

**BACTERIOLOGICAL AND PHYSICOCHEMICAL IMPACT  
ASSESSMENT OF INDUSTRIAL AND DOMESTIC  
WASTES ON RIVER SOKOTO**

**BY**

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ZARIA, NIGERIA**

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MICROBIOLOGY,  
FACULTY OF PHARMACEUTICAL SCIENCES,  
AHMADU BELLO UNIVERSITY,  
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**OCTOBER, 2017**

## DECLARATION

I hereby declare that the work presented in this thesis is original and was carried out by me under the supervision of Prof. Y.K.E. Ibrahim, Prof. J.O. Ehinmidu and Dr. B.A. Tytler of the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

No part of the thesis has previously been submitted for any degree or qualification, and all references used herein are duly acknowledged.

---

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## CERTIFICATION

This thesis titled “Bacteriological and Physicochemical Impact Assessment of Industrial and Domestic Wastes on River Sokoto” meets the regulations governing the award of degree of Doctor of Philosophy, Ahmadu Bello University, Zaria, and is approved for its scientific contribution to knowledge and literary presentation.

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## **DEDICATION**

Through thick and thin you followed me; I therefore dedicated this to you, Oh Allaah!

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My thanks go first and foremost to Almighty Allaah, the Lord of all worlds and all what they contain. May His endless blessings continue to shower on the gentle soul of the best of mankind, Prophet Muhammad (SAW), his households, his companions and those who follow his teachings until the day of judgement.

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## ABSTRACT

Bacteriological analysis of the water samples such as heterotrophic counts, coliform counts, Faecal Coliform/Faecal Streptococci (FC/FS) ratio were carried out following standard procedures. Gram-negative bacteria and Gram-positive Staphylococcal species were identified using ID 32E and Microbact 12S kits respectively. Susceptibility of the isolates to ceftazidime, ceftriaxone, cefuroxime, erythromycin, gentamicin, amoxicillin/clavulinate, cloxacillin, ampicillin, ciprofloxacin, ofloxacin and nitrofurantoin were carried out using the agar diffusion method. Susceptibility of the isolates to Dettol<sup>®</sup>, Savlon<sup>®</sup> and Izal<sup>®</sup> was also determined using agar dilution method. Resistant bacteria were subjected to molecular analysis to further ascertain their status. Physicochemical properties of the river water such as pH, temperature, electrical conductivity, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), total suspended solids (TSS), hardness, sulphate, chloride, nitrates and alkalinity were analysed using standard methods. Elemental analyses of water samples and bottom soil sediments were carried out using Atomic Absorption Spectrometer (AAS). Analysis of variance (ANOVA) was carried out on the elemental data to determine the level of impact of the wastes on the river water quality. The study revealed generally high levels of heterotrophic and coliform counts throughout the year with sampling point P1 around farmland having the highest counts and P4 on the stream that carried effluents from Sokoto Cement Factory, having the least. FC/FS ratios were generally very high (above 4.0). Of the 434 bacteria isolated, *E. coli* among the *Enterobacteriaceae* was highest (11.98%). *Pseudomonas aeruginosa* (7.37%) constituted the majority of non-*Enterobacteriaceae* Gram-negative organisms while *Staphylococcus aureus* (6.91%) was the highest among the Gram-positive organisms. The antibiotic susceptibility profiles showed that most of the *Enterobacteriaceae* isolates (□ 60%) were sensitive to ceftazidime, gentamicin, amoxicillin/clavulinate, the quinolones (ofloxacin,

cloxacillin, ciprofloxacin) and nitrofurantoin. Among the non-fermenting Gram negative isolates, *Elizabethkingia meningoseptica* were most susceptible to the various test antibiotics, ranging from 68.5% to 100% except with erythromycin, having 50% efficacy. *Staphylococcus cohnii*, among the Gram-positive isolates were the most susceptible to the various test antibiotics, ranging from 75% to 100% except with cefuroxime, cloxacillin, ciprofloxacin and ampicillin, having 50% efficacy. Multiple antibiotic resistance was shown by 91.30% of *Shigella flexneri*, 37.50% of *Pseudomonas aeruginosa* and 50% of *Staphylococcus cohnii*. The MIC values of the test disinfectants (Dettol<sup>®</sup>, Savlon<sup>®</sup> and Izal<sup>®</sup>) against the isolates showed that the mean MICs of Dettol<sup>®</sup> and Savlon<sup>®</sup> were higher for *Enterobacteriaceae* compare with other Gram-negative organisms with *Enterobacter aerogenes* having the highest values of 2.50 and 3.00 respectively. The  $\beta$ -lactam (*bla*<sub>TEM</sub>), virulence (*spvC*) and quinolones (*qnrS*) resistance genes were detected in *Pseudomonas aeruginosa* while aminoglycosides and quinolone resistance genes (*aacC3* and *qnrS*) were detected in *Klebsiella pneumoniae*. Physicochemical parameters show varying with changes in the season. Sampling point P1 had the highest values for DO, BOD, COD, TDS and TSS in the dry and rainy seasons while P4 had the highest electrical conductivity value in the rainy season. Concentration of nitrate, ammonia and chloride in the water samples increased and decreased for sulphate and phosphate in the rainy season. AAS analyses of water and bottom soil sediment samples showed that 18 elements were detected, of which five namely Fe, Ag, Cd, Hg and Pb had concentrations above WHO limits. One way ANOVA test of the elemental concentrations of water samples and bottom soil sediments showed that concentrations of Zn, Ag, Cd, Cr, Mn, Fe, Co and Pb were more in sediment than in water. Generally, water of River Sokoto failed both bacteriological and physicochemical tests and therefore not fit for domestic, industrial and agricultural purposes.

# **CHAPTER ONE**

## **INTRODUCTION**

### **1.1 General Introduction**

Of all natural resources available to man and vital to man's existence and survival, none is as abundant as water. Contaminated water jeopardizes both the physical and social health of all people. It is an affront to human dignity" (WHO, 2002).

Water is vital to the existence of all living organisms, but this valued resource is increasingly being threatened as human populations grow and demand more water of high quality for domestic purposes and economic activities. Globally, the rate of groundwater abstraction is increasing by 1% to 2% per year (WWAP, 2012). Water abstraction for domestic use, agricultural production, mining, industrial production, power generation, and forestry practices can lead to deterioration in water quality and quantity that impact not only the aquatic ecosystem, but also the availability of safe water for human consumption (UNEP, 2006).

In spite of the essential role played by water in supporting human life, it also has great potential for transmitting a wide variety of diseases and illnesses (Hutton, 1983). Indeed, understanding the impacts of contaminants on the environment, including the organisms which live in it, is rather complicated (Iscan, 2004).

The world's rivers are so badly affected by human activity that the water security of almost 5 billion people and the survival of thousands of aquatic species are reportedly threatened (Vaughan, 2010). Even the world's great rivers, such as the Yangtze, the Nile and the Ganges, are reportedly suffering serious biodiversity and water security stress (Vaughan, 2010).

It has been suggested that water pollution is the leading cause of deaths and diseases, worldwide (Pink, 2006). Disposing of sewage waste is a major problem with billions of people on the planet. According to 2013 figures from the World Health Organization (WHO), some 780 million

people (11 percent of the world's population) have no access to safe drinking water, while 2.5 billion (40 percent of the world's population) have no proper sanitation (hygienic toilet facilities); although there have been great improvements in securing access to clean water, relatively little progress has been made on improving global sanitation in the last decade. Sewage disposal affects people's immediate environments and leads to water-related illnesses such as diarrhea that kills 760,000 children under five each year (WHO, 2013). It has been reported that more than one-third of the global population (some 2.4 billion people) do not use improved sanitation facilities; of these, one billion people still practice open defecation (UNICEF/WHO, 2015).

Rapid increase in industrial development has created more awareness in interrelationship between pollution, environment and public health. Industrial development results in the generation of industrial effluents, and if untreated results in water, sediment and soil pollution. (Fakayode and Onianwa, 2002; Fakayode, 2005). Industrial wastes and emission contain toxic and hazardous substances, most of which are detrimental to human health (Ogunfowokan *et al.*, 2005; Jimena *et al.*, 2008; Rajaram *et al.*, 2008). Heavy metals from industrial processes are of special concern because they pollute water and cause chronic poisoning to aquatic animals (Ellis, 1989). While some heavy metals are purely toxic with no cellular role (Shi *et al.*, 2002), other metals are essential for life at low concentrations but become toxic at high concentrations (Badar *et al.* 2000). It is recognized that a considerable quantity of industrial, domestic and transportation wastes and by-products end up in waterways. The results had devastating consequences on environmental and biological systems hence it is important to constantly assess the risk factors on the ecosystem (Iscan, 2004).

Sokoto is a cosmopolitan town supposed to have regular potable water supply. Unfortunately, potable water supply is not only irregular but does not reach a large percentage of the populace.

This irregularity in potable water supply must have prompted the populace to seek for alternative sources of drinking water by resorting to well, sachet and even river water in order to meet their domestic water needs.

## **1.2 Wastewater and water pollution**

Industries are the major sources of pollution in all environments. Depending on the type of industry, various levels of pollutants can be discharged into the environment directly or indirectly through public sewer lines. Wastewater from industries includes employees' sanitary waste, process wastes from manufacturing, wash waters and relatively uncontaminated water from heating and cooling operations (Glyn and Gary, 1996).

Effluents from waste water treatment plants usually end up in surface water streams. These effluents usually contain small amounts of various contaminants but these harmful components accumulate over time in the river, especially in sediments (Cotmanet *al.*, 2001). High levels of pollutants in river water systems cause increase in biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), toxic metals such as Cd, Cr, Ni and Pb (Fakayode, 2005) and fecal coliform and hence make such water unsuitable for drinking, irrigation and aquatic life.

It is estimated that 5-20% of total water is used by industry (UNESCO, 2009). Industrial wastewaters range from high biochemical oxygen demand (BOD) from biodegradable wastes such as those from human sewage, pulp and paper industries, slaughter houses, tanneries and chemical industry. Others include those from metal coating shops and textiles, which may be toxic and require on-site physiochemical pre-treatment before discharge into municipal sewage system (Phiriet *al.*, 2005; UNESCO, 2009; Walakira and Okot-Okumu, 2011; Hussain and Prasad Rao, 2013).

### **1.2.1 People and water pollution**

Historically, the availability of water has long been an important factor in citing of human settlement and their development into towns and cities and the development of great civilizations. The Egyptians civilization flourished around the river Nile. In Nigeria, cities like Kaduna, Lagos, Makurdi and Aba depend very much on its rivers for various purposes. However, the rush by developing countries to industrialize has resulted in discharge of partially treated or raw wastes into the surrounding bodies of water since the development of treatment facilities cannot keep pace with the rate at which the wastes are generated by industries (Nwachukwuet *al.*, 1989). Industrial discharges, therefore contribute a larger portion of the flow of rivers during dry season, with the result that the water quality of rivers is further deminished.

The bacteria-laden river water in contact with the body during use may cause serious hazards to users due to bacteria contamination. Many bodies of water in Nigeria experience seasonal fluctuations, leading to a higher concentration of pollutants during the dry season when effluents are least diluted (Kanuet *al.*, 2006).

Historically, landfills have been the simplest form of eliminating urban solid wastes with minimum cost. They have been the most usual method for discarding solid wastes. However, landfills are considered authentic biochemical reactors that introduce large amounts of contaminants into the environment in the form of gas and leachates (Nolascoet *al.*, 2008).

Over the years, a considerable population growth has taken place in many African countries leading to large increase in urbanization, industrial and agricultural land use. Large quantity of pollutants of various types were thus discharged into receiving water bodies with resultant adverse effects on fisheries and other aquatic animals (Saadet *al.*, 1984).

### **1.3 Statement of Research Problem**

One of the most critical problems of developing countries is improper management of vast amount of wastes generated by various anthropogenic activities. More challenging is the unsafe disposal of these wastes into the ambient environment. Water bodies especially freshwater reservoirs are the most affected. This has often rendered these natural resources unsuitable for both primary and/or secondary usage (Fakayode, 2005).

Industrial effluent contamination of natural water bodies has emerged as a major challenge in developing and densely populated countries like Nigeria. Estuaries and inland water bodies, which are the major sources of drinking water in Nigeria, are often contaminated by the activities of the adjoining populations and industrial establishments (Sangodoyin, 1995). This is because river waters are the primary means for disposal of waste, especially the effluents, from closely located industries. These effluents pollute the water body by alteration of their physical, chemical and biological properties (Sangodoyin, 1991).

Water bodies are major receptacles of treated, untreated, or partially treated industrial wastes. The resultant effects on public health and the environment are usually great in magnitude (Osibanjo *et al.*, 2011).

Ekhaise and Anyasi (2005) had reported the unacceptable level of contamination of Ikpoba River, Benin city, due to brewery effluent discharge with the attendant hazard to human health. Similarly Ogedengbe and Akinbile (2010) reported the adverse effects of agro-industrial effluents from the factories in Oluyole Estate on the quality of Ona stream water. The downstream Asa River in Ilorin was polluted and its aquatic biota was bacteriologically contaminated and rendered unsafe for human and animal consumption (Kolawole *et al.* 2011). Tytler (2011) also reported that the two drainage streams which passed through locations in which several industries and human habitat are situated had negatively impacted the overall quality of River

Kaduna, bacteriologically, physicochemically and elementally. Rajiet *al.* (2010) earlier reported the microbial contamination of River Sokoto by pathogenic organisms.

River Sokoto is situated adjacent to the industrial area of the metropolis where industries such as Cement, Aluminium, Fertilizer, Foam and Tanning factories are located. The survey of the study area indicated that these factories discharge their effluents into the environment, which end up flowing into the river. The bank of River Sokoto harbours irrigation plots used for vegetable and other sundries farming within the metropolis. The waters of the river are also commonly used for domestic and recreational purposes. A study of the river water will assist the appraisal and suitability of River Sokoto water for the desired purposes and possibly suggest viable remediation efforts.

#### **1.4 Justification for the Study**

River Sokoto is a major source of water for domestic, agricultural and industrial uses in Sokoto metropolis.

- It is the source water for the water treatment plant that supplies pipe-borne water to the people in the metropolis.
- Residents in the locality use water from the river for domestic purposes.
- The river water is also used to irrigate adjoining farmlands where for cultivation of onion, sweet potato, maize, tomatoes and vegetables (some of which are often eaten raw).
- The factories in the locality use the river as source water for various purposes (such as washing of equipment and cooling of machinery).
- The river is also dredged for sand for the local construction industry.
- River Sokoto provides recreational facility (swimming) and fishing takes place throughout the year,

All these could pose serious health and environment hazards to the community if not addressed. The study will benefit residents by creating awareness on the bacteriological, physicochemical and biological elemental qualities of River Sokoto with a view to having good quality water.

### **1.5 Aim and Objectives of study**

Aim/Goal:

This study aims to evaluate the impact of industrial and domestic wastes on the bacteriological and physicochemical quality of River Sokoto with a view to creating awareness on the quality of the river water.

Specific objectives:

- a. Determination of the general bacteriological quality of the river water using standard methods. This included bacterial level and identification of contaminants.
- b. Determination of the antimicrobial profile (susceptibility and resistance profiles) of isolated organisms against some antibiotics and disinfectants by the agar diffusion and broth dilution methods.
- c. Determination of physicochemical characteristics such as pH, conductivity, total organic matter, BOD, OD, acidity, alkalinity and hardness of water samples from points on the river and its drainage streams during raining and dry seasons of the year using standard methods.
- d. Determination of the elemental composition of the water and bottom soil sediments using Atomic Absorption Spectroscopy (AAS).

### **1.6 Limitations and constraint**

This study is limited to assessing the bacteriological and some chemical quality of the section of River Sokoto around Sokoto Cement Factory where there are human settlements and irrigation farming.

Other Limitations include:

- Samples collection will be limited to the period from January to December, 2014.
- Effluents from the industries and factories not in operation at the time of the study will not be evaluated.
- The direct impact of the chemical and bacteriological pollution on the health of people living along the bank of the river will not be assessed.
- The influence of some elements, particularly heavy metals on the antimicrobial susceptibility profiles of the bacterial isolates will not form part of this study.

### **Constraint**

The expected constraint would be the collection of water samples during raining season when the river is full to capacity and this problem will be resolved by employing the services of residents who can paddle canoe.

## **CHAPTER TWO**

### **LITERATURE REVIEW**

#### **2.1 Water Pollution**

Water pollution is the introduction of physical, chemical, or biological agents into water that can degrade water quality and adversely affect the organisms that depend on the water. In other words, it is the physical, chemical, or biological changes in water quality that adversely affect living organisms. The quality of drinking water is an essential factor in determining human well-being. Polluted drinking water has been recognized as the cause for waterborne diseases that have claimed so many lives (Pink, 2006). Water pollution is the leading cause of death and diseases worldwide (Pink, 2006; West, 2006) and accounts for the death of 14,000 people daily (West, 2006). The problem of water pollution is worldwide affecting both developing as well as industrialized countries. In the United States, a National report on assessed waterways, classified 44% of lakes, 37% of rivers, and 32% of estuaries as polluted and unsafe for recreation due to toxic water pollutants (USEPA, 2002).

External pollutants can contaminate water sources in a number of ways, including industrial and municipal chemical or waste discharge, accidental chemical spills, or airborne deposition of particles carried by the wind (Environment Canada, 2009).

The major sources of water pollution are domestic wastes from urban and rural areas, and industrial wastes which are discharged into natural water bodies. The river and lakes near urban centres emit disgusting odours and fish are being killed in millions along the sea coasts. The flesh of some of the fish is unsafe for human consumption because of excessive levels of mercury and pesticides. The origin of these problems could be attributed to many sources and types of pollutions.

### **2.1.1 Classification of Wastewater Pollution**

There are three major sources of water pollution; namely: municipal, industrial and agricultural sources.

#### **2.1.1.1 Municipal Source of Wastewater**

Municipal wastewater contains sanitary sewage which is flushed from homes, businesses, institutions and industries through sink drains and toilets into city sewer systems. It is sometimes combined with stormwater from rain or melting snow draining off rooftops, lawns, parking lots and roads. The sewer system either takes the wastewater to a municipal wastewater treatment plant or releases it directly into a lake, river or ocean. Domestic wastewater consists of blackwater (excreta, urine and faecal sludge) and greywater (kitchen and bathing wastewater). The mix and composition will depend on the water supply and sanitation facilities available, water use practices and social norms (UN, 2015). Currently, roughly half of the world's population has no means of disposing of sanitary wastewater from toilets, and an even greater number lack adequate means of disposing of wastewater from kitchens and baths (Laugesen *et al.*, 2010). Municipal wastewater is one of the largest sources of pollution and it normally receives treatment before being released into the environment. The higher the level of treatment provided by a wastewater treatment plant, the cleaner the effluent and the lower the impact on the environment. Infection can result from direct exposure to untreated wastewater; exposure to wastewater can contaminate drinking-water, food and recreational water (UN, 2015).

#### **2.1.1.2 Industrial source of water pollution**

Among the possible classifications of industrial wastewaters, one distinguishes between diffuse industrial pollutants, such as those from mining and agri-industries, and end-of-pipe point discharges and mostly illegal discharges from tankers (UN, 2015). Industry is a huge source of water pollution. It produces pollutants that are extremely harmful to people and the environment.

It is estimated that 70% of industrial discharges in developing countries is dumped untreated (UNESCO, 2009). Many countries even lack a basic register of industrial discharges and are thus unable to quantify the problem aside from describing it as ‘bad’ or ‘severe’ or some other relative term. The number of places where industries discharge highly toxic substances using processes that are no longer used in other parts of the world raises great concern (UN, 2015). Among the pollutants from industrial sources are asbestos, lead, mercury, nitrates, phosphates, sulphur, oils and petrochemicals.

### **2.1.1.3 Agricultural source of pollution**

Agricultural water pollution is becoming a major concern not only in developed countries but also in many developing countries. The increasing use of fertilizers and pesticides and the specialization and concentration of crop and livestock production has had an increasing negative impact on water quality. Agriculture has long been recognized as an important source of non-point or diffuse water pollution. Key problems include microbial runoff from livestock or use of excreta as fertilizer. Domestic animals, such as poultry, cattle, sheep and pigs, generate 85% of the world’s animal faecal waste (Dufour *et al.*, 2012). Chemical runoff from pesticides, herbicides and other agrochemicals can result in contamination of surface and groundwater. In England, nitrate concentrations in water draining from much of the agricultural land are high, especially in arable systems which amply explain the high nitrate concentrations seen in many surface and ground waters (ADAS, 2007). While not solely attributable to agricultural pollution, nitrate is the most common chemical contaminants in the world’s groundwater and aquifers, and the mean nitrate concentrations have increased in the last decade in watersheds around the world (UNEP, 2010). A comparison of domestic, industrial and agricultural sources of pollution from the coastal zone of Mediterranean Countries found that agriculture was the leading source of

phosphorus compounds and sediment (UNEP, 2010). In Morocco, agricultural practices cause serious nitrate pollution in some areas of the country (Tagma *et al.*, 2009). The main agricultural water pollutants are nitrates, phosphorus and pesticides. Rising nitrate concentrations threaten the quality of water, while high pesticide use contributes substantially to indirect emissions of toxic substances. Increasing level of nitrates and phosphorus in surface waters reduce their ability to support plant and animal life and make them less attractive for recreation.

#### **2.1.1.4 Natural sources of water pollution**

Important natural sources of water pollution are surface run-off, seepage from ground water and swamp drainage. In urban areas, rain water is reported to be acidic (Venkateshwarlu and Satyanarayana, 2014). This is due to reaction between water droplets and atmospheric oxides of sulphur and nitrogen. The atmospheric sulphur dioxide ( $SO_2$ ) is always accompanied by a little amount of sulphur tri-oxide ( $SO_3$ ) which, under humid condition, reacts with water vapour to form sulphuric acid thus causing acid rain.

Leachates from animal excreta, decaying bodies of animals and plants, solid waste landfill sites and the decay of large quantities of organic matter in swamps or deep ponds also introduce appreciable amounts of soluble organics and microorganisms which in turn contaminate the adjacent ground water.

#### **2.1.2 Sources of water pollution**

Pollution of surface waters is often grouped into two based on their origin. These are:

##### **(a) Point Source Pollution**

This type of pollution originates from a known source e.g. industrial effluents, municipal sewage treatment plant, mining and land disposal sites.

##### **(b) Non-point Source Pollution**

This type of pollution comes from many different sources that are often difficult to identify e.g. agricultural runoff (pesticide, pathogens and fertilizer) and urban runoff.

Pollutants can be categorized into three main groups namely: biological pollutants, such as bacteria, protozoa or viruses; chemical pollutants, such as heavy metals, nutrients, pesticides and wastes and physical pollutants such as sediment, radioactive material, and heat (Alley, 2007).

### **2.1.3 Biological Pollutant**

Biological pollutant comprises of oxygen depleting substances (sewage) and pathogens. It is considered the greatest threat to human survival on earth. Each year, there are about 250 million cases of water-related diseases, which resulted in roughly 5 to 10 million deaths (Ahuja, 2009). Water containing microorganisms such as bacteria, viruses and protozoa as a result of improper treatment of effluents can cause humans and animals to become ill with gastrointestinal disorders.

Faecal coliforms and pathogenic microorganisms enter surface waters from many sources. Raw or inadequately treated sewage discharged into surface waters; excrement from wildlife; runoff from farm-animal feedlots and farmlands that have been fertilized with manure; and overflow of, and leaks from septic tanks can introduce pathogenic bacteria into surface and ground waters (Doran and Linn, 1979). The presence of micropollutants also endangers the reuse of treated wastewater, a generally proposed solution to achieve a sustainable water cycle management (Muñoz *et al.*, 2009). Humans become infected by drinking water or consuming food, including raw shellfish, contaminated with pathogens, or through recreational contact with water in form of bathing, boating, swimming, fishing (Rose *et al.*, 2001), or washing of clothes.

The use of water bodies as sinks for industrial and mining waste is a major water quality problem all over the world (Longe and Omole, 2008), and it is fatalistic to believe that water bodies have

an unlimited capacity for waste assimilation. The natural purification of polluted rivers is never fast and heavily polluted water may traverse long distances in days before a significant degree of purification is achieved (Chima *et al.*, 2010). The main contamination problems worldwide were faecal and organic pollution from untreated human waste and industrial by-products. Since the largest number of faecal coliform and faecal streptococci is always present in manure (Walley and Hawkes, 2003) then the presence of either of these microbe in a surface water sample is strong evidence of faecal contamination. The most serious water pollutions in terms of human health worldwide are pathogenic organisms such as *Pseudomonas* and *Salmonella*. The most important water-related diseases include typhoid fever, cholera, bacterial and amoebic dysentery, hepatitis, malaria, yellow fever, filariasis and schistosomiasis (Ezekwe *et al.*, 2013).

#### **2.1.3.1 Monitoring Biological Pollutants**

##### **(a) Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)**

Many microorganisms grow in the presence of organic and inorganic waste products which act as their food base. Hence, they decompose and utilize the substrates. However, when organic matters in a water body become excessive, it could lead to excessive bacterial production and consumption of dissolved oxygen that could impact the ecosystem. BOD and COD are among the parameters commonly used to measure organic pollution of a water body.

##### **(i) Biochemical Oxygen Demand (BOD)**

BOD is a measure of the amount of oxygen required by bacteria during aerobic decomposition of organic compounds in sewage. It is also represented by the amount of organic matter present in water or effluents. For more organic matter, more oxygen is required by bacteria for its decomposition. This results in release of organic nutrients in water bodies such as lakes, rivers, ponds, etc. resulting in death of fish.

The test involves filling of an air tight bottle with the water sample to the point of overflowing (Domini *et al.*, 2007). Dissolved oxygen is measured initially after dilution and after incubation at 20°C for the period of the test. It is recommended that the water should have a population of microorganisms that is capable of degrading the organic material in the water sample. Many unchlorinated domestic or industrial wastewaters have sufficient bacteria for this purpose. This population is called “the seed.” In carrying out the test, dilutions of the water samples are prepared such that the least dilution results in at least 50% dissolved oxygen depletion. The seed is also added to the actual samples and diluted so that ideally, the residual dissolved oxygen is at least 1 mg/l and the uptake at least 2 mg/l after 5 days. Since the greatest proportion of the oxygen content is depleted in 5 days, the BOD test run period is now set for 5 days, which is now denoted as BOD<sub>5</sub>.

When seed is used, the BOD<sub>5</sub> is computed as follows:

$$BOD_5 = \frac{D_1 - D_2}{P} - \frac{B_1 - B_2}{f} \dots\dots\dots\text{equation}$$

where,

*BOD*<sub>5</sub> = the Biochemical Oxygen Demand after 5 days in mg/l

*D*<sub>1</sub> = Dissolved oxygen (D.O.) of diluted sample immediately after preparation, mg/l

*D*<sub>2</sub> = D.O. of diluted sample after 5 days of incubation at 20°C, mg/l

*P* = Decimal volumetric fraction of sample used

*B*<sub>1</sub> = D.O. of seed control before incubation, mg/l

*B*<sub>2</sub> = D.O. of seed control after 5 days of incubation at 20°C, mg/l

*f* = ratio of seed in diluted sample to seed in seed control

**(ii) Chemical Oxygen Demand (COD)**

COD refers to the oxygen consumed by the oxidisable organic substances. The values of COD cannot be compared directly with that of BOD. The chemical oxidants such as potassium dichromate ( $K_2Cr_2O_7$ ) or potassium permanganate ( $KMnO_4$ ) are used to measure the level of oxidizable organic matter of water where the oxidants oxidise the constituents (or the hydrogen but not nitrogen). Then potassium iodide (KI) is added. The excess amount of oxygen reacts with KI and liberates iodine. The excess amount of oxygen liberates equal amount of iodine. By using starch indicator, iodine is titrated with sodium thiosulfate and amount is estimated (Dubey and Maheshwari, 2002). The COD (mg/l) is calculated as follows:

$$COD (mg/l) = \frac{8 C (V_B - V_A)}{V_S} \dots\dots\dots\text{equation}$$

Where,

$C$ = Concentration of titrant (mM/l)

$V_A$ = Volume (ml) of titrant used for control

$V_B$ = Volume (ml) of titrant used for water samples

$V_S$ = Volume (ml) of water sample taken.

**(iii) Total Organic Carbon and Total Carbon**

Determinations of organic carbon is of considerable value in assessing organic pollution for the investigation of polluted estuaries, tidal waters and marine (sea) water and effluents where other methods of estimating oxygen demand tend to be unreliable on account of the presence of high salt concentrations. For river water, wastewaters and effluents, however, determinations of DO, BOD and COD are sufficient and of importance (Ademoroti, 1996).

The TOC test does not provide as much information as BOD and COD tests provide. While the BOD test indicates the actual oxygen needed for biologically destroying the organics, and the

COD test, the chemical oxidation requirements for destruction, the TOC test indicates the total organic matter present and is independent of the oxidation state of the pollutant.

This test converts and removes inorganic carbon ( $\text{CO} + \text{CO}_2$ ) and measures total organic carbon by converting it into  $\text{CO}_2$  in a heated reaction chamber. The  $\text{CO}_2$  is measured using infrared methods (Alley, 2007).

An alternative determinant test to the TOC is the Total Carbon (TC) which eliminates the removal of inorganic carbon from the procedure. The problem with using a TC test as a biological treatment parameter is the presence of  $\text{CO}_2$  in the results.

### **b. Microbial Parameters**

The detection and enumeration of disease-causing organisms in surface waters is difficult, time consuming, and expensive; and for many of the pathogens, methods for their routine monitoring and isolation are nonexistent (Leonard, 2001). To predict their presence, relatively harmless organisms are required as indicators. Such indicators are required to possess the under-listed characteristics (WHO, 1996; Havelaar, 2001; Stevens *et al.*, 2003);

- Occur naturally and exclusively in the gastrointestinal tract and faeces of humans and other warm-blooded animals.
- Enter the water along with faecal materials and should be found in the presence of enteric pathogens.
- Survive longer than the enteric pathogens with which they occur
- Removable by water treatment to the same extent as pathogenic organisms, and
- Easier to isolate and identify than the enteric pathogens.

Some types of bacteria found in the gastrointestinal tracts of humans and other warm-blooded animals have traditionally been used as indicators of the occurrence of some pathogenic organisms.

***i. The coliform group***

The coliform group is made up of bacteria with defined biochemical and growth characteristics that are used to identify bacteria that are more or less related to faecal contaminants. The total coliforms represent the whole group, which multiply at optimum temperature of 37°C, while the thermotolerant coliforms are those that can grow at a higher temperature (44.2°C), of which *Escherichia coli* is an example that is specifically of faecal origin.

A finding of any coliform bacteria, whether thermotolerant or not, in water leaving the treatment works requires immediate investigation and corrective action, as this is an indication of contamination (Payment *et al.*, 2003).

***a. Total coliforms***

Coliform organisms, are not essentially (strictly) an index of faecal pollution or of health risk, but can provide basic information on source water quality. Total coliforms have long been utilized as a microbial measure of drinking water quality, largely because they are easy to detect and enumerate in water (Payment *et al.*, 2003).

They have traditionally been defined as Gram-negative, non-spore-forming rod-shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties, oxidase-negative, fermenting lactose at 35-37°C with the production of acid, gas, and aldehyde within 24-48 hours. They have also been defined based on the recognition that in order to ferment lactose, organisms must possess  $\beta$ -galactosidase activity.

Using this approach, total coliforms are defined as members of a genus or species within the family *Enterobacteriaceae* capable of growth at 37°C and possessing  $\beta$ -galactosidase.

Traditionally, total coliforms were regarded as belonging to the Genera *Escherichia*, *Citrobacter*, *Enterobacter*, and *Klebsiella*. The group includes many lactose-fermenting bacteria, such as *Enterobacter cloacae* and *Citrobacter freundii*, which can be found in both faeces and the environment (nutrient-rich waters, soil, decaying plant material) as well as in drinking water containing relatively high concentrations of nutrients. It also includes members of genera such as *Budvicia* and *Rahnella*, which are never found in mammalian faeces.

The presence of coliforms in un-piped or untreated water is tolerated because coliforms of non-faecal origin exist in natural water which is often difficult to differentiate. Coliforms are detectable by simple, inexpensive cultural methods that require basic routine bacteriology laboratory facilities, but well-trained and competent laboratory workers. They pose very little risk to the health of laboratory workers given good standards of laboratory hygiene (Payment *et al.*, 2003).

#### ***b. Thermotolerant ('faecal') coliforms***

The term 'faecal coliforms', although frequently employed, is not correct: the correct terminology for these organisms is 'thermotolerant coliforms' (Payment *et al.*, 2003). Thermotolerant coliforms are defined as the group of total coliforms that are able to ferment lactose at 44-45°C. They comprise the genus *Escherichia* and, to a lesser extent, species of *Klebsiella*, *Enterobacter*, and *Citrobacter*. Of these organisms, only *E. coli* is considered to be specifically of faecal origin, being always present in the faeces of humans, other mammals, and birds in large numbers and rarely, if ever, found in water or soil in temperate climates that has not been subject to faecal pollution (although there is the possibility of re-growth in hot environments) (Fujioka, 2001)

Thermotolerant coliforms are a less reliable index of faecal contamination than *E. coli* although under most circumstances and especially in temperate areas, in surface water, their concentrations are directly related to *E. coli* concentrations. Their use for water-quality examination is therefore considered acceptable when no other method is available. However, when methods for the simultaneous detection of thermotolerant coliforms and of *E. coli* are available, the *E. coli* detection method is preferred.

Due to high thermophilic background growth on the culture media and the potential multiplication of thermotolerant coliforms (and even *E. coli*) in the environment (Rivera *et al.*, 1988; Byappanahalli and Fujioka, 1998), the use of some microbial parameters to assess tropical source of water quality is problematic. *Clostridium perfringens* spores appear to be the most appropriate parameter for assessing faecal contamination in tropical climates (Fujioka, 2001). Thermotolerant coliforms are easily detectable and a variety of Internationally Standardised Methods and Media for their detection are available (ISO, 1990a; ISO, 1990b). These methods require basic routine bacteriology laboratory facilities and well-trained and competent laboratory workers.

## ***ii. Escherichia coli***

*Escherichia coli* is a taxonomically well-defined member of the family *Enterobacteriaceae*, and is characterised by possession of the enzymes  $\beta$ -galactosidase and  $\beta$ -glucuronidase. It grows at 44-45°C on complex media, ferments lactose and mannitol with the production of acid and gas, and produces indole from tryptophan. However, some strains can grow at 37°C but not at 44-45°C, and some do not produce gas. *E. coli* does not produce oxidase or hydrolyse urea. Complete identification of the organism is too complicated for routine use, but a number of tests

have been developed for rapid and reliable identification with an acceptable degree of accuracy (Payment *et al.*, 2003).

*E. coli* is abundant in human and animal faeces, and in fresh faeces, it may attain concentrations of  $10^9$  cfu per gram. It is found in sewage, treated effluents, and all natural waters and soils subject to recent faecal contamination, whether from humans, wild animals, or agricultural activity (Payment *et al.*, 2003).

*E. coli* is widely preferred as an index of faecal contamination. It is also widely used as an indicator of treatment effectiveness although, as with the other coliform indicators, it is more sensitive to disinfection than many pathogens (in particular viruses and protozoa). The detection of *E. coli* in water leaving a treatment works is of the same significance as any other coliform organism, but its absence does not necessarily indicate that pathogens have been eliminated (Payment *et al.*, 2003).

Since *E. coli* is indicative of recent faecal contamination, consideration should be given to whether steps need to be taken to protect consumers once there is a positive finding. In the event of more than one related sample containing *E. coli*, or the recognition of other significant features such as treatment aberrations, the issue of advice to boil water intended for drinking may be considered appropriate. However, in many instances it may be acceptable to restrict the response to the taking of additional samples and sanitary inspection in order to assist interpretation of the initial results. If the water is a treated piped supply, a positive sample suggests that a failure or ingress has occurred, such as a breakdown in disinfection, treatment before disinfection has failed, or contaminated water has entered the system. Immediate action must, therefore, be taken to discover the source of contamination and to take appropriate steps

(which will depend on the level of contamination) to protect consumers until the problem is resolved (Payment *et al.*, 2003).

### ***iii. Enterococci and Faecal Streptococci***

These are chain-forming Gram-positive cocci that used to be placed in the genus *Streptococcus*. Faecal streptococci are those streptococci generally present in the faeces of humans and animals. They all possess the Lancefield group D antigen. A sub-group of the faecal streptococci, which is relatively tolerant of sodium chloride and alkaline pH, have been grouped under the genus *Enterococcus*. Most of the *Enterococcus* species are of faecal origin and can generally be regarded as specific indices of human faecal pollution for most practical purposes (Payment *et al.*, 2003).

Faecal streptococci are more resistant to stress and chlorination than *E. coli* and the other coliform bacteria (Gleeson and Gray, 1997). Although both faecal streptococci and enterococci remain in use as monitoring parameters in drinking water, enterococci appear likely to supplant faecal streptococci as the parameter of choice as they are clearly of faecal origin from warm blooded animals. Enterococci, as an index of faecal pollution, can also be used to complement *E. coli* in catchment assessment, in tropical climates (where *E. coli* is less appropriate because of the suspicion of multiplication) and in ground water source evaluation. Enterococci can also serve as an additional indicator of treatment efficiency. They are highly resistant to drying and thus may be valuable for routine control after new mains are laid or distribution systems are repaired, or for detecting pollution of groundwater or surface waters by surface run-off. In the UK, they have been used to assess the significance of doubtful results from other organisms (Gleeson and Gray, 1997).

#### ***iv. Bacteriophages***

Bacteriophages (also known simply as phages) are viruses that only infect bacteria. Some bacteriophages are comparable in size and behaviour to human enteric viruses and they are relatively easy to detect and enumerate. Various groups and types of bacteriophage, particularly those of coliform bacteria (coliphages) and those of *Bacteroides* spp., have been proposed as indices of faecal pollution (and possible enteric virus presence) and as indicators of treatment efficiency for both water and wastewater-treatment processes (Sobsey *et al.*, 1995; Grabow, 2001). The frequency of occurrence of coliphages in human and animal faeces varies, and sometimes they are detected in faeces at only low frequencies. In this respect, coliphages differ from bacterial indices of faecal contamination.

*Bacteroides* spp. outnumber the coliform group in human faeces (Gleeson and Gray, 1997), with *Bacteroides fragilis* being the most commonly found species. They are strict anaerobes and have not been shown to multiply in the environment. Bacteriophages of *Bacteroides* have been proposed as an index of faecal pollution as they are considered to be more resistant to natural inactivation and water treatment processes than bacterial indicators and have a decay rate similar to that of human enteric viruses. The draw-backs, however, are their low densities in raw waters (requiring concentration from large volumes) and the unreliable methods of detecting them in water. However, they have been found to be unreliable indicators of the presence of enteric viruses in water, because some coliphages can multiply in the aquatic environment (Seeley and Primrose, 1982; Borrego *et al.*, 1990). Moreover, enteric viruses have been found in water where coliphages were not found (Morinigo *et al.*, 1992). Coliphages are detectable by simple, inexpensive and rapid methods that can be applied in a basic routine bacteriology laboratory. *Bacteroides* bacteriophages, however, require facilities for anaerobic culture and require a greater

degree of expertise and laboratory resources. Some Internationally Standardised Methods exist (ISO, 1986a; ISO, 1986b; ISO, 1990a; ISO, 1990b). They are generally not considered to be a health risk for laboratory workers, although some of the host bacterial strains may be opportunistic pathogens (Payment *et al.*, 2003).

