BACTERIOLOGICAL ANALYSIS OF DRINKING WATER FROM SELECTED SOURCES WITHIN GUSAU METROPOLIS, ZAMFARA STATE, NIGERIA

BY

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ADM. NO: 1410301005

NOVEMBER, 2018

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A PROJECT

SUBMITTED TO THE

DEPARTMENT OF BIOLOGICAL SCIENCES

FEDERAL UNIVERSITY GUSAU

in partial fulfillment of the requirements

for the award of Degree of

BACHELOR OF SCIENCE

MICROBIOLOGY

NOVEMBER, 2018

DECLARATION

I hereby declare that this project is written by me and it has not been presented before in any institution for a Bachelor Degree except for quotations and summaries which have been duly acknowledged.

Babangida Abubakar

11/12/2018
Date

CERTIFICATION

This project entitled "Bacteriological analysis of drinking water from selected sources within Gusau Metropolis, Zamfara State, Nigeria" meets the regulation governing the award of Bachelor of Science of the Federal University Gusau, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to my father Alh. Abubakar Tambari Gidangoga for his caring and support, and to my beloved mothers Hajiya Hafsat Abubakar Tambari Gidangoga, and Hajiya Mariya Abubakar Tambari Gidangoga.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank God, Almighty for awarding me the opportunity, courage, ability, and wisdom to carry out this research. Indeed His mercies are new every morning, to Him be the Glory.

I would like to show my great appreciation to my project supervisor Malam Naibi Mohammed that worked tirelessly toward the success of this research, may Allah reward you abundantly Ameen.

I would also like to acknowledge and extend my heart felt gratitude to my beloved parents, Alh. Abubakar Tambari Gidangoga, Hajiya Hafsat Abubakar Tambari and Hajiya Mariya Abubakar Tambari Gidangoga for their endless support toward my education may God reward you with the most beautiful garden (Aljanna Firdausi) Ameen.

I wish to express my sincere gratitude to my brothers like Alh. Mustapha Abubakar, Alh. Samaila Abubakar, Late Mu'azu Abubakar, Kabiru Abubakar, Mannir Abubakar, Umar Abubakar, Mudassiru Abubakar and my sisters Samira Abubakar, Maryam Abubakar, Aisha Abubakar, and my lovely daughter Amina (Ikhram) Buhari to mention but few that contributed directly or indirectly, May God almighty reward you all.

In addition, I would like to express my special gratitude to my friends Babangida Abubakar kurya madaro, Abubakar Antaru Ashara, Aminu Usman, Shamsu Lawal Bnaira, and my best friends Adam Hamis Nasidi, Akilu Abdullahi Nazaure, Nasiru Abubakar and Salim Ahmad Ibrahim, May almighty Allah help you in all your endeavours, Ameen.

Finally, I would also like to show my great appreciation to my Head of Department Dr. (Mrs.) Sa'adatu Abdullahi Shinkafi, Departmental Administrative Officer, Aliyu Badamasi. And the entire staff of the Department of Biological Sciences that contributed directly or indirectly toward the completion of my research work, May almighty Allah reward you all Ameen.

TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

This research was aimed at determining bacteriological analysis of water from selected sources within Gusau Metropolis, Zamfara State, Nigeria. The water used are tap water, borehole water, and well water. A number of sample of six (6) was obtained from each source. The method used include spread plate, standard plate count, and biochemical test methods. The result indicated that for tap water Madawaki had the lowest count of coliform (1.0×10^5) cfu/ml, while Tudun wada had the highest count of coliform (6.1×10^5) cfu/ml. For borehole water Sabon gari had the lowest count of coliform (2.0×10^5) cfu/ml and Galadima had the highest count of coliform (8.0×10^5) cfu/ml . for well water Tudun wada had the lowest count of coliform (1.7×10^5) cfu/ml and Madawaki had the highest count of coliform (5.0×10^5) cfu/ml.

CHAPTER ONE

INTRODUCTION

The bacteriological Analysis of water is performed routinely by water utilities and many governmental agencies to ensure a safe supply of water for drinking, bathing, swimming and other domestic and industrial uses. The examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, particularly those that cause typhoid fever and bacillary dysentery. Since human faecal pathogens vary in kind (viruses, bacteria, protozoa) and in number, it would be impossible to test each water sample for each pathogen. Instead, it is much easier to test for the presence of nonpathogenic intestinal organisms such as *E. coli*. *E. coli* is a normal inhabitant of the intestinal tract and is not normally found in fresh water. Therefore, if it is detected in water, it can be assumed that there has been faecal contamination of the water. In order to determine whether water has been contaminated by faecal material, a series of tests are used to demonstrate the presence or absence of coliforms (Agunwamba, 2000).

Water has remained one of the prized natural resources of any nation and it occupies a permanent position among urban and rural dwellers. Water is the most important compound in the world; over 90% of the weight of any cell is composed of water and the chemical reaction associated with life is based on this compound (Agunwamba, 2000).

Water is a transparent and nearly colorless, odorless, and tasteless chemical substance that is the main constituent of Earth's streams, lakes, and oceans, and the fluids of most living organism. It covered about 70 percent of the human body, Its chemical formula is H_2O, meaning that its molecule contains one oxygen and two hydrogen atoms that are connected by covalent bonds. Strictly speaking, water refers to the liquid state of a substance that prevails standard ambient temperature and pressure; but it often refers also to its solid state (ice) or its gaseous state (steam or water vapor). It also occurs in nature as snow, glaciers, ice packs and ice bergs, clouds, fog, dew, aquifers, and atmospheric humidity. Water covers 71% of the earth's surface (Agunwamba, 2000).

