USMANU DANFODIYO UNIVERSITY, SOKOTO

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BACTERIOLOGICAL AND PHYSICOCHEMICAL ANALYSIS OF PUBLIC DRINKING WATER SOURCES IN SOKOTO METROPOLIS

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By

MAIMUNA ATTAHIRU
(10/211307006)

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DEDICATION

This research is wholly dedicated to my beloved husband Muhammad S. Mana, my children Fatima M. S. Mana, Ahmad M. S. Mana, Musa M. S. Mana and Imran M. S. Mana.

CERTIFICATION

This dissertation by Maimuna Attahiru (10211307006) has fulfilled part of the requirements for the award of the Degree of the Master of Science (Microbiology) of the Usmanu Danfodiyo University, Sokoto and is approved for its contribution to Knowledge.

_____ _____

Prof. S. B. Manga Date
(Head of Department)

_____ _____

Prof. S. E. Yakubu Date
Major Supervisor

_____

Dr. A. B. Rabah Date
Co – Supervisor

_____

External Examiner **Date**

_____

P.G Representative Date

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ABSTRACT

Bacteriological and physicochemical properties of drinking water (tap, well and borehole) in Sokoto metropolis were investigated to determine the level of contamination and the persistence of target organisms. A total of 270 samples comprising of 90 well water samples, 90 tap water samples and 90 boreholes water samples were obtained from some parts of Sokoto metropolis and analyzed using standard procedures. Total coliform count, heterotrophic plate count, physico-chemical properties feacal-coliform count and the presence of *Escherichia coli*, *Enterobacter* species, *Bacillus subtilis*, *Shigella* species, *Salmonella* species, *Citrobacter* species, *Staphylococcu saureus* and *Bacillus cereus* were determined. Biochemical identification showed that out of the 108 confirmed *Escherichia coli* isolates, 51(47%) were from well water and of the 42 *Enterobacter* species recovered 19 (45%) were from well water while5 (42%) out of 12 *Salmonella* species were also recovered from well water. The tap water had 26 (24%) *Escherichia coli*, 13(30%) *Enterobacter*, 3 (25%) *Salmonella* species, 5(71%) *Bacillus species* and other unidentified organisms while in contrast, borehole water had 31 (29%) *Eschericia coli*, 10 (24%) *Enterobacter*, 4 (33%) *Salmonella* species, 2 (26%) *Bacillus* and other unidentified organisms. No *Vibrio* species were isolated in all cases. The molecular analysis showed that out of the 79 isolates identified from biochemical analysis, 40 isolates were further subjected to molecular analysis using microgen GN-ID System (kit method).There were 13 (33%) inactive *Eechericia coli*, 5 (13%) active *Eschericia coli* and 10 (25%) were other organisms, while the remaining 12(30%) were not identified. The physicochemical properties show that the highest pH was in borehole water sample (6.73) while the lowest was in well water samples (5.7). The highest Electrical conductivity was in tap water samples (142.67 µs/cm), while the lowest was in well (5.8 µs/cm). The highest value of dissolved oxygen was in borehole water samples (8.83 mg/l) and the lowest was found in tap water samples (3.57 mg/l). The highest BOD was in well water samples (15.57 mg/l) while the lowest was in tap water samples (7.73). Therefore, adequate treatment of all waters and public health education are highly recommended.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Water is a common chemical entity that is essential for the survival of all known forms of life (Solanki *et al.,* 2012). The qualities of drinking water are powerful environmental determinants of health (WHO, 2010). Water plays an indispensable role in sustenance of life and it is a key pillar of health determinant, since 80% of diseases in developing countries are due to lack of good quality water (Chessbrough, 2006). Drinking water quality management has been a key pillar of primary prevention of infections for over one and half centuries and it continues to be the foundation for the prevention and control of water-borne diseases (WHO, 2010). Water is the most abundant natural resource; although, it is not readily available in the form required by man (Obi and Okocha, 2007). The provision of good quality household drinking water is often regarded as an important means of improving health (Essien and Olisah, 2010). Good quality water must be colourless, odourless, tasteless and free from faecal pollution (Ezeugwunne *et al.,* 2009; Omalu *et al.,* 2010). Water plays an important role in the prevention of diseases; taking eight glasses of water daily minimizes the tendency of colon cancer by 45% and bladder cancer by 50% as well as reducing the risk of other cancers (Oparaocha *et al.,* 2010*).*

In many developing countries, availability of good drinking water is a major and critical problem and· it is a matter of great concern to our societies depending on non-public water system (Umezuruike *et al.,* 2009). Increase in human population poses a great pressure on provision of safe drinking water especially in developing countries

(Umezuruike *et al.,*2009). Consequently, water-borne diseases such as cholera and typhoid often have their outbreak especially during dry season (Banu and Menakuru, 2010). Contaminated water is a global public health threat placing people at a risk of a host of diarrhoeal and other illnesses (Umezuruike *et al.,* 2009). Although water can contain undesirable chemicals (from natural sources), the major risk to human health is faecal contamination of water supplies. Serious ill health can be caused by water contaminated with faeces being passed or washed into rivers, streams, pools or being allowed to seep into wells or boreholes (Cheesbrough, 2006).

Water of good drinking quality is of basic importance to human physiology and man's continued existence depends very much on its good quality and availability (Umezuruike *et al.,* 2009). The provision of potable water to the rural and urban populations is necessary to prevent health hazards. Before water can be described as potable, it has to comply with certain physical, chemical and microbiological standards, which are designed to ensure that the water is palatable and safe for drinking (Umezuruike *et al.,*2009). Potable water is defined as water that is free from diseases-producing microorganisms and chemical substances deleterious to health (Umezuruike *et al.,*2009). Water can be obtained from a number of sources, among which are streams, lakes, rivers, ponds, rain, springs, taps and wells (Umezuruike *et al.,* 2009). Unfortunately, clean, pure and safe water only exists briefly in nature and is immediately polluted by prevailing environmental factors and other human activities. Water from most sources is therefore unfit for immediate consumption without some sort of treatment (Umezuruike *et al.,*2009).

Water in nature is seldom totally pure. Rainfall is for example, is contaminated as it falls to earth, as a result of combustion of fossil fuel which add sulphur compound

responsible for acid rain. Water that moves below the ground's surface undergoes filtration that removes most organisms(Tortora *et al.,* 2002). For this reason, water from springs and deep wells are generally of good quality. Contaminants ingested into water supply cause many diseases, such as typhoid fever, dysentery, cholera and gastro entritis. Examples of such pathogens are *Salmonella* species, *Shigella* species, *Vibrio cholerae* and *E. coli* (Tortora *et al.,* 2002; Oladipo *et al.,* 2009).Industrial and agricultural chemicals leached from the land, enter water ina great amount and they could be resistant to biodegradation. Apart from this, rural water often has excessive amounts of nitrite from microbial action on agricultural fertilizers (Tortora *et al.,* 2002; Oladipo *et al., 2009).*

To attain a safe water supply to various communities, an understanding of water that is bacteriologically and chemically certified is therefore imperative. Above all, to ensure that the bacteriological quality of drinking water is safe for human consumption, the Nigeria based National Agency for Food and Drugs Administration Control (NAFDAC) in association with the World Health Organization (WHO), recommended that potable water should not contain any microorganism that is known to be pathogenic and the coliform number per 100 ml of water must be zero. However, it may contain three coliforms per 100 ml of water sample in occasional samples (Oladipo *et al.,* 2009). The bacteriological quality of public drinking water is of paramount importance and its monitoring must be given highest priority. This is so because studies (Oparaocha *et al.,* 2010) have attributed several disease outbreaks to untreated or poorly treated water containing bacterial pathogens that have been isolated from public drinking water.

1.1 Justification for the Study

The bacteriological quality of drinking water is a concern to consumers, water suppliers, regulators and public health authorities. The potential of drinking water to transmit microbial pathogens to great number of people causing subsequent illness is well documented in many countries at all levels of economic development. The number of outbreaks that has been reported throughout the world demonstrates that transmission of pathogens by drinking water remains a significant cause of illnesses. However, estimate of illness based solely on detected outbreaks is likely to underestimate the problem. A significant proportion of water-borne illnesses are likely to go undetected by the communicable disease surveillance reporting systems. Therefore, it is of paramount importance to assess the microbiological standard of public water in order to improve sanitation and reduce the incidence of diseases transmission.

1.2 Aim and Objectives of the Study

The aim of this study was to determine the bacteriological quality of public drinking water (borehole, well and taps) and characterize (biochemical and molecular) pathogens isolated these from Sokoto metropolis. Therefore, it is made up of the following specific objectives

i. to determine the physicochemical properties of the drinking water samples;
ii. to enumerate aerobic and mesophilic faecal and non faecal coliform;
iii. To isolate and comparethe bacteriological standard, and to determine the total coliform count of public drinking water in Sokoto metropolis with WHO, NAFDAC and Nigerian Standard for Drinking Water Quality (NSDWQ) standards.

iv. To characterize bacteria from public drinking water within the Sokoto metropolis using both biochemical and molecular methods.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diversity and Uses of Aquatic Environments

Aquatic environment is a water surrounding area that comprises of fresh and marine waters. The (aquatic environment) components relate to living in, or located in beds or shores of a water body, including but not limited to all organic and inorganic matter, and living organisms and their habitant. About 70% of the Earth's surface is covered by water much of which occurs in the oceans, estuaries and freshwaters (ponds, streams, rivers and lakes) (Nollet, 2007). These waters are used for many purposes such as recreation and transportation, as sources of drinking water (sometimes without any form of treatment, particularly in developing countries), and for industrial activities and irrigation on farmlands. Surface waters are also important ecosystems from which finfish, shellfish, and macroalgae are harvested, and in which a variety of organisms are cultured (Nollet, 2007).

2.2 Diversity of Aquatic Bacteria

A diverse bacterial flora inhabits marine and freshwater environments where they are found in various microhabitats including water column, sediments, on submerged hard substrates, and on the surface or within the bodies of aquatic plants and animals (Todar, 2005). Aquatic bacteria serve important ecosystem functions that include transformations of nitrogen, carbon, and sulfur (Whitman and Flick, 1995). These bacteria may be divided into three groups on the basis of their mode of feeding: saprophytes (heterotrophs)that obtain nutrition from dead organic matter, photoautotrophs that are capable of synthesizing organic matter using sunlight as a source of energy, and chemolithotrophs

(chemoautotrophs) that can synthesize carbon using inorganic materials as energy source (Todar, 2005).

However, other unique types of metabolism are known to exist among photosynthetic bacteria, including photoheterotrophs and chemoheterotrophs. Some bacteria live symbiotically with other aquatic organisms as parasites and derive nutrition from their hosts. Others are commensals that obtain nutrition from their host, without causing any apparent harm to the host, or are mutual symbionts that benefit their hosts while relying on them to provide habitat (Vernam and Evans, 2000). Some of the bacteria are obligate aerobes; others are obligate anerobesor facultative anerobes. Furthermore, some are thermophiles (grow best at 50^0Cto 70^0C), mesophiles (grow best between10^0Cto 50^0C), or psychrophiles (live and grow at -10^0C to 20^0C) (Nollet, 2007). The majorities of bacteria found in surface waters are not disease-causing and perform indispensable functions in the food webs of aquatic ecosystems. They utilize dissolved organic matter, and thus serve as important trophiclinks helping to recycle and regenerate inorganic nutrients for phytoplankton production, and in the transfer of organic carbon in the ecosystem (Nollet, 2007).

However, some bacteria such as sulfate-reducing bacteria and iron bacteria, although nonpathogenic, cause nuisance to humans. The iron bacteria oxidize iron or manganese and can produce a brownish slime that accumulates inside plumbing fixtures and pipes causing discolouration of water and imparting unpleasant odours. Sulfur bacteria reduce sulfate to hydrogen sulfide that corrodes water pipes and causes water to have a rotten-egg odour. Furthermore, bacterial biofilms form on ship hulls and ocean-going vessels and facilitate the growth of other biofouling agents such as algae, mussels, and

barnacles, which can decrease the overall speed of the ship and increase fuel consumption (Vernam and Evans, 2000).

