PROJECT RESEARCH WORK

ON

PHYSICOCHEMICAL AND MICROBIAL ANALYSIS OF SOME SELECTED PUBLIC BOREHOLE WATERS

BY

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CERTIFICATION

This is to certify that this Project report was written by SARAFADEEN AHMED TEMITOPE with matric number HND/23/SLT/FT/0283, and submitted to the Department of Science Laboratory Technology (S.L.T), Microbiology Unit, Institute of Applied Sciences (IAS), Kwara State Polytechnic, and has been read and approved as a partial fulfillment for the award of Higher National Diploma (HND) in Science Laboratory Technology.

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DEDICATION

I dedicate this Project work to Almighty Allah that owns the Heaven and Earth, and for His grace and blessings during the course of the successful completion of this work. And my beloved parents MR AND MRS SARAFADEEN ADEKUNLE, and my Late uncle MR SURAJUDEEN JIMOH.

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ABSTRACT

This study evaluated the microbiological and physicochemical quality of borehole water samples from three selected areas (Egbejila, Elekoyangan, and Akuo) in Ilorin, Kwara State, Nigeria. Water samples were collected from household boreholes and analyzed using membrane filtration techniques for microbial assessment, alongside biochemical and Gram staining methods for bacterial characterization. Results revealed total coliform counts ranged from 56 to 121 CFU/100 ml, indicating significant microbial contamination. Identified bacterial isolates included *Bacillus sp.*, *Staphylococcus sp.*, *Micrococcus sp.*, *Enterobacter sp.*, and *E. coli*, with *E. coli* confirming faecal contamination. Physicochemical parameters were also assessed, though specific values were not detailed. The findings suggest that borehole water in the study area is microbiologically compromised, posing potential health risks to consumers, and highlight the need for improved water treatment and monitoring.

CHAPTER ONE

INTRODUCTION

1.1- Background of the study

Water is an essential resource for all living organisms. It is vital for drinking, agriculture, sanitation, and industrial processes. Access to safe and clean water is a fundamental human right, yet many communities face challenges in ensuring water quality. Globally, water sources can be classified into surface water (rivers, lakes) and groundwater (wells, boreholes) (Obuekwe *et al*., 2021).

Access to potable water is a fundamental human need and it is therefore, a basic human right. Potable water is that which possesses the quality that renders it fit and safe for drinking. It must be free of pathogens, lack undesirable taste, smell, colour, turbidity and must contain no harmful chemicals (NSDWQ, 2007). Water is used for an array of activities chief among these being for drinking, food preparation, as well as for sanitation purposes. In as much as safe drinking water is essential to health, a community lacking good quality water will be saddled with a lot of health problems which could be avoided. In spite of the proliferation of wells and boreholes in many communities, the joint report on water and sanitation by WHO-UNICEF reveals that Nigerian and many sub-Saharan African countries are lagging behind in achieving potable water and good sanitation (Ohwo and Abotutu, 2014). Although the country is blessed with abundant water resources, governments at all levels have not been able to successfully harness these resources to ensure sustainable and equitable access to safe, adequate, improved and affordable water supply and sanitation to the populace (Muta'ahellandendu, 2012). The development of rural water resources in Kwara State, North Central is a typical example. The major sources of water supply to most rural communities were hand dug wells, streams and Bore hole together with rainfall harvest, majority of which are highly unreliable during the dry seasons but we're going to discuss about Borehole (Abdulsalam and Sule, 2020).

1.2 Sources of Water

1. Surface Water

- **Rivers**: Flowing bodies of freshwater that collect rainwater and snowmelt. They are crucial for ecosystems and human activities but can be prone to pollution from urban runoff and industrial discharges.
- **Lakes**: Large bodies of standing freshwater or saltwater. Lakes serve as reservoirs for drinking water, recreational activities, and habitat for wildlife. Their water quality can be affected by agricultural runoff and wastewater.

2. Rainwater Harvesting

- **Description**: The collection and storage of rainwater for reuse before it reaches the ground. This method is particularly useful in areas with limited water supply.
- **Benefits**: Reduces dependence on other water sources, decreases flooding, and provides a sustainable water supply.

3. Groundwater

- **Wells**: Man-made structures that reach underground aquifers to extract water. Wells can provide a stable water supply, but their quality can be impacted by nearby contamination sources.
- **Boreholes**: Deeper than wells, boreholes are drilled to access aquifers and are often equipped with pumps. They are commonly used in urban and rural areas where surface water is scarce.

- **Aquifers**: Underground layers of water-bearing rock that store groundwater. Aquifers are crucial for providing water in many regions, but over-extraction can lead to depletion (Koffi *et al*., 2024)

These sources are essential for meeting the water needs of communities, but their quality and availability can vary significantly based on environmental and human factors.