A technique has been developed to use bacteria of the Bacteroides–Prevotella group as indicators of faecal pollution of water and marker organisms for tracking the sources of pollution (Bernhard and Field, 2000a; Bernhard and Field, 2000b). This method involves amplification of target DNA by PCR with primers specific for Bacteroides–Prevotella group, followed by terminal restriction fragment length polymorphism (t-RFLP) and length heterogeneity PCR fingerprinting of the community. The fingerprints obtained clearly differentiate between human- and cattle derived assemblages of those organisms, and between water samples contaminated with human and animal faeces, allowing in-situ source identification. On the other hand, this technique does not allow one to assess the degree of contamination as it only produces, like most PCR-based techniques, a signal indicating microbial community composition and not microbial abundance.

#### *v. Enteric viruses*

Discharges of sewage and human excreta constitute the main source of human enteric viruses in the aquatic environment and enteric viruses are always associated with human or animal faecal pollution. However, failure to detect them does not indicate the absence of faecal pollution because their occurrence in faeces is highly variable. They can survive for long periods in the environment and are quite resistant to treatment (WHO, 1996, 1997).

Their enumeration can be expensive and results can take several weeks to obtain if molecular methods are not used. Furthermore, many cannot be grown under laboratory conditions. Their detection requires a very well-equipped laboratory and highly trained personnel. In addition,

most enteric viruses are pathogenic (to human and animals), albeit at different levels of severity, and virus culture must only be carried out by suitably qualified staff in specialized laboratories with the proper bio-safety equipment and procedures (WHO, 1996, 1997).

***vi. Sulphite-reducing Clostridia and Clostridium perfringens***

Sulphite-reducing clostridia are obligate anaerobic, spore-forming organisms, of which the most characteristic, *Clostridium perfringens*, is normally present in faeces (although in much smaller numbers than *E. coli*). Except for *Clostridium perfringens*, Sulphite reducing clostridia are not exclusively of faecal origin and can be derived from other environmental sources. The spores can survive in water for very long periods and are quite resistant to disinfection. As *C. Perfringensis* faecally specific, unlike the other sulphite-reducing clostridia, it is the preferred parameter for water quality assessment. Clostridia are not, however, recommended for the routine monitoring of distribution systems because of their length of survival they may be detected long after (and far from) the pollution event, leading to possible false alarms.

The presence of *C. perfringens* in groundwaters in the absence of *E.coli* and enterococci points to pollution at some time in the past and suggests the source may be liable to intermittent contamination. Being relatively resistant to disinfection, *C. perfringens* spores must be removed by some form of filtration as terminal disinfection is unlikely to inactivate them. Their presence in finished waters, therefore, suggests deficiencies in treatment filtration processes. It has been proposed that the detection of *C. perfringens* spores in finished water may indicate the potential for protozoan cysts to have passed through the treatment process (WHO, 1996, 1997).

International Standardised Methods are available (ISO 1986a; 1986b) and methods for detection of clostridia are relatively easy to perform, even though a simple pasteurisation step is required for the enumeration of spores and strict anaerobic conditions are needed for *Clostridium*

*perfringens*. Clostridia detection only requires a basic routine bacteriology laboratory. They are not normally a health risk for laboratory workers but they can be pathogenic and if carelessly handled can give rise to food poisoning and wound infections (WHO, 1996, 1997).

**vii. *Pseudomonas aeruginosa* and *Aeromonas* spp.**

*Aeromonas* and *Pseudomonas* spp. are Gram-negative, rod-shaped, oxidase positive, non-spore-forming bacteria that are environmentally widespread, with some being opportunistic pathogens.

*Ps. aeruginosa* is commonly found in faeces, soil, water, and sewage but cannot be used as an index of faecal contamination, since it is not invariably present in faeces and sewage, and may also multiply in the enriched aquatic environment and on the surface of organic materials in contact with water. However, its presence may be one of the factors taken into account in assessing the general cleanliness of water distribution systems. Its presence may lead to deterioration in bacteriological quality, and is often associated with a rise in water temperature or low rates of flow in the distribution system, and consequent complaints about taste, odour, and turbidity (WHO, 2001).

*Aeromonas* shows no particular association with faecal pollution. Most drinking water treatment processes reduce the numbers of *Aeromonas* below detectable levels, but treated distributed water can contain larger numbers as a result of re-growth in mains and storage reservoirs. Re-growth of *Aeromonas* depends on the organic content of the water, temperature, the residence time in the distribution network and the presence of residual chlorine (WHO, 2001).

Neither *Pseudomonas* nor *Aeromonas* are indices of faecal pollution, but they may be useful in assessing re-growth in distribution systems. They are both detectable by simple and inexpensive cultural methods that can be applied in a basic routine bacteriology laboratory. They may, however, pose a health risk for laboratory workers as some strains of these bacteria are

pathogenic. *Ps. aeruginosa* is an opportunistic pathogen that mainly gives rise to superficial infections following contact with heavily contaminated water (but does not cause enteric infections by ingestion). Strains of *Aeromonas* have been implicated in enteric infection but there is no strong evidence that the strains found in water distribution systems are of these types and lead to enteric infection (WHO, 2001). *Aeromonas* strains may also cause wound infections.

#### ***viii. Protozoan parasites***

*Cryptosporidium* oocysts and *Giardia* cysts are associated with human and animal faecal sources including amphibians, birds, and mammals, although the species capable of infecting man are restricted to warm-blooded hosts. However, the failure to detect cysts or oocysts does not constitute an indication of the absence of faecal pollution, as their numbers in faeces are highly variable. They can survive for very long periods in the environment and are quite resistant to treatment. They are sometimes found in treated water, usually in low numbers, and when found in filtered supplies suggest deficient coagulation-filtration processes. Viability is difficult to assess but even if non-viable their presence is an indicator of deficient physical treatment and the potential for viable (oo)cysts to be present at some time. Continuous sampling has some value in detecting short-term perturbations in treatment. As with enteric viruses, many species are pathogenic and their isolation and enumeration is expensive and requires a very well equipped laboratory with the proper bio-safety equipment and procedures, and highly trained personnel (Payment *et al.*, 2003).

#### ***ix. Other Microorganisms***

Other microorganisms such as bifidobacteria, candida/yeasts and acid-fast bacteria, have been considered in the past as potential parameters of drinking water quality. None of these has been

widely accepted and they are not recommended as parameters for routine drinking water evaluation (WHO, 2001).

#### **x. Limitations of use of Fecal Coliforms as Indicators of Pathogenic Organisms**

- ✓ Fecal coliforms are poor indicators of the presence of pathogenic viruses and some protozoan such as *Cryptosporidium* and *Giardia* in surface waters.

Studies indicate that fecal coliforms are not reliable predictors of the presence or absence of pathogenic viruses in marine waters and shellfish (Gerbaet *al.*, 1979; LaBelle *et al.*, 1980; Goyalet *al.*, 1984; Burkhardt *et al.*, 2000). Although Mackowiak *et al.*, (1976) reported outbreaks of hepatitis A following the consumption of oysters harvested from waters approved for shellfish harvesting based on fecal coliform bacteria levels, the relationship between the presence of enteroviruses and fecal coliform bacteria has not been very strong (Lipp *et al.*, 2001).

Consumption of oysters harvested from waters approved for shellfish harvesting based on fecal coliform bacteria levels has been associated with outbreaks of hepatitis A (Mackowiak *et al.*, 1976). The poor association observed between fecal coliforms and enteric viruses and protozoa in the marine environment has been suggested to be due to the differences in their survival rates in water. In seawater, some viruses survive longer than indicator bacteria. In fact, in marine sediments where viruses can accumulate (LaBelle *et al.*, 1980), viruses can survive for several months (Goyalet *al.*, 1984). Lipp *et al.*, (2001) found that none of the indicator bacteria (faecal coliforms, enterococci, *Cl. perfringens*) was significantly associated with *Giardia* or *Cryptosporidium* in a subtropical estuary in Florida. In an estuarine system in Australia, Ferguson *et al.* (1996) noted that *Cl. perfringens* was better than fecal coliforms or fecal streptococci as indicator of fecal pollution, and it was the only indicator organism that showed significant correlation with the presence of *Giardia*.

- ✓ Faecal coliforms are not good indicators of the presence of pathogenic bacteria (e.g., *Vibrio cholerae*, *V. vulnificus*, and *V. parahaemolyticus*) that are naturally occurring in surface waters.

The occurrence, distribution, and abundance of *Vibrio* spp. are more related to physicochemical factors, such as temperature and salinity, and the abundance of some zooplanktonic organisms with which they are associated in surface waters (Colwell and Huq, 2001) than to fecal coliforms. Moreover, under some environmental conditions (e.g., low temperatures of 15°C), *Vibrio cholerae*, *V. vulnificus*, and *V. parahaemolyticus* are known to occur as viable, nonculturable forms (Oliver, 1995; Oliver *et al.*, 1995).

- ✓ False positive results of fecal coliform bacteria analysis have been obtained that may be caused by a variety of different organisms including *Klebsiella* and coliforms from sources other than humans and animals.

The reliability of using fecal coliform as an indicator of fecal contamination in tropical waters has been questioned as these bacteria can grow and multiply in the environment (Chao *et al.*, 2003).

- ✓ The number of faecal coliforms in water alone cannot be used reliably to determine the source (human or nonhuman) of indicator bacteria.

Faecal coliform/fecal streptococci (FC/FS) ratios were used for many years in an attempt to differentiate non-human and human sources of faecal coliforms. FC/FS ratios above 4.0 were used to indicate human sources, while ratios below 0.7 indicated animal sources, and ratios between 0.7 and 4 indicated a mixture of human and animal sources (Geldreich and Kenner 1969; Geldreich, 1976; Manafi, 1998).

These ratios are currently considered unreliable because some faecal coliforms multiply in effluents and in the environment especially in tropical environments. Moreover, faecal streptococci survive longer in the environment than faecal coliforms (Manafi, 1998).

Determining the source of faecal coliform bacteria is important for two reasons.

- First, it can be used to plan, reduce, or eliminate the source of pollution.
- Second, knowing the source of the fecal coliforms will enable the assessment of the extent of risks of acquiring pathogenic diseases since enteric bacteria from human sources pose a different risk than those from farm animals and wildlife (Tytler, 2011).

## **2.1.4 Chemical Pollutants**

### **2.1.4.1 Chemical Elements Found in Water and their Effects**

#### **(i) Aluminium**

Aluminium has appeared to be associated with the brain lesions characteristic of Alzheimer disease, and in several ecological epidemiological studies the incidence of Alzheimer disease has been associated with aluminium in drinking-water (Shovlin *et al.*, 1993). There is a need for further studies. However, a concentration of aluminium of 0.2 mg/litre in drinking-water provides a compromise between the practical use of aluminium salts in water treatment and discoloration of distributed water. Aluminium is present in all waters to some degree. It only represents a health hazard if there is a mishap in the water treatment process.

#### **(ii) Ammonia (NH<sub>3</sub>, NH<sub>4</sub><sup>+</sup>)**

Ammonia (NH<sub>3</sub>) gas, usually expressed as Nitrogen, is extremely soluble in water. It is the natural product of decay of organic nitrogen compounds. Ammonia finds its way to surface supplies from the runoff in agricultural areas where it is applied as fertilizer. It can also find its way to underground aquifers from animal feed lots. Ammonia is oxidized to nitrate by bacterial action. A concentration of 0.1 to 1.0 ppm is typically found in most surface water supplies, and is expressed as N. Ammonia is not usually found in well water supplies because the bacteria in the soil converts it to nitrates. The concentration of ammonia is not restricted in drinking water standards (WHO, 2003).

#### **(iii) Antimony**

Reported concentrations of antimony in drinking-water are usually less than 4 µg/litre. Estimated dietary intake for adults is about 0.02 mg/day. Where antimony-tin solder is replacing lead solder, exposure to antimony may increase in the future. The provisional guideline value for

antimony has therefore been set at a practical quantification level of 0.005 mg/litre (WHO, 2006).

#### **(iv) Arsenic**

Arsenic in drinking water is a global threat to health, potentially affecting about 140 million people in at least 70 countries worldwide (Ravenscroft *et al.*, 2008). It is considered by some researchers to have more serious health repercussions than any other environmental contaminant (Smith and Steinmaus, 2007). Arsenic occurs naturally in soils and rocks, with typical concentrations of about 2-10 mg/kg. Igneous rocks tend to have low arsenic content, while shales, coals and volcanic rocks have higher levels. Arsenic is often found near deposits of sulfide minerals and ore deposits of metals such as tin and gold. In unconsolidated sediments, arsenic is primarily found in fine fractions, associated with metal oxides (especially iron) and to a lesser degree, clay minerals.

Arsenic can occur in drinking water at levels up to several mg/L, either as the reduced species, arsenite (As III) or the oxidized form, arsenate (As V). Arsenite is uncharged under natural conditions and as such is more mobile than arsenate.

Contamination can occur in surface water, but is more common in groundwater. Rainwater contains negligible amounts of arsenic. Inorganic arsenic is acutely toxic. Large doses—far higher than are found in water—cause rapid deterioration and death. Slow exposure, as in low-level water contamination causes several long-term effects. The effects of arsenic poisoning, known as arsenicosis, can take a number of years (typically 5 – 20) to develop. Arsenic exposure via drinking water can cause cancer in the skin, bladder and kidney, as well as skin changes such as hyperkeratoses (hard patches) and pigmentation changes. It has been estimated that one in ten people who drink water containing >500 µg of arsenic per litre may ultimately die from cancers of the lung, bladder and skin (Cantor, 1997). WHO guideline value for arsenic is 0.01mg/L

**(v) Barium**

Barium occurs naturally in rock, with an average of 250 mg/kg in continental crust. It is positively charged in water ( $Ba^{2+}$ ) and typically occurs at less than 0.1 mg/L, though natural concentrations in groundwater can exceed 1 mg/L. There is no evidence that barium is carcinogenic, but chronic exposure can cause hypertension in humans. WHO has set the guideline value of 0.7 mg/L for barium (UNICEF, 2008).

**(vi) Beryllium**

Beryllium is found infrequently in drinking-water and only at very low concentrations, usually less than 1 µg/litre. Beryllium has been shown to interact with DNA and cause gene mutations, chromosomal aberrations, and sister chromatid exchange in cultured mammalian somatic cells, although it has not been shown to be mutagenic in bacterial test systems. There are no suitable oral data on which to base a toxicologically supportable guideline value. However, the very low concentrations of beryllium normally found in drinking-water seem unlikely to pose a hazard to consumers (WHO, 2003).

**(vii) Bicarbonate**

The Bicarbonate ion is the principal alkaline constituent in almost all water supplies. Bicarbonate and/or carbonate concentrations dominate the anions in surface water (Wetzel, 2001). Alkalinity in drinking water supplies seldom exceeds 300 mg/l.

**(viii) Boron**

Boron is usually present in drinking-water at concentrations below 1 mg/litre, but some higher levels have been found as a result of naturally occurring boron. The total daily intake of boron is estimated to be between 1 and 5 mg. The guideline value for boron in drinking water is 0.3 mg/litre (WHO, 2003).

**(ix) Cadmium**

Cadmium levels in drinking-water are usually less than 1 µg/litre. The daily oral intake is 10-35 µg. There is evidence that cadmium is carcinogenic by the inhalation route, but no such clear evidence by the oral route. The guideline value set for cadmium is 0.003 mg/litre (WHO, 2003).

**(x) Calcium and Magnesium**

Calcium (Ca) and magnesium (Mg) are among elements in water. Where the natural level is high (>200mg/ litre), the water is 'hard' and does not lather easily with soap. High levels are believed to be generally beneficial to health. The protective effect on the cardiovascular system may be due to the greater solubility of harmful trace elements, such as lead, in soft water (WHO, 2003).

**(xi) Chloride**

No health-based guideline value is proposed for chloride in drinking-water. However, chloride concentrations in excess of about 250 mg/litre can give rise to detectable taste in water.

**(xii) Chlorine**

Chlorine is the most commonly used agent for the disinfection of water supplies. Chlorine is a strong oxidizing agent capable of reacting with many impurities in water including ammonia, proteins, amino acids, iron, and manganese. The amount of chlorine required to react with these substances is called the chlorine demand. Chlorine in the form of a solid is calcium hypochlorite. When chlorine is added to water, a variety of chloro-compounds are formed. An example of this would be when ammonia is present, inorganic compounds known as chloramines are produced. Chlorine also reacts with residual organic material to produce potentially carcinogenic compounds, the Trihalomethanes (THM's): chloroform, bromodichloromethane, bromoform, and chlorodibromomethane. THM regulations have required that other oxidants and disinfectants be considered in order to minimize THM formation. The other chemical oxidants examined are:

potassium permanganate, hydrogen peroxide, chloramines, chlorine dioxide, and ozone. No matter what form of chlorine is added to water, hypochlorite, hypochlorous acid, and molecular chlorine will be formed, the reaction lowers the pH, thus making the water more corrosive and aggressive to steel and copper pipe (WHO, 2006).

### **(xiii) Chromium**

Chromium is a dietary requirement for a number of organisms. This however only applies to trivalent chromium. Hexavalent chromium is very toxic to flora and fauna. Chromium (III) oxides are only slightly water soluble therefore concentrations in natural waters are limited.  $\text{Cr}^{3+}$  ions are rarely present at pH values over 5, because hydrated chromium oxide ( $\text{Cr}(\text{OH})_3$ ) is hardly water soluble. Chromium is largely bound to floating particles in water.

The human body contains approximately 0.03ppm of chromium. Daily intake strongly depends upon feed levels, and is usually approximately 15-200 $\mu\text{g}$ , but may be as high as 1mg. Chromium uptake is 0.5-1%, in other words very small. The placenta is the organ with the highest chromium amounts. Chromium (III) toxicity is unlikely, at least when it is taken up through food and drinking water. Hexavalent chromium is known for its negative health and environmental impact, and its extreme toxicity. It causes allergic and asthmatic reactions, is carcinogenic and is 1000 times as toxic as trivalent chromium. Health effects related to hexavalent chromium exposure include diarrhoea, stomach and intestinal bleedings, cramps, and liver and kidney damage. Hexavalent chromium is mutagenic. The lethal dose is approximately 1-2 g. Most countries apply a legal limit of 50 ppb chromium in drinking water. A professional illness in chromium industries is chromium sores upon skin contact with chromates. Chromium trioxide dust uptake in the workplace may cause cancer, and damage the respiratory tract (WHO, 2006).

#### **(xiv) Copper**

Copper levels in drinking-water are usually low at only a few micrograms per litre. Copper is an essential element, and the intake from food is normally 1-3 mg/day. Acute gastric irritation may be observed in some individuals at concentrations in drinking-water above 3 mg/litre. Liver disease has been reported in India (Nayak and Chitale, 2013), for example, due to copper pipes leading from a well.

For drinking-water, a provisional health-based guideline value of 2 mg/litre is suggested. This concentration should also contain a sufficient margin of safety for bottle-fed infants, because their copper intake from other sources is usually low (WHO, 2003).

#### **(xv) Cyanide**

Effects on the thyroid and particularly the nervous system were observed in some populations as a consequence of the long-term consumption of inadequately processed cassava containing high levels of cyanide. A guideline value of 0.07 mg/litre is considered for cyanide in drinking water (WHO, 2003).

#### **(xvi) Fluoride**

Fluoride in most groundwaters occurs as the fluoride anion ( $F^-$ ). Waters with high fluoride content are found mostly in calcium-deficient ground waters in many basement aquifers, such as granite and gneiss, in geothermal waters and in some sedimentary basins. Groundwaters with high fluoride concentrations occur in many areas of the world including large parts of Africa, China, the Middle East and Southern Asia - India, Sri Lanka (WHO, 1996).

Fluoride is found in vegetables, fruit, tea and other crops although drinking water is usually the largest contributor to the daily fluoride intake. Fluoride is also found in the atmosphere, originating from the dusts of fluoride-containing soils, from gaseous industrial wastes, from the

burning of coal fires in populated areas and from gases of volcanic activity. Thus fluoride, in varying concentrations, is freely available in nature. The WHO guideline value for fluoride is 1.5mg/litre, with a target of between 0.8–1.2mg/l to maximise benefits and minimise harmful effects. Acceptable levels depend on climate, volumes of water intake and the likely intake of fluoride in other sources. Fluoride is sometimes added to drinking-water to prevent dental caries. Level above 1.5 mg/l will cause fluorosis which is the pitting of tooth enamel and deposits in bones (WHO, 2003).

**(xvii) Iron**

Iron is found in natural fresh waters at levels ranging from 0.5 to 50 mg/litre. Iron may also be present in drinking-water as a result of the use of iron coagulants or the corrosion of steel and cast iron pipes during water distribution. Iron is an essential element in human nutrition. In drinking-water a guideline value of about 2 mg/litre which does not present a hazard to health is suggested. No health-based guideline value for iron in drinking water is proposed (WHO, 2003).

**(xviii) Lead**

Lead is present in tap water to some extent as a result of its dissolution from natural sources, but primarily from household plumbing systems containing lead in pipes, solder, fittings, or the service connections to homes. It is a general toxicant that accumulates in the skeleton. Infants, children up to six years of age, and pregnant women are most susceptible to its adverse health effects. Lead is toxic to the central and peripheral nervous systems, its effect can be subencephalopathic neurological and behavioural. The guideline value for lead is 0.01 mg/litre (WHO, 2003).

**(xix) Manganese**

Manganese is an essential trace element with an estimated daily nutritional requirement of 30-50µg/kg of body weight. Evidence of manganese neurotoxicity has been seen in miners following prolonged exposure to dusts containing manganese. There is no convincing evidence of toxicity in humans associated with the consumption of manganese in drinking-water, but only limited studies are available. The provisional guideline value of manganese in drinking water is 0.5 mg/litre (WHO, 2003).

**(xx) Mercury**

Mercury is present in the inorganic form in surface and ground waters at concentrations usually less than 0.5µg/litre. The kidney is the main target organ for inorganic mercury, as methyl-mercury mainly affects the central nervous system. The guideline value for total mercury is 0.001 mg/litre (WHO, 2003).

**(xxi) Molybdenum**

Concentrations of molybdenum in drinking-water are usually less than 0.01 mg/litre. However, in areas near mining sites molybdenum concentrations as high as 200µg/litre have been reported. No data are available on the carcinogenicity of molybdenum by the oral route. A guideline value of 0.07 mg/litre is set for molybdenum (WHO, 2003).

**(xxii) Nickel**

The concentration of nickel in drinking-water is normally less than 0.02 mg/litre. Nickel, as both soluble and sparingly soluble compounds, is now considered as a human carcinogen in relation to pulmonary exposure. The health-based guideline value for drinking-water is 0.02 mg/litre (WHO, 2003).

**(xxiii) Nitrate and nitrite**

Naturally occurring nitrate levels in surface and ground water are generally a few milligrams per litre. In many ground waters, an increase of nitrate levels has been observed owing to the intensification of farming practice. Concentrations can reach several hundred milligrams per litre. Experiments suggest that neither nitrate nor nitrites act directly as a carcinogen in animals, but there is some concern about increased risk of cancer in humans from the endogenous and exogenous formation of *N*-nitroso compounds, many of which are carcinogenic in animals. The guideline value for nitrate-nitrogen is 10 mg/litre. However, this value should not be expressed on the basis of nitrate-nitrogen but on the basis of nitrate itself, which is the chemical entity of concern to health, and the guideline value for nitrate is therefore 50 mg/litre (WHO, 2003).

**(xxiv) Potassium**

Potassium is an alkaline metal closely related to sodium. It is seldom that one sees it analyzed separately on a water analysis. Potassium is not a major component in public or industrial water supplies. However, it is essential in a well balanced diet and can be found in fruits such as bananas.

**(xxv) Radon**

Radon occurs naturally in ground water, particularly where there are granite rocks. It is a radioactive gas, which is released from water when it comes to the surface. In rocky areas it can present in houses at high concentrations, increasing the risk of lung cancer (WHO, 2006).

**(xxvi) Selenium**

Selenium levels in drinking-water vary greatly in different geographical areas but are usually much less than 0.01 mg/litre. Selenium is an essential element for humans and forms an integral part of the enzyme glutathione peroxidase and probably other proteins as well. Most selenium

compounds are water soluble and are efficiently absorbed from the intestine. Except for selenium sulfide, which does not occur in drinking-water, experimental data do not indicate that selenium is carcinogenic. The drinking-water guideline value for selenium is 0.01 mg/litre (WHO, 2006).

**(xxvii) Silica**

Silica ( $\text{SiO}_2$ ) is an oxide of silicon, and is present in almost all minerals. It is found in surface and well water in the range of 1 - 100 mg/l. Silica is considered to be colloidal in nature because of the way it reacts with adsorbents. Silica is not listed in the Primary or the Secondary Drinking Water Standards issued by the United States Environmental Protection Agency (USEPA, 1989).

**(xxviii) Silver**

Silver has occasionally been found in ground, surface, and drinking-water at concentrations above 5µg/litre. Levels in drinking-water treated with silver for disinfection may be above 50µg/litre. The only obvious sign of silver overload is argyria, a condition in which skin and hair are heavily discoloured by silver in the tissues. The low levels of silver in drinking water, generally below 5µg/litre, are not relevant to human health with respect to argyria. On the other hand, special situations exist where silver salts may be used to maintain the bacteriological quality of drinking-water. Higher levels of silver, up to 0.1mg/litre could be tolerated in such cases without risk to health. No guideline value is proposed for silver in drinking water (USEPA, 1992).

**(xxix) Sodium**

Sodium salts are widely found in the environment. Some waters contain a naturally high level, including water reclaimed from the sea. This is an increasingly important issue for communities drawing their water supply near coasts, and for arid regions. Water with high sodium levels may raise blood pressure slightly, and this is of particular concern in people with heart, liver, kidney

and other diseases where salt intake has to be restricted. Although concentrations of sodium in potable water are typically less than 20 mg/litre, they can greatly exceed this in some countries. No firm conclusions can be drawn concerning the possible association between sodium in drinking-water and the occurrence of hypertension. Therefore, no health-based guideline value is proposed. However, concentrations in excess of 200 mg/litre may give rise to unacceptable taste (WHO, 2003).

#### **(xxx) Sulphate**

Sulphates are discharged into water in industrial wastes and through atmospheric deposition; however, the highest levels usually occur in ground water and are from natural sources. Sulphate is one of the least toxic anions; however, catharsis, dehydration, and gastrointestinal irritation have been observed at high concentrations. No guideline is proposed for sulphate. However, because of the gastrointestinal effects resulting from ingestion of drinking-water containing high sulphate levels, it is recommended that health authorities be notified of sources of drinking-water that contain sulphate concentrations in excess of 500 mg/litre (USEPA, 1989).

#### **(xxxi) Uranium**

Uranium is introduced into water supplies as a result of leaching from natural sources, from mill tailings, from emissions from the nuclear industry, from the combustion of coal and other fuels, and from phosphate fertilizers. Uranium accumulates in the kidney, and nephropathy is the primary induced effect in humans and animals. At doses that are not high enough to destroy a critical mass of kidney cells, the effect is reversible, as some of the lost cells are replaced. The WHO recommends a provisional guideline value in drinking water of 2µg/litre (WHO, 2003).

### **(xxxii) Zinc**

Zinc is an essential trace element (daily requirement for adult men = 15-20 mg/day) found in virtually all food and potable water in the form of salts or organic complexes. Although levels of zinc in surface and ground water normally do not exceed 0.01 and 0.05 mg/litre, respectively, concentrations in tapwater can be much higher as a result of dissolution of zinc from pipes. It was concluded that, the derivation of a health-based guideline value is not required at this time. However, drinking-water containing zinc at levels above 3 mg/litre may not be acceptable to consumers (WHO, 2003).

#### **2.1.4.2 Test for Chemical Constituents**

Pollution of water bodies with heavy metals from variety of sources is becoming a matter of global concern (Itah, 1998; Muhammad *et al.*, 2014). There is also concern about the possible long-term health hazards which may arise from the presence of trace concentrations of impurities in drinking water (USEPA, 2002). There are several other chemical contaminants which may be naturally occurring or man-made, having known effects on the health of the consumers.

The chemical content of drinking water can be tested following the under-listed procedures (APHA, 1998):

- i. Preliminary Treatment of Water Sample:
  - 100ml aliquot of the water sample is evaporated to dryness in a beaker of known weight.
  - 0.2g of the sediment is digested using nitric acid, perchloric acid and hydrofluoric acid mixture at a ratio of 6:1:5 and heated on a water bath until appearance of white fumes.
  - The mixture is filtered and made up to 100ml with distilled water.

- ii. Analysis of the water samples using Atomic Absorption Spectrophotometric (AAS) method or other analytical method listed below.

#### **2.1.4.3 Analytical techniques and instrumentation**

The following analytical techniques are recommended by WHO (2003).

##### ***Volumetric titration method***

In this method, chemicals are analyzed by titration with a standardized titrant. The titration endpoint is identified by either the development of colour resulting from the reaction with an indicator, change of electrical potential or change of pH value (WHO, 2003).

##### ***Colorimetric method***

Colorimetric methods are based on measuring the intensity of colour of a coloured target chemical or reaction product. The optical absorbance is measured using light of a suitable wavelength. The concentration is determined by means of a calibration curve obtained using known concentrations of the determinant (WHO, 2003).

##### ***Electrode methods***

For ionic materials, the ion concentration can be measured using an ion-selective electrode. The measured potential is proportional to the logarithm of the ion concentration (WHO, 2003).

##### ***UltraViolet (UV) absorption method***

Some organic compounds absorb ultraviolet light (wavelength; 190-380nm) in proportion to their concentration. UV absorption is useful for qualitative estimation of organic substances, because a strong correlation may exist between UV absorption and organic carbon content (WHO, 2003).

##### ***Atomic Absorption Spectrometry (AAS)***

The Atomic Absorption Spectrometry method is used for determination of metals. It is based on the phenomenon that the atom in the ground state absorbs the light of wavelengths that are

characteristic to each element, when light is passed through the atom in the vapour state. Since this absorption of light depends on the concentration of atoms in the vapour, the concentration of the target element in the water sample is proportional to the measured absorbance. There are mainly two types of AAS: Flame or Electrothermal ionization based systems (WHO, 2003).

***a. Flame AAS method***

In the Flame Atomic Absorption Spectrometry method, a sample is aspirated into a flame and atomized. A light beam from a hollow cathode lamp of the same element as the target metal is radiated through the flame and the amount of absorbed light is measured by the detector. This method is relatively highly sensitive and free from spectral or radiation interference by co-existing elements. However, it is not suitable for simultaneous analysis for many elements, because the light source is different for each target element (WHO, 2003).

***b. Electrothermal AAS method***

Electrothermal Atomic Absorption Spectrometry Method is based on the same principle as Flame Atomic Absorption Spectrometry Method but an electrically heated atomizer or graphite furnace replaces the standard burner head for determination of metals. In comparison with Flame Atomic Absorption Spectrometry, Electrothermal Atomic Absorption Spectrometry gives higher sensitivities and lower detection limits and a comparatively small sample volume. Electrothermal Atomic Absorption Spectrometry suffers from more interference through light scattering by co-existing elements and requires a longer analysis time than Flame Atomic Absorption Spectrometry (WHO, 2003).

***Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP–AES) Method***

The principle of this method for determination of metals is as follows: An ICP source consisting of a flowing stream of argon gas is ionized by an applied radio frequency. A sample aerosol is

generated in a nebulizer and spray chamber and then carried into the plasma through an injector tube. A sample is heated and excited in the high-temperature plasma. The high temperature of the plasma causes the atoms to become excited. On returning to the ground state, the excited atoms produce ionic emission spectra. A monochromator is used to separate specific wavelengths corresponding to different elements, and a detector measures the intensity of radiation of each wavelength. A significant reduction in chemical interference and multi-element determination of metals can be achieved. ICP – AES has similar sensitivity to Flame Atomic Absorption Spectrometry or Electrothermal Atomic Absorption Spectrometry (WHO, 2003).

### ***X-Ray Fluorescence (XRF) Spectroscopy***

XRF spectroscopy is an example of ICP-AES. It is the reverse of absorption spectrophotometry. With this technique, molecules are induced to emit light, which they do at energies characteristic of their structure, and at intensities proportional to the sample concentration. This method works on the principle that metallic elements emit x-rays at their characteristic energies when bombarded by a high energy x-ray source. The method is useful for both qualitative and quantitative analyses of metals (Student Encarta Encyclopedia, 2007).

### ***Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) method***

In ICP-MS, elements are atomized and excited as in ICP-AES, then passed to a mass spectrometer. Once inside the mass spectrometer, the ions are accelerated by high voltage and passed through a series of ion optics, an electrostatic analyzer (ESA), and finally a magnet. By varying the strength of the magnet, ions are separated according to mass/charge ratio and passed through a slit into the detector which records only a very small atomic mass range at a given time. By varying the magnet and ESA settings, the entire mass range can be scanned within a relatively short period of time. Like in ICP-AES, multi-element determination of metals can also be achieved (WHO, 2003).

### ***Chromatography method***

Chromatography is a separation method based on the affinity difference between two phases, the stationary and mobile phases. A sample is injected into a column, either packed or coated with the stationary, and separated by the mobile phase based on the difference in interaction (distribution or adsorption) between compounds and the stationary phase. Compounds with a low affinity for the stationary phase move more quickly through the column and elute earlier. The compounds that elute from the end of the column are determined by a suitable detector. The following are the common ones (WHO, 2003).

#### ***a. Ion Chromatography***

An ion exchanger is used as the stationary phase, and the eluent for determination of anions is typically a dilute solution of sodium hydrogen carbonate and sodium carbonate. Colorimetric, Electrometric or titrimetric detectors can be used for determining individual anions.

In suppressed ion chromatography, anions are converted to their highly conductive acid forms and in the carbonate-bicarbonate, eluent anions are converted to weakly conductive carbonic acid. The separated acid forms are measured by conductivity and identified on the basis of retention time as compared with their standards (WHO, 2003).

#### ***b. High-Performance Liquid Chromatography (HPLC)***

HPLC is an analytical technique using a liquid mobile phase and a column containing a liquid stationary phase. Detection of the separated compounds is achieved through the use of absorbance detectors for organic compounds and through conductivity or electrochemical detectors for metallic and inorganic compounds (WHO, 2003).

### ***c. Gas Chromatography (GC)***

In GC, gas is used as the mobile phase and the stationary phase is a liquid that is coated either on an inert granular solid or on walls of a capillary column. When the sample is injected into the column, the organic compounds are vapourized and moved through the column by the carrier gas at different rates depending on differences in partition coefficients between the mobile and stationary phases. The gas exiting the column is passed to a suitable detector such as Flame Ionization (FID), Electron Capture (ECD) and Nitrogen-phosphorus. Since separation ability is good in this method, mixtures of substances with similar structure are systematically separated, identified and determined quantitatively in a single operation (WHO, 2003).

### ***d. Gas Chromatography (GC)/Mass Spectrometric (MS) Method***

GC/MS method is based on the same principle as the GC method, using a mass spectrometer (MS) as the detector. As the gas emerges from the end of the GC column opening, it flows through a capillary column interface into the MS. The sample then enters the ionization chamber where a collimated beam of electrons impact the sample molecules causing ionization and fragmentation. The next component is a mass analyzer, which uses a magnetic field to separate the positively charged particles according to their mass. Several types of separating techniques exist and the most common are quadrupoles and ion traps. After the ions are separated according to their masses, they enter a detector (WHO, 2003).

### ***e. Purge and Trap Packed – Column Gas Chromatography (GC)/Mass Spectrometric (MS) Method***

This method is applicable to the determination of various purgeable organic compounds that are transferred from the aqueous to the vapour phase by bubbling purge gas through a water sample at ambient temperature. The vapour is trapped with a cooled trap. The trap is heated and

backflushed with the same purge gas to desorb the compounds onto a gas chromatographic column. It uses the same principles as in GC or GC / MS (WHO, 2003).

### **2.1.5 Physical Pollutants**

Physical pollutants are products of pollution caused by colour (change), suspended solids, foaming, temperature conditions or radioactivity. Physical pollutants include:

- suspended solids
- immiscible liquids
- discharges that result in changes to the temperature or flow rate of the receiving water
- substances that impart a taste, odour or colour to the water.

#### **Suspended solids**

Various industries produce suspended material (or particulates) in their effluents, and this has several consequences. All solids tend to reduce light penetration, so the growth of plant life in watercourses is inhibited. This will have secondary effects on food chains. One of the most important effects on animals is the damage to fish gills. Prolonged exposure to high levels of suspended solids ( $50 \text{ mg l}^{-1}$  and above) is likely to lead to sublethal changes due to respiratory distress, and adverse growth and development (Au *et al.*, 2004). Some suspended solids can also cause harmful effects when soluble toxic components present in them are dissolved into the water by biological or chemical action.

#### **Immiscible liquids**

Immiscible liquids may be present as oils, greases or tarry substances, often in the form of an emulsion (a colloidal suspension of one liquid in another, as in mayonnaise). They may affect turbidity in the same way as suspended solids. However, emulsions are not likely to settle to the bed of the river. Frequently they float on the surface and adhere to vegetation at the waterline.

Some immiscible liquids are decomposed slowly by aquatic microorganisms. Many oils and tars are slightly soluble in water and thereby impart tastes and odours to it. Oil is generally less dense than water and will spread over the surface to form an extremely thin, often visible film; a small quantity of oil is therefore likely to pollute a large area. Oil is one of the more serious pollution problems. As an example, there are now around 3000 pollution incidents involving oil and fuels every year in England and Wales (Environment Agency, 2013).

### **Discharges contributing to a temperature change**

Industrial effluents are frequently discharged at temperatures different from those of the receiving river. Almost invariably the effluent is warmer than the river, since water is widely used for carrying away heat. A small increase in the temperature of a clean, fast-flowing stream may not affect the ecosystem adversely. Provided oxygen is plentiful, plant and animal populations may be altered slightly but remain in a balanced state. Species indigenous to warmer climates may become established in a heated portion of a river. However, heated effluents are usually discharged to watercourses that are already polluted to some degree, so the polluting effects are compounded. A heightened biochemical oxygen demand (BOD) on the river water due to a sewage discharge upstream may be exacerbated by raising the temperature. Any animal or plant that dies as a result of the heat or greater oxygen deficit is decomposed by bacteria, which decrease the oxygen level even more (OU, 2016).

### **Discharges causing variations in flow rate**

Variations in the flow of a river can result from excessive abstraction or from intermittent discharges of relatively large volumes of effluent, as when settling ponds (which are used to remove particulates from effluents in the ceramic industry, for example) are emptied. There are, however, maximum limits that must be adhered to. Since the organisms that become established

in a river will be those best suited to its conditions, sudden and repeated fluctuations in the rate of flow will mean that only those organisms that can withstand the changes will survive. Plants growing in silt deposits on the bed of a stream will be destroyed when the silt is washed away by a sudden increase in flow. When the flow falls, organisms that are dependent on a high dissolved oxygen concentration will die if the river reverts to a series of near-stagnant pools(OU, 2016).

### **Substances causing taste, odour and coloration**

Very low concentrations of some chemical compounds will produce unpleasant tastes and odours, or will taint the flesh of fish living in water contaminated by them. Interaction between substances may produce tastes that are apparent at concentrations well below those at which either substance is individually detectable. An ‘antiseptic’ taste of chlorinated tap water is obvious if the raw water supply contains phenols, since this results in the formation of chlorophenols. (Phenolic compounds can occur naturally in lowland rivers). Unpleasant tastes and smells, usually earthy or sulfurous in nature, can also occur naturally from decaying vegetation. The ecological effect of colour will depend on its light-absorptive properties in relation to the spectral requirements of algae and plants (i.e. which wavelengths of light they need). Many rivers are naturally coloured (e.g. those draining peat are light brown due to humic and fulvic acids) and yet are able to support biota, including trout. The ecological effects of colour are usually minimal compared with other factors(OU, 2016).