1.1 Importance of Water

i. Water Helps Your Body Remove Waste:

Adequate water intake enables your body to excrete waste through perspiration, urination, and defecation. The kidneys and liver use it to help flush out waste, as do your intestines. Water can also keep you from getting constipated by softening your stools and helping move the food you've eaten through your intestinal tract (Edzwald, 2013).

ii. Water Prevents You From Becoming Dehydrated:

Your body loses fluids when you engage in vigorous exercise, sweat in high heat, or come down with a fever or contract an illness that causes vomiting or diarrhea. If you're losing fluids for any of these reasons, it's important to increase your fluid intake so that you can restore your body's natural hydration levels (Edzwald, 2013).

iii. Water Transports substances and Metabolisms:

Water is essential for proper digestion, nutrient absorption and chemical reactions. The carbohydrates and proteins that our bodies use as food are metabolized and transported by water in the bloodstream (Edzwald, 2013).

1.2 Statement of the Problems

Serious ill-health can be caused by water becoming contaminated by faeces being passed or washed into rivers, streams, pools or being allowed to sieve into wells and bore-holes. Supplies of drinking water contaminated with sewage or excreted matter from man and animals cause diseases such as typhoid fever, cholera, diarrhoea, compylobacteriosis, amoebiasis and even helminthiasis.

The bacteriological analysis of water can confirm whether a water supply has been faecally contaminated.

1.3 Justification

Presence of microorganisms especially pathogenic ones cause contamination of the water which would lead to water borne diseases, therefore this research was conducted in other to identify which source is safer for human consumption and also to discourage the members of the community from drinking it.

1.4 Aim of the Study

To evaluate the bacteriological quality of Borehole water, Well water, and Tap water within Gusau Metropolis.

1.5 Objectives of the Study

1. To isolate and identify bacteria in water samples.
2. To estimate the number of coliforms present in water samples.

1.6 Hypothesis

H_0= there is no presence of coliform bacteria in the water samples used.

H_o= there is low coliform load in the water samples examined.

CHAPTER TWO

LITERATURE REVIEW

The health of any community fully depends on the accessibility of adequate and safe water. Hence, water is predominantly essential for life, health and for human self-respect. Therefore, in addition to community health benefits, all people have the right to safe and adequate water retrieved in equitable manner for drinking, cooking, personal, and domestic hygiene. In this case, both adequacy and safety of drinking water are equally important to reduce the incidence of water-related & water borne health problems especially diseases like diarrheal (Bharti and Katyal 2011).

A possible contamination source that carries threats to drinking water quality are open field defecation, animal wastes, plants, economic activities (agricultural, industrial and businesses) and even wastes from residential areas as well as flooding situation of the area. Any water sources, especially older water supply systems, hand dug wells; pumped or gravity-fed systems (including treatment plants, reservoirs, pressure break tank, pipe networks, and delivery points) are vulnerable to such contamination. Particularly systems with casings or caps that are not water tight are most vulnerable. This is particularly true if the water sources are located close to surface runoff that might be able to enter the source. Additional way by which pollution reaches and enters a water supply system is through overflow or infiltration by flood water and inundation of water commonly contain high levels of contaminants (Haylamichael and Moges, 2012).

The fitness of community extremely depends on the availability of safe and adequate water for drinking, domestic use, and personal hygiene. If public health is to be improved and maintained through provision of safe and adequate water supply the major five key elements are vital which includes quantity, quality, cost, coverage, and continuity. Most of the time the occurrence of communicable diseases in the country is related with water supply conditions in the locality. Infectious diseases affected by changes in the water supply condition are categorized as follows (Addisie, 2012).

i. Those spread through drinking water (water borne diseases, such as typhoid, cholera, gastroenteritis etc.)

ii. Those transferred through aquatic vectors (water based diseases, such as schistosomiasis)

iii. Those spread by insects that depend on water (water related diseases, such as malaria and yellow fever)

iv. Those diseases produced by the lack of adequate water for personal hygiene (water washed diseases, such as scabies and trachoma.

Based on the morbidity records, there is still a high incidence of communicable diseases which most of the time is related to water supply conditions in the country among which about 60% of the top ten diseases are relate to poor quality and scarcity of household water consumption (UNICEF, 2008).

2.1 Coliforms as Indicator of Bacteriological Quality of Water

Coliforms are gram negative, rod shaped, non-spore forming bacteria that ferment lactose at 35-37^{0}C within 24-48 hours to produce acid and gas

(Nester *et al..*, 2001). Coliforms are facultative, anaerobic, gram negative, non-sporing, rod shaped bacteria that ferment lactose with gas formation within 48hrs at 35^{0}C, and are members of the family enterobacteriaceae. These bacteria make up about 10% of the intestinal microorganisms of humans and other animals and have found wide spread use as indicator organisms, the coliform group include *Escherichia coli, Enterobacter aerogenes* and *Klebsiella pneumoniae* (Willey *et al.*, 2011).

According to APHA (1998), coliform bacteria are commonly used indicator of sanitary quality of food and water. These bacterial groups are not normally the cause of serious illness, they are easy to isolate and culture and their presence is used to indicate the presence of other pathogenic organisms probably of faecal origin. These faecal pathogens could be bacteria, viruses or protozoa and many multicellular parasites. Typical genera of coliform are *Citrobacter, Enterobacter, Hafnia, Klebsiella, Serratia* and faecal coliform that consist of *Escherichia coli*. Due to impracticality to test water source for all pathogens related to water borne diseases as a result of complexity and time consuming nature (Lehloesa and Muyiwa, 2000) indicator organisms are used (Grabow *et al.*, 2000). The subgroups of coliform are the total and faecal coliform.

