer *et al*., 2010; Yamazaki and Fukasawa, 2011; Pavlovic *et al*., 2011).

Enteroinvasive Escherichia coli (EIEC) bacteria are human enteric pathogens that have been identified worldwide. EIEC causes invasive disease that may result in severe illness in otherwise healthy persons (Escher *et al*., 2014) Transmission of EIEC is by the fecal–oral route, and contaminated food or water are the usual vehicles of infection. EIEC outbreaks are rare in Europe; the only recently reported EIEC outbreak in western Europe was in Italy in 2012 (Escher *et al*., 2014), and no outbreaks have been reported in the United Kingdom or other parts of northern Europe (Newitt *et al*., 2016).

Enteroaggregative *E. coli* (EAEC)

EAEC is the second most common cause of travelers' diarrhoea after ETEC in both developed and developing countries. EAEC are commonly recognized as a cause of endemic and epidemic diarrhea worldwide and recently, has been shown to cause acute diarrheal illness in newborns and children in industrialized countries. This organism has

also been associated with persistent diarrhoea. Diarrhoea caused by EAEC is often watery, but it can be accompanied by mucus or blood (Harrington *et al*., 2006; Weintraub, 2007; Croxen and Finlay, 2010).

Diffusely adherent *E. coli* (DAEC)

DAEC is a heterogenous group that generates a diffuse adherence pattern on HeLa and HEp-2 cells and has been linked with the watery diarrhea that can become persistent in young children in both developing and developed countries; as well as recurring urinary tract infections (Servin, 2005; Croxen and Finlay, 2010). The consequences of this persistence are unknown, but several observations have suggested a potential role in the development of chronic inflammatory intestinal disease (Servin, 2005). In addition, some studies have implicated DAEC strains as diarrheal agents only in children older than six months, depending on the study, and in adults (Ochoa *et al*., 2009; Opintan *et al*., 2010).

2.6 *E. coli* O157:H7 Infections

2.6.1 Virulence factors of *E. coli* O157:H7

Escherichia coli O157:H7 cause acute hemorrhagic diarrhea with abdominal cramps that in its severe cases result in hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Zhu and Hill, 2013). Young children, immunocompromised individuals and the elderly are more susceptible to hemorrhagic colitis and are at higher risk for the illness to progress to more severe complications (FDA, 2012). *E. coli* O157:H7's ability to induce injury in humans is the result of its ability to produce numerous virulence factors, most notably Shiga toxin (Stx), which is one of the most potent toxins known to man (Suh *et al*., 1998; Johannes, 2010). Shiga toxin has

multiple variants (e.g., *Stx1, Stx2*, *Stx2c*), and acts like the plant toxin ricin by inhibiting protein synthesis in endothelial and other cells (Sandvig, 2002). Endothelial cells line the interior surface of blood vessels, and are known to be extremely sensitive to *E. coli* O157:H7, which is cytotoxigenic to these cells (Sandvig, 2002).

In addition to Shiga toxin, *E. coli* O157:H7 produces numerous other putative virulence factors, including proteins which aid in the attachment and colonization of the bacteria in the intestinal wall and which can lyse red blood cells and liberate iron to help support *E. coli* metabolism (Allerberger *et al*., 1996). Additional potential virulence factors are encoded in the chromosome and on a ca. 60-MDa plasmid found in all EHEC strains of serotype O157:H7.

The EHEC genome contains the same locus of enterocyte effacement (LEE) as the EPECs and the intimate attachment of EHEC to host cells occurs through interaction between an adhesin called intimin (eaeA), and Tir (translocated intimin receptor). This intimate attachment induces the characteristic attaching and effacing lesions (A/E), but the initial adherence of EHEC to colonocytes is not well defined (Johnson and Nolan, 2009; Croxen and Finlay, 2010; Viazis and Diez-Gonzalez, 2011). The ability of STEC to produce A/E lesions is sufficient to cause non-bloody diarrhoea but Shiga toxin is essential for the development of bloody diarrhoea, HC, and HUC (Nataro and Kaper, 1998). Another toxin found in many STEC/EHEC isolates is the enteroaggregative heat-stable enterotoxin1 (EAST1) and usually two copies of the astA gene is present in the chromosome (Nataro and Kaper, 1998; Viazis and Diez-Gonzalez, 2011). The significance of this carriage in the pathogenesis of EHEC is unclear, but it has been suggested that some of the non-bloody

diarrhoea in persons infected with these strains might be due to the production of this toxin (Nataro and Kaper, 1998; Gyles, 2007). *E. coli* O157:H7 produces fimbriae which might aid intestinal adherence (Cordonnier *et al*., 2017).

Acid tolerance has been identified as a major virulence determinant of this organism (Gorden and Small, 1993; Wang and Doyle, 1998a). Acid tolerance enhances the ability of the cell to survive the acidic conditions of the initial segments of the gastrointestinal tract. This enables the organism to go on to colonize the gut (Paton and Paton, 1998). The pathogenic *E. coli* strains use a complex multistep mechanism of pathogenesis involving a number of virulence factors which, depending upon the pathotype, consists of attachment, host cell surface modification, invasin, a variety of toxins, and secretion systems which eventually lead toxins to the target host cells (Kaper *et al*., 2004).

2.6.2 Modes of Transmission of *E. coli* O157:H7

E.coli O157:H7 is transmitted through the faecal-oral route, by food and water and from person to person. Cattle are known to be the main reservoir for STEC O157, (making it zoonotic), and transmission is by consumption of raw or undercooked contaminated beef and other bovine food products (Jay *et al*., 2004; Kassenborg *et al*., 2004). In a study by Mead *et al*. (1997), cross-contamination of food items occurred when food handlers did not wash their hands after handling raw ground beef. This lapse was suggested as the source of infection rather than the direct ingestion of undercooked hamburgers. Outbreaks linked to consumption of unpasteurized apple juice (DHMH, 2012); and fermented hard salami (CDC, 1995) have illustrated the notable ability of *E. coli* O157:H7 to grow in foods of low pH under conditions where other pathogens would not survive. Fresh vegetables such as

lettuce have been the incriminated vehicle in several outbreaks (Warriner and Namvar, 2010; Pérez Rodríguez *et al*., 2011). Environmental contamination with this pathogen, such as vegetables that have been in contact with *E. coli* O157:H7–contaminated manure (fertilizer) or polluted irrigation water during stages of production and processing, has been pointed out as possible routes of transmission (Oliveira *et al.*, 2012).

Also, outbreaks related to consumption of contaminated water (Licence *et al*., 2001; Olsen *et al*., 2002), swimming in a crowded lake (Ackman *et al*., 1997) and to the use of surface water for recreational purposes (Bruneau *et al*., 2004) have been reported. An outbreak in May 2000 in Walkerton, Canada, was related to the consumption of faecally contaminated drinking water. This resulted in an estimated number of 2300 disease cases with seven being fatal (Hrudey *et al*., 2003). *Campylobacter jejuni* and STEC O157 were identified as the main pathogens responsible for the disease cases and STEC O157 was responsible for the deaths.

A low infectious dose is also consistent with waterborne transmission. This has been documented in several reports. In one outbreak of haemolytic colitis (HC) and haemolytic uraemic syndrome (HUS), *E. coli* O157:H7 was isolated from 21 individuals who swam in the same freshwater lake in Oregon (Keene *et al*., 1994). In The Netherlands, STEC O157 was isolated from approximately 6% of the cattle and 4% of the sheep (Heuvelink *et al*., 1998a, b), which makes it reasonable to assume that water contaminated with cattle or sheep faeces harbours STEC O157.

It has been shown that *E. coli* O157:H7 and other Stx-positive *E. coli* strains are perhaps the most dangerous enteric pathogens that clinical microbiologists in developed countries are

likely to encounter. At least three cases of laboratory-acquired infections with *E. coli* O157:H7 have occurred with serious consequences including renal failure and hemorrhagic colitis (Booth and Rowe., 1993; Burnens *et al*., 1993; Rao *et al*., 1996). No laboratory accident or obvious breach in technique was evident in any of the cases. This level of biohazard is due to the extremely low dose required for infection and the potent effects of Stx, which are not limited to the intestine. The low infectious dose and the expression of Stx are reminiscent of *S. dysenteriae* I infection factors, which most microbiologists in developed countries are unlikely to encounter. There also exists the potential for careless workers to transport the organisms home, where young children could be infected (Nataro and Kaper, 1998).

2.6.3 Emergence of *E. coli* O157:H7 in Africa

The first ever reported outbreak of *E. coli* O157:H7 infections in Africa was that which occurred in Swaziland in 1992 (Effler *et al*., 2001). It was characterized by bloody diarrhoea with severe abdominal pains. The source was traced to a river water contaminated with cattle faeces and carcasses. Two other outbreaks occurred in Central African Republic and in Cameroon in 1996 and 1997 respectively (Germani *et al*., 1997; Cunin *et al*., 1999). Although outbreaks of *E. coli* O157 in Africa have been few to date, available information indicates that the pathogen has wide geographic distribution. Since the 1992 outbreak in Swaziland, culture-proven *E. coli* O157 diarrhoeal illness has been reported from multiple locations, including Kenya, Nigeria, Côte d'Ivoire, and Central Africa Republic (Sang *et al*., 1996; Akinyemi *et al*., 1998; Germani *et al*., 1998; Dadie *et al*., 2000).

It is important to understand the complex interactions of environmental and behavioural factors that enabled *E. coli* O157:H7 to emerge in Africa. In their account of the 1992 *E. coli* O157 outbreak in Swaziland, Southern Africa, Effler *et al*., (2001), noted from data available to them, that carriage of *E. coli* O157 was due to cattle, cattle deaths secondary to drought, and heavy rains that resulted in contamination of surface water were important factors contributing to the emergence of *E. coli* O157 in Africa. However, because *E. coli* O157 is not detected by the usual methods used to isolate and identify traditional enteric bacterial pathogens, and because microbiology laboratories in many countries in Africa do not routinely test for this pathogen, *E. coli* O157 infections may go unrecognized (WHO, 1995; Wittenberg, 1999).

2.6.4 Survival of *E. coli* O157:H7 in the environment

Factors that affect the survival of faecal microbes in the environment include solar radiation, pH and temperature (Wang and Doyle, 1998b; Hughes, 2003); assimilable organic carbon and phosphorus content of drinking water (Lehtola *et al*., 1999); as well as bacterial antagonism in soil and predation by protozoa in water (Dixon, 2000).

E. coli O157:H7 has the ability to survive at refrigeration temperatures and under harsh environmental conditions. The organismis capable of surviving at pH 2.0 for 2-5 hours as well as become acid-adapted within a very short time, under extreme conditions (deJonge *et al*., 2003; Grant, 2004; Pérez Rodríguez *et al*., 2011). This unusual hardy characteristic enables this pathogen to survive the human stomach for several weeks to months in acidic foods (Price *et al*., 2000). Acid tolerance is thought to increase the pathogen's resistance to other stresses such as heat, radiation and antimicrobial agents (Buchanan and Doyle, 1997).

Moreover, *E. coli* O157:H7 is a relentlessly evolving organism, constantly mutating and acquiring new characteristics, including virulence factors that make the emergence of more dangerous variants a constant threat (Robins-Brown, 2005; Manning *et al*., 2008).

2.7 *Cryptosporidium*

Cryptosporidium is an obligate parasite belonging to the order apicomplexa with a complex life cycle involving both asexual and sexual reproduction, which it completes within an individual host (monoxenous). It is a genus of parasitic protozoa causing diarrheal illness in humans and animals. Currently more than twenty species of *Cryptosporidium* have been identified, infecting a wide range of hosts. Transmission is via an environmentally robust oocyst stage, which is infectious when excreted and can survive for weeks to months in fresh water.

2.7.1 Cryptosporidiosis

Cryptosporidium parasites are the causative agents of cryptosporidiosis in humans and animals (Checkley *et al*., 2015; Feng and Xiao, 2017). These diarrheal pathogens not only impede the growth of children, but are also associated with fatality in toddlers aged 12–23 months (Kotloff *et al*., 2013; Sow *et al*., 2016; GBDDD Collaborators, 2017). The Global Enteric Multicenter Study (GEMS) to identify the aetiology and population-based burden of paediatric diarrhoeal disease in sub-Saharan Africa revealed that *Cryptosporidium* is second only to rotavirus as a contributor to moderate-to-severe diarrhoeal disease during the first 5 years of life (Kotloff *et al*., 2013). In addition, *Cryptosporidium* can cause opportunistic infection with prolonged, life-threatening diarrhea in AIDS patients (Chen *et al*., 2002; Checkley *et al*., 2015).

Cryptosporidium oocysts are transmitted via the faecal-oral route. Potential sources of transmission include person to person through direct or indirect contact, animal to animal, animal to human, human to animal, waterborne from humans or animals through drinking water or recreational water and food-borne from contamination of water used in food preparation and manufacture or from food handlers. *Cryptosporidium* has been widely reported in various animals, including wildlife, zoo animals, laboratory animals, farm animals, and house pets (Wang *et al*., 2011; Xu *et al*., 2016); urban wastewater (Liu *et al*., 2011); surface drinking water sources (Xiao *et al*., 2012; Hu *et al*., 2014) and even in treated recreational waters (i.e. swimming pool waters) (Xiao *et al*., 2017). Its low infectious dose, prolonged survival in moist environments, protracted communicability, and extreme chlorine tolerance make *Cryptosporidium* ideally suited for transmission through drinking or recreational water (such as swimming pools) that has been contaminated with *Cryptosporidium* (Hlavsa and Xiao, 2017). Transmission can also occur through eating contaminated food, contact with infected people (for example, while providing care or during oral-anal sex) or animals, or contact with fecally contaminated surfaces. Studies have demonstrated that respiratory cryptosporidiosis may occur commonly in both immunocompromised and immunocompetent individuals and that *Cryptosporidium* may also be transmitted via respiratory secretions (Sponseller *et al*., 2014). Several studies also suggest that flies may play an important role in the mechanical transmission of *Cryptosporidium* including human infectious species (El-Sherbini and Gneidy, 2012; Adenusi and Adewoga, 2013; Zhao *et al*., 2014).

Profuse watery diarrhoea is the most common clinical feature of cryptosporidiosis in both immunocompetent and immunosuppressed mammals, including humans (Current and

Garcia, 1991). Secondary signs of infection in humans include abdominal pains, low grade fever, nausea, vomiting and weight loss (Current and Garcia, 1991; O'Donoghue, 1995). The severity and longevity of *Cryptosporidium* infections are directly related to the immune status of the host (Clark, 1999). Clinical signs are generally more chronic and severe in the immunocompromised and are not always confined to the gastrointestinal tract. Extra intestinal infection of the respiratory tract (Casemore, 1990), pancreatic duct, gallbladder and biliary tree have all been documented in human immunodeficiency virus infected patients (Hunter and Nichols, 2002). Most healthy hosts develop immunity after infection. In immunosuppressed hosts however, like HIV infected humans, recovery is difficult and severe dehydration can lead to death (Hunter and Nichols, 2002).

2.7.2 *Cryptosporidium* Oocysts in Water Supplies

Surface water becomes contaminated through the discharge of untreated and treated sewage and run-off of farm manure (Hunter and Thompson, 2005). Large rivers and lakes often receive both agricultural run-off and treated and untreated domestic wastewater. Oocysts of *Cryptosporidium* are ubiquitously present in the aquatic environment. They have been found in most surface waters, their concentration being related to the level of faecal pollution or human use of the surface water (Hansen and Ongerth, 1991; LeChevallier *et al.,* 1991). Several studies showed that the main sources of protozoan contamination of surface water were the intrusion of animal feces or wastewater due to heavy rains, (Gorham and Lee, 2016: Sterk *et al*., 2016; Moreira and Bondelind, 2017).

Several characteristics of *Cryptosporidium (*oo)cysts facilitate the waterborne transmission: compared to other protozoan parasites, the oocysts of *Cryptosporidium* are relatively small

(4-6 μm). Due to their small size, slow settling velocity, and resistance to environmental stress, oocyst contamination can persist in water for a long time (Loganthan *et al*., 2012). In addition, due to their small size, they are less efficiently removed during soil passage in bank filtration and in rapid or slow sand filtration. Filtration processes are important barriers for (oo)cysts in water treatment. Before the discovery of the sensitivity of the (oo)cysts to UV, the attention of the water industry was focused on (oo)cysts removal by filtration process and especially upgrading filter design and operations to optimise (oo)cyst removal. Full scale conventional treatment with coagulation, floc removal and rapid granular filtration removes >2.3 logs. Other filtration processes such as slow sand filtration and diatomaceous earth filtration give similar removal efficiencies for (oo)cysts. Pressure driven membrane processes (microfiltration, ultrafiltration, nanofiltration and reverse osmosis) are playing an important role in drinking water production in the United States and in Europe. These processes are being used in water treatment for multiple purposes including control of disinfection by-products, pathogen removal, clarification, and removal of inorganic and synthetic organic chemicals (Plutzer, 2008).

The state in which (oo)cysts occur in water (suspended or attached to particles) is relevant for water treatment (sedimentation, filtration) and (oo)cysts readily attach to particles in sewage effluent (Medema *et al*., 2006). Assavasilavasukul *et al*. (2008) showed that *Cryptosporidium* oocyst removal in conventional treatment was dependent on initial pathogen concentrations, with lower pathogen removals observed when lower initial pathogen spike doses were used. In addition, higher raw water turbidity appeared to result in higher log removal. Disinfection with chlorine and chloramines has always been an important barrier for waterborne pathogens, but they are not effective against the

Cryptosporidium oocysts. Chlorine dioxide treatment may result in inactivation, but the required product of concentration and contact time is still high especially at low temperatures (Medema *et al*., 2006). Ozone is the most potent chemical against (oo)cysts, although the effectiveness of ozone also reduces at lower temperatures and the Ct values required are high (Bukhari *et al*., 2000). Chemical disinfectants cannot be dosed too high concentrations, because toxic by-products are formed by the reaction with compounds in the water, such as trihalomethanes by chlorine, nitrite by monochloramine, chlorite by chlorine dioxide and bromate by ozone.

Exposure of (oo)cysts to multiple disinfectants has been shown to be more effective than to any of the disinfectants alone. The multiple stresses that oocysts encounter in the environment and during treatment might limit the infectivity of the oocysts (Belosevic *et al*., 1997; Medema *et al*., 2006). Using animal infectivity, experiments showed that oocysts are sensitive to UV; and, short-duration pulsed-UV light is an effective disinfection measure for *C. parvum* (Bukhari *et al*., 2004; Rochelle *et al*., 2004; Lee *et al*., 2008).

Post treatment contamination is a significant hazard. When the water in the distribution system or in storage containers is contaminated, no barriers are in place to prevent ingestion of infectious oocysts. Post treatment contamination may occur through infiltration of contaminants in the distribution system through leaks (during surges), in open distribution reservoirs or other open connections and during construction and repair. Cross connection and back siphonage may draw water from toilets or sewers into the distribution network. Storage tanks used in houses (i.e. in sand pipe systems) may also become contaminated.

Several outbreaks of cryptosporidiosis have been caused by post treatment contamination (Medema *et al*., 2006).

(Oo)cysts can survive for months or years in surface water and in soil (Robertson *et al*., 1992; Kato *et al*., 2004). The infectivity of these parasites is high, and varies between isolates. The large number of cattle and sheep in watersheds and the excretion of high numbers of oocysts make the infected animal important sources of water contamination. This is essential for the management of animal farming in watersheds. Unlike coccidian parasites and helminths, *Cryptosporidium* does not require a period of maturation of (oo)cysts after shedding with faeces. They are immediately able to infect a new host (Plutzer, 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Equipment and Instruments, Bacteriological Media, Reagents and chemicals, and Antibiotic Discs used in this study are presented in Appendix I

3.2 The Study Area

The study was on River Kaduna which runs through Kaduna metropolis (Lat. 10.52 N, Long. 7.44 E). River Kaduna is one of the major tributaries of River Niger; it took its source from Jos Plateau. It runs across Kaduna State and discharges into the Niger River near Wuya in Niger State. The River, as it flows through the city, was divided into three distinct reaches namely: the upper, middle and lower reaches by Alayande and Agunwamba (2010). This study was carried out in the lower reaches of the river which extends from downstream of the main Kaduna Bridge, to the Eastern Byepass Bridge. This reach flows through the Zango, Kudenda Industrial layout, Kakuri, Nasarawa, Abattoir, and Moslem burial ground areas. Hydraulic structures along this reach include the Railway Bridge, the Kaduna South Waterworks, three intake pumping stations belonging to the Nigerian Breweries Plc, the United Nigerian Textile and Arewa Textiles; and the Western Bypass Expressway Bridge. This reach is usually characterized by very low flows and almost no flow at all at the peak of the dry season. Many industrial effluents and agricultural and domestic wastes are discharged into this segment of the river. Physical developments are fast emerging in the floodplains within this reach especially around Zango, Angwan Muazu, Kakuri, and Kudenda industrial layout. The Kaduna South Waterworks at Barnawa with a

capacity of 35,000m^3/day (Plant Manager, Kaduna South Water works, March, 2013) is one of the two waterworks on the river in Kaduna. This study was carried out from a point upstream of the Kaduna South waterworks to 100 meters after the confluence of River Kaduna with the River Romi.

For the purpose of this study, there are five major drains that discharge into the River Kaduna. These are

a. Makera Drain: This drain contains effluents from textile mills and bottling companies (referred in this study as DQ).
b. Kakuri Drain: This drain contains effluents from textile mills and a fertilizer company (referred to as KK).
c. Nasarawa Drain: This drain consists only of domestic effluents (referred to as NS).
d. Rafin Juji Drain (Kudenda drain): This drain contains effluents from breweries, flour mills and glass and chemical plants (here referred to as KU).
e. Romi Drain: This drain contains effluents from Kaduna refinery, paper factory, Peugeot automobile plant, bottling and brewing companies (referred to as RM).

Map of the study area is shown in Figure 3.1

Sampling Points

The following sampling points were selected along the river for pollution studies:

1. Upstream of the Kaduna South Waterworks under the bridge at Kachia Road, near the Ahmadu Bello Stadium, referred to as WB. The point of sampling is close to the water

works intake. The water here has passed through built-up areas, cultivated land and motor mechanic workshops, but not industries.

2. Three points at the outfall of each of the above mentioned drains into River Kaduna as follows:

i. Ten metres before the outfall of the drains into the river
ii. Ten metres after the outfall of the drain into the river
iii. The drain just before it empties into the river

3. One hundred meters (100m) downstream of confluence of River Kaduna and Romi drain.

Geographical coordinates, (latitude (°N) and longitude (°E), of the sampling points were recorded at the commencement of field work using a Global Positioning System (GARMIN GPS 12XL) (Table 1).

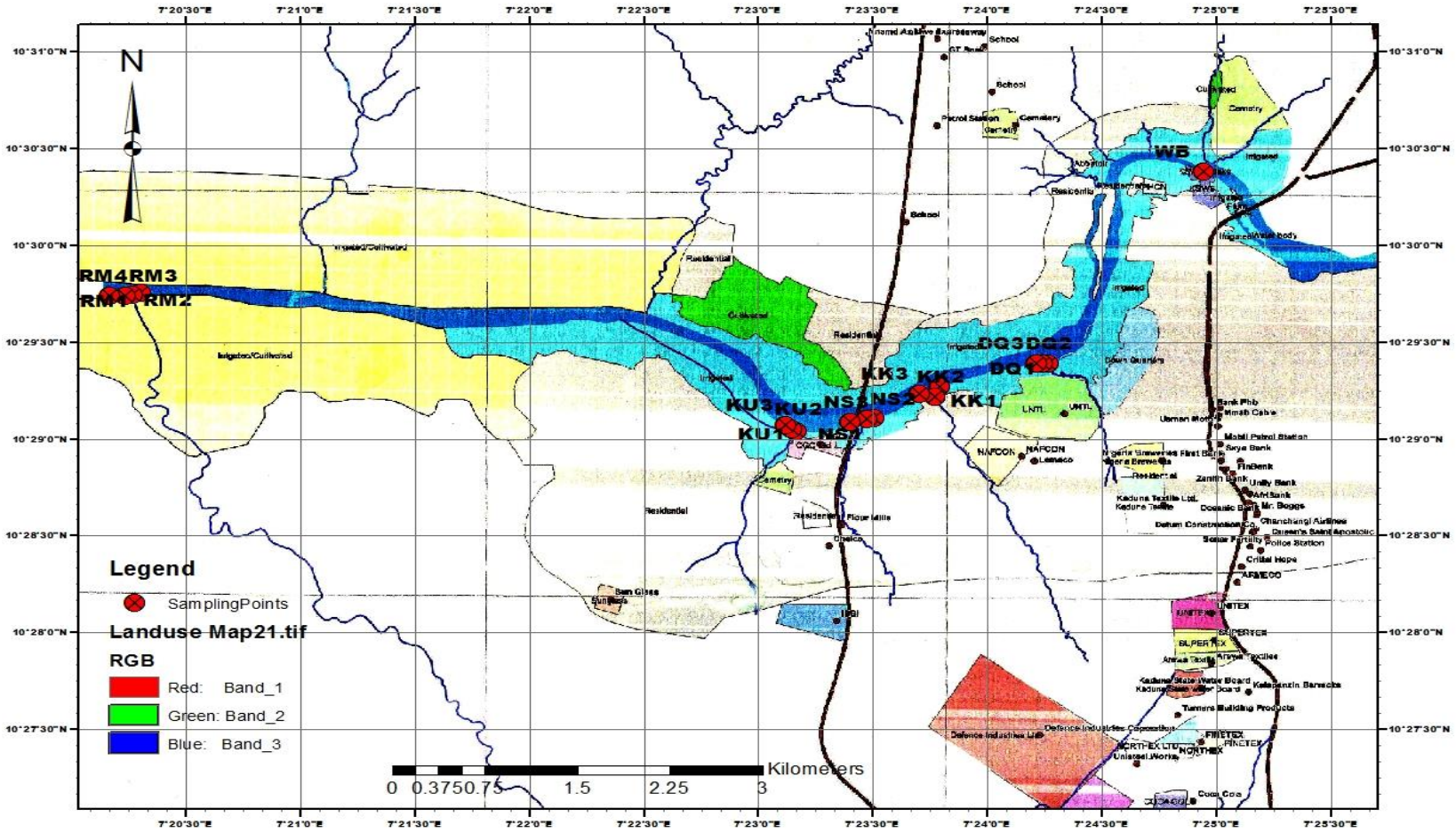


Figure 3.1: Map of the study area

WB=Waterboard (near Kaduna South waterworks intake); DQ=Makera drain; KK=Kakuri drain; NS=Nasarawa drain; KU=Kudenda drain; RM=River Romi

Table 3.1: Coordinates of the sampling points

Sampling point	Latitude (N)	Longitude (E)	Sampling point	Latitudde (N)	Longitude (N)
WB	10.30119	007.25122	NS3	10.28845	007.23568
DQ1	10.29121	007.24451	KU1	10.28845	007.23280
DQ2	10.29183	007.24445	KU2	10.28856	007.23238
DQ3	10.29204	007.24413	KU3	10.28875	007.23235
KK1	10.29030	007.23932	RM1	10.29482	007.20463
KK2	10.29031	007.23915	RM2	10.29442	007.20474
KK3	10.29018	007.23887	RM3	10.29477	007.20422
NS1	10.28866	007.23605	RM4	10.29472	007.20380
NS2	10.28855	007.23599			

3.3 Collection of Samples

Water samples were collected monthly from seventeen points from November, 2014 to October, 2015 covering both rainy and dry seasons. Sampling, preservation and storage were carried out according to standard methods (APHA, 1998). A total of 204 water samples were collected. The samples for bacteriological analyses were collected aseptically in pre-sterilized plastic bottles (sterilization was achieved using UV sterilization unit). One litre of water sample was collected in clean plastic containers for physicochemical analysis. Another one litre was collected for dissolved oxygen (DO) and biochemical oxygen demand (BOD) tests, approximately 100ml each for heavy metal and bacteriological analysis respectively (the sample for heavy metals analyses was acidified with 1ml concentrated NHO_3), 250ml in clean glass bottles for oil and grease determination, and 10 litres for *Cryptosporidium* investigations. The samples were transported in ice-packs to the Microbiology Laboratory of the National Water Resources Institute (NWRI), Kaduna.

