DETECTION OF ENTEROPATHOGENIC BACTERIA IN PUBLIC DRINKING WATER SOURCES IN SOME PARTS OF GUSAU MUNICIPAL

BY

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DECLARATION

I hereby declare that the work presented in this thesis is the original and was carried out by me at the Department of Microbiology and Public Health Engineering Laboratory Department of Water Resources and Environmental Engineering, Ahmadu Bello University, Zaria, that no part of this thesis has previously been submitted for any degree or qualification, and that all references used herein are duly acknowledged.

……

Garba Ibrahim

CERTIFICATION

This thesis titled Detection of enteropathogenic bacteria in public drinking water sources in some parts of Gusau municipal, Zamfara state Nigeria meets the regulations governing the award of degree of Master of Science, (Microbiology) Ahmadu Bello University, Zaria, and is approved for its scientific contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my father Alh. Garba Bazamfare Nasarawa Zurmi and my mother late Bilkis Garba whose loving memory remains indelible in my mind may her gentle soul rest in perfect peace. Ameen**.**

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ABSTRACT

This study was aimed at determining the level of contamination, seasonal distribution as well as the persistence of target organisms in well, tap and packaged water. A total of 180 well water samples, 60 tap water samples and 60 packaged water samples respectively were obtained from some parts of Gusau Municipal, the samples were analysed using standard procedures. Total coliform count, Heterotrophic plate count, some physico-chemical properties and presence of *Escherichia coli*, *Salmonella* species *Vibro* species and *Shigella* species were determined for two seasons. All the water samples had coliform and heterotrophic counts higher than the recommended standard for drinking water. The number in both cases was higher in the wet season than the dry season. Biochemical and serological identification showed that out of the 63 confirmed *Escherichia coli* isolates, 41 (45.4%) were from well water and of the 41 *Salmonella* species recovered, 31 (36.6%) were from well water, while 20 (22.2%) *Shigella* species were also recovered from well water. *Pseudomonas* species isolated from well water accounted for 44 (48.8%) of the isolates while 22 (24.4%) *Klebsiella* species, 13 (14.3%) *Proteus* species and 38 (42.2%) other unidentified isolates respectively. The tap water had 14 (23.3%) *Escherichia coli*, 9 (14.9%) *Salmonella* species, 12(20%) *Shigella* species and 32 (36.6%) *Klebsiella* species while in contrast packaged water had 8 (13.3%) *Escherichia coli*, 2 (1.6%) *Salmonella* species, 5 (8.3%) *Shigella* species and 14 (23.3%) *Klebsiella* species. No *Vibrio* species were isolated in all cases. All the well waters failed to meet the minimum requirements for nitrate and 5 day Biochemical Oxygen Demand. Result of susceptibility test showed that *Escherichia coli* isolates were susceptible to Gentamycin, Pefloxacin, Chloramphenicol, Tarivid, Augmentin, Streptomycin and Sprofloxacin , but resistance to Amoxacilin was observed: *Salmonella* isolates showed susceptibility to Gentamycin, Ciprofloxacin, Tarivid, Sparfloxacin, Amoxacilin, Streptomycin, Chloramphenicol and Augementin, resistance to Septrin and Perfloxacin was however observed. The isolates of

Shigella were susceptible to Perfloxacin, Streptomycin, Sprofloxacin, Tarivid, Amoxacin and Augmentin. Adequate treatments of all waters, public health education and proper antibiotics use are highly recommended.

CHAPTER ONE

INTRODUCTION

Water is the life wire of the body because it sustains life. It is a critical part of human diet, however many water sources in developing countries are unhealthy because they contain harmful physical, chemical and biological agents. The World Heath Organization (WHO) estimated that up to 80% of ill-health in developing countries is water and sanitation related (Cheesbrough, 2000). High incidence of childhood diarrhoea, helminthiasis trachoma and the overall high mortality rates are associated with poor environmental sanitation (Admassu, *et al.*,2004)

Globally disease that are waterborne and sanitation related infections are some of the major contributions to disease burden and mortality (Hunter, 2003). On a global scale, 25,000 people die each day as a result of poor water quality. Water related diseases such as cholera, diarrhoeal diseases, dracunculosis (Guinea worm), filariasis, malaria, onchocerciasis (River blindness), schistosomiasis (Bilharziasis), trypanosomiasis (Sleeping sickness), and typhoid still represent the single largest cause of human morbidity and mortality(Redmont, 2004). Therefore, inadequate water and sanitation services adversely affect the health and socioeconomic development of a community (Nanan, *et al.*,2000).The proportion of people with access to adequate water and sanitation has not increased due to population growth, insufficient continued investment, insufficient systems, and lack of training and spare parts to maintain systems in working order

(Cheesbrough, 2000). This account for the increased incidence of fatal and life threatening gastrointestinal illness majority of which occur in the rural areas of developing countries.

The World Health Organization estimated that up to 80% of all sickness and diseases in the world is caused by inadequate sanitation, polluted water or unavailability of water (Admassu, *et al.*, 2004). The main burden of disease in this category falls on the poorest societies and on children under 5years (Hunter, 2003). In fact, children bear about 68% of the global burden (Bartram, 2003). Surface water is frequently contaminated with faecal bacteria from non point sources such as domestic and wild animal defaecation, malfunctioning septic trenches, storm water, drainage and urban runoff, and from point sources such as industrial sources contamination (Ahmed, *et al.*, 2005). Defaecation from domestic animals may further contribute pathogens such as *Escherichia coli* 0157: H7 and *Cryptosporidium* species.

Most mortality and morbidity associated with water related diseases in developing countries is directly or indirectly due to infectious agents (Cheesbrough, 2000). These biological agents include enteropathogens which are waterborne agents that inhabit the gastrointestinal tract of humans and other warm blooded animals, and include the enteric bacteria and the *Vibrio* species. It has been reported that various human enteric pathogens such as *Salmonella* species, *Shigella* species and hepatitis A, have been found in surface waters as a result of human faecal contaminations (Ahmed, *et al.*, 2005). The distributions of many of

these pathogens vary substantially from one country to another (Hunter, 2003). These organism cause disease such as typhoid fever that represent the single largest cause of human morbidity and mortality (Redmont, 2004). And cholera which is prevalent in 80 countries and causes an estimated 120,000 deaths each year (World Health Organization, 2001).

Waterborne outbreaks of enteric disease have resulted either when public drinking water supplies were not adequately treated after contamination with surface water or when surface waters contaminated with enteric pathogens have been used for recreational purpose (Johnson, *et al.*, 2003). The grazing of cattle and land application of animal waste may lead to the occurrence of enteric pathogens in nearby surface and ground waters (Yang, *et al.*, 2004). Hubbard *et al.* (2004) reported that grazing animals and pasture production can affect water quality both positively and negatively, this potential can be a serious threat to public health. Thus the fate and transport of pathogenic micro-organism that are shed from cattle operations must be understood to evaluate and possibly mitigate the contamination of water supplies (Yang, *et al.,* 2004).

Water quality in many lakes and rivers has been impaired by the presence of high levels of faecal coliform bacteria, which is indicative of contamination by faeces Hubbard *et al.* (2004). In addition to nutrient loading of subsurface and surface waters, on-site disposal systems, such as septic tanks and injection wells are known to be a source of microbial contamination of ground water (Paul, *et al.*, 1995). Faecal coliforms have been widely used as indicator of the microbiological

quality of surface and ground waters (Ahmed, *et al.,* 2005). Byamukama *et al.* (2005) reported that indicator bacteria such as faecal coliform and *Escherichia coli* have been used for further assessment of faecal pollution and possible water quality deterioration in various fresh water sources.

Faecal coliform counts and *Escherichia coli* detection in particular remains the major and most reliable tool in assessment of the health risks pose by pathogens in water (Umoh, *et al.,* 2006). The presence of enteropathogens in public water source remains an obstacle and a threat to public health, therefore identification of major sources of faecal bacteria whether human or animal is necessary for improved management of surface water quality and the minimization of public health risk associated with such contamination (Ahmed, *et al.*,2005).

Statement of the problem

On a global scale, pathogenic contamination of drinking water sources poses the most significant health risk to humans. There have also been countless numbers of disease outbreaks and poisoning through out history resulting from exposure to untreated or poorly treated drinking water. However, significant risks to human health may also result from exposure to non pathogenic toxic substances such as lead and other heavy metals contaminants that are often globally ubiquitous in waters from which drinking water is derived. (Ritter, *et al.*, 2002).

Justification

Little or no research at all has been conducted on the quantification of these organisms in drinking water in the study area. Besides, in May (2005), there was outbreak of gastroenteritis in Gusau which resulted to the death of quite a number of people. The outbreak was suspected to be caused by enteropathogenic bacteria, thus the need to conduct research in order to examine whether these organisms are still present in alarming number in the drinking water sources.

Objectives

The study is designed to achieve the following objectives:

1. To detect enteropathogenic bacteria in public drinking water sources, compare the occurrence of these organisms from different sources and also to determine some physicochemical parameters.
2 Determine the level of contamination with respect to the water sources.
3. To characterize the isolates biochemically and serologically for easy identification.
4. To determine the antimicrobial susceptibility of the target isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 Enteric Organism

Enteric bacteria are aerobes and facultative anaerobes, non sporing and motile or non motile, oxidase negative; reduce nitrate to nitrite and ferment glucose with acid production (Cheesbrough, 2000). They comprised the major aetiologic agents of sporadic and epidemic diarrhoea both in children and adults (Kahali, *et al.*,2004).They have traditionally been used as indicators of faecal contamination in source drinking and recreational waters, however research has demonstrated that these bacteria may not be appropriate indicators of pathogenic viruses and protozoa. Thus the need for a reliable index or indicator organism for these pathogens has been widely recognised (Cole, *et al.,* 2003). Waterborne outbreaks of enteric diseases are a major public health concern, yet monitoring and identifying the disease causing pathogens from water samples remain difficult (Smith, *et al.,* 2003). Therefore detection of aetiologic agents of diarrhoea is important for the therapeutic aspects and for implementing appropriate control strategy (Kahali, *et al.,* 2004).

2.1.1 Shigella organism

Shigellae are gram negative, non lactose fermenting, non motile bacilli of the familly Enterobacteriaceae, classified into 4 species namely, *Shigella dysenteriae, Shigella flexneri, Shigella boydii and Shigella sonnei* (Clemens, *et al.,* 1992). They are the causative agents of Bacillary dysentery, a severe

inflammation of the colon which is endemic throughout the world with more than 95% of the 150 million annual cases and almost all the 600,000 death occurring in the developing world (Sansonetti, *et al.,* 1999). Diarrhoeal diseases kill more than 5 million children each year in the developing world, and about 10% of these deaths are due to bacillary dysentery caused by the 4 serotypes of *Shigella* species (Maurelli, 1992). Bacillary dysentery is endemic through-out the developing world but also occurs in sporadic cases and outbreaks of varying magnitude world wide and majority of these infections are caused by the members of the *Shigella* genus (Szakal, *et al.,* 2001). Shigellosis is primarily a paediatric disease, with more than half of all infections occurring in children between 1 and 10 years of age (Murray, *et al.,* 1998).

The role of food and water as a vehicle for the transmission of this organism is well documented and represent a significant public health threat (Szakal, *et al.,* 2001). Endemic disease in adult is common in male homosexuals and household contact of infected children. *Shigella* is a highly infectious microorganism with as few as 10 bacterial cells capable of causing diseases in adult volunteers. The low infectious dose makes the spread of shigellosis rapid in communities where sanitary standards and levels of personal hygiene are low. After an individual has been in contact with infected faeces or has ingested *Shigella* contaminated food or beverages, some of the bacteria survive gastric acidity, transit through the small intestine and reach the colon where they invade

the mucosa causing an acute destructive recto colitis with fever, intestinal cramps and emissions of mucopurulent and bloody stools (Sansonetti, 1999).

The essential pathogenic process is invasion of the mucosal epithelial cells by induced phagocytosis, escaped from the phagocytic vacuoles, multiplication and spread within the epithelial cell cytoplasm and passage to the adjacent cells (Murray, *et al.*,1998). Extremely high numbers of polymorphonuclear leucocytes (P.M.N) in stools is characteristics of this disease that strongly resemble the acute form of ulcerative colitis (Sansonetti, 1999). In many regions of the developing world, the HIV/AIDS epidemic intersects with spread of shigellosis leading to a more severe clinical manifestation of *Shigella* infection or recurrent intestinal disease and bacteraemia (Clemens, *et al.*, 1999).

2.1.2 *Escherichia coli*

This is a very common member of the intestinal microflora of both humans and warm blooded animals. It is a commensal or opportunistic pathogen implicated in acute Urinary tract infections [U.T.I] and gastrointestinal tract infections (Swie- cicka, *et al.,* 2003). Enteropathogenic *Escherichia coli* (EPEC) and a subset of the shiga toxin producing *Escherichia coli* (STEC) Known as Enterohaemorrhagic *Escherichia coli* (EHEC) represent two of the five pathotypes of diarrhoeagenic *Escherichia coli* recognized at present (Hornitzky, *et al.*,2005). Enteropathogenic and Enterohaemorrhagic *Escherichia coli* (EPEC and EHEC

respectively) are important causes of severe diarrhoeal disease in the developing and developed worlds (Higgins, *et al.*, 2005).

The genetic diversity of diarrhoeagenic *Escherichia coli* isolated from both healthy and clinically affected humans (diarrhoea, bloody diarrhoea (BD) and haemolytic uremic syndrome (H.U.S) continues to increase as more epidemiological studies of humans rely on the use of molecular tools such as Polymerase chain reaction (P.C.R) to detect virulence genes (Hornitzky, *et al.*,2005). The ability to cause attachment and effacing lesions among the intestinal epithelium is a hallmark of some groups of diarrhoeagenic *Escherichia coli* (Higgins, *et al.,* 2005). Pathogenicity is largely determined by gene-encoding virulence factors (vfs) such as adhesins, toxins, and polysaccharide surface coatings (Kuntaman, *et al.,* 2005).

The pathobiology of EPEC and EHEC is the subject of intensive research; however most of the existing information on the epidemiology of these organisms has been derived from clinical reports, and therefore little is known regarding the prevalence and survival of these categories of *Escherichia coli* in the environment (Higgins, *et al.,* 2005).

2.1.3 *Vibrio cholerae*

Cholera has historically occurred in periodic epidemics, with the most severe epidemics limited to a few countries namely, Bangladesh, India and Countries in Africa and South America (Huq, *et al.*, 2005). It continues to threaten

large portions of the world's population mainly in underdeveloped and poor countries where it is spread via the faecal-oral route (Osario and Camilli, 2003). Seven major pandemics of cholera have occurred since 1817 resulting in thousands of deaths and major socio economic changes (Murray, *et al.,* 1998). Interestingly therefore, it is one of the few bacterial diseases known for its pandemicity (Huq, *et al.,* 2005).