2.1.1 Total Coliforms

These are defined as aerobic or facultative anaerobic, gram negative, non-spore forming rod-shaped bacteria which ferment lactose and produce gas at 35^0C (APHA, 1998). Bacteria belonging to faecal coliform include those of faecal origin such as *Escherichia coli* while total coliforms are those bacteria that may come from other sources other than faecal origin such as *Klebsiella* spp., *Citrobacter* spp., *Serratia* spp. and *Enterobacter* spp. which are majorly obtained from water rich in nutrient, soil, decaying vegetation and drinking water with high levels of nutrients (WHO, 1996).

2.1.2 Faecal Coliforms

Coliforms are aerobic or facultativaely anaerobic, Gram negative, non spore forming, rods capable of fermenting lactose with production of acid and gas within 48hrs of being placed in appropriate media at 35^0c.

The coliforms group of bacteria is used as an indicator of pollution and includes many environmental species of bacteria such as *Escherichia coli, Klebsiella pneumonia, Enterobacter aerogenes, Citrobacter freundii and serratia liquifaciens* found in the soil, on fruits, leaves, grains and runoff water (Godfrey, 2003).

Coliform bacteria of faecal origin are referred to as faecal coliforms and grow at higher temperature (44.5^0c). The detection of this group of organisms which could be found in faeces of humans, animals and birds, indicate contamination from faecal sources (Godfrey, 2003).

According to Doyle and Erickson (2008), faecal coliform is defined as a facultative anaerobic, rod-shaped, gram negative, non-spore forming bacteria which produce acid and gas from lactose within 48 hours at 44 ± 0.5^{0}C. They appear in great quantities in the intestine and faeces of humans and animals, the presence of faecal coliforms in water often indicates recent faecal contamination, meaning there is greater risk that pathogens are present. Increased in their level offer a warning of water treatment failure, break in integrity of the distribution system and most possibly contamination with pathogens (Doyle and Erickson, 2008).

Faecal coliform bacteria are also known as thermotolerant coliform or presumptive *E. coli* (APHA, 1998). Coliform group includes organisms such as *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter*, which are not exclusively of faecal origin (APHA, 1998). *Escherichia coli* are mainly of faecal origin from birds, humans and other warm blooded animals (WHO, 1996). Hence, faecal coliform are considered to be a more specific indicator of the presence of faeces (Maier *et al.*, 2000). They are used to indicate unacceptable microbial water quality and are used as indicator in place of *E. coli* (SABS, 2001). Faecal coliform presence in a water sample shows the possible presence of other pathogenic bacteria such as *Salmonella* spp., *Shigella* spp., pathogenic *E. coli*, *Vibrio cholera*, *Klebsiella* spp and *Campylobacter* spp. associated with water borne diseases (DWAF, 1996).

2.1.3 *Escherichia coli*

It is the globally accepted indicator of faecal pollution in food and water (Edberg *et al.*, 2000). It is a gram negative, rod shaped bacterium and chiefly an inhabitant of the intestines of warm blooded animals and humans which is used to indicate recent faecal pollution of water samples (Rice *et al.,* 1991). The evaluation of potable water supplies for coliform bacteria is important in determining the sanitary quality of drinking water. High levels of coliform counts indicate a contaminated source, inadequate treatment or post treatment deficiencies (Mathew *et al.*, 1984). It has been reported that drinking water supplies have a long history of association with a wide spectrum of microbial infections (Grabow *et al.*, 2000).

2.1.4 Limit of Coliforms in Drinking Water

According to American Public Health Association, APHA (1998), the presence of total coliform bacteria is unacceptable because they are group of bacteria with common characteristics used to indicate unacceptable water quality. High level of coliform counts is usually an indication of water contamination. When non-coliform bacteria counts are in excess of 200 colonies/100ml of sample, they obscure or prevent the growth of coliform bacteria. When total coliform bacteria or high non-coliform bacteria counts are present in drinking water, such water needs to be checked for contamination problems (APHA, 1998).

The Total Coliform Rule (TCR), a National Primary Drinking Water Regulation (NPDWR) was published in New York in 1989 and became effective in 1990. The rule

mandated both a health goal (Maximum Çontaminant Level Goal, or MCLG) and legal limits (Maximum Contaminant Levels MCLs) for the presence of total coliform in drinking water. Environmental Protection Agency set the MCLG for total coliform at zero because there have been waterborne disease outbreaks in which research discovered very low levels of coliform, so any level show some form of health risk. Samples analyzed for either faecal coliform or *E. coli*, if one analysed sample is positive for *E. coli* faecal coliform, the system has an acute MCL violation (EPA, 2002). The MCLG for total coliform bacteria is zero. The MCL for total coliform was formulated as an indicator for potential presence of sewage in the water supply. Since the total coliform comprises faecal coliform and *Escherichia coli* and the *Escherichia coli* is a major inhabitant of humans and animals intestines. Presence of *Escherichia coli* or other faecal coliform in water is an indication that such water is actually contaminated with human sewage or animal waste. By convention that most indicator coliform bacteria are harmless, sewage may contain disease-causing organisms (EPA, 2002).

2.2 The Concept of Indicator Organisms

Faeces contain large number of organisms which include ***Escherichia coli, streptococcus faecalis***, (faecal streptococci) and ***clostridium perfringens***. The organisms form part of the normal flora of the intestinal tract (Cheesbrough, 2010).

A useful way, therefore of determining whether a water supply is faecally polluted and could possible contains enteric pathogens dangerous to health is to test for the presence of normal fecal organisms. To search directly in water sample for the presence of enteric pathogens is impractical for routine control purpose. Testing for normal fecal organisms

as indicator for faecal pollution is not easy to do, but also a reliable way of determining whether water is bacteriologically safe to drink. If no faecal bacteria are detected in water sample, it is probable that enteric pathogens (usually present in much smaller numbers) are also absent (Cheesbrough, 2010).