Fish samples were bought from the fishermen at the river banks monthly from upstream and downstream, and conveyed to the NWRI laboratory in ice packs and stored overnight in the freezer. The fish samples were later taken to the Biological Sciences Department of Ahmadu Bello University (ABU), Zaria for identification and documentation. Five of the more commonly occurring fish samples were then selected for the analyses. Fifty-seven (57) fish samples were used for the study. Vegetable samples were bought at source directly from the farmers and placed in clean polythene bags, transported to the Water Quality Laboratory of the NWRI and stored in the refrigerator until analysed. The vegetable samples were obtained in clean polythene bags on alternate months from upstream and

downstream and transported to the laboratory in ice packed coolers. A total of fifty-six (56) vegetable samples were analyzed.

Digestion of all the samples was done in the Water Quality laboratory of NWRI while analysis for heavy metals using the Atomic Absorption Spectrophotometer (AAS) was carried out at the Multi–User Science Research Laboratory (MUSRI), ABU, Zaria.

3.3.1 Preparation of water samples for heavy metals analyses

Water samples (N=204) for heavy metals analyses were collected in plastic bottles, filtered through Whatman filter paper (No. 1) and then acidified to pH 1.5 with concentrated nitric acid (APHA, 1998). One hundred milliliters (100ml) was transferred to a 250ml beaker and 5ml concentrated nitric acid was added and then refluxed in a closed chamber for 30 minutes. This was allowed to cool and the clear supernatant transferred to a 100ml volumetric flask and the volume made up with distilled water. The concentration of the heavy metals in the digest was determined with the Atomic Absorption Spectrophotometer (Varian, model AA240FS) as follows:

The analysis began with selection and adjustments of various units of the machine (i.e. lamp selection, wavelength selection, slit adjustment and flame adjustment) and the machine was standardized by aspirating deionized water to get zero absorbance. A blank was prepared (a solution which contained no analyte element). Standard solutions (a series of calibration solutions containing known amounts of analyte element) were prepared from a stock solution (APHA, 1998). Next, the blank and standard solutions were atomized in turn and the response for each solution was measured. A calibration graph (absorbance vs. concentration) was plotted showing the response obtained for each solution. Thereafter, the

sample solution was atomized and the responsemeasured. The concentration of the sample was then determined from the calibration curve, based on the absorbance obtained for the unknown.

3.3.2 Preparation of fish samples for heavy metals analyses

Fish samples (N=57) were processed according to the method described by Agbozu *et al*. (2007). Whole samples of fish were oven dried and pulverized into fine textures. Two grams (2g) of oven-dried and pulverized samples were emulsified with 2ml deionized water in a digestion flask and digested with a mixture of 10 mL concentrated nitric acid and 2 mL concentrated perchloric acid. The contents of the flask were digested gently and slowly, by heating in a water bath till the contents were almost dry. It was then set aside to cool. The digestates were filtered into a 50 mL volumetric flask and made up to mark with distilled water. Aliquots of the filtrates were used to estimate the concentrations of the various metals by means of an Atomic Absorption Spectrophotometry (Varian, model AA240FS) as described for the water samples above.

3.3.3 Preparation of vegetable samples for heavy metals analyses

Edible portions of the vegetable samples were washed with distilled water and air dried. Digestion was carried out according to the method described by Akan *et al.* (2013) as follows: The vegetable samples were weighed to determine the fresh weights and dried in an oven at 80^{o}C for 72 hours to determine their dry weights. The dried samples were pulverized in a mortar and the resulting powder digested by weighing 0.5g of oven-dried ground and sieved (<1mm) powder into an acid-wash porcelain crucible and placing it in a muffle furnace at 500^{o}C for four hours. The crucibles were removed from the furnace and

cooled. Ten milliliters (10mL) of 6M HCl was added and covered and subjected to heat on a steam bath for 15 minutes. Another 1mL of HNO_3 was added and evaporated to dryness by continuous heating for 1h to dehydrate silica and completely digest organic compounds. Finally, 5 ml of 6M HCl and 10ml of water were added and the mixture was placed on a steam bath for complete dissolution. The mixture was cooled and filtered through a filter paper (Whatman no. 541) into a 50ml volumetric flask and made up to mark with distilled water. Determination of Pb, Cd, Zn and Cr was made directly on each final solution using Atomic Absorption Spectrophotometer (Varian, model AA240FS) as described for the water samples above.

3.4 Identification and detection of *Cryptosporidium* oocysts

3.4.1 Concentration of Cryptosporidium from water: Chemical Flocculation

Raw water samples (with high turbidity) were collected into 10 L containers and transported immediately to the laboratory to perform chemical flocculation for the recovery of *Cryptosporidium* oocysts in accordance with Vesey *et al*. (1993). Ten litres of the sample were mixed with 100 mL of 1 M calcium chloride ($CaCl_2$) and 100 mL of 1 M sodium hydrogen carbonate (NaHCO3) by continuous stirring on a magnetic stirrer. The pH was adjusted to 10 using approximately 10 mL of 1 M NaOH. The water sample was then allowed to rest undisturbed at room temperature overnight. (This enabled flocculation and the formation of a calcium carbonate precipitate. The precipitate adsorbs and pulls particulates in water including protozoa to the bottom of the container to form a dense, stable pellet within 4 hours). After this time, the supernatant was discarded by vacuum aspiration and the calcium carbonate floc residue was dissolved by adding 300 mL of 10% sulphamic acid, giving a concentrate containing the (oo)cysts. The floc was fully dissolved

when the effervescing stopped. Then the pH was re-adjusted to 6.5 using approximately 100 mL of 1 M NaOH. The dissolved flocculate was decanted into 0.5 L plastic centrifuge tubes. The container was rinsed twice with 200 ml (100 ml each time) of 0.1 % Tween 20. The rinses were added to the centrifuge bottles. The bottles were centrifuged at 2,500 rpm for 20 min, and then the supernatant was aspirated to just above the pellet. The pellet was resuspended in the remaining supernatant and pooled into a 50 mL Falcon centrifuge tube. The first centrifuge tube was rinsed into the second with 20ml0.1% Tween 20 and the sample centrifuged at 2,500 rpm again for 20 min (S70D centrifuge). The supernatant was aspirated just above the pellet and the pellet resuspended in the remaining supernatant. A portion (about 1/3 of the pellet volume) of the pellet was prepared for microscopic examination.

3.4.2 Preparation of Samples for coccidia detection using floatation method

The pellet for microscopic examination was placed in a universal bottle and a plastic rod was used to squash it. Five milliliters (5ml) of zinc sulphate/sucrose mixture (Hansen and Perry, 1994) was added and mixed well. The mixture was sieved through a mesh into a centrifuge tube and more of the floatation medium was added until a convex meniscus was formed. A cover slip was gently placed on the preparation and left for 3-5 min. The cover slip was removed and placed on a clean glass slide and examined for coccidia oocysts. About 2-3ml of the preparation was withdrawn from the topmost layer of every tube that had coccidia, transferred into a clean tube, washed twice (by centrifugation and decanting) and concentrated further.

3.4.3 Sample Staining by the Modified Ziehl-Neelsen (mZN) Method

A thin smear was prepared on a microslide by using two drops of the pellet from 3.4.2, air-dried, fixed with methanol for 5 minutes and stained by the modified Ziehl-Neelsen (mZN) technique (Casemore, 2004). The smears were stained with carbol-fuchsin (0.34% fuchsin and 45% w/v phenol) for 30 minutes and washed with distilled water. The smears were then decolorized with 1% hydrochloric acid-alcohol (70%) for one minute and counterstained with 1% methylene blue for one minute. The slides were rinsed, air-dried and examined microscopically with the oil immersion (x100) objectives.

3.5 Isolation and Biochemical Identification of *Escherichia coli* and *Escherichia coli* O157:H7

Isolation and identification of *E. coli* O157:H7 was carried out using the method described by LeJeune *et al.* (2001). Twenty milliliters (20ml) of water sample was mixed with equal volume of double strength tryptic soy broth (TSB) and incubated at 44.5^{o}C for 24 h. After the incubation period, 10-fold serial dilution of the broths was performed in TSB. About 0.2ml of the 10^{-3}dilution was spread on Eosin Methylene Blue agar plates and incubated at 44.5 ^{o}C for 24 h. All the colonies with characteristic greenish metallic sheen were purified and subjected to Gram-staining, and standard biochemical tests using Microgen^{TM} GnA-B-ID System according to the manufacturer's instructions. All confirmed *E. coli* isolates from the biochemical tests were screened on Sorbitol MacConkey agar plates (SMAC, OXOID, Basingstoke England) supplemented with cefixime-tellurite ($SMAC_{CT}$). After incubation at 37^{o}C for 24 h all non-sorbitol (colourless) colonies were recorded as presumptive aquatic *E. coli* O157:H7 (Singleton, 1997). Further confirmation of *E. coli* O157:H7 was achieved by

testing for agglutination with *E. coli* O157:H7 antisera (Microgen), according to the manufacturer's instructions.

3.5.1 Antibiotic Susceptibility Testing

The antibiotic susceptibility patterns of theisolates from the water samples which were colourless on $SMAC_{CT}$ and agglutinated with antiserum specific for *E. coli* O157:H7were determined by the disc diffusion method of Cheesbrough (2000). An overnight ($37^{o}C$ for 18 hr) culture of each isolate was prepared in a nutrient agar plate. Five mililiters (5 ml) of sterile physiological saline and 0.5 McFarland turbidity standard solutions were prepared for standardization of inocula. Using a sterile wire loop 3-5 distinct colonies of similar appearance from nutrient agar were emulsified with the 5ml sterile physiological saline. In a good light match the turbidity of the suspension was compared to the turbidity standard of 0.5 McFarland and the emulsified saline suspensions were then inoculated on Mueller Hinton agar at angle 60^{o} across the plate using a sterile cotton swab to ensure even distribution and confluent growth. The plates were allowed to dry for 5 minutes with the plates closed in a properly disinfected laminar flow cabinet, before placing the sensitivity discs of the various antibiotics aseptically (using sterile forceps) on the dried inoculated agar surface. The plates were then allowed 30 minutes pre-diffusion time on the bench before they were incubated at 37 ^{o}C for 18 hours. After overnight incubation, the plates were examined for the zones of inhibition. The zones were then measured. The result was interpreted using the interpretation criteria by Cheesbrough (2000). The isolates were reported as sensitive and resistant to the various antibiotics depending on the size of the zones of inhibition.

3.5.2 Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR Index was determined according to the method of Krumperman (1983) and Paul *et al.* (1997) by dividing the number of antibiotics to which the isolates were resistant, by the total number of antibiotics tested.

$$MARIndex = \frac{Number\ of\ antibiotics\ to\ which\ resistant}{Total\ number\ of\ antibiotics\ tested}$$

3.5.3 Molecular Analysis

Bacteria cell preparation: The preparation of the bacterial cells was carried out using the method described by Dubey (2009): The components of Luria and Bertani broth were compounded as follows; peptone (10 g), NaCl (5g), 1N NaOH (10 ml), yeast extract (5 g), distilled water (1litre), pH 7.0 adjusted with NaOH solution and sterilized at 121^{o}C for 15mins. Prior to this, plates of MacConkey agar inoculated with the isolates were incubated at 37^{o}C for 18-24 h. Single colonies were picked from the pure cultures on MacConkey agar plates, inoculated into 5ml Luria and Bertani (LB) broth and incubated at 37^{o}C for 18-24 h. Bacterial cells were then harvested into Eppendorff's tubes by centrifugation at 4^{o}C, 8000 rpm (6800 xg) in a refrigerated microcentrifuge for 30 seconds. The supernatants were decanted and the cells harvested.

Genomic DNA extraction: Genomic DNA extraction was carried out using the method described by DNeasy Blood and Tissue Handbook (2006). The harvested cell pellets were dislodged and 20 µl of proteinase K was added and mixed thoroughly by vortexing. The mixture was further incubated at 56^{o}C for 3hrs and vortexed occasionally using a thermo-mixer until the cells were completely lysed and properly mixed to prevent clogging of the

DNeasy Mini spin column. Two hundred microliter (200 µl) of buffer AL was added to the sample, and mixed thoroughly by vortexing. Two hundred microliter (200 µl) of absolute ethanol (96-100 %) was added and mixed again by vortexing to yield homogenous solution. This mixture was then pipetted into DNeasy Mini spin column placed in a 2 ml collection tube and centrifuged at 8000 rpm for 1 min. The flow-through and collection tube was then discarded. The DNeasy Mini spin column was then transferred into a new 2 ml collection tube and 500 µl of AW1 buffer was added and centrifuged again at 8000rpm for 1 min before the flow-through was also discarded. For the third time, DNeasy Mini spin column was placed in 2ml collection tube and 500 µl AW2 buffer was added and centrifuged at 14000 rpm for 3 min to dry the DNeasy membrane in other to remove the residual ethanol that might interfere with subsequent reactions. The flow-through and collection tube were removed and discarded carefully so that the column does not come into contact with the flow-through, resulting in carryover of ethanol. The used DNeasy Mini spin column was now placed in a clean 2 ml micro-centrifuge tube and 200 µl buffer AE was introduced onto the DNeasy membrane to elute, and increase the final DNA concentration and yield in the eluate. The column was then incubated at room temperature for 1min before being centrifuged at 8000 rpm for 1 min. The elution of the membrane was then repeated twice to ensure increased overall DNA yield in the same microcentrifuge tube.

Agarose gel electrophoresis: The eluates were subjected to agarose gel electrophoresis to ascertain that genomic DNA was actually extracted according to the protocol described by Lee *et al.* (2012). One percent (1 %) agarose gel was used to resolve the genomic DNA. The agarose gel was prepared by combining l g of agarose in ten times concentration (10x)

of Tris acetate ethylene diamine tetra acetate (2ml 10x TAE) buffer and 98ml distilled water in a 250 ml beaker flask and subjected to heat in a microwave for 2 minutes until the agarose was dissolved. Twenty microliter (20 µl) of 5mg/ml ethidium bromide was added to the dissolved agarose solution as dye and mixed. The gel was then poured onto a mini horizontal gel electrophoresis tank and the casting combs inserted at the red bands to ensure easy view of the well, while filling the genomic DNA. It was then allowed to gel for 30 minutes.

The casting combs were carefully removed after the gel was completely formed. One time concentration (1x) TAE electrophoresis buffer was then added to the reservoir until the buffer covered the agarose gel. Five microliter (5 µl) of gel tracking dye (bromophenol blue) was added to 15 µl of each sample with gentle mixing. Twenty microliter (20 µl) of the sample was then loaded onto the wells of the gel. The mini horizontal electrophoresis gel each was then covered and the electrodes connected running from cathode (-) to anode (+). Electrophoresis was carried out at 100mV for 45 minutes to allow easy separation of sample based on molecular weight. At the completion of the electrophoresis, the gel was removed from the buffer (Tris) and the gel viewed under a trans-illuminator UV light of wavelength 302 nm. The band pattern of the DNA fragments was then photographed with a Polaroid camera and documented using an electrophoresis gel documentation system. Electrophoresis was employed to identify the number of plasmid copies present in different isolates. However, standard DNA molecular weight marker (1 kb) was used to estimate the genomic DNA size.

Polymerase Chain Reaction (PCR): Amplification of resistant DNA fragments was carried out using Dream Taq™ DNA polymerase, which is an enhanced multiplex PCR *Taq* DNA polymerase, optimized for all standard PCR applications as described in DNeasy Blood and Tissue Handbook (2006). Dream Taq™ PCR master mix (2x) was vortexed (for 30 sec) and centrifuged for 30 seconds at 8000 rpm after thawing. The thin walled PCR tube was then placed on an ice pack and the following components were added for each isolate for single reaction: (a) Dream Taq™ PCR master mix (b) Forward primers (c) reverse primers (d) template DNA, Taq buffer, dNTP (e) the nuclease – free water. The samples were vortexed gently and spinned down. The primers used for PCR detection of the antibiotic resistance genes and virulence genes (VGs) and other relevant information are listed in Tables 3.2 and 3.3.

DNA Sequencing: Genomic DNA sequencing of the *E. coli* O157:H7 isolates was carried out using the Sanger *et al.* (1973) method. PCR was carried out using a cocktail mixture [3.5µl of 2x master mix from Promega Company, 0.5 µl of 5 pMol forward primer (AGAGTTTGATCMTGGCTCAG) of 16S rRNA and 5pMol reverse primer (AAGGAGGTGWTCCARCCGCA) of 16S rRNA, 3 µl of 25 ng/µl of the *E. coli* O157:H7 extracted DNA and 2.5 µl of H_2O] to form a 10 µl PCR cocktail. The following PCR conditions were used: initial denaturation period, 94^oC for 5min; denaturation period, 94^oC for 30 sec.; annealing temperature, 56^oC for 30 sec.; extension period, 72^oC for 45 sec., at 36 circle times; final extension period of 72^oC for 7 min and holding temperature of 10^oC (Table 3.3). The amplicon (1500 bp) from the reaction was compared with a standard 100bp plus ladder from Invitrogen Company; and was observed on 1.5 % agarose gel. The PCR

product was further purified using alcohol purification protocol. Twenty microliters (20 µl) of absolute ethanol was added to each of the PCR products and incubated at room temperature for 15 minutes and later spinned down at 10,000 rpm for 15minutes. The supernatant was decanted and 40 µl of 70 % ethanol added and votexed for 5 min, before the supernatant was decanted for the second time.

The pellet was air dried and 10 µl of sterile distilled water was added before the amplicon was observed on 1.5 % agarose. The product from the purification process was loaded onto a 3130xl genetic analyzer from Applied Biosystems to give the sequences. The results were evaluated using Geneious software.

3.6 Analysis of data

All descriptive statistical analyses (Mean, standard deviation) were computed using Statistical Analysis System Software (S.A.S 9.0, 2008). The interaction between season and type was analysed using a factorial layout. The Tukey's procedure for mean comparison was used to rank the means after a significant effect ($P<0.05$) was observed.

Table 3.2:Primers for antibiotic resistance genes

S/N	Primer name	Sequence (5′→3′)	Target gene(s) or region	PCR product size (bp)	Annealing temp (°C)	References	Antibiotics Resistance To
1	TEM-F	ATTCTTGAAGACGAAAGGGC	bla_{TEM}	1,150	60	**Belaaouaj *et al.*, (1994)**	Beta-lactams
	TEM-R	ACGCTCAGTGGAACGAAAAC					
2	SHV-F	CACTCAAGGATGTATTGTG	bla_{SHV}	885	52	**Pitout *et al.*, (1998)**	
	SHV-R	TTAGCGTTGCCAGTGCTCG					Cephalosporins,
4	CTX-M-F	CGCTGTTGTTAGGAAGTGTG	DQ303459	569	60	Bali *et al.*, (2010)	
	CTX-M-R	GGCTGGGTGAAGTAAGTGAC					
6	TetA-F	GTAATTCTGAGCACTGTCGC	*TetA*	937	62	**Guardabassi *et al.*, (2000).**	Tetracycline
	TetA-R	CTGCCTGGACAACATTGCTT					
7	TetB-F	CTCAGTATTCCAAGCCTTTG	*TetB*	416	57		
	TetB-R	CTAAGCACTTGTCTCCTGTT				**Guardabassi *et al.*, (2000).**	
9	DfrXII-F	GGTGSGCAGAAGATTTTTCGC	*dfrA12*, *dfrA13*	319	60	**Navia *et al.*, (2003)**	
	DfrXII-R	TGGGAAGAAGGCGTCACCCTC					
10	Sul-F	TGGTGACGGTGTTCGGCATTC	*sul1*	789	63	**Mazel** *et al.*, (2000)	Sulphomethonidaz ole
	Sul-R	GCGAGGGTTTCCGAGAAGGTG					
11	Sul2-F	CGGCATCGTCAACATAACC	*sul2*	722	50	**Maynard *et al.*, (2003)**	
	Sul2-R	GTGTGCGGATGAAGTCAG					

Table 3.3**:** Primers for Virulence Genes

S/N	Primer	Forward	Reverse	References
1	Stx2 (482bp)	TTCTTCGGTATCCTATTCCC	ATGCATCTCTGGTCATTGTA	Olsvik and Strockbine (1993)
2	Stx1 (513bp)	CAGTTAATGTGGTGGCGAAG	CTGTCACAGTAACAAACCGT	Olsvik and Strockbine (1993)

CHAPTER FOUR

4.0 RESULTS

4.1 Physicochemical and faecal coliforms analysis

Results of physicochemical and faecal coliforms analysis are presented in Appendices II and III, respectively, only as background information, while monthly concentration of heavy metals are presented in appendix IV.

4.2 Heavy metals

4.2.1 Heavy Metal Contamination of the Water Samples

Table 4.1 presents the mean heavy metal concentrations in water samples at the different sampling points. The levels of lead (Pb) and cadmium (Cd) showed no significant statistical differences ($P \geq 0.05$) between the sampling points. The mean concentrations of zinc (Zn) and chromium (Cr) were significantly statistically different ($P \leq 0.05$) between the sampling points. Mean levels of Zn ranged from 0.007 to 0.269 mg/l. The highest concentrations were observed at KK2 (0.269 mg/l) followed by KU2 (0.267 mg/l) represented on Fig. 4.1 as NAF2 and KUD2 respectively. These differ significantly ($P \leq 0.05$) from the rest of the sampling points. On the other hand, the lowest value was obtained at 100m downstream (0.007 mg/l).

High Cr levels were recorded at KU1, 2 and 3 (0.212mg/l, 0.240mg/l and 0.260mg/l respectively), KK2 (0.251mg/l) and RM2 (0.275mg/l). They differed significantly ($P \leq 0.05$) from concentrations at all the other sampling points. The lowest concentration of Cr was recorded at Wb and DQ1 (0.058mg/l and 0.079mg/l respectively). Some guideline values

for water for various purposes are included in Table 4.1 for comparison with the mean values obtained in this study.

Comparison of heavy metal concentrations at the main sampling locations (Table 4.2) revealed significant statistical different ($P \leq 0.05$) values at the different locations. The highest value of Pb obtained at Romi, RM, (0.25mg/l) was highly statistically different ($P \leq 0.01$) from those at DQ, KU, KK, and NS which were similar (0.17-0.18mg/l) and the lowest value which was obtained at WB (0.15). High values of Cr were recorded at KU (0.26mg/l), RM (0.23mg/l) and NS (0.21mg/l). They differed significantly ($P \leq 0.05$) from values at KK (0.16mg/l), DQ (0.15mg/l) and WB (0.07mg/l).

The concentrations of metals in the water samples from the sampling locations based on seasonal variation are presented in Table 4.3. The results indicate that Cr and Pb differ significantly ($P \leq 0.05$) during the wet and dry seasons. The levels (mg/l) of both metals were higher during the dry season (Cr 0.22 ± 0.02; Pb 0.22 ± 0.02) than the wet season (Cr 0.11 ± 0.02; Pb 0.13 ± 0.01) respectively.