### **Monitoring Physical Pollutants**

#### ***Rainfall events***

Rainfall events are one of the most important causes of degradation in source water quality affecting surface waters and ground waters. Rainfall drives the movement of pathogens into and through water bodies and can move soil, re-suspend sediments, cause overflow of combined and

poorly maintained sewers, degrade groundwater through infiltration and so on. Forecasting and rainfall detection systems such as radar, hydrographic monitoring equipment and remote sensing can now be used to provide authorities with advanced warnings of upcoming rainfall events that might influence water quality and treatment. Although not a measure of faecal loading, rainfall events are useful in predicting deterioration in source water quality and permit appropriate precautionary measures to be taken to safeguard treated water quality (Payment *et al.*, 2003).

### ***Flow***

Measurement of flow of surface waters as well as flow during drinking water treatment provides important information regarding the availability and production of quality water. Low flow in surface waters may lead to biological degradation and higher concentrations of pollutants due to reduced dilution of discharges. During treatment, changes in flow can adversely affect coagulation and sedimentation processes, while filtration rate and contact time with disinfectant are key factors in the production of safe drinking water. Flow is easily measured using continuous on-line measurements (Payment *et al.*, 2003).

### ***Colour***

Colour in drinking water may be due to the presence of coloured organic matter, *e.g.* humic substances, metals such as iron or manganese, or highly coloured industrial wastes. Treatment removes much of the suspended matter. Source waters high in true colour can be treated to remove colour by oxidation with ozone and adsorption onto activated carbon.

Changes in colour from that normally seen can provide warning of possible quality changes or maintenance issues and should be investigated. They may, for example, reflect degradation of the source water, corrosion problems in distribution systems, change in performance of adsorptive treatment processes (such as activated carbon filtration) and so on. It is simply and cheaply

measured using a spectrophotometer or simple colorimeter or using visual comparison with known standards (Payment *et al.*, 2003).

### ***pH***

The pH of water affects treatment processes, especially coagulation and disinfection with chlorine-based chemicals. Changes in the pH of source water should be investigated as it is a relatively stable parameter over the short term and any unusual change may reflect a major event. pH is commonly adjusted as part of the treatment process and is continuously monitored (Payment *et al.*, 2003).

### ***Solids***

Water always contains a certain amount of particulate matter ranging from colloidal organic or inorganic matter that never settles to silts, algae, plankton or debris of all kinds that can settle quite rapidly. Various methods have been devised to identify or measure these solids. In raw water storage reservoirs and other large bodies of water, discs can be used to measure the depth of water through which the disc remains visible (*i.e.* transparency). Suspended solids can be measured indirectly as turbidity.

Methods for the measurements of solids are well described (APHA, 1998) and may involve simple procedures such as filtration, evaporation and/or drying at specified temperatures, and weighing. Results are reported in mg/l. Conductivity assays can be used to reflect total dissolved solids concentrations and can be applied rapidly on-line, although conductivity mainly reflects the mineral content.

Many of the tests methods for solids are inexpensive, some can be undertaken in-field or on-line, most require average skill, and others can be performed routinely by many laboratories providing data within hours (Payment *et al.*, 2003).

### ***Turbidity***

Turbidity is a measure of suspended solids. It is probably the most generally applicable and widely used non-microbial parameter that can provide the most significant data throughout the water abstraction and treatment process. It is not associated specifically with faecal material, but increases in turbidity are often accompanied with increases in pathogen numbers, including cysts or oocysts. Turbidity is often determined by measuring the amount of light scattered by the particulate matter in the water using a nephelometer calibrated. Instruments for measuring turbidity are calibrated in Nephelometric Turbidity Units (NTU). Nephelometers are available as online continuous turbidity meters and they can provide precise data on variations in water treatment efficiency. Waterworks using filtration should be able to achieve values of 0.5 NTU or less. Regulations in various countries specify values from 0.1 to 5 NTU in final treated water (WHO, 1997).

The turbidity of water affects treatment processes and especially disinfection with chlorine-based chemicals. Turbidity of surface water sources may be heavily influenced by rainfall events or algal growth and treatment processes should be tailored to respond to such changes. Most ground waters have a relatively stable turbidity and any change reflects a major event that needs to be investigated and corrected by tailoring the treatment to the incoming water quality (Waite, 1997). Turbidity is also a good measure of the extent to which treatment processes remove suspended matters. Test methods are inexpensive, require average skill, and are performed routinely by most laboratories (WHO, 1997).

### ***Particle Size Analysis***

Particles in water are distributed over a wide range of size. Various instruments have been developed to enumerate and size particles in water. These instruments measure the passage of

particles in a sensing zone where each is counted and sized according to the electronic pulse generated. This pulse is proportional to the characteristics of the size and shape of the particle.

Particle counting can provide a general index of removal effectiveness and as such is a good quality control parameter for filtration. However, factors other than size (such as electric charge on the particles) may affect removal processes. Particle size monitors are available as on-line instruments, however, the equipment is expensive and it requires a greater level of skill than turbidity analysis (WHO, 1997).

Microscopic particulate analysis provides detailed microscopic information on the nature of particulates in water. Biological particles (cysts, diatoms, fungi, zoo-plankton and phytoplankton) and inorganic particles are described and enumerated. It is useful to identify contaminants in groundwater, providing information on the nature and likely origins of its contamination. Groundwater influenced by surface water will contain a significant amount of algae and other particles not normally found in protected groundwater. It is mainly of value as a research and investigational tool rather than for routine monitoring. The analysis requires well-trained skilled personnel, is time-consuming and is performed by few laboratories (WHO, 1997).

### ***Disinfectant Residual Concentration***

Chlorine is the most widely used disinfectant in water treatment. For the majority of bacterial pathogens, and some viruses, terminal disinfection is the critical control point of treatment and proper measurement and control of disinfectant dose and contact time (alongside pH and turbidity) is imperative. The measurements of disinfectant dose, residual obtained and the time of contact are primary data that provide a minimal level of quality control of treated water and disinfectant residual concentration during and after disinfection is a required measurement at

most water treatment works. Simple and inexpensive colorimetric tests using titration methods or kits are available for manual determination by relatively low skilled personnel (WHO, 1997).

### ***Organic Matter***

Data on the level of organic matter in treated water provide an indication of the potential for the re-growth of heterotrophic bacteria (including *Pseudomonas* and *Aeromonas*) in reservoirs and distribution systems. Organic matter can be measured as Total Organic Carbon (TOC), Biochemical Oxygen Demand (BOD) or Chemical Oxygen Demand (COD). BOD is primarily used with wastewaters and polluted surface waters, and TOC is the only parameter applicable to drinking water. Measurement of these three parameters requires basic laboratory facilities and adequately trained personnel. The TOC data provide information on the amount of matter present in the water and it may be useful to measure the amount of organic material available to support bacteriological growth (WHO, 1996, 1997).

## **2.2 Treatment of Water Pollution**

The major sources through which water gets polluted can be categorized into municipal, industrial, and agricultural. Waste water from any of these sources therefore need to be treated before it is reused, poured into a body of water or on land.

### **2.2.1 Municipal wastewater treatment**

Municipal water pollution consists of waste water from homes and commercial establishments. Including the preliminary treatment, which is done to screen out, grind up, or separate debris, the basic methods of treating municipal wastewater fall into three stages: primary, secondary and tertiary treatment.

### **a. Preliminary treatment**

As wastewater enters a treatment facility, it typically flows through a step called preliminary treatment. A screen removes large floating objects, such as rags, cans, bottles and sticks that may clog pumps, small pipes, and downstream processes. The screens vary from coarse to fine and are constructed with parallel steel or iron bars with openings of about half an inch, while others may be made from mesh screens with much smaller openings. Screens are generally placed in a chamber or channel and inclined towards the flow of the wastewater. The inclined screen allows debris to be caught on the upstream surface of the screen, and allows access for manual or mechanical cleaning. Some plants use devices known as comminutors or barminutors which combine the functions of a screen and a grinder. These devices catch and then cut or shred the heavy solid and floating material. In the process, the pulverized matter remains in the wastewater flow to be removed later in a primary settling tank (USEPA, 2004).

### **b. Primary wastewater treatment**

With the screening completed and the grit removed, wastewater still contains dissolved organic and inorganic constituents along with suspended solids. The suspended solids consist of minute particles of matter that can be removed from the wastewater with further treatment such as sedimentation or gravity settling, chemical coagulation, or filtration. Pollutants that are dissolved or are very fine and remain suspended in the wastewater are not removed effectively by gravity settling. When the wastewater enters a sedimentation tank, it slows down and the suspended solids gradually sink to the bottom. This mass of solids is called primary sludge. Various methods have been devised to remove primary sludge from the tanks. Newer plants have some type of mechanical equipment to remove the settled solids from sedimentation tanks. Some plants remove solids continuously while others do so at intervals (USEPA, 2004).

### **c. Secondary wastewater treatment**

After the wastewater has been through Primary Treatment processes, it flows into the next stage of treatment called secondary. Secondary treatment processes can remove up to 90 percent of the organic matter in wastewater by using biological treatment processes. The two most common conventional methods used to achieve secondary treatment are attached growth processes and suspended growth processes. In attached growth (or fixed film) processes, the microbial growth occurs on the surface of stone or plastic media. Wastewater passes over the media along with air to provide oxygen. Attached growth process units include trickling filters, biotowers, and rotating biological contactors. Attached growth processes are effective at removing biodegradable organic material from the wastewater. Bacteria, algae, fungi and other microorganisms grow and multiply, forming a microbial growth or slime layer (biomass) on the media. In the treatment process, the bacteria use oxygen from the air and consume most of the organic matter in the wastewater as food. As the wastewater passes down through the media, oxygen-demanding substances are consumed by the biomass and the water leaving the media is much cleaner. However, portions of the biomass also slough off the media and must settle out in a secondary treatment tank. Similar to the microbial processes in attached growth systems, suspended growth processes are designed to remove biodegradable organic material and organic nitrogen-containing material by converting ammonia nitrogen to nitrate unless additional treatment is provided. In suspended growth processes, the microbial growth is suspended in an aerated water mixture where the air is pumped in, or the water is agitated sufficiently to allow oxygen transfer. Suspended growth process units include variations of activated sludge, oxidation ditches and sequencing batch reactors. The suspended growth process speeds up the work of aerobic bacteria and other microorganisms that break down the organic matter in the

sewage by providing a rich aerobic environment where the microorganisms suspended in the wastewater can work more efficiently. In the aeration tank, wastewater is vigorously mixed with air and microorganisms acclimated to the wastewater in a suspension for several hours. This allows the bacteria and other microorganisms to break down the organic matter in the wastewater. The microorganisms grow in number and the excess biomass is removed by settling before the effluent is discharged or treated further. Now activated with millions of additional aerobic bacteria, some of the biomass can be used again by returning it to an aeration tank for mixing with incoming wastewater (USEPA, 2004).

#### **d. Tertiary or “advanced” treatment**

In some receiving waters, the discharge of secondary treatment effluent would still degrade water quality and inhibit aquatic life. Further treatment is needed. Treatment levels beyond secondary are called advanced treatment. Advanced treatment technologies can be extensions of conventional secondary biological treatment to further stabilize oxygen-demanding substances in the wastewater, or to remove nitrogen and phosphorus. Advanced treatment may also involve physical-chemical separation techniques such as adsorption, flocculation/precipitation, membranes for advanced filtration, ion exchange, and reverse osmosis. In various combinations, these processes can achieve any degree of pollution control desired. As wastewater is purified to higher and higher degrees by such advanced treatment processes, the treated effluents can be reused for urban, landscape, and agricultural irrigation, industrial cooling and processing, recreational uses and water recharge, and even indirect augmentation of drinking water supplies. Nitrogen is usually removed by nitrification followed by de-nitrification. Like nitrogen, phosphorus is also a necessary nutrient for the growth of algae. Phosphorus reduction is often needed to prevent excessive algal growth before discharging effluent into lakes, reservoirs and

estuaries. Phosphorus removal can be achieved through chemical addition and a coagulation-sedimentation process - a process used to increase the removal of solids from effluent after primary and secondary treatment. Solids heavier than water settle out of wastewater by gravity. With the addition of specific chemicals, solids can become heavier than water and will settle (USEPA, 2004).

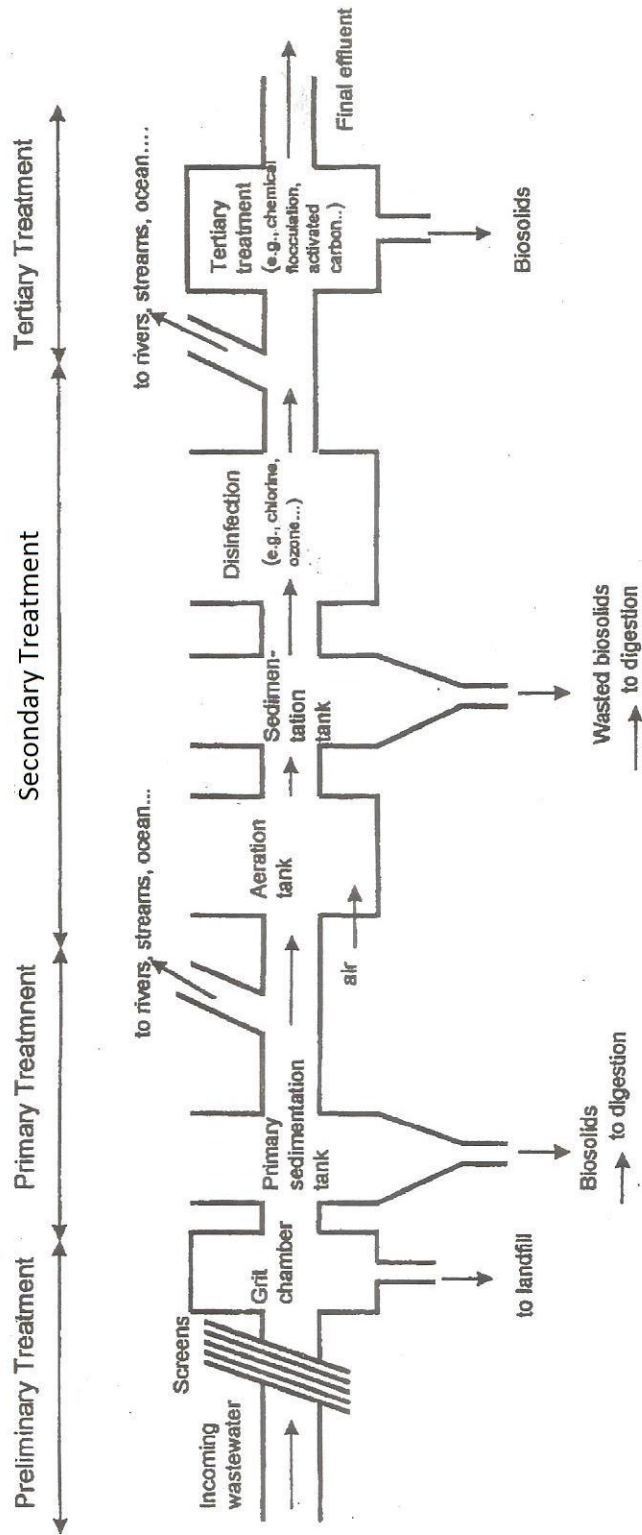


Figure 2.1: Wastewater treatment plant. Adapted from Bitton (2005).

### **2.2.2 Industrial wastewater treatment**

Industrial wastewater treatment involves all processes used in treating wastewater produced as a by-product of industrial or commercial activities. Some industries manufacture or use complex organic and inorganic chemicals and even heavy metals including pesticides, pharmaceuticals, paints and dyes, petrochemicals, detergents, plastics, paper pollution, etc. Waste waters can be contaminated by feedstock materials, by-products, product material in soluble or particulate form, washing and cleaning agents, solvents and added value products such as plasticisers. Treatment facilities that do not need control of their effluent typically opt for a type of aerobic treatment, i.e. aerated lagoons (Kashiwaya and Yoshimoto, 1980). Innovative processes for treating industrial wastewater containing heavy metals often involve technologies for reduction of toxicity in order to meet technology-based treatment standards (Barakat, 2011).

### **2.2.3 Agriculture wastewater treatment**

Non point sources (NPS) of sediment and nutrients, primarily in Agricultural runoff, have been identified as the major cause of surface water quality degradation. It was estimated that NPS pollutants account for 73 percent of the total biochemical organic demand (BOD), 83 percent of bacterial load, and 92 percent of suspended sediments in waterways in the United States (Clarke, 1993). The US EPA estimated that 57 percent of the impaired lake square miles, 64 percent of impaired river miles, and 19 percent of the impaired estuarine square miles are caused by discharges from agricultural lands (USEPA, 1987). Wastes from commercial feeders are contained and disposed off on land; their main threat to natural waters, therefore, is from runoff and leaching. Control may involve settling basins for liquids, limited biological treatment in aerobic and anaerobic lagoons, and a variety of other methods (Krantz and Kifferstein, 2005).

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1 Study Area**

Sokoto River is a tributary of the River Niger. It has its source near Funtua in the south of Katsina State. It flows north-west passing Gusau in Zamfara State. Further downstream, the river enters Sokoto State where it passes by Sokoto and is joined by the Rima River. Turning south, it flows through Birnin Kebbi in Kebbi State and about 120 kilometers south of Birnin Kebbi, it reaches its confluence with the Niger River (Hartenbach and Schuol, 2005). Sokoto metropolis has distinct weather conditions consisting of wet and dry seasons with mean annual rainfall ranging from 350mm to 670mm and temperature of 37-40°C during dry season.

The segment of River Sokoto used in the study is situated adjacent to Kalanbaina industrial area of the metropolis where industries such as cement, aluminum, fertilizer, foam and tanning factories are found. The preliminary survey of the study area showed that these factories discharged their effluents into the environment and such discharge ended up flowing into the river.

Residents along the river bank farm crops such as vegetables and use water from the river to irrigate the crops. Water from River Sokoto is also used for domestic and recreational purposes. Six sampling points were chosen namely; a point close to farmland (P1), a point about 1.5 kilometres away from P1 (P2), a point close to residents along the riverside about 2 kilometres from P2 (P3), a point on stream drainage immediately from Sokoto Cement Factory (P4), a point on the stream just about to enter the river about 1.5 kilometres from P4 (P5), and a point 2 kilometres away from P5 on the river (P6).



**Figure 3.1: Map of Study area of River Sokoto showing sampling points**  
 Source: Author's field work, 2012

## **3.2 Materials**

### **3.2.1 Test samples**

- Water samples from various points of River Sokoto and the tributary in the study area
- Bottom sediments samples from the bed of the tributary and the main river

### **3.2.2 Chemicals**

- Hydrochloric acid (BDH, England)
- Salicylic acid (BDH, England)
- Sulphuric acid (BDH, England)
- Boric acid (BDH, England)
- Barium chloride crystal (BDH, England)
- Nitric acid (BDH, England)
- Manganese sulphate (BDH, England)

#### **3.2.2.1 Reagents**

Chemical reagents used include:

- Ethidium bromide dye
- Methyl orange indicator
- Bromophenol blue indicator
- Ferroin indicator
- Potassium chromate indicator
- Phenolphthalein indicator
- Potassium dichromate standard
- Ferrous ammonium sulphate

- Ammonium molybdate reagent
- Kovac reagent
- Eriochrome black indicator
- Stannous chloride reagent
- Sodium hydroxide
- Sodium thiosulphate
- Fast Blue reagent
- Silver sulphate solution
- Phosphate buffer solution
- Hydrogen peroxide solution
- Phenol chloroform
- Absolute ethanol (100%)
- Sodium acetate
- Distilled water

### **3.2.3Antibiotics**

The following eleven antibiotic discs from Oxoid Ltd., Basingstoke, England, were used:

- Ampicillin (10µg)
- Ceftazidime (30µg)
- Cefuroxime (30µg)
- Ceftriaxone (30µg)
- Erythromycin (30µg)
- Gentamicin (10µg)

- Amoxicillin/Clavulinate (30µg)
- Cloxacillin (5µg)
- Ciprofloxacin (5µg)
- Ofloxacin (5µg)
- Nitrofurantoin (300µg)

### **3.2.4 Disinfectants**

- Dettol<sup>®</sup> (Chloroxylenol 4.8% w/v), Ricketts & Coleman, England
- Izal<sup>®</sup> (Saponated cresol 7%), Medrich Laboratories, Bangalore, India
- Savlon<sup>®</sup> (Chlorhexidine 0.3% w/v/Centrimide 3% w/v), Johnson & Johnson, England

### **3.2.5 Bacteriological Media and Other Items**

- MICROBACT 12S kit, Oxoid Ltd, Basingstoke, England
- ID 32E kit, BioMerieux, SA, France
- QIAamp DNA mini kit, Qiagen K.K, Tokyo, Japan
- Taq DNA polymerase, Courtaboeuf, France
- Primers
- DNA ladder, Alameda, USA
- Aesculin-Azide Agar, Oxoid Ltd, Basingstoke, England
- Mineral oil, Oxoid Ltd, Basingstoke, England
- Azide dextrose Broth, Oxoid Ltd, Basingstoke, England
- Brilliant Green Lactose Broth, Lab M, Bury, England
- Deoxycholate Citrate Agar, Lab M, Bury, England

- EC Medium, Lab M, Bury, England
- Endo Agar, Lab M, Bury, England
- Centrimide Agar, Lab M, Bury, England
- Selenite-F Broth, Oxoid Ltd, Basingstoke, England
- Lauryl tryptose Broth, Lab M, Bury, England
- Luria Bertani (LB) Broth, Lab M, Bury, England
- MacConkey Agar, Lab M, Bury, England
- Salmonella-Shigella Agar, Lab M, Bury, England
- Mannitol Salt Agar, Lab M, Bury, England
- Mueller-Hinton Agar, Lab M, Bury, England
- Nutrient Agar, Lab M, Bury, England
- Peptone water, Oxoid Ltd, Basingstoke, England
- Plate count Agar, Lab M, Bury, England
- Triple Sugar Agar, Lab M, Bury, England

### **3.2.6 Equipment**

- Conductivity and Temperature meter, ADWA AD 32, Mauritius
- Electronic pH meter, Jenway 3015, Staffordshire, UK
- Fast Sequential Atomic Absorption Spectrometer, Model VARIAN AA 240 FS, Australia
- Laboratory digital balance, Adams, USA
- Heating mantle, Gallenkamp, England
- Incubator, Gallenkamp, England
- Flame Photometer, Jenway FPF 7, UK

- Spectrophotometer, Optima SP 300, UK
- Vacuum pump, Edwards, England
- Centrifuge, Gallenkamp, England
- Kjeldahl apparatus, Edwards, England
- Reflux apparatus, Edwards, England
- Programmable Thermal Controller, PCT-100, M.J. Research Inc, USA
- Electrophoresis gel tank, Italy
- UV Light, Gel Doc, Italy

### **3.2.7 Glass Wares**

- BOD incubation bottles
- Burettes; 25ml and 50ml
- Volumetric flasks; 100ml, 250ml and 500ml
- Test tubes
- Durham tubes
- Eppendorff tubes
- Porcelain crucibles
- Petri-dishes
- Pasteur pipettes.

### **3.3 Methods**

#### **3.3.1 Sample Collection**

##### *a. Water Samples for Bacteriological Analyses*

Water samples for bacteriological analysis were collected in dry, heat sterilized 100ml amber bottles. Water samples at each sampling point on the river were taken at a depth of about 25cm in a counter current direction. Filled to three quarter of its capacity, the bottles were quickly covered to avoid contamination. The samples were transported to the laboratory in an ice-box for analyses within four hours of collection. This procedure was repeated at each sampling point on a monthly basis from January to December, 2014.

##### *b. Water Samples for Physicochemical Analyses*

Surface water samples for physicochemical analyses were collected mid-stream at depths 20–30 cm directly into clean 1 litre plastic bottles that were washed and rinsed with 50% nitric acid solution and further rinsed in distilled water to remove all impurities. Being filled to capacity to prevent entrapment of air, the bottles were closed and taken for analyses. Samples were collected six times, that is, in January, April, June, August, September and November.

##### *c. Samples for Elemental Analyses*

Water and bottom soil sediments were used for elemental analyses. Surface water samples were gently collected in clean plastic containers (as described in physicochemical analyses above) while the bottom samples were packed with soil sediments. Water samples were taken in August, 2014 while bottom soil sediments were taken in dry season (January, 2014) to allow access to the river bed.

### **3.3.2 Bacteriological Analyses**

Bacteriological analyses of water samples were carried out at the Veterinary Microbiology Laboratory, Usmanu Danfodiyo University, Sokoto. They included total bacteria count, coliform counts, isolation and characterization of bacteria contaminants and their susceptibilities to some antibiotics and disinfectants.

#### ***a. Preparation of Media***

Media were prepared following strictly the manufacturers' instructions as contained on the instruction's leaflets. When not needed immediately, prepared media were stored in refrigerator at 4°C.

#### ***b. Heterotrophic (Standard) Plate Count***

Heterotrophic plate count was conducted using the pour plate method as described in Standard Methods (APHA, 1998). Ten-fold serial dilution of the water samples were prepared in sterile distilled water. From  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  dilutions, 1ml were taken and transferred aseptically onto prepared and labeled sterile petri-dishes. Twelve milliliter (12ml) of sterile molten Plate Count Agar (Lab M, England) at 45°C was poured into the plates and properly mixed to ensure evenly distribution of the water samples in the agar media. Duplicates of each dilution were similarly prepared and were all allowed to set at room temperature. All plates were incubated at 35°C for 48 hours. Colonies produced on the agar were counted and the values were multiplied by the dilution factors to get the actual number in colony-forming units per milliliter (cfu/ml).

#### ***c. Coliform Counts***

Coliform counts were determined by multiple-tube dilution technique which involved three stages namely: Presumptive, Confirmed and Completed tests.

***i. Presumptive test:*** Ten-fold serial dilutions of water samples were prepared in sterile distilled water. Decimal volumes (1ml, 0.1ml and 0.01ml) of each dilution of sample was aseptically transferred to quintuplicate of 10ml sterile Lauryl tryptose broth fermentation tubes containing inverted Durham tubes and incubated at 35°C. The tubes were examined for accumulation of gas in the Durham tubes after 24 to 48 hours. Coliform organisms were presumed if gas accumulation was observed in the inverted tubes within 48 hours.

***ii. Confirmed test:*** All primary fermentation tubes showing gas accumulation after 24 to 48 hours were subjected to confirmation test. The tubes were gently shaken and one loopful of culture was transferred aseptically to a fermentation tube containing 10ml of Brilliant Green lactose broth (Lab M, England) with inverted Durham tubes. The tubes were incubated at 35°C for 48 hours. Formation of gas in the inverted tubes within 48 hours confirmed coliform bacteria.

***iii. Completed test:*** This involved double confirmation in Brilliant Green lactose broth for total coliforms and EC medium (Lab M, England) for faecal coliforms. One loopful of culture from the primary fermentation tube was again taken and placed in the Brilliant Green lactose broth and incubated at 35°C for 24 hours. At the same time, one loopful was also taken and placed in the EC medium containing inverted Durham tubes and incubated in a water bath at 44.5°C for 24 hours. Accumulation of gas in the inverted tubes after 24 hours confirmed the presence of faecal coliforms.

All brilliant green lactose broth fermentation tubes showing gas production were streaked on to Endo agar as soon as gas was produced, in a manner to obtain discretely separated colonies using the curve of a bent inoculating needle. The plates were incubated at 35°C for 24 hours. All typical colonies (golden-green, nucleated, with or without metallic sheen) were transferred into Lauryl tryptose broth and nutrient agar slant and incubated at 35°C for 24 hours. The agar slants

corresponding to the tube showing gas production after 24 hours of incubation were subjected to Gram staining and microscopic examination. Observation of Gram-negative, non-spore-forming, rod-shaped bacteria with production of gas in lauryl tryptose broth within 48 hours confirmed coliform bacteria.

#### ***d. Faecal Streptococci Count***

The multiple tube dilution technique as described in Standard Methods (APHA, 1998) was used. Serial dilutions of water samples were made from  $10^{-1}$  to  $10^{-3}$ . Decimal volumes of 1ml and 0.1ml of each dilution were aseptically transferred to quintuplicates of 10ml aliquots of sterile Azide dextrose broth (Oxoid, England) and incubated at 35°C. They were examined for turbidity between 24 to 48 hours. Tubes showing turbid growth were confirmed by streaking on Aesculin-azide agar (Oxoid, England) and incubated at 35°C for 24 hours. Any plate which showed brownish-black colonies with brown halo was further confirmed by a negative catalase test.

The estimation of coliform (total and faecal) and faecal streptococci counts was determined by making reference to standard tables for computation of Most Probable Number and reported as MPN/100ml. The value obtained was multiplied by the dilution factor to get the actual level of the bacteria in the water.

#### ***e. Selected General Biochemical Tests***

- i. Catalase test:* This test was used to differentiate between catalase producing Staphylococci and non-catalase producing Streptococci species. The procedure described by Cheesbrough (2006) was employed. 3% hydrogen peroxide solution was poured on cultures of isolates grown on agar slants. Rapid evolution of gases indicates catalase production.

- ii. *Oxidase test*: This was performed by smearing colonies of the isolates on filter paper soaked with oxidase reagent (Cheesbrough, 2006). Development of blue-purple colour within few seconds indicates positive test.
- iii. *Coagulase test*: This test differentiates between coagulase producing *Staphylococcus aureus* and non-coagulase producing members of the Staphylococci like *S. epidermidis* and *S. saprophyticus*. The slide method was employed using human plasma (Cheesbrough, 2006). With the aid of microscope, formation of floccules was observed. Only coagulase positive isolates produced clumping.

#### ***f. Isolation and Identification of selected Water-borne Bacteria***

One milliliter (1ml) of stock samples were mixed with 9ml of buffered peptone water as pre-enrichment and incubated at 35°C for 24 hours. The fresh culture was then streaked on several selective media: Mannitol Salt Agar, Cetrimide Agar, MacConkey Agar, Salmonella-Shigella Agar and Deoxycholate Citrate Agar (Lab M, England).

##### *i. Isolation of Staphylococcus species*

Isolates growing on Mannitol Salt Agar with distinct golden yellow pigmentation were sub-cultured on Nutrient agar slant for final identification using the MICROBACT 12 S identification kit (Oxoid Ltd, England).

##### *ii. Isolation of Streptococcus species*

Isolates with turbid growth in Azide dextrose broth (Oxoid Ltd, England) at 35°C for 24 hours were further streaked on Aesculin azide agar (Lab M, England) and incubated at 35°C for 48 hours. Catalase negative, brownish-black colonies with brown halo were identified as *Streptococcus faecalis* or *Enterococcus faecalis* as recently called.

*iii. Isolation of Bacillus species*

Large (4 - 10µm) isolates on heterotrophic plate count agar were Gram stained. Gram positive, spore-bearing, rod-shaped isolates were identified as *Bacillus* species.

*iv. Isolation of Pseudomonas species*

Isolates showing green or brownish pigments on Cetrimide agar were Gram stained. Those that were Gram negative were placed on Nutrient agar slant for final identification using ID 32E kit (BioMerieux, France).

*v. Isolation of Salmonella species*

One milliliter (1ml) of the pre-enriched culture was added into 9ml Selenite-F broth for selective enrichment as recommended by Kent *et al.* (1981). This was plated out on Salmonella-Shigella agar and Deoxycholate citrate agar (Lab M, England) simultaneously. All non-lactose fermenters were isolated on Nutrient agar slant for final identification using the ID 32 E kit.

*vi. Isolation of Shigella species*

Two millilitres (2ml) of the pre-enriched culture were inoculated onto 8ml Selenite-F broth and incubated at 37°C for 24 hours. A loopful of the broth culture was streaked on MacConkey agar (Lab M, England) and incubated at 37°C for 24 hours. All non-lactose fermenters were isolated on Nutrient agar slant for final identification using the ID 32 E kit.

*vii. Identification of Enterobacteriaceae and Other non-fastidious Gram negative Rods*

The ID 32 E strip, a standardized system for the identification of Enterobacteriaceae and other non-fastidious Gram-negative rods, which uses 32 miniaturized biochemical tests was used following strictly the manufacturers' procedures. Young culture (18 to 24 hours old) isolated on selective media was inoculated into 2ml of 0.85% NaCl to produce an homogenous suspension with a turbidity equivalent to 0.5 McFarland standard. Using a sterile electronic pipette, 55µl of

the suspension was carefully dispensed into each cupule of the strip. Cupules of Ornithine decarboxylase (ODC), Arginine dihydrolase (ADH), Lysine decarboxylase (LDC), Urease (URE), L-Arabitol (LARL), Galacturonate (GAT), 5 Ketogluconate (5KG) were covered with 2 drops of sterile mineral oil (Oxoid, England). The strip was labeled on the elongated flap of the strip, covered and incubated at 37°C for 24 hours in aerobic condition. A humid atmosphere was created in the incubator by placing a receptacle containing a small volume of water to prevent the tests from drying out. On removal from the incubator, 1 drop of Kovac's (James') reagent (Oxoid, England) was added to Indole (IND) cupule before reading the result on the strip. The result was recorded in the result sheet provided by the manufacturer. This was converted to 11-digit numerical figures which were fed into APIWEB (BioMerieux, France) identification software which gives the name of the organism. The outline of the process is presented in Figure 3.2.

#### *viii. Identification of Staphylococcus species*

To prepare the inoculums, 2-5 isolated colonies were picked from an 18-24 hour pure culture and emulsified in 3ml of staphylococcal suspending medium. This was mixed thoroughly to prepare a homogeneous suspension. In order to inoculate, a test strip was removed from its foil pouch and placed in the holding tray and then labeled appropriately. The lid was removed from the test strip and a sterile Pasteur pipette was used to add 4 drops (100 µl) of the bacterial suspension to each well. Well number 7, arginine was overlaid with 2 drops of Mineral Oil. The lid was replaced and the test strip incubated for 18 - 24 hours at 37°C aerobically. (To test its purity, 1 drop of the inoculum was placed onto TSA agar, a non-selective medium, and incubated at 37°C for 18 – 24 hours. Tests with growth indicating that the inoculum was not pure were repeated).

One drop of Fast Blue reagent was added to well number 12 of the test strip after incubation. Colour changes were noted and recorded on report form provided by the manufacturer of the strip. These were converted to numeric codes which were entered into Microbact software for identification interpretation. The outline of the process is presented in Figure 3.3.

METHODOLOGIE / PROCEDURE / METHODIK / TECNICA / PROCEDIMENTO /  
ΔΙΑΔΙΚΑΣΙΑ / METOD / METODE/ METODYKA

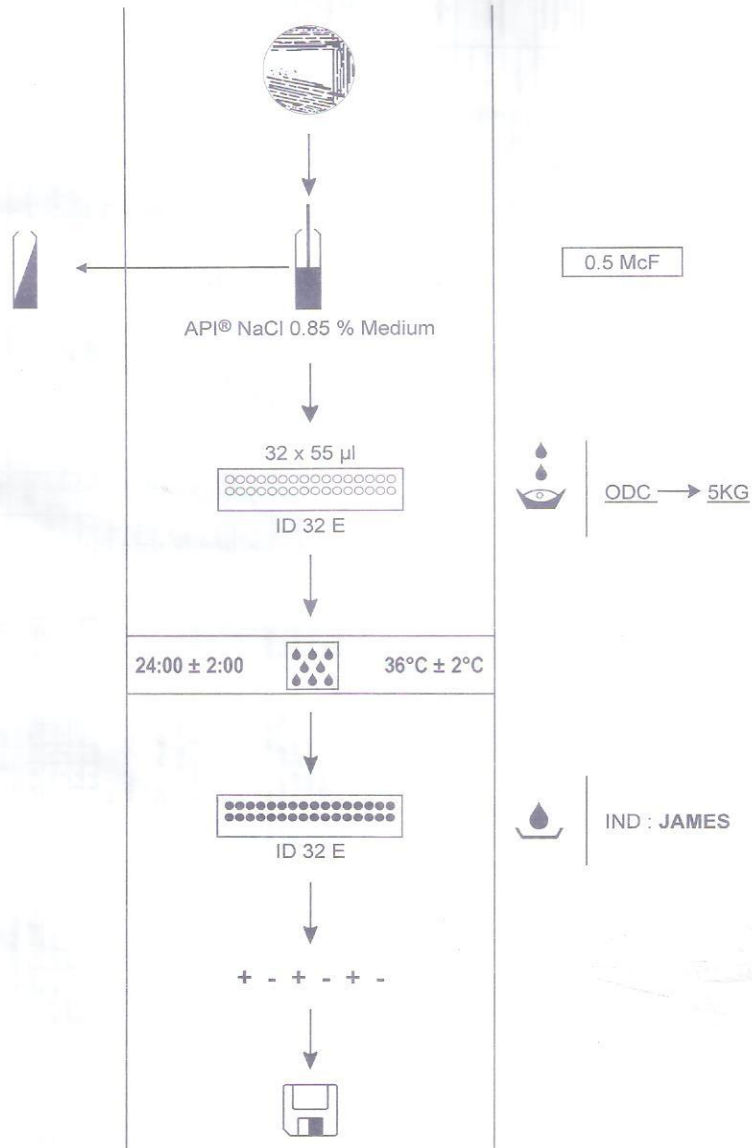


Fig. 3.2: Schematic illustration of method of identification using the ID 32 E strip (BioMérieux, SA, France)

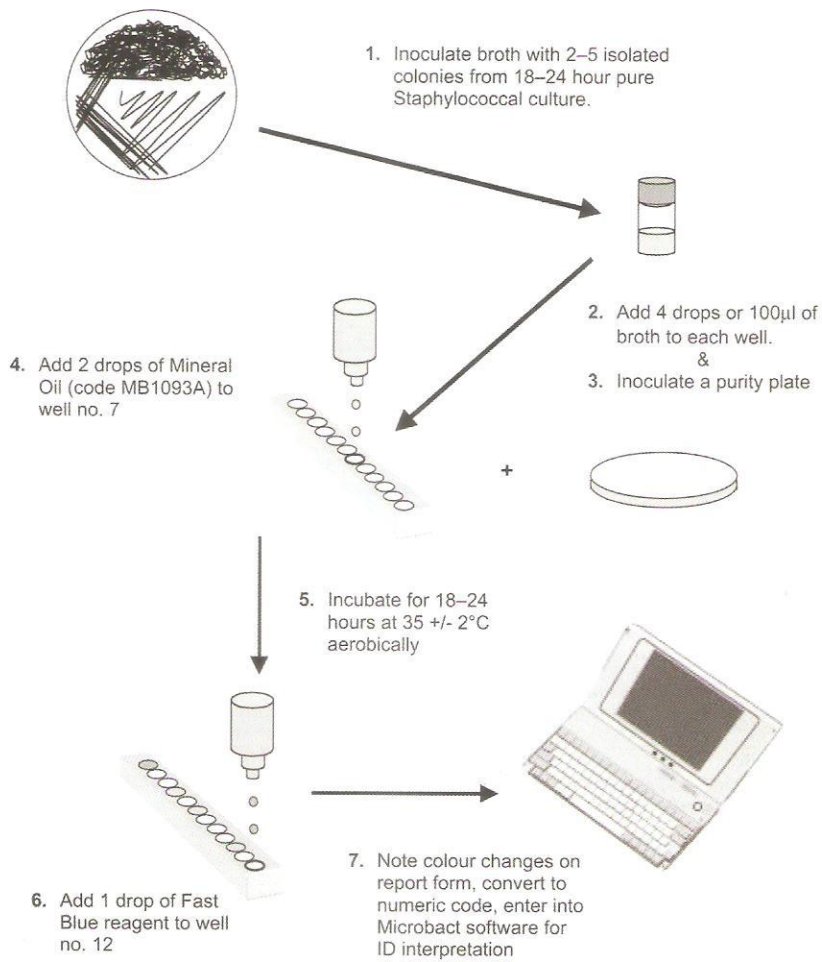


Fig. 3.3: Schematic illustration of method of identification using the Microbact Staph 12S strip (Microbact, Oxoid Ltd., Basingstoke, England).