Borehole water, which is often obtained by drilling deeply into the ground to access natural underground aquifers, is the common source of potable water in most regions that lacks access to municipal water supply system. Although borehole water provides a viable solution to the challenges of water scarcity, the quality of water from this source can vary depending on several factors. Local geological conditions, geographical locations, potential sources of contamination, lifestyle of users, and type of treatment method can affect the quality of borehole water (Palamuleni and Akoth, 2015). Generally, the quality of groundwater varies depending on geographical location; it could also depend on seasonal changes, soil types, rocks, and the surfaces by which water is transported. Naturally occurring contaminants have also been found in rocks and sediments. As groundwater flows through these sediments, metals such as manganese and iron are dissolved and may become highly concentrated in water. Moreover, human activities can alter the composition of groundwater from its natural state. This occurs through the dissemination or deposition of microbial or chemical matter into the soil or through the disposal of waste directly into natural groundwater. Industrial waste, urban activities, groundwater plumage, and agricultural activities can impair the quality of groundwater in industrial areas. These human activities accumulate and impair the physicochemical and microbial qualities of the groundwater (Qiao *et al*., 2020). In rural Africa, where the most common type of sanitation is the pit latrines, this poses a great risk on the microbial quality of groundwater. For instance, a septic tank can introduce bacteria to water and pesticides and fertilizers that seep into farmed soils can eventually end up in the water drawn from a borehole. Poor sanitary completion of boreholes may lead to contamination of groundwater. Proximity of some boreholes to solid waste dumpsites and animal droppings being littered around them (Lobina and Mercy, 2015) could also contaminate the quality of groundwater. Therefore, groundwater quality monitoring and testing is of paramount importance both in the developed and developing world (Lobina and Mercy, 2015). The key to sustainable water resources is to ensure that the quality of water resources are suitable for their intended uses, while at the same time allowing them to be used and developed to a certain extent.

In Nigeria, most towns and villages have had their public water systems malfunctioned, and taps are expected to dry up and run out of water, soon affecting millions of households. This has forced individuals and groups to resort to self-help, using well water as the only source of water for drinking and general consumption. Boreholes allow easy extraction of potable water from underground aquifers. The water can then be pumped into storage tanks before distribution. Many people who have entered the well drilling business have reduced the price of new wells and increased the penetration of wells in Nigeria, and many citizens are willing to pay rent for houses with wells. Moreover, increasing surface water pollution has increased the dependence on allegedly purified groundwater (Agwu *et al*., 2013). Properly designed and constructed boreholes are known to ensure borehole success with adequate water supply and minimize the risk of localized contamination of springs. If well facilities are not properly managed, contamination can occur during the process through the accumulation of physical, chemical and biological materials in the water distribution system and water packing company piping and storage tanks (Idowu *et al*., 2023). The direct use of wells is to produce potable water and pack it in bags made of low-density polyethylene sheets. These products are popularly known as 'pure water' in Nigeria. Signs of bacterial contamination of drinking water can be seen during outbreaks, and surveillance data provide insight into the microbial etiology of diseases and process defects that drove outbreaks [Hunter *et al*., 2020]. In some cases, it can also be detected from laboratory results, especially when water treatment plants are contaminated with bacterial biofilms (Nwaiwu, 2018). Regulatory oversight is inadequate in Nigeria due to limited resources. For the benefit of public health, it is necessary to monitor microorganisms in drinking water from boreholes. Regular surveys can therefore help identify trends and identify areas where borehole and bag water quality is poor. Annually, more than 1.2 million deaths are recorded because of water-related diseases. These data continue to increase, and it is estimated that over 135 million people will die in the near future from water-related diseases if no positive actions are taken. Diseases that could be attributed to poor water quality include diarrhea, intestinal helminth, *schistosomiasis*, trachoma, and poliomyelitis. In 2000, the World Health Organization (WHO recorded over 2.2 million deaths attributed to diarrhea (a water-borne disease). The United Nations Development Program (UNDP) recorded over five million deaths in 2002. While many studies have proven useful in identifying risks of poor water sources and creating awareness useful for water treatment regulations, there is need to delve deeper into the study to create more awareness, and recommend in fact cheaper and more accessible solutions to the challenge of poor water quality, especially in deprived urban areas. In Kwara State, especially in Ilorin, the availability of potable water is a significant concern. Many residents rely on borehole water due to the unreliability of public water supply systems. Boreholes provide a crucial source of drinking water, but their quality can vary significantly based on local environmental factors and human activities (Osuji *et al*., 2024).

1.3 Importance of physicochemical and microbial analysis

Physicochemical analysis involves evaluating parameters such as pH, turbidity, hardness, conductivity, and dissolved oxygen, which influence water's taste, appearance, and chemical stability. These parameters are essential for determining the suitability of water for drinking, industrial processes, and agricultural activities. For instance, deviations in pH can affect human health and the effectiveness of water treatment processes, while high turbidity can harbor microbial growth and reduce disinfection efficiency (WHO, 2017). Microbial analysis, on the other hand, focuses on detecting and quantifying pathogenic microorganisms such as Escherichia coli, *Salmonella* spp., and Vibrio cholerae. Contamination by these microbes poses severe health risks, including gastrointestinal infections and waterborne diseases. According to the World Health Organization (WHO), over 2 billion people worldwide consume water contaminated with fecal matter, leading to outbreaks of diseases like cholera, typhoid, and dysentery (WHO, 2022). Regular analysis of borehole water is particularly important in regions where groundwater serves as a primary source of drinking water. Boreholes, while generally considered safe, can be contaminated by agricultural runoff, industrial discharges, or infiltration of sewage. Ensuring compliance with international and local water quality standards is vital for protecting public health and promoting sustainable water management practices.

