COMPARATIVE ANALYSIS OF MICROBIAL LOAD OF TWO MAJOR WATER PRODUCTION UNITS IN UGBOWO, BENIN CITY, NIGERIA

BY

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UNIVERSITY OF BENIN, BENIN CITY.

SEPTEMBER, 2023

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

SEPTEMBER, 2023

CERTIFICATION

This is to certify that this project work was carried out by **Julian Chibuzor AGEH (Miss)** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

________________________________ ________________________

DR. (MRS.) R. ADAMS **DATE**

(Project Supervisor)

APPROVAL

This project work was carried out by J**ulian Chibuzor AGEH (Miss)** in partial fulfillment of the award of a Bachelor of Science, B.Sc (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

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PROF. (MRS.) F. I. AKINNIBOSUN **DATE**

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty, for bringing me this far in life. I am truly grateful.

ACKNOWLEDGEMENT

I want to first appreciate God for His mercies and grace that has brought me so far in life. To him be all the glory and adoration.

To my supervisor, DR. (MRS.) R. ADAMS for being very patient, and for her guidance, advice and corrections I say thank you.

My sincere appreciation and gratitude goes to my parents. Mr. Victor and Mrs. Theresa Ageh, for always believing in me and for their unending love and support.

I cannot end this without appreciating my beloved friend Master Uzoma Blessing, who has stood by me through thick and thin. I thank you for your support since 100 lelev till now, and for being a true definition of a friend. God bless you.

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ABSTRACT

Water covers 71% of the earth surface and is essential for all the living beings. Nearly all metabolic processes take place in the presence of water. Clean water has a number of health benefits which include enhancing appetite control, metabolism, increase energy levels and aid in heart rate. Microorganisms are a crucial component of water. They are not only in charge of nutrient cycling, but they can also contribute to a number of diseases that are water-borne. In this study, the prevalence of pathogenic bacteria and fungi in water samples from two major distributors in Ugbowo, Benin City, Nigeria was evaluated. Six bacteria genera in total were isolated from both water samples namely, *Proteus* sp, *Salmonella* sp, *Shigella sonnei*, *Enterobacter* sp, *Pseudomonas* sp and *Escherichia coli* all of which are known to cause unpleasant human diseases when consumed in large quantities. The fungal isolates obtained from both water samples sin this study were, *Mucor* sp, *Penicillium citrinum*, *Penicilium oxalicum*, and aflatoxigenic *Aspergillus fumigatus*. The total heterotrophic bacteria counts obtained from the distribution companies ranged from $4.70x10^4$±5.60 CFU/ml to 7.85 $x10^4$±2.80 CFU/ml. The fungal counts obtained also ranged from $2.24x10^4$±2.82 CFU/ml to $3.00x10^4$±1.41 CFU/ml. It was also observed in this study that the isolated bacteria demonstrated resistance to some of the antibiotics in the antibacterial susceptibility testing, suggesting the presence of multi drug resistant strains. Studies should be conducted to test the efficacy of alternative antimicrobial agents to human pathogenic bacteria and fungi

CHAPTER ONE

1.0 INTRODUCTION

Water covers 71% of the earth surface and is essential for all the living beings. Nearly all metabolic processes take place in the presence of water. Clean water has a number of health benefits which include enhancing appetite control, metabolism, increase energy levels and aid in heart rate. Water also aids in keeping the inside and keeps a steady temperature flowing harmony. Microorganisms are a crucial component of water. They are not only in charge of nutrient cycling, but they can also contribute to a number of diseases that are water-borne (Ravet and Brailowsky, 2014). Most prevalent illnesses induced by diaorrhea, dysentery, gastroenteritis and other gastrointestinal illnesses, eye, ear and pyogenic infections, yellow fever, malaria, and skin diseases, dyspepsia, dengue fever, schistosomiases, and infections of the urinary tract (Bharti *et al*., 2003).

Many pathogenic microorganisms such as *Salmonella*, *Spirochete*, *Rickettsia*, *Escherichia coli*, *Shigella*, *Enterobacter*, *Klebsiella*, *Citrobacter*, intestinal hepatits viruses, noroviruses and viruses that cause gastroenteritis such as, Enteroviruses, and parasites. Water also contains helminthes, or flatworms (Bharti *et al*., 2003) and various mould species such as *Aspergillus* and *Penicillium* species which produce substances that are toxic when consumed (Hageskal *et al*., 2006). These fungi are not just responsible for the negative health, but they also produce a bad taste and smell and cause several water quality issues (Dogget, 2000). Water borne *Escherichia coli* for example; is transmitted by direct contact with sick farm or domestic animals or faeces, as well as through contaminated drinking and recreational water (Besser, 1999).