### ***g. Determination of the Antibiotics Susceptibility Profiles of the Isolates***

The disc diffusion method described in the Clinical and Laboratory Standards Institute (CLSI, 2009) was employed for the evaluation of the susceptibilities of the isolates to various test antibiotics while the well diffusion method was employed for the disinfectants. Susceptibilities of the isolates were tested against eleven (11) antibiotics namely, ampicillin (10µg), cefuroxime (30µg), ceftazidime (30µg), ceftriaxone (30µg), erythromycin (30µg), gentamicin (10µg), amoxicillin/clavulinate (30µg), cloxacillin (5µg), ciprofloxacin (5µg), ofloxacin (5µg) and nitrofurantoin (300µg). All discs used were obtained from Oxoid Ltd (England). The disinfectants used were Dettol<sup>®</sup> (chloroxylenol, 5% v/v), IZAL<sup>®</sup> (saponated cresol, 0.5% v/v) and Savlon<sup>®</sup> (chlorhexidine/cetrimide, 6% v/v).

### ***i. Agar Disc Diffusion Test***

The procedure in the Clinical and Laboratory Standards Institute (CLSI) standard for the modified Kirby-Bauer method (CLSI, 2009) was used. Overnight cultures of the bacterial isolates on nutrient agar was suspended in sterile normal saline (0.9% w/v NaCl) and adjusted to a turbidity equivalent to 0.5 McFarland Standard. The culture was spread evenly on the surface of dried Mueller Hinton agar with sterile cotton swab. The antibiotic discs under test were placed firmly on the surface of the agar using sterile forceps. The plates were allowed to stand for an hour to enable the antibiotics diffuse into the agar and incubated thereafter at 35°C for 24 hours (Mill-Robertson *et al.*, 2003). The diameters of zone of inhibition were recorded and the result interpreted using CLSI guidelines (CLSI, 2009). Isolates were classified as;

- Sensitive, if the observed diameter of zone of inhibition was equal to or greater than the CLSI recommended diameter (mm) for sensitive category.

- Resistant, if the observed diameter of zone of inhibition was less than or equal to the CLSI resistant limit (mm) and
- Intermediate, if the observed diameter of zone of inhibition was between resistant and sensitive limits set by CLSI.

#### ***h. Determination of Multiple Antibiotics Resistance (MAR) Index***

Method of Krumperman(1983) and Paul *et al.*(1997) was employed. The Multiple Antibiotic Resistance (MAR) index was determined for each resistant bacterium isolate by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics tested.

#### ***i. Determination of Minimum Inhibitory Concentration (MIC) of the Disinfectants against the Isolates***

The MICs of the disinfectants used against the isolates were determined using the serial dilution technique(Bloomfield 1994).

Stock solutions of the disinfectants were prepared. Twelve (12) tubes containing 1ml sterile peptone water were serially arranged. Into tube 1 and 2 was introduced 1ml of the disinfectant from the stock. Serial dilution was carried out from tube 2 to the last tube in such a way that each new dilution has 50% less of the disinfectant. Standardized overnight culture of the isolates (containing approx.  $10^6$  cfu/ml) were then inoculated into the tubes and incubated at 35°C for 24 hours. Positive and negative controls were equally set up. The MICs of the disinfectants were then determined based on the patterns of growth in the tubes after incubation.

#### ***ii. Agar Well Diffusion Method***

Test organisms were streaked on Mueller Hinton agar as described in Agar disc diffusion test. Sterile cork borer No. 5 (8mm) was used to make holes on the agar with the bottom partly covered with molten agar to prevent draining of the disinfectants. Electronic micropipette was

used to dispense 40µl of the test disinfectants into the holes and allowed to diffuse for 1 hour. Controls were equally set up. All plates were incubated at 37°C for 18-24 hours.

The diameters of the zones of inhibition were measured with ruler and recorded in millimeter (mm). The zones of inhibition of concentrations equivalent to the MIC, half MIC, one and half and twice MIC were then determined on Mueller Hinton agar from which a graph of concentration against diameters of zones of inhibition were plotted. The isolates were classified as sensitive or resistant to the disinfectant if the zone of inhibition against the used concentration is greater or less than zones obtained against the MIC concentration respectively.

### **3.3.3 Molecular Analysis of resistant organisms**

The molecular characterization of resistant isolates was carried out at DNA Labs, Kaduna and it involved the following steps.

#### **Bacterial cell preparation**

Bacterial cells were prepared using the method described by Dubey (2009). Single colonies were picked from freshly grown isolates on MacConkey plate, inoculated into 5ml Luria Bertani (LB) broth medium and incubated overnight at 37°C for 18 – 24 hours. Young cells were then harvested in eppendorff tube by centrifugation at 4°C, 8000rpm (6800xg) in a refrigerated micro-centrifuge for 30 seconds. The supernatants were decanted and cells harvested.

#### **DNA extraction**

DNA extraction of the bacterial cells was carried out by using a DNA extraction kit (QIAamp DNA mini kit; Qiagen K.K., Tokyo, Japan). Heating block was first set at 60°C before starting the extraction. Into a 2ml tube was added 200µl of the bacterial cells in liquid medium. Four hundred microliter (400µl) of lyses buffer and 10µl of proteinase K were added to the sample and the tube was placed on heat block at 60°C for minimum of 1 hour. Four hundred microliter

(400 µl) of phenol chloroform (1:1) was added to the lysate and vortexed briefly. The mixture was spinned at 10000rpm for 10 minutes to separate the phases. The upper layer was carefully removed with a pipette without taking the white interphase which contained the DNA. For the second time, 400µl of chloroform was added to the remainder and vortexed briefly. The mixture was spinned again at 10000rpm for 10 minutes to separate the phases and the upper layer was carefully removed without taking the white interphase. To the remainder, was added 400µl of 100% ethanol and 20µl of 3M sodium acetate. This was mixed by inverting the tube several times and the tube was incubated at  $-20^{\circ}\text{C}$  overnight. On the following day, the tube was spinned at maximum speed for 10 – 30 minutes in refrigerated centrifuge and the ethanol was removed. Four hundred microliter (400µl) of 70% cold ethanol was added and spinned at maximum speed for 5 minutes at  $4^{\circ}\text{C}$  to precipitate the DNA because DNA is not miscible in alcohol. This step was repeated again to get more DNA precipitated and the salt totally removed. All traces of ethanol was removed by spinning the tube for 30 seconds at high speed and the DNA was dry out by leaving the tube open for 3 – 10 minutes. The pellet was re-suspended in 50µl sterile water for further analysis. Presence of DNA was confirmed by electrophoresing on an agarose gel containing ethidium bromide dye and checking under UV light.

### **Polymerase chain reaction**

Amplification of resistant DNA fragments was performed using Applied Biosystems Taq DNA polymerase (Courtaboeuf, France) which is a Multiplex PCR kit. For reaction set-up, the PCR tube was placed on an ice pack and the following items were added to each isolate for single reaction: (a) Taq polymerase master mix (b) primers (forward and reverse) (c) template DNA and (d) distilled water. Five primers with known molecular weights were used to amplify DNAs from thirteen bacterial isolates to determine which bacteria gave PCR amplicons of the same size

as the primer. Primers used in the multiplex PCR are listed in Table 3.1 below. The set-up was loaded on a PCR machine, PTC-100, Programmable Thermal Controller (M J. Research, Inc., USA). Thermocycling conditions for 35 cycles except for pre-denaturation and final extension were as follows: pre-denaturation at 95°C for 5 minutes; denaturation at 95°C for 30 seconds; optimized annealing temperature for 30 seconds at 52°C; extension for 1 minute at 72°C followed by a final extension at 72°C for 5 minutes. PCR products were resolved by gel electrophoresis. The process was repeated for other isolates.

**Table 3.1: PRIMERS FOR ANTIBIOTIC RESISTANCE GENES**

| S/<br>N | Primer<br>name | Sequence (5'→3')           | Target<br>region         | PCR<br>product<br>size (bp) | Anneali-<br>ng temp<br>(°C) | References                                | Antibiotics<br>resistance To |
|---------|----------------|----------------------------|--------------------------|-----------------------------|-----------------------------|---|------------------------------|
| 1       | TEM-F          | ATTCTTGAAGACGAAAGGGC       | <i>bla<sub>TEM</sub></i> | 1,150                       | 60                          | Belaouaj et al.,<br>(1994)                | Beta-lactams                 |
|         | TEM-R          | ACGCTCAGTGGAACGAAAAC       |                          |                             |                             |   |                              |
| 2       | <i>spvC</i> -F | ACTCCTTGCACAACCAAATGCGGA   | <i>spvC</i>              | 577                         | -                           | Ziemer and<br>Steadham (2003)             | -                            |
|         | <i>spvC</i> -R | TGTCTTCTGCATTTCCGCCACCATCA |                          |                             |                             |   |                              |
| 3       | SHV-F          | CACTCAAGGATGTATTGTG        | <i>bla<sub>SHV</sub></i> | 885                         | 52                          | Pitout et al., (1998)                     | Cephalosporins               |
|         | SHV-R          | TTAGCGTTGCCAGTGCTCG        |                          |                             |                             |   |                              |
| 4       | AacC3-F        | CACAAGAACGTGGTCCGCTA       | <i>aac(3)-III</i>        | 185                         | 60                          | Van de Klundert and<br>Vliegthart, (1993) | Gentamicin                   |
|         | AacC3-R        | AACAGGTAAGCATCCGCATC       |                          |                             |                             |   |                              |
| 5       | QnrSm-F        | GCAAGTTCATTGAACAGGGT       | <i>qnrS</i>              | 428                         | 54                          | Cattoir et al., (2007)                    | Quinolone                    |
|         | QnrSm-R        | TCTAAACCGTCGAGTTCGGCG      |                          |                             |                             |   |                              |

### **Agarose gel electrophoresis**

To confirm that DNA has actually been extracted, the DNA suspensions were subjected to agarose gel electrophoresis as follows: One percent (1%) agarose gel was prepared by dissolving 1g of agarose powder in 2ml of ten times concentration of Tris acetate ethylene diamine tetra acetate (TAE) buffer and 98ml distilled water in a 250ml beaker. This was heated in a microwave to dissolve the agarose. The gel was stained with 20 $\mu$ l of 5ml ethidium bromide dye and mixed. The gel was cast onto a gel electrophoresis tank with the combs inserted at the red bands to ensure easy view of the well and was allowed for 30 minutes to solidify. The casting combs were removed and 5 $\mu$ l of gel tracking dye (bromophenol blue) plus 15 $\mu$ l of the PCR products(making 20 $\mu$ l) were gently mixed and loaded into the well with the standard (Bioneer, 100 bp Plus DNA Ladder, Alameda, USA). The electrophoresis tank was covered and the electrodes connected to the power source for separation of sample based on molecular weight from cathode (-) to anode (+) at 100 mV for 45 minutes. At the completion of the electrophoresis, the gel was removed and viewed under a trans-illumination UV light (Gel Doc, Italy). The band pattern of the DNA fragments was then pictured and documented.

### **3.3.3 Physicochemical Analysis of the Water Samples**

Physicochemical analysis of water samples was carried out at the Central Laboratory, Usmanu Danfodiyo University, Sokoto.

#### ***a. pH Determination***

Electronic pH meter (Jenway 3015, Staffordshire, UK) with a combined electrode was used to read pH values. Known buffer solutions of pH 4 and pH 9 were prepared and used to calibrate the equipment. Having immersed the electrode in 25ml of water sample, the pH readings(2 readings per sample) of the water sample were taken after stabilization.

***b. Conductivity Measurement***

Conductivity values ( $\mu\text{S}$ ) of the water sample were measured using conductivity meter (ADWA AD 32, Mauritius). The electrode after being washed with distilled water was placed in a beaker containing 25ml of water sample. Two sets of readings were taken for each sample.

***c. Temperature Measurement***

The temperatures of the water samples were measured using a temperature measuring meter(ADWA AD 32, Mauritius). Readings were taken by dipping the electrode in the water on site and after stabilization. An average of two separate readings was taken as temperature reading per site.

***d. Determination of Total Solids***

Total Solid of the water samples was determined using weighing method (APHA, 1998). The initial weight (W1) of an evaporating dish was measured using a laboratory digital balance (Adams, USA) after being heated in a hot air oven at  $105^{\circ}\text{C}$  for 1 hour and cooled in a desiccator. 100 ml of water sample was added into the dish and boiled over a steam bath to dryness. The residue in the dish was further heated for one hour in an oven at  $105^{\circ}\text{C}$ , cooled and weighed until successive weighing gave the same result and this was recorded (W2). Total solid in the sample was calculated using the formula:

$$\text{Total Solid (mg/l)} = (W2 - W1) \times 100 \dots\dots\dots\text{equation}$$

***e. Determination of Total Suspended Solids***

Whatman filter paper rinsed in distilled water was dried in an oven at  $105^{\circ}\text{C}$  for one hour and cooled in a desiccator. Its weight (W1) was determined using a weighing digital balance. 100 ml of water sample was filtered through the paper and dried at  $105^{\circ}\text{C}$  for one hour. The weight

(W2) of filter paper containing the residue was recorded and the total suspended solids calculated using the formula: Total Suspended Solids (mg/l) = (W2 – W1) × 100.....equation

**f. Determination of Total Dissolved Solids**

Amount of total dissolved solids (TDS) of the water sample was determined by subtracting the values of the total suspended solids (TSS) from the corresponding total solids (TS) of the samples. This is represented mathematically thus:

$$\text{TDS (mg/l)} = \text{TS} - \text{TSS} \dots\dots\dots\text{equation}$$

**g. Determination of chlorides Level**

Chloride levels of the water samples were determined by argentometric method (APHA, 1998). One milliliter (1ml) of potassium chromate indicator was added into 50 ml of water sample and titrated with silver nitrate solution, until a brick red colour appeared. The blank titration was also carried out by replacing the water sample with distilled water. Chloride values were calculated using equation

$$\text{Chloride (mg/l)} = \frac{100 \times 0.05 \times (B - A)}{\text{Vol. of sample (ml)}} \dots\dots\dots\text{equation}$$

where, A = Volume of silver nitrate for water sample

B = Volume of silver nitrate for blank.

**h. Determination of Total Alkalinity**

Alkalinity values of water samples were determined by titration methods (APHA, 1998). To 50 ml of the water samples in clean 150 ml conical flask were added 3 drops of phenolphthalein indicator. The samples were titrated with 0.05M H<sub>2</sub>SO<sub>4</sub>, until the colour disappeared. To the colourless solution, 3 drops of methyl orange indicator were added and titrated further until the colour change from yellow to permanent reddish or orange red colour and the titre values were recorded and used to compute the alkalinity.

$$\text{Total Alkalinity (mg/l) CaCO}_3 = \frac{100 \times 0.05 \times A}{\text{Vol. of sample (ml)}} \dots\dots\dots \text{equation}$$

where, A = Volume of sulphuric acid used.

***i. Determination of Phosphate Content***

Phosphates were determined by colorimetric method (APHA, 1998). To 2ml aliquot of the water sample in a 25 ml volumetric flask was added one drop of phenolphthalein indicator followed by 2 ml of ammonium molybdate and then 1 ml of freshly diluted stannous chloride solution.

These were made up to 25 ml volume with distilled water and mixed thoroughly. After 5 - 6 minutes and before 20 minutes, the colour intensity (absorbance) was measured at a wavelength of 660 nm in a Spectrophotometer (OPTIMA SP 300, U.K.). Phosphate values were determined using equation:

$$\text{Phosphate (PO}_4\text{)mg/l} = \frac{A \times 0.61 \times D}{\text{Atomic weight of Phosphate}} \dots\dots\dots \text{equation}$$

where, A = Absorbance,

D = Dilution factor.

***j. Determination of Sulphate Content***

Sulphate contents of water samples were determined by Gravimetric/Turbidimetric method using BaCl<sub>2</sub> as precipitant. 50ml of the sample were measured into a 250ml beaker, and diluted to 150 ml with distilled water. 1ml HCl (concentrated) and 4 drops of methyl orange indicator were added. 10 ml of 10% Barium chloride solution were added and then boiled for 5 minutes. These were left overnight and then filtered using filter-paper. Distilled water was used to rinse the filter paper to make it free from chloride. The filter paper was dried at 80 °C in an oven, ignited at 800°C in a furnace (Lenton furnaces, England) for 1 hour, cooled in a desiccator and weighed.

The igniter cooling and weighing were repeated to give constant value. Sulphate content of the water sample was then calculated using equation:

$$\text{Sulphate (SO}_4\text{) mg/l} = \frac{\text{Weight of BaSO}_4 \times 411.5}{\text{Vol. of sample (ml)}} \dots \dots \dots \text{equation}$$

***k. Determination of Ammonia and Nitrate***

These values were determined by modified Kjeldahl method (Bremner and Mulvaney, 1982). For nitrate, 50 ml of water sample and 4 ml of salicylic acid/sulphuric acid were added in the digestion flask and swirled thoroughly to achieve homogenous mixture. 0.5 g of sodium thiosulphate was added and the mixture was heated cautiously until frothing has ceased. 1.1 g of potassium sulphate catalyst mixture was added and heated until the digestion mixture became clear. The mixture was boiled for up to 2 hours. It was ensured the temperature did not exceed 400° C. The digested mixture was allowed to cool and 20 ml distilled water was added slowly while shaking. 10 ml of boric acid was added, diluted with 20 ml distilled water and the flask was placed under the condenser of the distillation apparatus. 20 ml of sodium hydroxide was added through the funnel of the apparatus.

About 50 ml of condensate was distilled and a few drops of Boric acid indicator added. This was titrated with 0.01 mol/L sulphuric acid to a violet end point. The titre values were recorded and used to calculate nitrate content.

$$\text{Nitrate (NO}_3\text{) mg/l} = \frac{T_v \times 100}{\text{Vol. of sample (ml)}} \dots \dots \dots \text{equation}$$

where Tv = titre value

Ammonia values were similarly determined without adding Potassium sulphate catalyst mixture using:

$$\text{Ammonia (HN}_3\text{) mg/l} = \frac{T_v \times 100}{\text{Vol. of sample (ml)}} \dots \dots \dots \text{equation}$$

where Tv = titre value.

***l. Determination of Level of Hardness***

Titration method was used to determine total hardness of the water samples (APHA, 1998). The water sample was thoroughly shaken and 25 ml was taken and diluted to 50 ml with distilled water. 2 ml of Phosphate buffer solution was added to bring the pH of the water sample to 10. Three (3) drops of eriochrome black indicator was also added. This was titrated against 0.01M EDTA to a blue colour end point. Total hardness was then calculated using:

$$\text{Total Hardness (mg/l)} = \frac{T_v \times 0.01 \times 1000}{\text{Vol. of sample (ml)}} \dots \dots \dots \text{equation}$$

where, Tv = titre value and  
0.01 = EDTA Concentration.

***m. Determination of Dissolved Oxygen (DO)***

Levels of dissolved oxygen (DO) were determined using Azide modification of Winkler's method (APHA, 1998). 200 ml of water sample was carefully transferred into a 300 ml BOD bottle. 1 ml of manganese sulphate solution was added followed by 1 ml of alkaline azide reagent. The resulting mixture was titrated against 0.025 N sodium thiosulphate to the end point where there was colour change. The titre value was recorded as DO.

***n. Determination of Biochemical Oxygen Demand (BOD)***

Biochemical Oxygen Demand, the amount of oxygen required by bacteria to decompose organic matter in an aerobic condition, was determined using Azide modification of Winkler's method. The procedure in determining dissolved oxygen above was repeated and the DO recorded on day one was named Do. Another BOD bottle was similarly prepared and incubated at 20°C for 5 days in the dark. On the completion of 5 days, the bottle was decanted of water and to the precipitate

was added 2 ml of orthophosphoric acid. This was shaken gently and titrated with sodium thiosulphate to the end point where there was change in colour. The titre value was recorded as dissolved oxygen on day five ( $D_5$ ). BOD was then calculated as difference between the dissolved oxygen on day one and that on day five using the formula:

$$\text{BOD (mg/l)} = \frac{f(D_5 - D_0)}{P} \dots \dots \dots \text{equation}$$

where,  $D_0$  = dissolved oxygen of sample immediately after preparation

$D_5$  = dissolved oxygen of sample after 5 days of incubation

f = ratio of seed in diluted sample to seed in seed control

P = Decimal volumetric fraction of sample used.

***o. Determination of Chemical Oxygen Demand (COD)***

Chemical oxygen demand (COD) was determined as described in Standard Methods (APHA, 1998). To 50 ml of the water sample in a reflux flask was added 10 ml potassium dichromate solution with 1g mercuric sulphate and thoroughly mixed. Four sterile glass beads were added to control the boiling of the solution. 10 ml concentrated sulphuric acid containing silver sulphate was added carefully through the open end of the condenser and mixed by swirling. The reflux apparatus was run for 1 hour and allowed to cool. The flask was removed and its content diluted to 150 ml with distilled water. To the resulting solution was added 3 drops of ferroin indicator. This was titrated with standard ferrous ammonium sulphate to an end point where blue-green colour just changed to reddish-brown. A blank with 50 ml distilled water in place of water sample was treated equally and the chemical oxygen demand (COD) was then calculated using the equation:

$$\text{COD (mg/l)} = \frac{8C(V_B - V_A)}{V_S} \dots \dots \dots \text{equation}$$

where,  $V_A$  = volume of ferrous ammonium sulphate used for blank

$V_B$  = volume of ammonium sulphate used for water sample

$V_S$  = volume of water sample taken and

$C$  = molarity of ferrous ammonium sulphate.

### **3.3.5 Elemental Analyses of Water and Bottom Soil Sediments of River Sokoto**

Elemental analysis of the water samples and bottom soil sediments collected in clean plastic containers, from various sampling points of River Sokoto and its tributary were carried out with Atomic Absorption Spectrometer (AAS) at the Multi-User Science Research Laboratory (MUSRL), Ahmadu Bello University, Zaria, according to the method of Martin *et al.*, (1994). Water and soil sediment samples were first subjected to acidification by adding 3 drops of nitric acid to 100ml of the sample.

To determine the concentration of metal ion in the water sample, a cathode lamp with the cathode made of the metal to be tested for, emits light of a certain frequency. The light produced by the lamp passed through the water sample and vapourized. The degree of light absorption (absorbance) is proportional to the concentration of the metal in the sample and this is measured by a photomultiplier tube. The absorbance was then compared to that of a series of diluted standard solutions in order to determine the concentration by using a calibration graph which is a straight-line graph. Elements other than Na and K were determined with Fast Sequential Atomic Absorption Spectrometer, VARIAN AA 240 FS. Bottom soil sediments were also similarly analysed. Na and K were determined with Flame Photometer JENWAY, Model-FPF 7.

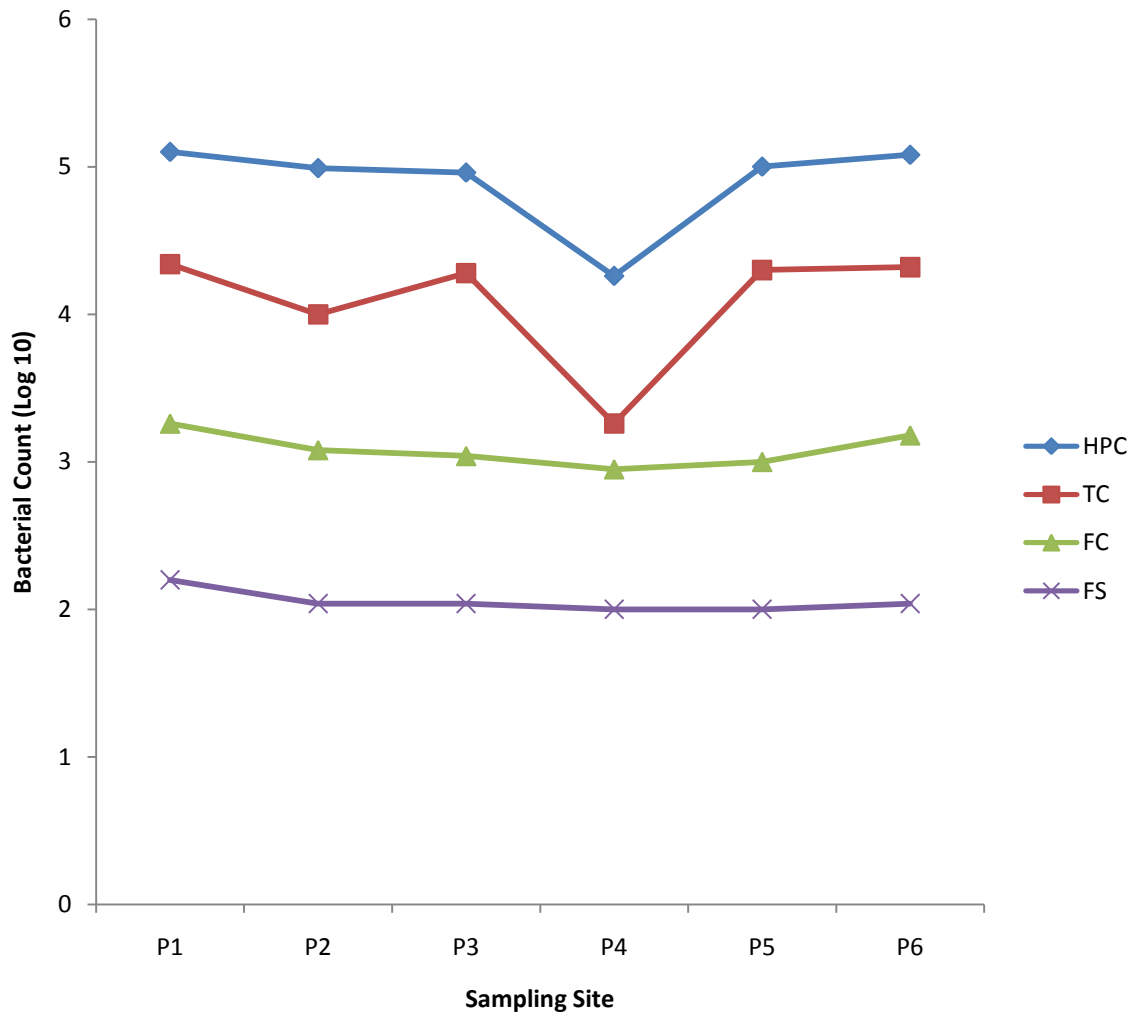
## CHAPTER FOUR

### RESULTS

#### 4.1 Bacteriological Analysis of Water Samples from River Sokoto

The heterotrophic plate counts (HPC), total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS) counts of water samples collected from River Sokoto and its drainage stream for the sampling period between January and December, 2014 are presented in Fig 4.1 – 4.4. Figure 4.1 represents bacteria counts in mid-dry season of 2013 and 2014, figure 4.2 is a plot of counts in May 2014 representing the early onset of the rain, figure 4.3 counts in mid-rain season and figure 4.4 in November 2014 are counts of the onset of dry season. They represent different periods of the two seasons (dry and rainy seasons). As shown in the figures, site P1 recorded the highest heterotrophic and total coliforms counts during the sampling periods with highest count in July, 2014. On the other hand, lowest heterotrophic and coliform counts were recorded at site P4; this is the point where effluents from Sokoto Cement Factory entered into River Sokoto.

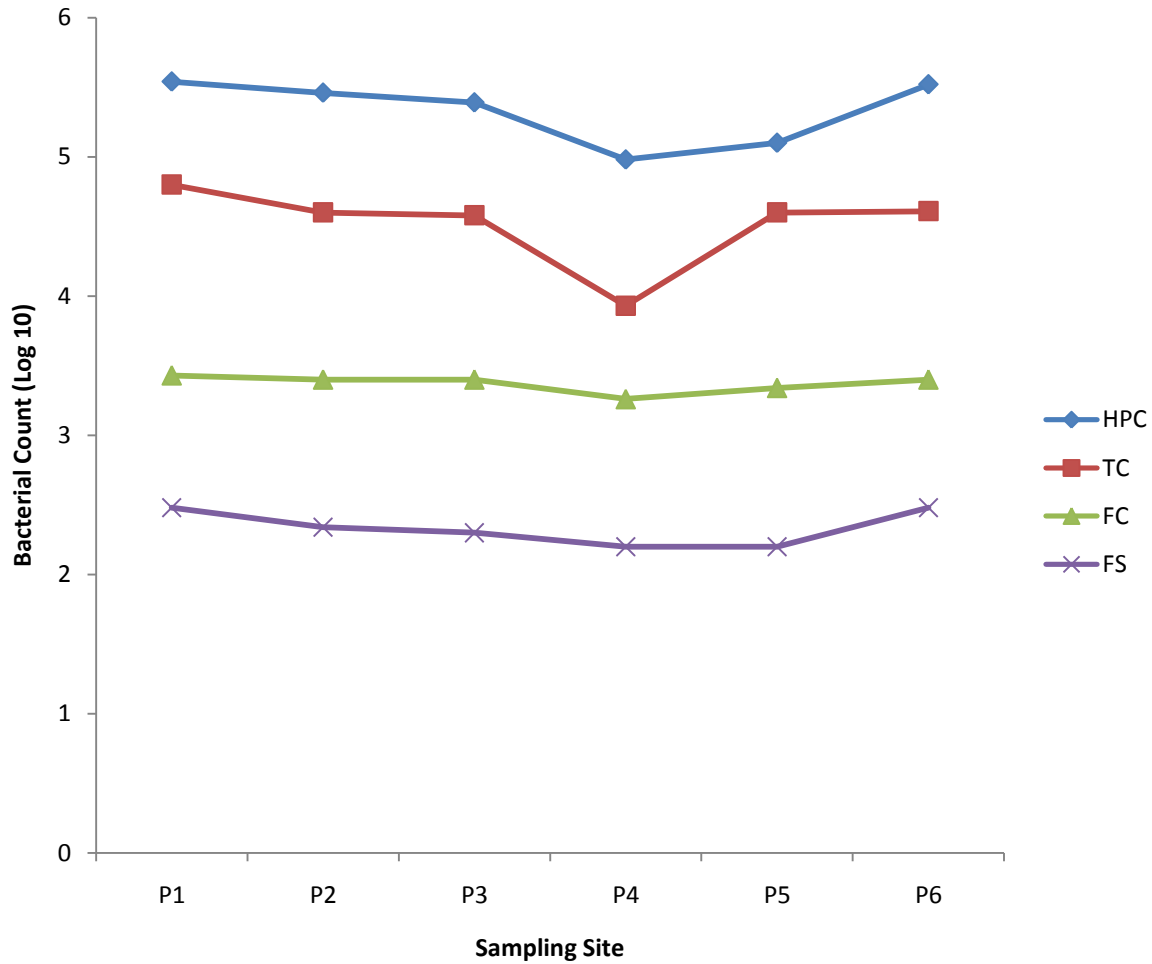
One way ANOVA Post Hoc test on Table 4.1 shows general high level heterotrophic bacterial counts. P4 samples have values significantly lower than the values in P3 used as control (site close to residents) while P1 samples relatively have values significantly higher than P3 samples through out the year.



**Key:**

- |  |  |
|--|--|
| P1 = Site 2 km away from farmland                | P6 = Site 2 km away from P5 on the river |
| P2 = Site close to farmland                      | HPC = Heterotrophic Plate Count          |
| P3 = Site close to residents                     | TC = Total Coliform Count                |
| P4 = Site on stream drainage from Cement Factory | FC = Faecal Coliform Count               |
| P5 = Site on stream drainage when entering river | FS = Faecal Streptococci Count           |

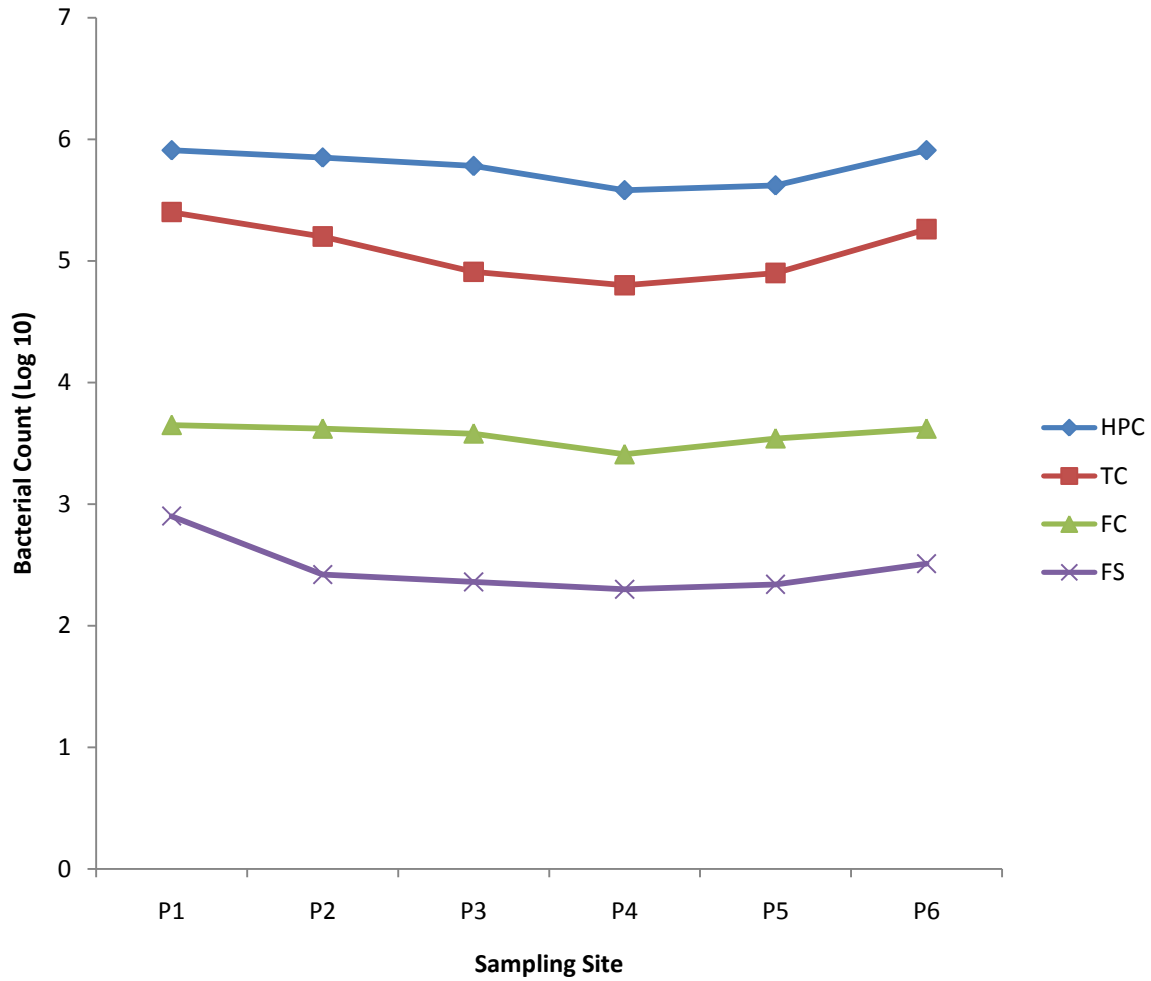
**Fig 4.1:** Heterotrophic Bacteria and Coliform Counts of River Sokoto Water at Different SamplingPoints in January, 2014.



Key:

|  |  |
|--|--|
| P1 = Site 2 km away from farmland                | P6 = Site 2 km away from P5 on the river |
| P2 = Site close to farmland                      | HPC = Heterotrophic Plate Count          |
| P3 = Site close to residents                     | TC = Total Coliform Count                |
| P4 = Site on stream drainage from Cement Factory | FC = Faecal Coliform Count               |
| P5 = Site on stream drainage when entering river | FS = Faecal Streptococci Count           |

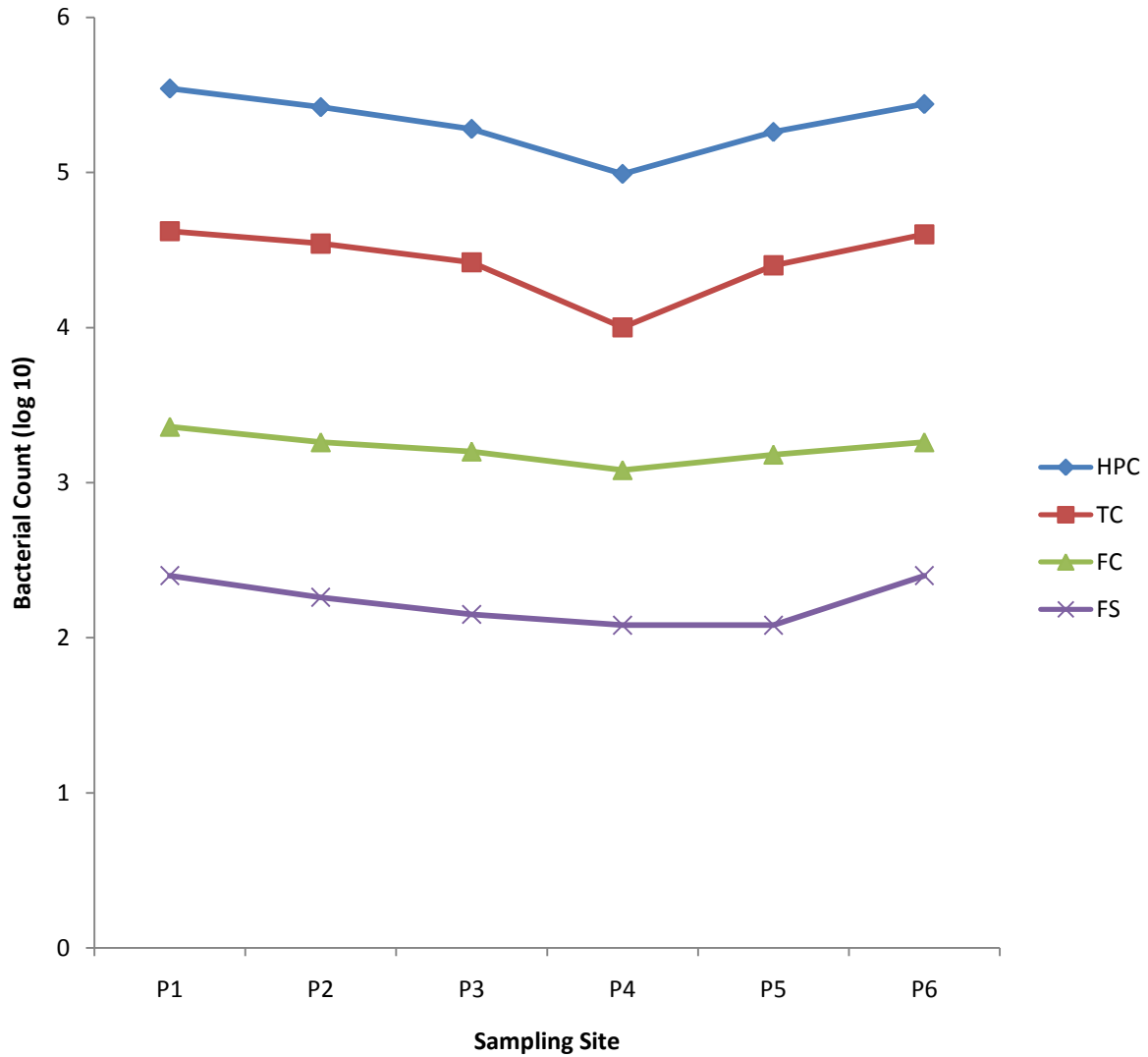
**Fig 4.2:** Heterotrophic Bacteria and Coliform Counts of River Sokoto Water at Different Sampling Points in May, 2014.