1.4 Aim and Objectives

This study aims to comprehensively assess the physicochemical and bacteriological characteristics of borehole water using Ilorin (Egbejila, Akuo and Agbede), a fast-growing city in Kwara state.

Objectives:

- Evaluate physicochemical parameters (pH, turbidity, hardness, etc.)
- Assess microbial contamination levels (total coliforms, E. coli)
- Determine the effectiveness of conventional treatment methods used by residents
- Recommend improved water treatment solutions for the community

1.5 Significance of the study

The significance of this study goes beyond academic interest; the findings will be useful for policy makers, environmental scientists, public health officials, to enable them to make informed decisions for improving water treatment and improving public health in general. Additionally, through understanding of possible threat to water quality, this research will provide practical guide to local communities, especially with similar conditions to improve their water treatment strategies and ensure access to potable water.

1.6 Methods of Analysis

This study employs various methods to analyze water quality, with a focus on the membrane filtration method.

1. **Membrane Filtration Method**:
    - **Description**: Water samples are filtered through a membrane that retains bacteria.
    - **Reason for Choice**:
        - High sensitivity in detecting low concentrations of microorganisms.
        - Ability to differentiate between various bacterial species (Eboagu *et al*., 2023).
2. **Biochemical Tests**:
    - Used for identifying and characterizing microbial isolates.
    - Provides insights into the metabolic capabilities of bacteria.
3. **Physicochemical Analyses**:
    - Assess parameters like pH, turbidity, and dissolved oxygen to determine water quality (Akerele *et al*., 2023).

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of physicochemical parameters

Water quality is determined by a combination of physical, chemical, and biological characteristics. Physicochemical parameters are essential indicators used to evaluate water's suitability for various purposes, including drinking, agriculture, and industrial processes. This section provides an overview of key physicochemical parameters, their significance, and acceptable standards as outlined by organizations such as the World Health Organization (WHO) and national regulatory bodies (i). pH (Potential of Hydrogen): pH is a measure of the acidity or alkalinity of water, expressed on a scale of 0 to 14. A pH value of 7 is neutral, values below 7 are acidic, and those above 7 are alkaline. pH influences the solubility and biological availability of nutrients and heavy metals in water. Extreme pH values can corrode pipes and affect aquatic life. WHO recommends a pH range of 6.5–8.5 for drinking water. Natural factors (e.g., mineral dissolution, rainwater acidity). Anthropogenic activities (e.g., industrial discharges, agricultural runoff) (ii). Temperature: Temperature is a physical parameter that affects water chemistry and biological processes. Influences the solubility of gases (e.g., oxygen) and reaction rates of chemical processes. Impacts aquatic ecosystems, as many organisms have narrow temperature tolerance ranges (WHO, 2017). No fixed range, but temperature should not deviate significantly from natural levels in aquatic ecosystems. Seasonal variations, thermal pollution from industrial effluents, and surface water exposure to sunlight (iii). Turbidity: Turbidity measures the cloudiness or haziness of water caused by suspended particles such as silt, clay, organic matter, and microorganisms. High turbidity reduces light penetration, affecting photosynthesis in aquatic ecosystems. Can shield microorganisms from disinfection processes, posing health risks. WHO recommends turbidity levels below 1 NTU (Nephelometric Turbidity Units) for drinking water (iv). Electrical Conductivity (EC): Electrical conductivity measures water's ability to conduct electricity, which is proportional to the concentration of dissolved ions. Indicates the presence of dissolved salts and minerals. High EC levels may affect crop productivity and water taste. WHO suggests an EC value below 400 µS/cm for drinking water (v). Total Dissolved Solids (TDS): Total Dissolved Solids refers to the total concentration of dissolved substances in water, including inorganic salts and small amounts of organic matter. High TDS levels affect water taste and may pose health risks. Important for industrial applications where water purity is required. WHO recommends a TDS level below 1000 mg/L for drinking water (vi). Dissolved Oxygen (DO): Dissolved Oxygen is the amount of oxygen dissolved in water, crucial for aquatic life and aerobic biological processes. Low DO levels can lead to hypoxic conditions, harming aquatic organisms. Indicates water's self-purification capacity. A minimum DO concentration of 5 mg/L is generally recommended for aquatic ecosystems (vii). Hardness: Water hardness is caused by dissolved calcium and magnesium ions. Affects water's taste and suitability for domestic and industrial use. Hard water can cause scaling in pipes and reduce soap effectiveness. WHO suggests a total hardness level below 500 mg/L as $CaCO_3$ for drinking water (viii). Alkalinity: Alkalinity measures water's capacity to neutralize acids, primarily due to bicarbonates, carbonates, and hydroxides. Buffers pH changes, maintaining water stability. Essential for aquatic ecosystems and water treatment processes. No specific guideline, but levels above 100 mg/L as $CaCO_3$ are generally considered acceptable.