Table 4.1: Mean heavy metal concentration in water samples at the different sampling points

		Heavy metal (mg/l) Mean±SE			
Sampling points	N	Pb	Cd	Zn	Cr
DQ1	12	0.139±0.029	0.017±0.003	0.040±0.008^{c}	0.079±0.027^{c}
DQ2	12	0.193±0.032	0.017±0.003	0.163±0.024^{b}	0.140±0.024^{b}
DQ3	12	0.180±0.035	0.016±0.003	0.043±0.006^{c}	0.116±0.028^{b}
KU1	12	0.251±0.046	0.016±0.003	0.023±0.006^{c}	0.212±0.055^{a}
KU2	12	0.272±0.043	0.018±0.003	0.267±0.074^{a}	0.240±0.051^{a}
KU3	12	0.216±0.044	0.017±0.028	0.091±0.028^{b}	0.260±0.060^{a}
KK1	12	0.165±0.037	0.015±0.003	0.021±0.0039^{c}	0.121±0.028^{b}
KK2	12	0.222±0.038	0.017±0.025	0.269±0.047^{a}	0.251±0.063^{a}
KK3	12	0.211±0.039	0.017±0.029	0.022±0.002^{c}	0.106±0.027^{b}
NS1	12	0.223±0.04	0.014±0.028	0.068±0.024^{b}	0.170±0.034^{b}
NS2	12	0.229±0.044	0.016±0.035	0.174±0.046^{b}	0.152±0.051^{b}
NS3	12	0.238±0.045	0.015±0.003	0.031±0.016^{c}	0.175±0.076^{b}
RM1	12	0.210±0.055	0.015±0.029	0.027±0.004^{c}	0.147±0.049^{b}
RM2	12	0.206±0.058	0.016±0.003	0.060±0.012^{c}	0.275±0.055^{a}
RM3	12	0.196±0.057	0.017±0.003	0.012±0.002^{d}	0.184±0.049^{b}
100m	12	0.205±0.060	0.017±0.003	0.007±0.001^{d}	0.118±0.057^{b}
WB	12	0.178±0.034	0.022±0.005	0.05±0.009^{c}	0.058±0.017^{c}
P-value		0.08	0.46	0.03	0.01
LOS		NS	NS	*	*
FAO (1985)		5.0	0.01	2.0	0.1
FMEnv (1991)		<1	<1	<1	<1

^{a,b,c}=Means under the same trait with different superscripts differ significantly (P<0.05); LOS-Level of significance; NS-Not significant; WB, DQ, KU, KK, NS, RM, 100m-Sampling locations; 1,2,3-Sampling points within each sampling location; N-Number of samples;

WB= close to Kaduna South Water Board intake; DQ= Down Quarter (Makera area); KK=Kakuri drain; NS=Nasarawa drain; KU=Rafin Juji drain (Kudenda); RM=Romi drain; 1 and 3=Ten metres before and after the discharge of contents of the drain into River Kaduna, respectively; 2=the drain just before it empties into the river; 100m=100m downstream after Rivers Kaduna/Romi confluence; *=significant

Table 4.2: Mean heavy metal concentration in water samples at the locations (pooled results)

		Heavy metal (mg/l) Mean±SE			
Parameters	N	Pb	Cd	Zn	Cr
DQ	36	0.17 ± 0.02^{b}	0.02 ± 0.005^{a}	0.08 ± 0.01^{a}	0.15 ± 0.02^{b}
KU	36	0.19 ± 0.04^{b}	0.001 ± 0.0001^{b}	0.09 ± 0.03^{a}	0.26 ± 0.05^{a}
KK	36	0.18 ± 0.03^{b}	0.02 ± 0.004^{a}	0.01 ± 0.03^{c}	0.16 ± 0.04^{b}
NS	36	0.18 ± 0.02^{b}	0.02 ± 0.003^{a}	0.12 ± 0.03^{a}	0.21 ± 0.06^{a}
RM	36	0.25 ± 0.06^{a}	0.02 ± 0.002^{a}	0.07 ± 0.02^{b}	0.23 ± 0.05^{a}
100m	12	0.22 ± 0.09^{a}	0.019 ± 0.006^{a}	0.04 ± 0.002^{c}	0.04 ± 0.002^{c}
WB	12	0.15 ± 0.03^{c}	0.02 ± 0.004^{a}	0.06 ± 0.009^{b}	0.07 ± 0.01^{c}
P-value		0.007	0.01	0.008	0.004
Los		**	**	**	**

^{a,b,c}- Means under the same trait with different superscripts differ significantly ($P<0.05$); Los-Level of significance; WB, DQ, KU, KK, NS, RM -Sampling locations; N-Number of samples; **-Highly significant
WB= close to Kaduna South Water Board intake; DQ= Down Quarter (Makera area); KK=Kakuri area; NS=Nasarawa bridge; KU=Kudenda; RM=Romi; 1 and 3=Ten metres before and after the discharge of contents of the drain into River Kaduna, respectively; 2=the drain just before it empties into the river; 100m4=100m after Rivers Kaduna-Romi confluence.

Table 4.3: Seasonal variation of heavy metals concentration of the sampling locations of the river

Heavy metal (mg/l)			p-value	Los
Mean±SE	Season	Wet		
	Dry			
Cr	0.22±0.02[a]	0.11±0.02[b]	0.003	***
Pb	0.22±0.02[a]	0.13±0.01[b]	0.002	***
Cd	0.02±0.002	0.02±0.002	0.84	NS
Zn	0.08±0.01	0.09±0.02	0.62	NS

[a,b]-Means under the same trait with different superscripts differ significantly (P<0.05); Los-Level of significance; NS-Not significant; ***-Highly significant

4.2.2 Contamination of Vegetable Samples

Some leafy vegetables grown along the Kaduna river banks were tested for their heavy metals levels. ANOVA of the mean concentrations of these metals in the vegetables revealed significant statistical differences ($P \leq 0.05$) among the metals in the vegetable samples. Chromium was detected in all the vegetable samples, the highest values ((0.94 mg/kg) being recorded in amaranthus followed by jute (0.93 mg/kg), (0.80 mg/kg) in lettuce and (0.72 mg/kg) in cabbage and cucumber, respectively. Conversely, cucumber (0.77 mg/kg) and cabbage (0.74 mg/kg) had higher concentrations of Pb, compared with jute (0.43mg/kg), amaranthus (0.18mg/kg) and lettuce (0.14mg/kg). Lettuce had the higher concentration of Cd than cabbage (0.05mg/kg), cucumber (0.02mg/kg), jute (0.03mg/kg) and amaranthus (0.04mg/kg). Zinc levels in the samples were significantly different ($P \leq 0.001$) from one another. The highest level was recorded in amaranthus (0.75mg/kg) and the lowest in cabbage (0.18mg/kg) as shown in Table 4.4.

The mean heavy metal concentration of the vegetable samples based on the location they were propagated (whether upstream or downstream) revealed that Cr and Pb levels were highly significantly different ($P \leq 0.05$) in the vegetables while Cd and Zn showed no significant difference ($P \geq 0.05$). Higher Cr levels were obtained at the downstream sampling sites with respect to all the vegetables tested as shown in Table 4.5. Lead was higher in jute obtained at the upstream than the downstream sampling sites.

Table 4.4: Comparison of mean heavy metal concentration in some vegetables cultivated along the banks of River Kaduna

		Heavy metal (mg/kg) Mean±SE			
Vegetable	N	Cr	Pb	Cd	Zn
Cabbage	10	0.72 ± 0.004^{c}	0.70 ± 0.04^{a}	0.05 ± 0.02^{b}	0.18 ± 0.01^{d}
Cucumber	10	0.72 ± 0.001^{c}	0.77 ± 0.01^{a}	0.02 ± 0.005^{c}	0.60 ± 0.02^{b}
Jute	12	0.93 ± 0.02^{a}	0.43 ± 0.06^{b}	0.03 ± 0.002^{c}	0.39 ± 0.03^{c}
Lettuce	12	0.80 ± 0.05^{b}	0.14 ± 0.008^{c}	0.11 ± 0.05^{a}	0.45 ± 0.04^{c}
Amaranthus	12	0.94 ± 0.01^{a}	0.18 ± 0.006^{c}	0.04 ± 0.0009^{b}	0.75 ± 0.09^{a}
p-value		0.0001	0.01	0.01	0.001
Los		**	*	*	**
FAO/WHO (2011)		2.3	0.3	0.2	20

[a,b,c]-Means under the same trait with different superscripts differ significantly ($P<0.05$); Los-Level of significance; N-Number of samples; *-significant; **-Highly significant

Table 4.5: Spatial variation of heavy metal concentration in vegetable samples

		Heavy metal (mg/kg) Mean±SE			
Vegetable	Location	Cr	Pb	Cd	Zn
Cabbage	US	0.71^{c}±0.003	0.70^{a}±0.06	0.06±0.003	0.23±0.004
	DS	0.73^{b}±0.006	0.70^{a}±0.07	0.05±0.02	0.13±0.004
Cucumber	US	0.69^{c}±0.02	0.76^{a}±0.02	0.02±0.002	0.61±0.02
	DS	0.75^{b}±0.009	0.79^{a}±0.01	0.03±0.01	0.58±0.03
Jute	US	0.86^{b}±0.02	0.66^{b}±0.08	0.02±0.004	0.42±0.06
	DS	1.00^{a}±0.07	0.20^{c}±0.009	0.03±0.0006	0.35±0.02
Lettuce	US	0.69^{c}±0.08	0.12^{d}±0.003	0.03±0.0006	0.46±0.04
	DS	0.92^{a}±0.02	0.16^{c}±0.01	0.19±0.10	0.44±0.07
Amaranthus	US	0.91^{a}±0.02	0.15^{c}±0.006	0.04±0.001	0.88±0.08
	DS	0.97^{a}±0.01	0.20^{c}±0.004	0.04±0.001	0.63±0.17
p-values		0.008	0.001	0.08	0.47
Los		***	***	NS	NS

[a,b,c]-Means under the same trait with different superscripts differ significantly (P<0.05); Los-Level of significance; ***-Highly significant, NS-Not significant, Los-Level of significance; US-Upstream; DS-Downstream

The seasonal variations in the mean values of heavy metals in vegetable samples are presented in Table 4.6. Generally, significant differences were not observed in the concentration of the metals during the dry and wet seasons except for Cr and Pb which were higher in the wet season for jute and cabbage, respectively.

4.2.3 Contamination levels of heavy metals in fish samples

Fish samples obtained from different segments of the river were purchased directly from the fishermen and tested for the presence of heavy metals. All fish samples tested contained the metals in varying concentrations (Table 4.7). With the exception of Cd, the mean concentrations of the metals were significantly different ($P \leq 0.05$) among the fish samples. Chromium levels in *Bagrus bayad* (0.99 mg/kg) and *Oreochromis niloticus* (0.93 mg/kg) were higher than in *Synodontis vermiculatus* (0.88 mg/kg) and *Labeo senegalensis* (0.81 mg/kg). The lowest concentration of Cr was recorded in *Clarias angularis* (0.70 mg/kg).

Lead levels in *O. niloticus* (0.39 mg/kg) and *L. senegalensis* (0.35 mg/kg) were not significantly different from each other but were higher than the levels found in *S. vermiculatus* (0.20 mg/kg), *C. angularis* (0.16 mg/kg) and *B. bayad* (0.14 mg/kg) whose values were also not significantly different from each other. There was no significant difference in the level of Zn found in *O. niloticus*, *L. senegalensis* and *B. bayad* with concentrations between 1.95 and 2.53 mg/kg; but lower concentrations were observed in *S. vermiculatus* (1.06 mg/kg) and *C. angularis* (1.25 mg/kg) which also did not differ significantly from each other. The results are presented in Table 4.7.

Table 4.6: Seasonal variation of heavy metal concentration in vegetable samples from River Kaduna

		Heavy metal (mg/kg) Mean±SE			
Vegetable	Season	Cr	Pb	Cd	Zn
Cabbage	DRY	0.722±0.006[c]	0.655±0.074[b]	0.078±0.029	0.179±0.05
	WET	0.724±0.005[c]	0.764±0.053[a]	0.021±0.002	0.187±0.018
Cucumber	DRY	0.722±0.020[c]	0.774±0.012[a]	0.016±0.004	0.587±0.003
	WET	0.717±0.026[c]	0.778±0.019[a]	0.035±0.013	0.614±0.017
Jute	DRY	0.889±0.019[b]	0.409±0.097[c]	0.025±0.003	0.361±0.043
	WET	0.978±0.102[a]	0.449±0.077[c]	0.028±0.0003	0.411±0.042
Lettuce	DRY	0.857±0.016[b]	0.149±0.011[d]	0.185±0.101	0.314±0.048
	WET	0.749±0.093[c]	0.138±0.013[d]	0.035±0.016	0.591±0.015
Amaranthus	DRY	0.948±0.021[a]	0.185±0.009[d]	0.036±0.002	0.807±0.597
	WET	0.931±0.016[a]	0.174±0.009[d]	0.037±0.001	0.699±0.08
p-values		0.008	0.001	0.08	0.47
Los		**	**	NS	NS

[a,b,c]-Means under the same trait with different superscripts differ significantly (P<0.05); **-Highly significant; NS-Not significant, Los-Level of significance

Table 4.7: Heavy metal concentration of fish samples caught in River Kaduna

		Heavy metal (mg/kg) Mean±SE			
Fish species	N	Cr	Pb	Cd	Zn
Bagrus bayad	11	0.99a±0.06[a]	0.14b±0.02[b]	0.03±0.003	1.95a±0.34[a]
Oreochromis niloticus	12	0.93a±0.08[a]	0.39a±0.01[a]	0.03±0.003	2.53a±0.45[a]
Synodontis vermiculatus	12	0.88b±0.06[b]	0.20b±0.07[b]	0.03±0.003	1.06b±0.21[b]
Labeo senegalensis	11	0.81b±0.09[b]	0.35a±0.12[a]	0.02±0.004	2.19a±0.39[a]
Clarias angularis	11	0.70b±0.09[b]	0.16b±0.01[b]	0.04±0.007	1.25b±0.21[b]
p-value		0.05	0.02	0.09	0.03
Los		*	*	NS	*
FAO (1983)		12-13[ɪ]	0.5	0.5	30

[a,b]-Means under the same trait with different superscripts differ significantly (P<0.05); *-significant, Los-Level of significance; NS-Not significant; N-Number of samples; [ɪ]-US FDA (1993a).

The results of the metal analysis of the fish samples based on the location they were caught show that Pb and Cd concentrations were significantly different statistically ($P \leq 0.05$) for some of the fish samples while Cr and Zn showed no significant difference (Table 4.8). Levels of Pb were higher in the upstream samples of *O. niloticus* and *S. vermiculatus* but showed no difference at the locations for *B. bayad*, *L. senegalensis* and *C. angularis*. Cadmium concentration, on the other hand was higher at the downstream location for *L. senegalensis* as indicated in Table 4.8.

Results of the analysis of seasonal variations in the heavy metal contamination of the fish samples using student's t-test are presented in Table 4.9. Significant statistical differences were recorded in the concentrations of Pb in some of the fish samples during the dry and wet seasons. The metal concentrations in the fish species were in the order Zn>Cr>Pb>Cd. Levels of Pb were higher in *Oreochromis niloticus* and *Labeo senegalensis* during the wet season than the dry season, and slightly higher in the dry season for *S. vermiculatus* and *C. angularis*. Lead concentration in *B. bayad* was similar for both seasons.

4.2.4 Constellation Plot of the sampling points

Figure 4.1 shows the constellation plot of the spatial structure of sampling points. Three major memberships were formed: 1st membership includes WB, DQ3, NAS3, NAF1 and NAF3; 2nd include DQ1, KUD1, NAS1, DQ2, KUD3, ROM2, ROM1, ROM3 and ROM4; while the 3rd group included NAF2, NAS2 and KUD2.

4.3 Prevalence of *Cryptosporidium* oocysts in water samples from Kaduna River

Cryptosporidium oocysts were detected in water samples from some of the sampling sites. Of the 204 water samples analyzed 22 (10.78%) showed presence of coccidian oocysts with

the wet mount out of which 7 (3.4%) had oocysts of *Cryptosporidium* species (Fig. 4.2). The result indicated that apart from the WB location, coccidia were found in some of the water samples obtained from all the locations.

Table 4.10 shows the incidence of oocysts in the water samples from the various locations. *Cryptosporidium* oocysts were identified in 2 (0.98%) water samples each from DQ and KK, with the highest number of samples, 3(1.47%) containing oocysts obtained at RM. No oocysts were identified at WB, NS and KU sites.

The result also showed that in relation to the period of sample collection, all the oocysts, 7(100%) were recovered in the rainy season, specifically five (5) in the month of May 2015 and two (2) in October 2015 (Fig. 4.3).

Table 4.8: Spatial variation in the mean heavy metal concentration of the fish samples

		Heavy metal (mg/kg) Mean±SE			
Fish species	Location	Cr	Pb	Cd	Zn
Bagrus bayad	US	0.96±0.09	0.16±0.03[c]	0.06b±0.003	1.89±0.44
	DS	1.03±0.09	0.13±0.03[c]	0.05b±0.02	1.99±0.54
Oreochromis niloticus	US	0.77±0.11	0.49±0.19[a]	0.02c±0.002	2.48±0.68
	DS	1.08±0.08	0.29±0.06[b]	0.03b±0.01	2.57±0.66
Synodontis vermiculatus	US	0.83±0.12	0.24±0.14[b]	0.02c±0.004	1.11±0.37
	DS	0.93±0.02	0.16±0.02[c]	0.03b±0.0006	1.00±0.24
Labeo senegalensis	US	0.63±0.13	0.39±0.18[b]	0.03b±0.0006	2.39±0.70
	DS	1.01±0.07	0.30±0.15[b]	0.19a±0.10	1.96±0.27
Clarias angularis	US	0.59±0.15	0.16±0.02[c]	0.04b±0.001	1.40±0.41
	DS	0.79±0.11	0.16±0.02[c]	0.04b±0.001	1.13±0.20
p-values		0.59	0.03	0.02	0.98
Los		NS	*	*	NS

[A,b,c] -Means under the same trait with different superscripts differ significantly (P<0.05); *-significant; Los-Level of significance; NS-Not significant; US-Upstream; DS-Downstream

Table 4.9: Seasonal variation of mean heavy metal concentrations in fish samples from River Kaduna

		Heavy metal (mg/kg) Mean±SE			
Fish	Season	Cr	Pb	Cd	Zn
Bagrus bayad	DRY	0.93±0.07	0.15±0.04[c]	0.04±0.002	2.26±0.62
	WET	1.05±0.10	0.14±0.02[c]	0.03±0.005	1.68±0.37
Synodontis vermiculatus	DRY	0.84±0.04	0.25±0.14[c]	0.03±0.005	1.07±0.23
	WET	0.92±0.12	0.15±0.03[d]	0.04±0.002	1.04±0.38
Clarias angularis	DRY	0.78±0.12	0.17±0.01[c]	0.04±0.002	1.19±0.22
	WET	0.63±0.14	0.16±0.02[d]	0.04±0.003	0.86±035
Oreochromis niloticus	DRY	1.01±0.10	0.35±0.15[b]	0.03±0.005	2.71±0.76
	WET	0.84±0.12	0.43±0.14[a]	0.03±0.003	2.34±0.55
Labeo senegalensis	DRY	0.93±0.08	0.26±0.14[c]	0.02±0.006	2.63±0.56
	WET	0.65±0.17	0.45±0.20[a]	0.02±0.006	1.67±0.46
p-values		0.32	0.02	0.92	0.98
Los		NS	*	NS	NS

[a,b,c]-Means under the same trait with different superscripts differ significantly (P<0.05); *-Significant; Los-Level of significance; NS-Not significant;

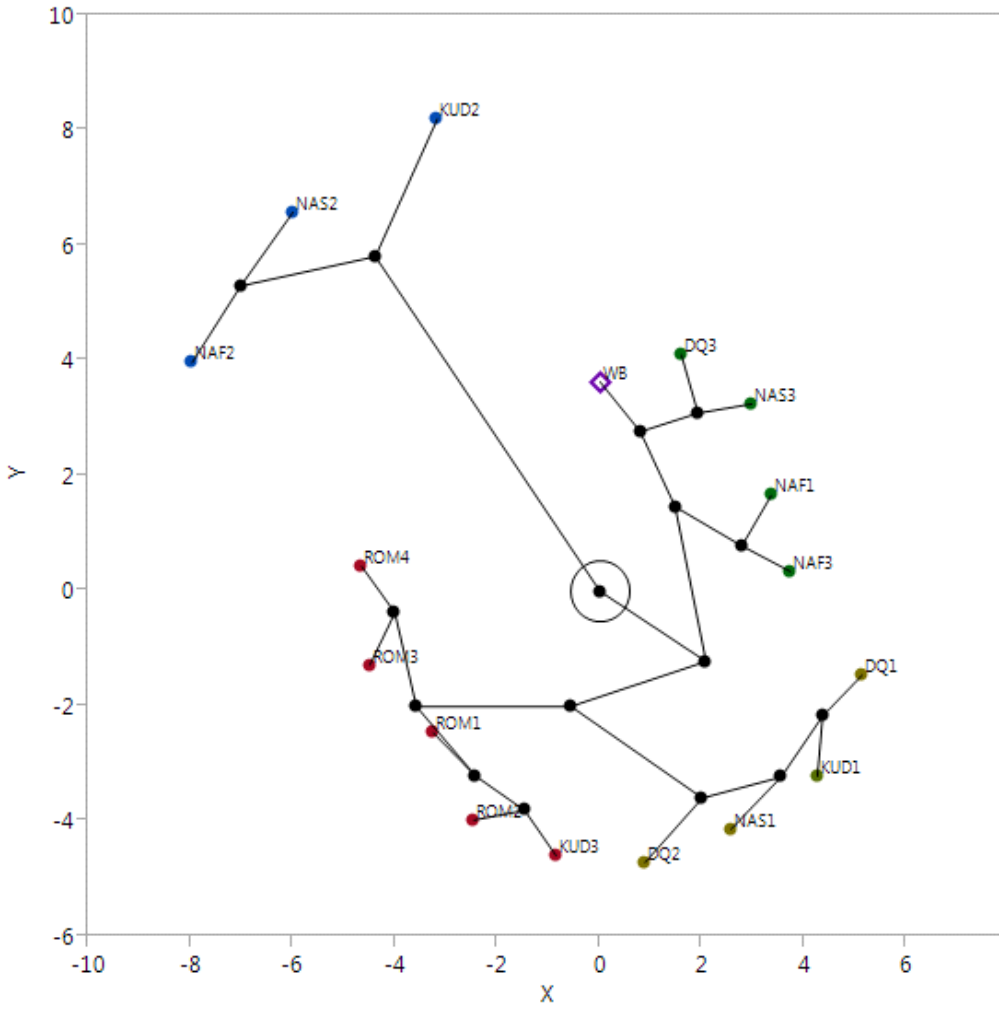


Figure 4.1: Constellation Plot of the sampling points

Key: WB=close to Kaduna South Water Board intake; DQ= Down Quarter (Makera area); NAF-(KK)Kakuri drain; NAS- (NS)Nasarawa drain; KUD- (KU) Rafin Juji drain (Kudenda); ROM- (RM)Romi drain; 1 and 3=Ten metres before and after the discharge of contents of the drain into River Kaduna, respectively; 2-the drain just before it empties into the river; 100m1-00m downstream after Rivers Kaduna/Romi confluence

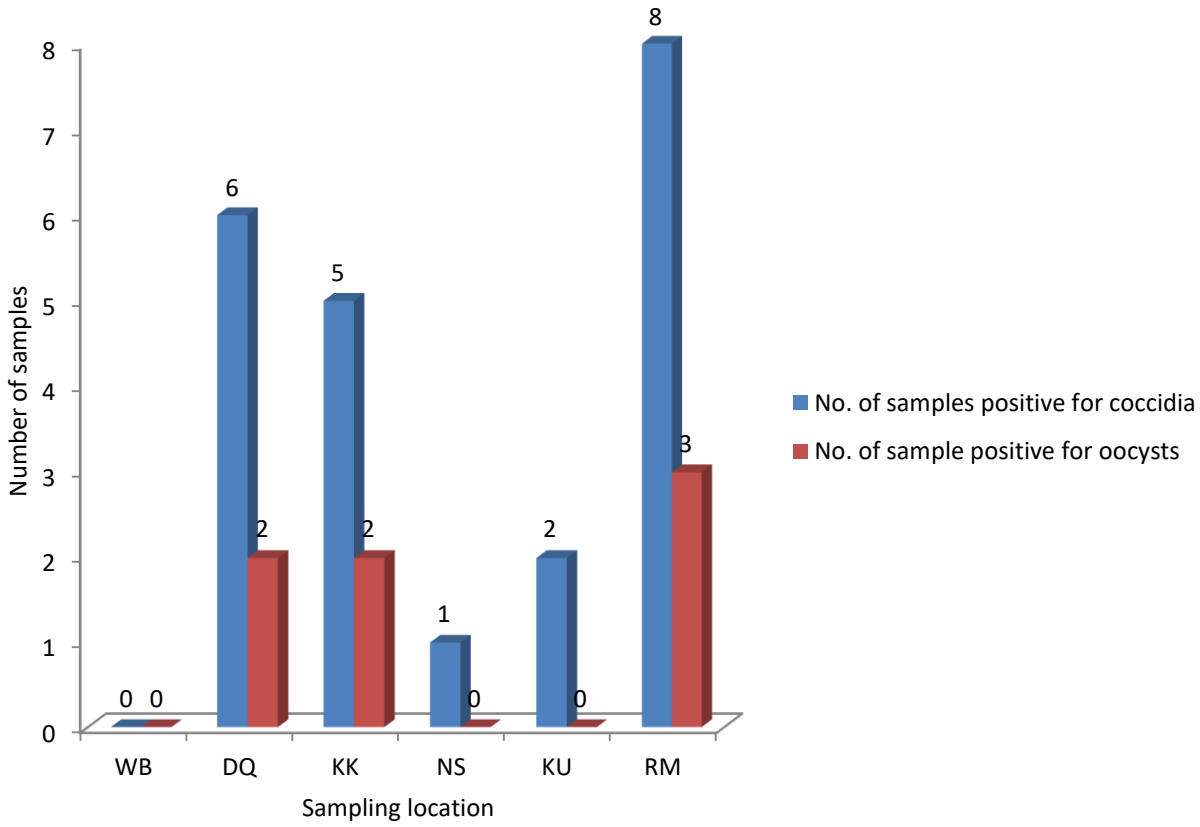


Fig. 4.2: Incidence of coccidia and *Cryptosporidium* oocysts in water samples obtained from the different sampling locations