Vibrio cholerae is indigenous to the aquatic environment. However, contaminated water supplies in some parts of the world have caused selected pathogenic clones of the species to become dominant in epidemics. Thus cholera continues to be an important cause of morbidity and mortality in many areas in Asia, Africa and Latin America (Jiang, *et al.,* 2000). It causes the acute diarrhoeal disease called cholera that has been known at least since, the times of Hippocrates (Osorio and Camilli, 2003). Cholera caused by *Vibrio cholerae* is a major disease that has caused great fear since the first recorded pandemics because of the frequency of death and the rapidity with which it occurs (Salim, *et al.,* 2005).

In the recent history of cholera, most major epidemics originated in coastal regions including both the south American epidemic that began in the coastal region of Peru spreading to 21 countries including Mexico, and the new 0139 outbreak in India and Bangladesh (Osario and Camilli, 2003). Approximately 200 O-antigens have been distinguished serologically but only 01 and 0139 have been implicated in epidemics and pandemic cholera isolates (Salim, *et al.,* 2005). The incidence of cholera is estimated to exceed 5 million cases each year thus

cholera is an ancient disease in the midst of modern resurgence (Jiang, *et al.*, 2000). Infact, the benefit derived from cholera epidemic was the recognition of the role of contaminated water in the spread of the disease and the need to improve community sanitation systems so that the disease could be controlled.

Horizontal gene transfer which has been demonstrated in *Vibrio cholerae* has been proposed as a mechanism for the emergence of new pathogenic strains and recent studies have confirmed such mechanism (Colwell, *et al.*, 2003). Many workers have recently dealt with the mechanism by which *Vibrio cholerae* adheres to different surfaces inside and outside human host, in general, the adhesive process appears to be multifactorial with contribution from a variety of different cell surfaces and secreted component (Pruzzo, *et al.*, 2003).

Environmental isolates of *Vibrio* have been shown to harbour genotypes associated with virulence, thus supporting the hypothesis that the natural population of *Vibrio cholerae*, including *Vibrio cholerae* non 01, non 0139, isolates can serve as a precursor for new pathogenic or epidemic strains, thus *Vibrio cholerae* poses a public health risk ranging from limited outbreaks to epidemics when it is ingested via untreated water (Colwell, *et al.*, 2003).

Although the autochthonous existence of *Vibrio cholerae* in the aquatic environment of cholera endemic regions has been fully established the triggering factor or factors initiating cholera epidemics in one or several different geographical locations at approximately the same time remains elusive. Hence

cholera is well recognized as a “waterborne” disease, with close linkage of the population dynamics of the causative agent with selected physical, chemical and biological parameters of the natural waters (Huq, *et al.*, 2005). The heightened awareness on the importance of the species under the genus *Vibrio* has led to a renewed interest in the study of their pathogenicity and taxonomy (Monsalud, *et al.*, 2003). The pathogenicity of *Vibrio* species has generated considerable impetus for more precise methods of enumeration and for clearer understanding of factors that affect public health (Koh, *et al.*, 1994).

The ecology of *Vibrio cholerae* is intimately coupled with its attachment to particles, and these interactions are considered important in its transmission from aqueous environment to humans (Long, *et al.*, 2005). Despite the ubiquity of *Vibrio* species the ability to determine the presence of these species in the environment with a degree of efficiency has been hindered by the culture techniques relied upon for detection (Colwell, *et al.*, 2003).

2.1.4 Salmonella organism

Salmonellae are considered one of the most important causal agents of food and water borne illness in developing countries. It causes enteric fever leading to substantial illness and death in many parts of the world (Ochiai, *et al.*, 2005). These organisms are gram negative, non spore-forming aerobic rods, frequently motile and grow readily on ordinary media (Tabukum, *et al.*, 1996). The genus comprises a number of serotypes most of which are considered potential

human pathogens but only a reduced number of serotypes have been associated with human infections (Martinez-urtaza, *et al.*, 2004). They are found in virtually all animals including poultry livestock, domestic animals, birds and humans and most infections result from the ingestion of contaminated food product and in children from direct faecal-oral spread (Murray, *et al.*,1998). The best known member of the *Salmonella* genus is *Salmonella typhi*, the organism responsible for typhoid fever, but many of its near relatives including *Salmonella typhimurium*, *Salmonella dublin*, and *Salmonella enteritidis* are also gaining notoriety as formidable pathogens (Pell, 1997).

Transmission of Salmonellae to humans typically occurs by ingesting meat, dairy products, and other foods contaminated by animal faeces or by cross contamination from food contaminated with Salmonellae (Wright, *et al.*, 2005). In addition to links to food borne disease, outbreaks from *Salmonella* species contamination of water have been documented (Pell, 1997). Although the pathogenicity of *Salmonella* is not fully understood, colonization and invasion of the pathogen on/into intestinal epithelium cells play important role in causing animal and human salmonellosis, thus several genes, including invasions (inv,) are involved in the invasiveness of *Salmonella* (Chen, *et al.*,2001). The economic cost of Salmonellae has been estimated at close to $1 billion/yr (₦32 billion) /yr (Pell, 1997).

2.2 Water quality

Drinking water is an important resource all around the globe, nevertheless little research has focused on identifying the bacteria in water distribution system or how treatment techniques alter the composition (Martiny, *et al.,* 2005). Access to adequate water supply is not only a fundamental need and human right; it also has considerable health and economic benefits to households and individuals (http://www.childinfo.org/areas/water). Water has been called "the next oil" the commodity over which most future wars will be fought. According to 2000 United Nations World Water Development Report, water related diseases killed more than 3 million people and affected more than 2 billion, particularly children under 5 years. Since 1990, the number of people without access to safe water sources has remained constant at approximately 1.1 billion, of whom approximately 2.2 million die of waterborne diseases each year. In developing countries, population growth and migrations strain existing water and sanitary infrastructure and complicate planning and construction of new infrastructure (Mintz, *et al.*, 2001)

Water is essential to life. We drink it, raise crops and livestock with it, clean our bodies and environment with it, and play in it. When it is contaminated with human or animal wastes, however, water carries illness and death. Approximately 1.1 billion persons, or one sixth of the world's population, lack access to safe water sources, and many more lack access to safe water (Vitzhum and Atkins, 2006). Furthermore within the next 20 years, the amount of water available per capita worldwide is expected to drop by a third (Fields, 2003). It is a vital resource necessary for all aspect of human and ecosystem survival and health, but in recent years, new alarms have been sounded about

the growing water scarcity and contamination and the likely inability to meet the water requirement of rapidly growing population (Gleick, 1995. http://www.aaas.org /international/ela/ fisheries/gleick).

Important diseases that can be transmitted by the waterborne route include cholera, typhoid fever, amoebic and bacillary dysentery, and other diarrhoeal diseases; these diseases, which cause an estimated 2,187,000 deaths worldwide each year, account for most water-associated morbidity and mortality. Other contributors include; the water-washed diseases (such as scabies, trachoma), caused by poor personal hygiene and preventable through improved access to safe water; the water-based diseases, caused by parasites found in intermediate organisms living in water (such as dracunculiasis, schistosomiasis); and the water-related diseases, caused by insect vectors that breed in water (e.g., dengue fever and malaria). Pollution of surface and ground waters from animal waste is of growing environmental concern (Hubbard, *et al.,* 2004). It was established that Humans contaminate water sources through poorly processed sewage effluents malfunctioning septic tanks and seepage from sanitary landfill (Olson, 2000). Human sources of faecal pollution constitute the most serious health risk because of the high likelihood of the presence of human pathogens (Bower, *et al.,* 2005).

During the last decade new problems that are driven by environmental concerns and socio-economic aspects have emerged, expanding agriculture and manufacturing business not only increasing the use of water but also contributing to pollution of valuable sources of surface and ground water

(http://www.Childinfo. org/areas/water). Untreated or inadequately treated municipal sewage is a major source of ground and surface water pollution in the developing countries, the organic materials that are discharged with municipal waste into water courses use substantial oxygen for biological degradation thereby upsetting the ecological balance of rivers and lakes. It also carries microbial pathogens that are the cause of the spread of intestinal diseases (http://www. edugreen.teri.res.in/explore/water/health.html). Domestic and wild animals contaminate water by defecation in unprotected surface water, through runoff and as a result of seepage of water through soil that contains an excessive amount of animal faeces (Olson, 2000).

Ground water is a major water resource which is vulnerable to depletion and pollution by point and non-point sources such as intensive agriculture and irrigation, especially when the irrigation water is of low quality (Bouwer, *et al.*, 1999). High loading rates of sediments, nitrogen, phosphorus and pathogens to soils and water can occur from animal operations such as grazing (Hubbard, *et al.*, 2004).

Regulatory agencies and environmental microbiologists have suggested that the heterotrophic bacterial counts in finished drinking water should not exceed 500 cfu/ml. Mainly to reduce interference with the detection of coliform bacteria (Payment, *et al.,* 1994). According to most regulations, a 100ml sample of drinking water should not contain any coliform bacteria (Hanninen, *et al.,* 2003). The United State Environmental Protection Agency sets limits on the allowable

concentration of coliform bacteria in drinking water (0 cfu in 100ml) and primary contact water (200 cfu in 100ml). It is unclear how often well water or surface water on farms is checked for contamination (Gagliardi *and* Karns, 2000). Since in rural areas such sources are used for domestic use, including consumption. Recent studies have also demonstrated that underground water system is increasingly vulnerable to both micro and heavy metal contamination (Colwell, *et al.,* 2001). It was established that concentrations of Nitrogen in excess of 10mg/l in the Nitrate form render ground water unsuitable for humans; high nitrogen concentrations entering streams or lakes may also contribute to eutrophication (Hubbard, *et al.*, 2004).

The chemical disinfection of drinking water to control microbial contamination has been one of the most successful public health measures ever undertaken. However in the 1970s, the chemical disinfection of drinking water was discovered to produce disinfection by products (DBPs) when chlorine is used as disinfecting agent, the volatile trihalomethanes (THMs) and non volatile haloacetic acids (HAAs) are the most abundant groups of DBPs formed; trichloroacetic acids, (TCAA) one of the two major HAAs in water, is a mouse liver carcinogen (USEPA, 1992). The U.S Environmental Protection Agency (USEPA) has classified TCAA as a possible human carcinogen (USEPA, 2001). And introduced regulations for the maximum contaminant levels of TCAA, among other DBPs in drinking water (Calafat, *et al.,*2003). Route of exposure to volatile compounds are ingestion, dermal absorption and inhalation, and pathways can include any activity

involving chlorinated water such as ingestion of tap water, swimming, bathing and showering (Whitaker, *et al.,* 2003).

Microbial water quality assessment and the rapid detection of pathogenic bacteria remains a great challenge in ecotoxicology, public health maintenance and more recently bio-terrorism prevention (Maynard, *et al.,* 2005). Therefore identifying sources of faecal pollution is important for assessing the public health risk and devising water management strategies (Bower, *et al.s,* 2005).

2.3 Waterborne pathogens

World wide, the most common bacterial diseases transmitted through water are caused by *Shigella, Salmonella*, Enterotoxigenic *Escherichia coli* (ETEC). *Campylobacter jejuni* and *Vibrio cholerae* (Singh and Mcfeters 1992). Many different viral, bacterial and parasitic diseases have been associated with water borne transmission (WHO 1996[a], Tibbets, 2000, Gale, 2001: Hunter, 2003). Some of these diseases have been spread through drinking water and others by recreational water polluted with excreta of humans and other animals that are either sick or healthy carriers. The infections contribute significantly to the global burden (Pruss, et *al*, 2002); and according to Payment and Riley (2002), many of these infections remain untreated and cause premature death in children and adults in the developing countries (WHO, 1996[b], Yassin, 2000).

Cholera, the classical waterborne disease has caused severe pandemics and still represents a serious problem, causing repeated epidemics especially in

developing countries due to poor sanitation, water shortage, natural disasters (like floods) and wars (Stewart-Tull, 2001; Payment and Riley, 2002; Hunter, 2003). According to Stewart –Tull (2001), 80,000 Rwandan refugees died in Zaire in 1994 from cholera. The aetiobiologic agent of cholera is *Vibrio cholerae* serogroup 01, which contains two serotypes based on variations in the O antigen factors, namely, Ogawa (factors A and B) and Inaba, factors A and C (WHO, 1996[a]; Stewart-Tull, 2001). The first report of classical cholera in Nigeria was in the 1970 and 1971 epidemics affecting Lagos, the Mid-West State, Kano and Zaria (Schram, 1972). That was during the seventh pandemic 1961 – 1975 (Stewart-Tull, 2001). Nowadays, epidemics are frequently reported in overcrowded Nigerian cities including Lagos and Kano upon water shortage, deterioration of sanitary conditions and ingestion of contaminated water or food (Idika, 1999).

Typhoid fever poses a serious health problem to the developing world. The annual incidence of this disease is estimated to be 20 million cases, resulting in more than 700,000 deaths (Mills-Roberson, *et al,* 2003). Most cases are the result of infection with *Salmonella typhi*, but colonisation with *Salmonella paratyphi* A., B. or C, results in paratyphoid fever, a contagious condition similar to typhoid fever (WHO, 1996[a]).

Non bacterial infectious agents that can be transmitted in water include fungi and protozoa (*Cryptosporidium*) (Hubbard, *et al.,* 2004). *Cryptosporidium* is an important waterborne pathogen that infects the gastro-intestinal or respiratory tract of mammals including humans (Ochiaie, *et al.,* 2005). It is an intestinal

protozoan parasite that continues to be an important cause of water borne gastro-intestinal disease worldwide (Sturbuam, *et al.,* 2002). The enteric protozoa *Giardia* and *Cryptosporidium* species are well documented causes of waterborne diseases (Rose, *et al.,* 1989). These microbes have been of increasing concern to the drinking water industry; and both organisms are associated with several waterborne outbreaks after contamination of the source water by faecal material of human or animal origin (Rimhanen-finne, *et al.,* 2001). Human enteric viruses occur in ground water and are thought to be responsible for a significant proportion of infectious gastroenteritis related to ground water consumption (Borchardt, *et al.,* 2004).

Surface water is frequently contaminated with faecal bacteria (Ahmed, *et al.,* 2005). When surface runoff or leaching occurs due to excessive irrigation or rainfall, contamination of water resources by enteric bacteria may result. These same bodies of water are often used for sources of drinking water or for recreational activities; therefore elevated concentrations of enteric bacteria pose a potential health hazard (Hubbard, *et al.,* 2004).

To ensure safe recreational water and a continued supply of portable water, frequent monitoring of both raw water sources and finished products for the presence of indicator organisms is very important, these procedures will establish baseline data against which microbiological quality can be compared during waterborne epidemics or unusual circumstances. Also, periodic examination will help to establish a system or protocol that can be activated without significant

delay, in emergency situations, including sudden disease outbreaks. More importantly, the detection of any organism not previously observed or a marked increased in the number of a specific pathogen in the source water may provide an early indication of possible health risks (Singh *and* Mcfeters, 1992).