**Key:**

- |  |  |
|--|--|
| P1 = Site 2 km away from farmland                | P6 = Site 2 km away from P5 on the river |
| P2 = Site close to farmland                      | HPC = Heterotrophic Plate Count          |
| P3 = Site close to residents                     | TC = Total Coliform Count                |
| P4 = Site on stream drainage from Cement Factory | FC = Faecal Coliform Count               |
| P5 = Site on stream drainage when entering river | FS = Faecal Streptococci Count           |

**Fig 4.3:** Heterotrophic Bacteria and Coliform Counts of River Sokoto Water at Different Sampling Points in July, 2014.



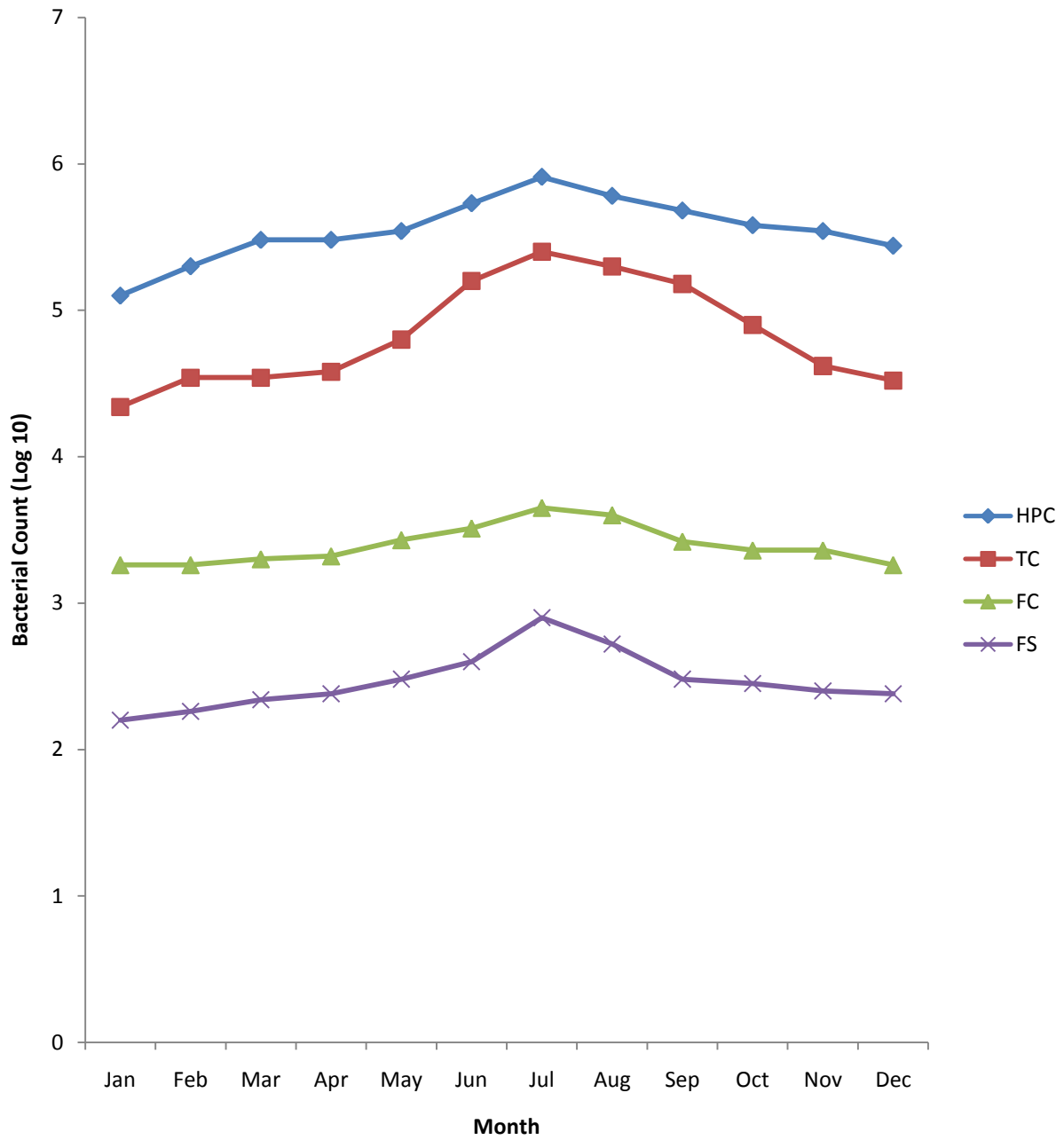
Key:

- |  |  |
|--|--|
| P1 = Site 2 km away from farmland                | P6 = Site 2 km away from P5 on the river |
| P2 = Site close to farmland                      | HPC = Heterotrophic Plate Count          |
| P3 = Site close to residents                     | TC = Total Coliform Count                |
| P4 = Site on stream drainage from Cement Factory | FC = Faecal Coliform Count               |
| P5 = Site on stream drainage when entering river | FS = Faecal Streptococci Count           |

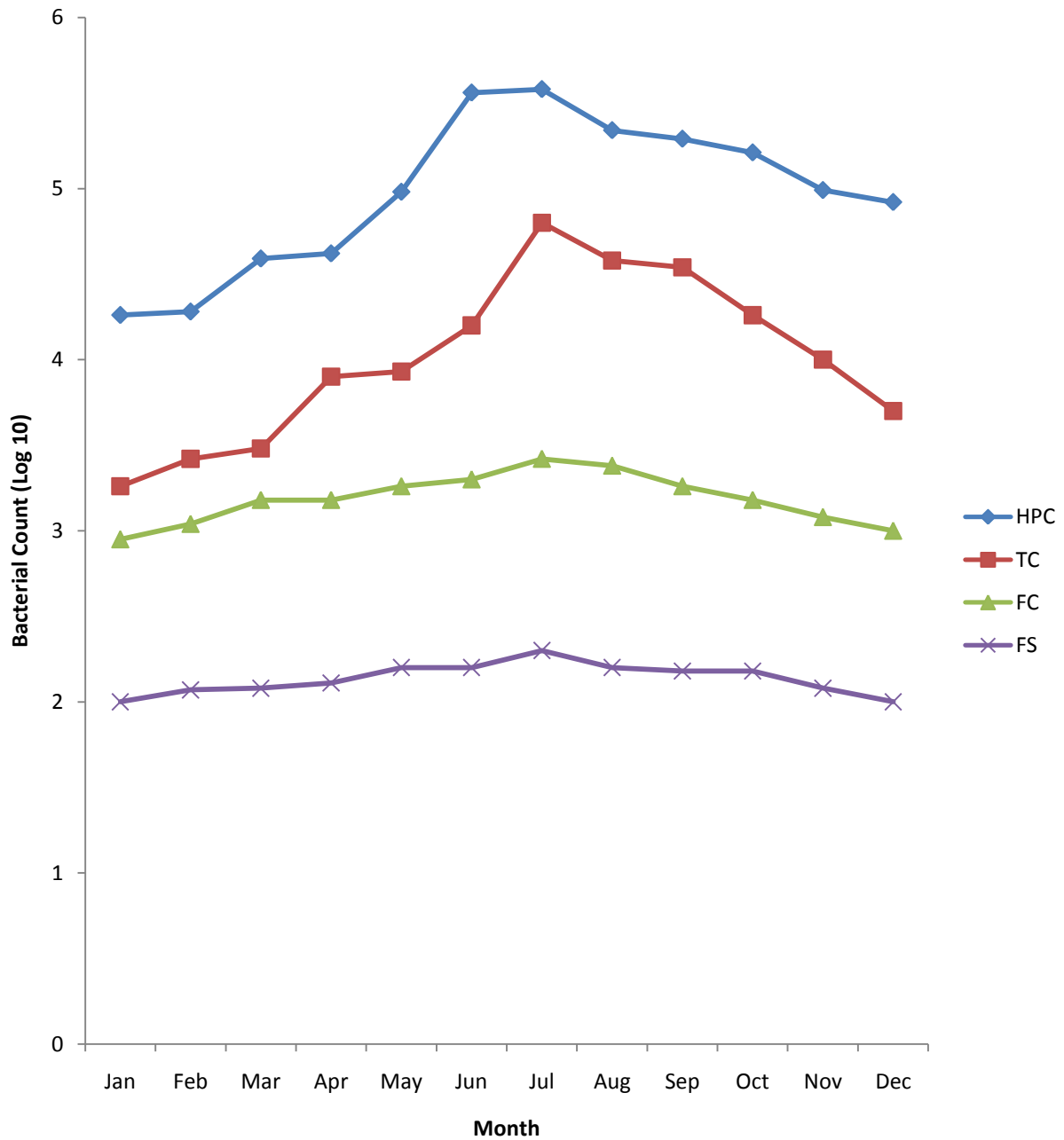
**Fig 4.4:** Heterotrophic Bacteria and Coliform Counts of River Sokoto Water at Different SamplingPoints in November, 2014.

### **Monthly Microbial Counts**

Monthly microbial counts at the different sampling points presented in Figs 4.5 and 4.6, showed that the month of July (which was the peak of rainfall) recorded highest values for HPC, TC, FC, and FS, while the month of December/January (which is the peak of dry season) had lowest counts. The increase in microbial counts observed in the rainy season was more pronounced for HPC and TC, and also at sampling point P<sub>4</sub>, compared with values at P<sub>1</sub>.



**Fig 4.5:** Monthly Bacteria count at P1 with highest Faecal Coliform from January to December, 2014.



**Fig 4.6:** Monthly Bacteria count at P4 with least Faecal Coliform from January to December, 2014.

**Table 4.1:** One Way ANOVA Post Hoc Test of Mean Heterotrophic Bacteria Count at different sampling sites on River Sokoto

| Site | Mean ± Standard Deviation        |                                  |                                  |                                |                                 |                                |                                  |                                |                                 |                                  |                                 |                                 |
|------|----------------------------------|----------------------------------|----------------------------------|--------------------------------|---------------------------------|--------------------------------|----------------------------------|--------------------------------|---------------------------------|----------------------------------|---------------------------------|---------------------------------|
|      | Jan                              | Feb                              | Mar                              | Apr                            | May                             | Jun                            | Jul                              | Aug                            | Sep                             | Oct                              | Nov                             | Dec                             |
| P1   | 130000 ±<br>18027.8 <sup>+</sup> | 200000 ±<br>52915.0 <sup>+</sup> | 300000 ±<br>50000.0 <sup>+</sup> | 30200 ±<br>2000.0 <sup>+</sup> | 345000±<br>5000.0 <sup>+</sup>  | 53600 ±<br>6557.4 <sup>+</sup> | 820000±<br>20000.0 <sup>+</sup>  | 60200 ±<br>2645.8 <sup>+</sup> | 475000±<br>5000.0 <sup>+</sup>  | 380000 ±<br>20000.0 <sup>+</sup> | 349000 ±<br>6000.0 <sup>+</sup> | 275000 ±<br>5000.0 <sup>+</sup> |
| P2   | 98000<br>±2000.0                 | 113000±2<br>000.0 <sup>-</sup>   | 280000 ±<br>.0 <sup>+</sup>      | 12800<br>±2000.0 <sup>-</sup>  | 291000 ±<br>1000.0 <sup>+</sup> | 39200<br>±2000.0 <sup>+</sup>  | 703330±<br>5773.5 <sup>+</sup>   | 48900<br>±3464.1 <sup>+</sup>  | 380000±<br>20000.0 <sup>+</sup> | 276000±4<br>000.0                | 264000±4<br>000.0 <sup>+</sup>  | 132000±2<br>000.0               |
| P3   | 92000±20<br>00.0 <sup>C</sup>    | 182000<br>±2000.0 <sup>C</sup>   | 165000<br>±5000.0 <sup>C</sup>   | 19700 ±<br>.0 <sup>C</sup>     | 248330 ±<br>6506.4 <sup>C</sup> | 38033±5<br>77.4 <sup>C</sup>   | 605670<br>±6027.7 <sup>C</sup>   | 42000<br>±10000. <sup>C</sup>  | 302000±<br>3605.6 <sup>C</sup>  | 264000±4<br>000.0 <sup>C</sup>   | 192000±<br>.0 <sup>C</sup>      | 120000±<br>10000.0 <sup>C</sup> |
| P4   | 18000±30<br>00.0 <sup>-</sup>    | 19000±20<br>00.0 <sup>-</sup>    | 39000±80<br>00.0 <sup>-</sup>    | 42000 ±<br>.0 <sup>-</sup>     | 95000 ±<br>1000.0 <sup>-</sup>  | 360000 ±<br>.0 <sup>-</sup>    | 384000<br>±2000.0 <sup>-</sup>   | 220000 ±<br>.0 <sup>-</sup>    | 196000±<br>2000.0 <sup>-</sup>  | 162000±<br>.0 <sup>-</sup>       | 98000<br>±2000.0 <sup>-</sup>   | 85000±.0 <sup>-</sup>           |
| P5   | 100000 ±<br>18000.0              | 145000 ±<br>13228.8              | 81000±<br>59101.6 <sup>-</sup>   | 28600<br>±4000. <sup>+</sup>   | 126330 ±<br>1527.5 <sup>-</sup> | 36833±5<br>77.4 <sup>-</sup>   | 420000 ±<br>17320.5 <sup>-</sup> | 302000<br>±2000.0 <sup>-</sup> | 300000±<br>.0                   | 194000±4<br>000.0 <sup>-</sup>   | 180000±5<br>000.0 <sup>-</sup>  | 110000±<br>10000.0              |
| P6   | 120000 ±<br>20000.0 <sup>+</sup> | 175000 ±<br>22912.9              | 282000 ±<br>19078.8 <sup>+</sup> | 28200<br>±2000. <sup>+</sup>   | 332000 ±<br>2000.0 <sup>+</sup> | 48600 ±<br>1000.0 <sup>+</sup> | 805330<br>±7571.9 <sup>+</sup>   | 573000<br>±2645.8 <sup>+</sup> | 426000±<br>5291.5 <sup>+</sup>  | 300000±<br>21794.5 <sup>+</sup>  | 275000±<br>10000.0 <sup>+</sup> | 180000±<br>20000.0 <sup>+</sup> |

Key

<sup>C</sup>=control variable

<sup>+</sup> = significantly higher than control

<sup>-</sup> = significantly lower than control

Blank = no significant difference

Level of significance = P ≤ 0.05

### **Faecal Coliform and Faecal Streptococci Analysis of the Water Samples**

The FC/FS value which is often used to predict the likely source of faecal contamination of rivers and waste waters, shows that the major contamination source of River Sokoto was human (Table 4.2). The values varied at each site from one month to another and also from one sampling site to another. For example, at P<sub>1</sub>, January recorded highest value with July having the lowest ratio. On the other hand, comparison of FC/FS ratios on monthly values, showed that lowest values were obtained in three of the six sites in the month of January and the highest values were obtained in the month of July in four of the sampling sites.

**Table 4.2:** Monthly Ratio of Faecal Coliform and Faecal Streptococci at various sampling points on River Sokoto, 2014

| <b>FC/FS Ratio at Sampling Sites</b> |             |              |              |              |              |             |
|--------------------------------------|-------------|--------------|--------------|--------------|--------------|-------------|
| <b>Month</b>                         | <b>P1</b>   | <b>P2</b>    | <b>P3</b>    | <b>P4</b>    | <b>P5</b>    | <b>P6</b>   |
| January                              | 11.25       | 10.91        | 10.00        | 9.00         | 10.00        | 13.64       |
| February                             | 10.00       | 10.67        | 13.33        | 9.32         | 11.67        | 11.25       |
| March                                | 9.09        | 10.00        | 11.33        | 12.50        | 13.08        | 10.00       |
| April                                | 8.68        | 10.00        | 10.00        | 11.54        | 11.25        | 10.00       |
| May                                  | 9.00        | 11.36        | 12.50        | 11.25        | 13.75        | 8.33        |
| June                                 | 8.00        | 12.00        | 13.18        | 12.50        | 15.00        | 9.68        |
| July                                 | 5.63        | 16.15        | 16.52        | 13.00        | 15.91        | 13.13       |
| August                               | 7.55        | 14.55        | 12.86        | 15.00        | 12.50        | 11.00       |
| September                            | 8.67        | 11.50        | 12.22        | 12.00        | 12.22        | 8.62        |
| October                              | 8.21        | 10.00        | 10.63        | 10.00        | 10.67        | 7.86        |
| November                             | 9.20        | 10.00        | 11.43        | 10.00        | 12.50        | 7.20        |
| December                             | 7.50        | 11.33        | 10.00        | 10.00        | 12.00        | 9.00        |
| <b>Mean</b>                          | <b>8.57</b> | <b>11.54</b> | <b>12.00</b> | <b>11.34</b> | <b>12.55</b> | <b>9.98</b> |

## Distribution of Bacterial Isolates

Table 4.3 presents the distribution of the bacteria isolated from the water samples of the river. A total of 434 bacteria organisms were isolated comprising nineteen different species. Among the *Enterobacteriaceae*, *Escherichia coli* had the highest percentage (11.98%) followed by *Enterobacter aerogenes* and *Klebsiella pneumoniae subspecies pneumonia*. *Pseudomonas aeruginosa* constituted the majority of non-*Enterobacteriaceae* Gram-negative organisms. *Staphylococcus aureus* (6.91%) was the highest among the Gram-positive organisms followed by *Staphylococcus saprophyticus* (5.99%). Other isolates in significant numbers are *Streptococcus faecalis*, *Bacillus subtilis*, *Elizabethkingia meningoseptica* and *Aeromonas sobria*. Some Gram negative and Gram positive bacteria of aquatic habitat like *Elizabethkingia meningoseptica*, *Klebsiella oxytoca*, *Providencia rettgeri*, *Raoultella ornithinolytica*, *Staphylococcus cohnii subspecies urealyticus* and *Staphylococcus chromogenes* were also isolated.

**Table 4.3:** Distribution and Percentage Frequency of Bacteria organisms isolated from Water Samples collected from River Sokoto and its Drainage Stream

| Organisms   | Number Isolated | % Frequency |
|---|-----------------|-------------|
| <b><i>Enterobacteriaceae</i></b>                    |                 |             |
| <i>Klebsiella pneumoniae subspecies pneumoniae</i>  | 38              | 8.76        |
| <i>Klebsiella oxytoca</i>                           | 13              | 3.00        |
| <i>Enterobacter cloacae</i>                         | 26              | 5.99        |
| <i>Enterobacter aerogenes</i>                       | 38              | 8.76        |
| <i>Salmonella typhi</i>                             | 28              | 6.45        |
| <i>Shigella flexneri</i>                            | 23              | 5.30        |
| <i>Escherichia coli</i>                             | 52              | 11.98       |
| <i>Providencia rettgeri</i>                         | 13              | 3.00        |
| <i>Raoultella ornithinolytica</i>                   | 26              | 5.99        |
| <b>Non-Enterobacteriaceae Gr-ve org</b>             |                 |             |
| <i>Elizabethkingia meningoseptica</i>               | 26              | 5.99        |
| <i>Pseudomonas aeruginosa</i>                       | 32              | 7.37        |
| <i>Aeromonas sobria</i>                             | 24              | 5.53        |
| <b>Gram positive isolates</b>                       |                 |             |
| <i>Staphylococcus aureus</i>                        | 30              | 6.91        |
| <i>Staphylococcus saprophyticus</i>                 | 26              | 5.99        |
| <i>Staphylococcus epidermidis</i>                   | 13              | 3.00        |
| <i>Staphylococcus cohnii subspecies urealyticus</i> | 4               | 0.92        |
| <i>Staphylococcus chromogenes</i>                   | 4               | 0.92        |
| <i>Streptococcus faecalis</i>                       | 10              | 2.30        |
| <i>Bacillus subtilis</i>                            | 8               | 1.84        |
| <b>Total</b>  | <b>434</b>      | <b>100</b>  |

### **Antibiotic Susceptibility Profiles of the Isolated Bacteria**

Tables 4.4 – 4.6 present the results of antibiotic sensitivity of the isolates to eleven antibiotics. Data presented in Table 4.4 show that most of the *Enterobacteriaceae* isolates (□ 60%) were sensitive to ceftazidime, gentamicin, amoxicillin/clavulinate, the quinolones (ofloxacin, ciprofloxacin) and nitrofurantoin. They were in most cases, less susceptible to the inhibitory activities of cefuroxime, erythromycin and ampicillin. There were notable variations among the various species in their susceptibility to the antibiotics. For example, while most of the *E. coli* isolates were generally susceptible to the cephalosporins, percentage of *Klebsiella* and *Salmonella* isolates susceptible to the same cephalosporins were significantly lower.

**Table 4.4:** Antibiotic Sensitivity Profiles of some *Enterobacteriaceae* Isolates from River Sokoto and its Drainage Stream

| Antibiotics                 | Percentage Sensitive (%) |               |               |               |               |               |               |               |               |
|-----------------------------|--------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
|                             | ECL<br>(n=52)            | KLO<br>(n=38) | KLP<br>(n=13) | ETC<br>(n=26) | ETA<br>(n=38) | SLT<br>(n=28) | SGF<br>(n=23) | PVR<br>(n=13) | RLO<br>(n=26) |
| Ceftazidime                 | 80.77                    | 52.45         | 53.77         | 90.77         | 84.95         | 53.57         | 84.21         | 76.15         | 50.00         |
| Ceftriaxone                 | 75.00                    | 50.00         | 50.00         | 61.54         | 60.53         | 50.00         | 52.17         | 69.23         | 50.00         |
| Cefuroxime                  | 69.23                    | 28.36         | 30.77         | 50.31         | 42.37         | 32.14         | 30.45         | 53.85         | 41.54         |
| Erythromycin                | 44.23                    | 50.00         | 53.85         | 50.00         | 46.84         | 39.29         | 52.17         | 53.85         | 50.00         |
| Gentamicin                  | 75.00                    | 50.00         | 76.92         | 53.85         | 86.84         | 85.71         | 73.91         | 76.92         | 34.62         |
| Amoxicillin/<br>Clavulinate | 68.39                    | 89.47         | 92.31         | 100.00        | 97.37         | 58.00         | 78.87         | 92.31         | 90.77         |
| Cloxacillin                 | 55.00                    | 71.05         | 69.23         | 80.77         | 84.21         | 53.57         | 61.30         | 76.92         | 65.39         |
| Ampicillin                  | 40.31                    | 55.26         | 53.85         | 80.77         | 78.95         | 50.00         | 52.49         | 76.92         | 60.46         |
| Ciprofloxacin               | 78.85                    | 64.21         | 92.31         | 50.00         | 50.00         | 82.14         | 82.14         | 38.46         | 80.77         |
| Ofloxacin                   | 80.77                    | 71.05         | 92.31         | 63.16         | 63.16         | 78.57         | 78.57         | 38.46         | 84.62         |
| Nitrofurantoin              | 34.62                    | 86.84         | 84.62         | 61.54         | 36.84         | 92.86         | 73.91         | 100.00        | 38.46         |

**Key**

ECL = *Escherichia coli*

KLO = *Klebsiella oxytoca*

KLP = *Klebsiella pneumoniae*

ETC = *Enterobacter cloacae*

ETA = *Enterobacter aerogenes*

SLT = *Salmonella typhi*

SGF = *Shigella flexneri*

PVR = *Providencia rettgeri*

RLO = *Raoultella ornithinolytica*

Among the non-fermenting Gram-negative isolates (Table 4.5), *Elizabethkingia meningoseptica* were most susceptible to the various test antibiotics, with percentage ranging from 68.5% to 100% except with erythromycin, having 50% efficacy. The other isolates (*Aeromonas sobria* and *Pseudomonas aeruginosa*) were relatively less susceptible, particular to the cephalosporins, erythromycin and ampicillin.

**Table 4.5:** Antibiotic Sensitivity Profiles of Non-fermenting Gram Negative Organisms Isolated from River Sokoto and its Drainage Stream

| Antibiotics             | Percentage Sensitivity (%) |               |               |
|-------------------------|----------------------------|---------------|---------------|
|                         | ELM<br>(n=26)              | ARS<br>(n=24) | PSE<br>(n=32) |
| Ceftazidime             | 96.15                      | 50.00         | 56.25         |
| Ceftriaxone             | 80.77                      | 50.00         | 50.00         |
| Cefuroxime              | 69.23                      | 40.00         | 45.88         |
| Erythromycin            | 50.00                      | 29.17         | 33.33         |
| Gentamicin              | 80.77                      | 79.17         | 90.63         |
| Amoxicillin/Clavulinate | 100.00                     | 58.33         | 60.00         |
| Cloxacillin             | 79.23                      | 40.63         | 52.00         |
| Ampicillin              | 68.46                      | 29.17         | 46.88         |
| Ciprofloxacin           | 92.31                      | 83.33         | 71.63         |
| Ofloxacin               | 84.62                      | 75.83         | 71.63         |
| Nitrofurantoin          | 76.92                      | 62.50         | 46.88         |

Key

ELM = *Elizabethkingia meningoseptica*    ARS = *Aeromonas sobria*    PSE = *Pseudomonas aeruginosa*

The susceptibility profiles of the Gram-positive isolates are presented in Table 4.6. *Staphylococcus cohnii* were the most susceptible to the various test antibiotics, with percentage ranging from 75% to 100% except with cefuroxime, cloxacillin, ciprofloxacin and ampicillin, having 50% efficacy. The other isolates (*Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Staphylococcus chromogenes*, *Streptococcus faecalis* and *Bacillus subtilis*) were relatively less susceptible, particular to the cefuroxime, cloxacillin and ampicillin.

**Table 4.6:** Antibiotic Sensitivity Profiles of Gram Positive Bacteria Isolated from River Sokoto and its Drainage Stream

| Antibiotics                 | Percentage Sensitivity (%) |               |              |               |              |               |              |
|-----------------------------|----------------------------|---------------|--------------|---------------|--------------|---------------|--------------|
|                             | STA<br>(n=30)              | STS<br>(n=26) | STC<br>(n=4) | STE<br>(n=13) | SCH<br>(n=4) | STF<br>(n=10) | BAS<br>(n=8) |
| Ceftazidime                 | 90.00                      | 88.62         | 100.00       | 100.00        | 50.00        | 50.00         | 50.00        |
| Ceftriaxone                 | 90.00                      | 84.46         | 75.00        | 84.62         | 50.00        | 30.00         | 30.00        |
| Cefuroxime                  | 56.67                      | 50.00         | 50.00        | 61.54         | 25.00        | 20.00         | 25.00        |
| Erythromycin                | 50.00                      | 73.08         | 75.00        | 53.85         | 75.00        | 50.00         | 50.00        |
| Gentamicin                  | 83.33                      | 57.69         | 75.00        | 84.62         | 75.00        | 90.00         | 75.00        |
| Amoxicillin/<br>Clavulinate | 80.00                      | 80.77         | 100.00       | 76.92         | 75.00        | 90.00         | 50.00        |
| Cloxacillin                 | 56.67                      | 38.62         | 50.00        | 38.77         | 50.00        | 60.00         | 37.50        |
| Ampicillin                  | 40.00                      | 34.46         | 50.00        | 30.46         | 25.00        | 50.00         | 25.00        |
| Ciprofloxacin               | 63.33                      | 80.77         | 50.00        | 61.54         | 50.00        | 60.00         | 50.00        |
| Ofloxacin                   | 93.33                      | 92.31         | 75.00        | 92.31         | 100.00       | 70.00         | 50.00        |
| Nitrofurantoin              | 83.33                      | 80.77         | 100.00       | 76.92         | 75.00        | 80.00         | 75.00        |

Key

STA = *Staphylococcus aureus*

STS = *Staphylococcus saprophyticus*

STC = *Staphylococcus cohnii*

STE = *Staphylococcus epidermidis*

SCH = *Staphylococcus chromogenes*

STF = *Streptococcus faecalis*

BAS = *Bacillus subtilis*

### **Multiple Antibiotic Resistance Indices of the Isolates**

Multiple Antibiotic Resistance Index (MARI) is used to determine the degree of antibiotics usage in an area. MARIs for isolated organisms are presented in Table 4.7. Based on the eleven test antibiotics, isolates among *Enterobacteriaceae* showed excessive multiple drug resistance with highest percentages of  $\text{MARI} \geq 0.2$ , ranging from 51.9% in *E. coli* to 91.3% in *Shigella flexneri*. On the other hand, partial multiple drug resistance was shown for non-fermenting Gram negative isolates (*Elizabethkingia meningoseptica*, *Aeromonas sobria* and *Pseudomonas aeruginosa*) with *Pseudomonas aeruginosa* having the highest percentage (37.5%). Gram positive organisms, in their own case, exhibited moderate multiple antibiotic resistance to the test antibiotics with  $\% \text{MARI} \geq 2$  ranging from 46.2 in *Staphylococcus epidermidis* to 50% in *Staphylococcus cohnii*.

**Table 4.7:** MARI of Isolated Organisms from Water samples collected from River Sokoto and its DrainageStream

| <b>Organisms</b>  | <b>% with MARI<math>\geq</math> 0.2</b> |
|---|---|
| <i>Escherichia coli</i> (n = 52)                            | 51.92                                   |
| <i>Klebsiellapneumoniae subspecies pneumonia</i> (n = 38)   | 31.58                                   |
| <i>Klebsiellaoxytoca</i> (n = 13)                           | 7.69                                    |
| <i>Enterobacter cloacae</i> (n = 26)                        | 42.31                                   |
| <i>Enterobacteraerogenes</i> (n = 38)                       | 60.53                                   |
| <i>Salmonella typhi</i> (n = 28)                            | 35.71                                   |
| <i>Shigella flexneri</i> (n = 23)                           | 91.30                                   |
| <i>Providencia rettgeri</i> (n = 13)                        | 46.15                                   |
| <i>Raoultella ornithinolytica</i> (n = 26)                  | 38.46                                   |
| <i>Elizabethkingia meningoseptica</i> (n = 26)              | 11.54                                   |
| <i>Aeromonas sobria</i> (n = 24)                            | 16.67                                   |
| <i>Pseudomonas aeruginosa</i> (n = 32)                      | 37.50                                   |
| <i>Staphylococcus aureus</i> (n = 30)                       | 46.67                                   |
| <i>Staphylococcus saprophyticus</i> (n = 26)                | 30.77                                   |
| <i>Staphylococcus cohnii subspecies urealyticus</i> (n = 4) | 50.00                                   |
| <i>Staphylococcus epidermidis</i> (n = 13)                  | 46.15                                   |
| <i>Staphylococcus chromogenes</i> (n = 4)                   | 25.00                                   |
| <i>Streptococcus faecalis</i> (n = 10)                      | 10.00                                   |

### Susceptibility of Isolates to Some Disinfectants

Table 4.8 presents the mean minimum inhibitory concentrations (MICs) of isolated organisms against three commonly used disinfectants namely Dettol<sup>®</sup> (4.8% w/v chloroxylenol), Savlon<sup>®</sup> (0.3/3.0% w/v chlorhexidine/cetrimide) and Izal<sup>®</sup> (7% w/v saponated cresol) in the study area.

The table shows that majority of the *Enterobacteriaceae* had almost half of their total numbers resistant to all the three test disinfectants. Furthermore, the mean MICs of Dettol<sup>®</sup> and Savlon<sup>®</sup> were higher for *Enterobacteriaceae* compare with other Gram negative organisms with *Enterobacteraerogenes* having the highest values followed closely by *Pseudomonas aeruginosa*. Values of Izal<sup>®</sup> for all the isolated organisms were close without much difference with Gram positive organisms (*Staphylococcus epidermidis*) having the least value. The MIC values of the test disinfectants against isolated organisms were generally lower than the recommended use concentrations of the disinfectants {Dettol<sup>®</sup> (5.0% v/v), Savlon<sup>®</sup> (6.0% v/v) and Izal<sup>®</sup> (0.5% v/v)}.

Table 4.9 shows the percentages of bacteria isolates resistant to the test disinfectants at recommended use concentrations – Dettol<sup>®</sup> (5.0% v/v), Savlon<sup>®</sup> (6.0% v/v) and Izal<sup>®</sup> (0.5% w/v). Isolates among *Enterobacteriaceae* were generally more resistant to the test disinfectants, but a Gram positive organism (*Staphylococcus epidermidis*) had the highest percentage resistance (53.85%) to Dettol<sup>®</sup>. On the other hand, non- *Enterobacteriaceae* Gram negative organisms (*Elizabethkingia meningoseptica*, *Aeromonas sobria* and *Pseudomonas aeruginosa*) were relatively less resistant to the test disinfectants. Gram positive organism (*Streptococcus faecalis*) recorded the least percentage resistance (10%) to Savlon<sup>®</sup>.

**Table 4.8:** Minimum Inhibitory Concentrations of Test Disinfectants on Isolates from River Sokoto and its Drainage Stream

| Organisms                                  | Mean MICs of Disinfectants (for 50% of Isolates) |                                |                              |
|--|--|--------------------------------|------------------------------|
|  | Dettol <sup>®</sup> (5.0% v/v)                   | Savlon <sup>®</sup> (6.0% v/v) | Izal <sup>®</sup> (0.5% w/v) |
| <b><i>Enterobacteriaceae</i></b>           |  |                                |                              |
| <i>Klebsiellapneumoniae</i> (n = 38)       | 0.63± 0.15                                       | 0.19 ± 0.82                    | 0.13 ± 0.43                  |
| <i>Klebsiellaoxytoca</i> (n = 13)          | 0.63 ± 0.47                                      | 0.75 ± 0.56                    | 0.25 ± 0.47                  |
| <i>Enterobacter cloacae</i> (n = 26)       | 0.63 ± 0.06                                      | 1.50 ± 0.22                    | 0.13 ± 0.12                  |
| <i>Enterobacteraerogenes</i> (n = 38)      | 2.50 ± 0.07                                      | 3.00 ± 0.38                    | 0.25 ± 0.09                  |
| <i>Salmonella typhi</i> (n = 28)           | 0.16 ± 0.11                                      | 1.50 ± 0.31                    | 0.25 ± 0.08                  |
| <i>Shigellaflexneri</i> (n = 23)           | 0.63 ± 0.05                                      | 0.38 ± 0.07                    | 0.13 ± 0.10                  |
| <i>Escherichia coli</i> (n = 52)           | 0.63 ± 0.04                                      | 0.75 ± 0.09                    | 0.13 ± 0.05                  |
| <i>Providencia rettgeri</i> (n = 13)       | 0.16 ± 0.07                                      | 1.50 ± 0.10                    | 0.13 ± 0.04                  |
| <i>Raoultella ornithinolytica</i> (n = 26) | 0.31 ± 0.01                                      | 1.50 ± 0.11                    | 0.25 ± 0.02                  |
| <b>Non-Enterobacteriaceae Gr-ve org</b>    |  |                                |                              |
| <i>Eliz.meningoseptica</i> (n = 26)        | 0.31 ± 0.02                                      | 0.38 ± 0.07                    | 0.10 ± 0.03                  |
| <i>Pseudomonas aeruginosa</i> (n = 32)     | 1.25 ± 0.02                                      | 3.00 ± 0.06                    | 0.25 ± 0.03                  |
| <i>Aeromonas sobria</i> (n = 24)           | 0.31 ± 0.02                                      | 1.50 ± 0.07                    | 0.13 ± 0.02                  |
| <b>Gram positive isolates</b>              |  |                                |                              |
| <i>Staph aureus</i> (n = 30)               | 0.63 ± 0.01                                      | 0.20 ± 0.05                    | 0.25 ± 0.01                  |
| <i>Staph saprophyticus</i> (n = 26)        | 0.16 ± 0.04                                      | 0.20 ± 0.10                    | 0.13 ± 0.03                  |
| <i>Staph epidermidis</i> (n = 13)          | 0.31 ± 0.01                                      | 0.38 ± 0.03                    | 0.10 ± 0.01                  |
| <i>Staph cohnii</i> (n = 4)                | 0.63 ± 0.04                                      | 0.75 ± 0.03                    | 0.25 ± 0.08                  |

|                                  |             |             |             |
|----------------------------------|-------------|-------------|-------------|
| <i>Staph chromogenes</i> (n = 4) | 0.63 ± 0.10 | 0.75 ± 0.24 | 0.10 ± 0.31 |
| <i>Strep faecalis</i> (n = 10)   | 0.16 ± 0.05 | 0.19 ± 0.06 | 0.13 ± 0.02 |
| <i>Bacillus subtilis</i> (n = 8) | 0.31 ± 0.01 | 0.75 ± 0.03 | 0.13 ± 0.01 |

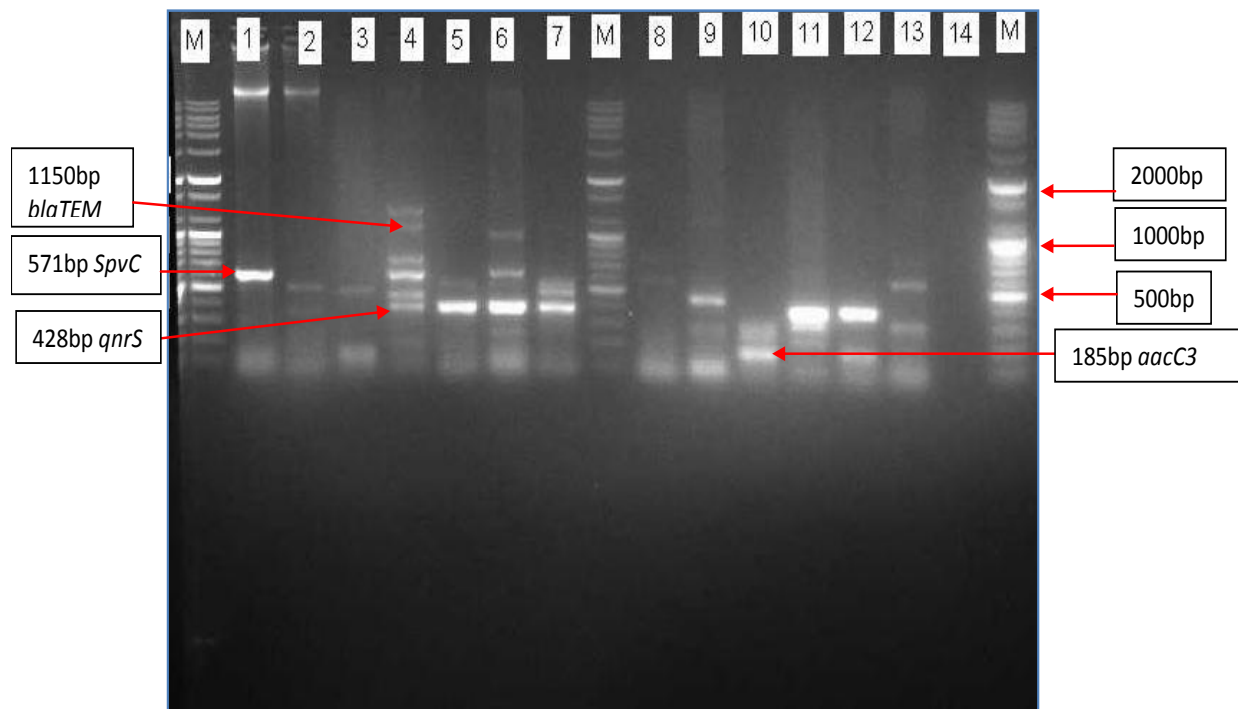
**Table 4.9:** Percentages of Isolates from River Sokoto and its Drainage Stream resistant to the Disinfectants

| Organisms                                    | Percentages of Isolates Resistant |                       |                     |
|--|-----------------------------------|-----------------------|---------------------|
|  | Dettol®<br>(5.0% v/v)             | Savlon®<br>(6.0% v/v) | Izal®<br>(0.5% w/v) |
| <b><i>Enterobacteriaceae</i></b>             |                                   |                       |                     |
| <i>Klebsiella pneumoniae</i> (n=3)           | 50.00                             | 42.00                 | 50.00               |
| <i>Klebsiella oxytoca</i> (n=13)             | 46.15                             | 38.46                 | 53.85               |
| <i>Enterobacter cloacae</i> (n=26)           | 47.37                             | 30.77                 | 38.46               |
| <i>Enterobacter aerogenes</i> (n=38)         | 50.00                             | 36.84                 | 42.11               |
| <i>Salmonella typhi</i> (n=28)               | 46.43                             | 42.86                 | 42.86               |
| <i>Shigella flexneri</i> (n=23)              | 43.48                             | 34.78                 | 52.17               |
| <i>Escherichia coli</i> (n=52)               | 48.08                             | 46.15                 | 50.00               |
| <i>Providencia rettgeri</i> (n=13)           | 46.15                             | 46.15                 | 46.15               |
| <i>Raoultella ornithinolytica</i> (n=26)     | 30.77                             | 26.92                 | 38.46               |
| <b>Non-Enterobacteriaceae Gr-ve org</b>      |                                   |                       |                     |
| <i>Elizabethkingia meningoseptica</i> (n=26) | 30.77                             | 26.92                 | 50.00               |
| <i>Pseudomonas aeruginosa</i> (n=32)         | 37.50                             | 25.00                 | 37.50               |
| <i>Aeromonas sobria</i> (n=24)               | 47.37                             | 41.67                 | 37.50               |
| <b>Gram positive isolates</b>                |                                   |                       |                     |
| <i>Staphylococcus aureus</i> (n=30)          | 46.67                             | 43.33                 | 43.33               |
| <i>Staphylococcus saprophyticus</i> (n=26)   | 38.46                             | 30.77                 | 30.77               |
| <i>Staphylococcus epidermidis</i> (n=13)     | 53.85                             | 46.15                 | 46.15               |

|   |       |       |       |
|---|-------|-------|-------|
| <i>Staphylococcus cohnii</i> (n=4)      | 50.00 | 25.00 | 25.00 |
| <i>Staphylococcus chromogenes</i> (n=4) | 50.00 | 25.00 | 25.00 |
| <i>Streptococcus faecalis</i> (n=10)    | 20.00 | 10.00 | 30.00 |
| <i>Bacillus subtilis</i> (n=8)          | 37.50 | 25.00 | 37.50 |

### Molecular Analysis of Resistant Bacteria

Results of molecular analysis of thirteen (13) resistant bacterial strains isolated from River Sokoto are presented in Plate 4.1 as shown below:



**Plate4.1:**Agarose gel electrophoresis (1%) used for the separation of multiplex PCR products.

M = molecular weightmarker (100 bp ladder; Bioneer, USA).