2.3 **Aquatic Pathogenic Bacteria**

Some bacteria, although naturally occurring, are known to cause diseases in humans, especially those with compromised immunity. For example, in the United States Gulf Coast areas, *Vibrio vulnificus* causes illness or even death in immune compromised individuals who consume bacteria-contaminated shellfish (Whitman and Flick, 1995; Shapiro *et al.,* 1998*;* Whitlock *et al*., 2002). In coastal waters of New England in the United States, *V*. *parahaemolyticus*, another naturally occurring bacterium, has been implicated in leg gangrene and endotoxin shock in humans (Roland, 1970). Several types of disease-causing viruses, protozoa, and bacteria are known to occur in sewage, human faces, and faecally contaminated waters (Feachem, *et al*1981: Mara and Feachem, 1999). Many of these pathogens such as *Vibrio cholerae*, *Salmonella* species*,* and *Campylobacter jejuni* originate directly from human and other warm-blooded animal sources, and are the causative agents of some of the most important waterborne diseases in the world, especially in developing countries where sanitation is generally poor and access to potable water is limited (Drasar and Forrest, 1996). Vibrio cholerae*,* for example, occurs naturally in freshwater and brackish waters in associations with planktonic organisms, and is responsible for cholera epidemics and the associated deaths that have occurred in many countries (Drasar and Forrest, 1996; Falade and Lawoyin, 1999).

Pathogenic microorganisms found in the guts of infected humans are excreted with faecal matter and are thus found in sewage and reclaimed water. Cysts produced by pathogenic protozoans*,* particularly *Cryptosporidium* and *Giardia,* are capable of surviving

under adverse environmental conditions (Nollet, 2007). The concentrations of these protozoans and human enteric viruses in sewage effluents, even after tertiary treatment, may be higher than the infective doses needed to cause diseases in humans.

Faecal coliforms and pathogenic microorganisms enter surface waters from many sources. These include raw or inadequately treated sewage discharged into surface waters, excrement from wildlife and runoff from farm animal feedlots and farmlands that have been fertilized with manure. The overflow of, and leaks from septic tanks can also introduce pathogenic bacteria into surface and ground waters. Humans become infected by drinking water or consuming food, including shellfish contaminated with pathogens; or through recreational contact with water in form of bathing, boating, swimming, fishing or washing of clothes (Doran and Linn, 1979).

2.4 Use of Bacteria as Indicators of Pathogenic Organisms in Water

The detection and enumeration of disease-causing bacteria in surface waters is difficult, time consuming, and expensive; and for many of the pathogens, methods for their routine monitoring and isolation are nonexistent or the costs for their isolation and enumeration are very prohibitive (Leonard, 2001). It is also impossible and impractical to identify all the enteric pathogenic bacteria present in the water at any particular time. Moreover, because of their low densities in surface waters, the absence of pathogenic organisms in tested water samples does not guarantee that the bacteria are not present in the water from which samples were collected. Therefore it is important to identify harmless bacteria that could be used as predictors of the presence of pathogenic organisms in groundwater, surface waters, or drinking water after treatment (Leonard, 2001).

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 in water. These indicator bacteria are total coliforms, faecal coliforms, *Escherichia coli,* faecal streptococci, and enterococci. A good type of indicator bacteria should occur naturally and exclusively in the gastrointestinal tract and faeces of humans and other warm-blooded animals. It should enter the water along with faecal materials and should be found in the presence of enteric pathogens. The indicator bacteria should also be able to survive longer than the enteric pathogens with which they occur and be removed by water treatment to the same extent as pathogenic organisms, and finally, it should be easier to isolate and identify than the enteric pathogens (WHO, 1996: Stevens *et al.,* 2003). Studies suggest that these traditionally used indicator bacteria meet the above requirements to varying degrees (Nollet, 2007).

2.5 **Total coliforms**

Coliforms are a group of Gram-negative, rod-shaped bacteria that are nonpathogenic and non-spore forming. The most common coliform genera are *Escherichia, Enterobacter, Citrobacter, Serratia* and *Klebsiella,* with *Eschericia coli* being the most abundant in the gut of humans and other warm-blooded animals. Coliform bacteria are identifiable by their ability to ferment lactose to produce acid and gas within 48hours, when incubated at 35°C. However, the development and use of media and commercial kits to detect coliforms based on specific enzymes (b-galactosidase) have expanded the definition of coliforms to include many genera of bacteria, some of which live primarily in the environment rather than in the gut of warm-blooded animals (Stevens *et al., 2003).*

Because they are found in the intestines of humans, domestic animals, and wild animals, coliforms are shed in faeces along with pathogenic organisms present in the gut of infected animals, and can be detected in water with relative ease; Total coliforms have been used by the US Public Health Service since 1914 as the standard for sanitary quality of water (LeChevallier, 1990). However, because some coliforms occur naturally in soils, aquatic environments including drinking water distribution systems, and plant matter where they can proliferate and because pathogenic organisms do occur in water and disease outbreaks have occurred even when coliforms are not present they are neither reliable indicators of faecal contamination nor indicators for the presence of pathogenic microorganisms (LeChevallier, 1990). In fact, many states in the United States have stopped using routine monitoring of total coliforms to determine whether faecal contamination of recreational waters has occurred (Nollet, 2007).

2.6 Faecal coliforms

Faecal coliforms (FC) are a subgroup of total coliforms consisting mainly of *E coli, Enterobacter* and some *Klebsiella.* They inhabit the intestines of warm-blooded animals. Because they can grow and ferment lactose at a relatively high temperature (45^0C), a characteristic that has earned them the name "thermotolerant coliforms", they can be differentiated from the other members of total coliforms (Neufeld, 1984). A high number of faecal coliforms in water suggests fecal contamination, which might have resulted in the introduction of pathogenic microorganisms in the water that presents potential health risks to individuals using the water. Faecal coliforms are better indicators of the presence of pathogenic bacteria in water than total coliforms, but their numbers alone cannot be used to tell whether faecal contamination is from human or nonhuman sources (Chao *et al.,* 2003). In addition, studies have shown poor associations between "swimmer-associated sickness"

and concentrations of faecal coliforms (Chao *et al.,* 2003). Moreover, there are indications that many members of faecal coliforms can grow and multiply in tropical and subtropical aquatic environments which undermine their significance as indicators of faecal contamination in such areas (APHA, 1998). Nevertheless, fecal coliforms have remained one of the indicators regularly monitored by many State agencies in the United States and in Europe to ensure that water bodies meet the established sanitary standards for drinking water sources and/or use in recreational activities (APHA, 1998). They are also used for classifying water used for growing shellfish as approved, conditionally approved, or restricted, in order to protect humans from consuming contaminated shellfish (International Shellfish Sanitation Conference, ISSC, 2002). Levels higher than established standards will result in closures of beaches and shellfish-harvesting waters, which may adversely affect tourism and the economy of the coastal areas.

2.7 ***Escherichia coli***

Escherichia coli are found in the intestines of humans and other warm-blooded animals where they perform important physiological functions (Conway, 1995). They are not normally found living in other environments, but have been reported to multiply in surface waters, especially in tropical environments (Todar, 2005). Several strains of *Eschericia coli* are usually non-disease causing, although illnesses such as septicemia and urinary tract infections have been reported (Cheesbrough, 2006), especially in immune compromised individuals. Some *Eschericia coli* strains (Example, *E. coli* 0157:H7) produce toxins that may cause diarrhea or even death in humans, particularly in elderly people and children (Todar, 2005).

A historical account of the use of *E. coli* as an indicator bacterium for faecal contamination can be found in Feng *et al.* (2002). *E. coli* was first proposed as an indicator species in 1892 (Feng *et al.,* 2002). But, it was only after the development of newer methods for rapid identification and differentiation of the species from the other members of the faecal coliform group that it officially came into use as an indicator species. Studies (Cabelli *et al*., 1982) suggest that *E*. *coli* is a more reliable indicator of faecal pollution and the occurrence of pathogens in water than faecal coliforms as a whole. A linear relationship has been reported between E.*coli* and enterococci counts in the marine environment and swimming-related gastroenteritis (Cabelli *et al.,*1982). In fact, USEPA (2003) recommended that *E. coli* or enterococci replace faecal coliform bacteria in State Water Quality Standards based on the study by Dufour (1984) that showed statistically significant relationship between E. *coli* and enterococci concentrations in freshwater and rates of swimming-related illness (Nollet, 2007).

2.8 **Faecal streptococci**

Faecal streptococci have been used as indicators of faecal contamination in water (Godfree *et al.,*1997). The group includes many species of bacteria in the genus *Streptococcus* such as *S. bovis, S. equines, S. avium, S. faceium* and *S. gallinarum* that are normally found in faeces and gut of warm-blooded animals. Unlike the coliform bacteria, they are Gram-positive and also tend to live longer in water than faecal coliforms. Hence, the ratios of faecal coliforms to faecal *streptococci*, which were used in the past to determine whether bacteria observed in water, were from human or non-human sources, were no longer considered to be reliable (Manafi, 1998).

2.9 **Enterococci**

Enterococci are a subgroup of the faecal streptococci that include *S. avium, S. faecium, S. gallinarum,* and *S. faecalis.* The group is found primarily in the gut of warm-blooded animals and generally do not grow in the environment hence, they are used as a bacterial indicator of faecal contamination of recreational surface waters (Leonard, 2001). They generally live longer in water than faecal coliforms and are preferred to fecal coliforms and faecal streptococci as indicators of illnesses associated with swimming and other recreational uses of freshwater and marine waters (Dufour, 1984). A linear relationship between *E. coli* and enterococci counts in the marine environment and swimming-related gastroenteritis was reported by Cabelli *et al.* (1982) and Leonard (2001). Therefore, a combined monitoring of *E. coli* and enterococci in water is believed to provide a higher degree of confidence in the estimated risk of faecal contamination as well as the presence of pathogens in water (Stevens *et al., 2003).*

2.10 ***Clostridium perfringens***

Clostridium perfringens is a spore-forming bacterium found in sewage and faeces of warm-blooded animals at high concentration (Davis *et al.,* 1977). They are anaerobic and rod shaped. Their spores make them more resistant to environmental stresses and to water disinfection than faecal coliforms and faecal streptococci. They also seem not to reproduce in the aquatic environment (Davis *et al.,* 1977). Hence, they have been used as indicators of faecal contamination and in tropical environments may be more preferred to faecal coliforms and enterococci. In an estuarine system in Australia, Ferguson *et al.* (1996) noted that C. *perfringens* was better than faecal coliformsor faecal streptococcias an indicator of faecal pollution, and was the only indicator that showed significant correlation with the

presence of *Giardia.* It has also been shown to be a suitable indicator of pathogenic protozoans and viruses in sewage-contaminated freshwater (Payment and Franco, 1993). However, their main drawback is that they are known to survive and accumulate in drinking water systems and the environment (Stevens, *et al*., 2003). Consequently, their presence in water does not always indicate a recent occurrence of faecal pollution.

2.11 Dynamics of Faecal Coliforms in Surface Waters in Relation to Environmental Factors

Implications for assessing faecal coliform levels in surface waters and adequate sampling and monitoring of indicator bacteria in surface waters require a good understanding of enteric bacterial distributions and the factors that influence their densities in water. Faecal coliform concentrations are not evenly distributed in surface waters. Their densities vary in relation to season, climate, tidal cycles and environmental factors such as temperature, salinity, turbidity, nutrients and solar radiation intensity. Faecal coliforms in surface waters peak after a rain event (Davis *et al.,* 1977; Seeley and Primrose, 1982). Subsequently, they decrease or disappear from the water column with time, through death and sedimentation processes, and may concentrate in sediments at high densities (Bergstein-Ben Dan and Stone, 1991: Gannon *et al.,* 1983). Coliform bacteria in sediments can be resuspended in shallow waters by tidal movements and winds dredging storm surge increased stream flow, and recreational activities such as boating (Crabill*et al.,*1999). Faecal coliforms also exhibit seasonality in their concentrations in surface waters due to seasonal patterns of precipitation and the associated runoff or due to seasonal variations in the recreational use of the water body (Crabill *et al.,*1999).

Climate variability associated with El Niño Southern Oscillation (ENSO) events also influences the concentrations of faecal coliform bacteria *(*Lipp *et al.,* 2001 Chigbu *et al.,*2004). During El Niño years, average faecal coliform bacteria densities are higher in surface waters than during El Niño years in some regions such as the United States Gulf coast. Demophoric growth within a watershed, coupled with a rise in the percentage of natural landscapes converted into impervious surface, has been shown to increase runoff carrying chemical pollutants and biological agents into coastal waters (Booth, 1991; Schueler, 1994). For example, a positive correlation between faecal coliform counts and percent impervious surface and watershed population was observed in the coastal areas of North Carolina (Mallin *et al.,*2001). The dynamics of faecal coliforms in coastal waters depends in part on bacterial loading from streams and rivers, mass transport and bacterial losses due to death and sedimentation. The rates at which they disappear from surface waters depend on many factors such as availability of nutrients, temperature, salinity and turbidity, degree of water mixing, solar radiation, predation and competition (Faust *et al.,* 1975; Auer and Niehaust, 1993).