2.4 Mode of transmission of waterborne diseases agents

There are a considerable number of human diseases which are transmitted via faecal contamination of water. They include cholera, dysentery paratyphoid fever, infectious hepatitis and gastroenteritis and many of these diseases are very common in warm tropical developing countries (Ogbondeminu, *et al.*, 1994). The main origin of human waterborne pathogens is the guts of humans and animals (Maynard, *et al.*, 2005). Drinking and recreational waters have also been linked to human cases of gastrointestinal diseases (Chalmers, *et al.*, 2000). Excreta disposal practices and water pollution are the leading causes of diseases worldwide even in industrialized societies which already have sophisticated water treatment technology in place, thus an increasing number of waterborne outbreaks have fuelled the debate concerning public health protection measures (Cifuentes, *et al.*, 2004).

Exposure to contaminated water through ingestion such as drinking water, recreation or irrigation is a significant mode of transmission of gastrointestinal infections (Umoh, *et al.*, 2006). Paul *et al.* (1995) have reported the outbreaks of hepatitis, gastroenteritis, and Norwalk virus dysentery attributed

to ground water contamination from septic tanks. Outbreaks have also been associated with swimming in a crowded lake, contaminated drinking water as well as surface water. Other modes of transmission include human-human contact, contact with inanimate surfaces and with animals or their faeces (Umoh, *et al.*, 2006).

Water based diseases are transmitted by drinking contaminated water, this is responsible for significant outbreaks of faecal-oral diseases such as cholera and typhoid fever and also included are diarrhoea, viral hepatitis A, cholera, dysentery, and dracunculiasis guinea worm.(http://www.childinfo.org/areas/water) The major sources of contamination of surface water are urban and farm runoffs, discharges from sewage treatment facilities, failing septic systems, wild life, farm animals and direct faecal contamination by humans and animals (Umoh, *et al.*, 2006).

The role of food and water as a vehicle for the transmission of these organisms is well documented and represent a significant public health threat (Szakal, *et al.,* 2001). Contamination of food and water by microorganisms from animal manures has become a topic of concern recently; non point-source contamination from manure may result from pastured animals, from roaming wild animals, or from manure intentionally spread on to fields as fertilizers or waste. While point sources of manure contaminations include animal feedlots, animal housing facilities, and manure storage areas such as lagoons. A point source may

also lead to non point-source spread manure or pathogens by runoff or leaching that spreads to fields and water supplies (Gagliardi *and* Karns, 2000).

Fresh water bodies polluted by faecal discharges from man and other animals may transport a variety of human pathogens (Ogbondeminu, *et al.,* 1994). In the urban areas water get contaminated in so many different ways, some of the most common reasons being due to leaky water pipe joints in areas where the water pipe and the sewage line pass close together. Sometimes the water gets polluted at source due to various reasons and mainly due to inflow of sewage into the source (http://www.Edu_green.teri.res.in/explore/water/health.html). Global exposures to faecal pollution is on the increase as a result of population growth, economic driving forces and weak institutions, thus in less developed countries, the excess burden of diarrhoea is likely to reflect multiple modes of transmission of pathogens (Cifuentes, *et al*., 2004).

2.5 Indicators of faecal pollution of water

Testing for the evidence of faecal contamination in water has been traditionally accomplished by detection or enumeration of faecal coliform (Cieben, *et al.,* 1995). Enumeration of total and faecal coliforms which are closely related to a bacterial group in the family Enterobacteriaceae has been used as an indicator of faecal contamination of water samples for over 100 years, however these traditional methods of detecting coliforms require a long detection time up to 96 hours and complicated procedures (Park, *et al.,* 1995). *Escherichia coli* are a

consistent and predominant facultative inhabitant of the human gastro-intestinal tract; its regular presence in the intestine and faeces of warm-blooded animals makes the bacterium an indicator of faecal pollution (Yang, *et al.*, 2004). Although the presence of *Escherichia coli* in water is rarely always associated with recent faecal pollution and is the preferred indicator organism for this purpose, conventional procedure for verification of faecal coliforms such as *Escherichia coli* when faecal contamination is questionable are very laborious and time consuming (Cieben, *et al.*, 1995). Bacterial, parasitic and viral pathogens in human faecal waste have the greatest potential to cause infection in other humans Hubbard *et al.* (2004) reported that other non bacterial infectious agents that can be transmitted in water include fungi and protozoa.

Faecal coliform and *Enterococci* are indicator organism used world wide to monitor water quality, they are used in microbial source tracking (MST) studies which attempt to assess the contribution of various host species to faecal pollution in water (Anderson, *et al.*,2005). Since indicator bacteria such as *Enterococci* and *Escherichia coli* are present in high abundance in the gastro-intestinal tracts of most warm-blooded animals, the detection of these organisms implies the presence of faecal contamination (Bower, *et al.*, 2005). Water contamination events often result from discharges from waste water treatment facilities, overflowing sanitary sewer systems, waste materials that find their way into domestic and industrial sewage and runoff of animal faecal matters during storm events (Maynard, *et al.*, 2005). Human sources of faecal pollution constitute a serious health risk because

of high likelihood of the presence of human pathogens. Zoonotic reservoirs of pathogens are also of high concern to public health.

The presence of faecal coliform in water indicates contamination per se, and may indicate that the sample is unsuitable for consumption, the specific detection of enteric pathogens like *Shigella* species*, or* enteroinvasive *Escherichia coli* is still necessary to recognise trace and prevent outbreaks (Szakal, *et al.*, 2001). Bacteria within contaminated water represented a highly diversified group; numerous *Salmonella* species*, Escherichia* species*, Shigella* species*, Yersinia* species, and *Klebsiella* species, within the Enterobacteriaceae are human waterborne pathogens found in domestic waste water (Maynard, *et al.*, 2005). Ahmed *et al.* (2005) reported that the use of indicator bacteria such as faecal coliform (fc) and *Escherichia coli* for the assessment of faecal pollution and possible water quality deterioration in various fresh water sources is widely used and accepted concept in temperate regions. It is equally an established technique used in the tropics to ascertain the extent of water pollution by faecal coliform. Unfortunately, the specificity of faecal coliforms to indicate faecal pollution can vary considerably depending on environmental conditions and the presence of industrial effluent (Cieben, *et al.*, 1995).

Strain of *Escherichia coli* in particular constitute an important set of waterborne pathogens composed of numerous Shiga toxin producing species and enterohaemorrhagic strains associated with portable recreational water (Maynard, *et al.*, 2005). Faecal anaerobes, such as *Bacteriodes* and *Bifidobacterium* have

long been suggested as alternative indicators to the faecal coliform group and they make up the majority of faecal bacteria in the gastro-intestinal tract of humans. They may be present at 1000 fold higher densities than the coliform group making these organisms highly sensitive indicators of faecal pollution (Bower, *et al.,* 2005).

The U.S Environmental Protection Agency recommends the use of *Escherichia coli*, a member of the faecal coliform group, as an indicator organism for recreational waters in fresh water bodies, and members of the genus *Enterococci* for both fresh water and salt water (Anderson, *et al.,* 2005).

2.6 Heterotrophic bacteria

Heterotrophs include bacteria, yeast and moulds that are part of the natural (non hazardous) microbes of water and organisms that are derived from diverse pollutant sources (Bhattacharya, 2004). Bacterial re-growth is often a problem in water distribution system as well as in domestic water filtration units (Payment, *et al.*, 1994). Heterotrophic count assay, formally known as the standard Plate Count assay, is used primarily to monitor heterotrophic bacteria load present in the water sample (http://www.mobio.com/services/services_view.plp?ld=9).

The media used for detection of heterotrophic bacteria have been formulated to promote the growth of a large number of bacteria, including those that have been stressed or have adapted to a low nutrient environment (Payment, *et al.,* 1994). However it is well known that the plate count technique significantly

underestimates the total number of cells present in a given environment including drinking water distribution systems and “overlooks” abundant lineages that could be hygienically relevant in portable water (Martiny, *et al.*, 2005). In many countries, current drinking water regulations require that water intended for human consumption should not contain pathogenic organism (Sreter, *et al.,* 2000).

Distribution pipe biofilms are also implicated in reducing the aesthetic (taste, colour, and odour) and microbiological quality of water through the continual detachment of biomass into the bulk water, the importance of biofilm process including the attachment, penetration and detachment of particles has been recognized in recent years and a range of studies investigating the fate of model pathogens and indicators such as coliform (LangMark, *et al.*, 2005). Thus there is a growing belief that some heterotrophic bacteria in drinking water are opportunistic pathogens and that their growth should be controlled (Payment, *et al.,* 1994).

2.7 Coliform organism

The overall microbial quality of water is determined on a routine basis by detecting the coliform group of bacteria that ferments lactose and constitutes a part of the normal mammalian intestinal flora, However, some members of genera such as *Klebsiella, Enterobacter*, and *Citrobacter* also have an extra intestinal prevalence and may gain entry into water from environmental sources (Singh and Mcfeters, 1992). Coliform bacteria including *Escherichia* species, *Klebsiella* species and *Enterobacter* species, are considered to be an indicator of faecal

contamination in feed and water with faecal transmitted food borne disease agents such as *Escherichia coli 0157 and Salmonella* species (Sanderson, *et al.,* 2005).

Testing for evidence of faecal contamination in water has been traditionally accomplished by detection and enumeration of faecal coliform (Cieben, *et al.,* 1995). Faecal coliform bacteria, predominantly *Escherichia coli* which originate primarily in the mammalian intestine, are detected by the ability to ferment lactose at 44.5 ^{0}C, they are usually long-term occupants of aquatic ecosystems. Thus their presence in water serves as a useful indicator of recent faecal contamination, which is the major source of many enteropathogenic diseases transmitted through water (Singh and Mcfeters, 1992). Faecal coliform bacteria and other bacterial indicators have been used by most water quality regulators in the United States for over a century as standard tools to measure faecal contamination and determine if a body of water is suitable for its designated use (such as fishing, drinking, recreational, industrial, wildlife preservation, etc,). These standards have helped to improve water sanitation and protect public health (Fong, *et al.,* 2005). According to most regulations, 100ml of sample of drinking water should not contain any coliform bacteria. A pre requisite for use of this volume is that the safety of the drinking water has been assessed by risk analysis and any possibility of faecal contamination thus is minimized (Hanninen, *et al.,* 2003).

2.8 Pathogens survival in the aquatic environment

Bacteria from the intestinal tract are under physiological stresses and gradually loose their ability to form colonies on differential and selective media. Thus their die-out rate depends on the water temperature, the effect of sunlight, the population of others bacteria presents and the chemical composition of the water (Presscott, *et al.*, 2002). For enteric bacteria, sea water is an extremes environment in which they are submitted to multiple physiological stresses, high osmolarity, low temperatures, nutrients starvation and oxidation through solar light irradiation. In addition to biological interactions, these factors have previously been considered responsible for the decay of enteric bacteria in natural marine waters (Munro, *et al.*, 1995). It has been shown that some faecal coliforms and faecal *Streptococci* can persist and even grow in certain environments, and free living coliforms may be indigenous to some tropical waters (Kreader, *et al.*,1995).

Accumulation of indicator bacteria and viruses in sediments is well documented and has been attributed to the sorption of microorganism to particles suspended in water, which then sediment out. The sediment may contain 100 to 1000 times as many faecal indicator bacteria as the overlying water. Therefore the period of pathogen survival and associated particle transport into water used for recreation and fisheries is of concern to regulatory authorities (Davies, *et al.*, 1995).

The physical, chemical and biological interactions in the aquatic environment create complex situations which may affect the pattern of residence and dispersion of allochtonous bacteria in different water bodies (Pereira and Alcantara, 1993). It has also been shown that, the persistence of faecal indicator bacteria in environmental water is affected by a complex array of physical, chemical, and biological factors (Anderson, *et al.*, 2005). Therefore the effects of different environmental factors on the persistence of enteric bacteria in natural waters are a matter of scientific interest with direct implications on public health (Pereira and Alcantara, 1993).

The presence of iron corrosion products have been reported by Appenzeller *et al.* (2005) to favour bacterial activity in drinking water networks, resulting in increases in both suspended bacteria or in increased occurrence and or cultivability of coliform bacteria. The large accumulations of bacterial cells observed on environmental ferric minerals and on corroded metallic structures maybe partially attributed to the surface properties of iron oxides. Their surface charge may promote attachment and colonization by microorganism. Several factors have also been proposed which considerably reduce the survival rates of faecal bacteria in the aquatic environment; sunlight is thought to be the single most important factor contributing to the death of these bacteria in the sea water. Other factors include high salinity, presence of toxic agents, predation and parasitism, as well as low nutrient concentrations. However faecal bacteria have been shown to survive and to a certain extent, even to grow in sediments which may protect them

from the influence of such factors as ultra violet radiation, high salinity, heavy metal toxicity and attack by bacteriophage. The faecal bacteria are able to obtain nutrients associated with the sediment particles, thus the observed persistence of bacteria at fairly constant levels in sediments may be the result of a balance between the rates of their growth versus predation (Davies, *et al.,* 1995).

It has been well documented that the survival of *Escherichia coli* can be dramatically decreased by high solar radiation (Perez-Rosas, *et al.,* 1988).

2.9 Antimicrobial resistance in *Escherichia coli, Shigella,* and *Salmonella* organism

Antibiotic-resistant bacteria and antibiotics are discharged in various amounts in the environment as a result of the increasing and often indiscriminate use of antibiotics in medical, veterinary and agricultural practices (Goni-Urriza, *et al.,* 2000). Antimicrobial agent resistance has been recognized as an emerging world wide problem, in both human and veterinary medicine, and its use is considered the most important factor for the emergence, selection and dissemination of antimicrobial agent resistant bacteria (Sayah, *et al.,* 2005; Berlin, *et al.,* 2005). Antimicrobial resistance in bacterial pathogens is a major impediment to successful therapy and in several instances, bacterial strains have arisen that are refractory to most available antimicrobial treatments (Lu, *et al.,* 2004). To estimate the extent of the antimicrobial resistance problem and to follow its evolution, surveillance programs have been established in many countries

worldwide and majority of these programs are dedicated to the surveillance of antimicrobial resistance in agents of zoonoses and indicator bacteria of the normal intestinal flora of animals, such as *Escherichia coli* and *Enterococcus* species (Boerlin, *et al.,* 2005). Several studies on antimicrobial susceptibility had been carried out with different resistance prevalence pattern (Adesiyin, *et al.,* 1993). In the case of *Escherichia coli*, resistance to streptomycin or spectinomycin is generally most prevalent (Boerlin, *et al.,* 2005). It has been suggested that human exposure to manure from animals given sub- therapeutic and therapeutic dosages of anti microbial agent is responsible for some cases of antimicrobial resistances; however this has not been scientifically documented (Olson, 2000).

For the last 10 years sporadically but regularly, the microbiology literature has reported on multi-drug resistant bacteria in geriatric hospitals, long-term care facilities and nursing homes, the emergence of these bacteria in such health care facilities has become a public health concern. Implementation of isolation precautions which is the principal measure recommended in acute-care hospitals to avoid cross transmission is often considered deleterious for the quality of life of elderly people living in long-term care facilities. Moreover, the elderly patients carrying MDR bacteria represent a reservoir of these bacteria in acute-care hospitals when they are transferred to them (Leflon-Guibout, *et al.,* 2004). Infact, it is being increasingly recognized that antimicrobial resistance develops at high frequency among bacteria in the food and animal production environment (Hayes, *et al.,* 2004). High prevalence of antimicrobial-resistant bacteria in commensal

flora contribute to the general increase and dissemination of bacteria resistance world wide and can be a source of resistance genes for enteric pathogens such as *Shigella* or *Salmonella* (Aubry- Damon, *et al.*,2004).