**Table 4.10:** Molecular Analysis of Resistant Bacteria Isolated from River Sokoto and its Drainage Stream

| Lane | Isolate                       | Number of Band | Band Size (bp)                   | Resistance Genes Detected                            |
|------|-------------------------------|----------------|----------------------------------|--|
| 1    | <i>Salmonella typhi</i>       | 2              | 571; □ 10,200                    | <i>spvC</i> ; Non-specific                           |
| 2    | <i>E. coli</i>                | 4              | 185; 300; 500; □10,200           | <i>aacC3</i> ; Non-specific                          |
| 3    | <i>Shigella flexneri</i>      | 2              | 185; 490                         | <i>aacC3</i> ; Non-specific                          |
| 4    | <i>Pseudomonas aeruginosa</i> | 6              | 428; 500; 571; 700; 1,150; 1,200 | <i>bla<sub>TEM</sub></i> ; <i>spvC</i> ; <i>qnrS</i> |
| 5    | <i>Staphylococcus aureus</i>  | 1              | 428                              | <i>qnrS</i>  |
| 6    | <i>Streptococcus faecalis</i> | 3              | 428; 500; 1000                   | <i>qnrS</i> ; Non-specific                           |
| 7    | <i>Bacillus subtilis</i>      | 2              | 428; 500                         | <i>qnrS</i>  |
| 8    | <i>Enterobacter cloacae</i>   | 1              | 185                              | <i>aacC3</i>   |
| 9    | <i>Klebsiella oxytoca</i>     | 2              | 300; 500                         | Non-specific   |
| 10   | <i>Staph saprophyticus</i>    | 2              | 185; 300                         | <i>aacC3</i> ; Non-specific                          |
| 11   | <i>Providencia rettgeri</i>   | 2              | 400; 428                         | Non-specific; <i>qnrS</i>                            |

|    |                               |   |               |                             |
|----|-------------------------------|---|---------------|-----------------------------|
| 12 | <i>Klebsiella pneumoniae</i>  | 2 | 185; 428      | <i>aacC3; qnrS</i>          |
| 13 | <i>Enterobacter aerogenes</i> | 3 | 185; 300; 600 | <i>aacC3</i> ; Non-specific |
| 14 | -                             | 0 | -             | Negative control            |

Table 4.10 shows the molecular analysis of resistant bacteria isolated from River Sokoto and its drainage stream. Aminoglycoside resistance genes (*aacC3*) were detected in the majority of the isolates namely *E. coli* (lane 2), *Shigella flexneri* (lane 3), *Enterobacter cloacae* (lane 8), *Staphylococcus saprophytica* (lane 10) and *Enterobacter aerogenes* (lane 13). Virulence resistance genes (*spvC*) were detected in *Salmonella typhi* (lane 1) while quinolones resistance genes were detected in *Staphylococcus aureus* (lane 5), *Streptococcus faecalis* (lane 6), *Bacillus subtilis* (lane 7) and *Providencia rettgeri* (lane 11). Three different resistance genes namely  $\beta$ -lactam (*bla<sub>TEM</sub>*), virulence (*spvC*) and quinolones (*qnrS*) were found in *Pseudomonas aeruginosa* (lane 4) while two resistance genes (*aacC3* and *qnrS*) were detected in *Klebsiella pneumoneae* (lane 12). However, non-specific resistance genes were detected in the majority of the isolates.

### Physicochemical Analysis of Water Samples

Results of the physicochemical tests carried out on water samples from six different points on River Sokoto during dry and wet seasons are presented in Tables 4.11, 4.12 and 4.13. Table 4.11 presents physicochemical data of water samples collected in January, 2014, which represents the dry season while Table 4.12 presents physicochemical values of water samples collected in August, 2014 (representing the rainy season).

The pH values in water samples were generally alkaline during the dry season in all the sampling points but slightly acidic during rainy seasons with the exception of sampling point, P4 which

was neutral. Sampling point, P1 recorded the highest values for dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) and total suspended solids (TSS) in both seasons. Sampling point, P4 recorded the highest electrical conductance value in rainy season but the least values of TDS and TSS in both dry and rainy seasons. Values of total hardness of water samples were generally higher in the rainy season than in the dry season with P4 having the highest value. There was increase in acidity and decrease in alkalinity from dry to rainy season. However, there was general increase in the values of electrical conductance, BOD, TS, TDS and hardness of water samples of the river which follows the trend of wastes discharged into the river after rainfall.

The concentration of nutrients namely nitrate, ammonia and chloride in the water samples increased and decreased for sulphate and phosphate from dry season to the rainy season with P4 having the highest value in both seasons, except for phosphate.

One way ANOVA Post Hoc test of physicochemical parameters of water samples from various points on river Sokoto in Table 4.13 shows that some parameters (pH, temperature, conductance, DO, BOD, phosphate, hardness and ammonia) depicted seasonal change. It further shows that pH and temperature follow the same trend in the dry season by being significantly higher in P1, P2, P4 and P5, but significantly lower in P6 compared with P3 (control). However, Nitrate values in other sampling points both in dry and rainy seasons were significantly higher than in P3, the control (Appendix B).

**Table 4.11:** Physicochemical Properties of Water samples from Different Sites of River Sokoto in January, 2014 (Dry Season)

| Parameter           | Values at Sampling Sites |              |              |              |              |              |
|---------------------|--------------------------|--------------|--------------|--------------|--------------|--------------|
|                     | P1                       | P2           | P3           | P4           | P5           | P6           |
| pH                  | 8.11 ± 0.08              | 8.06 ± 0.01  | 8.05 ± 0.01  | 8.37 ± 0.08  | 8.50 ± 0.09  | 8.02 ± 0.01  |
| Temperature (°C)    | 25.4 ± 0.09              | 25.5 ± 0.07  | 25.3 ± 0.69  | 25.6 ± 0.09  | 25.4 ± 0.20  | 25.4 ± 0.12  |
| Conductance (µS/cm) | 75.6 ± 0.12              | 96.7 ± 0.12  | 74.6 ± 0.15  | 201 ± 0.09   | 321 ± 0.15   | 96.4 ± 0.17  |
| D. O. (mg/L)        | 1.7 ± 0.11               | 1.0 ± 0.15   | 1.2 ± 0.09   | 1.2 ± 0.06   | 0.7 ± 0.12   | 0.9 ± 0.12   |
| B. O. D. (mg/L)     | 0.7 ± 0.12               | 0.4 ± 0.14   | 0.5 ± 0.13   | 0.3 ± 0.08   | 0.2 ± 0.09   | 0.4 ± 0.20   |
| C. O. D. (mg/L)     | 300 ± 0.32               | 150 ± 0.65   | 180 ± 0.74   | 230 ± 0.94   | 220 ± 1.08   | 230 ± 1.60   |
| Total Solids (mg/L) | 780 ± 0.78               | 580 ± 1.99   | 620 ± 1.05   | 240 ± 1.53   | 400 ± 1.80   | 600 ± 1.63   |
| TDS (mg/L)          | 500 ± 1.60               | 390 ± 3.27   | 470 ± 1.93   | 140 ± 0.99   | 260 ± 1.56   | 400 ± 3.41   |
| TSS (mg/L)          | 280 ± 2.49               | 190 ± 3.81   | 150 ± 2.37   | 100 ± 4.04   | 140 ± 1.60   | 200 ± 1.91   |
| Alkalinity (mg/L)   | 22 ± 0.68                | 21 ± 1.54    | 23 ± 1.30    | 28 ± 0.92    | 71 ± 1.49    | 11 ± 1.26    |
| Acidity (mg/L)      | 15 ± 0.85                | 25 ± 0.94    | 20 ± 1.34    | 5 ± 0.27     | 10 ± 1.04    | 15 ± 0.67    |
| Chlorides (mg/L)    | 35 ± 1.05                | 55 ± 1.30    | 40 ± 0.77    | 100 ± 1.15   | 50 ± 0.79    | 75 ± 0.66    |
| Sulphate (mg/L)     | 220 ± 1.19               | 190 ± 0.63   | 180 ± 0.71   | 195 ± 0.71   | 200 ± 0.83   | 210 ± 0.67   |
| Phosphate (mg/L)    | 0.11 ± 0.01              | 0.51 ± 0.05  | 0.24 ± 0.08  | 0.15 ± 0.01  | 1.77 ± 0.05  | 0.59 ± 0.06  |
| Hardness (mg/L)     | 161.6 ± 1.33             | 121.2 ± 0.38 | 151.5 ± 0.52 | 282.8 ± 0.61 | 184.8 ± 0.75 | 131.3 ± 0.40 |
| Nitrates (mg/L)     | 19.7 ± 0.52              | 9.8 ± 0.72   | 6.6 ± 0.06   | 10.3 ± 0.43  | 11.8 ± 0.64  | 12.7 ± 0.52  |

|                  |             |             |             |             |             |             |
|------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Ammonia-N (mg/L) | 0.46 ± 0.06 | 0.28 ± 0.04 | 0.35 ± 0.06 | 0.62 ± 0.05 | 0.84 ± 0.05 | 0.35 ± 0.06 |
|------------------|-------------|-------------|-------------|-------------|-------------|-------------|

**Key:**

DO = Dissolved oxygen; BOD = Biological oxygen demand; COD = Chemical oxygen demand

TDS = Total dissolved solids; TSS = Total suspended solids.

**Table 4.12:** Physicochemical Properties of Water samples from Different Sites of River Sokoto in August, 2014 (Rainy Season)

| Parameter           | Values at Sampling Sites |             |             |             |             |             |
|---------------------|--------------------------|-------------|-------------|-------------|-------------|-------------|
|                     | P1                       | P2          | P3          | P4          | P5          | P6          |
| pH                  | 6.24 ± 0.05              | 6.32 ± 0.01 | 6.36 ± 0.07 | 7.23 ± 0.04 | 6.80 ± 0.05 | 6.84 ± 0.02 |
| Temperature (°C)    | 29.1 ± 0.32              | 29.0 ± 0.03 | 28.2 ± 0.06 | 29.0 ± 0.03 | 29.2 ± 0.06 | 28.8 ± 0.06 |
| Conductance (µS/cm) | 470 ± 0.06               | 100 ± 0.04  | 90 ± 0.05   | 510 ± 0.03  | 120 ± 0.06  | 80 ± 0.05   |
| D. O. (mg/L)        | 0.10 ± 0.05              | 0.07 ± 0.01 | 0.08 ± 0.07 | 0.09 ± 0.01 | 0.07 ± 0.01 | 0.10 ± 0.05 |
| B. O. D. (mg/L)     | 0.51 ± 0.06              | 0.27 ± 0.05 | 0.20 ± 0.05 | 0.35 ± 0.01 | 0.43 ± 0.05 | 0.28 ± 0.01 |
| C. O. D. (mg/L)     | 1008 ± 0.54              | 480 ± 0.57  | 48 ± 0.04   | 32 ± 0.02   | 688 ± 0.58  | 720 ± 0.54  |
| Total Solids (mg/L) | 1300 ± 0.50              | 900 ± 0.40  | 900 ± 0.40  | 300 ± 0.16  | 900 ± 0.40  | 1100 ± 0.48 |
| TDS (mg/L)          | 800 ± 0.58               | 500 ± 0.58  | 500 ± 1.15  | 100 ± 1.42  | 500 ± 2.80  | 700 ± 5.77  |
| TSS (mg/L)          | 500 ± 2.31               | 400 ± 4.62  | 400 ± 4.04  | 200 ± 10.9  | 400 ± 4.04  | 400 ± 5.20  |
| Alkalinity (mg/L)   | 10 ± 1.15                | 8 ± 0.58    | 11 ± 2.03   | 13 ± 1.15   | 13 ± 1.73   | 7 ± 2.89    |
| Acidity (mg/L)      | 20 ± 1.15                | 60 ± 2.89   | 60 ± 4.04   | 20 ± 1.15   | 60 ± 3.46   | 60 ± 5.20   |
| Chlorides (mg/L)    | 60 ± 3.46                | 60 ± 5.77   | 80 ± 1.15   | 180 ± 3.46  | 110 ± 2.31  | 90 ± 4.04   |
| Sulphate (mg/L)     | 124 ± 2.31               | 82 ± 2.31   | 82 ± 1.73   | 124 ± 2.31  | 124 ± 2.89  | 82 ± 2.31   |
| Phosphate (mg/L)    | 0.21 ± 0.02              | 0.19 ± 0.05 | 0.21 ± 0.06 | 0.21 ± 0.03 | 0.21 ± 0.02 | 0.22 ± 0.02 |

|                  |              |              |              |              |              |              |
|------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Hardness (mg/L)  | 265.9 ± 0.06 | 350.4 ± 0.35 | 305.6 ± 0.64 | 850.3 ± 0.81 | 305.4 ± 0.43 | 230.5 ± 0.69 |
| Nitrates (mg/L)  | 14.2 ± 0.20  | 12.4 ± 0.23  | 8.6 ± 0.23   | 28.1 ± 0.15  | 24.3 ± 0.23  | 10.9 ± 0.58  |
| Ammonia-N (mg/L) | 0.82 ± 0.02  | 0.64 ± 0.02  | 0.98 ± 0.01  | 1.20 ± 0.11  | 1.10 ± 0.02  | 0.86 ± 0.01  |

Key:

DO = Dissolved oxygen; BOD = Biological oxygen demand; COD = Chemical oxygen demand

TDS = Total dissolved solids; TSS = Total suspended solids.

**Table 4.13:** One way ANOVA of Physicochemical parameters of water samples from six points on River Sokoto during dry and rainy seasons

| Parameter  | Dry Season |    |    |    |    |    | Rainy Season |    |    |    |    |    |
|------------|------------|----|----|----|----|----|--------------|----|----|----|----|----|
|            | P1         | P2 | P3 | P4 | P5 | P6 | P1           | P2 | P3 | P4 | P5 | P6 |
| pH         | +          | +  | C  | +  | +  | -  | +            | +  | C  | -  | -  | -  |
| Temp       | +          | +  | C  | +  | +  | -  | +            | +  | C  | +  | +  | +  |
| Conduct    | +          | +  | C  | +  | +  | +  |              |    |    |    |    |    |
| DO         | +          | -  | C  |    | -  | -  | +            | -  | C  | +  | -  | +  |
| BOD        | +          | -  | C  | -  | -  | -  | +            | +  | C  | +  | +  | +  |
| COD        | -          | -  | C  | -  | -  | -  | -            | -  | C  | -  | -  | -  |
| T. Solids  | -          | -  | C  | -  | -  | -  | -            | -  | C  | -  | -  | -  |
| TDS        | -          | -  | C  | -  | -  | -  | -            | -  | C  | -  | -  | -  |
| TSS        | -          | -  | C  | -  | -  | -  | -            | -  | C  | -  | -  | -  |
| Alkalinity | -          | -  | C  | -  | -  | -  | -            | -  | C  | -  | -  | -  |
| Acidity    | -          | -  | C  | -  | -  | -  | -            | -  | C  | -  | -  | -  |
| Chloride   | -          | -  | C  | -  | -  | -  | -            | -  | C  | -  | -  | -  |
| Sulphate   | -          | -  | C  | -  | -  | -  | -            | -  | C  | -  | -  | -  |
| Phosphate  | -          | +  | C  | -  | +  | +  |              | -  | C  |    |    | +  |
| Hardness   | +          | -  | C  | +  | +  | -  | -            | +  | C  | +  | -  | -  |
| Nitrates   | +          | +  | C  | +  | +  | +  | +            | +  | C  | +  | +  | +  |

Ammonia + - C + + - - C + + -

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Key

C= control variable

+ = significantly higher than control

- = significantly lower than control

Blank = no significant difference

Level of significance =  $P \leq 0.05$

### **Elemental Analysis of Water Samples from River Sokoto and its Drainage**

The results of AAS analysis indicating the concentrations of the analysed metals and mineral elements in water and bottom soil sediments for all sampling points in dry and rainy seasons are shown in Tables 4.14, 4.15 and 4.16.

Table 4.14 which presents the concentrations of elements in the water samples in dry season shows that the concentrations of Fe, Ag, Cd and Hg were generally high and above WHO limits. Elements such as Cr, Mn, Co, Ni and Sb were not detected in the water samples. Presence and levels of elements detected varied from one sampling point to another. For example, while Zn level was high and above WHO limit at P3, P4 and P6, it was much lower and below WHO limit at the other sampling points. Similarly, Mo and Pb were only detectable each in one of the six sampling points. The levels of Ca, Zn and Pb were significantly higher at P4, where the industrial effluents discharge into the river.

The concentration of elements in the water samples in rainy season is presented in Table 4.15. It shows very high concentrations of Hg above WHO limit in all the water samples. Furthermore, Mn and Pb were high in one sample each while Cr and Fe were high in more than one sample. However, Ni, Cu, Zn, Mo, Ag and Cd were within permissible limits in all samples and Sb was not detected.

Table 4.16 presents the concentrations of elements in the samples from bottom soil sediment of the river. Concentration values for Fe, Ag, Cd and Hg were generally high above WHO limits in all the samples while values for Cr, Mn, Sb, and Pb in some samples were lower and below WHO limit. Meanwhile, Pb was excessively high in P2, other elements (Cu and Zn) were below WHO limit and no Ni nor Mo was detected in the sediment samples.

All water and bottom soil sediment samples for all sampling points contained Hg, Ag, Cd and Fe with values significantly on the high side in the dry season. There was mostly higher concentration in water samples in dry season than in the rainy season.

The statistical analyses of the concentrations of elements in water and bottom soil sediment samples from various points on river Sokoto during dry and rainy seasons were depicted in Tables 4.17 and 4.18.

Table 4.17 shows the t-test analysis of water samples in dry and rainy seasons. The test indicated there were significant differences in Ca, Ni, Cu and Zn, no significant difference in Pb and differences in Na, Mg, K, Cr, Fe, Co, Ag, Cd, Sn, and Hg were highly significant. It was also shown in the table that Mn and Sb were not present in the water samples in the two seasons.

One way ANOVA test of the elemental concentrations of samples of water and bottom soil sediment from various points on river Sokoto during dry season was depicted in Table 4.18. The concentrations of majority of the elements (Mg, Ca, Cr, Mn, Fe, Co, Cu, Zn, Mo, Ag, Cd, Sb and Pb) vary in water and bottom soil sediment. While Ca, Mg and Mo were more in water, Zn, Ag, Cd, Cr, Mn, Fe, Co and Pb were more in sediment. Values of Na, K, and Hg were significantly lower than those in P3 (control) in water and bottom soil sediment in all sampling points. Ni was not detected both in the water and in the sediment.

**Table 4.14:** Concentration of Elements in Water from Different Sampling Points on River Sokoto in Dry Season

| Element | Concentration at sampling points (mg/L) |             |             |             |             |             | WHO Guideline (2003) [mg/L] |
|---------|---|-------------|-------------|-------------|-------------|-------------|-----------------------------|
|         | P1                                      | P2          | P3          | P4          | P5          | P6          |                             |
| Na      | 180.00±0.00                             | 230.00±0.00 | 180.00±0.00 | 200.00±0.00 | 180.00±0.00 | 200.00±0.00 | NP                          |
| Mg      | 28.90±0.01                              | 30.82±0.00  | 32.02±0.00  | 35.77±0.00  | 32.49±0.00  | 31.68±0.00  | NP                          |
| K       | 80.00±0.00                              | 90.00±0.00  | 60.00±0.00  | 70.00±0.00  | 70.00±0.00  | 50.00±0.00  | NP                          |
| Ca      | 83.02±0.00                              | 105.47±0.00 | 102.20±0.00 | 458.53±0.00 | 227.66±0.00 | 100.85±0.00 | NP                          |
| Cr      | 0.000±0.00                              | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.05                        |
| Mn      | 0.000±0.00                              | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.5                         |
| Fe      | 44.33±0.00*                             | 35.15±0.00* | 44.37±0.00* | 11.64±0.00* | 10.11±0.00* | 30.13±0.00* | 0.3                         |
| Co      | 0.000±0.00                              | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | NG                          |
| Ni      | 0.000±0.00                              | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.02                        |
| Cu      | 0.056±0.00                              | 0.505±0.00  | 0.306±0.00  | 0.173±0.00  | 0.736±0.00  | 0.064±0.00  | 2.0                         |
| Zn      | 0.076±0.00                              | 0.278±0.00  | 0.360±0.00* | 1.028±0.00  | 0.210±0.00  | 0.311±0.00* | 0.3                         |
| Mo      | 2.57±0.00*                              | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.07                        |
| Ag      | 3.60±0.00*                              | 3.53±0.00*  | 3.72±0.00*  | 3.26±0.00*  | 2.49±0.00*  | 2.57±0.00*  | 0.1                         |
| Cd      | 1.35±0.00*                              | 1.38±0.00*  | 1.43±0.00*  | 1.42±0.00*  | 1.34±0.00*  | 1.54±0.00*  | 0.003                       |
| Sn      | 96.41±0.00                              | 78.27±0.00  | 54.92±0.00  | 43.32±0.00  | 53.55±0.00  | 41.48±0.00  | NG                          |
| Sb      | 0.000±0.00                              | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.005                       |

|    |             |             |              |              |              |             |       |
|----|-------------|-------------|--------------|--------------|--------------|-------------|-------|
| Hg | 80.15±0.00* | 83.39±0.00* | 287.37±0.00* | 142.52±0.00* | 105.54±0.00* | 165.08±0.0* | 0.001 |
| Pb | 0.000±0.00  | 0.000±0.00  | 0.000±0.00   | 0.040±0.00   | 0.000±0.00   | 0.000±0.00  | 0.01  |

Key:

\* = Level above permissible limit.

NP = No value is proposed.

NG = Not mentioned in the WHO Guideline.

**Table 4.15:** Concentration of Elements in Water from Different Sampling Points on River Sokoto in Rainy Season

| Element | Concentration at sampling points (mg/L) |             |             |            |             |             | WHO Guideline (2003) [mg/L] |
|---------|---|-------------|-------------|------------|-------------|-------------|-----------------------------|
|         | P1                                      | P2          | P3          | P4         | P5          | P6          |                             |
| Na      | 31.00±0.00                              | 27.00±0.00  | 29.00±0.00  | 47.00±0.00 | 44.00±0.00  | 27.00±0.00  | NP                          |
| Mg      | 0.699±0.01                              | 0.707±0.00  | 0.706±0.00  | 0.728±0.00 | 0.745±0.00  | 0.780±0.00  | NP                          |
| K       | 62.00±0.00                              | 9.00±0.00   | 40.00±0.00  | 18.00±0.00 | 33.00±0.00  | 9.00±0.00   | NP                          |
| Ca      | 0.362±0.00                              | 0.378±0.00  | 0.342±0.00  | 1.496±0.00 | 0.948±0.00  | 1.573±0.00  | NP                          |
| Cr      | 0.063±0.00*                             | 0.135±0.00* | 0.149±0.00* | 0.044±0.00 | 0.061±0.00* | 0.076±0.00* | 0.05                        |
| Mn      | 0.000±0.00                              | 0.000±0.00  | 0.000±0.00  | 0.000±0.00 | 0.000±0.00  | 0.001±0.00  | 0.5                         |
| Fe      | 0.760±0.00*                             | 0.518±0.00* | 0.565±0.00* | 0.000±0.00 | 0.213±0.00  | 0.166±0.00  | 0.3                         |
| Co      | 0.044±0.00                              | 0.050±0.00  | 0.041±0.00  | 0.039±0.00 | 0.047±0.00  | 0.049±0.00  | NG                          |
| Ni      | 0.010±0.00                              | 0.005±0.00  | 0.007±0.00  | 0.014±0.00 | 0.001±0.00  | 0.010±0.00  | 0.02                        |
| Cu      | 0.014±0.00                              | 0.009±0.00  | 0.011±0.00  | 0.007±0.00 | 0.011±0.00  | 0.008±0.00  | 2.0                         |
| Zn      | 0.031±0.00                              | 0.032±0.00  | 0.070±0.00  | 0.036±0.00 | 0.027±0.00  | 0.054±0.00  | 0.3                         |
| Mo      | 0.024±0.00                              | 0.000±0.00  | 0.000±0.00  | 0.000±0.00 | 0.000±0.00  | 0.000±0.00  | 0.07                        |
| Ag      | 0.008±0.00                              | 0.000±0.00  | 0.000±0.00  | 0.000±0.00 | 0.000±0.00  | 0.000±0.00  | 0.1                         |
| Cd      | 0.002±0.00                              | 0.000±0.00  | 0.000±0.00  | 0.000±0.00 | 0.000±0.00  | 0.000±0.00  | 0.003                       |
| Sn      | 1.426±0.00                              | 1.165±0.00  | 0.036±0.00  | 0.014±0.00 | 0.032±0.00  | 0.012±0.00  | NG                          |

|    |             |             |             |              |             |             |       |
|----|-------------|-------------|-------------|--------------|-------------|-------------|-------|
| Sb | 0.000±0.00  | 0.000±0.00  | 0.000±0.00  | 0.000±0.00   | 0.000±0.00  | 0.000±0.00  | 0.005 |
| Hg | 2.989±0.00* | 1.828±0.00* | 1.411±0.00* | 1.732±0.00*  | 1.366±0.00* | 1.516±0.00* | 0.001 |
| Pb | 0.000±0.00  | 0.000±0.00  | 0.000±0.000 | 0.069±0.000* | 0.000±0.000 | 0.000±0.000 | 0.01  |

**Key:**

\* = Level above permissible limit.

NP = No value is proposed.

NG = Not mentioned in the WHO Guideline.

**Table 4.16:** Concentration of Elements in Bottom Soil Sediments from Different Sampling Points on River Sokoto in Dry Season

| Element | Concentration at sampling points (mg/L) |             |             |              |              |             | WHO Guideline (2003) [mg/L] |
|---------|---|-------------|-------------|--------------|--------------|-------------|-----------------------------|
|         | P1                                      | P2          | P3          | P4           | P5           | P6          |                             |
| Na      | 150.00±0.00                             | 160.00±0.00 | 100.00±0.00 | 200.00±0.00  | 220.00±0.00  | 210.00±0.00 | NP                          |
| Mg      | 26.04±0.00                              | 31.59±0.00  | 28.17±0.00  | 34.48±0.00   | 34.28±0.00   | 30.54±0.00  | NP                          |
| K       | 60.00±0.00                              | 70.00±0.00  | 30.00±0.00  | 70.00±0.00   | 70.00±0.00   | 70.00±0.00  | NP                          |
| Ca      | 68.11±0.00                              | 365.36±0.00 | 71.54±0.00  | 227.43±0.00  | 363.22±0.00  | 78.35±0.00  | NP                          |
| Cr      | 2.629±0.00*                             | 0.000±0.00  | 0.000±0.00  | 10.61±0.00*  | 0.000±0.00   | 1.453±0.00* | 0.05                        |
| Mn      | 0.000±0.00                              | 0.000±0.00  | 0.000±0.00  | 1.992±0.00*  | 0.411±0.00   | 0.000±0.00  | 0.5                         |
| Fe      | 53.15±0.00*                             | 50.87±0.00* | 94.00±0.00* | 137.38±0.00* | 129.54±0.00* | 76.59±0.00* | 0.3                         |
| Co      | 0.560±0.00                              | 0.007±0.00  | 0.000±0.00  | 0.000±0.00   | 0.000±0.00   | 0.000±0.00  | NG                          |
| Ni      | 0.000±0.00                              | 0.000±0.00  | 0.000±0.00  | 0.000±0.00   | 0.000±0.00   | 0.000±0.00  | 0.02                        |
| Cu      | 0.753±0.00                              | 0.505±0.00  | 0.591±0.00  | 0.884±0.00   | 0.533±0.00   | 0.383±0.00  | 2.0                         |
| Zn      | 0.424±0.00                              | 0.434±0.00  | 0.050±0.00  | 1.565±0.00   | 0.766±0.00   | 0.331±0.00  | 0.3                         |
| Mo      | 0.000±0.00                              | 0.000±0.00  | 0.000±0.00  | 0.000±0.00   | 0.000±0.00   | 0.000±0.00  | 0.07                        |
| Ag      | 7.139±0.00*                             | 5.182±0.00* | 6.314±0.00* | 3.322±0.00*  | 3.486±0.00*  | 3.554±0.00* | 0.1                         |
| Cd      | 1.337±0.00*                             | 1.276±0.00* | 1.167±0.00* | 1.214±0.00*  | 1.240±0.00*  | 1.252±0.00* | 0.003                       |

|    |              |              |              |              |              |              |       |
|----|--------------|--------------|--------------|--------------|--------------|--------------|-------|
| Sn | 0.000±0.00   | 39.21±0.00   | 46.07±0.00   | 69.95±0.00   | 76.07±0.00   | 84.36±0.00   | NG    |
| Sb | 7.534±0.00*  | 2.521±0.00*  | 3.716±0.00*  | 3.423±0.00*  | 0.000±0.00   | 0.406±0.00*  | 0.005 |
| Hg | 540.76±0.00* | 501.88±0.00* | 801.57±0.00* | 249.48±0.00* | 119.84±0.00* | 111.59±0.00* | 0.001 |
| Pb | 8.204±0.00*  | 10.428±0.0*  | 0.000±0.000  | 0.000±0.000  | 0.000±0.000  | 0.000±0.000  | 0.01  |

Key:

\* = Level above permissible limit.

NP = No value is proposed.

NG = Not mentioned in the WHO Guideline.

**Table 4.17:** The t-test Analysis of Elemental concentrations of water samples from different pointson River Sokoto in dry and rainy seasons

| Element | Mean ± SD       |               | Level of significance | t-value |
|---------|-----------------|---------------|-----------------------|---------|
|         | Dry Season      | Rainy Season  |                       |         |
| Na      | 195.00 ± 19.75  | 34.17 ± 8.95  | 0.000 □ □             | 18.17   |
| Mg      | 31.95 ± 2.26    | 0.73 ± 0.03   | 0.000 □ □             | 33.83   |
| K       | 70.00 ± 14.14   | 28.50 ± 20.72 | 0.002 □ □             | 4.05    |
| Ca      | 179.62 ± 146.37 | 0.85 ± 0.58   | 0.014 □               | 2.99    |
| Cr      | 0.00 ± 0.00     | 0.09 ± 0.05   | 0.001 □ □             | -4.73   |
| Mn      | 0.00 ± 0.00     | 0.00 ± 0.00   | -                     | -       |
| Fe      | 29.29 ± 15.28   | 0.37 ± 0.29   | 0.001 □ □             | 4.63    |
| Co      | 0.00 ± 0.00     | 0.05 ± 0.01   | 0.000 □ □             | -22.53  |
| Ni      | 0.00 ± 0.00     | 0.01 ± 0.01   | 0.010 □               | -3.16   |
| Cu      | 0.31 ± 0.27     | 0.01 ± 0.00   | 0.023 □               | 2.69    |
| Zn      | 0.38 ± 0.33     | 0.04 ± 0.02   | 0.033 □               | 2.47    |
| Ag      | 3.20 ± 0.54     | 0.00 ± 0.00   | 0.000 □ □             | 14.56   |
| Cd      | 1.41 ± 0.07     | 0.00 ± 0.00   | 0.000 □ □             | 47.18   |

|    |                |             |           |       |
|----|----------------|-------------|-----------|-------|
| Sn | 61.34 ± 21.61  | 0.44 ± 0.66 | 0.000 □ □ | 6.90  |
| Sb | 0.00 ± 0.00    | 0.00 ± 0.00 | -         | -     |
| Hg | 144.01 ± 77.76 | 1.81 ± 0.61 | 0.001 □ □ | 4.48  |
| Pb | 0.00 ± 0.00    | 0.00 ± 0.01 | 0.341     | -1.00 |

Difference is not significant at  $p > 0.05$

□ Difference is significant at  $p \leq 0.05$

□ □ Difference is highly significant at  $p < 0.01$

**Table 4.18:** One way ANOVA Post Hoc Test of Elemental Concentrations of Water samples and Bottom soil sediments from different sampling points on River Sokoto in dry season

| Element | Concentration in Water |    |    |    |    |    | Concentration in Soil Sediment |    |    |    |    |    |
|---------|------------------------|----|----|----|----|----|--------------------------------|----|----|----|----|----|
|         | P1                     | P2 | P3 | P4 | P5 | P6 | P1                             | P2 | P3 | P4 | P5 | P6 |
| Na      | -                      | -  | C  | -  | -  | -  | -                              | -  | C  | -  | -  | -  |
| Mg      | -                      | -  | C  | +  | +  | -  | +                              | -  | C  | -  | -  | -  |
| K       | -                      | -  | C  | -  | -  | -  | -                              | -  | C  | -  | -  | -  |
| Ca      | -                      | +  | C  | +  | +  | -  | +                              | -  | C  | -  | -  | -  |
| Cr      |                        |    |    |    |    |    | +                              |    | C  | +  |    | +  |
| Mn      |                        |    |    |    |    |    |                                |    | C  | +  | +  |    |
| Fe      | -                      | -  | C  | -  | -  | -  | -                              | -  | C  | +  | +  | -  |
| Co      |                        |    |    |    |    |    | +                              | +  | C  |    |    |    |
| Ni      |                        |    |    |    |    |    |                                |    |    |    |    |    |
| Cu      | -                      | +  | C  | -  | +  | -  | +                              | -  | C  | +  | -  | -  |
| Zn      | -                      | -  | C  | +  | -  | -  | +                              | +  | C  | +  | +  | +  |
| Mo      | +                      |    | C  |    |    |    |                                |    |    |    |    |    |
| Ag      | -                      | -  | C  | -  | -  | -  | +                              | -  | C  | -  | -  | -  |
| Cd      | -                      | -  | C  | -  | -  | +  | +                              | +  | C  | +  | +  | +  |
| Sn      | +                      | +  | C  | -  | -  | -  | +                              | +  | C  | -  | -  | -  |



is therefore imperative to study the river to know the impact of these factors on the river with the aim of improving its quality for the safety of the people.

The study on River Sokoto revealed that the heterotrophic and coliform counts increased with the onset of rainfall in May and started to decrease at the peak of rainfall in July. This is expected because early rainfalls are characterized with carrying heavy contaminants as runoff into the river which reduces as rainfall reduces. Similar phenomenon has been reported by other researchers (Venglovsky *et al.*, 2009; Tytler, 2011; Singh *et al.*, 2012). High level heterotrophic and coliform counts observed in this study agree with the findings of Tytler (2011) where adjoining two drainage streams from industrial and residential areas, impacted negatively the water quality of River Kaduna. While sampling point (P1) about 2 kilometers away from farmland had the highest heterotrophic and coliform counts, site P4 on the stream that carried effluents from Sokoto Cement Factory into the river had the least values throughout the year. Cement factories are not normally associated with large volumes of liquid waste. The statistical analysis results further revealed that there was generally no significant difference in the bacteria counts except at P4 versus P5 and P4 versus P6. Runoff from various tributaries carrying large amount of excrements from people residing around the river, might be responsible for high coliform counts in P1 while the least values recorded in P4 might be due to the fact that bacteria are not common components of the effluents from cement factories. Bacteria counts recorded in the river might have arisen from residents' defecations and compost manure used on farm located at the upstream, near the factories. Lack of proper sanitation in urban cities has been cited as the main cause of high bacterial pathogens in rivers traversing major world cities (Abraham, 2010). The preliminary survey of the study area showed that residents used to constantly defecate around the place and this might be the reason for the constancy in the faecal

streptococci counts. Unhygienic defecation on ground causing contamination of surface water has also been reported in other studies (Brooks *et al.*, 2009; Furtula *et al.*, 2013; Raji *et al.*, 2015). It was usual in the study area during rainfall to see runoffs heavily loaded with human excrements that were discarded by residents because of lack of latrine. The high level of faecal coliform observed in the river water poses a health risk to those using it especially for domestic purposes. Both faecal coliform and faecal streptococci have been reported to pose health hazards (Brooks *et al.*, 2009; Singh *et al.*, 2012; Furtula *et al.*, 2013).

In all the water samples, FC/FS ratios greater than 4.0 were obtained which is an indication of human contamination. Though the values obtained in this study were the highest, the results are in agreement with those of Singh *et al.* (2012) in Ganges River in India and Tytler (2011) in River Kaduna, Nigeria.

River Sokoto was highly contaminated with pathogenic bacteria such as *Klebsiella*, *Shigella*, *Salmonella*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. These organisms are pathogenic, likely products of human activities in and around the river and their presence in the river water suggest the level of poor sanitation practice among the residents in the river's proximity. Previous studies abound that have shown similar organisms isolated from rivers (Obi *et al.*, 2004; Tytler, 2011; Abednego *et al.*, 2013). Most of the *Enterobacteriaceae* isolates are water-borne pathogens capable of causing many water-borne diseases while the *Pseudomonas aeruginosa* isolates (opportunistic organisms), are known to cause various infections in people with cystic fibrosis, chronic obstructive lung disease or immune compromised people (Halls, 2004). Epidemiologic reports have associated use of raw river water with waterborne disease outbreaks. Hamner *et al.*, (2006) associated water-borne disease (including acute gastrointestinal disease, cholera, dysentery, hepatitis- A, and typhoid) with the use of Ganges River in India.