However, temperature and solar radiation are considered the most important abiotic factors (Esham and Sizemore,1998). For example, Xu *et al.*(2002)found that solar radiation and temperature and insulation combined, explained 31%, 78%, and 87%, respectively, of coliform bacteria die-off coefficients in a lagoon on a French Island (Noirmountier*),* in the Atlantic Ocean (George *et al.,* 2001). Predation by protozoans is a major biotic factor influencing faecal coliform death rates; it accounted for 47%-99% of the mortality in the Seine River, France (Menon *et al.,* 2003).

2.12 Problems Associated with Using Faecal Coliforms as Indicators of Pathogenic Organisms

Faecal coliforms are poor indicators of the presence of pathogenic viruses and some protozoans such as *Cryptosporidium* and *Giardia* in surface waters. Studies indicated that faecal coliforms are not reliable predictors of the presence or absence of pathogenic viruses inmarine waters and shellfish (Gerba *et al.,* 1979*;* Goyal *et al.,* 1984; Burkhardt *et al.,* 2000). Even where a significant relationship has been found between the presence of enteroviruses and faecal coliform bacteria, the relationship was not very strong. Consumption of oysters harvested from waters approved for shellfish harvesting based on faecal coliform bacteria levels has been associated with outbreaks of hepatitis A (Mackowiak *et al.,* 1976). The poor association observed between faecal coliforms and enteric viruses and protozoans in the marine environment might be due to the differences in their survival rates in water. In seawater, some viruses survive longer than indicator bacteria *(*Gerbaand Goyal*,* 1988). In fact, in marine sediments where viruses can accumulate, viruses can survive for several months. Lipp *et al*. (2001b) found that none of the indicator bacteria (faecal coliforms*,* enterococci*, Clostridium perfringens)* was significantly associated with *Giardia* or *Cryptosporidium* in a subtropical estuary in Florida. In an estuarine system in Australia, Ferguson *et al*. (1996) reported that C. *perfringens* was better than faecal coliforms or faecal streptococci as indicator of faecal 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 such as *Vibrio cholerae, V. vulnificus,* and *V. parahaemolyticus* that are

naturally occurring in surface waters. The occurrence, distribution, and abundance of this *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 than to feacal coliforms. Moreover, under some environmental conditions (such as low temperatures of 15°C), *Vibrio cholerae,* V. *vulnificus* and V. *parahaemolyticus* are known to occur as viable, non cultura bleforms (Oliver, 1995).

False positive results of facal 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 faecal coliform as an indicator of faecal contamination in tropical waters has been questioned as these bacteria can grow and multiply in the environment (Chigbu *et al.,* 2004).

The number of faecal coliforms in water alone cannot be used reliably to determine the source (human or nonhuman) of indicator bacteria (Geldereic, 1976). Faecal coliform/faecal streptococci (FC/FS) ratios were used for many years in an attempt to differentiate nonhuman and human sources of faecal coliforms such that FC/FS ratios above 4 indicated human sources, ratios below 0.7 indicated animal sources, whereas ratios between 0.7 and 4 indicated a mixture of human and animal sources *(*Geldreich*,* 1976*).* These ratios are currently considered unreliable because some faecal coliforms multiply in effluents and in the environment especially in tropical environments (Oliver, 1995). 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 faecal coliforms will enable us to assess the extent of risks of acquiring diseases since enteric bacteria from human sources pose a different risk than those from farm animals and wildlife (Manafi, 1998).

2.13 Bacterial source tracking

A new methodology currently under development to determine sources (including domestic animals, wild animals, humans) of bacteria is known as bacterial source tracking (BST), also referred to as microbiological source tracking (Parveen *et al.,* 1999). The microorganisms that have been used in BST are *Escherichia coli,* faecal streptococci, *bifidobacteria* and *Bacteroide-Prevotella.*

Bacterial source tracking methods can be placed into two major groups: (i) genetic fingerprinting, a molecular, genotypic method and (ii) antibiotic resistance, a biochemical, phenotypic method. The two methods require the development of large libraries of organisms and are costly and time consuming. In the case of ribotyping, a database of DNA fingerprints is created from bacteria including E. *coli* that are obtained from known sources such as humans and domestic animals. New bacterial isolates from unknown sources are subsequently compared with the already developed DNA fingerprints from known sources. The source of the bacteria is then determined based on the degree of similarity of the DNA belonging to the new bacterial isolates and the DNA fingerprint library (Olive and Bean, 1999).

Genetic fingerprinting is a culture-dependent technique that makes it possible to identify strains of bacteria recovered on solid or liquid medium. Details of ribotypingare

offered in the context of source tracking; however, this procedure can be employed in a similar fashion to identify specific bacterial strains. While genotypic techniques identify organisms on the basis of their genetic makeup, antibiotic-resistance testing employs the fact that different kinds of antibiotics are used in humans and animals. As a result, patterns of resistance to a variety of antibiotics in the natural bacterial populations symbiotic with these organisms will differ in the environmental isolates that are human derived and those that are farm animals or wildlife derived. In the case of antibiotic-resistance methodology, profiles of antibiotic resistance of faecal coliform bacteria from known sources are obtained and banked. The sources of new faecal coliform bacteria isolated from surface water are identified based on the degree of similarity and differences of their antibiotic-resistance characteristics to those from known sources (Wiggins *et al.,* 1999*).*

This approach was proposed in the 1990s for faecal streptococci and successfully applied to distinguish between point and nonpoint sources of microbial pollution (Parveen *et al.,* 1997). In those studies, environmental bacteria were grown on EMB agar, colonies identified as coliforms or streptococci were selected and tested for resistance to several antibiotics at various concentrations. Although this method is time consuming (it takes 4 days to obtain the results) and not necessarily very precise, it is nevertheless significantly cheaper than ribotyping (Whitlock *et al.,* 2002). Although BST can be used to tell whether a bacterial isolate is from human, wildlife, or farm-animal sources, at present it cannot be used to distinguish reliably between wildlife and farm-animal sources.

2.14 Other indicators of faecal contamination

Other organisms that have been considered as indicators of faecal contamination particularly from human sources include bacteriophages and coliphages. Bacteriophages, in

particular coliphages(viruses that infect coliforms*),* have been proposed as indicators of faecal pollution. 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 (Borrego *et al.,* 1990). Moreover, enteric viruses have been found in water where coliphages were not found *(*Morinigo *et al., 1992).*

Attempts have also been made to use coprostanol*,* a faecal sterol, as an indicator of faecal contamination. Coprostanolis found in the faeces of humans as a result of the breakdown of cholesterol by bacteria in the intestines. It is degraded in the marine environment, disappearing in about three weeks; hence, its concentrations in the sediment may be useful as an indicator of faecal contamination. Additionally, a significant relationship has been obtained between coprostanol and *E. coli* concentrations in tropical waters (Isobe *et al.,* 2002*).*

2.15 **Prevotella – Bacteroides detection**

Bacteroides group is a conglomeration of bacteria described in the late 19th century (Veillon and Zuber, 1898). It includes Gram-negative, anaerobic organisms associated with mammalian intestines. Molecular typing of the group resulted in its subdivision into three genera of Prevotella*,* Bacteroides and *Porphyromonas,* the former two being of the greatest interest in pollution source tracking (Allsop and Stickler, 1985). Bitton (2005) has listed a set of parameters of a good organism for pollution detection and source tracking. Organisms of the Prevotella– Bacteroides group satisfy all seven parameters; however, cultivation of the anaerobic organisms is a task too large for a laboratory charged with conducting routine analyses of water. Thus, the detection methodology to be employed needs necessarily to be culture independent. A technique has been developed recently to

use bacteria of the Prevotella – Bacteroides group as indicators of faecal pollution of water and marker organisms for tracking the sources of pollution (Bernhard and Fields, 2000). This method involves amplification of target DNA by PCR with primers specific for Prevotella – Bacteroides group, followed by terminal restriction fragment length polymorphism (t-RFLP) and length heterogeneity PCR fingerprinting of the community (Bernhard and Fields, 2000). The fingerprints obtained were clearly different 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 (Bernhard and Fields, 2000).

2.16 Use of heterotrophic plate count as a measure of water quality

Heterotrophic plate count (HPC), an estimate of the total number of viable microorganisms (yeasts, moulds, and bacteria) in water, is used routinely to assess the water quality, to determine whether changes have occurred in water during storage or distribution due to bacterial re-growth, or to monitor the efficiency of water treatment processes (Michiels and Moyson, 2000). Heterotrophic organisms cannot manufacture their own food; hence they rely on organic and inorganic materials from other sources for nutrition. Heterotrophic plate count therefore is estimated by counting the number of colonies on culture media. This estimate is not accurate, firstly, because the culture media used to enumerate heterotrophic organisms do not support the growth of the different types of heterotrophs equally. Secondly, some of the bacteria occur in viable but non culturable forms. It has been estimated that about 1 % of the total bacteria found by direct microscopy are obtained when HPC methods are used (Wagner, *et al*., 1993). Since the majority of

these microorganisms may be from sources (soils, vegetation) other than the gut of warm-blooded animals, HPC is not recommended for use in determining the safety or hygienic quality of drinking water with regards to the presence of pathogenic organisms. No guidelines with regards to drinking water are available for HPC and it is difficult to completely remove heterotrophic organisms from drinking water. According to Bitton, HPC bacterial concentrations in drinking water should not be more than 0.1cfu/mL (Nollet, 2007).

2.17 **Bacteriological water quality standards**

Drinking water standard: In the United States, the drinking water quality criteria and directives on its monitoring can be found in the National Primary Drinking Water Regulations (NPDWR, 2006). The presence or absence of total coliforms in public water systems is determined rather than the number of total coliforms. The frequency of monitoring total coliforms depends on the population of humans served by the distribution system. For example, the minimum number of 100mL samples analyzed for the presence of total coliforms per month ranges from one per month for a population of 25-1000 to 480 per month for a population of about 3.96 million or more (Nollet, 2007). No more than 5% of the samples tested can be positive for total coliforms. If this rule is violated, a repeat sampling is conducted within 24hours. If it is positive again for total coliforms, further analysis must be done to determine whether faecal coliforms and *E. coli* are present, and for compliance, none should be present. Otherwise, the maximum contaminant limit (MCL) is violated, which should be reported to the public and the State (Wagner, *et al*., 1993).

In Europe, the microbiological quality standard for drinking water for *E coli* and enterococciis 0/100mL (for unbottled water) or 0/250mL (for water in bottles or

containers). In addition, for waters in bottles or containers, *Pseudomonas aeruginosa* must not exceed 0/250mL, and HPC must not exceed 100/mL or 20/mL in samples incubated at 22°C or 37°C respectively (CD, 1998). The recreational water quality standards for the United States and the European Union are based on a number of microbiological parameters including total coliforms, faecal coliforms and enteroviruses (CD, 1998). The values for these standards are higher than those for drinking water. Also, values for swimming are higher than for partial body contact, such as boating or fishing. For example, the faecal coliform standard for swimming in the United States is a geometric mean number (200 cfu/100mL) whereas that for partial body contact is 1000 cfu/100mL(CD, 1998).

In the United States, the guideline for bacteriological quality monitoring in freshwater recommends that either *E. coli* or enterococci may be used for monitoring(CD, 1998). Shellfish such as clams, mussels, oysters, and scallops are filter feeders and tend to concentrate contaminants including bacteria, which may continue to grow in them. Consumption of oysters with pathogenic bacteria or viruses has resulted in gastrointestinal diseases (CD, 1998).

To reduce the risks of acquiring diseases due to consumption of contaminated oysters, the US Food and Drug Administration and the Interstate Shellfish Sanitation Conference (ISSC) formed the National Shellfish Sanitation Program (NSSP) that developed criteria for protecting shellfish-harvesting waters (ISSC, 2002). For example, the Mississippi ordinance requires a shellfish-harvesting area to be closed "when the geometric mean of the seawater from compliant sampling stations in the area exceed a faecal coliform most probable number (MPN) of 14 per 100ml and/or more than 10% of the samples exceed an MPN of 43 for a 5tube 3 dilution test"(ISSC, 2002).

2.18 Detection and Enumeration of Bacteria in Water

APHA (1998) states that bacteria in water can be detected and enumerated by plating and culturing in liquid media, by culturing on solid media, direct microscopic observation, or by use of molecular methods including gene probes and PCR. Methods used for culturing in liquid media are (a) the MPN method also known as the multiple tube fermentation technique and (b) the presence- absence (P/A) test. Cultivation on a solid media may be carried out using the viable plate count procedure such as (a) spread-plate technique, (b) pour-plate technique or (c) membrane filtration method (APHA, 1998).