The presence of bacteria resistant to antimicrobial agents in food animals has serious implications for veterinary and public health. Clinical treatment of infected animals together with the potential transmission of resistant zoonotic pathogens to humans through the food chain maybe affected by high backgrounds levels of resistance within the intestinal flora of food animals (Hoyle, *et al.*,2004).

Antimicrobial treatments are major factors for selection of resistance in the commensal flora of humans. Industrial animal farming is also associated with large scale antimicrobial use, which leads to a high level of colonization of animals with antimicrobial-resistant bacteria that can then contaminate the food and in turn humans, (Aubry-Damon, *et al.,* 2004).

The emergence of *Escherichia coli* isolates with multiple-antibiotic-resistant phenotypes, involving co-existence to 4 or more unrelated families of antibiotic, has been previously reported and considered a serious health concern (Saenz, *et al.,* 2004). The levels of antimicrobial agent resistance that have been reported range from 72% up to 100, and 87% for faecal and non faecal coliform respectively (Sayah, *et al.,* 2005). Resistances arise by mutation, influencing the target or efflux of antimicrobial agents or by the acquisition of resistance genes encoding antimicrobial or target alternation, or alternate pathways (Lu, *et al.,*

2004). Transference of resistance determinants by mobile genetic elements including plasmids, transposons, and gene cassettes in integrons and the alternation in mar locus regulation are important factors that can contribute to the increase in multi-resistant bacteria (Saenz, *et al.,* 2004). Substantial genetic and biochemical similarities exist between resistant determinants in antimicrobial agent producing *Actinomycetes* and resistance genes found in gram negative pathogens (Lu, *et al.*, 2004).

The spread of microbial drug resistance is a global public health challenge, which impairs the efficacy of antimicrobial agents and results in substantial increased illness and death rates and health care associated cost (Bartoloni, *et al.,* 2006). Antimicrobial drug resistance is increasing worldwide and is related to the rampant use of drugs, the use of left over drugs also tend to increase antimicrobial drug resistance in a community by exerting selective pressure in the commensal flora (McNulty, *et* al., 2006). Therefore the importance of monitoring resistance in commensals indicator bacteria, such as *Escherichia coli* has been recognized, and at this time, there is an international effort to organize meaningful schemes. However, many of these efforts concentrate on cross sectional prevalence surveys and do not address the dynamics of antimicrobial agent resistance within the commensal or host population (Hoyle, *et al.,* 2004).

Antimicrobial agents such as fluoroquinolones and third generation cephalosophorins such as ceftriaxone are commonly used to treat severe human

Salmonella infections. Resistance to this and other antimicrobial drugs as well as multi-drug resistance has increased over the last several decades, partly as a consequence of antimicrobial agents in companion animals (dogs and cats). The use of antimicrobial agents in companion animals such as dogs and cats may also contribute to the development of antimicrobial resistance in *Salmonella,* but the impact of this is unknown (Wright, *et al.,*2005).

Ampicilin was the drug of choice for the treatment of shigellosis until ampicilin resistant *Shigella* strains became widespread in several geographical areas. This high prevalence of resistance to ampicilin made trimethoprim-sulfamethaxazole the drug of choice, but despite its choice for therapy of shigellosis its use should be monitored in view of the high frequency of resistance to trimethoprim- sulfamethaxazole in isolates from travellers returning from all regions. In the same vein tetracycline alone is no longer useful for the treatment of shigellosis because majority of *Shigella* isolates found in diverse geographical locations are now resistant as a result of the intensive use of this antibiotic in developing countries (Vila, *et al.,* 1994).

2.10 Water quality management

Waterborne diseases such as cholera and typhoid fever have been on the increase thus there is a need for appropriate, cheap and feasible methods of detecting faecal contamination (Byamukama, *et al.,* 2000). It has been established that in many developing countries, improvement in water supplies has not been

supplemented by improvement in sanitation facilities; moreover, health education is rarely included in environmental hygiene programmes (Cheung, 1999). It was also observed that waterborne pathogens may cause acute or chronic disease thus investigation of water as a disease vector requires methods to assess the presence of pathogens in water sources and drinking water (Benson, *et al.*,2004). In order to attain zero levels of indicators and pathogens and low numbers of heterotrophic bacteria in drinking water treatment, changes in areas of water treatment, distribution and analytical methods are expected to be introduced (Singh and Mcfeters, 1992). Identifying sources of faecal pollution is thus important for assessing the public health risk and devising management strategies (Bower, *et al.*, 2005). Sanitation with good hygiene, acts as a fundamental "primary barrier" by ensuring that faecal matter is disposed of safely and does not spread in the environment (Admassu, *et al.,* 2004).

Sources of faecal contamination in water ways must therefore be identified in order to adequately address water quality problems and protect public health. Source detection provides direct evidence as to the origin of pollution by identifying indicator organism by host. Using an indicator to assess water quality is useful because indicator organism are easier to detect than actual pathogens, not only do the types of pathogenic bacteria, viruses, or protozoan present in contaminated water vary depending on the host source but pathogens are also often low in numbers and difficult to culture relative to indicator organisms (Mclellan, *et al.*,2003).

Enteropathogens usually appear intermittently in low concentrations in an aquatic environment that is not ideal for either their growth or extended persistence. Thus to ensure the good health of a community, there is a need for readily available methods to detect and enumerate pathogens from aquatic sources. Routine or periodic examination of water for pathogens is essential under special circumstances, such as waste water reclamation, water sources with a history of frequent contamination, or during and after actual waterborne outbreaks. However, several limitations render the detection of pathogens difficult .The detection of any enteric bacteria, even of low pathogenicity in the aquatic environment may serve as a warning of unsafe recreational water or a breach in the integrity of disinfection or distribution system of portable water (Singh and Mcfeters, 1992).

2.11 Water quality and physicochemical parameters

Human settlement and industries have long been concentrated along rivers, estuaries, and coastal zones owing to the predominance of waterborne trade (Radojevic and Bashkin, 1999). These urban agglomeration place enormous burdens on natural resource, the most notable of which is water (Mazari- Hiriart, *et al.,* 2005). Pollution of surface and ground waters from animal wastes is of growing environmental concern (Hubbard, *et al.,* 2004). It has been established that human use of water leads to degradation of water quality by introducing various polluting substances (Radojevic and Bashkin, 1999).

Though microbiological parameters are the most important in determining the safety of water it is also essential to measure several different physicochemical properties of water in order to understand the true nature and pollution level of different source water. Some of these parameters may have limited health significance but in general there are guidelines intended to assure chemically balanced water (WHO, 1996[a]).

2.11.1 Temperature

Temperature may be the most important single factor affecting the occurrence and behaviour of life in surface waters because it affects practically every physical factor that is of concern in water quality management. It alters the density, viscosity, vapour pressure, surface tension, gas solubility and the rate of gas diffusion (Ellis, *et al.*, 1993). It also had a significant impact on the density of microbiological indicators at the time of sample analysis. Holding temperature below 10^{o}C is generally considered acceptable for *Escherichia coli*, the routinely used microbiological indicator of water quality (Pope, *et al.*, 2003). It is likely that an increase in water temperature may bring about an increase in the total bacterial population, although there may well be a decrease in the diversity which point out that the majority of algae and protozoa are only capable of living within the narrow temperature ranges and that temperature variations can result in substantial changes in species dominance (Ellis, *et al.*, 1993).

2.11.2 pH

The pH of an aquatic system is an important indicator of water quality and the extent of pollution of the watershed areas (WHO, 1996[a]). In surface waters, pH is one of the most important water quality indices. The concentration of H^+ ions has great significance for chemical and biological processes occurring in natural water as it can influence the growth of water biota especially fish population (Radojevic and Bashkin, 1999). Many chemical reactions are controlled by pH and biological activity is usually restricted to a fairly narrow range of 6.5-8.5. Highly acidic or highly alkaline waters are undesirable because of corrosion hazards and possible difficulties treatment (WHO, 1996[a]).

2.11.3 Biochemical oxygen demand

Basically the biochemical oxygen demand is the amount of oxygen used by aerobic microorganism as they metabolize the complex unstable molecules of pollution. It is usually carried out over 5 day's incubation period, and was for a long time considered to be the basic parameter of water pollution control and surface water quality (Ellis, *et al.*, 1993). High BOD volume implies reduced oxygen concentration and is indicative of faecal pollutions. If there is too much organic pollution present and consequently, the BOD is too high, the Dissolve oxygen may become too low to support aquatic life (Radojevic and Bashkin, 1999).

2.11.4 Macro elements

Phosphorus normally present as phosphates is an important element in surface water quality, it is essential to life and in many situations it may be growth limiting elements (Ellis, *et al.*, 1993). Phosphorus and nitrates are important parameter in water quality assessment, High levels of phosphorus and nitrates increase the growth of vegetation in water and the Eutrophic standard (Jonnalagadda and Mhere, 2001). Relatively high concentrations of phosphorus may be present in some surface waters as a result of discharge of waste water treatment works effluents in which most of the phosphate may have been derived from domestic detergent, other phosphates may be present as a result of Agricultural run-off and organic phosphate is present in animal waste or in decaying cell material (Ellis, *et al.*, 1993).

Nitrate is a common contaminant in shallow ground water system (Smith, *et al.*, 1994). Concentrations of nitrogen in excess of l0mg/1 in the nitrate (NO_3) form render ground water unsuitable for drinking for humans (Hubbard, *et al.*, 2004). In the united states, the environmental protection agency has set a maximum contaminant level (MCL) for nitrate in drinking water of 0.71mM(10mg of NO3-N /litre) (Hu., *et al* 2000). Nitrate contamination in ground water resource originates mainly from the excessive use of fertilizer and uncontrolled land discharges of treated waste water thus it can cause health hazard to infants and pregnant women hence limiting the direct use of the ground water resource for human consumption in several parts of the world (Shrimali and Singh, 2001).

Nitrate is readily absorbed into the blood where it combines with the haemoglobin that carries oxygen and forms methaemoglobin, which can not carry oxygen. The reduced oxygen supply to the body tissues produces physical stress and when severe enough, nitrate poisoning is life threatening because of suffocation (Lamond, *et al*., 1997)www.oznet.ksu.edu/library/h20ql2/mf857.pdf.)

2.11.5 Eutrophication

Eutrophication is a natural and desirable process; however, human activities can greatly increase the presence of nutrients, leading to a cultural eutrophication, a major problem in many areas. (Radojevic and Bashkin, 1999). Principally eutrophication is considered to be a major disadvantage, in that it detracts from the amenity value of lakes so that boating and fishing can become less attractive and even difficult, Also and very importantly, when abstracted to be used in portable water supplies, the water of eutrophic lakes can create very considerable problems both as a result of clogging of sand filter and with the production of objectionable tastes and odours. There is also the real but limited danger to cattle and potentially to humans also of the presence of toxic cyanobacteria in algal blooms (Ellis, *et al.,* 1993).

Eutrophication can be solved by ensuring that waste water discharges into water bodies do not contain excessive nutrients by employing advanced methods

of waste water treatment capable of removing this substance (Radojevic and Bashkin, 1999).

CHAPTER THREE

MATERIALS AND METHOD

The study Area is Gusau municipal a relatively densely populated administrative and commercial centre. It is the capital of Zamfara state in the North western part of Nigeria. It is located on longitude 6^{o} and 7^{o} E and on latitude 12^{o} and 13^{o}N. It is sited on Pre-Cambrian basement complex, and also characterized by gently rolling highland topography associated with the old granite formation. Minor inselberg which rises to some 300 meters above undulating plains of the area also exist. The area is characterized by wet and dry season with annual rainfall between 750mm - 830mm. Wells, rivers, taps and packaged water serve as public drinking water sources. For the purpose of this study the area was divided into 10 sampling unit and 3 wells, 10 taps water point and 10 packaged water plant were designated for sampling in each of the area, the frequency of sampling ranged from 4- 5 times for each sampling point.

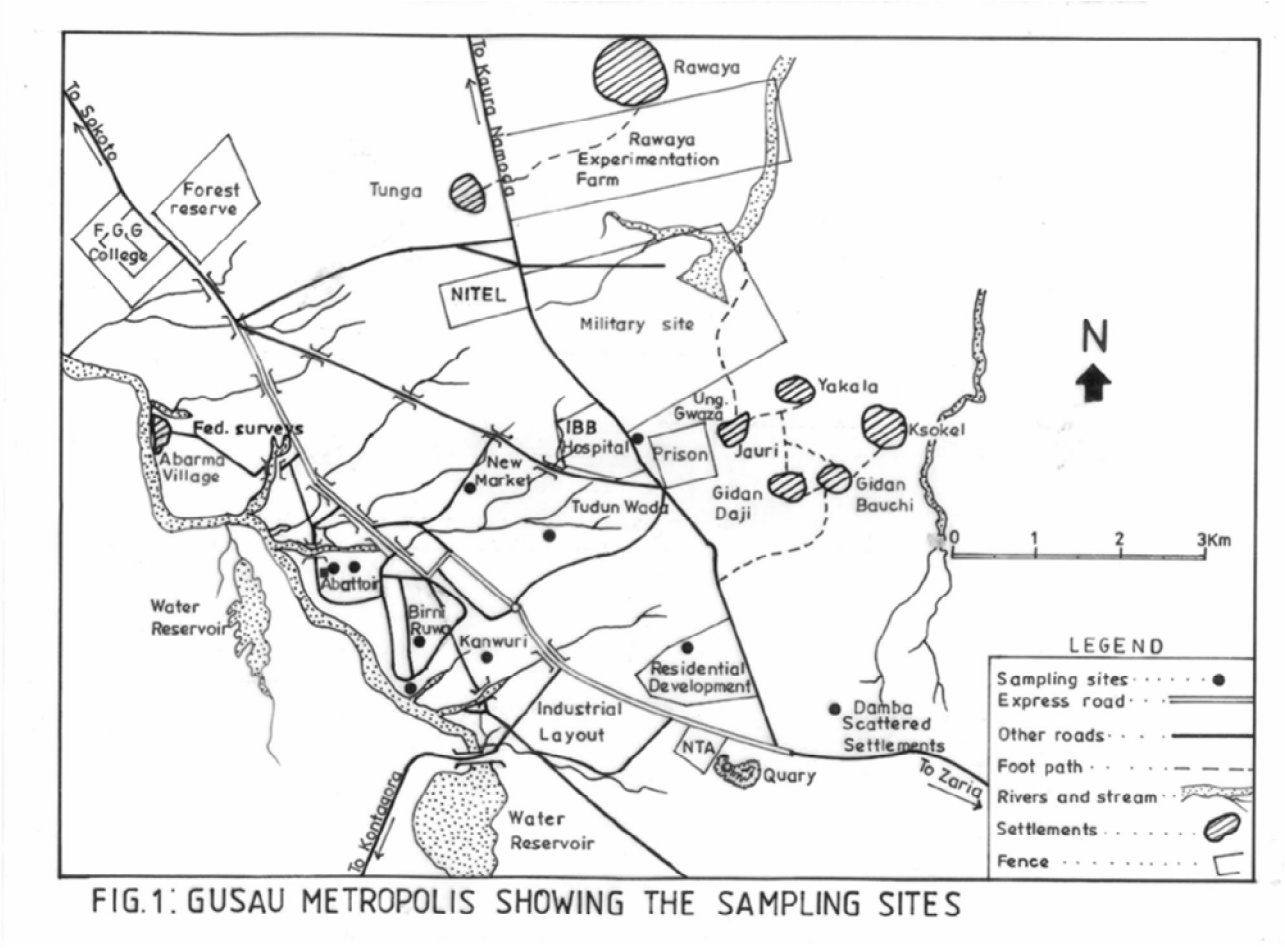


FIG.1: GUSAU METROPOLIS SHOWING THE SAMPLING SITES

3.1 Sample collection

3.1.1 Well Water

Sample from well water was collected in a sterile 250ml capacity bottle using “Guga”, the bottles were filled with the water and capped carefully and then labelled with a sample code number, transported to the laboratory in ice cold box and analysed within 6 hrs.