Criteria for an organism to be used as indicator organisms for the analysis of water should include the following:

i. Present in faecal contaminated water when enteric pathogens are present but in greater numbers.
ii. Incapable of growth in aquatic environment but capable of surviving larger than pathogens.
iii. Equally or more resistance to disinfection rather than pathogens.
iv. Easily and accurately enumerated.
v. Applicable to all types of water.
vi. Absent from non-contaminated water and exclusively associated with animals and human faecal wastes.
vii. Density of indicator should be directly correlated with the degree of faecal contamination (Ashbolt *et al*, 2013).

2.3 Water Borne Diseases

Many important human pathogens are maintained in association with living microorganisms other than humans, including many wild animals and birds. Some of these bacteria and protozoa pathogens can survive in water and infect humans. When water are used for recreation or are source of sea food that is consumed uncooked, an epidemic certainly exist (Prescott *et al.*, 2000).

2.3.1 Epidemic and Endemic Waterborne Disease

Poor water quality continues to be a major public health problem globally. According to the World Health Organization, diarrhoeal disease accounts for an estimated 4 % of the total global burden of disease measured in disability adjusted life years (DALYs) and around 1.8 million deaths every year. It has been estimated that almost 90% of that burden is attributable to unsafe water supply, sanitation and hygiene, mainly affecting children in developing countries. In developed countries, waterborne disease is no longer considered a constant threat. However, waterborne diseases have not been eradicated, and every year some waterborne outbreaks occur. Outbreaks caused by contaminated drinking water may have substantial public health impact and will cause large concern in the affected community (WHO, 2006).

In recent years, the importance of non-outbreak waterborne illness has gained renewed interest. The proportion of endemic gastrointestinal illness in the community that can be attributed to water is unknown. Probably, drinking water systems that fulfill the required standards can intermittently be contaminated by pathogens either through low-level

contamination of source water, inadequate water treatment or deterioration of water quality in the distribution system (Rice *et al.*, 1991). Although the concentrations of infectious organisms may be very low in these incidents, they may result in sporadic cases of illness that are not recognized or investigated as a possible outbreak (Craun and Calderon, 2006).

2.3.2 Water Borne Disease of Viral Origin

i. Hepatitis A (Infectious Hepatitis)

Usually is transmitted by faecal-oral contamination of food, drinks or shell fish that live in contaminated water and contains the virus in their digestive system (Prescott *et al.*, 2005).

Once in the digestive system, the viruses multiply within the intestinal epithelium, usually only mild intestinal symptoms. Occasionally, viraemia (presence of virus in the blood) occurs and the virus may spread to the liver. The viruses reproduce in the liver, enter the bile and are released in to the small intestine. This explains why feaces are so infectious (Prescott *et al.*, 2005).

ii. Poliomyelitis

Poliomyelitis, polio or infantile paralysis is caused by the polio virus, a member of the family Picornaviridae (Prescott *et al.*, 2005). The virus is very stable and can remain infectious for relatively long periods in food and water which are its main routes of transmission. Once ingested, the virus multiplies in the mucosa of the throat and/ or small intestine. From these sites, the virus invades the tonsils and lymph nodes of the neck and

terminal portion of the small intestine. Generally there are either no symptoms or a brief illness characterized by fever, headache, sore-throat, vomiting and loss of appetite. The virus sometimes enters the blood stream and causes viraemia. In minority of cases (less than 1%), the viraemia persist and the virus enters the central nervous system and causes paralytic polio (Prescott *et al.*, 2005)

2.3.3 Water Borne Diseases Caused by Protozoan Parasites

i. Giardiasis

Giardia lamblia is a flagellated protozoan that causes the very common intestinal disease Giardiasis. *Giardia lamblia* is worldwide in distribution, and it affects children more seriously than it does adult. In United State, this protozoan is the most common cause of epidemic water borne diarrheal disease (about 30000 cases yearly). Approximately 7% of the population are healthy carriers and shed cysts in their faeces. *Giardia lamblia* is endemic in child day care centers in the United State. Transmission is most frequent with cysts contaminated water supplies. Epidemic outbreaks have been recorded in wilderness areas, suggesting that humans may be infected from clean water with giardia harbored by rodents, beers, cattle or household pets. As many as 200million humans may be infected worldwide (Prescott *et al.*, 2000).

ii. Cryptosporidiosis

Cryptosporidiosis is caused by *cryptosporidium parvum* which is an intracellular parasite responsible for acute gastroenteritis and less frequently respiratory infection in humans. It is associated with giardia, the most commonly diagnosed gastro intestinal protozoan in

the world (Guy *et al.*, 2003)

Ninety percent (90%) of reported outbreaks of these pathogenic protozoans occur through water while 10% are related to food. Giardia and cryptosporidium have the potential for zoonotic transmission. Water borne outbreaks are associated with drinking water, wells, rivers, lakes and recreational swimming pools. The reported frequencies of occurrences of contamination of surface water with Giardia, cryptosporidium are from 60-96% in the United State and from 20-64% in Canada (Guy *et al.*, 2003).

Cryptosporidium parvum is a threat to water supplies because it is resistant to chlorine disinfections and the parasite is small and thus difficult to filter, and harbored by many animal species.

2.3.4 Water Borne Diseases of Bacterial Origin

i. Gastroenteritis

Escherichia coli is undoubtedly the best studied bacterium and the experimental organisms of choice for many microbiologist. It inhabits the colon of humans and other warm blooded animals, and it is quite useful in the identification of faecal contamination of water. *E. coli* circulate in the resident population, typically without causing symptoms due to the immunity afforded by previous exposure. Because many cells are needed to initiate infection, contaminated food and water are the major means by which they are spread (Prescott *et al.*, 2011).