Waterborne pathogens are continuously resurfacing as a result of social and environmental changes. For instance, estimated in the five to ten years to come, diarrhoea will account for about 2.4% of all cases worldwide in several developing nations. One of the many faecal-oral processes by which they can be passed from one person to another, or in some circumstances, from animals to people. All water samples obtained from a distribution system, including those gathered on the premises of the consumer should be ideally free of pathogenic microorganisms. Thus is not always possible in reality (Aik *et al*., 2020).

According to Ezeugwunne *et al*., (2009), good quality water is tasteless, colourless, odourless, and free of faecal contamination. In order to encourage healthy living among the residents of a specific geographic area, a consistent supply of clean, nourishing water is absolutely necessary. Despite four decades of efforts, the availability of safe and potable water in Nigeria's urban areas is still insufficient. Various governments have made numerous efforts since independence (Ajayi *et al*., 2008). The standard industrialized world model for delivering of safe drinking water and sanitation technology is however not affordable in most of the developing world. Given the repeated promises made by the world to the Millennium Development Goals (MDG) set for 2015, the significance of locally supplied inexpensive alternative drinking water projects, as well as their contribution to cost effectiveness has not been overlooked. It is crucial to emphasize sustainable access in both urban and rural areas of emerging countries. One of such local intervention in Nigeria where public drinking water supply is unreliable is drinking water sold in polythene sachets (Gandy, 2004).

Water in sachets is readily available and affordable but there are concerns about its purity. The integrity of the hygienic environment and conditions where the majority of the water in sachets are produced has been questioned. Apart from environmental contaminants, contamination from improper vendor handling also poses threats to the health of the ignorant consumers who drink often times without any proper cleaning of the sachets. Previous studies have identified handling as the source of infection in food and water-borne diseases in several countries. Water related diseases has now continued to be one of the major health problems globally (Dada, 2009).

1.1 AIM AND OBJECTIVES

The aim of the study was to evaluate the microbial load of drinking water from two major distribution companies in

The specific objectives of this research were to:

1. Obtain drinking water samples from the two sources
2. Isolate and identify the bacteria and fungi present in the water samples.
3. Compare the total microbial load of the water samples from the two sources.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE MAJOR GROUPS OF BIOPOLLUTANTS OF WATER

The water we drink, by definition as H_2O, meets the body's fundamental water demands and includes all required minerals and components. A mineral shortage is the most common cause of a variety of illnesses. It can lead to fluorine deficiency or endemic goiter disease. Fresh water, in addition to readily available chemical constituents, can contribute to the health of teeth. The expanded world of water is another living cosmos that is hidden from our gaze. Water has a wide range of effects on all living things, including humans. In the water, there are several amplified degrees of existence that are potentially toxic and can induce deadly infectious illnesses. Ignoring proper treatment might result in considerable losses.

It should be noted that pathogenic agents infected by polluted water produce a variety of diseases. According to the World Health Organization (WHO), drinking polluted water caused over 25,000 deaths per day in 2008. An individual who drank polluted water receives one hospital bed out of every four. Each year, contaminated water causes more than 80% of all diseases worldwide. Each year, dirty water causes over 5 billion diseases in Latin America, Asia, and Africa. In underdeveloped nations, food and water pollution is the leading cause of mortality (Fenwick, 2006).

It is clear that the process of purifying freshwater has significantly reduced the number of waterborne diseases in wealthy nations throughout the last century. In fact, this problem has been eradicated or effectively managed to some extent. However, in developing nations, further investment and allocation of resources are necessary. Budgeting is necessary due to the need for protecting water distribution networks from potential secondary contamination. Moreover, the expansion of modern conveniences and the process of transforming rural areas into cities demand the need for digging trenches and excavations. Reducing the emphasis on installing these infrastructure structures, like water distribution systems, could potentially have negative consequences for older equipment, such as water pipes that have ruptured due to excavation for new water, sewer, or telephone lines. (Medema *et al*., 2003).

The pipes reaching their shelf life limit, consequences that are not allowed, regular disruptions in the water distribution systems (leading to issues like water hammering and pipes breaking), which in turn causes negative pressure and contamination to be sucked into the water distribution systems, and the lack of precise control over water sterilization processes are problems that can rapidly spread contamination throughout the city in various ways. Microbial contamination of water can happen unintentionally, but a majority of contamination arises from inadequate attention given to the proper disposal of sewage. Municipal and household wastewater harbors diverse types of harmful microorganisms at high concentrations. While most of the microbes in the digestive system of humans cannot survive outside of the body for a long time, there are several reasons indicating that a significant number of harmful microbes may still be able to survive and infect humans.