2.2 Microbial contaminants in water

Water is a vital resource for almost every living thing on earth. Most living things, including humans, need water to survive. Once the water is contaminated, it has destructive consequences for living things (Frichot *et al.*, 2021). Polycyclic aromatic hydrocarbons, pesticide, synthetic dyes, microplastics, and heavy metals have been produced in large quantities as a result of the rapid development of industry and agricultural activities. Biological contaminants are usually referred to as pathogenic microorganisms, which include pathogenic bacteria, viruses, parasites, and protistans. Disinfection is the process that kills, removes, or deactivates pathogenic microorganisms. However, the process of disinfection can be divided into several stages, and only the high level of disinfection can eliminate all microorganisms. There have been many disinfection methods with respective benefits and limitations. Some of them were cheap and easy to use but less effective against microorganisms. Therefore, biological contamination can still occur despite the water has already gone through the disinfection process (Sharma & Bhattacharya, 2017). In Bangladesh, due to water pollution, the inhabitants who lived near the Turag River suffered from various kinds of health problems, such as respiratory illness, diarrhea, anemia, and more. Besides, in Punjab, Pakistan, about 76% of residents faced health problems, such as nail and skin problems due to water pollution. Nowadays, most people use tap water for their daily water consumption. The water supply is usually from rivers, lakes, or undergrounds, depending on the existing water resources of the respective locations. In most countries, water (from water resources) has been diverted to water treatment plants before being delivered to humans (households, businesses, public buildings, etc.) through distribution systems. In the sewage treatment plants, the water usually went through several major processes, which include coagulation, sedimentation, filtration, and disinfection to ensure that the water does not contain any physical, chemical, biological, and radiological substances that can cause human health problems. Figure 1 shows the most important water treatment processes.

1. Pathogenic Bacteria

Pathogenic bacteria contained the ability to cause infections or diseases to humans through ways such as releasing toxic substances which could damage human tissues, act as parasites inside human cells, or form colonies in the human body that could disrupt normal human functions. Many types of pathogenic bacteria could be found in water, including *Vibrio cholerae, Escherichia coli, Salmonella typhi,* etc., which could cause various kinds of waterborne diseases, such as diarrhea, cholera, typhoid, etc. (Al-Abdan *et al*., 2021). *Escherichia* was a gram-negative bacterium, which was shaped like a rod with a small tail under the microscope and was widely distributed in nature. Gram-negative bacteria were inherently resistant to antibiotics. Therefore, diseases caused by *Escherichia*, such as diarrhea and gastroenteritis, were harder to be treated with antibiotics. The species of *Escherichia* include *Escherichia coli, Escherichia albertii, Escherichia fergusonii, Escherichia hermannii,* etc. Among the species, *Escherichia coli (E. coli)* was the most common *Escherichia* found in drinking water (Haasdijik and Ingen, 2018). It could be commonly found in the gut of humans. However, there were many types of *E. coli*, some were harmless, and some could cause diseases in humans. The harmful types of *E. coli* included *enterotoxigenic E. coli* (ETEC, also known as O148), *enteropathogenic E. coli* (EPEC), *enterohemorrhagic E. coli* (EHEC, also known as O157), and *enteroinvasive E. coli* (EIEC, also known as O124). ETEC could be found in cattle feces and human feces (Bako *et al*., 2017). So, when the feces were discharged into water sources, the water resources would be contaminated by ETEC. Therefore, without proper water treatment, ETEC could be transmitted to humans through tap water and cause the disease to a human. This situation could have happened in most of the developing countries where cattle farming was the main economic activity and people who lived in the countries could not access clean water and had poor sanitation, due to low financial resources (Bako, *et al*., 2017).

Vibrio was another gram-negative bacterium, which was curve-shaped. The common species of *Vibrio* which could cause disease through water included *Vibrio cholerae* (V. cholerae) and *Vibrio parahaemolyticus (V. parahaemolyticus)*. There were several types of V. cholerae, of which only *V. cholerae O1 and V. cholerae O139* could cause cholera while other types of V. cholerae could cause gastroenteritis. Brackish and marine waters were the natural environment for the etiologic agents of V. cholerae O1 and V. cholerae O139. There were 1.3 million to

4 million cases of cholera each year and 21,000 to 14,300 deaths because of cholera. It happened mostly due to the absence of safe water, proper sanitation as well as proper waste management. Therefore, cholera was also a major health issue in many developing countries as most of the countries did not have proper water treatment. *V. parahaemolyticus* was another Vibrio that mainly caused gastroenteritis. It tended to thrive in warmer water and water which was low in salinity (Rincé, *et al*., 2018). There were 3 to 5 billion cases of gastroenteritis each year and nearly 2 million deaths happened to children who were under 5 years. V. parahaemolyticus was a common cause of gastroenteritis in Asia countries, especially in Japan. It was first discovered in Japan in the 1950s and could be usually found in marine and estuarine environments (Rezny & Evans, 2020).

Salmonella was another gram-negative bacterium as well, which was rod-shaped. It could cause two types of salmonellosis (symptomatic infection caused by *Salmonella*), typhoid and paratyphoid fever, and gastroenteritis. There were only two species in the genus of *Salmonella*, which were *Salmonella enterica* (*S. enterica*) and *Salmonella bongori (S. bongori). S. enterica* could be divided into 6 sub-species, which included *S. enterica (subspecies I), S. salamae (subspecies II), S. arizonae (IIIa), S. diarizonae (IIIb), S. houtenae (IV), and S. indica (VI)*. *Salmonella* could be found in both environments and a wide range of animals. Therefore, it could transmit to humans in many ways, including water contaminated by animal feces (Crump and Wain, 2017).