3.1.2 Tap Water

Water samples were collected from 10 different tap outlets in the study area. They were collected in 1-litre sterile containers, the samples were assigned a label and sample code number and transported to the laboratory in an ice pack box for further analysis.

3.1.3 Packaged water

Sixty 60 cl of packaged water from 10 different packaged water sales point were purchased, placed in an insulated cold box, transported to the laboratory and analyzed within 6 hours of collection.

3.2 Sample analysis

3.2.1 Preparation of Media

Each media used was prepared from commercially purchased powder according to the manufacturers instructions. Sterilization was done by autoclaving at 15Lb/sq inch at 121^{o}C for 15 minutes. All glass wares (pipettes and Petri dishes) were packed in canisters and sterilized in the hot air oven at 160^{o}C for 90 minutes.

Aseptic conditions maintained throughout sample collection and analysis and possible media contamination prevented.

3.3 Heterotrophic plate count

Ten fold serial dilutions of the water samples were prepared in sterile distilled water. The 10^1 and 10^6 dilutions were used. From these 0.1ml of the sample was aseptically transferred on to the centre of a prepared plate count Agar (Oxoid) a steriled glass rod was used to spread the water sample evenly on the surface of the media, the plates were made in duplicate and incubated (Gallen Kamp England Model IH – 150) at 37^{o}C for 24 hours. Colonies formed were counted as described in standard methods, and the result reported as cfu/milliliter and the value was multiplied by the dilution factor to get the corrected normalised cfu/ml.

3.4 Total coliform count

Ten fold serial dilutions of water sample were prepared in steriled distilled water, the 10^1 and 10^6 were used respectively, from these 0.1ml were spread aseptically on to duplicates plates of Eosin Methylene blue Agar (Oxoid) following standard procedure, the plates were incubated (Gallenkamp England mode/IH-150) at 37^{o}C for 24hours typical lactose fermenting colonies were counted and reported as cfu/ml and the value multiplied by the dilution factor to get the corrected normalized cfu/ml.

3.5 Isolation of *Escherichia coli*

Escherichia coli from the various sample were isolated by pre-enrichment using trypticase soya broth and high temperature methods of incubation described by LeJeune, *et al.* (2001). Twenty (20ml) of each water sample was combined with equal volume of sterile double strength trypticase soya broth in a container and incubated at 44.5^{o}C (Gallen Kamp England Model IH – 150) for 24hours. After enrichment a loopful of the broth was steaked on to Eosin Methylene blue Agar plates (OXOID) incubated at 44.5^{o}C for 24 hours. Mixed cultures were transferred to fresh E.M.B. Agar plates (Oxoid) to obtain pure colonies. About 4-5 typical lactose fermenting small almost black centre colonies were picked and confirmed gram negative using the standardized gram staining procedure (Claus 1992). Discrete colonies were picked and streaked on to slants of Nutrient Agar. Incubated (Gallenkamp England model IH - 150) at 37^{o}C for 24 hours and stored in a refrigerator for further analysis.

3.5.1 Isolation of *Salmonella*

Salmonellae were isolated by pre-enrichment using selenite – F broth (Oxoid) the broth tubes were incubated (Gallenkamp England Model IH -150) at 37^{o}C for 24 hours. A loopful of the Broth culture was streaked on to the surface of Deoxycholate citrate Agar (Oxoid) and incubated at 37 ^{o}C for 24 hours, purified colonies were picked and put on to the nutrient agar slant and refrigerated for further screening.

3.5.2 Isolation of *Shigella*

Five (5) milliliter of the water sample were inoculated on to Ten (10) millilitre Selenite F- broth, and incubated (Gallenkamp England Model IH – 150) at 37^{o}C for 24hours. After incubation a Loopful of the broth culture was streaked on to the surface of Deoxycholate Citrate Agar (Oxoid) and incubated at 37^{o}C for 24hours.

3.5.3 Isolation of *Vibrio* species

Five (5) ml of the water samples were inoculated into Ten (10) ml of triple strength alkaline peptone water and incubated at 37^{o}C for 24hours. After incubation 2-3 loopfuls of the enriched broth were inoculated on to Thiosulphate Citrate Bile Salt Agar and incubated (Gallenkamp England Mode/IH.150) at 37^{o}C for 24hours. *Vibrio* like colonies were further characterized and confirmed by a battery of biochemical test. Including final slide Agglutination using polyvalent 01 and 0139 antisera.

3.6 Biochemical identification

Lactose fermenting, small almost black with greenish metallic sheen colonies on Eosin Methylene Blue plates (Oxoid) were isolated, purified and subjected to IMVIC test as well as test on Kligler Iron Agar Slants. Isolates that gave the typical IMVIC test reaction ++ -- and gave A/A reaction on K.I.A. were isolated and stored on Nutrient Agar slant for further test.

Colourless colonies on Deoxycholate Citrate agar were aseptically picked and inoculated on Kligler Iron Agar Slants (K.I.A) and Urea agar slants, isolates from slants that gave reaction indicative of *Salmonella* species and *Shigella* species, that is urease negative, K/A and small blackening (H_2S) on K.I.A. and motility positive (*Salmonella* species). Urease negative, K/A no gas on K.I.A. and motility negative (*Shigella* species) were isolated and stored on Nutrient Agar slants for further test.

Yellowish colonies cultured on Thiosulphate citrate Bile salt agar and that gave a typical *Vibrio* species reaction biochemically were isolated and stored on nutrient agar slants for further screening.

3.7 Serological identification

Colonies that gave a typical *Salmonella* reaction following biochemical test were identified using slide Agglutination technique by *Salmonella* O Antisera poly A-1 and Vi, while isolates biochemically confirmed as *Shigella* were confirmed serologically using *Shigella* species antisera. And for *Vibrio* species slide Agglutination using polyvalent 01 and 0139 antisera was done to confirm the isolates as *Vibrios.*

3.8 Antibiotic sensitivity

Each of the isolates (*Salmonella, Shigella* and *Escherichia coli*) were subjected to antibiotic susceptibility testing using the bauer-kirby method that has

been standardized by NCCLS and evaluated by the methods of national committee for clinical laboratory standards (2002).

Isolates grown overnight on Nutrient Agar were suspended in sterile normal saline (0.9% w/v NaCl). Using a sterile wire loop until the turbidity was equivalent to 0.5 Mcfarland standards. A sterile non toxic cotton swabs dipped into the standardized innocula were used to streak the entire surface of Mueller Hinton Agar plates. Antibiotics disks (PS 003 G – VE, polytes lab. Enugu, Nigeria) were aseptically placed using a sterile forceps, and all plates incubated (Gallenkamp England model IH-150) at 37^{o}C for 24hours (Mills –Robertson, *et al.,* 2003).

The result was interpreted using NCCLS guidelines (see appendix iii) each isolate was classified as;

1. Sensitive if the observed zone of inhibition diameter was equal to or greater than NCCLS sensitive diameter (mm)
2. Resistant if the observed zone of inhibition diameter was less than or equal to the NCCL resistant diameter (mm) or
3. Intermediate if the observed zone of inhibition diameter fall within the intermediate range between the NCCLS resistant and sensitive limits.

3.9 Physicochemical analysis

Biochemical oxygen demand, electrical conductivity and nitrate were determined in the laboratory according to American Public Health Association Standard (1998). Temperature and pH were measured in-situ with Hanna

instrument HI 99/1000 pH metre dipped in the water sample at inclined position for about 5 minutes to allow for equilibrium temperature measurement. Temperature was recorded to the nearest 1.0^{o}C, while measurement of the pH was done to the nearest 0.01.

3.9.1 Electrical conductivity

The conductance of each water sample was determined using a conductivity set (MS Nory Lab). After rinsing the cell with a portion of each sample, it was filled with the sample and fitted. Upon a press of the test button, the read out which automatically corrected to 25.0^{o}C was reported as microsiemens per centimeter (µs/cm).

3.9.2 Nitrate

Using the phenoldisulphonic acid method, 100ml of samples poured into petridishes were evaporated to dryness on a steam bath. The residue in each dish was then dissolved with 2ml of phenoldisulphonic acid and transferred into 200ml conical flask. Six ml. of ammonium hydroxide was added and the mixture made up to 100ml with distilled water. This was then filtered into a beaker and the transmittance was read 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, 2ml, 3ml, 4ml and 5ml portions of the standard nitrate solution 90.7218g/l, KNO_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

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

N = concentration from standard curve

3.9.3 5- Day biochemical oxygen demand (BOD_5)

Using the titrimetric dilution method, duplicate BOD bottles (300ml) were filled with each sample or a dilution (10% dilutions of samples were made by dispensing 30ml into BOD bottles and making up to 300ml with distilled water). One set of seeded bottles were incubated at 20^oC and used after 5 days to determine the dissolved oxygen (DO_5) concentration. The other set was used to determine DO_0, within 4 hrs of sample collection. Briefly after, 2ml of manganese sulphate ($MnSO_4$) solution and 2ml of alkali – iodide azide reagent were added, the bottles were stoppered and inverted a few times for 2 minutes to ensure mixing of the content thoroughly. Another shaking followed floc formation and precipitation, after which the bottle was opened, the precipitates were allowed to settle (to approximately half the bottle volume leaving a clear supernatant above

the manganese hydroxide floc). Then 2ml of concentrated H_2SO_4 was added; the bottles were re-stoppered and contents mixed properly (as above) until dissolution was complete. Then 200ml were dispensed from each BOD bottle in to 250ml conical flask; 1ml of starch solution was added to give a blue – black solution that was titrated with 0.0021m sodium thiosulphate, to a colourless end point.

For the titration of 200ml sample, 1 ml of 0.0021M $Na_2S_2O_3$ = 1mg Do Per litre.

Where DO_0 = Initial concentration of dissolved oxygen

DO_5= final dissolved oxygen concentration

$$BOD_5 \text{ (mg/l)} = \frac{DOo - Do_5}{\text{Sample volume (ml)}} \times 300$$

3.9.4 Data Analysis

Data was analysed by Anova and correlation using Microsoft excell 2003 post for windows 1.1

CHAPTER FOUR

RESULTS

Two bacteriological parameters heterotrophic plate count (HPC/ML), Total colifom count (TCC/ML) and five physico-chemical parameters: Temperature, pH, Electrical conductivity, Nitrate –Nitrogen (NO_3 – N) and five days Biochemical Oxygen Demand (BOD_5) were determined for a total of 300 samples of water collected from three different sources (wells, tap and packaged water) on a weekly basis over a seven month period spanning September 2005 – March 2006, covering both wet and dry seasons. The breakdown is as follows, 90 samples from well during the wet season, another 90 samples collected from wells during dry season. 60 samples each collected during the wet and dry seasons from tap water outlets and 60 samples each collected from different packaged water sales point in both wet and dry seasons.

Table 1 shows the frequency of isolation of enteric bacteria in the water samples analysed during wet and dry seasons. It was observed that some organisms occurred more frequently than others. *Escherichia coli* and *Shigella* species were more prevalent in well water sources during the wet seasons, whereas *Salmonella, Pseudomonas, Klebsiella* and *Proteus* species occurred more frequently in well water during the dry season. However, *Shigella* species *and Escherichia coli* were more frequent in tap water sources in the wet seasons. The frequency of isolation of *Salmonella* and *Klebsiella* species from tap water source was lower during the wet season than the dry season. But as for the packaged water, low frequency of isolation of Enterics was recorded during both wet and dry season. In all the cases *Klebsiella* species had the highest prevalence accounting for 66.6% during the study period this was followed by *Pseudomonas* species with 48.8% isolation frequency. *Escherichia coli, Shigella* and *Salmonella* species had 46.6%, 43.3% and 36.6% isolation frequency respectively. No *Vibrio* species was isolated throughout the study period, probably because *Vibrio* species can exist in a viable but non-culturable state believed to be a complex physiological adaptation strategy that allows bacteria to remain viable for extended periods of time essentially in a survival stage (Rahman, *et al.,* 1994).

Table 1: Frequency occurrence of enteric bacteria in water sources analysed during wet and dry season

SOURCE	PATHOGENS ISOLATED	NUMBER ISOLATES WET SEASON (N=90)	NUMBER ISOLATES DRY SEASON (N=90)	TOTAL NO. ISOLATED IN WET AND DRY SEASON	% ISOLATED IN WET SEASON	% ISOLATED IN DRY SEASON	TOTAL ISOLATED IN WET AND DRY SEASON
Well	*Escherichia coli*	25	16	41	27.7	17.7	45.4
	Salmonella Spp.	14	16	30	15.5	17.7	33.2
	Shigella Species	11	9	20	12.2	10.0	22.2
	Pseudomonas	20	24	44	22.2	26.6	48.8
	Klebsiella Specie	10	12	22	11.1	13.3	24.4
	Proteus Species	5	8	13	5.5	8.8	14.3
	Others	18	20	38	20.0	22.2	42.2
Tap		(n=60)	(N=60)				
	Escherichia coli	08	06	14	13.3	10.0	23.3
	Salmonella	02	07	09	3.3	11.6	14.9
	Shigella Species	09	03	12	15.0	5.0	20.0
	Klebsiella Species	09	13	22	15.0	21.6	36.6
	Proteus Species	0	0	0	0.0	0.0	0.0
	Vibrio Species	0					
Packaged		(N=60)	(N=60)				
	Escherichia coli	05	03	08	8.3	5.0	13.3
	Salmonella	02	0	01	1.6	0	1.6
	Shigella Species	03	02	05	5.0	3.3	8.3
	Klebsiella Specie	09	05	14	15.0	8.3	23.3
	Vibrio Species	0	0	0	0.0	0.0	0.0

n = number of samples analysed

Table 2 indicates the mean values of water quality parameter for samples obtained from well, tap and packaged water and two bacteriological parameters. Result of correlation analysis indicated that there was significant correlation between 5 days biochemical oxygen demand and both heterotrophic plate count and total coliform count in both the wet and dry seasons (r= 0.9997) and (r = 1.0000). Also positive correlation between the value of nitrate and both heterotrophic plate count and total coliform count was observed (r = 0.9781 and 0.9792) at $P \leq 0.05$, No correlation was however observed between other parameters and both heterotrophic plate count and total coliform count. In the same vein, the temperature, electrical conductivity and pH for all the samples were within the WHO water quality standard see appendix IV. The 5 day biochemical oxygen demand and nitrate value for tap and packaged water were also within the drinking water quality standard (WHO, 1996[a]) during the wet season, in contrast to BOD_5 and nitrate value for well water which was above the limit for water quality standard. The values for heterotrophic plate count and total coliform count were higher for all the water samples analysed during wet and dry season surpassing the European Union, US Environmental Protection Agency and WHO drinking water guidelines for bacteriological quality. See Appendix (IV and V)

Table 2: Mean values of water quality parameters for water obtained from well, tap and packaged waters.