2.18.1 Viable Plate Count Procedure

Koster *et al.* (2003) state that variety of culture media have been developed using this method for culturing different types of microorganisms. However, the method results in selective culture of some microorganisms as no medium meets all the nutritional and physiological requirements of all microorganisms in water. Moreover, some microorganisms sometimes occur in viable, but non culturable forms in water samples, produce false negatives, or are underestimated if stressed during sampling or analysis. The following are the three techniques that may be used under this method: Spread-plate technique: The water sample (0.1-0.5mL) is simply spread uniformly on the surface of agar and then incubated at 37^0C for 24 hours. The incubation temperature and duration for bacterial growth depend on the bacteria of interest. This method is simpler than the pour plate technique described below as the plates can be prepared in advance. With this method, the morphology of the colonies can be easily distinguished (Koster *et al.,* 2003). The colonies can also be transferred with relative ease. Also, the bacterial cells in the samples are not heat-shocked due to exposure to media at 40°C contrary to the pour-plate technique.

Pourplate technique: For this method, the appropriate agar medium is melted and allowed to cool to about 40°C. Then, a specific volume of the sample, generally 1mL, is added into 100-15mm or 90-15mm sterile disposable Petri dishes. About 15-20mL of the medium is added into the plates with the samples, mixed very well, and allowed to solidify. Thereafter, the Petri dishes are incubated at the recommended temperatures and for the appropriate length of time depending on the bacteria of interest. If the target bacteria are present, colonies will grow on the surface of, and within the medium, which are then counted and reported as number of colonies per milliliter. A major drawback of the pourplate technique is that, it may shock bacteria in the samples and medium with a temperature of 44°C-46°C, which may influence their growth, and consequently the estimates of the number of bacteria in the sample (Koster *et al.,* 2003). Secondly, because the microorganisms are submerged in the media, the colonies often grow slowly and thirdly; the colonies growing in the medium can be difficult to transfer (Koster *et al.,* 2003*).*

Membrane Filtration Method: The water sample (generally 100mL) is filtered through a 0.45µm (sometimes 0.22µm) sterile filter membrane thereby retaining the organisms on the filter membrane surface. With face upward, the filter is then simply placed on the surface of a culture medium and incubated. Alternatively, before incubation, the filter may be placed on an absorbent pad that is sterile and saturated with broth. This method is suitable for examining relatively large volume of water with low turbidity (Koster *et al.,* 2003). The bacteria are also not heat-shocked in the process. However, the bacterial cells may be damaged or injured due to excessive filtration pressure (Nollet, 2007).

2.18.2 **Most Probable Number Method (Multiple Tube Technique)**

As the name suggests, this method involves the use of many tubes of liquid medium and serial dilutions to extinction of the water sample (0.001, 0.01, 0.1 and 1mL) to be tested. The number of replicates for each dilution ranges usually from 3 to 10, depending on the source of water to be analyzed. After incubation at the appropriate temperature and for a specified duration, the number of positive and negative tubes is scored based on characteristic changes in the medium (acid or gas production). Assuming a Poisson distribution of the bacteria, a statistical table is used to estimate the MPN of viable microorganisms in the original water sample (APHA, 1998). The advantages of this method are that it can be used to analyze all kinds of samples and results are easy to interpret requiring no special skills (Koster *et al.,* 2003*).*

The disadvantages of this method include, firstly, that it is selective for the growth of some microorganisms whose nutritional and physiological requirements have been met by the medium and incubation conditions used. Secondly, since the actual numbers of cells or bacterial colonies are not counted in this method, it is not as accurate as the plate-count procedure. Thirdly, the use of many tubes with liquid media makes it more labor-intensive than the plate-count technique (Koster *et al.,* 2003*).*

2.18.3 **Presence-Absence Test**

The objective of the presence-absence test is not to determine the concentration of microorganisms present in a sample, particularly drinking water sample, but to know simply whether the organism is present or absent. After inoculation in a suitable medium and incubation, a positive result is indicated by changes or growth in the medium. The test

is usually used to examine on a routine basis samples (100mL in a single culture container) from water treatment plants or distribution systems. It should be followed with further tests to determine the densities of the organism of interest, if a positive test is obtained (Nollet, 2007).

2.19 **Direct Epifluorescent Microscopy**

Kepner and Pratt (1994) state that It is well understood that a "universal growth medium" does not exist. Different physiological groups of microorganisms have different requirements for both electron donors and electron acceptors, both in terms of their nature and their concentration. As a result, any culture-based method only accounts for a small subset of the microbial community such as "heterotrophic" organisms or sulfate-reducing bacteria (Kepner and Pratt, 1994). Consequently, a technique for estimating the total number of bacterial cells regardless of their metabolic capabilities and physiological status was needed. Several direct count procedures have been developed in response to that need. Development of reliable membrane filters and fluorescent stains specific for DNA and RNA by the 1980s accelerated the adoption of this methodology (Kepner and Pratt, 1994).

Unlike samples destined for culture-based procedures, the samples collected for direct count need to be preserved immediately after collection. Preservatives used are generally formaldehyde or glutaraldehyde, applied at concentrations of about 2% v/v (Kepner and Pratt, 1994). It appears that preserved samples can be stored for an appreciable time before processing (Turley and Hughes, 1992). Preserved sample is filtered through a membrane filter under gentle vacuum, less than 80 mmHg as strong suction could disrupt cells. The pore size of the filter should be sufficiently small (0.22mm) to retain microbial cells, and filters for fluorescent detection are generally purchased prestained black. The

cells are stained with a fluorochrome. The two most commonly used stains are 4, 6-diamidino-2-phenylindole (DAPI) and Acridine Orange (AO) (Hobbie *et al.,* 1977). Both of those compounds bind nucleic acid molecules, DNA and RNA. A number of procedures have been developed for each one of dyes in question (stain, then filter versus filter first, then stain) and, apparently, those procedures are accepted equally. Staining is done under various conditions, with 100mg/L and 1-5min staining time used in most studies for AO and 0.01mg/L and 5min staining time for DAPI (Kepner and Pratt, 1994). Filters are then dried in the dimmed light and mounted with immersion oil. The bacterial cells trapped on the filter and stained with fluorescent dyes are then counted under the epifluorescent microscope.

In order to minimize investigator's bias and side-to-side variability of cell number on a filter, a number of fields (at least 10-20) are counted along the two perpendicular transects. Assuming Poison distribution of bacterial cells on filters at least 400 cells per filter need to be counted for a reliable (CI 95 less +10%) estimate of bacterial numbers (Kepner and Pratt, 1994). The resulting counts are calculated back to the bacterial numbers per unit water volume. The direct count methods are dependent on the ability of the investigator to recover bacteria from the sample, to observe stained particles, and to identify such particles as bacteria. Each one of these steps could limit the accuracy of the method, depending on the circumstances. Bacterial cell recovery generally presents no problem in water analysis setting. However, highly turbid waters can clog the filters with inorganic particles. Furthermore, such particles could obscure bacteria on their sides facing away from the microscope objective, presenting an observation problem. The accepted particles, therefore is to count bacteria on the side of a particle facing the objective lens and

then double the number (Kepner and Pratt, 1994). Identification is generally less of a problem than recovery and observation. However, the florescent techniques fails to precisely distinguish between alive and dead bacterial cell number on a filter, a number of fields (at least 10-20) are counted along the two perpendicular transects. Assuming Poisson distribution of bacterial cells on filters at least 400 cells per filter need to be counted for a reliable (eI 95 less than + $10^{\%}$) estimate of bacterial numbers (Kepner and Pratt, 1994). The resulting counts are calculated back to the bacterial numbers per unit water volume. The direct count methods are dependent on the ability of the investigator to recover bacteria from the sample, to observe stained particles, and to identify such particles as bacteria. Each one of these steps could limit the accuracy of the method, depending on the circumstances. Bacterial cell recovery generally presents no problem in water analysis setting. However, highly turbid waters can clog the filters with inorganic particles. Furthermore, such particles could obscure bacteria on their sides facing away from the microscope objective, presenting an observation problem. The accepted practice, therefore, is to count bacteria on the side of a particle facing the objective lens and then double the number (Kepner and Pratt, 1994). Identification is generally less of a problem than recovery and observation. However, the fluorescent technique fails to precisely distinguish between alive and dead bacterial cells. Although DNA and RNA complexes of AO fluoresce differently (green versus red), producing a difference between the cells that have a lot of RNA and thus are metabolically active and the ones that are not, this difference is rarely accounted for in the real-life testing situation.

A novel twist on fluorescent count is fluorescent in-situ hybridization (FISH). In this technique, fluorescent dyes are attached to short single stranded DNA fragments,

complementary to known sequences of 16S RNA. As a result, under the right conditions, only the cells of the phylogenetic group for which the probe was designed will be detected (Manz *et al.,* 1993). Although this method has been applied in microbial ecology studies, it is somewhat too expensive and time consuming to be performed in the process of routine water testing. Staining with fluorescent antibodies has been successfully applied for detection of pathogenic protists (Manz *et al.,* 1993).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Figure 1 shows the map of the study area-Sokoto metropolis. Sokoto State is located between longitudes 4° to 6° 400 north. It shares border with Niger Republic toward North, Zamfara State to the East and Kebbi State to the South and West. It has a population of 3,702,676 (National Population Commission, 2006). Sokoto State has a land area of about 28,232.37 km^2 (Sokoto State Government, 2000) with a mean annual rainfall ranging between 500mm to 1,300mm. The mean annual temperature is about 38.3^0C, the maximum daytime temperatures are under 40°C for most months of the year. The warmest months are usually between February and April, when daytime temperatures can exceed 45^0C. There are two major ethnic groups namely, Hausa and Fulani. Also, there are Zabarmawa as minority in the border of the local government areas (Sokoto State Government, 2009).

3.2 Collection of water Samples

Two hundred and seventy (270) water samples were randomly collected, 90 each from, wells, taps and boreholes at different locations within different geographical zones of Sokoto metropolis. These included Runjin Sambo, Arkilla, Rijiyar Shehu and Dambuwa for well. Mabera, Kanwuri, Kuffa and Tsohuwar Kasuwa for tap. Runjin Sambo, Sama Road, Gagi and Dundaye for borehole. Each sample was collected using sterile 250ml plastic bottles for each sample in triplicates. To ensure the sterility of the samples from boreholes and taps, the borehole taps was sterilized by means of lighters, after which the taps opened to flow for 1 minute and then the plastic bottle was filled with water up to 200ml leaving some space to allow shaking before analysis. The collected samples were delivered to laboratory within 30minutes of collection.