Key: WB-Waterboard; DQ-Downquarter; KK-Makera area; NS-Nasarawa; KU-Kudenda; RM-Romi

Table4.10: Incidence of *Cryptosporidium* oocysts in water samples obtained from the different sampling locations

Sampling location	No. of samples positive for oocysts	Percentage positive
WB	0	0
DQ	2	0.98
KK	2	0.98
NS	0	0
KU	0	0
RM	3	1.47
Total	**7**	**3.43**

Key: WB-Waterboard; DQ-Downquarter; KK-Makera area; NS-Nasarawa; KU-Kudenda; RM-Romi

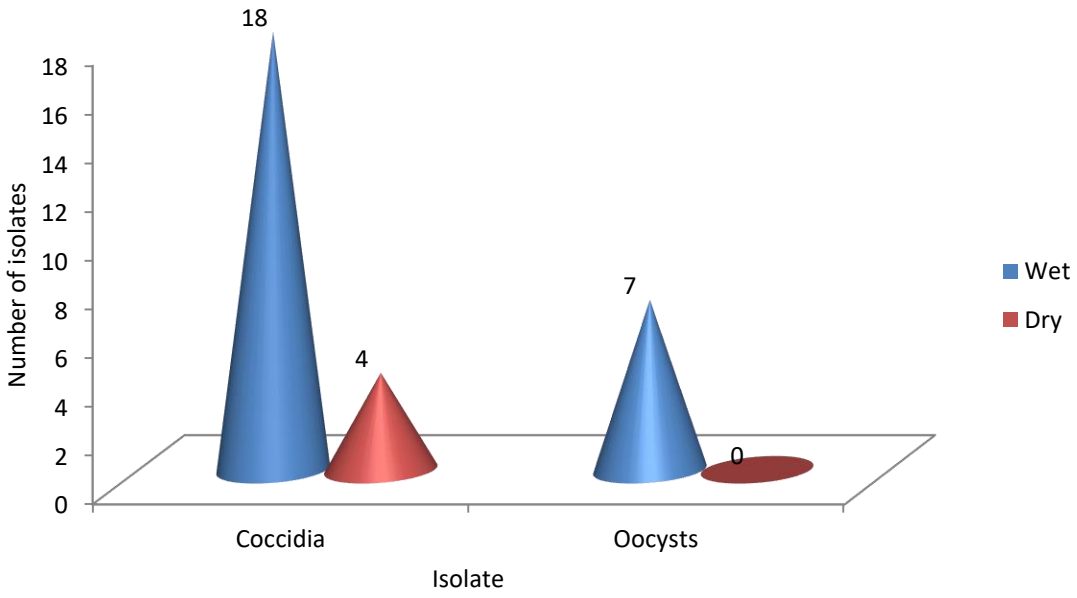


Fig. 4.3: Seasonal occurrence of the identified oocysts

4.4 *Escherichia coli* and *Escherichia coli* O157:H7

4.4.1 Isolation and Biochemical Identification of *E. coli* and *E. coli* O157:H7

Out of 118 bacterial isolates, 59 (50%) were confirmed to be *E. coli* while 59 (50%) belonged to other Enterobacteriacea (Appendix V). However, the total percentage of *E. coli* O157:H7 among the *E. coli* isolates was 25.4% (15/59) (Fig.4.4). Table 4.11 shows the prevalence of *E. coli* O157:H7 within the various sampling sites of Kaduna River, with Nasarawa 7(46.7%) having the highest occurrence.

4.4.2 Antibiotic Susceptibility Profile of the *E. coli* O157:H7 Isolates

The *E. coli* O157:H7isolates had resistance to the following antibiotics Amoxicillin 11(73%), Sulphametoxazole/Trimethoprim 10(66.7%) and Doxycycline 10(66.7%) but susceptible to Amikacin 15(100%), Nitrofurantoin 15(100%) and Ceftriazone 15(100%) (Table 4.12).

4.4.3 Multiple Antibiotic Resistance Index (MARI) and Resistance Pattern of *E. coli* O157:H7

The Multiple Antibiotic Resistance Index of the *E. coli* serotype O157:H7 isolates is presented in Table 4.13 which revealed that 13(86.7%) of *E. coli* O157:H7 had multiple resistance with MAR Index of 0.2 and above. The Table also shows the antibiotic resistance pattern of the *E. coli* O157:H7 isolates. All the isolates (100%) had resistance to one or more antibiotics. Four (26.7%) of the *E. coli* O157 isolates in this study exhibited resistance to three or more antibiotics.

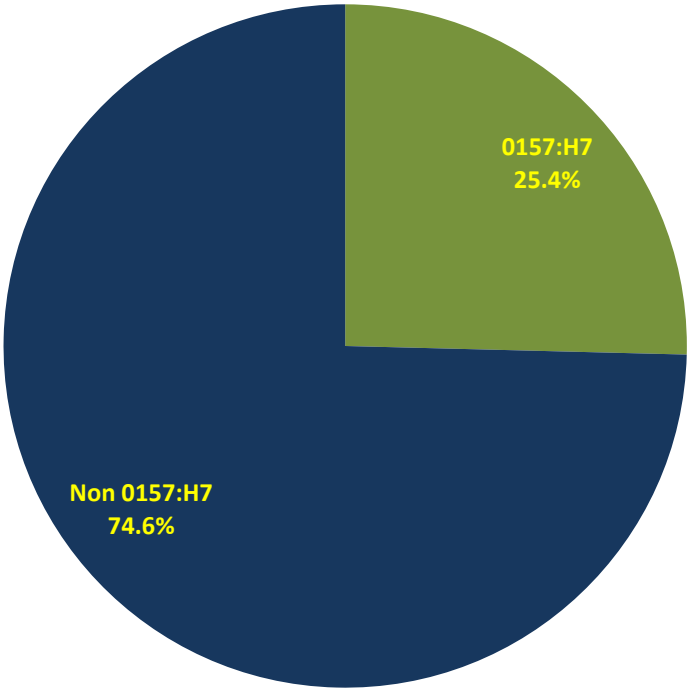


Fig.4.4: Incidence of *E. coli* O157:H7 among confirmed *E. coli* isolates

Table 4.11: Prevalence of *E. coli* O157:H7 isolated from River Kaduna

Location	Sampling Site	No. of *E.coli* O157:H7 Isolated (%)	Coded Isolate
Downquarter	DQ1	6 (40%)	DQ11
			DQ35
			DQ82
			DQ91
			DQ34
			DQ123
Romi	RM4, RM2	2(13.3%)	RM12
			RM94
Nasarawa	NS2	7(46.7%)	NS71
	NS1		NS79
	NS3		NS80
	NS3		NS85
	NS2		NS92
	NS1		NS101
	NS3		NS115

Key: Sampling site: 1 = 10 meters before the point of discharge of contents of the drain into the river; 2 = The drain just before it empties into the river (Confluence); 3 = 10 metres after the point of discharge of contents of the drain into the river; 4 = 100m downstream of Romi River-Kaduna River confluence; DQ=Downquarter drain; RM- Romi river; NS=Nasarawa drain

Table 4.12: Percentage of *E. coli* O157:H7 resistant to antibiotics used (n=15)

Antibiotic/Disc potency	No of isolates resistant to antibiotics	Percentage resistance (%)
Ceftriaxone(CRO)/(30µg)	0	0
Nitrofurantoin(F)/(300µg)	0	0
Amoxicillin(AmL)/(10µg)	11	73.3
Ofloxacin(OFX)/(30 µg)	0	0
Meropeneme(MEN)/(10 µg)	0	0
Ciprofloxacin(CIP)/(30 µg)	0	0
Streptomycin(S)/(25 µg)	4	26.7
Sulfamethoxazole/ Trimethroprim(SXT)/(25 µg)	10	66.7
Doxycycline(DO)/(30 µg)	10	66.7
Amikacin(AK)/(10 µg)	0	0

Table 4.13: Multiple Antibiotic Resistance Index (MARI) and Resistance Pattern of *E. coli* O157:H7 Isolates

No. of Isolates with Pattern	Antibiotic Resistance Pattern	Multiple Antibiotic Resistance Index	Percentage Occurrence (%)
1	SXT	0.1	6.66
1	AML	0.1	6.66
4	AML, DO	0.2	26.66
2	AML, SXT	0.2	13.33
3	SXT, DO	0.2	20
1	AML, S, SXT	0.3	6.66
3	AML, S, SXT, DO	0.4	20

Key: AML=Amoxicillin, DO=Doxycycline, S=Streptomycin, SXT=Sulphamethonidazole-Trimethoprim,

4.4.4 Molecular Characterization of Antibiotics Resistance Genes in *E. coli* O157:H7 Isolates

4.4.4.1 Genomic DNA extractionand characterization of extended spectrum beta lactamase genes

Plate I shows the Genomic DNA from the 15 *E. coli* O157:H7isolates. Plates II, III, and IV show the amplicons of extended spectrum β-lactamase genes (*TEM, SHV* and *CTX-M*). Resolution of the amplification products by agarose gel electrophoresis revealed that 8(53.3%) of the *E. coli* O157:H7 isolates were positive for the *TEM* gene, 8(53.3%) had the bla_{SHV} gene while $bla_{CTX\text{-}M}$ was found in 13(86.7 %) of the isolates as shown in Table 4.14.

4.4.4.2 Molecular Characterization of Trimethoprim Resistance Genes in *E. coli* O157:H7

Molecular detection of trimethoprim resistance gene revealed that 7(46.7%) of the *E. coli* O157:H7 isolates were positive for *DfrXII* with 319 base pair (Plate V and Table 4.14).

4.4.4.3 Molecular Characterization of Sulfamethoxazole Resistance Genes in *E. coli* O157:H7

Agarose gel electrophoresis of sulfamethoxazole resistance gene as shown in Plates VI, VII and Table 4.14 respectively, revealed that *Sul1* gene was present in 3(20%) of the *E. coli* O157 isolates. *Sul2* did not amplify in any of the isolates.

4.4.4.4 Molecular Characterization of Tetracycline Resistance Genes in *E. coli* O157:H7

Eleven (73.3%) of the isolates encoded the Tetracycline resistance gene, *TetA* while *TetB* was absent in all the isolates (Plates VIII, IX and Table 4.14).

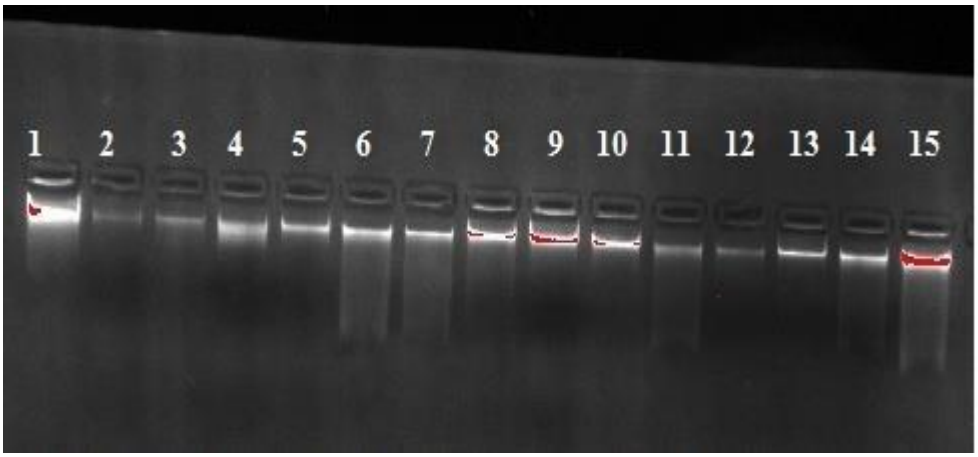


Plate I: Genomic DNA extracted from the *E. coli* O157:H7 isolates

Key:Lane 1= NS79; Lane 2= RM94; Lane 3= DQ91; Lane 4= RM12; Lane 5= DQ82;
Lane 6= NS101; Lane 7= NS85; Lane 8= NS115; Lane 9= DQ123; Lane 10= NS80;
Lane 11= NS71; Lane 12= NS92; Lane 13= DQ11; Lane 14= DQ34; Lane 15= DQ35

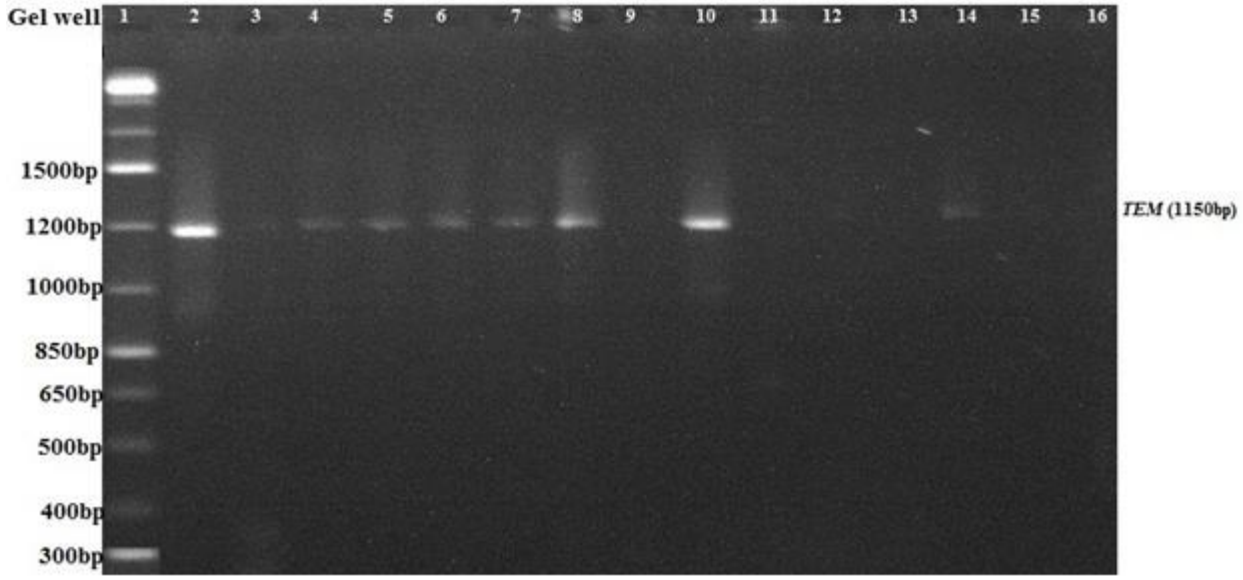


Plate II: Agarose Gel Photo of Amplicons of *TEM* gene from *E. coli* O157:H7 siolates

Key: Lane 1= 100bp Molecular weight marker; Lanes 2, 4-8, 10, 14= 1150bp

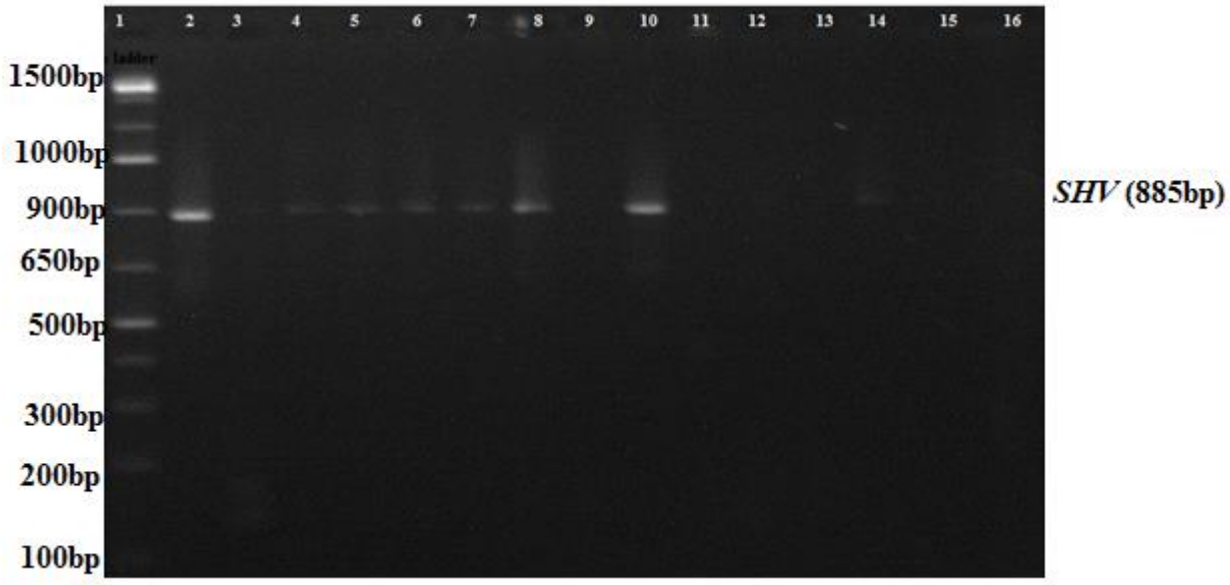


Plate III: Agarose Gel Photo of Amplicons of *SHV*gene from *E. coli* O157:H7 siolates

Lane 1= 100bp Molecular weight marker; Lanes 2, 4-8, 10, 14= 885bp

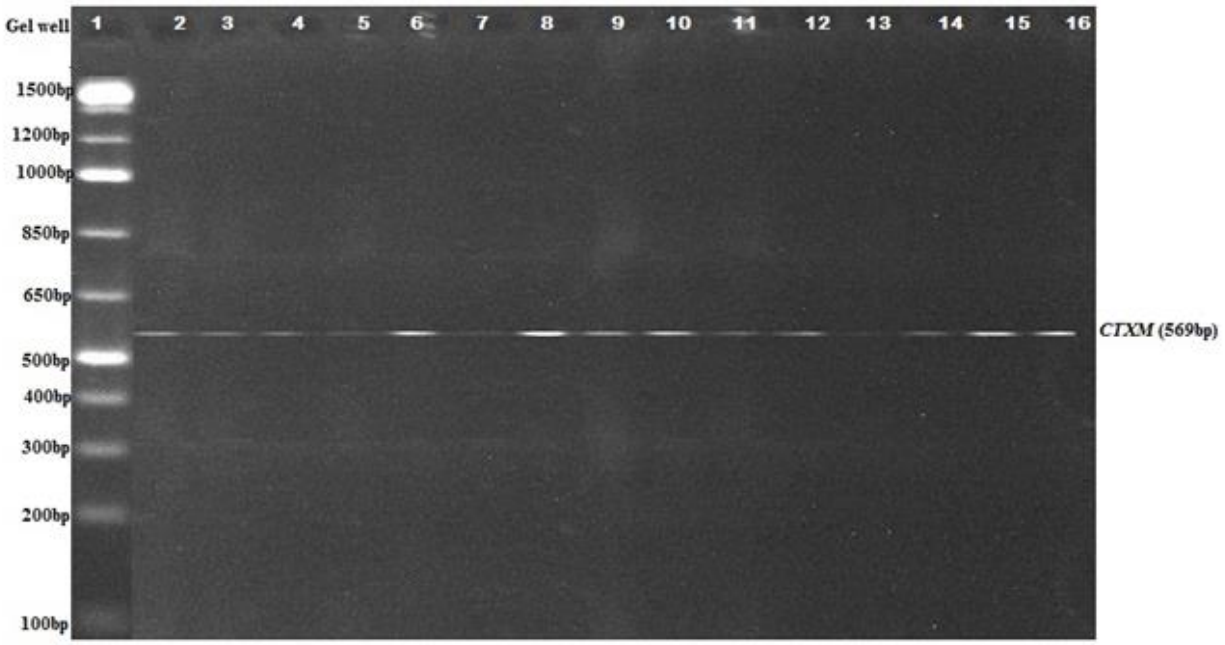


Plate IV: Agarose Gel Photo of Amplicons of *CTX-M*$_{15}$gene from *E. coli* O157:H7 isolates

Lane 1= 100bp Molecular weight marker; Lanes 2-6, 8-12, 14-16= 569bp

Table 4.14: Distribution of Some Antibiotic Resistance and Virulence Genes among the *E. coli* O157:H7 isolates

S/N	**Isolates**	**Gene Expressed**									
		TEM	*SHV*	*CTX-M$_{15}$*	*DfrXII*	*Sul1*	*Sul2*	*TetA*	*TetB*	*Stx-1*	*Stx-2*
1	NS79	+	+	+	-	-	-	+	-	+	-
2	RM94	-	-	+	+	-	-	+	-	+	-
3	DQ91	+	+	+	-	-	-	+	-	+	+
4	RM12	+	+	+	+	-	-	+	-	+	+
5	DQ82	+	+	+	-	-	-	-	-	+	+
6	NS101	+	+	-	-	-	-	+	-	+	-
7	NS85	+	+	+	+	+	-	-	-	+	+
8	NS115	-	-	+	+	-	-	+	-	+	+
9	DQ123	+	+	+	-	-	-	+	-	+	+
10	NS80	-	-	+	-	-	-	-	-	+	+
11	NS71	-	-	+	+	+	-	+	-	+	+
12	NS92	-	-	-	-	-	-	+	-	+	-
13	DQ11	+	+	+	-	-	-	+	-	+	+
14	DQ34	-	-	+	+	+	-	+	-	+	+
15	DQ35	-	-	+	+	-	-	-	-	+	-
	Percentage occurrence of genes	53.3%	53.3%	86.7%	46.7%	20%	0%	73.3%	0%	100%	66.7%

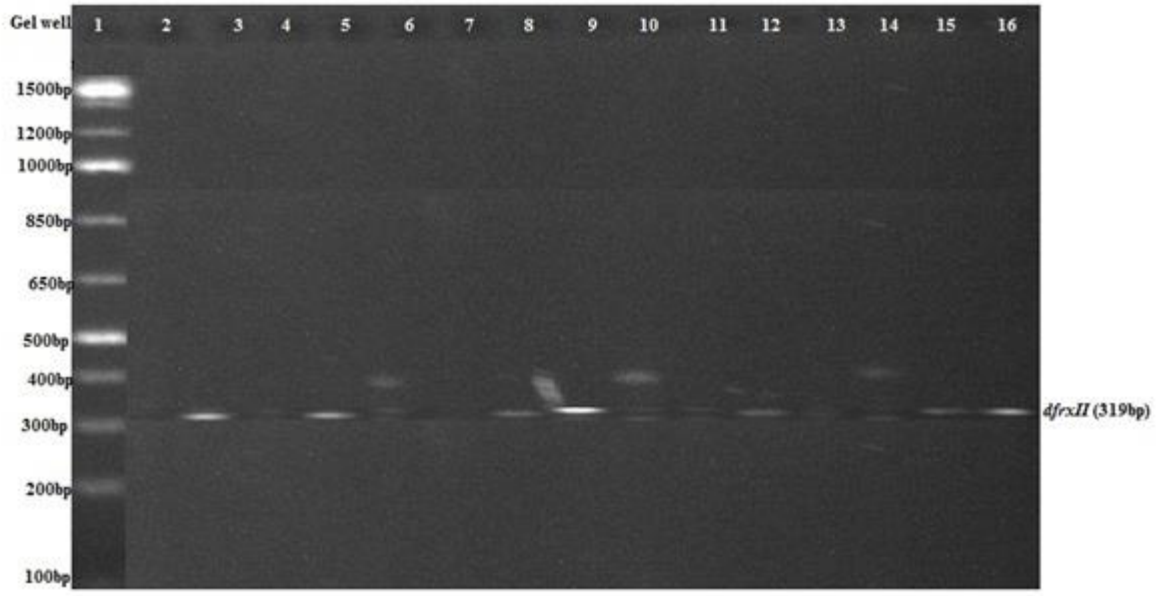


Plate V: Agarose Gel Photo of Amplicons of *DfrXII* genefrom *E. coli* O157:H7 isolates

Key: Lane 1= 100bp Molecular weight marker; Lanes 3, 5, 8, 9, 12, 15, 16= 319bp

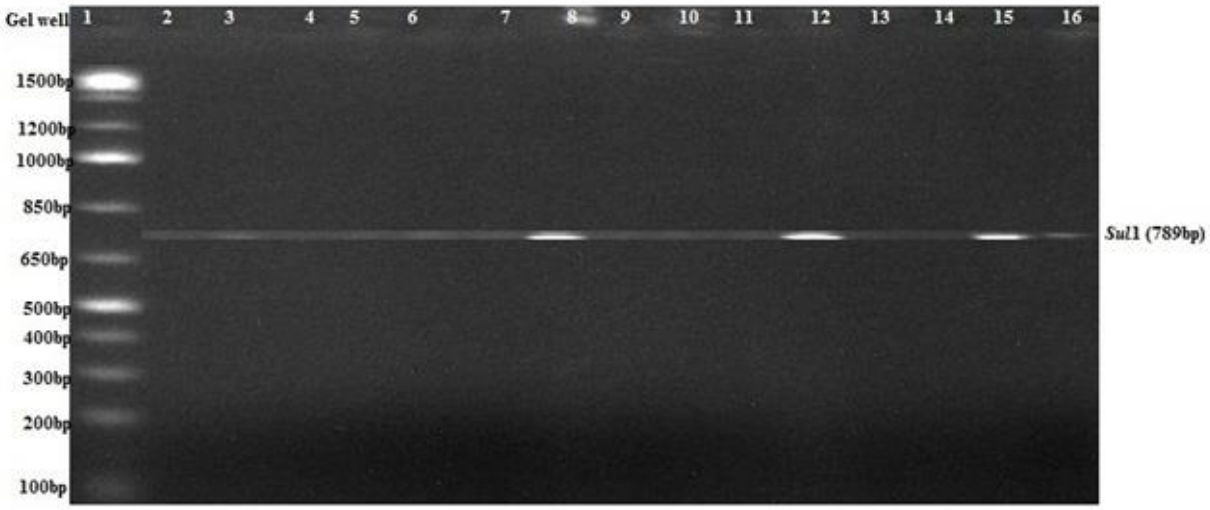


Plate VI: Agarose Gel Photo of Amplicons of *Sul1* gene from *E. coli* O157:H7 isolates