WET SEASON

PARAMETER	WELL (N=30) MEAN ± S.E.	TAP (N=10) MEAN ± S.E.	PACKAGED (N=10) MEAN ± S.E.
$WT^{O}C$	28.46 ± 0.25	25.80 ± 0.42	21.70 ± 0.71
EC (µs/cm)	511.60 ± 10.20	129.00 ± 3.48	141.00 ± 6.07
NO_3	70.23 ± 2.35	16.20 ± 2.07	1.28 ± 0.04
BOD_5	74.00 ± 4.22	0.78 ± 0.10	1.77 ± 0.25
pH	6.49 ± 0.05	6.84 ± 0.06	6. 89 ± 0.04
HPC/cfu/ml	$1.9 \times 10^6 \pm 1.7 \times 10^5$	$8.3 \times 10^3 \pm 4.5 \times 10^3$	$1.4 \times 10^4 \pm 4.7 \times 10^2$
TCC/cfu/ml	$9.6 \times 10^5 \pm 1.4 \times 10^5$	$8.6 \times 10^3 \pm 5.3 \times 10^3$	$5.6 \times 10^3 \pm 1.7 \times 10^3$

Key;

N= number of samples analysed

S.E= Standard error

$WT^{O}C$= Water Temperature

EC= Electrical conductivity (µs/cm)

NO_3= Nitrate

pH=Hydrogen ion concentration

BOD_5= Biochemical oxygen demand

HPC=Heterotrophic plate count

TCC=Total plate count

CFU=colony forming unit

Table 3 shows the mean values of some physiological and bacteriological water quality parameters. The temperature, pH and electrical conductivity were observed to be within the acceptable limit for drinking water guidelines for all the water samples. But nitrate value for the well water was higher than standard; in the same vein the heterotrophic plate count and total coliform count were higher than the drinking water guidelines (WHO, 1996[a])

Table 3: Mean values of water quality parameters for water obtained from well, tap and packaged waters in the dry season

DRY SEASON

PARAMETER	WELL (N=30) MEAN ± S.E.	TAP (N=10) MEAN ± S.E.	PACKAGED (N=10) MEAN ± S.E.
$WT^{O}C$	25.56 ± 0.24	25.50 ± 0.40	22.00 ± 0.81
EC (µs/cm)	561.00 ± 22.30	137.00 ± 3.64	150.00 ± 12.14
NO_3	83.22 ± 3.66	12.80 ± 1.44	127.00 ± 0.04
BOD_5	31.60 ± 3.19	0.79 ± 0.11	1.81 ± 0.26
pH	6.80 ± 0.06	6.85 ± 0.07	6. 94 ± 0.06
HPC/cfu/ml	$1.2 \times 10^6 \pm 9.7 \times 10^4$	$2.9 \times 10^2 \pm 1.1 \times 10^2$	$4.1 \times 10^4 \pm 1.5 \times 10^4$
TCC/cfu/ml	$1.4 \times 10^6 \pm 4.1 \times 10^2$	$7.6 \times 10^1 \pm 2.2 \times 10^1$	$2.7 \times 10^3 \pm 5.4 \times 10^2$

Key;

N= number of samples analysed

S.E= Standard error

WT^0C= Water Temperature

EC= Electrical conductivity (µs/cm)

NO_3= Nitrate

pH=Hydrogen ion concentration

BOD_5= Biochemical oxygen demand

HPC=Heterotrophic plate count

TCC=Total plate count

CFU= colony forming unit

Table 4 compares the mean values of heterotrophic plate count for the water samples in both wet and dry seasons. Well water had slightly higher value during wet season (1.9E6) than the dry seasons (1.2E6) the same thing was observed for tap water, in contrast to packaged water which was observed to have higher value during the dry season than the wet season. Result of ANOVA shows no significant difference between the mean heterotrophic count for all the water sources (P=0.4368).

Table 4: Comparison of mean values of Heterotrophic plate count for the wet and dry season for different water sources.

WATER SOURCE	WET SEASON MEAN ± S.E. IN CFU/ML	DRY SEASON MEAN ± S.E IN CFU/ML
Well (n = 30)	$1.9x10^6 \pm 1.7x10^5$	$1.2x10^6 \pm 9.7x10^4$
Tap (n = 10)	$8.3x10^3 \pm 4.4x10^3$	$2.9x10^2 \times \pm 1.1x10^2$
Packaged (n=10)	$1.4x10^4 \pm 4.7x10^2$	$4.0x10^4 \pm 1.5x10^4$

Key

N=no of sampling units

S.E= Standard error

CFU= colony forming unit

Table 5 compares the mean values of temperature, pH and electrical conductivity for the water samples analysed during the wet and dry seasons. It was observed that all he three physico-chemical parameters for all the samples were within the guidelines for drinking water during both wet and dry seasons. Result of two ways ANOVA showed that there was no significant variation between temperatures, (P value=0.0918 and 0.4287) for all the water sources during wet and dry seasons, pH for different water sources (P=0.2628). Also no significant variation in this parameter was observed across the seasons (P=0.3381). Significant difference between the mean electrical conductivity for the water source was observed across the seasons (P=0.0255 and 0.0374). The same thing for NO_3 (P=0.01139) however no significant variation was observed across the seasons (P=0.5880). Also there was no significant variation between BOD_5. For all water sources (P= 0.0866) as well as across the seasons (P=0.5944).

Table 5: Comparison of mean values of temperature, pH and electrical conductivity for the wet and dry season for different water sources.

PARAMETER	WATER SOURCES	WET SEASON MEAN ± S.E.	DRY SEASON MEAN ± S.E.
$WT^{O}C$	Well (n=30)	28.46 ± 0.25	25.56 ±0.24
	Tap (n=10)	25.80 ± 0.42	25.50+0.40
	Packaged (n=10)	21.70±2.24	22.00+0.81
pH	Well (n=30)	6. 49 ± 0.05	6. 80 ± 0.06
	Tap (n=10)	6.84 ± 0.06	6.85 ± 0.07
	Packaged (n=10)	6.94±0.04	6.94±0.06
Electrical	Well (n=30)	511.60 ± 10.20	561.00 +22.30
Conductivity	Tap (n=10)	129.00 ± 3.48	137.00+3.64
(µs/cm)	Packaged (n=10)	141.00±19.20	150.00+12.14

Key

N= number of samples analysed

S.E= Standard error

$WT^{0}C$= Water Temperature

EC= Electrical conductivity (µs/cm)

pH=Hydrogen ion concentration

Table 6 shows the mean values of nitrate and 5 day biochemical oxygen demand for water samples in wet and dry seasons. The mean nitrate value for well water (30Mg/l) was higher than the limit for drinking water see Appendix (IV) in contrast to the values for tap and packaged waters. The 5 day BOD for well water had also surpassed the drinking water guidelines see Appendix (IV and V). Whereas it was found to be within the drinking water guidelines in respect of tap and package water.

Table 6: Mean values of Nitrate and 5 Day Biochemical Oxygen Demand for the wet and dry season for different water sources

PARAMETER	WATER SOURCES	WET SEASON MEAN ± S.E.	DRY SEASON MEAN ± S.E.
Nitrate	Well (n=30)	70.23 ± 2.35	83.22 +0.01
	Tap (n=10)	16.20 ± 2.07	12.80+1.44
	Packaged (n=10)	1.28±0.04	1.27+0.04
BOD_5	Well (n=30)	74. 00 ± 4.22	31. 60 ± 3.19
	Tap (n=10)	0.78 ± 0.10	0.79 ± 0.11
	Packaged (n=10)	1.77±0.25	1.81±0.26

KEY

NO_3= Nitrate

BOD_5= Biochemical oxygen demand

N= Number of sampling area unit

Table 7 compares the mean values of total coliform count for the water samples in wet and dry seasons. It was observed that None of the water samples had count less than 2 cfu/ml thus the limits of the WHO drinking water guidelines was surpassed. Well water had the highest coliform count in the dry seasons than during the wet season, in contrast to tap and packaged water that had high coliform count during the wet seasons. However, result of two ways ANOVA revealed that there was significant difference (P=00386) between the mean total coliform counts for the water samples analysed, but no significant variation was observed across the seasons (P=0.4370).

Table 7: Comparison of mean values of Coliform Count for the wet and dry season for different water sources

WATER SOURCE	WET SEASON MEAN ± S.E.(CFU/ML)	DRY SEASON MEAN,S.E.(CFU/ML)
Well (n=30)	$9.5x10^5 \pm 1.4x10^5$	$1.4x10^6 \pm 4.2x10^5$
Tap (n=10)	$8.6x10^3 \pm 5.2x10^3$	$3.3x10^1 \pm 2.4x10^1$
Packaged (n=10)	$5.6x10^3 \pm 1.7x10^3$	$2.6x10^3 \pm 5.4x10^2$

S.E= Standard error

CFU/ML=Colony forming unit/ML.

N= Number of sampling area unit

Table 8 shows the mean values of some physico-chemical parameters in respect of well water during the wet season. It was observed that the temperature, pH and electrical conductivities were within the range of values for different water types see Appendix (IV). In the same vein both BOD_5 and nitrate value for all the wells had surpassed the standard for different water types. No significant variation was observed between physico-chemical parameters for all water sources (P=0.9021) across the seasons.

Table 8: Comparing mean values of physico-chemical parameters for the well water during wet season

WET SEASON

PARAMETER	TUDUNWADA (N=12) MEAN ± S.E.	KANWURI (N=10) MEAN ± S.E.	BIRNINRUWA (N=8) MEAN ± S.E.
WT ^{0}C	28.1±0.45	28.3±0.42	29.2±0.31
pH	6.36±0.09	6.53±0.09	6.63±0.09
EC µs/cm	1137.1±370.54	595.3±119.88	776.30±135.84
BOD_5	63.3±7.10	74.0±6.53	90.5±5.35
NO_3	59.9+6.11	62.49±5.66	66.5±5.29

N= number of samples analysed

S.E= Standard error

WT= Water Temperature

EC= Electrical conductivity (µs/cm)

NO_3= Nitrate

pH=Hydrogen ion concentration

BOD_5= Biochemical oxygen demand

Table 9 shows the mean values of some physico-chemical parameters for well water during the dry season. The temperature, pH and electrical conductivity for all the wells in Tudun Wada, Kanwuri, and Birnin Ruwa were within the standard for different water types. The relatively high electrical conductivity observed for well around Tudun Wada was the result of high electrolytes present which is a function of organic and inorganic pollution. Both BOD_5 and nitrate values were observed to be high for all the wells in Tudun Wada, Kanwuri and Birnin Ruwa surpassing the standard set for different water types.

Table 9: Comparing mean values of physico-chemical parameters for the well water during dry season

PARAMETER	TUDUNWADA (N=12) MEAN ± S.E.	KANWURI (N=10) MEAN ± S.E.	BIRNIN RUWA (N=8) MEAN ± S.E.
WT^0C.	25.08±0.34	26.4±0.11	25.25±0.49
pH	6.79±0.09	6.18±0.04	6.79±0.13
EC (µs/cm)	1018±287.12	776±41.88	675±146.34
BOD_5	27.2±3.04	39.8±2.47	28.13±4.12
NO_3	62.3±8.11	79.94±3.05	79.87±7.51

Key

N= number of samples analysed

S.E= Standard error

WT^0C = Water Temperature

EC (µs/cm)= Electrical conductivity

NO_3= Nitrate

pH=Hydrogen ion concentration

BOD_5= Biochemical oxygen demand

Table 10 reveals the mean values of physico-chemical parameter for the wet and dry seasons for different tap water. All the parameters except nitrate were within the acceptable limit for drinking water guideline in both the wet and dry seasons. Result of two way ANOVA shows no significant difference (P=0.9021) between those parameters for the two sampling area unit across the season.

Table 10: Comparing mean values of physico-chemical parameters during the wet and dry season for different Tap water sources.

	WET SEASON		DRY SEASON	
PARAMETER	TUDUN WADA (N=6) MEAN ± S.E.	BIRNIN RUWA (N=4) MEAN ± S.E.	TUDUN WADA (N=6) MEAN ± S.E.	BIRNIN RUWA (N=4) MEAN ± S.E.
WT.	26.3±0.56	25.0±0.41	25.33±0.66	25.75±0.25
pH	6.78±0.07	6.92±0.11	6.93±0.07	6.73±0.13
EC	133.3±4.21	122.5±4.78	136.6±4.94	137.5±6.29
BOD_5	0.761±0.15	0.807±0.16	0.755±0.17	0.842±0.17
NO_3	17.3±2.81	14.5±3.30	12.0±1.86	14.0±2.44

Key

N= number of samples analysed

S.E= Standard error

WT^0C= Water Temperature

EC (μs/cm) = Electrical conductivity

NO_3= Nitrate

pH=Hydrogen ion concentration

BOD_5= Biochemical oxygen demand

Table 11 shows the comparison of the mean values of total coliform count for the wet and dry seasons for different tap water sources. Birnin Ruwa tap water was observed to have high coliform count than Tudun water tap water, during the wet season. While Tudun Wada tap water had high coliform count compared to Birnin Ruwa tap water during the dry season. In all the area units, none of the waters were within the WHO drinking water guidelines. Equally, result of two way ANOVA revealed no significant difference (P=0.5031) between the mean total coliform counts in the sampling area unit for the wells across the seasons. Similarly no significant variation (P=0.4616) was observed for tap water across the seasons.