Although the vast majority of *E. coli* strains are non-pathogenic members of the intestinal microbiota , some strains cause diarrheal disease by several mechanisms: six categories or strains of diarrheagenic *E. coli* are now recognized : *enterotoxigenic E. coli (ETEC)* , *enteropathogenic E. coli (EPEC), enteroinvasive E.coli (EIEC), enterohemorrhagic E.coli (EHEC), enteroaggregative E. coli (EAEC)* , and diffusely adhering *E. coli (DAEC)* (Prescott *et al.*, 2011).

ii. Cholera

Cholera is an acute diarrheal disease caused by infection of the intestine with Gram negative, comma-shaped bacterium *vibrio cholerae.* It is transmitted by ingesting food or water contaminated by faecal material from infected individuals. The infection is usually mild or without symptoms in most healthy adults but sometimes can be severe. The disease is characterized by profuse watery diarrhea, vomiting and leg cramps (Prescott *et al.*, 2011).

2.4 Water Pollution

Water pollution refers to the contamination of water bodies often as a result of human activities. In defining pollution, we generally look at the intended use of water, how far it departs from the norm, its effect on public health, its ecological impacts. From public health or ecological view, a pollutant is any biological, physical or chemical substances that in identifiable excess is known to be harmful to other desirable living organism. Water pollutants include excess amount of heavy metals, certain radioactive isotopes, faecal coliform bacteria, phosphorus, nitrogen, sodium and other useful (even necessary)

elements as well as certain pathogenic bacteria and viruses. In some instances, materials may be considered as pollutant to a particular segment of the population although not harmful to the segments. For example excessive sodium as a salt is not generally harmful, but it is to some peoples who must restrict salt intake for medical reasons (Prescott, 2000).

It is fundamental principle that the quality of water determines its potential uses. The major uses of water today are for agriculture, industrial processes, and domestic (household) supply. Water for domestic use must be free from constituents harmful to health, such as insecticides, pesticides, pathogens and heavy metals concentration, and should not damage plumbing or household appliances. The quality of water required for industrial purposes varied widely depending on the process involved.

Some process may require distilled water, others simply needed water that is not highly corrosive or that is free from particles that could clog or otherwise damage the equipment. Because most vegetation is tolerant to a wide range of water quality, agricultural water may vary widely in physical, chemical and biological properties (Botkin and Keller, 2000).

Many different processes and materials may pollute surface water or ground water. All segments of our society (urban, rural, industrial, and agriculture) may contribute to the problems of water pollution. Most of the sources result from runoff and leaks or seepage of water pollutants in to the surface water or ground water. Pollutants are also transferred by air and deposited in water bodies, increasing population also as well as placing more demands on our finite water resources As a result, it can be expected that sources of

drinking water in some location will degrade in future (Prescott, 2000).

2.4.1 Types of Water Contamination

According to Viman *et al.* (2010) point and non-point source are the two types of water pollution. Industrial impacts on water can be severe when toxic chemicals are dumped or accidentally spilled into water ways. This type of pollution is being called point source pollution. Industries must have special permits to discharge waste materials into waterways, usually having to first treat the water ways. Severe fines and penalties may result from non-compliance. Point source pollution has effects on ecosystems: Pollutants can come a specific source such as pipe that discharges used water or other materials from a factory into a water body such discharges can be harmful to the aquatic ecosystem and can affect the forest tree species surrounding the body of water, Pollutants can also come from large areas such as agricultural fields that have been covered with fertilizers or pesticide. Fertilizer and pesticides residue can run off or wash into streams and rivers or seep into soil contaminating underlying groundwater at the surface it can also reach trees, Pollutants can contaminate our drinking water source reduce oxygen levels which can kill fish and other wildlife (Viman *et al.*, 2010).

Non-point source pollution is a much bigger problem, it occurs when rainfall, snowfall, or irrigation runs over land or through the ground and picks up pollutants and deposits them in bodies of water. Toxic construction materials like paint, solvents, acids, and glues can also pollute water. In urban areas, rainfall that lands on non-permeable street, sidewalks and parking lots creates runoff, carrying pollutants into streams. Lawn and garden chemicals like herbicides, insecticides, and fertilizers can seep into groundwater or end up

in waterways. Toxic solvents, paints, oils and cleaners often get poured down the drain rather than being disposed properly (Viman *et al.*, 2010).

2.4.2 Sources of Microbial Contamination of Water

The most dangerous form of water pollution occur when faecal contaminants like *Escherichia coli* enter the water supply and also through the faecal-oral route of transmission. Microbial contaminants in water supply are the source of many diseases such as typhoid fever, cholera, bacillary dysentery, and so on; Examples of such microbial contaminants are *Salmonella* spp, *Shigella* spp, *Escherichia coli* (Edema *et al.*, 2001). Source of water contamination could have a wide effect on the community because it can introduce new pathogens in the home environment (Sobsey, 2002). Several studies have reported that the microbiological quality of water deteriorates after collection, during transport and during storage at the point-of-use due to secondary contamination factors (El Attar *et al.*, 1982). Due to the distances, unavailability and shortage of piped water supplies inside most households in many developing countries of the world; people revert to storage of their drinking water as an alternative means (Sobsey, 2002). Microbial contaminations within the home occur through numerous routes, the most important transmission routes include water, food, person-person contact, unhygienic behaviour (i.e. through faeces) and storage conditions increased the risk of contamination which can lead to infectious diseases (Roberts *et al.*, 2001).

2.5 Bacteriological Testing Of Water

Escherichia coli count is the most useful test for the detecting of faecal contamination of water supplies in water quality analysis. Two principal techniques are available for counting coliforms (Cheesbrough, 2010).

i. Membrane filtration techniques.