Residents around River Sokoto use water from the river for similar purposes. Therefore, isolation of these pathogenic bacteria from River Sokoto could be the cause for rampant cases of water borne infections in the study area as reported earlier by Raji and Ibrahim (2011).

High counts of *Staphylococcus aureus* isolates might be as a result of constant interaction of humans with the river for different purposes as the organisms are commensals of human skin. This study showed *E. coli* as the most dominant bacteria isolated, an observation that is in agreement with those of Tytler (2011) in River Kaduna, Nigeria and Abednego *et al.* (2013) on Nairobi River in Kenya. Chitanand *et al.* (2010) have also reported similar results in India. Generally, members of the *Enterobacteriaceae* family have been incriminated to cause waterborne infections in the previous studies and these infections thrive in the study area (Raji and Ibrahim, 2011). While *E. coli* causes gastrointestinal disorders like diarrhea, *Salmonella* and *Shigella* are involved in typhoid fever and diarrhea infections respectively. The presence of *Raoultella ornithinolytica* and *Elizabethkingia meningoseptica* in River Sokoto is a new finding- such organisms have never been reported in rivers in this part of the world.

Observation of resistant strains of *E. coli*, *Klebsiella spp*, *Salmonella* and *Shigella spp* to ampicillin in the study area calls for urgent attention. Some level of resistance exhibited by *Providenciarettgeri* to gentamicin in this study was expected as it has been reported in earlier study by Cornaglia *et al.* (1995) that *P. rettgeri* and *P. stuartii* were generally resistant to gentamicin and tobramycin but susceptible to amikacin. However, *Shigella* and *Providencia* were generally susceptible to ceftazidime and nitrofurantoin respectively, which is consistent with the findings of Nakamura *et al.* (2005). *Raoultella ornithinolytica* (formerly called *Klebsiella ornithinolytica*) belongs to the family of *Enterobacteriaceae* which are Gram-negative and capable of causing bacteremia, pneumonia and urinary tract infections. Reports in the

literaturesuggest that most clinical isolates of *R. ornithinolytica* were resistant to several classes of antimicrobials (Al-Hulu *et al.*, 2009). This study corroborates the above assertion that most of the *R. Ornithinolytica* isolates in this study exhibited resistance to the various test antibiotics such as ampicillin, amoxicillin/clav, ceftazidime, ceftriaxone, ciprofloxacin, ofloxacin, cloxacillin, cefuroxime, nitrofurantoin, erythromycin and gentamicin.

The low resistance of *S. faecalis* isolates to gentamicin in this study is in agreement with those of Furtular, *et al.* (2013). It is also consistent with other previous reports (Butaye *et al.*, 2001; Butaye *et al.*, 2002; Petsaris *et al.*, 2005). Emergence of antibiotic resistance among *P. aeruginosa* isolates resulting in severe infections has been variously reported (Olayinka *et al.*, 2004; Tytler, 2011). It was reported that in Zaria and Enugu about 60%, 42%, 33% of clinical isolates of *Pseudomonas spp*, *Escherichia spp*, and *Klebsiella spp* respectively were multidrug resistant (Olayinka *et al.*, 2004). When MAR index is greater than 0.3, it indicates high level infectious disease in an area where antibiotics are frequently used. Some of the isolated organisms in this study demonstrated some degree of general resistance to all the test antibiotics and exhibited multi drug resistance with some of the antibiotics, suggesting the study area to be of high antibiotic usage.

The results of the study have shown that the isolated organisms were not only resistant to antibiotics but also the test disinfectants. Savlon when compared with Dettol was seen to have recorded the least percentage resistance to *Streptococcus faecalis*. This on one hand might be due to the fact that Dettol<sup>®</sup> was more frequently used than Savlon in the study area and has resulted in the isolates being resistant to Dettol<sup>®</sup> and it might be due to combination (synergistic) effect of chlorhexidine and cetrimide in Savlon<sup>®</sup>, on the other hand. Improvement in antimicrobial efficacy due to synergistic effect has been reported (Ibrahim and Alere, 1998). Therefore, efficacy of

Savlon<sup>®</sup> over other disinfectants has made it an antimicrobial of choice to minimise the menace of resistant strains in the study area.

Plasmids harbor genes coding for specific functions including virulence factors and antibiotic resistance that permit bacteria to survive the hostile environment found in the host and resist treatment (Ramirez *et al.*, 2014). In the molecular analysis carried out in this study, *Salmonella* virulence plasmid genes (*spvC*) were detected in the *Salmonella typhi* isolates analysed. These virulence genes must have been the determinant genes for the resistance earlier observed for *Salmonella typhi* isolates in the antibiotic susceptibility test. *Salmonella* plasmid virulence genes have been detected in various *Salmonella* species (Carlson *et al.*, 1999; Ziemer and Steadham, 2003; Carattoli, 2003). The  $\beta$ -lactam (*bla*<sub>TEM</sub>) and quinolone (*qnrS*) resistance genes detected in the test *Pseudomonas aeruginosa* isolate and; the aminoglycoside (*aacC3*) and quinolone (*qnrS*) resistance genes detected in the test *Klebsiella pneumoniae* isolates, is not new. In several geographical regions, including Argentina, the USA and Southeast Asia, plasmid mediated quinolone resistance (PMQR) determinants have been reported at high prevalence in both human and animal isolates and are often associated with the expression of extended-spectrum  $\beta$ -lactamases (ESBLs) (Park *et al.*, 2006; Deepak *et al.*, 2009; Zhang *et al.*, 2012; Andres *et al.*, 2013; Liu *et al.*, 2013; Shaheen *et al.*, 2013). The presence of quinolone resistance genes in *Staphylococcus aureus*, *Streptococcus faecalis*, *B. subtilis* and *Providencia rettgeri*; and aminoglycoside resistance genes in *Staph saprophyticus* may account for the relative high level of resistance of the isolates to the test antibiotics, earlier reported in this study. Non-specific resistance genes were detected in *Klebsiella oxytoca* and as additional resistance gene in other isolates. This is as a result of the fact that the primers used in this study are not all-encompassing and other resistance determinant genes, not captured in the study may have been responsible.

Physicochemical parameters of the water samples for dry and rainy seasons show that the dissolved oxygen (DO) values (ranging from 0.7 – 1.7 in dry season and 0.07 – 0.10 in rainy season) and the biochemical oxygen demand (BOD) values (ranging from 0.2 – 0.7 in dry season and 0.2 – 0.51 in rainy season), were within guideline permissible limit for natural water (Appendix C). This finding agrees with the result of Singh *et al.* (2012) on the study of Ganges river. The BOD is the amount of oxygen required to oxidise biologically oxidisable substances in water and the DO is the amount of oxygen dissolved in river water. The DO is being replenished by atmospheric re-aeration and through photosynthesis. This process of self-purification is negatively affected in the presence of excess organic substrate in the water and finally stopped with the addition of sewage or organic wastes. This explains the low DO values recorded in this study and also justify the high level heterotrophic bacteria counts.

The high levels of chemical oxygen demand (COD) and conductance in the dry season compared with the rainy season is in consonance with the findings of similar results to those of Tytler (2011). The COD is the amount of oxygen required for the chemical oxidation of organic matter with the help of strong chemical oxidants. The COD will always have higher value than the BOD because it measures the amount of oxygen required to oxidise both biologically oxidisable and biologically non-oxidisable substances. High level of COD and conductance in the river water may be as a result of decrease in rainfall in dry season with resultant increase in concentration of electrolytes and other elements in the river water (Khan *et al.*, 2012).

The values of total dissolved solid (TDS) and total suspended solid (TSS) in the dry season in this study were within permissible limit recommended for surface water. However, in the rainy season, the TSS values were higher due to floating materials like fine silt carried by rainwater. Hardness is the sum of polyvalent metallic ions in water. Calcium and magnesium are

the principal components, and hard waters are most common in groundwater, especially when derived from limestone, dolomite or chalk aquifers. Total hardness values showed that the river water was generally hard during the rainy season and this might be due to the topography of the study area.

Not only was the water at sampling point P4 very hard, it also contained chloride at a value above permissible limit of 150 mg/L recommended for surface water. Sampling point P4 received effluents directly from Sokoto Cement Factory and this might be responsible for the high chloride and hardness values which decreased along the stream at point P5 as the water flowed into the river. High level chloride can have negative effects on aquatic organisms and plants by increasing species mortality and stress plant respiration to affect quality of river water. The concentrations of other nutrients such as sulphate and phosphate were within permissible limits during dry and rainy seasons except for nitrate and ammonia which had values high above permissible limit recommended for surface water. High level of nitrate and ammonia might be as a result of extensive farming with fertilizers that takes place at the bank of the river.

As shown by the AAS analysis, the water and bottom soil sediment samples for all sampling points contained Hg, Ag, Cd and Fe in relatively high values and much higher in the soil sediments. Similar finding was reported by Muhammad *et al.* (2014) in River Bunsuru, Northwestern Nigeria and attributed it to the fact that the discharge of heavy metals into rivers by domestic and industrial activities causes their rapid association with particulates and incorporation into bottom sediment.

Raji *et al.* (2010) also reported the presence of heavy metals and other elements in relatively high concentrations in the drinking waters in the same geographical zone. Muhammad *et al.* (2014) found the concentration of Pb in the bottom sediment and floodplain samples of River Bunsuru

Northwestern Nigeria to be significantly high during the rainy season sampling, while Cu values were consistently high for the rainy and dry seasons. The result of this study also has lead (Pb) concentrations in the bottom soil sediments at two sampling points P1 ( $8.204 \pm 0.0007$  mg/L) and P2 ( $10.428 \pm 0.00$  mg/L) with values very high. Elevated concentration of heavy metals in aquatic environment is a good indicator of man-induced pollution (Davies *et al.*, 1991). The abnormally very high value of lead in P2 could cause lead poisoning. This may be attributed to massive lead pollution of the river in 2010 from mining sites in Zamfara State from where River Sokoto has its source (Muhammad *et al.*, 2014). Presence of lead in sediments at whatever concentration poses threat to fishes in aquatic environment because of the bottom feeding habits of the aquatic organism (Muhammad *et al.*, 2014).

Though, other elements such as Cr, Co, Ni and Sn were found in water and bottom soil sediment samples at values very low level, long term accumulation of these elements may pose health hazards to the people in the locality (Cotman *et al.*, 2001). The presence of chromium and other elements in soaps and detergents used for washing and bathing in the river could be responsible for this.

The result shows that human contaminations are the major sources of contamination in contrast to the previous studies (Sangodoyin, 1995; Fakayode, 2005; Kanu *et al.*, 2006) which have shown that industrial effluents are responsible for the contamination of river water. There was low impact of industrial effluents on the pollution rate of River Sokoto because majority of the factories were not functioning in the course of this study as was similarly experienced by Tytler (2011) in his study on River Kaduna.

## CHAPTER SIX

### CONCLUSIONS AND RECOMMENDATIONS

#### Conclusions

The findings of the study showed that River Sokoto is bacteriologically and chemically contaminated and not fit for domestic use, drinking, farming and recreational activities.

- (a) There were high levels of heterotrophic and coliform bacteria and FC/FS ratios of the river water due to constant contamination with human wastes.
- (b) There was seasonal variation in the heterotrophic bacterial levels with the highest values at the peak of rainfall.
- (c) The distribution of the bacteria isolated from the river showed a wide range of pathogenic organisms, of which high proportion were enteric bacteria with *E. coli* having the highest proportion. This further indicates human wastes contamination.
- (d) Many of these pathogenic bacteria, especially *Shigella flexneri*, *E. coli*, *Staphylococcus aureus* etc. were multi-drug resistant to commonly used antibiotics in the study area.
- (e) PCR analysis of some of the antibiotic resistant isolates further confirmed occurrence of resistant bacteria strains in the study area.

- (f) Water of River Sokoto contained heavy metals namely Hg, Cd, Ag and Pb at high concentrations and Cr, Co, Ni and Snat low concentrations which have been reported to cause chronic diseases such as high blood pressure, anemia, Mina mata disease, cancer of lung, skin, bladder and kidney, skin lesions (hyperkeratosis) and pigmentation changesand therefore may pose health risks to the people using the water for various purposes.
- (g) Human contaminations are the major sources of contamination in this study rather than what were mostly reported in previous studies (Sangodoyin, 1995; Fakayode, 2005; Kanu *et al.*, 2006) that industrial effluents are responsible for the contamination of river water.

## **Recommendations**

1. The findings of the study have implicated human wastes contamination as the major source of contamination of the Sokoto River and therefore, residents in the study area should be provided with standard latrines and also be enlightened on observation of regular sanitation practices.
2. Water from the river should be properly boiled or disinfected before being used for domestic purposes.
3. Fishes from pathogenic bacteria-ladened River Sokoto and irrigated vegetables from farmlands around the river should be properly cooked before eating to prevent bacterial infections.
4. Antibiotic susceptibility profile of pathogenic organisms should be carried out positively prior to therapy.

5. People and industries in the study area should be enlightened on proper waste management and disposal.
6. Government should conduct regular surveillance and monitoring of the river to ensure good quality of the river water.

## **REFERENCES**

- Abednego, M.M., Mbaruk, A.S., John, N.M., and John, M.M. (2013). Water-borne bacterial pathogens in surface waters of Nairobi River and health implication to communities downstream Athi River. *International Journal of Life Science & Pharm Research* 3(1): 4 – 10.
- Abraham, W.R. (2010). Megacities as source for pathogenic bacteria in rivers and their fate downstream. *Intl. J. Microbiol*, 10 (13): 1155.
- ADAS (Agricultural Development Advisory Services). (2007). Diffuse nitrate pollution from agriculture – strategies for reducing nitrate leaching. ADAS report to Defra – supporting paper D3 for the consultation on implementation of the Nitrates Directive in England.
- Ademoroti, C.M.A. (1996). Standard Methods for Water and Effluents Analysis. Foludex Press Ltd. Ibadan, pp. 52-53.
- Ahuja, S. (2009). Overview. In: *Handbook of Water Quality and Purity* 1<sup>st</sup>Edn. pp. 1-16
- Ajayi S.O. and Osibanji O. (1981). Pollution studies on Nigeria Rivers 11; Water quality of some Nigerian Rivers. *Environ. Pollut. Series 2*: 87-95
- Al-Hulu, S.M., Al-Charrakh, A.H., Al-Saadi, M.A.K. (2009) Isolation and characterization of *Raoultella ornithinolytica* from clinical specimens in Hilla city, Iraq. *Med J Babylon* 7: 42-47.
- Alley, R.E. (2007). Pollution Classifications. In: *Water Quality Handbook* 2<sup>nd</sup>Edn McGraw-Hill Publishing Co., New York pp. 24-51.

- Andres, P.; Lucero, C.; Soler-Bistue, A.; Guerriero, L.; Albornoz, E.; Tran, T.; Zorreguieta, A.; Galas, M.; Corso, A. and other authors (2013). Differential distribution of plasmid-mediated quinolone resistance genes in clinical enterobacteria with unusual phenotypes of quinolone susceptibility from Argentina. *Antimicrob Agents Chemother* **57**: 2467–2475.
- APHA (American Public Health Association). (1998). *Standard Methods for the Examination of Water and Wastewater*, 20th edn. Washington, D.C.
- Au, D.W.T., Pollino, C.A., Wu, R.S.S., Shin, P.K.S., Lau, S.T.F. and Tang, J.Y.M. (2004) Chronic effects of suspended solids on gill structure, osmoregulation, growth, and triiodothyronine in juvenile green grouper *Epinephelus coioides*, *Marine Ecology Progress Series*, 266: 255–64.
- Badar, U.; Ahmed, N.; Beswick, A. J.; Pattanapitpaisal, P. and Macaskie, L.E. (2000). Reduction of Chromate by microorganisms isolated from metal contaminated sites of Karachi, Pakistan. *Biotechnology Letters*, 22: 829-836.
- Barakat, M.A. (2011). New trends in removing heavy metals from industrial wastewater, *Arabian Journal of Chemistry* **4**: 361-377.
- Belaouaj, A., Lapoumeroulie, C, Canica, M. M., Vedel, G., Nevot, P., Krishnamoorthy, R. (1994). Nucleotide sequences of the genes coding for the TEM-like  $\beta$ -lactamases IRT-1 and IRT-2 (formerly called TRI-1 and TRI-2). *FEMS Microbiology Letters* **120**: 75-80.
- Bernhard, A.E. and Field, K.G. (2000a) Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA markers from fecal anaerobes. *Appl. Environ. Microbiol.* **66**: 1587-1594.
- Bernhard, A.E. and Field, K.G. (2000b) A PCR assay to discriminate human and ruminant feces based on host differences in Bacteroides-Prevotella 16S ribosomal DNA. *Appl. Environ. Microbiol.* **66**: 4571-4574.
- Bitton G. (2005). *Wastewater Microbiology*, Third Edition. John Wiley & Sons, Inc., Publication, Hoboken, New Jersey. Pp 221.
- Bloomfield, S. F. (1994). Methods for Assessing Antimicrobial Activity. In: Denyer, S. P. and Hugo, W. B. ed. *Mechanisms of Action for Chemical Biocides: Their Study and Exploitation*. Blackwell Scientific Publications, Oxford, pp. 1-22.

- Borrego, J.J., Cornax, R., Morinigo, M.A., Martinez-Manzares, E., and Romero, P. (1990) Coliphages as an indicator of fecal pollution in water: their survival and productive infectivity in natural aquatic environments. *Water Research*. **24**: 111-116.
- Bremner, J.M. and Mulvaney, C.S. (1982): Methods of soil analysis, part 2 Chemical and Microbiological properties, 595-624
- Brooks, J.P., Adeli, A., Read, J.J. and McLaughlin, M.R. (2009) Rainfall simulation in greenhouse microcosms to assess bacterial-associated runoff from land-applied poultry litter. *J. Environ. Qual.* **38**: 218–229.
- Burkhardt, W., Calci, K.R., Watkins, W.D., Rippey, S.R. and Chirtel, S.J. (2000) Inactivation of indicator microorganisms in estuarine waters. *Water Research*. **34**: 2207-2214.
- Butaye, P., Devriese, L.A., Haesebrouck, F. (2001) Differences in antibiotic resistance patterns of *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from farm and pet animals. *Antimicrob. Agents Chemother.* **45**: 1374–1378.
- Butaye, P., Baele, M., Devriese, L.A., Haesebrouck, F. (2002). Comparison of susceptibility to antimicrobials of the enterococcal species isolated from pigeons (*Columba livia*). *Microb. Drug Resist.* **8**: 215–218.
- Byappanahalli, M.N. and Fujioka, R.S. (1998) Evidence that tropical soil environment can support the growth of *Escherichia coli*. *Water Science and Technology* **38(12)**: 171-174.
- Cantor, K. P. (1997). Drinking Water and Cancer. *Cancer Causes Control*, **8**: 292-308.
- Carattoli, A. (2003) Plasmid-mediated antimicrobial resistance in *Salmonella enterica*. *Curr. Issues Mol. Biol.* **5**: 113-122.
- Carlson, S.A., Bolton, L.F., Briggs, C.E., Hurd, H.S., Sharma, V.K., Fedorka-Cray, P.J. and Jones, B.D. (1999). Detection of multiresistant *Salmonella typhimurium* DT 104 using multiplex and fluorogenic PCR. *Molecular and Cellular Probes* **13**: 213-222.
- Cattoir, V., Poirel, L., Rotimi, V., Soussy, C. J. and Nordmann, P. (2007). Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. *J Antimicrob Chemother.* **60**: 394–397.
- Chao, K.K., Chao, C.C. and Chao, W.L. (2003). Suitability of the traditional microbial indicators and their enumerating methods in the assessment of fecal pollution of subtropical freshwater environments. *J. Microbiol. Immunol. Infect.* **36**: 288-293.

- Cheesbrough, M. (2006). *Medical Laboratory Manual for Tropical Countries*. 2<sup>nd</sup> Edn. University Press, Cambridge, pp 200-360.
- Chima, G. N., Nwaugo, V. O., and Ezekwe I. C. (2010). Impacts of Rock Quarrying on Akwukwu Tributary of the Ivo River in Ishiagu Ebonyi State, Nigeria. *Journal of Applied Environmental Sciences*, 6(2): 68-73.
- Chitanand, M.P., Kadam, T. A., Gyananath, G., Totewad, N.D., Balha, D.K. (2010). Multiple antibiotic resistance indexing of coliforms to identify high risk contamination sites in aquatic environment. *Indian J Microbiol*, **50**: 216–220.
- Clarke, R. (1993). *Water: The International Crisis*. Massachusetts Institute of Technology Press.
- Clinical and Laboratory Standards Institute (CLSI) (2009). Methods for dilution antimicrobial susceptibility testing for bacteria that grew aerobically. Approved Standard M7-A10. Wayne, PA.
- Colwell, R.R. and Huq, A. (2001) Marine ecosystems and cholera. *Hydrobiologia* **460**: 141-145.
- Cornaglia G., Frugoni S., Mazzariol A., Piacentini E., Berlusconi A., Fontana R. (1995) Activities of oral antibiotics on *Providencia* strains isolated from institutionalized elderly patients with urinary tract infections. *Antimicrob. Agents Chemother.* **39**: 2819–2821
- Cotman M; Zagorc-Koncan J. and Droic A. (2001) Study of impacts of treated wastewater to the Krka River, Slovenia. *Water Sci. Technol*; 44(6): 47-54.
- Davies, C.A.; Tomlinson, K. and Stephenson, T. (1991). Heavy metals in river Tees estuary sediments. *Environ. Technol.*, **12**: 961-972.
- Deepak, R. N.; Koh, T. H. and Chan, K. S. (2009). Plasmid-mediated quinolone resistance determinants in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae* in a large Singapore hospital. *Ann Acad Med Singapore* **38**: 1070–1073.
- Domini, C. E., Vidal, L. and Canals, A. (2007). Main Parameters and Assays Involved with Organic Pollution of Water. In: L.M.L. Nollet (Ed.), *Handbook of Water Analysis*. Marcel Dekker, New York, pp. 337-366.
- Doran, J.W. and Linn, D.M. (1979). Bacterial quality of runoff water from pasture land. *Appl. Environ. Microbiol.* **37**: 985-991
- Dubey, J.P. (2009). *Toxoplasmosis of Animals and Humans*, Second ed. CRC Press, Boca Raton, FL.

- Dubey, R.C. and Maheshwari, D.K. (2002). Practical Microbiology. S. Chand & Company Ltd. Ram Nagar, New Delhi, pp. 327-328.
- Dufour, A.; Bartram, J.; Bos, R. and Gannon V. (2012). Animal waste, water quality and human health. IWA Publishing, London.
- Ekhaise, F.O. and Anyasi, C.C. (2005). Influence of breweries effluent discharge on the microbiological and physicochemical quality of Ikpoba River, Nigeria. *African Journal of Biotechnology* 4 (10): 1062-1065.
- Ellis, K.V. (1989). Surface water pollution and its control. Macmillan press Ltd, Hound mill, Basingstoke, Hampshire RG, London, pp 3-18, 97,100,101 and 208.
- Environment Agency (2013). *What is Inland Oil Pollution? Key Issues as the Environment Agency Sees It*. Retrieved on 6 June 2015 from [www.environment-agency.gov.uk/research/library/position/41233.aspx](http://www.environment-agency.gov.uk/research/library/position/41233.aspx)
- Environment Canada (2009). *Water Pollution* webpage. Retrieved on September 18, 2013 from <http://www.ec.gc.ca/eau-water/default.asp?lang=En&n=E86BC86A-1>.
- Ezekwe, I.C.; Ezekwe, A.S. and Endoro, O.P. (2013). Biological contaminants in the River Nun and environmental ethics of riverside communities in the Niger Delta: The case of Amassoma, Bayelsa, Nigeria. *Estud Biol.* 35(84): 67-75.
- Fakayode, S. and Onianwa, P. (2002) Heavy Metal Contamination of Soil, and Bioaccumulation in Guinea Grass (*Panicum maximum*) around Ikeja Industrial Estate, Lagos, Nigeria. *Environmental Geology*, **43**, 145-150. <http://dx.doi.org/10.1007/s00254-002-0633-9>.
- Fakayode, S.O. (2005) Impact Assessment of Industrial Effluent on Water Quality of the Receiving Alaro river in Ibadan Nigeria. *African Journal of Environmental Assessment and Management*, **10**, 1-13.
- Ferguson, C.M., Coote, B.G., Ashbolt, N.J. and Stevenson, I.M. (1996) Relationships between indicators, pathogens and water quality in an estuarine system. *Water Res.* **30**: 2045-2054.
- Fujioka, R. S. (2001). Monitoring coastal marine waters for spore-forming bacteria of faecal and soil origin to determine point from non-point source pollution. *Water Science and Technology* 44(7), 181-188.
- Furtula, V., Jackson, C.R., Farrell, E.G, Barrett, J.B., Hiott, L.M. and Patricia A. Chambers, P.A. (2013). Antimicrobial resistance in *Enterococcus* spp. isolated from environmental

- samples in an area of intensive poultry production. *Int. J. Environ. Res. Public Health*.10: 1020-1036.
- Geldreich, E.E. (1976) Fecal coliform and fecal streptococcus density relationships in water discharges and receiving water. *CRC Crit. Rev. Environ. Control*.6: 349-369.
- Geldreich, E.E. and Kenner, B.A. (1969) Concepts of faecal Streptococci in stream pollution.*J. Water Pollut. Control Fed.* **41**: 336-352.
- Gerba, C.P., Goyal, S.M., LaBelle, R.L., Cech, I. and Bodgan, G.F. (1979) Failure of indicator bacteria to reflect the occurrence of enteroviruses in marine waters. *Am. J. Public Health* **69**: 1116-1119.
- Gleeson, C. and Gray, N. (1997).*The Coliform Index and Waterborne Disease*. E and FN Spon, London. pp194.
- Glyn, H.J. and Gary, W.H. (1996) Environmental Sciences and Engineering Prentice Hall International Inc. pp778.
- Goyal, S.M., Adams, W.N., O'Malley, M.L. and Lear, D.W. (1984) Human pathogenic viruses at sewage sludge disposal sites in the Middle Atlantic region.*Appl. Environ. Microbiol.***48**: 758-763.
- Grabow, W. O. K. (2001) Bacteriophages: Update on application as models for viruses in water. *Water SA*. 27(2): 251-268.
- Halls, N.A. (2004) Microbiological contamination control in pharmaceutical clean rooms. Published by CRC Press, p.3
- Hamner, S., Tripathi, A., and Mishra, R.K. (2006). The role of water use patterns and sewage pollution in incidence of water-borne/enteric diseases along the Ganges River in Varanasi, India. *International Journal of Environmental Health Research*, 16 (2):113–132.
- Hartenbach, A. and Schuol, J. (2005). Bakolori Dam and Bakolori Irrigation Project—Sokoto River, Nigeria.*Americ. J. Pub. Health*, 76, 407-410.
- Havelaar, A., Blummenthal, U.J., Strauss, M., Kay, D. and Bartram, J. (2001). Guidelines the current position. In: *Water quality: Guidelines Standards and Health* (ed. Fewtrell, L. and Bartram, J.) World Health Organization Water Series, IWA Publishing, London (UK) 17-41.

- Hellweger, F.L. and Masopust, P. (2008). Investigating the fate and transport of *Escherichia coli* in the Charles River, Boston, using high-resolution observation and modeling. *J. Am. Wat. Res. Assoc.* **44(2)**: 509-522.
- Hussain, M and Prasad Rao, T.V.D (2013). Effect of Industrial Effluents on Surface Water Quality – A Case Study of Patancheru, Andhra Pradesh, India, *Current World Environment*, **8(3)**: 445-454.
- Hutton, L. G. (1983). Field Testing of Water in Developing Countries. Medmenham: Water Research Centre.
- Ibrahim, Y.K.E. and Alere, A.O. (1998) Evaluation of the hygienic hand disinfection properties of some commonly used hospital antiseptics. *Nig. J. Pharm. Sci.* **6(1)**: 643-71.
- Iscan, M. (2004) Hazard identification for contaminants. *Toxicology*; 205(3): 195-9.
- International Organization for Standardization (ISO) 6461-1 (1986a). *Water quality - Detection and enumeration of the spores of sulphite-reducing anaerobes (clostridia) - Part 1: Method by enrichment in a liquid medium*. International Organization for Standardization, Geneva, Switzerland.
- International Organization for Standardization (ISO) 6461-2 (1986b). *Water quality – Detection and enumeration of the spores of sulphite-reducing anaerobes (clostridia) - Part 2: Method by membrane filtration*. International Organization for Standardization, Geneva, Switzerland.
- International Organization for Standardization (ISO) 9308-1 (1990a). *Water Quality - Detection and enumeration of coliform organisms, thermotolerant coliforms and presumptive Escherichia coli -Part 1: Membrane filtration method*. International Organization for Standardization, Geneva.
- International Organization for Standardization (ISO) 9308-2 (1990b). *Water Quality - Detection and enumeration of coliform organisms, thermotolerant coliforms and presumptive Escherichia coli -Part 2: Multiple tube (most probable number) method*. International Organization for Standardization, Geneva.
- Itah A. Y. (1998). Creating Public Awareness in Environmental and Public Health Education: The Nigerian Situation. *39<sup>th</sup> Annual conference Proceedings of Science Teachers' Association (STAN)*, 186-189.

- Jimena, M.G.; Roxana O.; Catiana Z.; Margarita H.; Susana M. and Ines-Isla, M (2008). Industrial effluents and surface waters genotoxicity and mutagenicity evaluation of a river of Tucuman, Argentin,*Journal of hazardous Material*, **155(3)**: 403-406.
- Kanu, I.; Achi, O.K.; Ezeronye, O.U. and Anyanwu, E.C. (2006). Seasonal variation in bacterial heavy metal biosorption in water samples from Eziam River near soap and brewery industries and the environmental health implications. *Int. J. Environ. Sci. Tech.*, 3 (1): 95-102.
- Kashiwaya, M. and Yoshimoto, K. (1980). Tannery Wastewater Treatment by the Oxygen Activated Sludge Process. *Journal of Water Pollution Control Federation*, 52(5): 999–1007.
- Khan, R.M., Jadhav, M.J. and R. Ustad (2012). Physicochemical analysis of Triveni lake water of Amravati district in (MS) India. *Bioscience Discovery*, **3(1)**: 64-66.
- Kolawole, O.M; Kolawole, T.A.; Olayemi, A.B. and Okoh, A.I. (2011).Assessment of water quality in Asa River (Nigeria) and its indigenous *Clariasgariepinus* fish.*Int J. Environ Res Public Health*. 8(11): 4332–4352.
- Krantz, D. and Kifferstein, B. (2005).Water Pollution and Society.*Journal of Environmental Law Practice*, 4(1): 1-2.
- Krumperman, P. H. (1983) Multiple Antibiotics Resistance Indexing *Escherichia coli* to identify risk sources of faecal contamination of food. *Appl. Environ. Microbiol.***46**: 165-170.
- Kent, P. T., Thomason, B. M. and Morris, G. K. (1981) Salmonella in Foods and Feeds. In: *Review of Isolation Methods and Recommended Procedures*. Atlanta, GA: Public Health Service, CDC. p 6.
- LaBelle, R.L., Gerba, C.P., Goyal, S.M., Melnick, J.K., Cech, I. and Bogdan, G.F. (1980) Relationships between environmental factors, bacterial indicators, and the occurrence of enteric viruses in estuarine sediments. *Appl. Environ. Microbiol.***39**: 588-596.
- Laugesen, C.H.; Fryd, O.; Koottatep, T. and Brix, H. (2010). Sustainable wastewater management in developing countries: New paradigms and case studies from the field. ASCE Press, Virginia, USA.
- Leonard, D.L. (2001). National Indicator Study: Is an International Approach Feasible? *J. Shellfish Res.* **20**: 1293-1298.

- Lipp, E.K., Kurz, R., Vincent, R., Rodriguez-Palacios, C., Farrah, S. and Rose, J.B. (2001). Theseasonal variability and weather effects on microbial fecal pollution and enteric pathogens in a subtropical estuary. *Estuaries***24**: 266-276.
- Liu, B. T., Liao, X. P., Yue, L., Chen, X. Y., Li, L., Yang, S. S., Sun, J., Zhang, S., Liao, S. D. and Liu, Y. H. (2013). Prevalence of  $\beta$ -lactamase and 16S rRNA methylase genes among clinical *Escherichia coli* isolates carrying plasmid-mediated quinolone resistance genes from animals. *Microb Drug Resist.* **19**: 237–245.
- Longe, E. O. and Omole, D. O. (2008).Analysis of Pollution Status of River Illo, Ota, Nigeria.*The Environmentalist*, 28: 451-457.
- Mackowiak, P.A., Caraway, C.T. and Portnoy, B.L. (1976) Oyster-associated hepatitis: lessons from the Louisiana experience. *Am. J. Epidemiol.* **103**: 181-191.
- Manafi, M. (1998) New approaches for the fast detection of indicators, in a particular enzyme detection methods (EDM). *OECD Workshop on Molecular Methods for Safe Drinking Water*, pp. 1-16.
- Martin, T.D., Creed, J.T. and Brockhoff, C.A. (1994). EPA Method 200.2; Sample Preparation Procedure for Spectrochemical Determination of Total Recoverable Elements, Methods for the Determination of Metals in Environmental Samples, Supplement I, EPA/600/R-94/111, EPA, Cincinnati, OH.
- Mill-Robertson, F., Crupper, S. S., Addy, M. E., and Mensah, P. (2003). Antibiotic Resistance and Genotyping of Clinical Group B *Salmonella* Isolated in AccraGhana. *Journal of Applied Microbiology.* 94: 289-294.
- Morinigo, M.A., Wheeler, D., Berry, C., Jones, C., Munoz, M.A., Cornax, R. and Borrego, J.J. (1992) Evaluation of different bacteriophage groups as fecal indicators in contaminated natural waters in Southern England. *Water Res.* **26**: 267-271.
- Mthembu, M.S. (2008). The usefulness of multiple antibiotic resistance (MAR) indexing technique in differentiating faecal coliform bacteria from different sources. Thesis (Msc), University of Zululand.
- Muhammad, H.L.; Shehu, R.A. Bilbis, L.S. and Dangoggo, S.M. (2014). Analyses of selected heavy metals and mineral elements in pollution prone aquatic environments of North-Western Region of Nigeria.*Asian Journal of Biological Sciences.***7(6)**: 252-261.

- Muñoz, I., Rodríguez, A., Rosal, R., Fernández-Alba, A.R. (2009). Life Cycle Assessment of urban wastewater reuse with ozonation as tertiary treatment. A focus on toxicity-related impacts. *Sci. Tot. Environ.*, **407**: 1245–1256.
- Nakamura, T. and Takahashi, H. (2005). Screening of antibiotics resistance to *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* by an advanced expert system. *J Infect Chemother* **11**: 288-92.
- Nayak, N.C. and Chitale, A.R. (2013) Indian childhood cirrhosis (ICC) & ICC-like diseases: The changing scenario of facts versus notions. *Indian J Med Res*, 137(6): 1029 – 1042.
- Nolasco, D.; Lima, R.N.; Hernandez, P.A. and Perez, N.M. (2008) Non-controlled biogenic emissions to the atmosphere from Lazareto landfill, Tenerife, Canary Islands. *Environ Sci Pollut Res Int*; **15(1)**: 51-60.
- Nwachukwu, S.U.; Akpata, T.V.I. and Essien, M.E. (1989). Microbiological assessment of industrial sewage of Agbara Industrial Estate in Ogun State. *International Journal of Ecology and Environmental Sciences*, **15**: 109-115.
- Obi, C.L., Bessong, P.O., Momba, M.N.B., Potgieter, N., Samie, A. and Igumbor, E.O. (2004). Profiles of antibiotic susceptibilities of bacterial isolates and physicochemical quality of water supply in rural Venda communities, South Africa. *Water SA* **30(4)**: 515-519.
- Ogedengbe, K and Akinbile, C.O. (2010). Comparative analysis of the impact of industrial and agricultural effluent on Ona Stream in Ibadan, Nigeria. *New York Science Journal*. 3(7): 25-33.
- Ogunfowokan, A.O.; Okoh, E.K; Adenuga, A.A. and Asubiojo, O.I (2005). An assessment of the impact of point source pollution from a university sewage treatment oxidation pond on a receiving stream – a preliminary study. *Journal of Applied Sciences*, **5(1)**: 36 – 43.
- Olayinka, B.O., Olonitola, O.S., Olayinka, A.T. and Agada, E. A. (2004) Antibiotic susceptibility pattern and multiple antibiotic resistance index of *Pseudomonas aeruginosa* urine isolates from a University Teaching Hospital. *Afr J Clin Exp Microbiol* **5(2)**: 198-202.

- Oliver, J.D. (1995). The viable but non-culturable state in the human pathogen *Vibrio vulnificus*. *FEMS Microbiol.Lett.***133**: 203-208.
- Oliver, J.D., Hite, F., McDougald, D., Andon, N.L. and Simpson, L.M. (1995). Entry into, and resuscitation from, the viable but non-culturable state by *Vibrio vulnificus* in an estuarine environment.*Appl.Envir.Microbiol.***61**: 2624-2630.
- Osibanjo, O.; Daso, A. P. and Gbadebo, A.M. (2011). The impact of industries on surface water quality of River Ona and River Alaro in Oluyole Industrial Estate, Ibadan, *Nigeria Afr. J. Biotechno.* **10 (4)**: 696-702.
- The Open University (OU), (2016) Effects of pollutants on the aquatic environment. <http://www.open.edu/openlearn/nature-environment/effects-pollutants-on-the-aquatic-environment/content-section-6>. Retrieved on 24 November, 2016.
- Park, C. H., Robicsek, A., Jacoby, G. A., Sahm, D. and Hooper, D. C. (2006). Prevalence in the United States of *aac(69)-Ib-cr* encoding a ciprofloxacin-modifying enzyme. *Antimicrob Agents Chemother.* **50**: 3953–3955.
- Paul, S., Bezbaruah, R. L., Roy, M. K. and Ghosh, A.C. (1997). Multiple Antibiotic Resistance (MAR) index and its reversion in *Pseudomonas aeruginosa*.*Letters in Applied Microbiology***24**: 169-171.
- Payment, P.; Waite, M. and Dufour, A (2003). Introducing parameters for the assessment of drinking water quality. In: Dufour, A.; Snozzi, M.; Koster, W. *et al.*, edn. IWA Publishing, pp 47-77.
- Petsaris, O., Mischczak, F., Gicquel-Bruneau, M., Perrin-Guyomard, A., Humbert, F., Sanders, P., Leclercq, R. (2005). Combined antimicrobial resistance in *Enterococcus faecium* isolated from chickens. *Appl. Environ. Microbiol.***71**: 2796–2799.
- Phiri, O.; Mumba, P.; Moyo, B. H. Z. and Kadewa, W. (2005) Assessment of the impact of industrial effluents on water quality of receiving rivers in urban areas of Malawi.*Int. J. Environ. Sci. Tech.***2**: 237-244.
- Pink, D. H. (2006). Investing in Tomorrow’s Liquid Gold. Retrieved on September 18, 2013 from <http://finance.yahoo.com/columnist/article/trenddest/3748>
- Pitout, J.D.D., Thomson, K.S., Hanson, N.D., Ehrhardt, A.F., Moland, E.S. and Sanders, C.C. (1998)  $\beta$ -lactamases responsible for resistance to expanded-spectrum cephalosporins in

- Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* isolates recovered in South Africa. *Antimicrobial Agents and Chemotherapy* **42**: 1350–1354.
- Rajaram, T. and Ashutost, D. (2008). Water pollution by industrial effluents in India: Discharge scenario and case for participatory ecosystem specific local regulation. *Envr. J.*, **40(1)**:56-69.
- Raji, M.I.O. and Ibrahim, Y.K.E. (2011). Prevalence of waterborne infections in Northwest Nigeria: A retrospective study. *Journal of Public Health and Epidemiology* **3(8)**: 382-385.
- Raji, M.I.O.; Ibrahim, Y.K.E. and Ehinmidu, J.O. (2010). Bacteriological quality of public water sources in Shuni, Tambuwal and Sokoto towns in North-Western Nigeria. *Journal of Pharmacy and Bioresources*, **7(2)**: 55-64.
- Raji, M.I.O., Ibrahim Y.K.E., Tytler, B.A., Ehinmidu, J.O. (2015). Faecal Coliforms (FC) and Faecal Streptococci (FS) ratio as tool for assessment of water contamination: A case study of River Sokoto, Northwestern Nigeria. *The Asia Journal of Applied Microbiology* **2(3)**: 27-34.
- Ramirez, M.S.;Traglia, G.M.;Lin, D.L.; Tran, T. and Tolmasky, M.E. (2014). Plasmid-mediated antibiotic resistance and virulence in Gram-negatives: the *Klebsiella pneumoniae* paradigm. *Microbiol Spectr.* **2(5)**: 1–15.
- Ravenscroft, P.; Brammer, H and Richards, K. S. (2008). Arsenic pollution: a global synthesis. Blackwell-Wiley.
- Rivera, S. C., Hazen, T. C. and Toranzos, G. A. (1988). Isolation of fecal coliforms from pristine sites in a tropical rain forest. *Applied and Environmental Microbiology* **54(2)**:513-517.
- Rose, J.B., Epstein, P.R., Lipp, E.K., Sherman, B.H., Bernard, S.M. and Patz, J.A. (2001). Climate variability and change in the United States: potential impacts on water and foodborne diseases caused by microbiologic agents. *Environ. Health Perspect.* **109**:Suppl 2, 211-221.
- Saad, M.A.H.; El-Rayis, O. and Ahdy, H. (1984). Status of nutrients in Lake Mariut, a delta lake in Egypt suffering from intensive pollution. *Mar Pollut Bull*, **15(1)**: 408-411.
- Sangodoyin, A.Y. (1991). Groundwater and surface water pollution by open refuse dump in Ibadan, Nigeria. *Journal of Discovery and Innovations*, **3(1)**: 24-31.