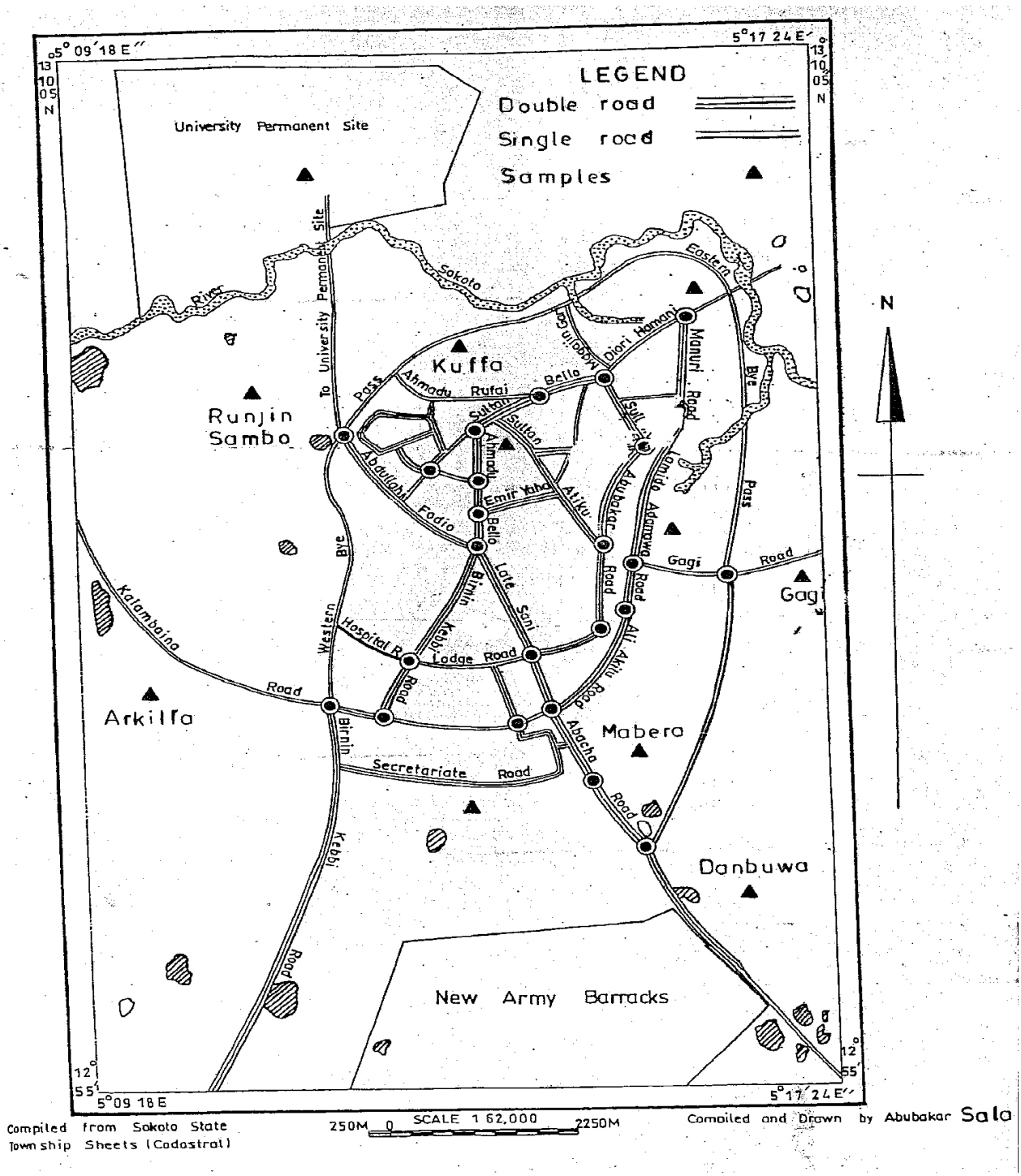

Figure 1: A map of Sokoto Metropolis Showing Sample Locations

3.2.1 Collection of water samples from taps

External fittings from the tap, such as anti- splash nozzle or rubber tube were removed (if any). The outside nozzle of the tap was cleaned using sterile cotton wool. The tap was turning on full, and the water was allowed to run to waste for at least one minute. This allowed time for the nozzle of the tap to be flushed and any stagnant water in the service pipe to be discharge. The tap was sterilized by means of cigarette lighter. The tap was allowed to cool by running the water to waste for 30 seconds. The sampling bottles were then filled 200ml leaving some space to allow shaking before analysis and using waterproof marker the bottles were numbered with the sample code number (WHO, 1996).

3.2.2 Collection of water samples from wells

The cap and he cover of the sterile samples bottle were removed aseptically, for those wells that have water nearer, the mouth of the sample bottles were dipped into the wells with about 25ft depth for those well that were very deep, “guga” was use to fetch water samples into the sterile sample bottles. For wells in Dambuwa, it was close to gutter and as the time of sample collection, the well was not covered. The sample bottles covers were carefully replaced and the sample bottles were then labeled with the sample code number.

3.2.3 Collection of water samples from boreholes

The hand pump was continuously operated for five minutes. The mouth of the pump was heated using a lighter and some quantities of water were pump to waste. The sample of the water was collected aseptically by allowing the water from the pump to flow directly into the sterile bottle, and carefully replacing the bottle cap and cover.

3.3 Bacteriological Analysis

The bacteriological analysis was carried out using the multiple tube fermentation procedure (WHO, 1996, NSDWQ, 2007).

3.3.1 Multiple Tube Fermentation

i. **Presumptive Test**: Nine tubes (3 each) and inverted vials containing lactose broth were inoculated with pre-measured portions of the sample (10ml, 1.0ml and O.lml). The tubes were incubated at 35°C for 48hours, production of gas in any of the inverted vial within 48hours constituted a positive test. The absence of gas in all of the vials constituted a negative test.

ii. **Confirmation Test**: A sterilized wire loop was used to transfer a drop of culture from each positive tube to a tube containing brilliant green lactose bile broth (BGLBB). Gas formation in any vial within 48hours at 37°C was considered as positive test. The presence of gas in all inoculated tubes is a positive test; that is the organisms which produced the gas in the presumptive tubes are likely to be coliform.

iii. **Completed Test**: This involved streaking plates prepared with Eosin Methylene blue (EMB) agar with culture from one of the positive presumptive tubes. Development of typical coliform colonies with or without a green metallic (Sheen) after 24 hours incubation at 37°C constituted a positive test. No growth signified a negative test.

To perform complete test, a typical colony from an EMB plate was transferred on to a nutrient agar plate, and into a tube with inverted vial, containing lactose broth. An agar plate and broth tubes were incubated for 24 hours at 37°C. In tubes where no gas was

produced in the inverted vial at the end of the 24hours period, the test was considered negative. (Nollet, 2007).

3.4 Gram's staining

Gram's straining was carried out as described by Nollet (2007). The smear was allowed to air-dry and then passed over a flame in order to be fixed. After fixing, the smear was covered with a primary dye (crystal violet) and washed with water after one minute. The slide was covered with Lugol's iodine and again washed with water after the same period. Acetone-alcohol was used to decolorize the smear and washed immediately with water. This was followed by application of safranin (secondary dye) and left for 30 seconds and later washed with water. Back of the slide was cleaned with cotton wool and allowed to air-dry. The slides were examined microscopically using oil immersion objective lens.

3.5 Total heterotrophic Count

Agar was prepared according to manufacturer's standard and then held at 44 - 46^0C in water bath. Serial dilutions were prepared using 0.1% peptone water. One (1) milliliter of the sample was transferred to a sterile empty petri dish. Agar was melted by heating In boiling water and then allowed to cool at 44 – 46^0C. Approximately, 15ml of agar medium was poured into the petri dish containing the sample. The sample and agar were mixed thoroughly by rotating the plate several times, clockwise, then anticlockwise. When the medium has solidified the plates were inverted and incubated at 35^0C for 48hrs. Following the incubation period, the colonies were counted using colony counter and hand tally.

3.6 Molecular Identification of isolates Using Microgen GN-ID System (Kit Method)

Microgen GN-ID was used, it employs 12 (GNA) or 24 (GNA+B) standardized biochemical substrates in microwells to identify the family enterobacteriaceae and other non-fastidious Gram negative Bacilli (Oxidase-negative and positive)(Microgen™, 2011).

The adhesive tape was removed and all the positive reactions were recorded in the forms provided with the aid of the colour chart included in the booklet. Two drops of Kovac's reagent was added to well 8. The result was read and recorded after 60 seconds. Formation of a red colour indicated a positive result. A drop of VP I reagent and 1 drop of VP II reagent were added to well 10 and was read and recorded after 15 minutes. Formation of a deep pink colour indicates a positive result. One (1) drop of TDA reagent was added to well 12 and was read after 60 seconds. Formation of cherry red colour indicated a positive result. Finally, for the oxidase positive organisms, the nitrate reduction test was performed on well 7 after reading the OPNG result. One (1) drop of nitrate a reagent and 1 drop of Nitrate B reagent was added to the well and read after 60 second. The development of a red colour indicates that nitrate has been reduced to nitrite. The results were recorded on the forms (Microgen™, 2011).

3.7 Identification of bacterial Isolates

On the microgen GN-ID A+B forms the substrates have been organized into triplets (set of 3 reactions) with which each substrate assigned a numeric value 1,2 or 4). The sum of the positive reactions for each triplet forms a single digit of the octal code that was entered into the microgen identification system software (MID-50) which generated a report of the five most likely organisms in the selected database (Microgen™, 2011).

3.8 Physicochemical Analysis of the water samples

3.8.1 Electrical Conductivity

The conductance of each water sample was determined using a conductivity testing set (MS Nory lab). After rinsing the cell with a portion of each sample, it was filled with 50ml of the sample and fitted, upon a press of the test button, the read out which automatically corrected to 25^{o}C was reported as microsiemens per centimeters (µs/cm) (APHA, 1998).

3.8.2 Nitrate

Using the phenoldisulphonic acid method(APHA, 1998), 100ml of samples poured into Petridishes were evaporated to dryness on a steam bath. The residue in each was then dissolved with 2ml of phenoldisulphonic hydroxide was added and the mixture made up to 100ml with distilled at 410 nm using Cecil spectrophotometer. Distilled water was used as blank, and the readings were compared with the standard curve estimate obtained.

Standard curves for the preparation of the standard curve. 1ml, 2mI, 3mI, 4mI, and 5mI, portions of the standard nitrate solution 90.7218g/1,$KN0_3$ were each diluted and made up to 50ml. These were treated the same as the samples. Distilled water was used as blank.

Nitrate (N) calculated using the relation

$$N\ (mg/I^{-1}) = \frac{N \times 1000}{\text{Sample vol (ML)}}$$

N = concentration from standard curve (APHA 1998).

3.8.3 Determination of pH

pH of the effluent samples was determined using pH meter (Model 3015, Jenway, U.K). A 20ml of the sample was placed in a beaker. A buffer solution of pH 7.0 was used

to standardize the pH meter. The electrode of the pH meter was inserted into the sample and the pH readings were taken.

3.8.4 Determination of temperature

This was determined at the point of sample collection. This was done by dipping the bulb of mercury-in-glass thermometer into the sample and the reading recorded (APHA 1998).

3.8.5 Determination of dissolved oxygen (DO)

Dissolved oxygen was determined by measuring 50ml of the effluent sample plus 1ml of $MnSO_4$ solution into bottles containing 1ml of alkali-iodine solution and shaken thoroughly. When the precipitate had settled, most of the clear liquid were decanted and 2ml concentrated H_3PO_4 was added together with some water to dissolve the precipitate. The released iodine was titrated against 0.025M sodium thiosulphate solution. The dissolved oxygen was reported in mg/L and compared with the value of air saturated water at the same temperature of the sample collected. The dissolved oxygen was calculated as follows: for 200ml used, 1ml of thiosulphate is equal to 1mg/L DO (APHA, 1998).

3.8.6 Biochemical oxygen demand (BOD)

The effluent sample was seeded with 2ml distilled water. Dissolved oxygen in the sample was determined and recoded as D_1 .Then a screw – capped bottle was filledd to the brim with the remainder of the diluted sample, sealed and incubated in the dark for five days at 20^{o}C. Lastly, dissolved oxygen determination was carried out on this incubated sample allowing for dilution of the sample and recorded as D_2. Biochemical oxygen

demand is the difference between the two determined dissolved oxygen levels (APHA, 1998).

3.9 **Statistical analysis**

Descriptive statistical analysis were used to establish relationships and variations among the data obtained using SPSS (Version 14) statistical package.

CHAPTER FOUR

4.0 RESULTS

4.1 Physicochemical properties of Wells Water Samples

The physicochemical properties (pH, electrical conductivity, temperature, dissolve oxygen, nitrate and biochemical oxygen demand) of water samples from wells are presented in Table 4.1. The pH of the water samples from wells ranged from 5.17±0.058mg/l in Rijiyar Shehu to 5.83±0.058mg/l in Arkilla (Table 4.1). The electrical conductivity also ranged from 5.70±1.0µs/cm in Rijiyar Shehu to 92.3±1.53mg/l in Runjin Sambo, while temperature of the water samples at the time of the analysis ranged from 22^{o}C in Dambuwa to 28^{o}C in Arkilla, Runjin Sambo and Rijiyar Shehu. The dissolved oxygen of all the other samples ranged between 6.13±0.058mg/l in Arkilla to 7.5±0.10mg/l in Rijiyar Shehu. The nitrite content of all the water samples ranged between 2.30±0.17mg/l in Rijiyar Shehu to 4.30±0.10mg/l in Arkilla, while the Biochemical oxygen demand of all the water samples ranged between 11.47±0.15mg/l in Arkilla and 15.57±0.00mg/l in Dambuwa with the water samples from Dambuwa having the lower biochemical oxygen demand of11.47±0.15mg/l, while Arkilla having the highest biochemical oxygen demand of 15.57±0.00mg/las shown in (Table 4.1).