Membrane filtration test is a technique that, 100ml water sample or diluted samples is filtered through a membrane filter. The membrane, with coliform organisms on it, is then cultured on a pad of sterile selective broth containing lactose and indicator. After incubation, the number of coliforms colonies can be counted, this gives presumptive number of *Escherichia coli* in 100ml water sample (Cheesbrough, 2010).

ii. Multiple tube / most probable number (MPN) techniques.

Multiple tube / most probable number (MPN) technique is technique that 100ml water sample distributed (five 10ml amounts and one 50ml amounts) in bottles of sterile selective culture broth containing lactose and an indicator. After incubation the number of bottles in which fermentation with acid and gas production has occurred is counted. The lactose fermented by the coliform in the water. By reference to probability tables, the most probable number of coliforms in the 100ml water sample can be estimated (Cheesbrough, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.0 Study Area

The research was conducted in Gusau Zamfara State..

3.1 Methods

The methods and materials used in this research are designed to meet its objectives. Equally, coliforms identification was carried out using fecal coliform organisms as indicator, spread plate method and standard plate count was used in this research.

3.1.1 Sample size

The samples were collected from different electoral wards of Gusau, six samples was collected from each ward, which make total of about 30 samples.

3.1.2 Collection of water samples

Water samples for microbial analysis were collected in sterile bottles and care was taken to prevent accidental contamination of the sterile bottle or water sample during sampling and transportation to the water testing laboratory.

3.1.3 Collecting a Sample from a Tap

Procedure

i. Any external fittings from the tap were removed.

ii. The outside nozzle of the tap was disinfected with flame to remove any dirt/grease which has been collected.

iii. The tap was turned on full, and the water allowed to run for a while to clear the pipes. This allows time for the nozzle of the tap to be flushed and any stagnant water in the service pipe to be discharged.

iv. The sample bottle was filled from a gentle flow of water, and the cap of the bottle was replaced.

3.1.4 Collecting a Sample from a Borehole

Procedure

The tap was continuously operated for at least 5 minutes, letting the water flush the fittings and pipes. The outside nozzle of the borehole tap was flamed to remove any grease which might have accumulated on it. The tap was turned on fully and water allowed running for one minute to ensure that the nozzle of the tap was flushed and any stagnant water in the service pipe discharged. The sample was collected aseptically by allowing the water from the pump to flow directly into the sterile bottle. Then the bottle cap was carefully replaced and covered.

3.1.5 Collecting a Sample from a Well

Procedure

i. The sterile sample bottle was tied on to a length of rope or strong string.

ii. The cap was removed and lower the bottle in to well.

iii. When no more air bubbles rise to the surface, the bottle was raised out of the well and the cap was replaced.

3.2 Media Preparation

3.2.1 Nutrient Agar

Nutrient agar medium was prepared according to manufacturer's instruction by weighing twenty eight (28g) grams of the powder using weighing balance, which was transferred into conical flask containing 1000ml of distilled water, covered with cotton wool and aluminum foil, shaken thoroughly and heated to dissolve completely. The solution was further autoclaved at 121^0C for 15 minutes, allowed to cool to 47^0C and poured into sterile plates to solidify (Cheesbrough, 2010).

3.2.2 Eosin Methylene Blue (EMB) Agar

Forty (40) grams of eosin methylene blue agar was dissolve into 1000mls of distilled water, it was then heated and process for autoclaving at 121^0C for 15 minutes, the media was allowed to cool and dispense into clean petri dishes to solidify. The solidified EMB plates were then subjected to sterility test by incubating inoculated and un-inoculated plates together at 37^0C for 24 hours (Cheesbrough, 2010).

3.3 Analysis of Water Samples.

This was done using spread plate method

3.3.1 Spread Plate Technique

Spread plate technique is the method of isolation and enumeration of microorganisms in a mixed culture and distributing it evenly. The technique makes it easier to quantify bacteria in a solution.

3.3.2 Procedure

Serial dilution of all the samples was prepared on to the center of an agar plate.0.1ml of sample was inoculated The glass spreader was flame over the Bunsen burner The sample was spread over the surface of agar using the sterile glass spreader The plate was incubated at 37°C for 24 hours to observe colonies The colony forming unit (CFU) of the sample was counted manually And then the colonies obtained were subcultured on a plate containing eosin methylene blue agar (EMB).

3.3.3 Coliform count

Standard total coliform count was performed by inoculating the sample in to the plates containing eosin methylene blue agar and the plates were incubated at 37^0C for 24hours to observe colonies.

3.4 Gram staining

Gram staining procedure was carried out from the subculture of EMB positive plate above as follows:

A thin smear of a colony was made with a drop of sterile distilled water on a previously cleaned microscope slide and allowed to air dry. The smear was heated, fixed by passing over the Bunsen burner flame 3 times. The smear was flooded with crystal violet dye and allowed to stand for one minute, after which the dye was rinsed off with slow running tap water. Gram iodine was added to cover the smear for another one minute and rinsed with slow running tap water. The smear was then decolorized with acetone briefly and then the smear was counter stained with safranin and allowed to stand for another one minute before rinsing with slow running tap water.

The stained smear was allowed to air dry and observed using oil immersion objective lens x100. The purple colour indicate Gram positive bacteria while pinkish colour indicate Gram negative, Gram negative spore forming rods indicate fecal coliform.