Lane 1= 100bp Molecular weight marker; Lanes 8, 12, 15 = 789bp

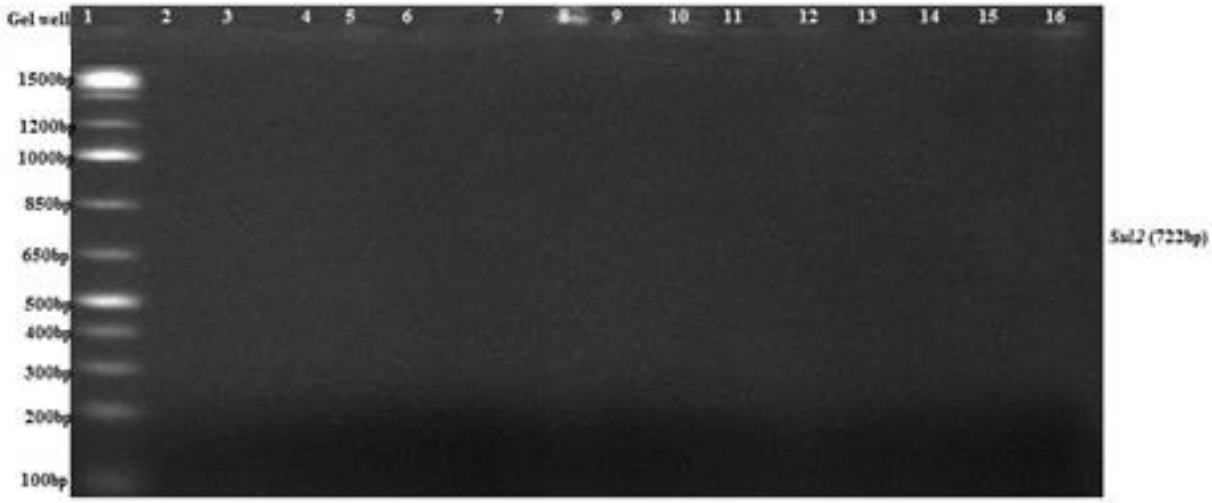


Plate VII: Agarose Gel Photo showing no *Sul2* gene amplified

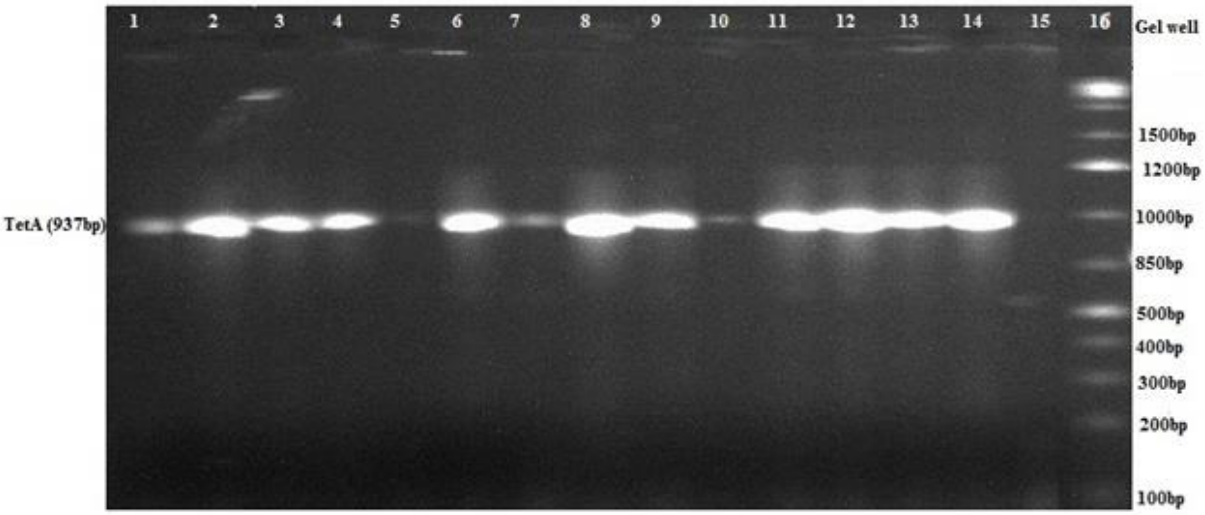


Plate VIII: Agarose Gel Photo of Amplicons of *TetA* gene from *E. coli* O157:H7isolates

Lanes 1-4, 6, 8, 9, 11-14 = 937bp; Lane 16= 100bp Molecular weight marker

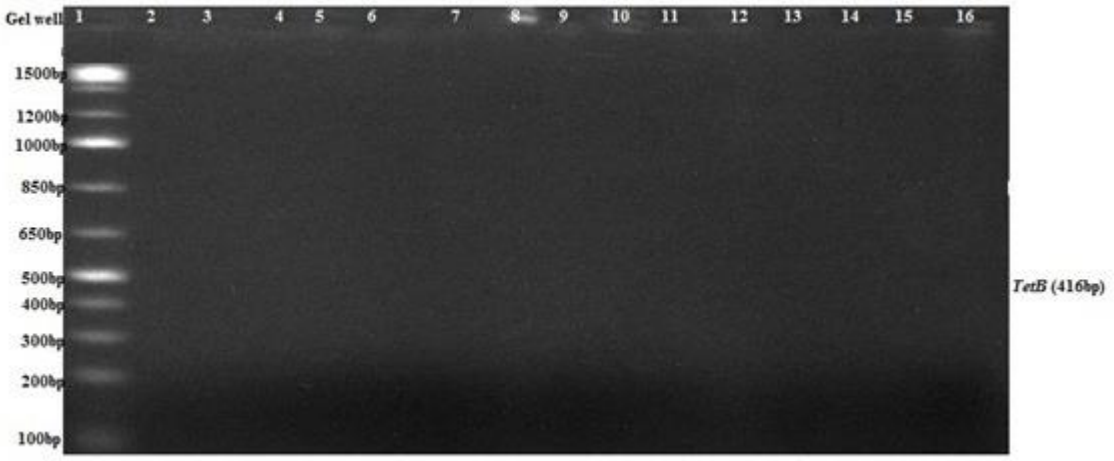


Plate IX: Agarose Gel Photo showing no *TetB* gene amplified

4.4.5 Molecular Characteristic of Virulence genes in the *E. coli* O157:H7 Isolates from surface water samples

Molecular detection of virulence properties showed that all the *E. coli* O157:H7 isolates from the water samples evaluated contained *Stx1*(100%) with 10(66.7%) of the isolates harboring the *Stx2* gene with 482bp. The results are represented in Plates X, XI and Table 4.14.

4.4.6 Sequencing of *E. coli* O157:H7 isolates from water sources

Plate XII shows the PCR amplification of 3 of the most resistant *E. coli* O157:H7isolates from water samples. The test used 16S rRNA primer. The base pair for this *E. coli* O157:H7amplified at a position that corresponds with 1650 bp band when compared with 1kb ladder.

4.4.6.1 BLAST Analysis of Sequenced *E. coli* O157:H7 isolates from Kaduna River water samples

BLAST of the generated nucleotide sequence on the NCBI data base showed thatthe isolates had 99 % identity with *E. coli* O157:H7 strain WAB.1892 with Accession Number AM184233.1 and maximum score of 1805.

4.4.6.2 Phylogenetic Study of the Sequenced *E. coli* O157:H7 isolates

Phylogeny of the sequenced *E. coli* O157:H7 isolates from water samples (Fig. 4.5) revealed that the isolates evolved from the same ancestral node but the time taken for evolutional rate of changes to occur in them varied from one isolate to another.

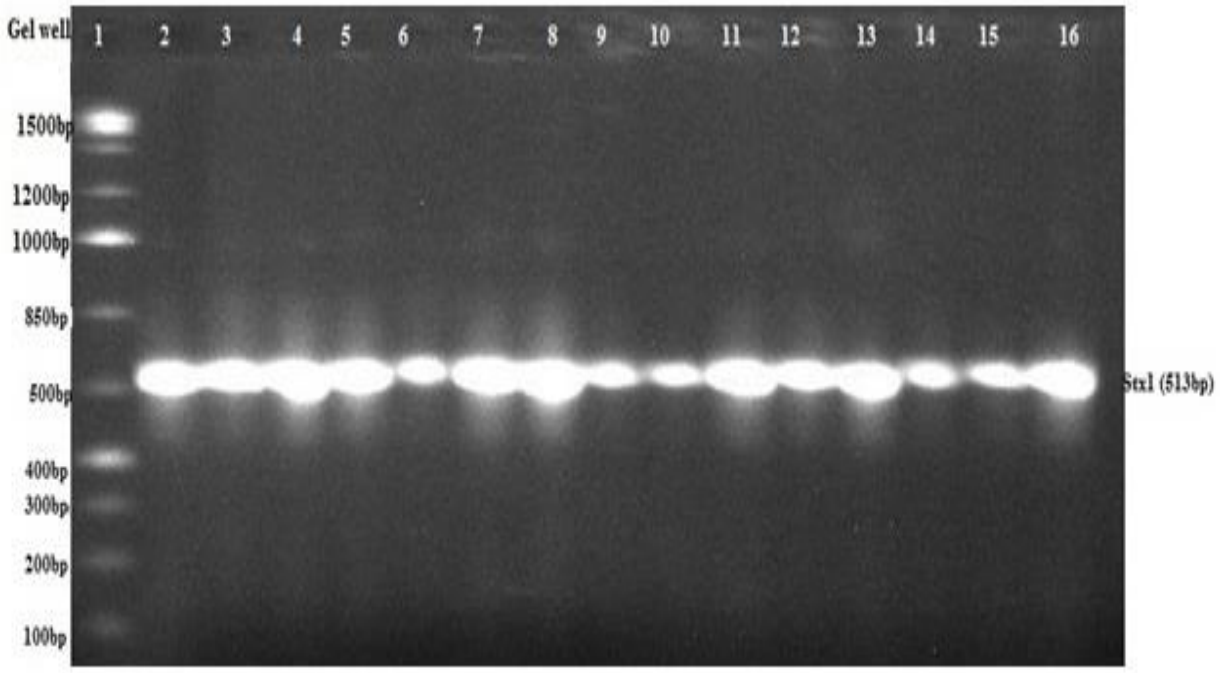


Plate X: Agarose Gel Photo of Amplicons of *Stx1* gene from *E. coli* O157:H7 isolates

Key: Lane 1= 100bp Molecular weight marker; Lanes 2-16= 513bp

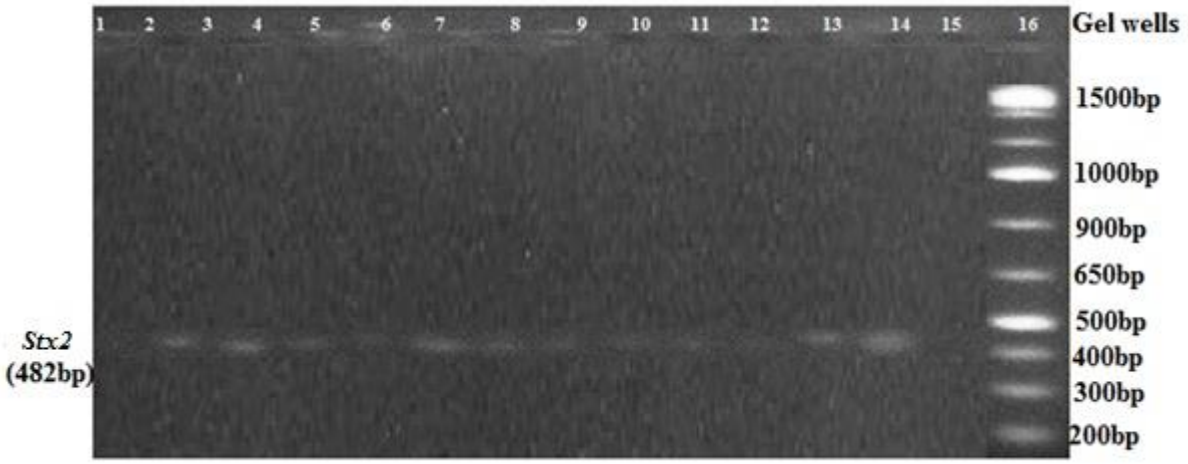


Plate XI: Agarose Gel Photo of Amplicons of *Stx2* gene from *E. coli* O157:H7 isolates

Key: Lanes 3-5, 7-11, 13, 14 = 482bp; Lane 16= 100bp Molecular weight marker



Plate XII: Agarose Gel Photo of Amplicons of 16S rRNA gene from *E. coli* O157:H7 isolates

Lane 1=1kDa protein ladder

Lane 2=DQ91

Lane 3=DQ82

Lane 4=NS115

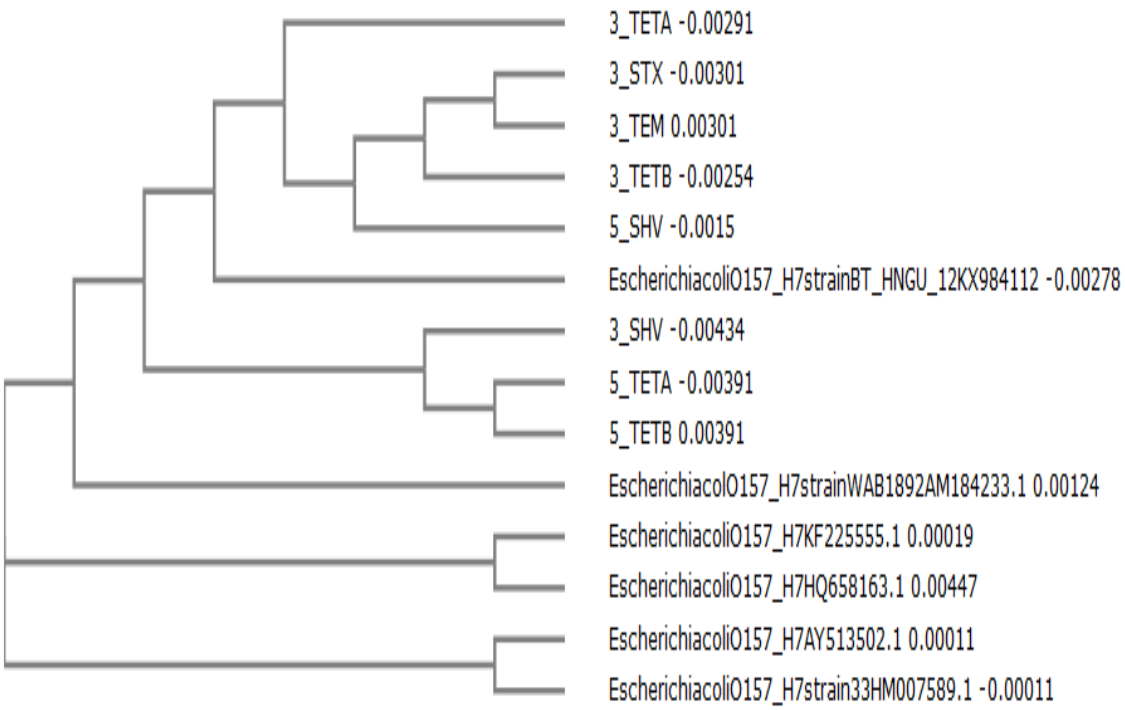


Fig.4.5: Neighbour-joining Phylogenetic Tree for *E. coli* O157:H7 isolates from the water samples sequences

Key:

3=DQ91

5=DQ82

CHAPTER FIVE

5.0 DISCUSSION

5.1 Heavy Metal concentration in water samples

Heavy metals have been used as indices of pollution because of their high toxicity to human, animal and aquatic life (Omoigberale and Ogbeibu, 2005). The concentrations of the metals obtained from samples in this study were generally higher in the water from the drains than the receiving river water. This could be because the drains convey effluents (which are mostly untreated) from industries and factories as well as wastes from households into the river. Once in the river, these wastes and effluents are expected to be diluted by the larger volume of water. In addition, a study by Amundsen *et al.* (2011) demonstrated that majority of the metal emissions are deposited near the pollution source, and only moderate amounts of the heavy metal contaminants are transported to long distances. Oguzie and Okhagbuzo (2010) made a similar observation in their study of Ikpoba River in Benin City, Nigeria. The heavy metal concentrations obtained from the raw water were compared with various raw water quality guidelines including FAO (1985) and FMEnv (1991). The results showed that the Cd and Cr levels obtained in this study were slightly above the safe limits recommended for irrigation water (FAO, 1985). The metals were generally within the limits for discharge of effluents into surface water as recommended by FMEnv (1991).

The mean concentrations of lead (Pb) were more or less uniform at all the sampling points, showing slight increases at the outfall of the drains. Lead is ubiquitous, and can come from a variety of items in the environment. Items such as paint, cosmetics, human medicines,

food supplements, pesticides, batteries, and petroleum-based fuels are sources of lead pollution (Stohs and Bagchi, 1995; CDC, 2016; National Academies of Sciences, Engineering, and Medicine, 2017). This pattern of spread of Pb in this study concurs with the report of Oguzie and Okhagbuzo (2010) in which it was observed that no significant differences existed between the concentrations of Pb at the different stations sampled along the Ikpoba River. However, they observed that Pb concentrations varied from station to station. The range of Pb concentration at the different sampling points in this study was between non-detectable and 0.76mg/l with an average of 0.208 mg/l which is within the limit of <1 mg/l recommended by FMEnv (1991). Mahre *et al*. (2007) recorded a range of Pb levels along River Kaduna as being 0.09mg/l – 0.62mg/l, which is close to that obtained in this study. The mean concentrations of Pb in this study were higher than the total mean concentrations of Pb in water (0.110mg/l) and effluents (0.125mg/l) along Ikpoba river (Oguzie and Okhagbuzo, 2010); and the mean values of 0.025mg/l and 0.14mg/l reported for Ogba River (Oguzie and Igwegbe, 2007) and New Calabar River (Abu and Egenonu, 2008), respectively. Lead and its compounds are present in municipal and industrial wastes and are transported into urban rivers through flood run-off water. High Pb concentration in some surface water samples have been attributed to the use of leaded petrol in vehicles. High lead levels in surface water may be due to vehicular deposition. Dry deposition of particulate lead on water bodies is capable of increasing the Pb level of surface waters. Lead is a potential killer especially in children. In some villages in Gummi and Bukkuyum Local Government areas of Zamfara state, more than 400 deaths were reported due to lead poisoning (Galadima *et al*., 2011). Moreover, a relationship has been established between Pb in human body and the increase of blood pressure in adults (Maihara and Fávaro, 2006).

Like lead (Pb), no significant difference (P≥0.05) was observed in the concentration of cadmium (Cd) in all the sampling points. Cadmium levels in this study ranged between non-detectable and 0.035 mg/kg, with an average of 0.017 mg/kg which exceeds the 0.01 mg/l safe limit recommended by FAO (1985). However, the average value obtained is lower than the values (0.040mg/l) reported by Obasohan and Oronsaye (2000) and Anim-Gyampo *et al*. (2013); the mean levels (0.043 and 0.072mg/l in water and effluents, respectively), reported by Oguzie and Okhagbuzo (2010); and the (0.08-0.10mh/l) recorded for River Kaduna by Mahre *et al.* (2007). The possible sources of Cd in the surface water may be from atmospheric fall-out, fossil fuel combustion and release of sediment-bound metal (Dan *et al.,* 2014). Mahre *et al*. (2007) remarked that the levels of Cd in River Kaduna water (0.08-0.10mg/l) and sediment samples were about fifty times higher than the WHO standard. These workers were of the opinion that high levels of heavy metals in river Kaduna could be traced to high influxes from industrial wastes including the immense urban run-off.

Zinc concentration varied with the sampling points, with levels ranging from below detection limit to 0.665mg/l and an average of 0.08mg/l. This is below the FAO and FMEnv regulatory limits respectively (FAO, 1985; FMEnv, 1991). This concentration of Zn (0.08mg/l) is comparable to the value (0.085mg/l) reported for Ikpoba River by Oguzie (1999). The Zn values obtained in this study are also comparable to those (0.0-0.63mg/l) reported in Warri River by Wogu and Okaka (2011) but lower than those (ND -2.76) obtained along Kaduna River by Yusuf *et al*. (2008). From literature these rivers seem to have something in common as they all receive large scale wastes from industrial, agricultural and domestic sources. This lower concentration of Zn could be as a result of

reduced industrial activities following shot down of some industries, self purification activities of the river as well as adsorption of ions and bioaccumulation of the metals.

The following sampling points were selected for the study: WB = Upstream of the Kaduna South Waterworks intake point; DQ = Makera drain; KK = Kakuri drain; NS = Nasarawa drain; KU = Kudenda drain; RM = Romi drain; The numbers '1' or '3' attached to the sampling points represent ten meters before and after the drains, respectively; while the number '2' represents the drain just before it empties into River Kaduna.The highest zinc levels recorded at KU2 and KK2 can be explained by the fact that these drains convey effluents from a glass factory, plastic factory and textile mills. The sources of Zn in the environment are industries involved in smelting, electro-galvanizing, mining, metallurgy and in the production of pesticides, rubber, plastics and various alloys. Domestic waste, shipyard, automotive and industrial effluents also release good amounts of Zn into the aquatic environment (Garcia and Millan, 1998). Zinc is also present in mixed effluents like dungs, poultry droppings and fertilizers which get flushed into inland water bodies through flood run-offs.

Chromium concentrations were from below detection limits to 0.978mg/l with an average of 0.165mg/l. The average value exceeded the 0.1 mg/l recommended by FAO (1985); but is within the regulatory limit of FMEnv (1991). The higher Cr concentrations recorded at KU, KK2 and RM2 than at other points may be attributed to the nature of the waste conveyed through the drains into the river. Romi river receives wastes from the automobile and petrochemical plants, KK2 receives wastes from the textile mills, and wastes from a glass making factory are channeled through the KU drain. Chromium is known to be a

major component of the waste products of these industries (FMEnv, 1991). The highest Cr concentration recorded at all sites at KU is supported by the study of Nwaedozie *et al*. (2011) which recorded high levels of all the metals studied in river sediment of KU. According to Nwaedozie *et al*. (2011), Kudenda site (i.e Rafin juji drain) is the area that receives the highest quantities of effluents along the river course. This is evidenced from the fact that the water in this portion of the river is perpetually coloured. Ali *et al*. (2005) observed high Cr values (0.03-3.04mg/l) at the Makera drain, and inferred that the observation may be due to the presence of chromium in soaps and detergents used for washing at home; and dyes from textile mills. However, Ugoji and Aboaba (2004) reported much lower values (0.255mg/l) similar to some of the mean values obtained in this study, in textile effluent in Lagos. Beetseh (1985) had indicated the presence of high levels of zinc and cadmium in some effluent of some factories in Kaduna. Chromium is an important trace metal required to stabilize the blood glucose levels. This in turn controls the chances of diabetes, by the efficient use of insulin. It aids the breakdown of lipids in the body. However, ingestion of high doses of Cr can cause irregular heartbeat, stomach distress, itching and flushing in human. In addition, chromium can cause ulceration in the stomach and small intestine, as well as liver and kidney damage (Jarup, 2003; Chen *et al*., 2014).

Result of seasonal variation of heavy metals concentrations showed that Pb and Cr varied highly significantly in the wet and dry seasons. Higher levels of both metals were recorded during the dry season. This is probably due to concentration of the contaminants as a result of reduced water volume during this period. In the wet period the intense rains cause an increase in the river flow, producing a dilution of the contaminants. When the river flow decreases during the warm period, the concentrations of contaminants begin to increase,

and simultaneously, a concentrating effect of the dissolved pollutants occurs in the water due to water evaporation (Olías *et al.,* 2004). Our result concurs with the observation of Yusuf *et al.* (2008) that Kaduna river water quality was not adversely affected during the wet season because of the heavy rains and the resultant high dilution but deteriorated during the dry season. In addition, Oguzie and Okosodo (2008) stated that during the dry season, the action of wind becomes more pronounced in the transport of hazardous substances which bioaccumulate in inland water bodies. The results are at variance with the findings of Godwin *et al.* (2004) and Dan *et al.* (2014) who recorded higher values of these metals in the wet season, an occurrence which the authors attributed mainly to surface run-offs and anthropogenic inputs.