Table 11: Comparison of Mean values of Total Coliform Count for the wet and dry season for different tap water sources

WATER SOURCE	WET SEASON MEAN ± S.E.CFU/ML	DRY SEASON MEAN ± S.E.CFU/ML
Tudun Wada (n = 6)	$1.2x10^3 \pm 9.6x10^2$	$4.8x10^1 + 4.0x10^1$
Birnin Ruwa (n = 4)	$1.9x10^4 + 1.2x10^4$	$1.0x10^1 + 1.2x10^0$

Key

SE = Standard error

CFU=Colony forming unit

N=Number of sampling area unit

Table 12, shows the comparison of the mean values of total coliform count during the wet and dry seasons in respect of well water. Tudun Wada wells had the highest coliform count in the wet season compared to Birnin Ruwa and Kanwuri well waters, the same case was observed in the dry season, in all the cases all the wells had coliform count that surpassed the limit for drinking water. Equally, result of two ANOVA revealed no significant difference (P =0.0866) between the mean total coliform counts in the sampling area unit for the wells across the seasons (P=0.5944)

Table 12: Comparison of mean values of total Coliform Count for the wet and dry season for different well water sources

WATER SOURCE	WET SEASON MEAN ± S.E.(CFU/ML)	DRY SEASON MEAN ± S.E.(CFU/ML)
Tudun Wada (n=12)	$1.3x10^6+1.9x10^5$	$1.5x10^6+7.4x10^5$
Birnin Ruwa (n=10)	$8.7x10^5+2.3x10^5$	$5.2x10^5+1.1x10^5$
Kanwuri (n=10)	$7.6x10^5+2.2x10^5$	$6.3x10^5+1.9x10^5$

Key

SE = Standard error

CFU=Colony forming unit

N=Number of samples analysed

Table 13 shows the antibiotics susceptibility patterns of *Escherichia coli,* isolated from different water source using 10 antibiotics which include Septrin (30µg) Chloramphenicol (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (10 µg), Amoxacilin (30 µg), Augmentin (30 µg), Gentamycin (10 µg), Perfloxacin (30 µg), Tarivid (10 µg), Streptomycin (30 µg).

The table indicates that the highest resistance was shown to Amoxacilin with 30 isolates representing 50% of the isolates resistance to the antibiotic. The highest susceptibility was shown to Gentamycin and Perfloxacin with 100% (60) and 91.6% (55%) isolates susceptible to these antibiotics respectively, in the same vein, 88.3% (53), 83.3% (50), 80% (48), 76.6% (46) and 75% (45) susceptibility was recorded for Chloramphenicaol, Tarivid, Augmentin, Streptomycin and Sparfloxacin respectively. Intermediate susceptibility was shown to Septrin with 35% (21) isolates sensitive to it.

Table 13: Antibiotics Susceptibility Pattern of *Escherichia coli* Isolates from water sources (n=60)

S/N	ANTIMICROBIAL AGENT	SYMBOL	DISC CONTENT (µG)	SUSCEPTIBILITY PATTERN					
				number resistant	%	Number intermediate	%	number Sensitive	%
1.	Septrin	SXT	30	10	(16.6)	21	(35)	29	(48.3)
2.	Chloramphenicol	CH	30	7	(11.6)	0	(0.0)	53	(88.3)
3.	Sparfloxacin	SP	10	12	(20.0)	3	(5.0)	45	(75)
4.	Ciprofloxacin	CPX	10	17	(28.3)	3	(5.0)	40	(66.6)
5.	Amoxacilin	AM	30	30	(50.0)	5	(8.3)	25	(41.6)
6.	Augmentin	AU	30	12	(20.0)	0	(0.0)	48	(80.0)
7.	Gentamycin	GN	10	0	(0.0)	0	(0.0)	60	(100)
	Perfloxacin	PEF	30	0	(0.0)	5	(8.3)	55	(91.6)
8.	Tarivid	OFX	10	8	(13.3)	2	(3.3)	50	(83.3)
9.	Streptomycin	S	30	14	(23.3)	0	(0.0)	46	(76.6)

N=Number of isolates

Table14 shows the antibiotics susceptibility patterns of *Salmonella* species isolated from different water source using 10 antibiotics which include Septrin (30µg) Chloramphenicol (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (10 µg), Amoxacilin (30 µg), Augmentin (30 µg), Gentamycin (10 µg), Perfloxacin (30 µg), Tarivid (10 µg), Streptomycin (30 µg).The table shows that pattern of susceptibilty *Salmonella* isolates to antibiotics, the highest resistance was shown to Septrin 48/7% (20) and Perfloxacin 46.3% (19). The highest susceptibility was observed for Gentamycin 92.6% (38), Ciprofloxacin 82.9% (34), Tarivid 75.6% (31),73% (30 each for Sparfloxacin and Amoxacilin respectively, 68% (28) were susceptible to Streptomycin, 58.5% (24) and 51.2% (21) were susceptible to Chloramphenicol and Augmentin respectively, the least susceptibility was shown to Septrin and Perfloxacin with 43.9% (18) of the isolates susceptible, some few isolates were found to show intermediate susceptibility with 19.5% (8) and 17.0% (7) sensitivity to Sparfloxacin and Augmentin respectively.

Table 14: Antibiotic Susceptibility Pattern of *Salmonella* Isolates from water sources (n=41)

S/N	ANTIMICROBIAL AGENT	SYMBOL	DISC CONTENT (μG)	SUSCEPTIBILITY PATTERN					
				number resistant	%	Number intermediate	%	number Sensitive	%
1.	Septrin	SXT	30	20	(48.7)	3	(7.3)	18	(43.9)
2.	Chloramphenicol	CH	30	15	(36.5)	2	(4.8)	24	(58.5)
3.	Sparfloxacin	SP	10	0	(0.0)	8	(19.5)	30	(73.1)
4.	Ciprofloxacin	CPX	10	2	(4.8)	5	(12.1)	34	(82.9)
5.	Amoxacilin	AM	30	8	(18.5)	0	(0.0)	30	(73.1)
6.	Augmentin	AU	30	13	(31.7)	7	(17.0)	21	(51.2)
7.	Gentamycin	GN	10	3	(7.3)	0	(0.0)	38	(92.6)
8.	Perfloxacin	PEF	30	19	(46.3)	4	(9.7)	18	(43.9)
9.	Tarivid	OFX	10	9	(21.9)	1	(2.4)	31	(75.6)
10.	Streptomycin	S	30	11	(26.8)	2	(4.8)	28	(68.2)

N=Number of isolates

Table 15 show the antibiotics susceptibility patterns of *Shigella* species isolated from different water source using 10 antibiotics which include Septrin (30µg) Chloramphenicol (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (10 µg), Amoxacilin (30 µg), Augmentin (30 µg), Gentamycin (10 µg), Perfloxacin (30 µg), Tarivid (10 µg), Streptomycin (30 µg).The table shows the susceptibility pattern of *Shigella* species. isolates, the highest resistance was shown to Septrin 62.5% (25), 45% (18), 37.5% (15) and 30% (12, resistance was observed for Amoxacilin, Augmentin and Chloramphenicol respectively. Moderate resistance was shown to Ciproflacxacin 20% (8) Gentamycin 25% and Tarivid 25% (10). Another 12.5% (5) and 7.5% (3) of the isolates resistance to Sparfloxacin and Perfloxacin. No resistance at all was shown to Perfloxacin.

The highest susceptibility was observed for Perfloxacin 100% while 92.5% (37), 75% (30), 70% (28) and 62.5% (25) of the isolates susceptible to Streptomycin, Sparfloxacin, Gentamycin, Ciprofloxacin and Chloramphenicol respectively.

Table 15: Antibiotic Susceptibility Pattern of *Shigella* Isolates from water sources (n=40)

S/N	ANTIMICROBIAL AGENT	SYMBOL	DISC CONTENT (μG)	SUSCEPTIBILITY PATTERN					
				number resistant	%	Number intermediate	%	number Sensitive	%
1.	Septrin	SXT	30	25	(62.5)	5	(12.5)	10	(25.0)
2.	Chloramphenicol	CH	30	12	(30.0)	13	(32.5)	25	(62.5)
3.	Sparfloxacin	SP	10	5	(12.5)	5	(12.5)	30	(75.0)
4.	Ciprofloxacin	CPX	10	8	(20.0)	4	(10.0)	28	(70.0)
5.	Amoxacilin	AM	30	18	(45.0)	2	(5.0)	20	(50.0)
6.	Augmentin	AU	30	15	(37.5)	5	(5.0)	20	(50.0)
7.	Gentamycin	GN	10	10	(25.0)	0	(12.5)	30	(75.0)
8.	Perfloxacin	PEF	30	0	(0.0)	0	(0.0)	40	(100)
9.	Tarivid	OFX	10	10	(25.0)	8	(20.0)	22	(55.0)
10.	Streptomycin	S	30	3	(7.5)	0	(0.0)	37	(92.5)

N=Number of isolates

CHAPTER FIVE

DISCUSSION

Several studies have reported that surface and groundwater contamination by faecal pathogens generally occur through surface run-off, leaching and direct faecal deposition into the water bodies via several livestock production activities like confined animal feedlot, free range system, abattoir wastes, and land spreading of manure (Kuczynska, *et al.*,2005; Timpe and Fulhage, 1995). The contamination of the water bodies/source is mainly from either a point source which is a single identifiable source of pollution or a non-point source which is the major threat to ground water pollution (Bouwer, *et al.*, 1999). Such contamination brings the threat of infection for people who use the water for drinking, bathing or watering fruits and vegetable. In Nigeria some of the major sources of water are open and shallow wells, streams and even ponds. Also animals/livestock are reared within compounds and households of owner and are allowed to roam freely in search for food hence they consequently serve as sources of faecal contamination of water sources, (Tabukum , *et al.*,1996).

Coliform bacteria have been widely used as indicator of the microbiological quality of surface and ground waters (Ahmed, *et al.*, 2005). Thus the presence of coliforms is an index of the bacteriological quality of water, the isolation of coliforms especially *Escherichia coli*, from the water sources is attributable to contamination by material of human and animal origin and this is health significance as these organisms have generally been

reported as causative agent of gastroenteritis in human. The occurrence of *Salmonella, Klebsiella* and *Shigella* species which have the ability to cause a variety of human diseases, gave further evidence of bacterial contamination of the water sources, and this may have potential human hazard (Ogbondeminu, *et al.,* 1994). The possibility of other unidentified water borne pathogens like viruses, *Giardia intestinalis*, *Campylobacter* species, *Entamoeba histolytica*, *Cryptosporidium* species and some chemicals cannot be ruled out as the media used selected essentially the Enterobacteriaceae. Furthermore epidemiological data on water borne infections in this community is not available in this study.

The frequency of isolation of *Escherichia coli* and *Shigella* species was high during the wet season because of pollution of the well waters which is in agreement with the findings of Kisteman, *et al*. (2002). Who demonstrated that substantial shares of the total microbial load in water courses and drinking water reservoir in Germany resulted from rainfall and extreme run off events. The high Total *Escherichia coli* count in the wet season is also in agreement with a similar study by Lejeune, *et al.* (2001) which reported highest *Escherichia coli* count in summer, high count in spring and least count in winter in livestock drinking water microbiology analysis of livestock water trough. In the same manner these organism have been isolated in tap and packaged water which is also attributable to contamination of the water. The tap water which ought to be treated must have been contaminated as a result of

non treatment or poor water treatment or as a result of leaky water pipes which are buried in municipal drainage systems thus getting contaminated, conversely the packaged water might have been contaminated during packaging, some of these bacteria represent potential threats to human health including bacteria causing common diseases such as acute gastroenteritis, urinary tract infections and nosocomial infections. The effects arising from contact with such water vary depending on the volume of the water ingested by an individual and the individual's immune status, with the children and elderly being the most susceptible (Mazari-hiriart, *et al.*, 2005).

Vibrio cholerae or any of its species was not isolated throughout the study period probably because in response to extreme environmental condition, the bacteria may enter a dormant state which has been designated the viable but non culturable state, since the organisms can not be recovered by using traditional culture media. It has been hypothesised that it is in the viable non culturable state that *Vibrio cholerae* survives in the environment during inter epidemic periods, equally interaction with plankton appears to play an important role in the ecology of the microorganism and to facilitate persistence mainly in response to low temperature and reduced nutrients concentrations (Binsztein, *et al.*, (2004).

It can be seen that none of the waters studied, met the international standards. For drinking water, heterotrophic plate count should not exceed 500

cfu/ml and for indicator bacteria 0 cfu/ml should be found in the 90^{th} percentile of samples therefore this waters are not suitable for human consumption.

Statistically significant difference ($p<0.05$) existed between the mean total Coliform count of the water sources, thus indicating the extent of continuous pollution from point source as well as diffuse sources relative to tap and packaged waters. No significant difference was however noted across the seasons, most probably because the factors responsible for the contamination of the waters always exist; these factors include sewage pollution, leaky pipe joints traversing municipal drainage system and hand contamination during handling and processing of packaged water (Table 7).

The High total Coliform bacteria observed for well during the dry season were due to the fact that the water volume is decreased and the likely proliferation of the organism in contrast to the wet season when the volume of the water is increased thus there was dilution and dispersion of the organism.

Temperature

The measured well water temperature reflected changes corresponding to the seasons. With high values recorded during the wet seasons. Values recorded during the dry season were generally lower with the least occurring during the harmattan period, Nov – Jan, the highest temperature recorded during the study period was 30^{oc}, while the least was 24^{oc}. However

no correlation was observed between temperatures and heterotrophic plate count and total Coliform count.

The temperature profile for tap water did not show any significant variation in both the dry and wet seasons most probably because the pipes are buried in the soil. In contrast to the packaged water in which case the temperature was found to be as low as 19^{oc}, perhaps because of storage in the cold box. Although no apparent correlation existed between temperature and heterotrophic plate count and total Coliform count, studies have shown that the survival of enteric bacteria is reduced at higher temperature (Wang and Doyle, 1998).

pH

Though not definitive, the pH of an aquatic system is an important indicator of water quality and to certain extent measures the extent of pollution in the water shed area. Unpolluted water normally shows a neutral pH, the range of mean values observed in the study was 6.49 – 6.94. However, no significant correlation existed between pH values and total Coliform count and Heterotrophic plate count for different water in both the wet and dry seasons (Table 2 and 3).

Conductivity

The specific conductance yields a measure of waters capacity to convey an electric current, a property related to the total concentration of ionized substances in water the temperature at which the measurement is made, the nature of the various dissolved substances and their actual and relative concentration. It can be used as an approximate measure of the total concentration of inorganic substances in water. Ions that have a measure of influence on the conductivity of water are Na^{+},H^{+},k^{+},mg^{2+},Ca^{2t},cl^{-},NO_3^{-} and SO_4^{2-}. It is an important measurement in waters meant for various uses such as irrigation, drinking, and food industry, the mean electrical conductivity for well observed was high during the dry season (Table 5). The Difference recorded across the seasons is attributable to the fact that during the dry season there is usually low volume of water in the wells, thus concentration of inorganic ions becomes high and the low concentration of ions during the wet seasons is ascribed to the increased volume of water and therefore dilution of the inorganic ions.

Biochemical Oxygen Demand

The Measurement of BOD has long been the basic means of determining the degree of pollution in aquatic systems. The high the level of Biochemical Oxygen Demand the higher the level of contamination of a water body. The significantly positive correlation between the heterotrophic plate count and Total Coliform Count and Biochemical Oxygen Demand ($P < 0.05$)

indicate that all the water body analysed are rich in pollutants. It could be seen that the mean Biochemical Oxygen Demand for well water remained high relative to dry season because of increased pollution resulting from storm events.