3.5 Biochemical Test

Biochemical tests are the tests used for the identification of bacteria species based on the differences in the biochemical activities of different bacteria. The types of biochemical reactions each organism undergoes act as a thumbprint for it is identification.

i. Indole Test

The colonies from the subculture nutrient agar slant was taken and inoculated onto prepared peptone water (5ml) in Bijou bottles and incubated at 37^0C for 24hrs. After 24hrs of incubation 2-3 drops of Kovac's reagent was added and gently shaken. A positive

reaction was indicated by the development of rose pink colour in the reagent layer above the broth within one minute. Negative reactions was indicated when the indole reagent retained its yellow colour. The test is based on the principle that if an organism elaborates the enzyme tryphtophanase, it should be indole positive when grown in a medium containing the amino acid tryptophan. Tryptophan is usually present in the digest of various proteins of animals and plants provided the process of digesting the protein does not destroy it. The enzyme tryptophanase split tryptophan into indole, pyruvic acid and ammonia. However the formation of indole is inhibited in the presence of fermentable sugar.

In addition of kovac's indole reagent, the indole compound reacts with p- dimethyl amino benzaldehyde to form a red compound. P- dimethylaminobenzaldehyde is the active agent of the kovac's indole reagents.

ii. Methyl Red – Voges-Proskauer Test

5ml of methyl red- Vogues Proskauer Test (MR-VP) broth was inoculated with subculture Colonies and incubated for 48-72hrs at 37^0c. After this period of incubation, 1ml of the broth was transferred to a small serological tube, to this small quantity, 2-3 drops of methyl red was added. The development of red colouration on addition of the indicator signified a positive result while a yellow colour shows a negative result.

To the rest of the broth in the original tube, 15 drops of 5% alpha- napthol in ethanol was added followed by 5 drops of 40% potassium hydroxide (KOH). This was mixed by shaking, the caps was placed in a slopping position. The development of a red colour

[illegible] within 1 hour would indicate VP positive test. No colour [illegible] VP negative tests.

[illegible] Test

[illegible] agar slant was prepared in bijou bottles in accordance with [illegible] and positive colonies obtained from the subculture NA slants [illegible] the slants and incubated at 37°c for 24-48hr with the bijou bottles [illegible]. The development of a deep blue colour indicated positive result. The [illegible] is based on the fact the simmon's citrate agar medium contains the [illegible] bromothymol blue, and sodium citrate as the sole carbon source. Only [illegible] can metabolize citrate would grow in the medium since the medium has no [illegible] that can be readily used for growth.

[illegible] of citrate creates an alkaline condition in the medium and the [illegible] changes to an alkaline colour blue. The neutral colour of bromothymol blue is [illegible] the uninoculated medium and medium inoculated with citrate negative [illegible] would remain green.

CHAPTER FOUR

RESULTS

The analyses were carried out for all the thirty (30) samples using fecal coliforms as indicator organisms. The results obtained therefore are presented in the following tables.

Table 4.1: Number of coliforms obtained per ml of each sample.

Tap water	CFU/ml
MY	5.0×10^5
TW	6.1×10^5
SG	5.0×10^5
GD	1.8×10^5
MDW	1.0×10^5

The table portrays sample MDW as having the lowest count of coliform per ml and sample and TW with the highest coliform count per ml.

KEYS:

MY= MAYANA

TW= TUDUN WADA

SG= SABON GARI

GD= GALADIMA

MDW= MADAWAKI

Table 4.2: Number of coliforms obtained per ml of each samples,

BOREHOLE	CFU/ml
SB	2.0×10^5
GB	8.0×10^5
MDB	7.0×10^5

The table shows that sample SB as having the lowest count of coliform per ml and sample GB with the highest count of coliform count units per ml.

KEYS:

MY= MAYANA

TW= TUDUN WADA

SG= SABON GARI

GD= GALADIMA

MDW= MADAWAKI

Table 4.3: Number of coliforms obtained per ml of each samples,

WELL	CFU/ml
MW	3.0×10^5
TW	1.7×10^5
SW	4.0×10^5
GW	2.0×10^5
MDW	5.0×10^5

The data from table above indicates that sample TW as having the lowest count of coliform per ml and sample MDW with the highest count of coliform count units per ml.

KEYS:

MY= MAYANA

TW= TUDUN WADA

SG= SABON GARI

GD= GALADIMA

MDW= MADAWAKI

CHAPTER FIVE

DISCUSSION

From the results obtained above, for tap water, (Table 4.1) Madawaki, generally had low counts of coliforms (1.0×10^5) cfu/ml, SG, GD, MY, had cfu/ml between (1.8×10^5-5.0×10^5) while TW had higher coliform count (6.1×10^5) cfu/ml. which failed to meet the standard set by WHO and NAFDAC guidelines. This could be ascribe to remote faecal contamination which may have resulted from seepage of faecal material into the sources from latrines or septic tanks which are not located far enough (Agunwanba, 2000).

For Borehole water (Table 4.2) SG, had the lowest counts of coliforms (2.0×10^5) cfu/ml, MDW, had (7.0×10^5) cfu/ml, while GD, had the highest count of coliforms (8.0×10^5) cfu/ml. which did not meet the standard set by WHO. This could be as a result of location of the water sources in accordance with world health organization recommendation of at least 30 meter away from latrines so as to prevent seepage of faecal material into the sources (Agunwanba, 2000).

For well water (Table 4.3) TW, had the lowest count of coliforms (1.7×10^5) cfu/ml, MY, GD, SG, had coliform count between (2.0×10^5-4.0×10^5) cfu/ml while MDW, had the highest count of coliforms (5.0×10^5) cfu/ml. which exceeded the recommended limit set by WHO. this could be ascribed to intermittent faecal contamination which may have result from continuous seepage of faecal materials into the water sources from latrines or septic tank that are very closed (Agunwanba, 2000).

TW, (Tap water), GD, MDW, (Borehole water) and MDW, (well water) had high faecal coliform count which are mostly *Klebsiella spp* and *Escherichia coli* which may have entered the water from the soil, As such failed to meet the WHO drinking water standard of maximum 100 plate count ml^{-1}(1 X 10^2cfu/ml) and therefore unsuitability of these samples for human consumption.