River sediments usually serve as repository of elements in the aqueous environment. Although river sediment was not assessed for heavy metal concentration in this study, several studies have reported very high concentrations of metals in sediments (Anim-Gyampo *et al*., 2013; Omozokpia *et al*., 2015; Zhang *et al*., 2016; Chen *et al*., 2016). Many other workers have observed that bottom sediments contain higher concentrations of metals than the overlying water (Anim-Gyampo *et al*., 2013; Alinnor and Alagoa, 2014). Sediments act as important reservoirs or sinks for a wide range of contaminants including heavy metals from various sources (agricultural and wastewater discharges) and other pollutants in the aquatic environment (Stephens *et al*., 2001; Fernandes *et al*., 2008) including pesticides. When the water is disturbed the elements are resolubilized into the surrounding water (Gonzalez *et al*., 2000). Heavy metal contamination in sediment can affect the water quality and bioaccumulation of metals in aquatic organisms, resulting in potential long-term implication on human health and on ecosystem (Fernandes *et al*., 2008).

5.2 Heavy Metals in Vegetable Samples

Vegetables constitute essential components of the diet, contributing proteins, vitamins, iron, calcium and other nutrients. Vegetables usually augment the nutritive value of most of man's staple food, which are deficient in vitamins, proteins and minerals. Heavy metals varied among the vegetables which reflect the differences in their uptake capabilities and their translocation to edible portions of the plants. Different concentrations of the metals Pb, Cr, Zn and Cd were detected in all the five green leafy vegetable samples examined. These vegetables are known lead accumulators (Phuong *et al*., 1995; Szczyglowska *et al*., 2011; Uddin *et al.*, 2016). Leafy vegetables have been described by Singh *et al.* (2012) as prolific accumulators of heavy metals. The significant differences in metal concentrations among the different vegetables implied that different vegetable species had different abilities and capacities to take up and accumulate different metals.

Studies carried out by Seid-Mohammadi *et al.* (2014) showed that heavy metal concentration varied among different vegetable species. This may be attributed to differential absorption capacities of the vegetables for different heavy metals. Similarly, Mohammed and Rashed (2003) attributed the differences in heavy metal accumulation in different crops to their diverse morphological characteristics and selective uptake of metals by each crop. Vegetables can take up high amounts of essential and toxic metals from contaminated soils and irrigation water. The essential metals are required for the growth of the plant in small concentrations. Higher concentrations may cause toxicity (Gupta and Sinha, 2006).

On the other hand, Cd and Pb are non-essential elements. They are toxic and cause carcinogenic responses and teratogenic abnormalities in humans, even at very low concentrations (Nazar *et al*., 2012). Itanna (2002) observed that leafy vegetables tend to accumulate high amounts of Cd, Ni, and Pb due, not only to their large leaf areas and high transpiration rates, but also to their fast growth rates. Green leafy vegetables have the capability of accumulating Pb from the soil as well as from the atmosphere through their leaves. Lead has been reported as a severe cumulative body toxin which enters the body through food, air and water; and cannot be eliminated by washing the vegetables (Abbas *et al.*, 2010; Zamor *et al.*, 2012). The high levels of Pb in certain leafy vegetables may also occur due to their uptake from irrigation water, soil or industrial and vehicular emissions (Zamor *et al*., 2012).

In this study, ANOVA tests showed that variations in concentrations of Cd and Zn in vegetables were not significant ($P>0.05$) in different seasons and locations. This agrees with the observations of Singh and Kumar (2006) and Dadban *et al*. (2009) for various heavy metals in vegetables sampled in different seasons. However, significant differences were observed in the Cr concentrations of most of the vegetables between the upstream and downstream locations. Chromium levels were found to be higher in the vegetables at the downstream location. This is supported by the observed higher Cr concentrations in the water samples obtained from KU, NS and RM sites which represent the downstream in this study. It is logical that the more the heavy metal in the water that wets the soil the more the metals absorbed by the soil and the higher the heavy metal taken up by plants.

Lead is widespread in nature and could contaminate the vegetables from different sources (Rahman *et al.*, 2012). The observed higher Pb concentrations at the upstream for jute,

downstream for lettuce, and in the rainy season for cabbage, could be due to the fact that these plants are generally short with leaves closer to the ground and therefore were easily exposed to contaminated soil through irrigation water or run-off. Differences in metal accumulation by different crop species have also been attributed to the position of edible parts in relation to their distance from the roots (Mohammed and Rashed, 2003). Spatial difference in heavy metal contamination load of the different vegetables could be due to different levels of heavy metal contamination of soil, water and air through various polluting sources prevailing in the study areas. The cultural practices adopted by the farmers at different sites, like use of industrial and municipal effluents for irrigation, application of sewage-sludge and application of agro-chemicals might have caused differences in the level of contamination.

The results reported by Samarghandi *et al*. (2000) and Sharma *et al*. (2006) suggested that there were significant differences in the concentrations of heavy metals in vegetable samples between areas and seasons. Akan *et al*. (2009) reported high level of heavy metals in all the vegetable organs in the dry season than the rainy season which the authors attributed to the use of untreated effluents from tanneries and textile mills by farmers in the dry season. Chiroma *et al*. (2003) studied heavy metal contamination of vegetables on soils irrigated with sewage in Yola, Nigeria and reported the presence of high concentration of the metals (Fe, Zn, Cu, Mg, Mn and Pb) in the vegetables. This suggests that heavy metal contamination occurred and later accumulated in plants cultivated in the soil.

Cadmium concentration was the lowest of all the metals in the vegetables (Table 4.4). The highest noted was 0.11 mg/kg in lettuce and the lowest 0.02 mg/kg in cucumber. That not

withstanding, it is noted that low-intake of oral Cd may cause negative gastrointestinal, musculoskeletal, renal and neurological effects (Kumar *et al*., 2007). The most common toxic effects of cadmium in humans is renal failure, accumulation in the bone resulting in calcium loss and malfunctioning of peripheral and central nervous system (Castro-Gonzalez and Méndez-Armenta, 2008). Among all the green leafy vegetables tested no one particular vegetable had the highest capability of accumulating all the metals Cd, Pb, Cr and Zn.

5.3 Heavy Metals in Fish Samples

The study revealed that the mean heavy metal concentrations in the fish species were generally higher than those found in the surrounding water. This agrees with the observation that concentrations of heavy metals in fish tissues are always higher than those of water (Chale, 2002), and concurs with the finding of Anim-Gyampo *et al*. (2013). Bioaccumulation may be responsible for this observation. Ekpo *et al*. (2008) stated that this is due to the fact that the fishes bio-accumulate the metals over time. This could also be attributed to the metals being lipophilic; that is, they reside and accumulate in fatty tissues. In addition, Goodwin *et al*. (2003) and Osman *et al*. (2007) stated that fish accumulate toxic chemicals such as heavy metals directly from water and diet; and contaminant residues may ultimately reach concentrations hundreds or thousands of times above those measured in the water, sediment and food.

According to Dara (1995), chromium does not normally accumulate in fish and hence low concentrations were reported even from the industrialized parts of the world. The results in this study show mean Cr concentrations range of 0.70–0.99 mg/kg which is far lower than the US FDA guideline value of 12-13mg/kg. The observed concentrations of Cr in the fish

samples of Kaduna River might be due to the wastewater from various industries such as dying and tanning industries, textile mills, and run-off from upstream agricultural fields.The chromium concentrations obtained for Tilapia and Clarias in this study were similar to those obtained from Nkisa River in Imo State (Alinnor and Alagoa, 2014). The authors attributed heavy metal contamination of the river to crude oil pipeline leakages, domestic activities as well as metal works that go on near the river.

The mean level of Pb obtained in catfish in this study (0.16 mg/kg) was lower than that recorded for River Kaduna (0.56 mg/kg) (Nwaedozie, 1998) and Taylor Creek (0.87 mg/kg) (Agbozu *et al*., 2007). Lead level recorded in tilapia in this study was similar to the result obtained for the same fish species from Makoko River but lower than that of Carter River, both in Lagos, Nigeria (Bolawa and Gbenle, 2010). In a similar study, Alinnor and Obiji (2010) reported that Pb concentration in *Tilapia quineensis* found in Nworie River in Imo State, Nigeria, was below detection level.

Cadmium was virtually not bioaccumulated by the fish species in this study. This can be attributed to the fact that the fishes were conceivably able to excrete the metal at a rate that exceeded the uptake of the metal (Wangboje *et al*., 2013). The metal was also probably less bioavailable to the fish species compared to the other heavy metals in this study. The mean Cd concentrations were lower than those recorded for fish at the Taylor Creek (Agbozu *et al*., 2007) and tilapia in Nworie River (Alinnor and Obiji, 2010).

The mean levels of Zn in this study ranged between 1.06 and 2.53 mg/kg and were much lower than the values reported for *Synodontis membranaceus* (72.33mg/kg) and *Tilapia Zilli* (65.33mg/kg) obtained from the lower reaches of River Niger (Obodo, 2002).

However, the levels of the metals in the fish species studied were within the permissible limits set by FAO (1983).

This study indicates that heavy metals concentration in different fish species differ; this is in line with the report of Atuanya *et al.* (2011) that concentration of heavy metals varies with variation in fish species. The results of this study show that the mineral content of carnivorous fish (*B. bayad* which feeds on or near the bottom) was higher than that found in herbivorous fish (*O. niloticus* and *L. senegalensis*) followed by omnivorous fish (*S. vermiculatus* and *C. angularis*). This agrees with the report of Farkas *et al*. (2000) that the concentrations of elements in fish body could be related primarily to their feeding habits. Similarly, Jezieska and Witeska (2006) stated that interspecies differences in metal accumulation in fish may be related to living and feeding habits.

The result in this study is closely related to that of Yi and Zhang (2012) who reported that benthic carnivorous and euryphagous (feeding on a large variety of food) fishes had higher metal concentrations than phytoplankton and herbivorous fish. The finding in this study that the mineral content of carnivorous fish (*B. bayad* which feeds on or near the bottom) was higher than that found in herbivorous fish (*O. niloticus* and *L. senegalensis*) followed by omnivorous fish is at variance with the report of Ayeloja *et al.* (2014) who observed that the mineral content of herbivorous fish (Tilapia fish) was highest followed by omnivorous fish (catfish) and then piscivorous African pike (*H. odoe*). According to Rashed (2001), Pb and Cd concentrations can increase in fish tissues collected from freshwaters impacted by agricultural activities.

Heavy metals such as Pb, Cu, Zn and Cd are common trace metals found in chemical fertilizers (Anim-Gyampo *et al*., 2013), and fertilizers are being used extensively in the farm land all along the Kaduna river banks. Hence, there is the possibility of the heavy metals originating from the chemical fertilizers and all forms of pesticides utilized by the farmers being washed down into the river. Fish absorb metals through ingestion of contaminated food or by absorption by the gills from where they are transferred to man on consumption (Nwaedozie, 1998). Fish samples can be considered to be one of the most significant indicators in freshwater systems for the estimation of metal pollution levels (Rashed, 2001).

Constellation is a relationship matrix meant to cluster or group items in a robust way as to form memberships and minimize the bias in clustering. In order to discern the relationship among the sampling points based on their pollution load, the constellation plot was employed. Three membership clusters were distinctly formed. The first membership comprised those points which aligned with WB (which could be rightly called the control point since it did not receive industrial effluents). This group could be adjudged to be the less polluted of the three groups.

The second membership is classified as moderately polluted which is further divided into two arms. The third membership represented by NAF2, NAS2 and KUD2 is completely outcropped from the other members signifying that it is the most polluted. This is validated by the results of the heavy metal analyses which indicated that these points, being the outfalls of the drains into the river, recorded higher concentrations of the metals in most cases.

The segment of the River Kaduna under study share similar characteristics of land use intensification and population density. The observed results show that this approach could be used to design future spatial sampling strategy. This means that for rapid quality assessment studies only the representative sites from each cluster can be used. This will reduce the number of analyses and also the cost of risk assessment procedure.

5.4 Prevalence of *Cryptosporidium* oocysts in River Kaduna

Occurrence of *Cryptosporidium* oocysts in water samples from some sites along River Kaduna confirmed the presence of *Cryptosporidium* species in the river. The results obtained revealed that 3.4% of the water samples analysed contained *Cryptosporidium* oocysts. This is at variance with previous reports which noted that the oocysts of *Cryptosporidium* species were continually present in virtually all surface waters (Hansen and Ongarth, 1991), and those of Uneke and Uneke (2007) who reported very high concentration of *Cryptosporidium* oocysts in water samples from all the surface water sample sites. In addition, a study carried out in the China revealed the presence of *Cryptosporidium* oocysts in 82.7% of recreational lake water (Xiao *et al*., 2018). The sources of oocysts observed in this study may be human waste discharges and farm and free-ranging animals in the areas under study. Several studies have shown that main sources for protozoan contamination of surface water were the intrusion of animal feces or wastewater due to heavy rains (Gorham and Lee, 2016; Sterk *et al*., 2016; Moreira and Bondelind, 2017).

Farm animal and human sewage discharges are generally considered the major sources of surface water contamination with *Cryptosporidium* oocysts (Meinhardt *et al.,* 1996). In

addition, Casemore (1990) observed that there is usually high concentration of *Cryptosporidium* oocysts in the surface waters located in areas where there is inadequate waste disposal system. The low level of *Cryptosporidium* oocysts observed in this study could be as a result of the isolation method employed which involved flocculation and modified Ziehl-Neelsen staining technique. Currently, the monitoring of *Cryptosporidium* oocyst in water is largely performed by using the United States Environmental Protection Agency (USEPA) Method 1623, which involved filtration, isolation of (oo)cysts by immunomagnetic separation, immunofluorescence assay (Efstratiou *et al*., 2017).

Oocysts were detected more in the river water in May which marks the onset of the rainy season and less in October when the rains were tailing off. Oocysts were not detected in all the other months of the year. Variations in oocysts concentrations have been shown to be associated with and were highest when they were influenced by post rainfall run-off and showing decrease through the summer months (Hansen and Ongarth, 1991; Casemore, 1991). It is well established that millions of oocysts are usually excreted by an individual (approximately 10^6/ml of stool each day) during an acute stage of cryptosporidial infection and patients with AIDS are known to excrete approximately 10^{10} oocysts per day (Casemore, 1990). Hence the first rains are expected to wash all these into the surface water body. This may explain the occurrence of oocysts in more water samples in the month of May. In the dry season there is usually little or no water to flush the human or animal faeces containing the oocysts from the surfaces or environment into the water bodies and at the peak of the rains, they are dispersed and washed off as rivers flow downstream (Casemore, 1991). Precipitation is thought to be a strong seasonal driver for cryptosporidiosis in tropical countries (Lal *et al*., 2012). Our findings concur with some studies in parts of

Africa which reported a higher prevalence of Cryptosporidium just before, or at the onset of the rainy season (Siwila *et al*., 2011; Tellevik *et al*. 2015).

Oocysts are infective stage of life cycle released via feces from infected hosts to the environment in large amount. When present in the environment, they are liable for transmission of disease to animals and humans (Shi *et al.,* 2010). The results of this study suggest that apart from people coming in contact with the water by way of recreation or vocation (e.g fishermen), vegetables and fruits harvested from the farms especially during the dry season may be contaminated with *Cryptosporidium* oocysts, since water at the river is often used for irrigation of these farms. As a result, consumers of farm produce especially raw fruits and vegetables commonly sold in the markets must take necessary precautions before consuming such items to avoid cryptosporidial infection.

5.5 Identification of *Escherichia coli* and *Escherichia coli* O157:H7 in River Kaduna

One hundred and eighteen (118) *E. coli* isolates were obtained from 204 water samples. Biochemical identification of the *E. coli* isolates using Microbat Gram-negative *Bacillus* (GNB) Identification System 12E (Microgen) showed that 59 (50%) of the isolates were *E. coli.* Out of this number, 15 (25.4%) were PCR-confirmed *E. coli* O157:H7. This means that *E. coli* O157:H7 was detected in 7.4% (15) of the surface water samples tested. The detection of *E. coli* O157:H7 at a relatively high frequency in this river water is a cause for public health concern about the water source that serves as raw water treated for drinking, recreation, irrigation and herd watering. This is more so as most of the commercial crops irrigated with the river water like cabbage, lettuce, amaranthus, are usually eaten uncooked or only partially cooked. A likely source of contamination of aquatic systems with *E. coli*

O157:H7 is cattle manure and agricultural run-off. The seriousness of this finding can be explained by the fact that *E. coli* O157: H7 can be transmitted from irrigation water to lettuce and its subsequent internalization in plant tissues which protects the pathogen from the action of sanitizing agents (Solomon *et al*., 2002). Our finding is not surprising since it is well documented that cattle is the chief reservoir of *E. coli* O157 (Renter *et al*., 2003; LeJeune *et al*., 2004).

The segment of River Kaduna studied is strongly influenced by cattle excrement occasioned by fertilizer and organic manure from agricultural land, *in situ* herd watering, sewage, and discharges from the abattoir and industrial wastes. The prevalence of 7.4% *E. coli* O157:H7 agrees with Heijnen and Medema (2006) who isolated *E. coli* O157:H7 in 7.4% (2/27) of surface water samples, higher than the 2.1% reported by Chigor *et al.* (2010) for Kubanni Dam in Zaria, Kaduna State, but lower than the 10.1% (8) reported by Tanaro *et al.* (2010). *E. coli* O157:H7 illnesses have been reported (though not in outbreak proportions) in southern Nigeria since 1994 (Ogunsanya *et al*., 1994; Olorunshola *et al*., 2000). *E. coli* O157:H7 is dangerous because of its resistance to low pH (~2.5), which allows its passage through the stomach, its low infective dose (as few as 10 cells); and its high pathogenicity (Tilden *et al*., 1996). Following tissue invasion, it may even cause death (Ritchie *et al*., 2003). Moreover, *E. coli* is a microorganism that is proficient at horizontal gene transfer that could transmit its resistance genes to other facultative or obligate pathogenic bacteria (El-Shatoury *et al.*, 2015). Eighty percent (12) of the *E. coli* O157:H7 were recorded in the wet season and 86.7% were isolated from the upstream locations, specifically Nasarawa (NS) (46.7%) and Makera (DQ) (40%). Heavy rains resulting in surface run-off and eventual contamination of surface water may be responsible for the higher prevalence

during the wet season. A lot of reports have associated the infection of *E. coli* O157:H7 around the world with having contact with infected cattle and/or eating undercooked beef, cider, cheese, vegetables, fruits or other products (Crump *et al.,* 2002; Robinson *et al.,* 2004; Currie *et al*., 2018; CDC, 2018).

5.5.1 Antibiotic Susceptibility

According to Jorgensen and Ferraro (2009), the goal of antimicrobial susceptibility testing of significant bacterial isolates is to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. The results of antibiotics susceptibility tests showed that the isolates were susceptible to amoxicillin (26.7%), streptomycin (73.3%), sulfamethoxazole-trimethoprim (33.3%), doxycycline (33.3%) and 100% susceptibility each for amikacin, nitrofurantoin, ceftriaxone, ofloxacin, ciprofloxacin, and meropeneme, respectively. This implies that amoxicillin, sulph-trimeth and doxycycline were the least active. The susceptibility of the isolatesto ciprofloxacin and amoxicillin observed in this study concurs with the finding of El-Shatoury *et al*. (2015) who reported that *E. coli* O157:H7 isolates from water sources showed sensitivity (100%) to ciprofloxacin but were resistant to amoxicillin (100%). *E. coli* O157:H7 isolated from water samples in this study were found to show high level of resistance to some of the antibiotics used. Over 66% of the *E. coli* O157 isolates exhibited resistance to doxycycline. Ram *et al*. (2008) also reported that 57.8% of *E. coli* isolates exhibited resistance to tetracycline. Other studies have also reported occurrence of resistance to antimicrobials of tetracycline class in surface water isolates of *E. coli* (Obi *et al.,* 2004; Begum *et al.,* 2005; Qadri *et al.,* 2005; Sayah *et al.,* 2005).

Antimicrobial resistance is a natural phenomenon that bacteria use to protect themselves against competitors (Kümmerer, 2004). Emergence of, and increase in antimicrobial resistance in the areas studied could arise from overuse or abuse of antimicrobial agents in agriculture and human medicine. In addition, urban wastes from urban settlements and activities may have contributed to the menace. This was also the view of Ibekwe *et al.* (2011) who opined that the use of a variety of personal care products in household settings, e.g., pharmaceutical compounds such as antibiotics for different therapeutic and subtherapeutic end points in urban environments may contribute more substantially to emergence of antimicrobial resistance in the environment than previously thought. Earlier report by Webster *et al.* (2004) has equally demonstrated that *E. coli* isolates from urban areas/point source have resistance to more antibiotics than rural/nonpoint source isolates possibly because of greater exposure to antibiotics.

Antibiotic resistant bacteria (ARB) in rivers may originate from anthropogenic sources, such as hospitals, municipal, and aquaculture effluents (Cabello, 2006; Watkinson *et al.*, 2007; Prado *et al.*, 2008); in addition, they could occur naturally, since many acquired resistance mechanisms originated in producers of antibiotics, such as actinomycetes (Martinez, 2008). Both anthropogenic and naturally occurring ARB in water environments may compromise human health. The misuse of antibiotics in medicine and agriculture has influenced the frequency and spread of antibiotic resistant bacteria in many aquatic environments (Laroche *et al*., 2010; Ribeiro *et al*., 2012).

According to Ram *et al*. (2008) urban sewage, waste waters from poultry and dairy farms, hospital wastes, animal and human carcasses contribute antimicrobial agent residues to

river Gomti in India. Antibiotic resistance accounts for hundreds of thousands of deaths annually (Review on Antimicrobial Resistance, 2014), and its projected increase has made the World Health Organization recognize it as a major global health threat (WHO 2014).

All the *E. coli* O157:H7 isolates (100%) were resistant to one or more antibiotics. More than 26% of the isolates exhibited multidrug resistance to 3 or more antibiotics. Three isolates (20%) were resistant to four antibiotics including amoxicillin, doxycycline, sulphamethoxazole/trimethoprim and streptomycin. It is noteworthy that three of the four multidrug resistant isolates (75%) identified in our study were from the same location. So also is the MAR-index at ≥ 3. The location is the Nasarawa area where water quality is highly influenced by anthropogenic activities and waste discharges from mainly residential areas. The observed antibiotic resistance may be attributed to the impact of industrial and human activities on the bacterial isolates within these sites. There are a number of reports which suggest that heavy metal contamination in natural environments can have an important role in the maintenance and propagation of antibiotic resistance (Su *et al*., 2014; Lu *et al*., 2015; Na *et al*., 2018). Lin *et al.* (2004) have demonstrated the role played by industrial and human activities on the antibiotic resistance distribution of bacterial isolates in the environment. In addition, Goni-Urizza *et al.* (2000) found a correlation between antibiotic resistant bacteria in rivers and the input of urban effluents.

Multiple antibiotic resistant indices (MARI) value of 0.2 (arbitrary) is used to differentiate between low- and high-health risk, and MARI greater than 0.2 suggests that (a) strain(s) of bacteria originate(s) from an environment with high contamination or antibiotics usage (Christopher *et al*., 2013). The MARI estimates obtained for some of the isolates from our

study sites were greater than 0.2, suggesting that the isolates originated from environments with high use or contamination of antibiotics. The high MARI values obtained in this study could be due to the exposure of the isolates to antibiotics pressure, which might have resulted from inappropriate use of antibiotics among the population in the study area, and may lead further to increase in the development of multidrug resistance overtime if appropriate measures are not put in place. This might be an indication that a large proportion of the bacterial isolates have been pre-exposed to several antibiotics. Although antimicrobial resistance is a natural phenomenon, it is being propagated by misuse of antimicrobial medicines, inadequate or inexistent programmes for infection prevention and control (IPC), poor-quality medicines, weak laboratory capacity, inadequate surveillance and insufficient regulation of the use of antimicrobial medicines (Ndihokubwayo *et al*., 2013; WHO, 2015); and low patient compliance, self-medication, and potentially sub-therapeutic prescription by health workers are some of the factors influencing multiple antibiotic resistance (Chukwu *et al.,* 2011). In addition, the presence of MAR *E.coli* strains in the water samples may be attributed to poor sanitary and poor hygienic conditions of the sampling sites, poor hygienic condition of the people living around the sampling sites, contamination by domestic animals and industrial activities around the sites, inadequate waste disposal facilities and refuse/sewage disposal around the sampling sites (Oluyege *et al.,* 2014). Moreover, when manure produced in agriculture is applied to land, pollutants such as antimicrobial compounds, resistant bacteria or resistance genes concentrate and mobilize in soil and often end up in ground or surface water through runoff (Duriez and Topp, 2007; Ribeiro *et al*., 2012). Multi antimicrobial agent-resistant *E. coli* have been

detected in food, clinical and surface water samples in developing countries (Qadri *et al*., 2005; Alam *et al*., 2006; Ram *et al*., 2007).

5.5.2 Molecular characterization of Antibiotic Resistance genes in *E. coli* O157:H7

Molecular characterization of ESBL genes in *E. coli* O157:H7isolates showed that *CTX-M* (86.7 %) were the predominant ESBL genes among the *E. coli* O157:H7in the water samples, although sulfhydryl variable (*SHV*) (60.0 %) and Temoneira (*TEM*) (53.3) genes were equally high. These findings are in line with the report of Lei *et al.* (2013) who reported that bla_{CTX-M} is the most widely distributed gene encoding extended-spectrum β-lactamases in human globally. Approximately two-thirds of antibiotics administered to humans are β-lactams (Lachmayr *et al*., 2009). Our result also concurs with that of Adefisoye and Okoh (2016) who recorded high detection of ^{bla}*TEM* conferring resistance to β-lactam at a frequency of 56.4%. The study of Lu *et al.* (2010) had demonstrated the presence of a rich reservoir of diverse ESBL-producing bacteria and ESBL genes, in particular, the ^{bla}*CTX-M* gene, in an urban river sediment environment. Since people could be exposed to river water through aquatic products or direct interaction the presence of antibiotic resistant bacteria producing these genes in urban river could pose a significant risk to public health. Moreover, the antibiotic resistant bacteria may transfer the antibiotic resistance genes to other pathogens through horizontal gene transfer (Baquero *et al.*, 2008). This way, antibiotic resistance is prone to spread and become more difficult to control and eliminate.