Nitrate

Nitrate is a common contaminant in shallow ground water systems (Smith, *et al.,* 1994). It generally occurs in trace quantities in surface waters but may attain high levels in some ground waters. The significant difference observed between the water sources ($p<.0.05$) (Table 6) was not unexpected, in that well were observed to be more frequently contaminated relative to the tap and packaged water. Rainfall and storm events are some of the factors responsible for the increased Nitrate level observed, the relatively high Nitrate value observed for well water during the dry season is because of the decreased water volume thus increased concentration of inorganic ions such as NO_3 and PO_4. For both the well and tap waters the value of Nitrate surpassed the WHO drinking water guidelines, this high concentration of Nitrate is indicative of pollution, thus accounting for significant correlation between Total coliform count and Heterotrophic plate count. Previous studies have shown that high concentration of Nitrates cause methaemoglobinaemia in infants when they are fed with this water and that the mortality risks is very high when the NO_3 concentration exceeds 10mg/l of NO_3 – N (WHO 1996[b], Tredoux, 2002).

The concentration of NO_3 observed for tap water which ought to have been treated may be attributed to non-treatment or even leaky water pipe joints that traversed municipal drainage system.

The response of the *Escherichia coli* Isolates from the analyzed water sources (Table 13) indicated strong resistance to amoxacilin with 50% (30) of the isolates showing resistance to it, though a strong antibiotic, this may be the result of indiscriminate use of ampicilin and amoxacilin by people in most cases without prescription, this may be responsible for the development of plasmid mediated resistance to antibiotics. The sensitivity of the isolates to chloramphenicol, sparfloxacin, ciprofloxacin, augmentin, gentamycin, perfloxacin, tarivid and streptomycin is not unexpected because they all belong to the β-lactam class of antibiotics known for their broad spectrum of activity against a wide range of bacteria particularly Enterobacteriaceae. This is in agreement with findings by Edberg, *et al.*, (1986) that environmental isolates were susceptible to most antibiotics because resistance transfer (factor) transfer is thought to be much less efficient in the environment where microorganism are widely separated by distance as compared with human intestine where there may be as 10^{11} /g bacteria resistant to antibiotics. Besides, the relative cost of these antibiotics makes indiscriminate use almost impossible, thus minimizing the problem of plasmid mediated resistance.

Table 14 shows the responses of *Salmonella* species isolated from the various water sources to different antibiotics, it was observed that 48.7% (20)

of the isolates were resistant to Septrin while 46.3% (19) were resistant to Perfloxacin, this may be the result of plasmid mediated resistance, significant susceptibility was shown to Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxicillin, Augmentin, Gentamycin, Perfloxacin, Tarivid and Streptomycin all of which are broad spectrum antibiotics belonging to the β-lactam class. The susceptibility 0f *Salmonella* to amoxicillin contradicts studies conducted by Guiltermo, *et al.* (2000). And Gazouli, *et al.* (1998) who reported widespread appearance of *Salmonella* species resistant to antibiotics such as Cotrimoxazole and Ampicillin that have long been used to treat salmonellosis. Gentamycin and Chloramphenicol have been found to be effective against *Salmonella* and *Shigella* organism which corroborate the findings by Urio, *et al*. (2001)

Table 15 shows the responses of *Shigella* species isolates to antibiotics. It was seen that 62.5% (25) were found to show resistance to Septrin while 45% (18) were found to be resistant to Amoxacilin, most probably this may be plasmid mediated. The Significant susceptibility observed for Sparfloxacin, Ciprofloxacin, Gentamycin, Perfloxacin and Streptomycin may be the result of potency of these classes of β-lactam antibiotics.

Conclusion/Recommendations

The water used for public consumption is unsafe for drinking, because none of the waters sampled met the international standards for drinking water.

The presence of indicator bacteria in the waters calls for reassessment of water treatment methods particularly for tap and packaged water. As for well water, the siting of all the wells, their construction and condition was poor, thus there is the need for construction of the wells far away from municipal sewage and drainage systems, provision of protective covering can also minimize the possibility of contamination especially during rainfall, and storm events. Although the susceptibility pattern of the isolates from the environmental waters didn't show multi drug resistance there is the need for proper use of antibiotics.

There is the need to develop cheap and effective small-scale water disinfection methods for making well water safe for drinking, thus the appropriateness of water storage, solar water disinfection, boiling and chlorination as points of use (small-scale) methods for making well water safe for drinking. Hand washing before processing and packaging water particularly packaged can lead to a significant reduction in water related gastroenteritis which is in agreement with the findings that hand washing with soap can lead to a marked reduction in diarrhoea without improving water quality even among malnourished children who are at increased risk of death from diarrhoea. However access to safe water remains a prerequisite for maintaining hygiene and to further reducing diarrhoea disease, 88% of which is attributed to unsafe water supply, inadequate sanitation and hygiene (Bartram, 2004).

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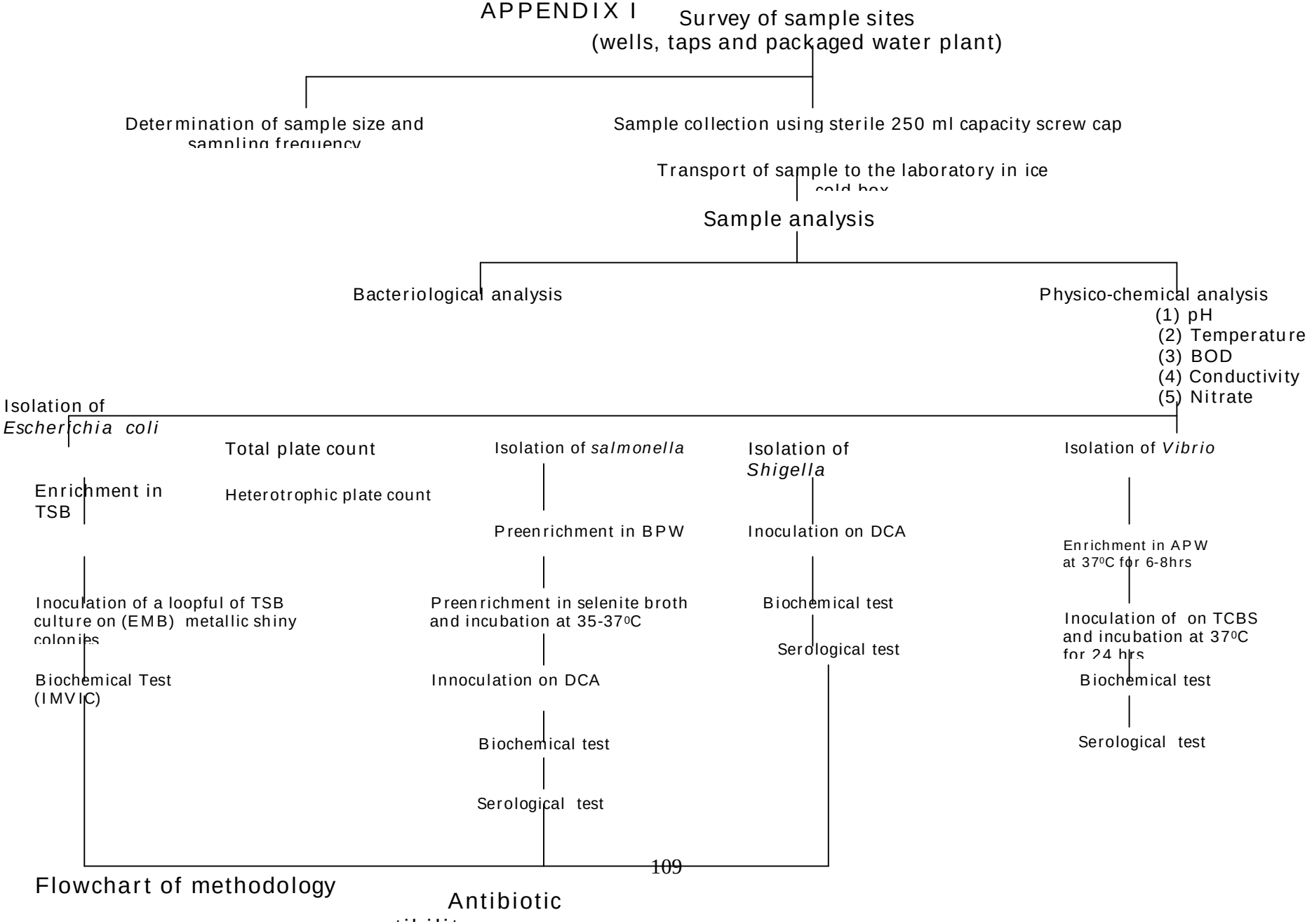
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APPENDIX I



Flowchart of methodology

APPENDIX II

Microbial pathogens linked to drinking water or recreation water contact

Organism	Disease	Transmission	Clinical features
Bacteria			
Vibrio cholerae	Cholera	Drinking water	Watery diarrhoea may be severe
Salmonella spp	Salmonellosis	Occasional outbreaks with drinking water	Diarrhoea, colicky, abdominal pain and fever
Salmonella typhi	Typhoid	Drinking water	Fever, malaise and abdominal pain with high mortality
Shigella spp	Shigellosis (Bacillary dysentery)	Both drinking and recreational water	Diarrhoea frequently with blood loss
Campylobacter spp	Campylobacteriosis	Both drinking and recreational water	Diarrhoea frequently with blood loss
Enterotoxigenic E. coli		Drinking water	Watery diarrhoea
Enterohaemorhagic Escherichia coli		Drinking water and recreational water contact	Bloody diarrhoea and haemolytic uraemic syndrome in children
Yersinia spp	Yersiniosis	Drinking water	Fever, diarrhoea and abdominal pain
Francisella tularensis	Tularaemia	Drinking water	Typhoid-like or mucoutaneous with suppurative skin lesions
Helicobacter pylori		Drinking water	Gastritis that can progress to gastric cancer
Mycobacterium spp (Not. *M. tuberculosis*)	Various	Potable water systems in hospitals, some recreation	Varies, includes respiratory disease, wound infections, skin disease
Viruses			
Hepatitis A and hepatitis E viruses	Viral hepatitis	Drinking and recreation water contact	Hepatitis
Various, esp. Norwalk-like viruses	Viral gastroenteritis	Drinking and recreational water contact	Vomiting and diarrhoea
Enteroviruses	Various, including	Drinking and recreational water	Various

	poliomyelitis	contact	
Microbial pathogens linked to drinking water or recreation water contact			
Organism	**Disease**	**Transmission**	**Clinical features**
Helminthes			
Schistosoma spp	Schistosomiasis	Contact with surface water infected with free swimming cercariae	Urinary and intestinal damage bladed cancer
Dracunculus medinensis	Dracunculiasis	Drinking water	Painful ulcers on lower limbs and feet
Protozoa			
Giardia duodenalis	Giardiasis	Faecal oral spread through drinking water or recreational water	Diarrhoea pain, weight loss and failure to thrive
Cryptosporidium parvum	Cryptosporidiosis	Faecal oral spread through drinking water or recreational water	Diarrhoea, often prolonged
Cyclospora cayetanensis	Cyclosporiasis	Faecal oral spread through drinking water	Diarrhoea, may be severe dysentery
Toxaplasma gondi	Toxoplasmosis	Drinking water contaminated by feline animals	Glandular fever, foetal damage in pregnant women
Free-living amoeba	Amoebic meningoencepalitIs	Aspiration of infected surface water into nose	Fatal encephalitis
Entamoeba histolytica	Amoebiasis	Faecal-oral spread through drinking water	Dysentary with diarrhoea may be severe
Algae			
Cyanobacteria	Various	Toxins in drinking water or direct contact with surface blooms	Dermatitis, hepatitis, respiratory symptoms, potentially fatal
Pfessteria psicicida	Estuary-associated syndrome	Toxins in water	Respiratory and eye irritation, deficiencies in learning and memory and acute confusional states

APPENDIX III

Antimicrobial Inhibition Zone Size Interpretative Chart

			Diameter of Zone of Inhibition in mm			
Antimicrobial agent	**Symbol**	**Disk content (µg)**	**Resistant mm or less**	**Intermediate (mm)**	**Sensitive (mm)**	**Quality control limit**
Amoxacilin	Amp.	25	13	14 – 16	17	18 – 24
Sparfloxacin	Cef	20	14	15 -17	18	20 – 26
Chloramphenicol	Chl	10	12	113 – 17	18	21 – 27
Ciprofloxacin	Cip	5	15	16 – 20	21	30 – 40
Cotrimoxazole	Cot	50	10	11 - 15	16	23 – 29
Gentamycin	Gen	10	12	13 – 14	15	19 – 26
Perfloxacin	Pef	30	12	13 – 16	17	28 – 35
Tarivid	Ofx	10	13	14 – 18	19	22 – 28
Streptomycin	S	30	14	15 – 16	17	20 – 25
Augmentin	Aug	30	15	16 – 20	21	30 – 40

* Adapted from National Committee for Clinical Laboratory Standards (NCCLS) Zone Size Interpretive Chart (2002)

* Based on results obtained using Mueller – Hinton Agar and *E. coli* (ATCC 25922) as positive control

APPENDIX IV

Characteristics of water sources and water standards

Characteristics	Typical quality lowland River	ECa Guide limits for surface waters used as raw water for drinking water **	WHO Guidelines for drinking water (Action level)
pH	6	5.5 – 9.0	6.0 – 8.5
Temperature		22	
Conductivity	700	1000	
Total dissolved solids			1000
Total suspended solids			25
Chloride	50	200	250
Nitrate-Nitrogen	2		10
Biochemical oxygen demand	4	< 5	
Phosphate-phosphorous		1[b]	0.05[b]
Total coliforms	2.0×10^4	5.0×10^3	< 1
Faecal coliforms[C]		2.0×10^3	< 1
Heterotrophic count			≤ 500

KEY

*Adapted from Tebbut (1990) and WHO (1996[a])

** Raw water for normal full physical and chemical treatment with disinfection

[a] Abbreviations:EC, European Community: WHO, World Health Organisation

[b] Limits set by the American Food and Drug Administration (FDA)

[c] WHO Standard for water used in irrigation of food crops consumed raw,<1000 faecal coliforms/100ml

APPENDIX V

Typical pH ranges in different waters

Water type	pH
Unpolluted surface waters	6.5 – 8.5
Polluted waters	3.0 – 12.0
Unpolluted rainwater	4.6 – 6.1
Acidic rainwater	2.0 – 4.5
Sea waters	7.9 – 8.3
Swamp waters	5.5 – 6.0
Ground waters	≤ 2.0 – 12.0
Mine drainage waters	1.5 – 3.5
Industrial and municipal waste waters	≤ 1.0 - ≥ 12.0

Conductivity of some types of water

Water type	**Conductivity** (μs/cm)
Freshly distilled water	0.5 – 2
Aged distilled water	2 – 4
Rain water	3 – 60
Drinking water	50 – 1500
Waste water	> 10,00

Classification Of Surface Water Quality Based On Biochemical Oxygen Demand values

Degree of pollution	**BOD_5 ($mgO_2\ L^{-1}$)**
Very clean	< 1.0
Clean	1.1 – 1.9
Moderately polluted	2.0 2.9
Polluted	3.0 3.9
Very polluted	4.0 -10.0
Extremely polluted	> 10

Source: Radojevic and Bashkin Practical Environment Analysis