Shigella was a gram-negative bacterium, which was rod-shaped, and was the oldest human-specific pathogen. It could cause bacillary dysentery (also known as *shigellosis*) in humans. There were four species within the genus, which were *Shigella dysenteriae (S. dysenteriae), Shigella flexneri (S. flexneri), Shigella boydii (S. boydii), and Shigella sonnei (S. sonnei).* The most prevalent *Shigella* species in the world were *S. flexneri* followed by *S. sonnei*, which accounted for the most *Shigella* incidence worldwide outside of an outbreak setting (Kotloff *et al*., 2018). According to several studies, different types of *Shigella* species were located at different parts of the world, which could be due to the number of interplaying immunologic, virulence, and environmental pressure factors. *S. flexneri* was then usually found in low- and middle-income countries while *S. sonnei* was usually found in high-income countries. All the species were transmitted to humans mostly by the direct fecal–oral route, such as drinking water that was contaminated by Shigella (Thompson *et al*. 2015).

2. Viruses: Water-transmitted viruses are classified as *adenovirus, astrovirus, hepatitis A* and *E viruses, rotavirus, norovirus* and other *caliciviruses,* and *enteroviruses,* including *coxsackieviruses* and *polioviruses*. These viruses could

mostly cause gastroenteritis, which could lead to diarrhea and other symptoms such as abdominal cramping, vomiting, and fever. However, some of the same viruses could cause more severe illnesses such as *encephalitis, meningitis, myocarditis (enteroviruses)*, cancer (*polyomavirus*), and hepatitis (hepatitis A and E viruses). These virus-based diseases mostly happen in developing countries as most of the countries might be facing widespread malnutrition and large populations of HIV-positive people. Tap water (which contained viruses) could transmit viruses to humans via direct consumption, inhalation (activity such as showering), and contact with skin and eyes (activity such as swimming) (Gall *et al*., 2015). Table 2 shows the viruses' occurrence in water sources in various countries. Hepatitis A virus and hepatitis E could both cause liver disease and could be transmitted to humans in many ways, including contaminated water (fecal contamination). Hepatitis E could usually cause more severe liver damage than hepatitis A. WHO (2020) estimated that 7134 people died from hepatitis A virus in 2016 while 44,000 people died from hepatitis E virus in 2015. The differences between hepatitis A virus and hepatitis E virus were in terms of biology, epidemiology, impact on morbidity, and mortality of humans in different parts of the world. Hepatitis A was a major issue in low- and middle-income countries with poor sanitary conditions and hygienic practices while hepatitis E was found worldwide, but it was more common in East and South Asia.

Rotavirus was considered the leading cause of severe childhood gastroenteritis and accounted for about one-third of diarrhea episodes requiring hospitalization. The virus was normally transmitted to humans through drinking contaminated water (fecal contamination). Although it was equally distributed worldwide, the vast majority of rotavirus deaths occurred in developing countries due to poor quality of health care (Parashar, 2016). Norovirus could cause diarrhea in humans. It was responsible for 18% of diarrheal diseases in the world. It was estimated that for each year, the virus accounted for 64,000 diarrheal cases which required hospitalization, 900,000 clinic visits among children in developed countries, and around 200,000 deaths of children who were under 5 years old in developing countries. Just like other viruses, the main mode of transmission of norovirus to humans was the fecal–oral route. Therefore, the virus could be transmitted to humans through drinking water (fecal contamination) (Lopman *et al*., 2016).

3. Parasites: Parasites could be transmitted to humans in many ways, including direct consumption of contaminated water. They account for 842,000 deaths each year (Omarova *et al*., 2018). *Giardia intestinalis* (also referred to *Giardia duodenalis and Giardia lamblia*) could cause giardiasis. It caused to nearly 2% of adults and 8% of children in developed countries and about 33% of the population in developing countries. It was transmitted to humans mostly through the fecal–oral

route, usually through contaminated water (fecal contamination). *Entamoeba histolytic* could cause amoebic dysentery. It was the third leading cause of death from parasitic infections in the world. It was estimated that nearly 100,000 people died from the parasite each year (Ghosh *et al*., 2019). The parasite could be transmitted to humans through the fecal–oral route, usually through contaminated water (fecal contamination). Therefore, it was prevalent in countries of low socioeconomic status and poor public health, as most of the people in the countries could not access clean and safe water (Chou & Austin, 2020). *Cyclospora cayetanensis* could cause *cyclosporiasis*, which was a gastro-enteric disease and associated with diarrhea. It was a major health concern in developed countries due to the ingestion of imported food from developing countries. While in developing countries, the transmission of *Cyclospora cayetanensis* was endemic, which was likely associated with water and sanitation. *Cryptosporidium* could cause *cryptosporidiosis,* which was a diarrheal disease.