4.2 Physicochemical properties water samples from boreholes in Sokoto metropolis

The physicochemical parameters of water samples from boreholes within Sokoto metropolis were analyzed. Table 4.2 indicated that the temperature of all the water samples at the time of the analysis was 26^{o}C, while the pH of all the water samples ranged between 5.90±0.10 in Dundaye to 6.73±0.006 in Gagi. The electrical conductivity of all the water samples range Between 43.0±1.00µs/cm in Gagi to 98.3±1.54µs/cm in Runjin

Table 4.1: Physicochemical properties of water samples from wells in Sokoto metropolis

	SAMPLE SITES				STANDARDS		
	Arkilla	Rijiyar Shehu	Runjin Sambo	Dambuwa	WHO (1996)	NAFDAC (2000)	NSDWQ (2007)
pH	5.83±0.058	5.17±0.058	5.43±0.058	5.7±0.10	7.0-8.5	6.5-8.6	6.5-8.5
EC(µs/cm)	7.60±1.0	5.80±1.0	92.3±1.53	22.0±1.0	1000	1000	1000
Temp. (°C)	28.0	28.0	28.0	22.0	35.0	29.0	25.0
DO (mg/l)	6.13±0.058	7.50±0.10	6.90 ±0.10	6.50±0.10	9.0	9.0	9.0
Nitrite (mg/l)	4.30±0.10	2.30±0.17	3.57±0.058	2.80±0.10	45.0	45.0	0.02
BOD (mg/l)	11.47±0.15	14.53±0.15	13.90±0.10	15.57±0.00	5.0	5.0	5.0

Key:

pH – power of hydrogen

EC- Electrical conductivity

DO – Dissolved Oxygen

BOD – Biochemical Oxygen Demand

WHO – World Health Organization

NAFDAC – National Agency For Food and Drug Administration and Control

NSDWQ – National Standard for Drinking Water Quality

Table 4.2: Physicochemical analysis of water samples from boreholes in Sokoto metropolis

	SAMPLE SITE				STANDARDS		
	Sama Road	Runjin Sambo	Gagi	Dundaye	WHO (1996)	NAFDAC (2000)	NSDWQ (2007)
pH	6.67±0.06	6.27 ±0.6	6.73±0.06	5.90±0.10	7.0-8.5	6.5-8.6	6.5-8.5
EC(µs/cm)	80.0±1.0	98.3 ± 1.54	43.0±1.00	78.0±1.0	1000	1000	1000
Temp. (°C)	26.0	26.0	26.0	26.0	35.0	29.0	25.0
DO (mg/l)	7.63±0.15	7.83 ±0.06	8.83 ± 0.06	7.60±0.10	9.0	9.0	9.0
Nitrite (mg/l)	1.90±0.10	2.43±0.06	1.47±0.06	1.43±0.06	45.0	45.0	0.02
BOD (mg/l)	14.87±0.15	14.80±0.17	11.57±0.12	13.13±0.05	5.0	5.0	5.0

Key:

pH – power of hydrogen

EC- Electrical conductivity

DO – Dissolved Oxygen

BOD – Biochemical Oxygen Demand

WHO – World Health Organization

NAFDAC – National Agency For Food and Drug Administration and Control

NSDWQ – National Standard for Drinking Water Quality

Sambo while the dissolved oxygen ranged between 7.60±0.10mg/l in Dundaye to 8.83±0.06mg/l in both Gagi and Runjin Sambo. The nitrite content of all the water samples ranged between 1.43±0.06mg/l in Dundaye to 2.43±0.06mg/l in Runjin Sambo. Biochemical oxygen demand ranged between 11.57±0.12mg/l in Gagi to 14.87±0.15mg/l in Sama Road (as shown Table 4.2).

The results of physicochemical analysis of water samples from taps obtained within Sokoto metropolis are shown in Table 4.3. The pH of the water samples ranged between 6.13±0.60 in Kuffa to 6.57±0.06 in Tsohuwar Kasuwa. The electrical conductivity of all the water samples ranged between 104.67 ± 1.53µs/cm in Tsohuwar Kasuwa to 142.67 ± 2.04µs/cm in Kuffa. Temperature of all the water samples at the time of the analysis was 29^{o}C, while dissolves oxygen ranged between 3.57±0.06mg/l in Kuffa to 5.13±0.15mg/l in Tsohuwar Kasuwa. Nitrite content of all the water samples ranged between 1.53±0.15mg/l in Kuffa to 2.40±0.10mg/l in Tsohuwar Kasuwa, while biochemical oxygen demand of all the water samples ranged between 6.37±0.115mg/l in Kuffa to 9.83±0.153mg/l in Kanwuri (Table 4.3).

4.3 **Bacteriological properties of water samples in Sokoto metropolis**

Table 4.4 shows the results of bacteriological examination of drinking water from wells in Sokoto Metropolis. Water samples from Dambuwa had the minimum heterotrophic count of 2.07 x 10^6 cfu/ml while the minimum count was in Rijiyar Shehu with 1.33 x 10^6 cfu/ml. The highest total coliform count was recorded in Dambuwa which had 3.14 x 10^6cfu/ml while the lowest count was in Rijiyar Shehu having 1.3x 10^6cfu/ml. Faecal coliform count of the entire water sample ranged between 2.0 x 10^6cfu/ml and 6.0cfu/ml.

Table 4.3: Physicochemical properties of water samples from taps in Sokoto Metropolis

	SAMPLE SITE				STANDARDS		
	Tsohuwar Kasuwa	Mabera	Kanwuri	Kuffa	WHO (1996)	NAFDAC (2000)	NSDWQ (2007)
pH	6.57±0.06	6.47 ±0.06	6.30±0.10	6.13±0.60	7.0-8.5	6.5-8.6	6.5-8.5
EC(µs/cm)	104.67±1.53	112.00±2.00	123.33±2.52	142.67±2.04	1000	1000	1000
Temp. (°C)	29.0	29.0	29.0	29.0	35.0	29.0	25.0
DO (mg/l)	5.13±0.15	4.13 ±0.12	4.73 ± 0.15	3.57±0.06	9.0	9.0	9.0
Nitrite (mg/l)	2.40±0.10	2.10±0.10	1.77±0.15	1.53±0.15	45.0	45.0	0.02
BOD (mg/l)	9.27±0.152	7.73±0.115	9.83±0.153	6.37±0.115	5.0	5.0	5.0

Key:

pH – power of hydrogen

EC- Electrical conductivity

DO – Dissolved Oxygen

BOD – Biochemical Oxygen Demand

WHO – World Health Organization

NAFDAC – National Agency For Food and Drug Administration and Control

NSDWQ – National Standard for Drinking Water Quality

The highest faecal count was in Dambuwa 6.0 x 10^6cfu/ml while the lowest occurred in Runjin Sambo with 2.0 x 10^6cfu/ml. Isolates included *Bacillus mycoides* and *Salmonella* species in Arkilla, *Shigella* and *Salmonella* species in Rijiyar Shehu, *Staphylococcus aureus*, *S. epidermis* and *Eschericia coli* in Runjin Sambo, and finally *Salmonella* species in Dambuwa (Table 4.4).

The bacteriological analysis of drinking water from boreholes in Sokoto metropolis\ shows that the total heterotrophic counts of all water samples ranged between 1.7 x 10^6cfu/mlin Sama Road to 1.7 x 10^6cfu/ml in Gagi. The higher total coliform count was recorded in Sama Road with 2.67 x 10^6cfu/ml and the lower was in Runjin Sambo with 1.33 x 10^6cfu/ml with no total coliform count recorded in Gagi and Dundaye. High faecal coliform count was recorded in "Sama" Road with 2.0 x 10^6cfu/ml and the lower was in Runjin Sambo with 1 x 10^6cfu/ml with no faecal coliform count recorded in Dundaye and Gagi. Isolates included *B. firmus, S. pyogenes, Citrobacter* species,*S. aureus*, *Bacillus cereus* and *Proteus* species (Table 4.5).

Similarly, the bacteriological properties of drinking water samples from taps within Sokoto metropolis are presented in Table 4.6. The highest total heterotrophic count was recorded in "Kuffa" with 3.57 x 10^5cfu/ml and the lowest recorded in Tsohuwar Kasuwa with 1.20 x 10^6cfu/ml. The highest total coliform count was recorded in Kuffa with 2.33 x 10^6cfu/ml and the lowest was recorded in Tsohuwar Kasuwa with 1.00 x 10^6cfu/ml; while the faecal coliform count ranged between 2.0cfu/ml in Tsohuwar Kasuwar to 1.0cfu/ml in Kuffa with no feacal coliform count recorded in Kanwuri and Mabera. The isolates included *Salmonella* species, *Hafnia* species, *Enterobacter* species, *Proteus* species, *Bacillus alvei, Bacillus subtilis* and *Shigella* species(Table 4.6).

Table 4.4: Bacteriological properties of water from wells in Sokoto metropolis

Sample site	Total heterotrophic count (cfu/ml)	Total coliform count (cfu/ml)	Faecal coliform count (cfu/ml)	Isolates
Arkilla	2.00×10^6	2.19 ± 1.0	3	*Bacillus mycoides, Salmonella*species
Rijiyar Shehu	1.33×10^6	1.3 ±0.1	3	*Shigella* species, *Salmonella*species
Runjin Sambo	7.30×10^5	2.13±0.2	2	*S. aureus, S. epidermis, E. coli*
Dambuwa	2.07×10^6	3.14±1.10	6	*S. aureus, Salmonella* species
WHO	0/100ml	0/100ml	0/100ml	
NSDWQ	10/100ml	10/100ml	0/100ml	

Key:

WHO – World Health Organization

NSDWQ – National Standard for Drinking Water Quality

Cfu – Colony Forming Unit

Table 4.5: Bacteriological properties of water samples from boreholes in Sokoto metropolis

Sample site	Total heterotrophic count (cfu/ml)	Total coliform count (cfu/ml)	Faecal coliform count (cfu/ml)	Isolates
Sama Road	1.67×10^6	2.67±0.00	2	*B. firmus, S.pyogenes, Citrobacter* species *and S. aureus*
Runjin Sambo	1.22×10^6	1.33±1.154	1	*Bacillus cereus*
Gagi	1.73×10^6	0.00	0	*Proteus* species*, B. cereus* and *S.aureus*
Dundaye	1.33×10^5	0.00	0	*Staphylococcus aureus,*
WHO	0/100ml	0/100ml	0/100ml	
NSDWQ	10/100ml	10/100ml	0/100ml	

Key:

WHO – World Health Organization

NSDWQ – National Standard for Drinking Water Quality

Cfu – Colony Forming Unit

Table 4.6: Bacteriological properties of water samples from taps in Sokoto Metropolis

Sample site	Total heterotrophic count cfu/ml	Total coliform count cfu/ml	Faecal coliform count cfu/ml	Isolates
Tsohuwar Kasuwar	1.20×10^6	1.00 ±0.2	2	*Salmonella* species
Mabera	3.00×10^5	1.14 ±0.0	0	*Hafnia* species, *Enterobacter* species, *Proteus* species
Kanwuri	1.37×10^6	1.67 ±1.2	0	*Salmonella* speciesand *Bacillus. alvei*
Kuffa	3.57×10^5	2.33 ±1.3	1	*B. subtilis* and *Shigella* species
WHO	0/100ml	0/100ml	0/100ml	
NSDWQ	10/100ml	10/100ml	0/100ml	

Key:

WHO – World Health Organization

NSDWQ – National Standard for Drinking Water Quality

Cfu – Colony Forming Unit

Result obtained shows that there were 12 non-active *E. coli,* 5 active and 10 other bacteria which are *Klebsiella, Salmonella,* Klebsilla*, Citrobacter, Marganella, Diversus, Proteus, Yersinia, Enterocolitica* and *Citrobacter* species. The remaining 13 isolates were not identified. The predominant species was *E. coli,* which occurred sixteen (16) times in this study. All the fourty (40) isolates shows negative response from Hydrogen Sulphide (H_2S) except isolate number thirty one (31) which was positive, for TDA and Citrate thirty six (36) isolates were negative and four (4) were positive, for Mannitol thirty eight (38) were positive and two (2) were negative, for Xylose thirty two (32) were positive and eight (8) were negative, for Urase thirty six (36) negative and four were positive and for Glucose thirty seven (37) were positive and three (3) were negative(Table 4.7).