Human health is at risk when consuming water and food that have been contaminated, using untreated water for bathing or showering, participating in water-related activities, and ultimately, utilizing contaminated water for farming and industrial activities. Biopollutants are compounds that lead to water pollution, either by being released by living organisms or by the organisms themselves being present in the water. When these biopollutants enter drinking water, they lead to the spread of diseases that are transmitted through water. The diseases can be categorized into three groups: Pathogenic microorganisms, viruses, and parasites and protists. The presence of these organisms in water can be caused by either the water being contaminated or the water getting infected during the piping process. The contamination could potentially be introduced into the distribution network. There are several reasons that can be listed for the disruption of the network line flow, including broken lines caused by excavation, the installation of additional connections, outdated network infrastructure, and a decrease in pressure especially during power outages (Grabow, 1996).

2.1.1 Pathogenic Bacteria

This collection of microorganisms can cause infections by either directly entering the body through drinking water or indirectly through the consumption of contaminated food. In developing nations, the leading cause of death is attributed to disease-causing microorganisms. Pathogenic microorganisms cause severe illnesses like cholera and typhoid, and although they pose a lesser threat, they are also accountable for a significant occurrence of infantile diarrhea. In developing nations, diseases such as diarrhea and internal infections are the leading factors contributing to mortality in both urban and rural areas. Certain diseases that come from water include cholera, which is caused by *Vibrio cholerae*, and bacillary dysentery, which is caused by *Shigella dysenteriae* (Medema *et al*., 2003).

Transmission of cholera can potentially happen between individuals; however, the primary mode of spreading the disease is through the environment, specifically water. The salmonellosis epidemic can be transmitted through food, although it can also be transmitted through water in some cases. Regarding the diseases mentioned earlier, apart from water, there are alternative methods of transmitting infection such as direct contact between individuals, carriers, and food sources (Popoff, 2005).

2.1.2 Pathogenic Viruses

Some viruses may survive in the human alimentary canal, as well as the mouth and larynx. These elements will be expelled via feces, which can be found in waste water and polluted waterways. Of fact, their sheer presence does not imply that they are hazardous to people. Polio virus spread in water was seldom documented. The reason for this is that this virus becomes diluted in water, making separation harder. Although the cause of infectious hepatitis is unclear, epidemiological data suggests that epidemics of this illness have spread around the world via polluted water (Gantzer *et al*., 1998).

2.1.3. Parasites

Protists and parasites can also be conveyed to the human body by direct drinking of polluted water, resulting in sickness. The most common parasitic and protistal disorders include amoebic dysentery produced by *Entamoeba histolytica*, giardiasis or flagellate diarrhoea caused by *Giardia lamblia*, and balantidiasis caused by *Balantidium coli*. It is also possible that it will infect people through contaminated food (Marshall *et al*., 1997).

2.2 SOURCES OF WATER SUPPLY

2.2.1 Surface Water and Groundwater Sources for Drinking Water

Any body of water that is exposed to the atmosphere and susceptible to runoff from the land is referred to as surface water. As a result, it is quite likely to include bacteria that can cause illness and in some cases, more severe and even fatal diseases. A significant section of the surface in some places includes a variety of chemicals and pathogens and needs to be purified. On the other hand, soils and rocks hide groundwater. Compared to surface water, it is thought to be less sensitive to sedimentation. But its abstract demands drilling and pumping equipment, neither of which are often viable options or available particularly in emerging nations (Tufenkji *et al*., 2002).

The choice of the best accessible sources of water is the most crucial stage in supplying a region with safe drinking water. The easier and less sensitive it is to convert source waters into safe drinking water, the better. Freshwater availability fluctuates both geographically and momentarily. Freshwater that is replenishable on earth typically takes the form of unbalanced distribution of surface water. Groundwater is dispersed more evenly, even if a large portion of its fossilized water. The amount of water used in a specific area is influenced not just by the availability of natural groundwater but also by the population and a regions land use as well as practical considerations (Tufenkji *et al*., 2002).

In the developed countries, the municipality is obliged by law to supply the consumers with high-quality water, while in the developing countries, this is not always valid. Hence, the economy of a community determines the attitudes and funding toward water development and treatment. The local climate also plays a significant role due to influences on evaporation rates and practices such as lawn watering and cooling requirements. Additionally, cultural values, actions, policies, and laws of national governments also have an impact on water use. Finally, the issue of ownership of the resource which is linked to government influences can be an important factor. Municipal water supply systems include facilities for storage, transmission, treatment, and distribution. The design of these facilities depends on the quality of the water, the particular needs of the user or consumer, and the quantities of water that must be processed. In certain cases, seawater can also be used as a drinking water source through the process of desalination (Katsanou and Karapanagioti, 2019).