Trimethoprim-sulfamethoxazole and β-lactam antibiotics are commonly used for the treatment of gastroenteritis and UTIs (Shaohua *et al.,* 2001). As such, *dfrXII* genes that

code for resistance to streptomycin and trimethoprim and, *sul1* and *sul2* genes that code for resistance to sulfamethoxazole were evaluated in this study. The result showed that *dfrXII* genes were present in 10 (66.7 %) of the isolates, *sul2* genes were absent in all the isolates while 4(26.7%) had *sul1* gene. This contradicts the result of Silva de Faria *et al*. (2016) and Suzuki *et al.* (2015) that Sulfonamide-resistance genes (*sul1* and *sul2*) were present in all water samples tested and agrees with the report of Colomer-Lluch *et al*. (2014) which demonstrated that the *sul1* gene was more prevalent in water samples in Tunisia. Anthropogenic point sources of pollution have been reported to increase the abundance of *sul1* in river water and sediments (Berglund *et al.*, 2015). Sediment stores *sul* and *tet* genes for a long time in non-contaminated areas (Tamminen *et al.,* 2011; Muziasari *et al*., 2014), whereas water samples indicate present status. The abundance of antibiotic resistance bacteria in surface waters suggests their continuous input into the environment.

Study on tetracycline resistance showed that 13(86.7%) of the isolates of *E. coli* O157:H7 had *tetA* gene compared with *tetB* gene which did not amplify in any of the isolates. These resistance genes were however higher than those observed by Stoll *et al.* (2012) who reported 18% and 7% *tet*(*A)* in water samples collected from Australia and Germany, respectively. Similarly, Lyimo *et al.* (2016) equally reported an overall prevalence in tetracycline-resistant isolates with *tet(A)* being predominant (57.4 %) followed by *tet(B)* (24.0 %). Ibekwe *et al.* (2011) observed that tetracycline resistance was by far the most common type of resistance observed in *E. coli* isolates associated with wastewater treatment plants and this was linked to human origin. Tetracycline resistance genes are located on mobile genetic elements, and can be transmissible between bacteria (Roberts, 2005). Moreover, *tet(A),* which is plasmid mediated, encodes an active efflux pump that

functions against all tetracycline drugs (Lyimo *et al*., 2016). Agerso and Sandvang (2005) and Adesoji *et al*. (2015) have demonstrated the successful horizontal transfer of *tet(A)* genes by conjugation experiments on account of the gene being present on a broad-host plasmid(s) that is capable of moving between genera. Although sulfonamides are widely used in veterinary medicine, their use is even more widespread in human medicine (SKÖLD, 2000). The prevalence of resistance to specific antibiotics or antibiotic classes within a microbial population may be explained by the presence of mobile genetic elements within the population encoding that antibiotic resistance (Thavasi *et al*., 2007).

5.5.3 Molecular charaterization of shiga toxin genes in *E. coli* O157:H7

Shiga toxin producing *Escherichia coli* (STEC) is an important group of zoonotic human diarrheal pathogen associated with a wide spectrum of clinical conditions ranging from non-blood and bloody diarrhea to hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Paton and Paton, 2002; Dutta *et al*., 2011; Feng *et al*., 2011). The most important component of their virulence is the production of one or both Shiga-like toxins (*Stx*) I and II (O'Brien and Holmes, 1987). The *E. coli* O157:H7 isolates from River Kaduna were screened for shiga-like toxins. The results showed that both *stx1* and *stx2* genes coding for Shiga-like toxin producing strains (STEC) occurred in the isolates with a frequency of 100% and 73.3%, respectively. The results were higher than those of Obi *et al*. (2004) who reported 2% occurrence each for both toxins in river sources of South Africa. Similarly, Rivas *et al*. (2006) and Tanaro *et al*. (2010) recorded one *E. coli* O157 strain each which harbored the *stx2* gene from water sources. Detection of STEC in the magnitude observed in our study gives serious concern for public health considering its growing number of animal reservoirs. Though cattle and sheep are regarded as the principal reservoir of STEC,

it is also prevalent in gastrointestinal tract of other domestic animals like pigs, dogs and cats (Hazarika *et al*., 2007). Besides, a study from India showed presence of STEC in pigeon droppings (Jomezaden *et al*., 2009).

In this study, most of the *E. coli* O157:H7 isolates which encoded for virulence genes were obtained during the rainy season. This demonstrates a potential risk of infection from *E. coli* carrying virulence genes (VGs) in storm water. The prevalence of VGs in the isolates collected during the dry periods suggests that there is always the presence of pathogenic *E. coli* in the surface water. It does suggest however, that the risk of contracting infection may increase after the rains as a result of run-off. In their studies of surface water samples (drinking water, for irrigation, or other purposes) from the Indian rivers, Ram *et al*. (2007); Ram *et al*. (2008) and Ram *et al*. (2009) have demonstrated the presence of antibiotic-resistant shiga toxin and enterotoxin producing *E. coli*. The authors considered that such a finding could be an important health concern due to the risk of developing waterborne outbreaks.

The majority of strains of this (*E. coli* O157:H7) organism produce *Stx2*, some produce both *Stx1* and *Stx2*, and a few produce *Stx1* only (Law, 2000). Although the presence of a single or multiple VGs in an *E. coli* strain does not necessarily indicate that a strain is pathogenic unless that strain has the appropriate combination of VGs to cause disease in the host (Boerlin *et al*., 1999), the detection of the stx1 and/or stx2 gene(s) may indicate the presence of other Shiga toxin-producing E. coli in the environment, which in itself would be of importance in regard to public health protection and outbreak response (Mull and Hill, 2009). The *Stx* genes, encoded by lambda-like bacteriophages, cancirculate among

hundreds of different *E. coli* strains (Schmidt, 2001) and integrate into many sites in the O157 genome (Ohnishi *et al*., 2002; Shaik and Tarr, 2003). Previous studies have observed correlations between specific *Stx* genes and disease, particularly for *stx2* and *stx2c* (Jelacic *et al*., 2003; Persson *et al*., 2007), although it has not been suggested that having both variants together may increase virulence. El-Jakee *et al.* (2009) stated that from the 14 *E. coli* O157 strains isolated from different water sources in Egypt and characterized by monoplex PCR, eight (57.1%) isolates carried stx1 and four (28.6%) possessed stx2 gene. Shiga toxins cause severe illness by affecting the lining of the intestine (Virginia Bioinformatics Institute, VBI, 2009).

5.5.4 Sequencing of the isolates

Due to differences in pathogenicity expressed by different strains in disease outbreak, strains of medical interest are often classified below the species level by sequencing, serotyping, enzyme typing, identification of toxins or other virulence factors, or characterization of plasmids, protein patterns, or nucleic acids (Baron, 2003). To evaluate the identity of these isolates to the molecular level among identified *E. coli* isolated from water samples, sequencing of the isolates using 16SrRNA primer was carried out. The results showed that the isolates evaluated in this study were *E. coli* O157:H7, and there was a correlation between the biochemical identification method employed in the identification of the isolates and sequencing result. Further analysis of the two pairs of primers for amplifying the *Shigella* toxins (*stx1*, *stx2*) target genes in *E. coli* O157:H7 showed that the isolates harbored *Stx2* which had 89% identity relationship with *E. coli* O157:H7 strain WAB.1892 with Accession Number AM 184233.1. This study is in line with the finding of Tahamtan *et al*. (2010) in Iran who also observed the higher prevalence of the *Stx2* gene

than stx*1* in strain populations isolated from cattle. The presence of this gene among *E. coli* isolated from water sample in our environment indicates a risk alert of *E. coli* O157:H7 being shed by cattle (its reservoir) (Lim *et al.,* 2010) which nust have been drinking from the water sources, and appropriate measures are needed to prevent the spread of life-threatening waterborne diseases such as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) caused by this bacterium.

Problems associated with the presence of antibiotic-resistant bacteria have reached epidemic proportions in recent years, with cost estimates exceeding $4 billion in the United States alone per annum (Jones *et al.,* 2003). The ability of *E. coli* O157:H7 to cause human disease, colonize the bovine gastrointestinal tract, develop resistance and survive in the environment is based on its putative virulence plasmid called pO157 (Lim *et al.,* 2010). The pO157 shows a dynamic structure and includes different mobile genetic elements such as transposons, prophages, insertion sequences (IS), and parts of other plasmids, which have been reported to encode antibiotic resistance genes (Burland *et al.,* 1998).

The following antibiotic resistance genes: *SHV, TEM, TETA* and *TETB* were also evaluated in this study to substantiate the correlation between the phenotypic antibiotic resistance expressed by these isolates and their genomic constituents. The result showed that antibiotic resistant genes observed in *E. coli* O157: H7 in our environment also had significant percentage identity with those found in *E. coli* O157:H7 strain WAB.1892 with Accession Number AM 184233.1 as follow: extended spectrum beta lactamase genes; *SHV* had 99% and 89% identity relationship in isolates 3 and 5 respectively while *TEM* had 88% and 99%. These ESBL genes have been reported to induce resistance to β-lactam antibiotics in

clinical isolates (Komijani *et al.,* 2016). According to Anderson *et al.* (2003), antibiotic-resistant pathogens pose the threat of severe and costly animal health problems as it raises significant public health concern. It is worthy to note that isolates encoding ESBL genes could exhibit resistance to virtually all antibiotics which are not structurally related (Igwe *et al.*, 2013). The clinical significance of these isolates is that antimicrobial-resistant bacteria from water or food animals may colonize the human population via the food chain, contact through occupational exposure, or waste runoff from animal production facilities to humans (Witte, 1998).

Tetracycline resistance among human isolates has been attributed to co-selection via genetic linkage of resistance determinants or to the use of tetracycline to treat non-enteric bacterial infections in humans (Zhanel *et al.,* 1995). This broad-spectrum antibiotic which was known to be effective against both gram positive and gram negative organisms have recently lost its therapeutic efficacy due to acquisition of resistance gene (Igwe *et al.,* 2013). These *TETA* and *TETB* tetracycline resistant genes were also evaluated in this study. The results showed that *TETA* found in isolate 3 and isolate 5 had 88 and 99 percentage identity with *E. coli* O157:H7 strain WAB.1892 with Accession Number AM 184233.1 while *TETB* had 89% and 99% respectively. This study concurs with the finding of Bryan *et al.* (2004) who observed 97% identity relationship with the strains evaluated in his environment. These differences in percentages might be associated with genetic variation dynamics within the genome of the isolates as a result of mutation and because the emergence and dissemination of this resistance characteristics may complicate future therapeutic options that are being developed for treatment of HUS and HC, continued surveillance of emerging antimicrobial resistance among environmental and zoonotic food-

borne pathogens, especially *E. coli* O157:H7, is required to ensure public health (Schroeder *et al.,* 2002).

On drawing the phylogenetic tree relationship of the genes, the result showed that the isolates genome originated from the same ancestral node with *E. coli* O157:H7 strain WAB.1892 but with time isolate *E. coli* O157:H7 strain_H7strainBT-HNGU-12KX984112-0.00278 evolved. This could have been linked to exposure to environmental factors such as chemical, adaptation and evolutionary changes (Zhou *et al.,* 2010).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Industrial effluents, trade and municipal wastes and wastes from agricultural activities are channeled into River Kaduna mostly untreated via surface run-off and many numerous tributaries. It can be concluded that these wastes contributed high amounts of heavy metals including Cd, Cr, Pb and Zn to the river some of which (Cd and Cr) exceeded FAO guidelines recommended for agriculture. The presence of Cd and Cr in the river water at the concentrations observed presents a danger to the general public as the water is used to irrigate crops along the river banks.

The result shows that higher Cr levels were recorded in water samples at the downstream location. This corroborates the higher Cr levels observed in the vegetables obtained from the downstream location. This suggests that Cr accumulated from the upstream and became more concentrated at the downstream location. Lead concentrations in cabbage, cucumber and jute exceeded the FAO/WHO recommended safe limit. Heavy metals in vegetables pose a serious public health problem as the heavy metals cannot be degraded nor washed off the vegetables. The concentration of Cr, Cd, Pb, and Zn in the fish samples were within the FAO and US FDA guidelines. However, there is need for continuous monitoring of fish in River Kaduna to ensure the heavy metals concentration in the fish does not exceed the recommended guideline values.

The study established the presence of *Cryptosporidium* oocysts in River Kaduna. Precipitation seem to play an important role in the dislodging and distribution of the oocysts

in the aquatic environment. Apart from people coming in direct contact with the river water, the contaminated water is often used for irrigation of crops on the banks of the river.

River Kaduna serves as a source of water for recreation, irrigation, herd watering and the waterworks raw water intake source. *E. coli* O157:H7 was detected in the river at a relatively high frequency. This is of public health concern considering the fact that most of the commercial crops, like cabbage, lettuce, amaranthus and the likes irrigated with the river water are usually eaten uncooked or only partially cooked. All the *E. coli* O157:H7 isolates were found to be resistant to one or more antibiotics. In addition, the study established the presence of some antibiotic resistance genes (*SHV*, *TEM*, *CTX-M*, *TETA*) and virulence genes (*stx1* and *stx2*) in the river. These attributes possessed by the *E. coli* O157:H7 isolates would ensure continue transfer of resistance genes to other pathogenic bacteria.

The study shows that the stretch of the river that was studied was polluted and the vegetables and fish contaminated with the heavy metals Cr, Cd, Pb and Zn. *E. coli* O157:H7 and *Cryptosporidium* oocysts were distributed randomly along the river course.

6.2 Recommendations

1. Numerous laws and decrees promulgated by various administrations and governments in Nigeria to mitigate the impact of water pollution have not been adhered to. The responsible agencies such as the National Environmental Standards and Regulations Enforcement Agency (NESREA) and Kaduna Environmental Protection Authority (KEPA) should be strengthened to enforce compliance to existing guidelines and offenders should be sanctioned or punished appropriately.

2. NESREA and other environmental law enforcing agencies could adopt the ‘polluter pays’ principle where the person who caused or knowingly permitted the contamination of a site will be responsible to cover the cost of remediaton.
3. An integrated wastes treatment technology employing Best Management Practices (BMP) should be adopted by the industries within the river catchment to reduce the environmental impact of their wastes.
4. There is need for epidemiological studies on the immediate communities that are directly or indirectly associated with the river water (such as fishermen, farmers, canoe operators, inhabitants of nearby communities who use the water for various purposes and those who consume vegetables and fruits obtained from farms irrigated with the river water) in order to properly assess the health hazards of the pollutants.
5. Aggressive public enlightenment/education on proper antibiotic usage is needed to reduce the impact and spread of antibiotic resistance.
6. Owing to the risk of possible outbreak of waterborne diseases and the cumulative tendency of heavy metals in the population, there is need to create public awareness on the potential risk associated with surface water contact and produce consumption without prior treatment.

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APPENDICES

Appendix I: Materials used in the study

Equipment and Instruments

The following items of equipment were used for this study:

Incubator, Autoclave (Portable 230V and 1850W Adelphi MFG CO. Ltd, England), Refrigerator (Haier Thermocool), Microscope, Centrifuge (Eppendorf centrifuge 5417R), Magnetic stirrer, Analytical weighing balance, Vortexing machine (Touch plate Super Mixer, CAT No.1291 Lab-line instrument inc USA), Hot air oven (Baird and Tatlock (London) Ltd, Chad Well Essex, England), PCR Thermocycler (Techne TC-312), Gel Electrophoresis machine (Max Fill Scie-plas. ModelHU10 serial no 5237), Micropipette, Laminar air flow cabinet (PCR-8 recirculating laminar flow pre station Labcaire product 220/240v), Spectrophotometer (Varian, Model AA240FS), Photometer, pH meter, EC/TDS meter, ZR BashingBead™ Lysis Tube, Zymo-Spin™ IV Spin Filter, Collection Tube, Zymo-Spin™ IIC Column, Microwave oven, trans-illuminator UV light of wavelength 302 nm, Polaroid camera, 3130xl genetic analyzer, ZR Bashing Bead Lysis tube, Global Positioning System (GARMIN GPS 12XL)

Bacteriological Media

The following analytical bacteriological media were used: Eosine Methylene Blue (EMB), Nutrient agar (Merck, Germany), Sorbitol MacConkey agar (Oxoid Ltd., England), Mueller Hinton agar, Tryptic Soy Broth (TSB) (Merck, Germany), MacConkey broth (Bioteck Suffolk, UK), Brilliant Green Lactose broth, Peptone Water

Reagents and Chemicals

The following reagents and chemicals were used for this study; Nitric Acid, Hydrochloric acid, Perchloric acid, Methyl red, Barritts solution A, Barritts solution B, α-napthol, Acridine orange, TAE, Tris buffer, KOH, NaOH, Ethidium bromide, Glycerol, Glacia acetic acid, 6X loading dye, agarose gel (Schwarz/Mann, England), dettol and jik (Reckit Benckiser Ltd., Nigeria), neutralizing solution (Fermentas, UK), 96 % ethanol (BDH Chemical Ltd., England), Tween 20 (Riedel-deHaen, sigma-Aldrich, UK), Sulphamic Acid (NAFCO), Microgen^{TM} GnA-B-ID System, cefixime-tellurite, Microgen *E. coli* 0157 Agglutination test kit, wash solution (Fermentas, UK), elution buffer (Fermentas, UK), tris-acetate EDTA (TAE) (Sigma chemical Ltd.,England), ethidium bromide dye (Sigma chemical Ltd., England), 10mM Tris-HCl (Sigma chemical Ltd., England), re-suspension solution (Fermentas, UK), lysis solution (Fermentas, UK),Fungal/Bacterial DNA Binding Buffer, DNA Pre-Wash Buffer.

1.4: Antibiotic Discs

The following antibiotics where obtained from Oxoid LtD, UK:

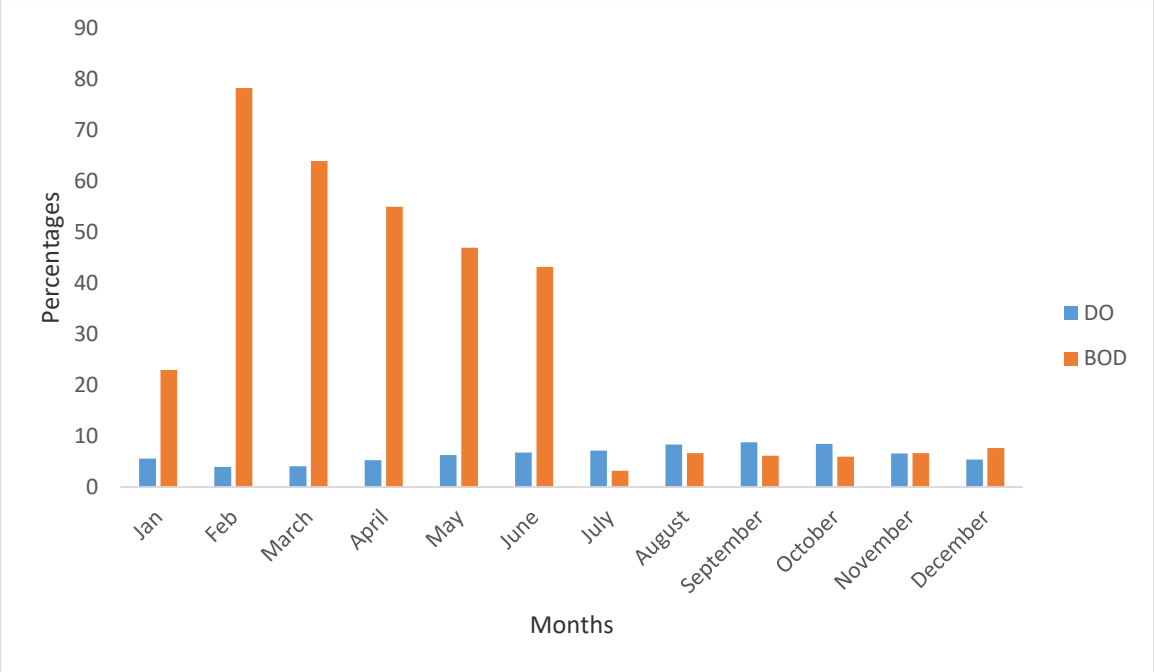
Amikacin (10 µg), Ceftriaxone (30 µg), Ofloxacin (30 µg), Ciprofloxacin (30 µg), Meropeneme (10 µg), Doxycycline (30 µg), Sulfamethoxazole/Trimethoprim (25 µg), Nitrofurantoin (300 µg), Amoxicillin (10 µg), Streptomycin (25 µg).

Appendix IIa: Results of Physiocochemical analysis of the river water samples

Table: Least squares means physiochemical parameters and macronutrients										
Location	Points	pH	Temperature	TDS	Oil & Grease	Nitrate	Iron	Manganese	Sulphate	Phosphate
DQ	1	8.20±0.36	27.2±0.70	60.9±10.16d	28.7±2.68c	1.06±0.21c	1.26±0.45c	0.28±0.11b	89.2±12.9b	2.98±1.13d
	2	8.05±0.57	26.8±0.68	540.6±64a	48.8±6.06b	0.8±0.13c	2.58±0.91c	0.25±0.10b	50.2±8.89c	11.1±5.47b
	3	8.37±0.52	26.8±0.73	145.6±21.3c	22.2±2.72c	10.1±5.89a	2.06±0.61c	0.54±0.30b	75.2±15.0b	7.4±3.35c
KU	1	7.47±0.22	26.5±0.63	75.7±26.2d	40.9±6.39b	1.25±0.27c	1.67±0.38c	0.53±0.28b	73.2±13.3b	11.2±5.57b
	2	6.92±0.37	26.7±0.68	344.3±38.9	87.2±15.8a	1.62±0.26c	10.9±3.37a	1.17±0.33a	94.2±16.0a	24.8±9.67a
	3	7.39±0.24	26.6±0.63	69.7±7.16d	23.0±4.94c	1.04±0.32c	5.52±1.87b	0.27±0.10b	68.9±14.3b	7.55±3.31c
KK	1	8.13±0.32	27.0±0.63	56.4±10.8d	39±11.8b	1.47±0.36c	2.93±0.48c	0.82±0.38b	97.1±14.7a	9.94±2.96b
	2	7.68±0.37	26.6±0.56	342.6±25.5b	41.9±8.8b	1.60±0.42c	1.88±0.88c	0.60±0.32b	40.9±6.74d	12.6±3.48b
	3	7.82±0.35	26.7±0.59	101.0±18.9c	26.2±2.5c	1.66±0.46c	2.78±0.48c	0.41±0.19b	58.4±12.5c	5.39±1.82c
NS	1	8.11±0.61	28.4±1.81	60.7±8.59d	21.4±3.32c	1.77±0.44c	2.13±0.48c	0.34±0.05b	107.2±17.1a	3.31±0.89d
	2	7.67±0.37	26.7±0.78	307.4±61.5b	18.7±2.56c	1.59±0.42c	11.5±3.19a	1.96±0.40a	97.9±13.7a	4.74±2.1d
	3	7.79±0.24	26.7±0.78	56.9±6.87e	52.0±6.69b	3.07±0.93b	4.09±1.31b	0.84±0.35b	98.9±14.3a	4.47±2.29d
RM	1	7.31±0.16	26.6±0.75	56.0±9.14e	23.2±2.79c	1.25±0.29c	1.72±0.30c	0.31±0.09b	64.8±13.5b	9.22±2.38b
	2	7.34±0.16	26.4±0.66	97.9±36c	28.2±4.78c	1.57±0.32c	1.37±0.19c	0.27±0.13b	44.9±12d	3.63±1.36c
	3	7.40±0.15	26.5±0.63	66.5±11.0d	21.7±2.04c	1.34±0.32c	0.65±0.11c	0.18±0.07b	60.2±12.2b	1.09±0.27e
	4	7.40±0.14	26.4±0.66	58.1±9.48e	23.6±3.25c	1.67±0.28c	0.52±0.08c	0.22±0.08b	41.2±7.4d	1.20±0.28e
P-value		0.24	0.99	0.0001	0.05	0.006	0.0001	0.0001	0.0001	0.0001
LOS		NS	NS	***	*	**	**	**	**	**

WB= close to Kaduna South Water Board intake; DQ= Down Quarter (Makera area); KK=Kakuri area; NS=Nasarawa bridge; KU=Kudenda; RM=Romi; points 1 and 3=Ten metres before and after the discharge of contents of the drain into River Kaduna, respectively; point 2=the drain just before it empties into the river; point 4=100m after Rivers Kaduna-Romi confluence, TDS=Total Dissolved Solids

Appendix IIb: Dissolve Oxygen (DO) and Biochemical Oxygen Demand (BOD) of the water samples



Appendix III: Feacal coliform counts (MPN/100ml) at the different sites along River Kaduna