Table 4.7: Molecular identification of *E. coli* and other *entero bacteriacaea* isolates from water (tap, borehole and well) using GNA - ID system in Sokoto metropolis

Isolate code	Lysine	Ornithine	H_2S	Glucose	Mannitol	Xylose	ONPG	Indole	Urease	Citrate	TDA	Octal Code	Final identification
I_1	+	+	-	+	-	+	+	+	-	+	-	7734	4720	*E. coli* inactive
I_2	+	+	-	+	-	+	+	+	-	+	-	2706	4360	Not identified
I_3	+	+	-	+	-	+	+	+	-	-	-	2411	4760	*Morganella morgaii*
I_4	+	-	-	+	-	+	+	+	-	+	-	2722	4760	*Citrobacter*
I_5	-	-	-	+	-	+	-	+	-	+	-	2515	4760	*Proteus mirabilis*
I_6	+	-	-	-	-	+	+	+	-	-	-	0704	4760	*Pantoeaagglo*
I_7	+	+	+	-	-	+	+	+	+	+	-	4760	6720	Not identified
I_8	+	-	-	+	-	+	+	+	-	+	-	2714	4360	*Yersinia*
I_9	+	-	-	+	-	+	+	+	+	+	-	2763	4744	*Citrobacter*
I_{10}	+	+	-	+	-	+	+	+	+	+	-	6763	4760	*E. coli* active
I_{11}	+		-		-	+		+		+		+	-		-	4720	*E. coli* inactive
I_{12}	+		-		-	+		+		-		+	-		-	6760	*E. coli* inactive
I_{13}	+		+		-	+		+		+		+	-		-	4760	Not identified
I_{14}	+		-		-	+		+		+		+	-		-	0720	*E. coli* inactive
I_{15}	-		-		-	+		+		-		+	-		-	2744	*E. coli* active
I_{16}	-		+		-	+		+		+		-	-		-	2620	*E. coli* inactive
I_{17}	-		+		-	+		+		-		+	-		-	6760	*E. coli* active
I_{18}	+		+		-	+		+		+		+	-		-	6720	*E. coli* active
I_{19}	-		-		-	+		+		+		+	-		-	0760	*E. coli* inactive
I_{20}	+		+		-	+		+		-		+	-		-	6720	Not identified
I_{21}	+		-		-	+		+		+		-	-		-	4740	*Klebsiella ozaerere*
I_{22}	+		-		-	+		+		+		+	-		-	4740	Not identified
I_{23}	+		-		-	+		+		+		+	-		-	4740	*Klebsiella ozaerere*
I_{24}	+		-		-	+		+		-		+	-		-	4720	*E. coli* inactive
I_{25}	+		+		-	+		+		-		-	-		-	6700	*Salmonella*
I_{26}	+		-		-	+		-		+		-	-		-	4720	*Klebsilla ozaerere*
I_{27}	+		-		-	+		+		-		+	-		-	4720	*E. coli* inactive
I_{28}	+		-		-	+		+		-		+	-		-	4720	*E. coli* inactive
I_{29}	+		-		-	+		+		+		-	-		-	4720	*E. coli* active
I_{30}	+		-		-	+		+		+		-	-		-	4720	*E. coli* inactive
I_{31}	+		-		-	+		+		-		+	-		-	4720	*E. coli* inactive
I_{32}	+		-		-	+		-		+		-	+		+	4360	Not identified
I_{33}	+		-		-	+		+		+		-	+		-	4760	*Morganella morgaii*
I_{34}	+		-		-	+		+		-		+	+		-	4760	*Citrobacter*
I_{35}	-		-		-	+		-		+		-	+		+	4760	*Proteus mirabilis*
I_{36}	+		-		-	+		+		+		-	-		-	4760	*Pantoeaagglo*
I_{37}	+		+		-	+		+		-		+	-		-	6720	Not identified
I_{38}	+		-		-	+		+		+		-	-		-	4360	*Yersinia*
I_{39}	+		-		-	+		+		+		+	-		+	4744	*Citrobacter*
I_{40}	+		-		-	+		+		+		-	+		-	4760	*E. coli* active

KEY:

-	=	No reaction
+	=	There is reaction
$I_1 – I_{40}$	=	Number of isolate
H_2S	=	Hydrogen sulphide
TDA	=	Triptophan Deaminase
OPNG	=	O – nitrophenyl – β – D – galactopyranoside.

CHAPTER FIVE

5.0 DISCUSSION

The result of physiochemical analysis of water show that the pH of all the water samples collected from wells does not comply with standard requirement (Table 4.1). Their average value (5.5) is less than the lower limits of the pH (6.5) recommended by WHO, NAFDAC and NSDWQ. Tap and bore hole water samples have an average level pH level of 6.4

The average value (31.9µs/cm) and (74.1µs/cm) of conductivity of all the water samples collected from wells and taps respectively were observed and was below the standard (1000µs/cm) recommended by NAFDAC (2000), WHO (1996) and NASDWQ (2007). This lower conductivity may be due to the presence of chlorides, phosphate and nitrite and this can cause corrosion in pipes. For the borehole water, the average conductivity of boreholes water sample was due to the presence of materials that ionize when washed into the water, ground water inflow can have the same effects depending on the bedrock they flow through. This high value is not suitable for certain species of fish or macro invertebrate. All the water samples collected from wells and boreholes have their average value of B.O.D (13.9 mg/ml for wells and 13.6 mg/ml for boreholes) to be higher than the recommended standard by NAFDAC, WHO and NSDWQ (5.0mg/l). The higher the level of BOD the higher the level of contamination of water body. These could lead to disease outbreak such as diarrhoea, cholera and typhoid. Also the aquatic life in the water body is threatened.

The heterotrophic plate count was high in well water particularly in Runjin Sambo 7.30×10^6cfu/ml it was so because at the time of sample collection, the location of the well

was noted to be close to a pit latrine. This could have attributed to high level of contaminant. Also, faecal coliform recorded was 6.0cfu/ml for the same sample. This implies that water is been contaminated by human or animal waste, these can cause short term effects, such as diarrhoea, cramps, nausea, headache or other symptoms. They may pose a special health risk for infants, young children and people with severely compromised immune system. Similarly, total coliform count was generally high, with in Dambuwa recording the highest count of 6.0x 10^6cfu/ml (well water), which was recorded as 3.14 x 10^6 cfu/ml it was the highest of all the water samples] as compared with the standard count of 10/100ml.The above result is not surprising as the neighbourhood where these wells are highly populated and the mouth of the wells is at the ground level. The presences of total coliform in water suggest a possible outbreak of such water-borne diseases as dysentery, cholera and typhoid fever if the water is consumed untreated.

Faecal streptococci presence in Sokoto water supplies can be attributed to both human and animal faecal contamination especially when one takes into consideration the way and manner animals are reared and grazed as well as the way our latrines are dug. This is in consonance with the findings of Geldreich (1976)where he reported that America's surface waters having faecal *Streptococci* ratio of <4, are predominantly from human source while the ratio of <0.7 mainly have been contaminated by the faeces of wild and domestic animals

The presence of *Shigella* could be attributed to the problems associated with treatment plant at the Sokoto water works as well as findings of Geldreich 1976where he had reported that tap water will only contain *Shigella* if it is untreated and drawn from a contaminated source. *Salmonella's* presence can be linked to the broken down pipes and

improper protection of reservoir and open wells (Johnson *et al*., 2003).This is as observed by Rahaman *et al*. (1986) who reported that presence of *Salmonella* in piped water supplies suggest a serious fault in the design and maintenance of the system. He also found that 9% of tap water samples in Auragabad (India) contained *Salmonella* due to leakages of polluted water into the distribution system.

Williams *et al.* (1970), Davis *et al.* (1977) and George *et al.* (2001) observed that contamination of stream by *Salmonella* may help to maintain infection among animals although stream pollution has undoubtedly contributed to some outbreaks. The frequent transmission of Salmonellosis has also been attributed to contaminated animal products(Johnson *et al.,* 2003). Several studies have indicated that *Enterobacter* is found in the intestinal tract of human and animals and in soils, sewage water and dairy. It is also associated with urinary tract infection, septicemia, and wound infection (Johnson *et al.,* 2003).

Similarly, it was observed (NPDWR, 2006) that ground water, which is very close to the surface is subject to greater chances of contamination from the surrounding areas than water which lies more deeply in the ground where the effect of filtration by the soil are greater. It was also observed that the distance from potential source of contamination such as latrines, cattle pens, refuse pits and hollows in the ground all may serve as potential source of pollution especially if the distance is not up to 30 meters or more (NPDWR, 2006). Other observed factors include, the sanitary protection of the lining or casing of the well, which should be complete and undamaged, the state of the slab or apron covering the water point, whether this is complete or is cracked and whether the water is allowed to run

off into adequate water disposal system and also the type of waste water disposal system, whether this is adequate and well away from the water point.

However, the types and numbers of microorganisms differ in both cases with borehole having more (e.g., in borehole there is the presence of *B. firmus, S. pyogenes, Citrobacter* species*, S. aureus, Bacillu cereus* and *Proteus*). It can also be seen that the coliform count of tap and well water is relatively high as compared to that of borehole. In summary therefore, the Sokoto water supplies can be said to be grossly contaminated resulting from different pollution sources.

In developed countries, the guidelines or standards for water supplies, have advocated zero coliform and zero faecal coliform countin a drinking water supply. However, in the developing countries where water supply is irregular, it has been stipulated that the coliform count should be less than 10 per 100mls. However, there are some suggested bacteriological criteria for drinking untreated water or unchlorinated water like that of wells and reservoir (LeChevallier, 1990). It has been suggested that a zero *E. coli* count is category A and it is regarded as excellent, while an E. *coli* count of 1-10 is placed in category B, which is regarded as acceptable but that regular sanitary checks on equipment is necessary. A count of 1-50 is in category C and is regarded as unacceptable (Morinigo *et al.,* 1992). It is also advisable to look for and correct structural faults and poor maintenance of pump and plant. Similarly, the equipment and source should be disinfected. Wherever the count is more than 50 it is placed in category D and the water is considered as grossly polluted and it is recommended that an alternative source is to be found and necessary repairs and disinfection of the taps and borehole must be made (Michiels *et al.,* 2000).

The contamination of the water bodies/source is from either a point source, which is single identifiable source pollution or a non-point source, which is the major threat to ground water pollution (Auer and Niehaus, 1993). Such contamination brings the threat of infection for people who use the water for drinking bathing or washing fruits and vegetables. In Nigeria some of the major sources of water are open and shallow wells, stream and even ponds (Essien and Olisah, 2010). Also animals/livestock are reared within compounds and households of owners and are allowed to roam freely in search for food; hence, they consequently serve as sources of faecal contamination of water sources (Auer and Niehaus, 1993). Several studies have reported that surface and ground water contamination by faecal pathogens generally occurs through surface run-off, leaching and direct faecal deposition into the water bodies via several livestock production activities like confined animal feed lot, free range system, abattoir wastes and land spreading of manure (Allsop and Stickler, 1985).

WHO (1996) found out that in nearly all epidemics of water-borne diseases, the bacteriological quality of the water was unsatisfactory and that there was an evidence of contamination or a failure of terminal disinfection. They also found out that during the passage of water from the treatment works to the consumer its bacteriological quality may deteriorate, and members of the coliform group may be present in inadequately treated supplies, or a result either of growth on unsuitable materials in contact with water (such as washers, packing materials, plastics and plasticizer).

5.1 Conclusion

The study attempted to determine the quality and portability of the water supplies; to verify the bacteriological quality of the various water supplies. The water used for public consumption is unsafe for drinking because none of the water samples analyzed met the national and international standards for drinking water. The presence of indicator bacteria in the waters calls for assessment of water treatment methods particularly for taps and borehole water. As for well water, the sitting of all the wells, their construction and condition was poor, thus there is need for construction of the wells for away from municipal sewage and drainage systems, provision of protective covering can also minimize the possibility of contamination.

5.2 Recommendations

The following recommendations are suggested:

1. Selection of Sokoto water source that meets expected aesthetic and organoleptic water quality parameters.
2. Health education to explain the importance of clean and adequate supply of water, and its relationship to possible disease outbreaks.
3. Full cooperation of the local community to protect water pollution and chemical contamination.
4. Surveying of drinking water supplies, including on-site inspections, and the frequent and regular bacteriological testing of water supplies.
5. If and when any coliform bacteria are noticed and found in water sources and supplies disinfection process should be instituted and the water bodies must be resampled from the same and related sites, to confirm original positive finding and

locate the possible source of contamination. Even when satisfactory results are obtained on re-test, the frequency of routine testing/examination should be reviewed monthly to ensure overall quality of the supply.

6. Animals that go out daily for grazing should be well controlled to avoid contamination of water supplies and sources, especially in lakes, ponds and rivers, which serve as alternative sources in cases of water scarcity.