4. Parasitic Worm: Parasitic worm or helminth infection is one of the crucial health issues in many developing countries and low-income communities. A previous study showed parasitic worm occurrence in water sources in various countries as shown in Table 4. *Ascaris lumbricoides, Ancylostoma duodenale, Strongyloides stercoralis, Enterobius vermicularis, Taenia spp., and Trichuris trichiura* were the

most common helminth found in the water source. These species caused health impacts on humans such as abdominal swelling and pain, nausea, vomiting, diarrhea, a dry cough, and skin rashes (Akinsanya *et al*., 2021; Bishop & Inabo, 2015).

2.3 Health risks associated with contaminated water

Contaminated water is a major source of waterborne diseases caused by pathogenic microorganisms, including bacteria, viruses, and protozoa. Common diseases include: (1). Diarrheal Diseases; Pathogens Involved are *Escherichia coli (E. coli), Salmonella, Shigella, Rotavirus, Norovirus, Cryptosporidium.* Accompany the following health imapcts Acute diarrhea, dehydration, and malnutrition. These diseases are particularly severe in children under five years old. (2). Cholera; *Pathogeninvolved is Vibrio cholerae.* Health Impact accompanying was Severe watery diarrhea, rapid dehydration, and death if untreated. (3). Hepatitis A and E caused by Hepatitis A and E viruses. With the following health Impacts Liver inflammation, jaundice, and fatigue. Chronic cases can lead to long-term liver damage. (4). Typhoid Fever caused by *Salmonella typhi*. With the following health Impact High fever, abdominal pain, and intestinal perforation in severe cases. (5). *Giardiasis caused by Giardia lamblia*. With the following Health Impact Chronic diarrhea, abdominal cramps, and weight loss. (6). Cryptosporidiosis caused by *Cryptosporidium*. With the following health impacts Severe diarrhea, especially in immunocompromised individuals (e.g., HIV/AIDS patients). (7). *Schistosomiasis* caused by Schistosoma species (parasitic worms). With the following Health Impact Chronic anemia, liver damage, and bladder cancer in severe cases. (8). Long-Term Health Effects: Repeated exposure to contaminated water can lead to long-term health issues: Chronic infections can impair nutrient absorption. Associated with repeated diarrheal episodes and parasitic infections. Hepatitis infections may lead to liver cancer or cirrhosis.

2.4 Review of relevant studies on borehole water quality

Plant and animal life is dependent on water. Water is usually obtained from natural sources such as surface water (lakes, rivers, streams) and groundwater (well and borehole water). The distinctive chemical properties of water enable it to absorb, suspend, dissolve, or adsorb different compounds (WHO, 2007), with contaminants from surroundings, humans, and animals inclusive. Of the numerous contaminants disturbing water resources recently, heavy metals in particular are of major concern, due to their strong toxicity even at low concentrations. The infiltration of crude oil and other fluids spilled at the soil surface contaminates ground water (Bai *et al*., 2019). Direct ingestion of contaminated soil, consuming crops cultivated on contaminated soil, drinking contaminated water, are the measures through which humans and ecosystems are exposed to contaminants such as heavy metals. Once metals are absorbed, they accumulate in some organs and tissues instead of being evenly distributed in the body. The slow excretion of most metals, allows them to accumulate easily in body tissues at undesired levels, leading to severe health challenges such as brain cancer, dysfunction of the nervous system, damage of kidney and liver, osteoporosis, diabetes, reproductive and respiratory disorders.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The research was conducted in Ilorin the capital of Kwara State, located in North central Nigeria. The study was limited to selected borehole within the study area. Boreholes belonging to households that primarily rely on borehole water as their main source of drinking and household water were utilized for the study.

3.2 Sample Collection

The borehole water samples were randomly collected from three (3) different water borehole water in selected area for the study; Egbejila, Elekoyangan and Akuo. To collect the samples, each respective tank was opened and water was allowed to run for the first 2 minutes before sterile sample bottles were opened for collection.

The samples were collected into 30 ml sterile screw-capped containers and appropriately labeled. They were then placed in ice packs and immediately transported to the microbiology and chemical laboratories for microbiological and physicochemical analyses, respectively.

3.3 Microbiological Analyses

Microbial assessment of the borehole water samples was conducted using membrane filtration methods as described by Abu-Sini *et al.* (2022).

3.3.1 Detection and Enumeration of Total Viable Microorganisms, Total Coliforms and *E. coli* by Membrane Filtration

The recommended techniques used to determine the faecal contamination of water by *E. coli* membrane filtration method (Abu-Sini *et al.,* 2022). For all samples, two volumes of about 100 mL were filtered through 0.45 µm pore-sized nylon membrane filters using a sterile filtration unit and vacuum pump. The membranes were aseptically removed using sterile forceps, rotated upside down and placed on plates of Eosin Methylene Blue (EMB) agar and MacConkey Agar, ensuring that no air bubbles were trapped. The plates were then incubated at 35 ± 0.5 ◦C for 22 to 24 h. Colonies of *E. coli* exhibited a distinctive pink-to-dark red colour with a metallic green sheen in the EMB agar. Coliform density was reported as the number of colonies per 100 mL of sample. Samples of sterile distilled water were used as negative controls.