- Sangodoyin, A.Y. (1995) Characteristics of control of industrial effluents-generated pollution. *Env. Mgt. & Health* 6: 15-18.
- Seeley, N.D. and Primrose, S.B. (1982). The isolation of bacteriophages from the environment. *J. Appl. Bacteriol.* **53**: 1-17.
- Shaheen, B. W., Nayak, R., Foley, S. L. and Boothe, D. M. (2013). Chromosomal and plasmid-mediated fluoroquinolone resistance mechanisms among broad-spectrum-cephalosporin-resistant *Escherichia coli* isolates recovered from companion animals in the USA. *J. Antimicrob Chemother*, **68**: 1019–1024.
- Shi, W., Becker, J., Bischoff, M., Turco, R. F. and Konopka, A. E. (2002). Association of Microbial Community Composition and Activity with Lead, Chromium and Hydrocarbon Contamination. *Applied and Environmental Microbiology*, **68(8)**: 3859-3866.
- Shovlin, M. G., Yoo, R. S., Crapper-McLachlan, D. R., Cummings, E., Donohue, J. M., Hallman, W. K., Khachaturian, Z., Orme-Zavaleta, J. and Teefy, S. (1993). *Aluminum in Drinking Water and Alzheimer's Disease: A Resource Guide*, Denver, Co.: American Water Works Association Research Foundation.
- Singh, J.; Sharma, S.; Nar, S. and Devi, S. (2012). Harnessing bacterial indicators along with physicochemical parameters to assess pollution in the Ganges River. *J. Pure Appl. Microbio.*, **7**: 1409-1415.
- Smith A. and Steinmaus, C. (2007). High concentrations of arsenic in drinking water result in the highest known increases in mortality attributable to any environmental exposure. Paper presented at the “Arsenic in drinking water, a global threat to health” session of the Royal Geographic Society Annual Conference, August 2007. [www.rgs.org](http://www.rgs.org)
- Smitha, P.G, Byrappa, K. and S.N. Ramaswamy (2007). Physico-chemical characteristics of water samples of Bantwal Taluk, South-Western Karnataka, India. *J. Environ. Biol.*, **28(3)**: 591-595.
- Sobsey, M. D., Battigelli, D. A., Handzel, T. R. and Schwab, K. J. (1995). *Male-specific Coliphages as Indicators of Viral Contamination of Drinking Water*. American Water Works Association Research Foundation, Denver, Colorado, pp 150.
- Stevens, M., Ashbolt, N. and Cunliffe, D. (2003). Recommendations to change the use of coliforms as microbial indicators of drinking water quality. National Health and Medical Research Council, Australian Government.

- Student Encarta Encyclopedia, 2007 edition. Microsoft Corporation, Redmond, Washington.
- Tagma, T.; Hsissou, Y.; Bouchaou, L.; Bouragba, L. and Boutaleb, S. (2009). Groundwater nitrate pollution in Souss-Massa basin (south-west Morocco). *African Journal of Environmental Science and Technology***3**: 301-309.
- Tytler, B.A. (2011). Impact Assessment of Industrial Effluents and Domestic Wastes on River Kaduna: Physicochemical & Bacteriological Considerations. PhD Dissertation submitted to the Postgraduate School, Ahmadu Bello University, Zaria, pp. 72-73.
- United Nations (UN) (2015). Wastewater Management: A UN-Water Analytical Brief. [http://www.unwater.org/fileadmin/user\\_upload/unwater\\_new/docs/UNWater\\_Analytical\\_Brief\\_Wastewater\\_Management.pdf](http://www.unwater.org/fileadmin/user_upload/unwater_new/docs/UNWater_Analytical_Brief_Wastewater_Management.pdf). Retrieved on 18/09/2016.
- United Nation Environment Programme (UNEP) (2006). Water Quality for Ecosystem and Human Health. United Nations Environment Programme. Global Environment Monitoring System/Water Programme, Canada, pp.8.
- United Nation Environment Programme (UNEP). (2010) Clearing the waters. A focus on water quality solutions. UNEP, Canada.
- United Nations Educational, Scientific and Cultural Organization (UNESCO). (2009). Water in a changing world. The United Nations World Water Development Report 3. UNESCO, Paris.
- United Nations Children's Fund (UNICEF). (2008). Handbook on Water Quality, UNICEF, New York.
- United Nations Children's Fund/World Health Organization (UNICEF/WHO) (2015). Progress on Sanitation and Drinking Water: 2015 Update and MDG Assessment. New York, Geneva. [http://www.wssinfo.org/fileadmin/user\\_upload/resources/JMP-Update-report-2015\\_English.pdf](http://www.wssinfo.org/fileadmin/user_upload/resources/JMP-Update-report-2015_English.pdf). Retrieved on 18/09/2016.
- United States Environmental Protection Agency (USEPA) (1987) Ambient Monitoring Guidelines for Prevention of Significant Deterioration. EPA 450/4-87-007. USEPA, Washington, DC.
- United States Environmental Protection Agency (USEPA) (1989) Drinking Water; National Primary Drinking Water Regulations; Filtration, Disinfection; Turbidity, *Giardia lamblia*, Viruses, Legionella and Heterotrophic Bacteria. *Federal Register*, **54**: 27486-27541.

- United States Environmental Protection Agency (USEPA) (1992) National Primary and Secondary Drinking Water Regulations– Synthetic Organic Chemicals and Inorganic Chemicals; National Primary Drinking Water Regulations Implementation, *Federal Register*, **57**: 31776-31849.
- United States Environmental Protection Agency (USEPA) (2002) The National Water Quality Inventory: Report to Congress for the 2002 Reporting Cycle-A Profile, Fact Sheet No. EPA 841-F-07-003. United States Environmental Protection Agency (EPA), Washington, DC.
- United States Environmental Protection Agency (USEPA) (2004) Primer for Municipal Wastewater Treatment Systems.EPA 832-R-04-001. United States Environmental Protection Agency (EPA), Washington, DC.
- Van De Klundert, J. and Vliegthart, J. (1993).PCR detection of genes coding for aminoglycoside-modifying enzymes. In: Persing *et al.* (editors) Diagnostic Molecular Microbiology: Principles and Applications. AMS Press, Washington, D.C., pp. 547-552.
- Vaughan, A. (2010). Human impact on world's rivers ‘threatens water security of 5 billion’. *The Guardian*, 29 September 2010.<https://www.commondreams.org/news/2010/09/29/human-impact-worlds-rivers-threatens-water-security-5-billion>. Retrieved on 24/10/2017.
- Venglovsky, J., Sasakova, N. and Placha, I. (2009). Pathogens and antibiotic residues in animalmanures and hygienic and ecological risksrelated to subsequent land application.*Bioresource Technology*. 100 (22):5386–5391.
- Venkateshwarlu, P. and Satyanarayana, J. (2014). Chemical Composition of Rainwater at an Urban Area during Southwest Monsoon. *International Journal of Modern Chemistry and Applied Science*, 1(1): 1 – 4.
- Waite, W.M. (1997). *Assessment of Water Supply and Associated matters in Relation to the Incidence of Cryptosporidiosis in Torbay in August and September 1995*. Drinking Water Inspectorate, London.
- Walakira, P. and Okot-Okumu, J. (2011). Impact of Industrial Effluents on Water Quality of Streams in Nakawa-Ntinda, Uganda, *J. Appl. Sci. Manage.* 15(2): 289-296.

- Walley, W. J. and Hawkes, H. A. (2003). A computer-based reappraisal of Biological Monitoring Working Party scores using data from the 1990 River Quality Survey of England and Wales.
- West, L. (2006). World Water Day: A Billion People Worldwide Lack Safe Drinking Water. Retrieved on September 18, 2013 from <http://environment.about.com/od/environmentalevents/a/waterdayqa.htm>
- Wetzel, R. G. (2001). Limnology, 3<sup>rd</sup> Edition. Academic Press, USA.
- World Health Organization (WHO) (1996). Guidelines for Drinking-water Quality. 2<sup>nd</sup> Edn., Vol 1, World Health Organization, Geneva, Switzerland.
- World Health Organization (WHO) (1997). Division of emerging and communicable diseases surveillance and control annual report – 1996. World Health Organization, Geneva.
- World Health Organization (WHO) (2001). Guidelines for Drinking water Quality. Addendum: *Microbiological agents in drinking water*. World Health Organization, Geneva.
- World Health Organization (WHO) (2002). Evaluation of the H<sub>2</sub>S method for detection of faecal contamination of drinking water, pp1-40.
- World Health Organization (WHO) (2003). Guidelines for Drinking-water Quality. 3<sup>rd</sup> Edn. Chapter 8, *DRAFT.*, World Health Organization, Geneva.
- World Health Organization (WHO). (2006) Guidelines for drinking-water quality incorporating first addendum. Vol. 1: Recommendations. 3<sup>rd</sup> Edn. World Health Organization, Geneva.
- World Health Organization (WHO). (2013). Fact sheet Number 330, World Health Organization, Geneva.
- World Water Assessment Programme (WWAP). (2012). The United Nations World Water Development Report 4: Managing Water under Uncertainty and Risk. Paris. <http://www.unesco.org/new/en/natural-sciences/environment/water/wwap/wwdr/wwdr4-2012/>
- Zhang, Y.; Yang, J.; Ye, L.; Luo, Y.; Wang, W.; Zhou, W.; Cui, Z. and Han, L. (2012). Characterization of clinical multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates, 2007-2009, China. *Microb Drug Resist*, **18**: 465–470.
- Ziemer, C. J. and Steadham, S. R. (2003). Evaluation of the specificity of Salmonella PCR primers using various intestinal bacterial species. *Letters in Applied Microbiology*, **37**: 463-469.

## APPENDICES

Mean Monthly Bacteria counts from January to December, 2014

Appendix A (i)

| January                          | Sampling Site                 |                               |                               |                               |                               |                               |
|----------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|                                  | P1                            | P2                            | P3                            | P4                            | P5                            | P6                            |
| Heterotrophic Plate Count (35°C) | 1.25±0.48x<br>10 <sup>5</sup> | 0.98±0.27x<br>10 <sup>5</sup> | 0.92±0.15x<br>10 <sup>5</sup> | 0.18±0.78x<br>10 <sup>5</sup> | 1.00±0.83x<br>10 <sup>5</sup> | 1.20±0.33x<br>10 <sup>5</sup> |
| Total Coliform                   | 22000                         | 10000                         | 19000                         | 1800                          | 20000                         | 21000                         |
| Faecal Coliform                  | 1800                          | 1200                          | 1100                          | 900                           | 1000                          | 1500                          |
| Faecal Streptococci              | 160                           | 110                           | 110                           | 100                           | 100                           | 110                           |

Appendix A (ii)

| February                         | Sampling Site                 |                               |                               |                               |                               |                               |
|----------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|                                  | P1                            | P2                            | P3                            | P4                            | P5                            | P6                            |
| Heterotrophic Plate Count (35°C) | 2.00±0.28x<br>10 <sup>5</sup> | 1.13±0.18x<br>10 <sup>5</sup> | 1.82±0.21x<br>10 <sup>5</sup> | 0.19±0.26x<br>10 <sup>5</sup> | 1.45±0.25x<br>10 <sup>5</sup> | 1.75±0.48x<br>10 <sup>5</sup> |
| Total                            | 35000                         | 29000                         | 12000                         | 2600                          | 28000                         | 30000                         |

|                     |      |      |      |      |      |      |
|---------------------|------|------|------|------|------|------|
| Coliform            |      |      |      |      |      |      |
| Faecal Coliform     | 1800 | 1600 | 1600 | 1100 | 1400 | 1800 |
| Faecal Streptococci | 180  | 150  | 120  | 118  | 120  | 160  |

Appendix A (iii)

| March                            | Sampling Site              |                            |                            |                            |                            |                            |
|----------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                                  | P1                         | P2                         | P3                         | P4                         | P5                         | P6                         |
| Heterotrophic Plate Count (35°C) | 3.00±0.40x 10 <sup>5</sup> | 2.75±0.39x 10 <sup>5</sup> | 1.65±0.21x 10 <sup>5</sup> | 0.39±0.47x 10 <sup>5</sup> | 1.20±0.45x 10 <sup>5</sup> | 2.82±0.30x 10 <sup>5</sup> |
| Total Coliform                   | 35000                      | 32000                      | 30000                      | 3000                       | 15000                      | 32000                      |
| Faecal Coliform                  | 2000                       | 1800                       | 1700                       | 1500                       | 1700                       | 2000                       |
| Faecal Streptococci              | 220                        | 180                        | 150                        | 120                        | 130                        | 200                        |

Appendix A (iv)

| April                            | Sampling Site              |                            |                            |                            |                            |                            |
|----------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                                  | P1                         | P2                         | P3                         | P4                         | P5                         | P6                         |
| Heterotrophic Plate Count (35°C) | 3.02±0.24x 10 <sup>5</sup> | 1.28±0.63x 10 <sup>5</sup> | 1.97±0.31x 10 <sup>5</sup> | 0.42±0.12x 10 <sup>5</sup> | 2.86±0.38x 10 <sup>5</sup> | 2.82±0.33x 10 <sup>5</sup> |
| Total Coliform                   | 38000                      | 35000                      | 20000                      | 8000                       | 35000                      | 36000                      |
| Faecal Coliform                  | 2100                       | 2000                       | 2000                       | 1500                       | 1800                       | 2100                       |

|                     |     |     |     |     |     |     |
|---------------------|-----|-----|-----|-----|-----|-----|
| Faecal Streptococci | 242 | 200 | 200 | 130 | 160 | 210 |
|---------------------|-----|-----|-----|-----|-----|-----|

Appendix A (v)

| May                              | Sampling Site                 |                               |                               |                               |                               |                               |
|----------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|                                  | P1                            | P2                            | P3                            | P4                            | P5                            | P6                            |
| Heterotrophic Plate Count (35°C) | 3.45±0.53x<br>10 <sup>5</sup> | 2.91±0.44x<br>10 <sup>5</sup> | 2.48±0.34x<br>10 <sup>5</sup> | 0.95±0.99x<br>10 <sup>5</sup> | 1.26±0.14x<br>10 <sup>5</sup> | 3.32±0.48x<br>10 <sup>5</sup> |
| Total Coliform                   | 63000                         | 40000                         | 38000                         | 8600                          | 40000                         | 41000                         |
| Faecal Coliform                  | 2700                          | 2500                          | 2500                          | 1800                          | 2200                          | 2500                          |
| Faecal Streptococci              | 300                           | 220                           | 200                           | 160                           | 160                           | 300                           |

Appendix A (vi)

| June                             | Sampling Site                 |                               |                               |                               |                               |                               |
|----------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|                                  | P1                            | P2                            | P3                            | P4                            | P5                            | P6                            |
| Heterotrophic Plate Count (35°C) | 5.36±0.62x<br>10 <sup>5</sup> | 3.92±0.35x<br>10 <sup>5</sup> | 3.80±0.60x<br>10 <sup>5</sup> | 3.60±0.56x<br>10 <sup>5</sup> | 3.68±0.28x<br>10 <sup>5</sup> | 4.86±0.72x<br>10 <sup>5</sup> |
| Total Coliform                   | 160000                        | 70000                         | 42000                         | 8800                          | 41000                         | 80000                         |
| Faecal                           | 3200                          | 3000                          | 2900                          | 2000                          | 2700                          | 3000                          |

|                     |     |     |     |     |     |     |
|---------------------|-----|-----|-----|-----|-----|-----|
| Coliform            |     |     |     |     |     |     |
| Faecal Streptococci | 400 | 250 | 220 | 160 | 180 | 310 |

Appendix A (vii)

| July                             | Sampling Site              |                            |                            |                            |                            |                            |
|----------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                                  | P1                         | P2                         | P3                         | P4                         | P5                         | P6                         |
| Heterotrophic Plate Count (35°C) | 8.20±0.26x 10 <sup>5</sup> | 7.00±0.96x 10 <sup>5</sup> | 6.08±0.10x 10 <sup>5</sup> | 3.84±0.72x 10 <sup>5</sup> | 4.20±0.68x 10 <sup>5</sup> | 8.05±0.22x 10 <sup>5</sup> |
| Total Coliform                   | 250000                     | 160000                     | 82000                      | 63000                      | 80000                      | 180000                     |
| Faecal Coliform                  | 4500                       | 4200                       | 3800                       | 2600                       | 3500                       | 4200                       |
| Faecal Streptococci              | 800                        | 260                        | 230                        | 200                        | 220                        | 320                        |

Appendix A (viii)

| August                    | Sampling Site              |                            |                            |                            |                            |                            |
|---------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                           | P1                         | P2                         | P3                         | P4                         | P5                         | P6                         |
| Heterotrophic Plate Count | 6.02±0.72x 10 <sup>5</sup> | 4.89±0.30x 10 <sup>5</sup> | 4.20±0.18x 10 <sup>5</sup> | 2.20±0.62x 10 <sup>5</sup> | 3.02±0.86x 10 <sup>5</sup> | 5.73±0.78x 10 <sup>5</sup> |

|                     |        |       |       |       |       |        |
|---------------------|--------|-------|-------|-------|-------|--------|
| (35°C)              |        |       |       |       |       |        |
| Total Coliform      | 200000 | 90000 | 80000 | 38000 | 40000 | 160000 |
| Faecal Coliform     | 4000   | 3200  | 2700  | 2400  | 2500  | 3300   |
| Faecal Streptococci | 530    | 220   | 210   | 160   | 200   | 300    |

Appendix A (ix)

| September                        | Sampling Site              |                            |                            |                            |                            |                            |
|----------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                                  | P1                         | P2                         | P3                         | P4                         | P5                         | P6                         |
| Heterotrophic Plate Count (35°C) | 4.75±0.60x 10 <sup>5</sup> | 3.80±0.57x 10 <sup>5</sup> | 3.02±0.48x 10 <sup>5</sup> | 1.96±0.56x 10 <sup>5</sup> | 3.00±0.86x 10 <sup>5</sup> | 4.26±0.71x 10 <sup>5</sup> |
| Total Coliform                   | 150000                     | 65000                      | 45000                      | 35000                      | 40000                      | 80000                      |
| Faecal Coliform                  | 2600                       | 2300                       | 2200                       | 1800                       | 2200                       | 2500                       |
| Faecal Streptococci              | 300                        | 200                        | 180                        | 150                        | 180                        | 290                        |

Appendix A (x)

| October                          | Sampling Site              |                            |                            |                            |                            |                            |
|----------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                                  | P1                         | P2                         | P3                         | P4                         | P5                         | P6                         |
| Heterotrophic Plate Count (35°C) | 3.80±0.54x 10 <sup>5</sup> | 2.76±0.43x 10 <sup>5</sup> | 2.64±0.35x 10 <sup>5</sup> | 1.62±0.62x 10 <sup>5</sup> | 1.94±0.74x 10 <sup>5</sup> | 3.00±0.40x 10 <sup>5</sup> |
| Total Coliform                   | 80000                      | 40000                      | 38000                      | 18000                      | 30000                      | 65000                      |

|                     |      |      |      |      |      |      |
|---------------------|------|------|------|------|------|------|
| Faecal Coliform     | 2300 | 1800 | 1700 | 1500 | 1600 | 2200 |
| Faecal Streptococci | 280  | 180  | 160  | 150  | 150  | 280  |

Appendix A (xi)

| November                         | Sampling Site              |                            |                            |                            |                            |                            |
|----------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                                  | P1                         | P2                         | P3                         | P4                         | P5                         | P6                         |
| Heterotrophic Plate Count (35°C) | 3.49±0.56x 10 <sup>5</sup> | 2.64±0.28x 10 <sup>5</sup> | 1.92±0.56x 10 <sup>5</sup> | 0.98±0.38x 10 <sup>5</sup> | 1.80±0.45x 10 <sup>5</sup> | 2.75±0.39x 10 <sup>5</sup> |
| Total Coliform                   | 42000                      | 35000                      | 26000                      | 10000                      | 25000                      | 40000                      |
| Faecal Coliform                  | 2300                       | 1800                       | 1600                       | 1200                       | 1500                       | 1800                       |
| Faecal Streptococci              | 250                        | 180                        | 140                        | 120                        | 120                        | 250                        |

Appendix A (xii)

| December                         | Sampling Site              |                            |                            |                            |                            |                            |
|----------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                                  | P1                         | P2                         | P3                         | P4                         | P5                         | P6                         |
| Heterotrophic Plate Count (35°C) | 2.75±0.87x 10 <sup>5</sup> | 1.32±0.16x 10 <sup>5</sup> | 1.20±0.94x 10 <sup>5</sup> | 0.85±0.62x 10 <sup>5</sup> | 1.10±0.68x 10 <sup>5</sup> | 1.80±0.56x 10 <sup>5</sup> |
| Total                            | 33000                      | 30000                      | 25000                      | 5000                       | 18000                      | 32000                      |

|                     |      |      |      |      |      |      |
|---------------------|------|------|------|------|------|------|
| Coliform            |      |      |      |      |      |      |
| Faecal Coliform     | 1800 | 1700 | 1200 | 1000 | 1200 | 1800 |
| Faecal Streptococci | 240  | 150  | 120  | 100  | 100  | 200  |

Appendix B (i): Physicochemical Properties of Water Samples in January 2014

| Parameter           | Values at Sampling Sites |      |      |       |       |      |
|---------------------|--------------------------|------|------|-------|-------|------|
|                     | P1                       | P2   | P3   | P4    | P5    | P6   |
| pH                  | 8.11                     | 8.06 | 8.05 | 8.37  | 8.50  | 8.02 |
| Temperature (°C)    | 25.4                     | 25.5 | 25.3 | 25.6  | 25.4  | 25.4 |
| Conductance (µS/cm) | 75.6                     | 96.7 | 74.6 | 201.0 | 321.0 | 96.4 |
| D. O. (mg/L)        | 1.7                      | 1.0  | 1.2  | 1.2   | 0.7   | 0.9  |
| B. O. D. (mg/L)     | 0.7                      | 0.4  | 0.5  | 0.3   | 0.2   | 0.4  |
| C. O. D. (mg/L)     | 300                      | 150  | 180  | 230   | 220   | 230  |
| Total Solids (mg/L) | 780                      | 580  | 620  | 240   | 400   | 600  |
| TDS (mg/L)          | 500                      | 390  | 470  | 140   | 260   | 400  |
| TSS (mg/L)          | 280                      | 190  | 150  | 100   | 140   | 200  |
| Alkalinity (mg/L)   | 22                       | 21   | 23   | 28    | 71    | 11   |
| Acidity (mg/L)      | 15                       | 25   | 20   | 5     | 10    | 15   |

|                  |       |       |       |       |       |       |
|------------------|-------|-------|-------|-------|-------|-------|
| Chlorides (mg/L) | 35    | 55    | 40    | 100   | 50    | 75    |
| Sulphate (mg/L)  | 220   | 190   | 180   | 195   | 200   | 210   |
| Phosphate (mg/L) | 0.11  | 0.51  | 0.24  | 0.15  | 1.77  | 0.59  |
| Hardness (mg/L)  | 161.6 | 121.2 | 151.5 | 282.8 | 184.8 | 131.3 |
| Nitrates (mg/L)  | 19.7  | 9.8   | 6.6   | 10.3  | 11.8  | 12.7  |
| Ammonia-N (mg/L) | 0.46  | 0.28  | 0.35  | 0.62  | 0.84  | 0.35  |

Key:

DO = Dissolved oxygen; BOD = Biological oxygen demand; COD = Chemical oxygen demand

TDS = Total dissolved solids; TSS = Total suspended solids.

Appendix B (ii):Physicochemical Properties of Water Samples in April 2014

| Parameter           | Values at Sampling Sites |      |      |       |       |      |
|---------------------|--------------------------|------|------|-------|-------|------|
|                     | P1                       | P2   | P3   | P4    | P5    | P6   |
| pH                  | 7.65                     | 7.63 | 7.62 | 7.78  | 7.82  | 7.58 |
| Temperature (°C)    | 27.8                     | 27.7 | 27.5 | 27.9  | 27.8  | 27.8 |
| Conductance (µs/cm) | 250.0                    | 94.2 | 72.8 | 200.0 | 212.0 | 94.8 |
| D. O. (mg/L)        | 1.2                      | 0.5  | 0.8  | 0.8   | 0.2   | 0.4  |
| B. O. D. (mg/L)     | 0.32                     | 0.25 | 0.30 | 0.25  | 0.5   | 0.25 |
| C. O. D. (mg/L)     | 280                      | 120  | 150  | 200   | 180   | 200  |
| Total Solids (mg/L) | 620                      | 550  | 600  | 200   | 360   | 570  |
| TDS (mg/L)          | 360                      | 350  | 400  | 150   | 250   | 330  |
| TSS (mg/L)          | 260                      | 200  | 200  | 50    | 110   | 240  |
| Alkalinity (mg/L)   | 24                       | 22   | 25   | 30    | 72    | 12   |
| Acidity (mg/L)      | 12                       | 23   | 20   | 7     | 11    | 13   |

|                  |       |       |       |       |       |       |
|------------------|-------|-------|-------|-------|-------|-------|
| Chlorides (mg/L) | 36    | 57    | 45    | 120   | 70    | 80    |
| Sulphate (mg/L)  | 230   | 180   | 160   | 220   | 225   | 170   |
| Phosphate (mg/L) | 0.15  | 0.55  | 0.26  | 0.18  | 1.80  | 0.64  |
| Hardness (mg/L)  | 160.2 | 120.3 | 150.4 | 280.6 | 181.5 | 128.1 |
| Nitrates (mg/L)  | 18.6  | 9.5   | 6.2   | 10.1  | 11.3  | 12.5  |
| Ammonia-N (mg/L) | 0.42  | 0.23  | 0.32  | 0.58  | 0.81  | 0.34  |

Key:

DO = Dissolved oxygen; BOD = Biological oxygen demand; COD = Chemical oxygen demand

TDS = Total dissolved solids; TSS = Total suspended solids.

Appendix B (iii): Physicochemical Properties of Water Samples in June 2014

| Parameter           | Values at Sampling Sites |       |      |       |       |      |
|---------------------|--------------------------|-------|------|-------|-------|------|
|                     | P1                       | P2    | P3   | P4    | P5    | P6   |
| pH                  | 7.08                     | 6.96  | 6.84 | 7.50  | 7.25  | 7.16 |
| Temperature (°C)    | 28.6                     | 28.8  | 28.0 | 28.9  | 28.9  | 28.5 |
| Conductance (µs/cm) | 480.0                    | 120.0 | 98.4 | 550.0 | 260.2 | 95.7 |
| D. O. (mg/L)        | 0.80                     | 0.35  | 0.40 | 0.40  | 0.09  | 0.32 |
| B. O. D. (mg/L)     | 0.42                     | 0.30  | 0.35 | 0.30  | 1.50  | 0.30 |
| C. O. D. (mg/L)     | 500                      | 300   | 500  | 110   | 200   | 450  |
| Total Solids (mg/L) | 800                      | 600   | 700  | 250   | 400   | 600  |
| TDS (mg/L)          | 480                      | 350   | 400  | 200   | 300   | 320  |
| TSS (mg/L)          | 320                      | 250   | 300  | 50    | 100   | 280  |
| Alkalinity (mg/L)   | 12                       | 10    | 14   | 15    | 20    | 8    |
| Acidity (mg/L)      | 22                       | 45    | 48   | 20    | 50    | 50   |

|                  |       |       |       |       |       |       |
|------------------|-------|-------|-------|-------|-------|-------|
| Chlorides (mg/L) | 48    | 58    | 60    | 140   | 100   | 85    |
| Sulphate (mg/L)  | 160   | 110   | 100   | 170   | 180   | 90    |
| Phosphate (mg/L) | 0.20  | 0.35  | 0.24  | 0.20  | 1.00  | 0.40  |
| Hardness (mg/L)  | 268.3 | 352.5 | 308.6 | 852.5 | 308.4 | 232.8 |
| Nitrates (mg/L)  | 15.4  | 10.2  | 7.5   | 18.6  | 20.1  | 10.5  |
| Ammonia-N (mg/L) | 0.64  | 0.52  | 0.75  | 0.96  | 0.91  | 0.68  |

Key:

DO = Dissolved oxygen; BOD = Biological oxygen demand; COD = Chemical oxygen demand

TDS = Total dissolved solids; TSS = Total suspended solids.

Appendix B (iv): Physicochemical Properties of Water Samples in August 2014

| Parameter           | Values at Sampling Sites |       |      |       |       |      |
|---------------------|--------------------------|-------|------|-------|-------|------|
|                     | P1                       | P2    | P3   | P4    | P5    | P6   |
| pH                  | 6.24                     | 6.32  | 6.36 | 7.23  | 6.80  | 6.84 |
| Temperature (°C)    | 29.1                     | 29.0  | 28.2 | 29.0  | 29.2  | 28.8 |
| Conductance (µs/cm) | 470.0                    | 100.0 | 90.0 | 510.0 | 120.0 | 80.0 |
| D. O. (mg/L)        | 0.10                     | 0.07  | 0.08 | 0.09  | 0.07  | 0.10 |
| B. O. D. (mg/L)     | 0.51                     | 0.27  | 0.20 | 0.35  | 0.43  | 0.28 |
| C. O. D. (mg/L)     | 1008                     | 480   | 48   | 32    | 688   | 720  |
| Total Solids (mg/L) | 1300                     | 900   | 900  | 300   | 900   | 1100 |
| TDS (mg/L)          | 800                      | 500   | 500  | 100   | 500   | 700  |
| TSS (mg/L)          | 500                      | 400   | 400  | 200   | 400   | 400  |
| Alkalinity (mg/L)   | 10                       | 8     | 11   | 13    | 13    | 7    |
| Acidity (mg/L)      | 20                       | 60    | 60   | 20    | 60    | 60   |

|                  |       |       |       |       |       |       |
|------------------|-------|-------|-------|-------|-------|-------|
| Chlorides (mg/L) | 60    | 60    | 80    | 180   | 110   | 90    |
| Sulphate (mg/L)  | 124   | 82    | 82    | 124   | 124   | 82    |
| Phosphate (mg/L) | 0.21  | 0.19  | 0.21  | 0.21  | 0.21  | 0.22  |
| Hardness (mg/L)  | 265.9 | 350.4 | 305.6 | 850.3 | 305.4 | 230.5 |
| Nitrates (mg/L)  | 14.2  | 12.4  | 8.6   | 28.1  | 24.3  | 10.9  |
| Ammonia-N (mg/L) | 0.82  | 0.64  | 0.98  | 1.20  | 1.10  | 0.86  |

Key:

DO = Dissolved oxygen; BOD = Biological oxygen demand; COD = Chemical oxygen demand

TDS = Total dissolved solids; TSS = Total suspended solids.

#### Appendix B (v): Physicochemical Properties of Water Samples in September 2014

| Parameter           | Values at Sampling Sites |      |      |       |       |      |
|---------------------|--------------------------|------|------|-------|-------|------|
|                     | P1                       | P2   | P3   | P4    | P5    | P6   |
| pH                  | 6.72                     | 6.81 | 6.86 | 7.45  | 7.01  | 7.20 |
| Temperature (°C)    | 29.9                     | 29.5 | 28.6 | 29.5  | 29.7  | 29.0 |
| Conductance (µs/cm) | 320.0                    | 95.2 | 84.8 | 250.0 | 118.5 | 79.5 |
| D. O. (mg/L)        | 0.68                     | 0.52 | 0.64 | 0.68  | 0.52  | 0.15 |
| B. O. D. (mg/L)     | 0.48                     | 0.22 | 0.16 | 0.27  | 0.34  | 0.23 |
| C. O. D. (mg/L)     | 872                      | 320  | 45   | 30    | 520   | 615  |
| Total Solids (mg/L) | 1000                     | 800  | 800  | 200   | 800   | 1000 |
| TDS (mg/L)          | 850                      | 580  | 560  | 120   | 570   | 420  |
| TSS (mg/L)          | 150                      | 220  | 240  | 80    | 230   | 580  |
| Alkalinity (mg/L)   | 13                       | 10   | 14   | 15    | 26    | 10   |
| Acidity (mg/L)      | 20                       | 40   | 38   | 18    | 30    | 35   |

|                  |       |       |       |       |       |       |
|------------------|-------|-------|-------|-------|-------|-------|
| Chlorides (mg/L) | 58    | 57    | 60    | 120   | 70    | 85    |
| Sulphate (mg/L)  | 130   | 110   | 120   | 140   | 135   | 115   |
| Phosphate (mg/L) | 0.20  | 0.25  | 0.22  | 0.21  | 0.85  | 0.45  |
| Hardness (mg/L)  | 205.1 | 280.6 | 265.2 | 680.7 | 260.5 | 185.3 |
| Nitrates (mg/L)  | 16.4  | 10.2  | 8.2   | 18.8  | 16.2  | 11.5  |
| Ammonia-N (mg/L) | 0.68  | 0.54  | 0.84  | 1.10  | 0.98  | 0.72  |

Key:

DO = Dissolved oxygen; BOD = Biological oxygen demand; COD = Chemical oxygen demand

TDS = Total dissolved solids; TSS = Total suspended solids.

Appendix B (vi): Physicochemical Properties of Water Samples in November 2014

| Parameter           | Values at Sampling Sites |      |      |       |       |      |
|---------------------|--------------------------|------|------|-------|-------|------|
|                     | P1                       | P2   | P3   | P4    | P5    | P6   |
| pH                  | 7.84                     | 7.96 | 8.00 | 8.35  | 8.24  | 8.02 |
| Temperature (°C)    | 27.8                     | 27.6 | 26.7 | 27.6  | 27.7  | 27.1 |
| Conductance (µs/cm) | 150.0                    | 90.8 | 70.1 | 180.0 | 118.0 | 79.2 |
| D. O. (mg/L)        | 1.50                     | 0.74 | 0.86 | 0.87  | 0.60  | 0.45 |
| B. O. D. (mg/L)     | 0.45                     | 0.20 | 0.12 | 0.25  | 0.32  | 0.21 |
| C. O. D. (mg/L)     | 810                      | 280  | 40   | 28    | 480   | 500  |
| Total Solids (mg/L) | 750                      | 550  | 550  | 180   | 600   | 800  |
| TDS (mg/L)          | 250                      | 240  | 242  | 60    | 255   | 310  |
| TSS (mg/L)          | 500                      | 310  | 308  | 120   | 345   | 490  |
| Alkalinity (mg/L)   | 18                       | 20   | 21   | 25    | 52    | 10   |
| Acidity (mg/L)      | 22                       | 30   | 25   | 10    | 20    | 22   |

|                  |       |       |       |       |       |       |
|------------------|-------|-------|-------|-------|-------|-------|
| Chlorides (mg/L) | 56    | 40    | 50    | 110   | 60    | 80    |
| Sulphate (mg/L)  | 180   | 140   | 130   | 160   | 170   | 180   |
| Phosphate (mg/L) | 0.18  | 0.45  | 0.24  | 0.18  | 1.20  | 0.50  |
| Hardness (mg/L)  | 182.4 | 165.8 | 192.5 | 425.3 | 210.9 | 145.2 |
| Nitrates (mg/L)  | 18.2  | 9.9   | 7.5   | 12.5  | 12.3  | 12.1  |
| Ammonia-N (mg/L) | 0.57  | 0.38  | 0.52  | 0.83  | 0.90  | 0.57  |

Key:

DO = Dissolved oxygen; BOD = Biological oxygen demand; COD = Chemical oxygen demand

TDS = Total dissolved solids; TSS = Total suspended solids.

Appendix C (i): Toleration standards for surface waters, which will be used for the preparation of drinking water [guidelines from Council Directive 75/440/EEC]

| <i>Substances</i>   | <i>Concentration (mg/l)</i> |
|---------------------|-----------------------------|
| Chlorides           | <150                        |
| Sulfates            | <250                        |
| Total salt          | <500                        |
| Electroconductivity | <1000                       |
| pH                  | 6.5-8.7                     |
| O2 saturation       | 70%                         |
| BOD5                | <5                          |
| COD                 | <8                          |
| NH4 <sup>+</sup>    | <0.6                        |
| Nitrites            | <100                        |
| Nitrates            | <0.1                        |
| Phosphates          | <7                          |

(ii): Usual water parameters range for irrigation use [“Waste water treatment and use in agriculture”, FAO 1992]

| <b>Substances</b>         | <b>Range</b> |
|---------------------------|--------------|
| Electroconductivity       | 0-3000 mS/cm |
| Total Dissolved Solids    | 0-2000 mg/l  |
| <b>Cations and anions</b> |              |
| Ca <sup>=2</sup>          | 0-400 mg/l   |
| Mg <sup>=2</sup>          | 0-60 mg/l    |
| Na <sup>=</sup>           | 0-920 mg/l   |
| CO3 <sup>-2</sup>         | 0-3 mg/l     |

|                                 |             |
|---------------------------------|-------------|
| HCO <sub>3</sub> <sup>-2</sup>  | 0-610 mg/l  |
| Cl <sup>-</sup>                 | 0-1065 mg/l |
| SO <sub>4</sub> <sup>-2</sup>   |             |
| <b>Nutrients</b>                |             |
| NO <sub>3</sub> <sup>-</sup> -N | 0-10 mg/l   |
| NH <sub>4</sub> <sup>-</sup> -N | 0-5 mg/l    |
| PO <sub>4</sub> -P              | 0-2 mg/l    |
| K <sup>=</sup>                  | 0-2 mg/l    |
| pH                              | 6.0-8.5     |
| B                               | 0-2 mg/l    |
| Sodium Adsorption Ratio         | 0-15 meq/l  |

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