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APPENDICES

APPENDIX A: BIOCHEMICAL CHARACTERISTICS OF SAMPLES COLLECTED FROM WELLS

Location Arkilla	Cultural Characteristics	Morphology	Cat	Lac	Glu	Suc	Man	HS	MR	VP	IND	Bacteria isolated
1.	Large and dry colonies	Gram positive rods with spore	+	-	A	A	A	-	-	+	+	*Bacillus*
2.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	-	*E. coli*
3.	Small rough edge and dry	Gram positive rods with spore	-	-	A	-	-	-	-	-	-	*Salmonella*
4.	Mucoid colonies	Gram negative rods without spore	+	AG	AG	A	AG	-	-	+	+	*Enterobacter*
5.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
6.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
7.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
8.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
9.	Mucoid colonies	Gram negative rods without spore	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*

10.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
11.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
12.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
13.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
14.	Mucoid colonies	Gram negative without spore	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
15.	Mucoid colonies	Gram negative without spore	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
16.	Mucoid colonies	Gram negative without spore	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
17.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
18.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
19.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
20.	Small and	Gram negative rods	-	AG	AG	-	+	-	+	-	+	*E. coli*

	smooth colonies	without spore										
21.	Mucoid colonies	Gram negative without spore	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
22.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	AG	-	+	-	+	*E. coli*

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	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	AG	-	+	-	+	*E. coli*
1												
2.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	AG	-	+	-	+	E. *coli*
3.	Large and dry colonies	Gram positive rods with spore	+	-	A	A	A	-	-	+	-	*Bacillus*
4.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	AG	-	+	-	+	E. *coli*
5.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	AG	-	+	-	+	E. *coli*
6.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	AG	-	+	-	+	E. *coli*
7.	Small rough edge	Gram positive cocci in	-	-	A	-	A	-	-	-	-	*Salmonella*

	and dry	chain										
8.	Mucoid colonies	Gram negative without spore	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
9.	Large and dry colonies	Gram positive rods with spore	+	-	A	A	A	-	-	+	-	*Basillus*
10.	Small and smooth colonies	Gram negative rods without spores	-	AG	-	+	-	+	-	+	-	*E. coli*
11.	Small and smooth colonies	Gram negative rods without spores	-	AG	-	+	-	+	-	+	-	*E. coli*
12.	Small and smooth colonies	Gram negative rods without spores	-	AG	AG	-	-	+		-	+	*E. coli*
13.	Small and smooth colonies	Gram negative rods without spores		AG	AG	A		-	+	-	+	*E. coli*
14.	Small and smooth colonies	Gram negative rods without spores		AG	AG	-	+	-	+	-	+	*E. coli*
15.	Small and smooth colonies	Gram negative without spore		AG	AG	-		-	-	+	-	*Enterobacter*
16.	Small and smooth colonies	Gram negative rods without spores		AG	AG	-		-	+	-	+	*E. coli*
17.	Small and smooth colonies	Gram negative rods without spores		AG	AG	-		-	+	-	+	*E. coli*

18.	Small and smooth colonies	Gram negative rods without spores		AG	AG	-		-	+	-	+	*E. coli*

Runjin Sambo

	Small and smooth colonies	Gram negative without spore	+	AG	A	AG	-	-	+	+	-	*Enterobacter*
1												
2.	Small and smooth colonies	Gram negative rods without spores	-	AG	Ag	-	+	-	+	-	+	*E. coli*
3.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4.	Greenish and flat colonies	Gram negative without spores	+	-	-	-	A	-	-	+	-	*Salmonella*
5.	Small and smooth colonies	Gram negative without spores	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
6.	Large and dry colonies	Gram negative without spores	+	-	A	A	A	-	-	+	+	*Bacillus*
7.												
8.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
9.	Small and smooth colonies	Gram negative without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*

10.	-	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
11.	Mucoid colonies	Gram negative without spore	+	AG	AG	A	AG	-	-	+	-	*Enterobecter*
12.	Dry and mucoid colonies	Gram negative without spore	+	AG	AG	A	A	-	-	+	+	*Klebsiella*
13.	Dry and mucoid colonies	Gram negative without spore	+	AG	AG	A	A	-	-	+	+	*Klebisella*
14.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
15.	-	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*

16.	Small rough edge and dry	Gram positive rods with spore	+	-	-	-	-	-	-	-	-	
17.	Large and dry colonies	Gram positive rods chain	-	-	A	-	-	-	-	-	-	*Salmonella*
18.	Dry and mucoid colonies	Gram negative without spore	+	AG	AG	A	A	-	-	+	+	*Klebsiella*
19.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
20.	-	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
21.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
22.	Large and dry colonies	Gram positive rods with spore						-	+	-	+	*E. coli*
23	Small rough edge and dry	Gram positive cocci in chain	-	-	A	-	-	-	-	-	-	*Salmonella*
24.	Mucoid colonies	Gram negative without spore	+	AG	AG	A	AG	-	-	+	-	*Enterobacte*
25.	Dry and mucoid colonies	Gram negative without spore	+	AG	AG	A	A	-	-	+	+	*Klebsiella*
26.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
1.	Large and dry colonies	Gram positive rods with spore	+	-	A	A	A	-	-	+	-	*Bacillus*

Dambuwa

2.	Mucoid colonies	Gram negative without spore	+	AG	AG	A	AG	-	-	+	-	*Enterobecter*
3.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
4.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
5.	-	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
6.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
7.	Mucoid colonies	Gram negative rods without spore	-	AG	AG	A	AG	-	-	+	-	*Enterobacter*
8.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
9.	Small and smooth colonies	Gram negative without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
10.	Small and smooth colonies	Gram negative without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
11.	Small and smooth colonies	Gram negative without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
12.	-	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
13	Mucoid colonies	Gram negative without spore	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
14	Mucoid colonies	Gram negative without	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*

APPENDIXB: BIOCHEMICAL CHARACTERISTICS OF BOREHOLE WATER SAMPLES

Location	Cultural Characteristics	Morphology	Cat	Lac	Glu	Suc	Man	HS	MR	VP	IND	Bacteria Isolated
Sama Road												
1.	Small and smooth colonies	gram negative rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
2.	Small and smooth colonies	gram negative rods without spores	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
3.	Small and smooth colonies	gram negative rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
4.	Small and smooth colonies	gram negative rods without spores	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
5.	Small and smooth colonies	gram negative rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
7.	Small and smooth colonies	gram negative rods without spores	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
8.	Small and smooth colonies	gram negative rods without spores	-	AG	AG -	+	-	+	-	+	E. coli	
9.	Large and dry colonies	gram negative rods with spores	+	-	A	A	A	-	-	+	+	*Bacillus*
10.	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	*NIL*
11.	Small and smooth colonies	gram negative rods without spore	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
12.	Small and smooth	gram negative rods	-	AG	AG	-	+	-	+	-	+	*E. coli*

	colonies	without spore										
15.	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NI	NIL	NIL	*NIL*
16.	Small and smooth colonies	Gram negative rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
17.	Small and smooth colonies	Gram negative rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
18.	Small and smooth colonies	Gram negative rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
Runjin Sambo												
1.	Small rough edge and dry	Gram positive cocci in chain	-	-	A	-	-	-	-	-	-	*Salmonella*
2.	Small and smooth colonies	Gram negative rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
3.	Small and smooth colonies	Gram negative rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
4.	Small and smooth colonies	Gram negative rods without spores	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
5.	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	*NIL*
6.	Small and smooth colonies	Gram negative rods without spores	-	AG	AG	-	+	-	+	+	+	*E. coli*
7.	Small and smooth colonies	Gram negative rods without spores	-	AG	AG	-	+	-	-	+	+	*E. coli*
8.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
9.	Small and smooth	Gram negative rods	-	AG	AG	-	+	-	+	-	+	*E. coli*

	colonies	without spore										
10.	Small rough edge and dry	Gram positive cocci in chain	-	-	A	-	-	-	-	-	-	*Salmonella*
11.	Mucoid colonies	Gram negative without spore	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
12.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
13.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
Gagi 1.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
2.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
3.	Small ad smooth colonies	Gram negative rods without spore	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
4.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
5.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
6.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
7.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	-	-	+	*E. coli*
8.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	-	-	+	*E. coli*
9.	Small and	Gram negative	-	AG	AG	-	+	-	+	-	+	*E. coli*

	smooth colonies	rods without spore										
10.	Mucoid colonies	Gram positive bacillus with spores	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
11.	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	*NIL*
12.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
13.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*

APPENDIX C: BIOCHEMICAL CHARACTERISTICS OF WATER SAMPLE FROM TAPS

Location	Cultural Characteristics	Morphology	Cat	Lac	Glu	Suc	Man	HS	MR	VP	IND	Bacteria Isolated
Tsohuwar Kasuwa												
1.	Large and dry colonies	Gram positive rods with spores	+	-	A	A	A	-	-	+	+	*Bacillus*
2.	Large and dry colonies	Gram positive rods with spores	+	-	A	A	A	-	-	+	+	*Bacillus*
3.	Small and smooth colonies	Gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
4.	Small and smooth colonies	Gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
5.	Small and smooth colonies	Gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
6.	Small and smooth colonies	Gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
7.	Small and smooth colonies	Gram negative rods without spores	+	AG	AG	-	+	-	+	-	+	*E. coli*
8.	Small and smooth colonies	Gram positive rods without spores	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*

9.	Large and smooth colonies	Gram positive rods without spores	+	-	A	A	A	-	-	+	+	*Bacillus*
10.	Small and colonies Colonies	Gram Positive rods without spores	+	AG	AG	A		AG	-	+	-	*Enterobacter*
11.	Small and smooth colonies	Gram positive rods without spores	AG	AG	-		+	-	+	-	+	*E. coli*
12.	Large and dry colonies	Gram positive rods without spores	+	-	A	A	A	-	-	+	+	*Bacillus*
13.	Small and smooth colonies	Gram positive rods without spores	AG	AG		-	+	-	+	-	+	*E. coli*
14.	Small and smooth colonies	Gram positive rods without spores	AG	AG		-	+	-	+	-	+	*E. coli*
15.	Large and dry colonies	Gram positive rods without spores	+	-	A	A		A	-	-	+	*Bacillus*
16.	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	*NIL*
17.	Small and smooth colonies	gram positive rods without spores	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
18.	Small and smooth colonies	Gram positive rods without spores	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
19.	Small and smooth colonies	Gram positive rods without spores	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
20	Small and smooth colonies	gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
21	Small rough edge and dry	gram positive rods without spores		-	-	A	-	-	-	-	-	*Salmonella*

Mabera 1.	Small and colonies	gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
2.	Small and colonies	gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
3.	Small and smooth colonies	Gram positive Rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
4.	Dry and mucoid colonies	gram positive rods without spores	+	AG	AG	A	A	-	-	+	-	*Klebsiella*
5.	Small and smooth colonies	gram positive rods without spores	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
6.	Dry and mucoid colonies	gram positive rods without spores	+	AG	AG	A	A	-	-	+	+	*Klebsiella*
7.	Small and irregular shape	gram positive rods without spores	+	AG	AG	A	AG	-	-	+	-	*Enterobcter*
8.	Small and smooth shape	gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
9.	Small and smooth shape	gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
10.	Small and smooth shape	gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
11.	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NI	NIL	
12.	Small and smooth colonies	gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	E.

13.	Small and smooth colonies	gram positive rods without spores	-		AG	AG	-	+	-	+	-	+	*E. coli*
14.	NIL	NIL		NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	*NIL*
15.	Small rough edge and dry	Gram positive cocci in chain	-		-	A	-	-	-	-	-	-	*Salmonella*
16.	Small and colonies	gram positive rods without spores		+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
17.	Small and smooth colonies	gram positive rods without spores	-		AG	AG	-	+	-	+	-	+	*E. coli*
18.	Small and smooth colonies	gram positive rods without spores	-		AG	AG	-	+	-	+	-	+	*E. coli*
19.	Dry and smooth colonies	gram positive rods without spores		+	AG	AG	A	A	-	-	+	+	*Klebsiella*
20.	Small and smooth colonies	gram positive rods without spores		-	AG	AG	-	+	-	+	-	+	*E. coli*
Kuffa 1.	Small and smooth colonies	gram positive rods without spores		+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
2.	Small and smooth colonies	gram positive rods without spores		+	AG	AG	A	AG	-	-	+	-	*Enterobacter*

3.	Small and smooth colonies	gram positive rods without spores	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
4.	Small and smooth colonies	gram positive rods without spores	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
5.	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	*NIL*
6.	Small and smooth colonies	gram positive rods without spores	-	AG	AG	-	+	-	-	-	+	*Ecoli*
7.	Greenish and flat colonies	gram positive rods without spores	+				A			+		*Pseuomonas aerimginse*
8.	Small and colonies	gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
9.	Small and colonies	gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
10.	Dry and irregular shape	gram positive rods without spores	-	A	AG	A	A	-	+	-	+	*Clostridium*
11.	Small and smooth colonies	gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
12.	Small and smooth colonies	gram positive rods without spores	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
Kanwuri 1.	Small and smooth colonies	gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*
2.	Small and smooth colonies	gram positive rods without spores	-	AG	AG	-	+	-	+	-	+	*E. coli*

3.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
4.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
5.	Small rough edge and dry	Gram positive cocci in chain	-	-	A	-	-	-	-	-	-	*Salmonella*
6.	Large and dry colonies	Gram negative rods without spore	+	-	A	A	A	-	-	+	-	*Bacillus*
7.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	-	-	+	*E. coli*
8.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	-	-	+	*E. coli*
9.	Mucoid colonies	Gram negative rods without spore	+	AG	AG	A	AG	-	-	+	-	*Enterobacter*
10.	Small rough edge and dry	Gram positive cocci in chain	-	-	A	-	-	-	-	-	-	*Salmonella*
11.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
12.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*
13.	Small and smooth colonies	Gram negative rods without spore	-	AG	AG	-	+	-	+	-	+	*E. coli*