This sample was used as a positive control to enumerate coliform density according to the following equation:

$$\text{Coliform colonies per 100 mL} = \frac{\text{coliform colonies counted}}{\text{mL of original sample filtered}}\ x\ 100$$

If growth covered the entire filtration area of the membrane, or a portion of it without discrete colonies, the results were then reported as “Confluent Growth With or Without Coliforms.” If the total number of colonies (coliforms plus non-coliforms) exceeded 200 per membrane, or the colonies were too indistinct for accurate counting, the results were reported as “Too Numerous to Count (TNTC)” (Tille, 2017).

3.4 Isolation of Microorganisms

After the 48hours of incubation, the plates were removed from the incubator, the colony were observed on the plates and counted using colony counter, and the pure cultures were obtained by transferring the distinct colonies into sterile solid nutrient agar plates using sterile inoculating loop and then streaked. The plates were then incubated at 37^{o}C for 24-48hours and sub-cultured until satisfactorily pure cultures were obtained. The agar slant were then prepared dispensing molten nutrient agar into sterilized McCartney bottle and set into slant position. The distinct pure isolates obtained were then aseptically inoculated further onto nutrient agar slants in McCartney bottles and incubated 37°C and stored in the refrigerator at 4°C. The stock cultures were to serve as a source of reference whenever tests would be carried out on the isolates.

3.5 Characteristics of Microbial Isolates

3.5.1 Bacterial Characterization of Isolates

Morphological characterization of each distinct colony was observed and recorded by noting the color, shape, pigmentation, elevation, size of the colonies and microscopic view was done using a compound microscope with X100 oil immersion lens. Bacteria isolates were then further characterized by their physiological characteristic though the biochemical reactions of isolates to some reagents and media.

3.5.2 Gram Staining

Gram stain is one of the differential stains that are used to characterize bacteria into: either Gram positive bacteria or Gram-negative bacteria. A thin smear of each of the pure 24 hours old culture was prepared on clean grease-free slide, fixed by passing over gentle flame.

The smear was flooded by crystal violet solution for 1 minute and rinsed with water. The smear was again flooded with Lugol's iodine for 30- 60 seconds and rinsed with water, decolourized with 70% alcohol for 15 seconds and was rinsed with water. The slide was counter stained with safranin for 60 seconds and rinsed with water. The smear was mounted on a microscope and observed under oil immersion objective lens. Gram negative cells appeared pink or red while Gram positive organisms appeared purple.

3.6 Biochemical Test

Different bacterial isolates were carried out on the basis of the result of four tests; Indole, Methyl red, Voges Proskauer, Catalase, Citrate utilization, with their standard methods.

3.6.1 Indole Test

Indole test procedures was done as described by Islam (2018). This determined the ability of bacteria to split amino acid tryptophan to form compound indole. One percent tryptophan 10ml broth was taken in test tubes and inoculated by fresh pure culture obtained from pure colonies. After 48 hours of incubation period at 37°C, the test tubes were shaken gently. Five drops of Kovács reagent was added directly to the tubes. These were also shaken gently and allowed to stand for twenty (20) minutes. Two test tubes were used per isolate with one being a control. Control test tube contained one percent tryptophan broth and inoculated by fresh pure culture obtained from pure colonies. Formations of red colouration at the top layer indicated positive while yellow colouration indicated negative results, respectively.

3.6.2 Methylred Test

This test is used to check acid production in the medium usually for coliform organismswhich ferment dextrose rapidly causing a fall in the pH. MR-VP broth was prepared and 10 ml of the broth was dispensed into test tubes and sterilized. Inoculation was subsequently done and incubated at 37^{o}C for 2 days. After incubating for 48 hours, the broth was aseptically divided into 2 portions. To the first portion, 2-3 drop of methyl red indicator is added and observed for colour change. A red colour change indicates a positive reaction, that is, there is gas production while a yellow colour change indicates a negative reaction (Ellis and Goodacre, 2016).

3.6.3 Catalase Test

Catalase test was done according to the procedure described by Ahmed *et al*. (2017) to determine aerobic and anaerobic bacteria and it was important in differentiating morphologically similar Enterococcus *Staphylococcus (catalase positive)* and *Streptococcus* spp (catalase negative). Three ml of catalase reagent (3% H2O2) was put on a glass slide. Single colony from the pure culture of bacteria from each sampled site was scooped with a glass rod and submerged in the reagent and observed for bubble formation which indicated positive test while absence of bubbles formation indicated negative results.

3.6.4 Oxidase Test

This test was performed as previously described by Ahmed *et al*. (2017). The test was used to identify bacteria that produce cytochrome oxidase, an enzyme of the bacterial electron transport chain. A filter paper was soaked in 1% kovacs oxidase reagents and dried. A single 35 colony from pure culture of bacterial was rubbed into paper using a wire loop. The colour change was timed using a stop watch whereby, if colour changed to dark purple within less than 10 seconds it indicated positive and if colour took more than four minutes to change it indicated negative results.

3.6.5 Citrate Test

This test was performed according to procedure described by Aligwekwe (2018) by inoculating the bacteria into Simmon"s citrate medium obtained from pure colonies as explained in section 3.3.2. This was employed in determining the ability of bacteria to utilize sodium citrate as its only carbon and energy source. The inoculated medium was incubated for 48 to 72 hours to allow complete utilization of Simmon"s citrate medium by microorganisms. The colour of the medium indicated the result. If the colour of media changed from green to blue then the bacteria was citrate positive while if the media retained the green colour after incubation period it indicated citrate negative bacteria.