The coliforms may also originated from the intestinal tract making the water unfit for human consumption. Wagner and Lanolx (2000) reported that, in most rural areas of developing countries, many ground water supplies are contaminated from the source like seepage pits, septic tanks which are located in their vicinities.

Some of the coliforms count obtained could be due to the fact more recently, viable microorganisms have been discovered down to a depth of 1000 to 1200 meters, and many pollutants that reach the subsurface will persist and may affect water quality of ground water for extended periods (Prescott, 2005). The presence of this bacterial counts and contamination with coliform bacteria could be attributed to poor hygienic practices observed in filtering and purification of the water in the water board. Therefore, there is very high tendency of having high number of pathogenic organisms which could lead to water borne diseases. This is in accordance with the result of Ashbolt (2004), who has reported that the poor personal hygiene of handlers and poor environment contribute significantly to the level of contamination of package water in developing countries. Due to the coliform count and the presence of *Escherichia coli* (a faecal coliform) in the samples, it is however failed to meet the WHO drinking water standard of zero coliform per 100mls and this underline the unsuitability of these samples for human consumption

going by WHO and NAFDAC recommendations and guidelines. This is because *Escherichia coli* is a faecal indicator bacterium indicating faecal contamination and presence of pathogenic organisms. This is in accordance with the result obtained by Olaoye and Onilude (2009) who reported poor microbiological quality of drinking water in Gaza which deviates from recommended limits of World Health Organization (WHO). However, absence of faecal coliform in the rest of the samples with zero coliform per 100mls could be attributed to better hygienic practices.

Detection of coliforms in drinking water show the danger of faecal pollution and consequent hazard of contracting diseases through pathogenic organisms. Disease causing organisms are transmitted via drinking water predominantly of faecal origin, but the coliform count would not constitute much concern without the detection of *Escherichia coli.* It has been reported that a typical enteropathogenic *Escherichia coli* is a leading cause of infantile diarrhoea in developing countries (Trabulsi *et al.*, 2002). The poor bacteriological quality of water samples recorded in this research project have been observed in developing countries by some researchers. In a study carried out by Obiri *et al* , (2003) on microbiological evaluation of drinking water in Ghana, the author reported that the water samples were of poor quality. According to them, the occurrence of these indicator organisms in water constitutes a serious threat to the community and they called for strict observance of good manufacturing practices by processors and handlers.

5.1 Conclusion

From all the water samples collected and analyzed *Escherichia coli* and *Klebsiella* species were isolated and identified, for tap water, Madawaki, generally had low counts of coliforms (1.0×10^5) cfu/ml, while TW had higher coliform count (6.1×10^5) cfu/ml. which failed to meet the standard set by WHO (Zero Coliform per 100ml).

For Borehole water SG had the lowest counts of coliforms (2.0×10^5) cfu/ml, while GD, had the highest count of coliforms (8.0×10^5) cfu/ml. which did not meet the standard set by WHO (Zero Coliform per 100ml) .

For well water TW had the lowest count of coliforms (1.7×10^5) cfu/ml, while MDW, had the highest count of coliforms (5.0×10^5) cfu/ml. which exceeded the recommended limit set by WHO (Zero Coliform per 100ml) .

This indicate the degree at which the faecal contamination occurred to routine analysis of borehole, tap and well water and may consider the water to be unsafe for human consumption however there were differences in the degree of the presence of faecal coliforms in these water sources. Though most untreated water supplies contain faecal bacteria but in the case of protected ground water such as tube wells, seal wells and boreholes are possibly achieved very low level of contamination that suggested guidelines described by World health organization that:

i. Zero *Escherichia coli* are excellent.
ii. 1-10 *Escherichia coli* is acceptable but need regular sanitary checkup.

iii. 10-50 *Escherichia coli* and above are unacceptable and grossly polluted as previously shown by most of the water sources which required correct structural faults, repairs and necessary sanitary measures.

5.2 Recommendations

i. The need to boil and filter water before consumption should be emphasized to prevent outbreak of waterborne disease.

ii. Construction of boreholes, taps, and well should be done in accordance with world health organization recommendation with regards to their location and depth.

iii. Legislations which regulate sewage disposal should be enforced to circumvent the effect of contamination of sub surface water by sewage.

iv. Community should fully participated in sanitation and encourage to participate workshop, seminars and public awareness on the danger associated with indiscriminate disposal of sewage and refuse.

v. The populace should be adequately informed on the need to protect potable water supply

vi. It is recommended that government should provide enough facilities to water agencies so as to ensure the provision of safe and reliable drinking water.

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APPENDIX

Biochemical Test Result

Isolate from Samples	Gram Reaction	Ind	MR	Cit	VP	Inference
GT	-ve short Rod	+	+	-	-	*E.Coli*
MT	-ve rod	-	-	+	+	*Klebsiella spp*
TT	-ve short Rod	+	+	-	-	*E.Coli*
ST	-ve rod	-	-	+	+	*Klebsiella spp*
MDT	-ve short Rod	+	+	-	-	*E.coli*
SB	-ve rod	-	-	+	+	*Klebsiella spp*
MDB	-ve short Rod	+	+	-	-	*E.coli*
GB	-ve rod	-	-	+	+	*Klebseilla spp*
MW	-ve short Rod	+	+	-	-	*E.coli*
GW	- ve rod	-	-	+	+	*Klebseilla spp*
SW	- ve rod	-	-	+	+	*Klebseilla spp*
MDW	- ve rod	-	-	+	+	*Klebseilla spp*
TW	- ve rod	-	-	+	+	*Klebseilla spp*