		DQ		KK			NS			KU			RM			
	WB	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	4
Nov	$7.1x10^{4c}$	$1.6 x10^{3d}$	$1.3 x10^{5b}$	$2 x10^{4c}$	$5 x10^{4c}$	$10.5 x10^{4c}$	$5 x10^{4c}$	$4.3 x10^{3d}$	$1.1 x10^{5b}$	$5 x10^{5b}$	$5 x10^{4c}$	$5 x10^{6a}$	$1.5 x10^{5b}$	$6.3 x10^{3d}$	$2.2 x10^{3d}$	$5.5 x10^{3d}$	$4.7 x10^{3d}$
Dec	$3.2 x10^{4c}$	$2.7 x10^{3d}$	$3.1 x10^{5b}$	$5.4 x10^{4c}$	$5.5 x10^{3d}$	$2.1 x10^{5b}$	$7.1 x10^{3}$	$5 x10^{4c}$	$1.3 x10^{5b}$	$1.1 x10^{6a}$	$5.5 x10^{3}$	$5.1 x10^{5b}$	$10.2 x10^{4c}$	$6.1 x10^{3d}$	$2 x10^{4c}$	$5 x10^{5b}$	$5.1 x10^{3d}$
Jan	$3.1 x10^{4c}$	$2.5 x10^{4c}$	$29.2 x10^{4c}$	$5.3 x10^{3d}$	$1.6 x10^{3d}$	$19.1 x10^{4c}$	$5.1x10^{3d}$	$5.3 x10^{3d}$	$1.1 x10^{6a}$	$7.1 x10^{4c}$	$4.2 x10^{3d}$	$3.2 x10^{5b}$	$6 x10^{5b}$	$5.5 x10^{3d}$	$3.5 x10^{3d}$	$4.5 x10^{3c}$	$4.1 x10^{3d}$
Feb	$16.5 x10^{3d}$	$1.9 x10^{3d}$	$1.6 x10^{5b}$	$2.5 x10^{5b}$	$1.2 x10^{3d}$	$9.5 x10^{4c}$	$4.7 x10^{4c}$	$3.5 x10^{3d}$	$1.2 x10^{6a}$	$6.7 x10^{4c}$	$4.4 x10^{3d}$	$4.1 x10^{6a}$	$9.8 x10^{4c}$	$4.7 x10^{3d}$	$2.6 x10^{3d}$	$6.6 x10^{3d}$	$5.3 x10^{3d}$
Mar	$2.3 x10^{4b}$	$2.1 x10^{3c}$	$3.9 x10^{4b}$	$1.7 x10^{3c}$	$3.2 x10^{3c}$	$8 x10^{4b}$	$4.1 x10^{3c}$	$2.4 x10^{3}$	$1.2 x10^{6a}$	$5.6 x10^{4b}$	$3.5 x10^{3}$	$3.1 x10^{4b}$	$7.5 x10^{4b}$	$4.9 x10^{3c}$	$2.2 x10^{3c}$	$5 x10^{4b}$	$5.2 x10^{3c}$
Apr	$1.9 x10^{4b}$	$2.5 x10^{3c}$	$13.5 x10^{3c}$	$1.5 x10^{3c}$	$2.6 x10^{3c}$	$10.3 x10^{3c}$	$11 x10^{4b}$	$3.1 x10^{3c}$	$9.8 x10^{4b}$	$5 x10^{5a}$	$3.7 x10^{3c}$	$2.2 x10^{5a}$	$2.5 x10^{4b}$	$2.1 x10^{3c}$	$3 x10^{4b}$	$7.6 x10^{3c}$	$6.1 x10^{3c}$
May	$6.8 x10^{4c}$	$1.1 x10^{4c}$	$1.7 x10^{4c}$	$2.5 x10^{3d}$	$19.5 x10^{3d}$	$24.5 x10^{4c}$	$4.1 x10^{4c}$	$6.3 x10^{3d}$	$2.1 x10^{6a}$	$10.1 x10^{4c}$	$1.5 x10^{4c}$	$4.1 x10^{5b}$	$5.7 x10^{4c}$	$1.3 x10^{4c}$	$1.1 x10^{4c}$	$1.4 x10^{4c}$	$9 x10^{4c}$
June	$10.3 x10^{4b}$	$5.8 x10^{4b}$	$33.1 x10^{4b}$	$4.8 x10^{4b}$	$3.6 x10^{4b}$	$75.4 x10^{4b}$	$4.1 x10^{4b}$	$2.2 x10^{4b}$	$6.9 x10^{5a}$	$38.5 x10^{4b}$	$8.2 x10^{4b}$	$8.3 x10^{5a}$	$1.2 x10^{5a}$	$6.6 x10^{4b}$	$1.5 x10^{4b}$	$3.2 x10^{4b}$	$2.2 x10^{4b}$
July	$8.2 x10^{4b}$	$3.8 x10^{4b}$	$42.1 x10^{4b}$	$1.3 x10^{4b}$	$25.5 x10^{3c}$	$54.3 x10^{4b}$	$32.5 x10^{3c}$	$5.1 x10^{4b}$	$5.6 x10^{5a}$	$7.3 x10^{5a}$	$6.5 x10^{4b}$	$6.6 x10^{5a}$	$10.2 x10^{4b}$	$6.4 x10^{4b}$	$1.3 x10^{3c}$	$2.6 x10^{4b}$	$2 x10^{4b}$
Aug	$5.6 x10^{3c}$	$2.2 x10^{4b}$	$17.5 x10^{4b}$	$11.5 x10^{3c}$	$2.1 x10^{4}$	$10.5 x10^{4b}$	$2.3 x10^{4b}$	$22.5 x10^{3c}$	$30.5 x10^{4b}$	$4.2 x10^{5a}$	$2.1 x10^{4b}$	$6.2 x10^{5a}$	$8.1 x10^{5a}$	$4.3 x10^{4b}$	$1.2 x10^{3c}$	$2.4 x10^{3c}$	$2 x10^{5a}$
Sept	$26.2 x10^{3d}$	$7.5 x10^{3d}$	$22.2 x10^{4c}$	$15.6 x10^{3d}$	$16.5 x10^{3d}$	$2.2 x10^{5b}$	$2.1 x10^{4c}$	$2.8 x10^{4c}$	$2.9 x10^{5b}$	$3.6 x10^{6a}$	$4.3 x10^{4c}$	$4.1 x10^{5b}$	$1.4 x10^{5b}$	$3.1 x10^{4c}$	$9.2 x10^{3}$	$1.4 x10^{4c}$	$1.5 x10^{4c}$
Oct	$8.8 x10^{4c}$	$10 x10^{4c}$	$1.8 x10^{4c}$	$3.8 x10^{4c}$	$1.1 x10^{4c}$	$2.7 x10^{5b}$	$4.1 x10^{4c}$	$4.2 x10^{4c}$	$4.3 x10^{5b}$	$5.5 x10^{5b}$	$5 x10^{5b}$	$3.6 x10^{6a}$	$1.0 x10^{5}$	$3.9 x10^{4c}$	$1.1 x10^{4c}$	$2.4 x10^{4c}$	$1.7 x10^{4c}$

WB= close to Kaduna South Water Board intake; DQ= Down Quarter (Makera area); KK=Kakuri area; NS=Nasarawa bridge; KU=Kudenda; RM=Romi; 1 and 3=Ten metres before and after the discharge of contents of the drain into River Kaduna, respectively; 2=the drain just before it empties into the river; 100m4=100m after Rivers Kaduna-Romi confluence

Appendix IV: Monthly concentration of heavy metals in water samples

Mean monthly concentration of Pb (ppm) in the water samples in the different locations

location	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
WB	0.297	0.256	0.068	0.113	0.292	0.004	ND	0.284	ND	ND	0.118	0.127
DQ												
1	0.333	0.089	0.055	0.078	0.281	0.022	ND	0.143	0.150	0.133	0.123	0.242
2	0.360	0.060	0.098	0.106	0.332	ND	0.023	0.210	0.198	0.167	0.158	0.267
3	0.395	0.046	0.081	0.065	0.332	ND	0.014	0.190	0.176	0.160	0.158	0.212
KK												
1	0.438	0.037	0.086	0.242	0.325	0.015	0.035	0.181	0.134	0.108	0.155	0.223
2	0.482	0.056	0.091	0.256	0.352	ND	0.037	0.203	0.195	0.231	0.158	0.256
3	0.498	0.035	0.101	0.245	0.331	ND	0.020	0.249	0.203	0.161	0.148	0.213
NS												
1	0.570	0.021	0.090	0.272	0.348	ND	0.033	0.237	0.191	0.154	0.172	0.243
2	0.562	0.040	0.101	0.299	0.354	ND	0.020	0.208	0.237	0.132	0.159	0.287
3	0.603	0.016	0.104	0.268	0.346	ND	0.033	0.263	0.252	0.197	0.180	0.246
KU												
1	0.622	0.040	0.100	0.267	0.333	ND	0.020	0.266	0.289	0.302	0.172	0.273
2	0.622	0.107	0.129	0.310	0.328	ND	0.037	0.263	0.303	0.331	0.205	0.302
3	0.645	0.027	0.084	0.186	0.174	ND	ND	0.261	0.188	0.222	0.178	0.278
RM												
1	0.742	0.056	0.132	0.103	ND	ND	0.008	0.282	0.312	0.232	0.200	0.251
2	0.750	0.060	0.110	0.144	0.023	ND	ND	0.278	0.324	0.287	0.200	0.249
3	0.727	0.065	0.118	0.123	0.002	ND	0.036	0.284	0.334	0.199	0.223	0.241
4	0.767	0.053	0.122	0.134	0.002	ND	0.061	0.283	0.346	0.206	0.227	0.255

WB= close to Kaduna South Water Board intake; DQ=Makera area; KK=Kakuri area; NS=Nasarawa bridge; KU=Kudenda; RM=Romi; Locations 1 and 3=Ten metres along the river before and after the discharge of contents of the drain into River Kaduna, respectively; 2=the drain just before it empties into the river; 4=100m after Rivers Kaduna/Romi confluence; ND=Non detectable

Appendix IV contd

Mean monthly concentration of Cd (ppm) in the water samples in the different locations

Location	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
WB	0.013	0.073	0.004	0.015	0.025	0.025	0.029	0.036	0.023	0.006	0.006	0.012
DQ												
1	0.008	0.020	0.005	0.010	0.029	0.027	0.030	0.034	0.024	0.005	0.006	0.010
2	0.007	0.007	0.005	0.026	0.029	0.027	0.031	0.022	0.032	0.009	0.004	0.009
3	0.005	0.003	0.006	0.022	0.029	0.028	0.034	0.025	0.028	0.004	0.006	0.007
KK												
1	0.007	0.003	0.006	0.008	0.027	0.030	0.032	0.026	0.018	0.009	0.008	0.008
2	0.013	0.003	0.007	0.018	0.028	0.025	0.030	0.026	0.021	0.017	0.009	0.012
3	0.012	0.002	0.007	0.021	0.030	0.023	0.033	0.028	0.019	0.012	0.007	0.010
NS												
1	0.009	0.003	0.009	0.008	0.026	0.026	0.032	0.024	0.009	ND	ND	0.008
2	0.007	0.003	0.009	0.027	0.028	0.030	0.032	0.027	0.010	0.005	0.001	0.008
3	0.008	0.003	0.009	0.028	0.028	0.026	0.031	0.026	0.012	0.006	0.001	0.007
KU												
1	0.008	0.004	0.005	0.017	0.028	0.028	0.031	0.026	0.021	0.019	0.003	0.007
2	0.008	0.005	0.006	0.022	0.027	0.028	0.033	0.027	0.022	0.023	0.004	0.008
3	0.006	0.004	0.011	0.020	0.028	0.028	0.032	0.024	0.019	0.021	0.005	0.009
RM												
1	0.008	0.005	0.010	0.009	0.025	0.027	0.034	0.024	0.020	0.005	0.006	0.009
2	0.005	0.005	0.006	0.009	0.026	0.027	0.035	0.026	0.020	0.009	0.007	0.011
3	0.009	0.004	0.007	0.010	0.027	0.029	0.034	0.028	0.021	0.010	0.007	0.012
4	0.014	0.005	0.007	0.012	0.029	0.028	0.033	0.028	0.023	0.007	0.006	0.012

WB= close to Kaduna South Water Board intake; DQ= Makera area; KK=Kakuri area; NS=Nasarawa bridge; KU=Kudenda; RM=Romi; Locations 1 and 3=Ten metres along the river before and after the discharge of contents of the drain into River Kaduna, respectively; 2=the drain just before it empties into the river; 4=100m after Rivers Kaduna/Romi confluence, ND=Non delectable

Appendix IV contd

Mean monthly concentration of Zn (ppm) in the water samples in the different locations

Location	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
WB	0.080	0.044	ND	0.027	ND	ND	ND	ND	ND	0.034	ND	0.128
DQ												
1	0.082	0.004	ND	0.039	ND	ND	0.043	ND	0.007	0.041	ND	0.102
2	0.060	0.293	0.199	0.211	ND	ND	0.122	0.284	0.072	0.140	0.071	0.083
3	0.063	ND	0.012	0.057	ND	ND	ND	ND	0.009	0.055	0.004	0.029
KK												
1	0.028	ND	ND	0.010	ND	ND	ND	ND	0.056	0.023	ND	0.012
2	0.543	0.020	0.111	0.349	ND	ND	ND	ND	0.202	0.123	0.051	0.431
3	0.024	ND	ND	0.021	ND	ND	ND	ND	0.031	0.032	0.003	0.024
NS												
1	0.020	ND	ND	0.023	ND	0.014	ND	ND	0.033	0.21	ND	ND
2	0.564	0.008	0.017	0.202	ND	ND	ND	ND	0.120	0.27	ND	0.202
3	0.022	ND	ND	0.011	ND	ND	ND	ND	0.021	0.02	ND	0.007
KU												
1	0.007	ND	ND	0.046	ND	ND	ND	ND	0.017	ND	ND	0.004
2	ND	0.665	0.496	0.545	0.591	0.047	0.045	ND	0.065	0.153	0.101	0.441
3	0.289	ND	0.002	0.064	ND	ND	ND	ND	0.014	0.022	ND	0.131
RM												
1	0.032	ND	ND	0.022	ND	ND	ND	ND	0.012	0.034	0.007	0.066
2	0.012	ND	ND	0.103	ND	ND	ND	ND	0.007	0.054	ND	0.057
3	0.008	ND	ND	0.018	ND	ND	ND	ND	0.002	0.007	ND	0.017
4	0.013	0.010	ND	0.003	ND	ND	ND	ND	0.012	0.010	ND	0.006

WB= close to Kaduna South Water Board intake; DQ= Makera area; KK=Kakuri Area; NS=Nasarawa bridge; KU=Kudenda; RM=Romi; Locations 1 and 3=Ten metres along the river before and after the discharge of contents of the drain into River Kaduna, respectively; 2=the drain just before it empties into the river; 4=100m after Rivers Kaduna/Romi confluence, ND=Non detectable

Appendix IV contd

Mean monthly concentration of Cr (ppm) in the water samples in the different locations

Location	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
WB	0.181	0.013	0.101	0.024	ND	ND	ND	0.021	0.028	0.102	0.031	0.147
DQ												
1	0.235	ND	0.096	0.014	ND	ND	ND	ND	0.008	0.027	0.075	0.205
2	0.319	0.059	0.153	0.132	ND	ND	ND	ND	0.062	0.133	0.021	0.267
3	0.298	ND	0.147	0.110	ND	ND	ND	0.012	0.021	ND	ND	0.128
KK												
1	0.318	ND	0.109	0.099	ND	ND	ND	ND	0.003	0.054	ND	0.098
2	0.679	ND	0.129	0.232	ND	ND	ND	0.067	0.035	0.087	ND	0.326
3	0.283	ND	0.067	0.122	ND	ND	ND	0.010	0.008	0.032	0.021	0.082
NS												
1	0.344	ND	ND	ND	ND	ND	ND	0.038	ND	0.112	0.018	0.247
2	0.402	ND	0.018	ND	ND	ND	ND	0.071	ND	0.201	ND	0.466
3	0.978	0.006	0.015	0.114	ND	ND	ND	0.030	ND	0.232	0.043	0.131
KU												
1	0.419	ND	ND	0.043	ND	ND	ND	0.004	ND	ND	ND	0.263
2	0.513	0.062	0.026	0.332	ND	ND	ND	0.013	0.076	0.164	ND	0.518
3	0.542	ND	0.079	0.352	ND	ND	ND	0.023	0.012	0.098	ND	0.347
RM												
1	0.558	0.039	0.024	0.130	ND	ND	ND	0.002	0.019	0.022	ND	0.424
2	0.556	ND	0.048	0.313	ND	ND	ND	ND	0.027	0.087	0.012	0.451
3	0.583	0.024	0.020	0.189	ND	ND	ND	ND	0.008	0.098	ND	0.430
4	0.626	0.013	0.023	0.046	ND	ND	ND	ND	ND	0.019	ND	0.436

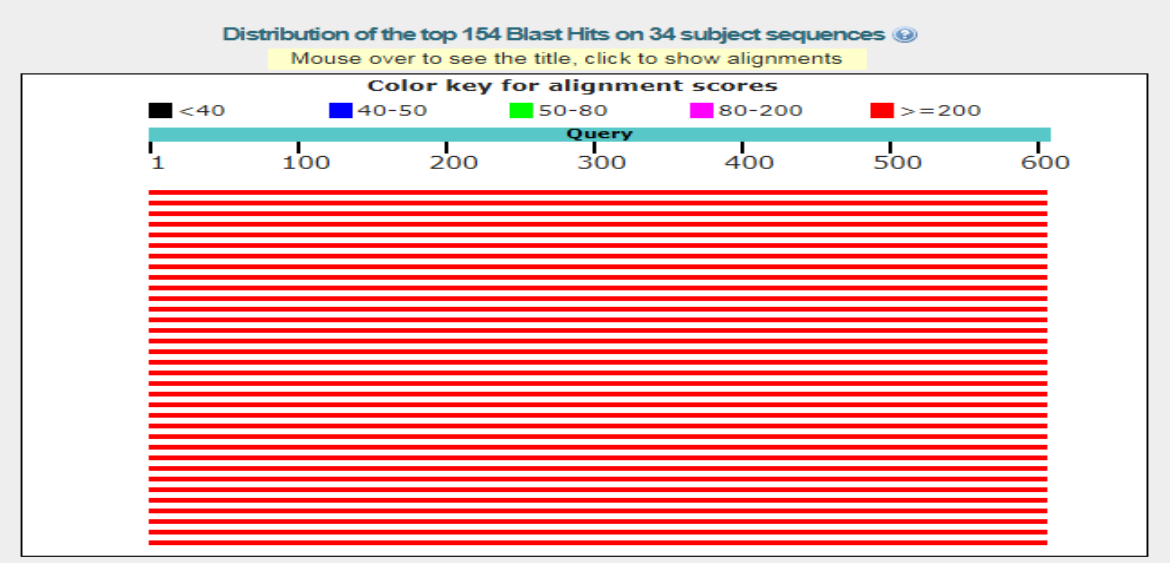
WB= close to Kaduna South Water Board intake; DQ= Makera Area; KK=Kakuri area; NS=Nasarawa bridge; KU=Kudenda; RM=Romi; Locations 1 and 3=Ten metres along the river before and after the discharge of contents of the drain into River Kaduna, respectively; 2=the drain just before it empties into the river; 4=100m after Rivers Kaduna/Romi confluence, ND=Non detectable

Appendix V: Distribution of enterobacteriaceae among the isolates

S/N	Organisms	Occurrence of isolates (n=118)	Percentage
1	*Escherichia coli*	31	25.4
2	*Escherichia coli* inactive	28	23
3	*Morganella morganii* biogroup 1	2	1.6
4	*Proteus mirabilis*	1	0.8
5	*Providencia rustigianii*	4	3.27
6	*Yersinia pseudotuberculosis*	4	3.27
7	*Shigella* serogroups ABC	3	2.45
8	*Providencia stuartii*	5	4.09
9	*Photohabdus luminescens*	1	0.8
10	*Hafnia alvei*	2	1.6
11	*Obesumbacterium proteus* biogroup 2	1	0.8
12	*Ewingell americana*	1	0.8
13	*Citrobacter freundii*	6	4.9
14	*Proteus vulgaris*	2	1.6
15	*Serratia mercenscens*	1	0.8
16	*Klebsiella ozaenae*	4	3.27
17	*Enterobacter aerogenes*	1	0.8
18	*Salmonella arizona*	9	7.37
19	*Serratia liquifasciens*	2	1.6
20	*Klebsiella oxytoca*	6	4.9
21	*Salmonella* species	2	1.6
22	*Citrobacter diversus/C.koseri*	1	0.8
23	*Providencia rettgeri*	1	0.8
	Total	118	100

Appendix VI: Blasting of the *E. coli* O157:H7 isolates from Kaduna River

The result shows the level of identity that the nucleotide sequence of the *E. coli* O157:H7 isolates from Kaduna River expressed when compared with other *E. coli* O157:H7 sequence on NCBI data base.



Sequences producing significant alignments:

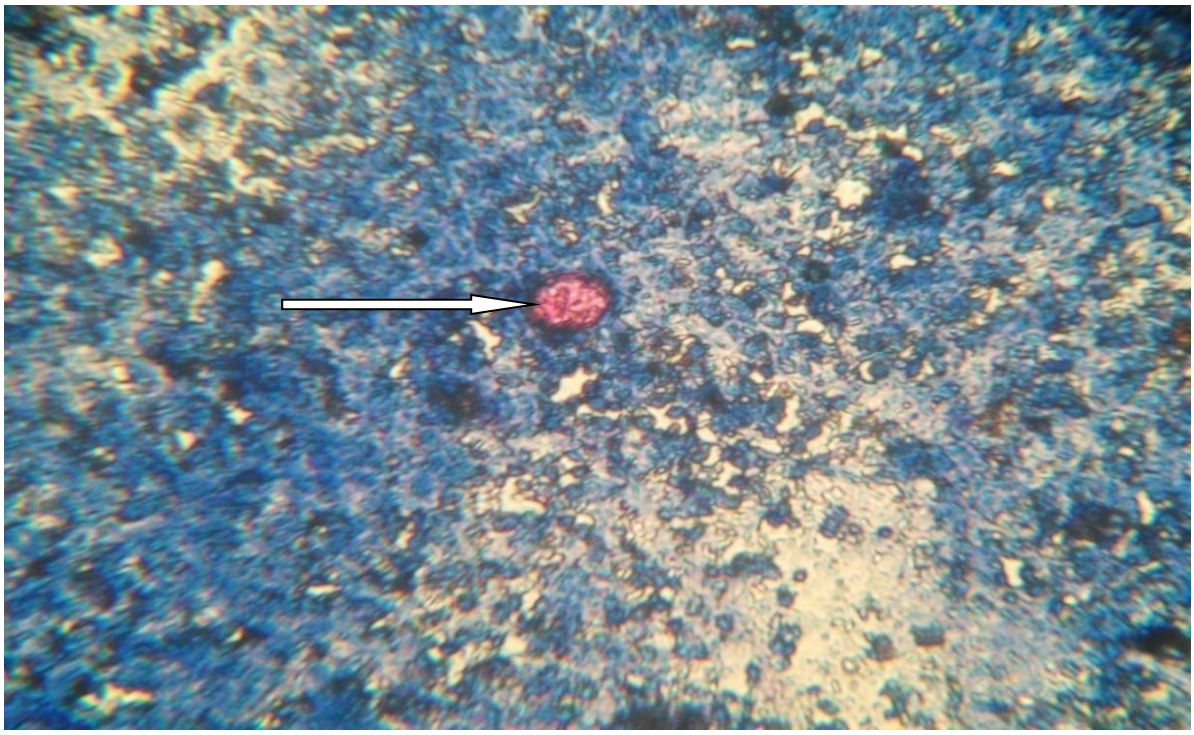
Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Escherichia coli O157:H7 partial 16S rRNA gene, strain WAB1892	1275	1275	100%	0.0	99%	AM184233.1
Escherichia coli O157:H7 strain CP017446.1 16S ribosomal RNA gene, partial sequence	1273	1273	99%	0.0	99%	MG663511.1
Escherichia coli O157:H7 strain FRIK2455, complete genome	1273	8914	99%	0.0	99%	CP015843.2
Escherichia coli O157:H7 strain 9234, complete genome	1273	8914	99%	0.0	99%	CP017446.1
Escherichia coli O157:H7 strain 8368, complete genome	1273	8897	99%	0.0	99%	CP017444.1
Escherichia coli O157:H7 strain 4276, complete genome	1273	8914	99%	0.0	99%	CP017442.1
Escherichia coli O157:H7 strain 3384, complete genome	1273	8892	99%	0.0	99%	CP017440.1
Escherichia coli O157:H7 strain 2159, complete genome	1273	8914	99%	0.0	99%	CP017438.1
Escherichia coli O157:H7 strain 2149, complete genome	1273	8914	99%	0.0	99%	CP017436.1
Escherichia coli O157:H7 strain 1130, complete genome	1273	8914	99%	0.0	99%	CP017434.1
Escherichia coli O157:H7 strain FRIK944, complete genome	1273	8914	99%	0.0	99%	CP016625.1
Escherichia coli O157:H7 strain FRIK2069, complete genome	1273	8914	99%	0.0	99%	CP015846.1
Escherichia coli O157:H7 strain FRIK2533, complete genome	1273	8914	99%	0.0	99%	CP015842.1
Escherichia coli O157:H7 strain JEONG-1266, complete genome	1273	8897	99%	0.0	99%	CP014314.1
Escherichia coli O157:H7 strain WS4202, complete genome	1273	8897	99%	0.0	99%	CP012802.1
Escherichia coli O157:H7 str. SS52, complete genome	1273	8893	99%	0.0	99%	CP010304.1
Escherichia coli O157:H7 str. EDL933, complete genome	1273	8897	99%	0.0	99%	CP008957.1
Escherichia coli O157:H7 str. SS17, complete genome	1273	8877	99%	0.0	99%	CP008805.1
Escherichia coli O157:H7 str. TW14359, complete genome	1273	8903	99%	0.0	99%	CP001368.1
Escherichia coli O157:H7 str. EC4115, complete genome	1273	8903	99%	0.0	99%	CP001164.1

Questions/comments

Appendix VII: *Cryptosporidium* oocyst (arrowed) as seen under the microscope



Appendix VIII: Colored water from Kudenda drain emptying into River Kaduna