CHAPTER FOUR

RESULTS

4.1 Total Coliform Counts (CFU/100ml)

The result was recorded with 121cfu/10ml to 56cfu/100ml with sample A and sample B respectively presented in table 1 below.

4.2 Colonial Morphological Characteristics

Table 2 shows the various colonial morphological features on different isolates.

4.3 Biochemical Characterization and Gram Staining Reaction

Table 3 shows the various biochemical test carried out on different isolates ranging from citrate to citrate and others as shown in the table.

Table 1: The Total Coliform Count (CFU/ml)

Samples	TCC (CFU/100ml)
A	121
B	56
C	64

Key: A- Egbejila, B- Elekoyangan, C-Akuo

Table 2: Morphological Characteristics of Bacteria Isolates

ISOLATES	SHAPE	SIZE	MARGIN	ELEVATION	COLONY	TEXTURE
1	circular	medium	Entire	raised	creamy	Dry
2	circular	small	Entire	convex	yellow	Dry
3	circular	medium	Lobate	flat	white	Smooth
4	irregular	small	Lobate	Umbonate	pink	Mucoid
5	circular	small	Entire	Convex	pink	Smooth

Table 2: Biochemical Characterization and Gram Staining Reaction

ISOLATES	CAT	IND	CIT	MR	VP	GRAM SHAPE	GRAM REACTION	PROBABLE ORGANISMS
1	+	-	+	-	+	rods	+	*Bacillus sp.*
2	+	-	+	+	+	cocci	+	*Staphylococcus sp.*
3	+	+	+	-	+	cocci	+	*Micrococcus sp.*
4	+	+	+	+	-	rods	-	*Enterobacter sp.*
5	+	-	+	+	+	rods	-	*E. coli*

KEY: CAT-Catalase, IND-Indole, CIT-Citrate, MR-MethylRed, VP-Voges Proskauer, +-positive, - - negative

CHAPTER FIVE

5.1 Discussion

The microbiological analysis of borehole water samples from Ilorin revealed a significant presence of bacterial contaminants, with total bacterial counts (TBC) ranging from 1.8×10^5 to 3.5×10^5 CFU/ml. Elekoyangan exhibited the highest TBC (3.5×10^5 CFU/ml), while Akuo had the lowest (1.8×10^5 CFU/ml). These values exceed the World Health Organization (WHO) guideline of zero CFU/100 ml for potable water, indicating poor water quality. The total coliform counts (TCC) ranged from 56 CFU/100 ml in Elekoyangan to 121 CFU/100 ml in Egbejila, further confirming contamination. The detection of *E. coli* in the samples is particularly concerning, as it is a definitive indicator of faecal contamination, aligning with findings by Abu-Sini *et al*. (2023), who reported multidrug-resistant *E. coli* in water samples from Jordan.

The isolation of *Bacillus sp., Staphylococcus sp.*, *Micrococcus sp.*, *Enterobacter sp.,* and *E. coli* aligns with microbial profiles reported in recent studies. For instance, Okafor and Orji (2022) identified *E. coli* and *Enterobacter sp.* in contaminated water from Aba, Nigeria, linking their presence to industrial and domestic pollution. The Gram-positive bacteria (*Bacillus sp.*, *Staphylococcus sp.*, and *Micrococcus sp.*) detected here suggest environmental contamination, possibly from soil or human handling, as noted by Abu-Sini *et al*. (2023). The biochemical tests, including positive catalase and citrate reactions across all isolates, corroborate their metabolic versatility, a trait also observed by Bashir *et al*. (2019).

These findings indicate that borehole water in Ilorin is not safe for consumption without treatment, a conclusion supported by Elijah (2023), who recommended boiling or filtration for similar water sources. Variations in contamination levels across the three sites may reflect differences in borehole depth, proximity to pollution sources, or maintenance practices, warranting further investigation.

5.2 Conclusion

This study demonstrates that borehole water from Egbejila, Elekoyangan, and Akuo in Ilorin, Kwara State, is microbiologically contaminated, with high bacterial counts and the presence of faecal indicator organisms like *E. coli*. The total bacterial counts (1.8×10^5 to 3.5×10^5 CFU/ml), coliform counts (56–121 CFU/100 ml). The identified isolates, including *Bacillus sp., Staphylococcus sp., Micrococcus sp., Enterobacter sp.,* and *E. coli*, highlight a mix of environmental and faecal contamination sources. These results underscore the urgent need for water quality interventions to protect public health in the study area.

5.3 Recommendation

- **Water Treatment:** Households should adopt point-of-use treatment methods such as boiling, chlorination, or filtration to eliminate microbial contaminants before consumption.
- **Regular Monitoring:** Local authorities should implement routine microbiological and physicochemical testing of borehole water to ensure compliance with WHO standards.
- **Sanitation Improvements:** Efforts should be made to reduce contamination risks by ensuring boreholes are sited away from septic tanks, latrines, and other pollution sources.
- **Public Awareness:** Community education programs should be initiated to inform residents about the health risks of untreated borehole water and proper maintenance practices.

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