2.2.2 Water Cycle and Climate Change

The sole naturally renewable supply of freshwater on a global scale is rainfall, which amounts to approximately 110,000 cubic kilometers per year. A significant portion of the moisture that falls as rain over land, about 70,000 km^3 per year, returns to the atmosphere through the processes of evaporation and transpiration by plants. The infiltration rates differ based on the land utilization, soil characteristics, moisture level, and the intensity and duration of rainfall. If the amount of precipitation exceeds the amount that can be absorbed into the ground, water will flow over the surface. Approximately 26% of the volume of this particular portion of the cycle, which amounts to 18,000 km^3 per year, is accessible for human use. Globally, the annual amount of water runoff, which includes the process of water infiltrating the soil and replenishing groundwater, is calculated to be 41,000 cubic kilometers per year. The scarcity of available water resources throughout the year is restricted to 12,500km^3/year due to geographical isolation and periodic occurrences like floods. Hence, approximately 54% of the available water flow and 23% of the overall replenishable resource (rainfall over land) are presently suitable for human utilization in various ways, as per estimates by Postel *et al*. in 1996.

Even though groundwater doesn't make up a large volume of water worldwide, it is an essential water source that can meet human needs, as it is included in the limited supply of freshwater. In terms of maintaining sustainability over a long period of time, the renewable resource holds the utmost importance. However, it can also be seen as a finite resource because it can be taken out at a faster pace than it is being replaced. Climate change causes the atmosphere to heat up, resulting in changes to the hydrologic cycle. Therefore, the quantity, timing, type, and strength of rainfall, water movement in watersheds, and the condition of aquatic and marine habitats can all be altered based on the region (Katsanou and Karapanagioti, 2019).

Despite the creation of various scenarios to forecast the potential consequences of climate change, there remains a lack of agreement on the specific quantitative impacts. Yet, there is a widespread consensus that numerous semi-arid or even arid regions will experience increased dehydration, leading to diminished replenishment of groundwater. In regions with high humidity, the recharge is projected to rise as a result of an increased occurrence of intense rainfall events. Efforts are being made to enhance our comprehension of the mechanisms behind climate change at a regional and local level. This will enable us to identify and implement measures to lessen its impact (Katsanou and Karapanagioti, 2019).

2.2.3 Surface Water

Surface water collects on the surface of the Earth, whether it is on the ground or within a flowing body of water such as a stream, river, lake, reservoir, or ocean. The catchment area refers to the overall land area that contributes surface runoff to a lake or river. The quantity of water is primarily determined by the volume of rainfall, along with factors such as the size of the watershed, the incline of the terrain, the composition of the soil and vegetation, as well as the manner in which the land is utilized. The fluctuation of a lake's water level is determined by the discrepancy between the amount of water flowing in and out of the lake in relation to its overall capacity. Cities often rely on surface water sources such as rivers and lakes to meet the high demands of their water supply systems due to the consistent and abundant supply of water they can provide. For smaller community water needs, people typically favor alternative water sources like wells or gravity systems supplied by springs, rather than relying on surface water. This is due to the likelihood of high expenses for treating and delivering surface water, as well as the unreliable nature of operation and maintenance (Katsanou and Karapanagioti, 2019).

2.2.3.1 Lakes

Lakes possess various characteristics including catchment area, input and output of water, nutrient levels, dissolved oxygen, contaminants, pH levels, sediment accumulation, and type, among others. The main factors that contribute the most to the lake's water supply include rainfall on the lake's surface, water flow from streams in the lake's surrounding area, underground water sources, and human-made sources from areas beyond the lake's drainage basin. The most important results include the evaporation occurring on the lake surface, the flow of surface water and groundwater, and any water that is taken out for human purposes. The water level of a lake experiences changes that are influenced by both weather patterns and variations in water extraction. Most lakes around the world are filled with freshwater. The water composition of these varies due to numerous factors. Freshwater lakes play a vital role as valuable natural assets that not only provide drinking water but also serve various other purposes. However, they encounter several issues with the decline in water quality as a result of human activities such as pollution, agricultural and fishing activities, and climate change (Mushtaq and Nee-Lala, 2017; Zhou *et al*., 2015).

2.2.3.2 Rivers and Streams

Rivers play a role in the water cycle. The movement of the water is influenced by several factors such as rainfall, the amount of water flowing over the surface, the movement of water between different areas, the flow of water beneath the ground, and the water pumped into and out of the system. The volume of water released from a river changes with the seasons and can also vary from year to year. The water quality of rivers and streams is affected by their fluctuating flow throughout different seasons in numerous countries. The river water's chemical makeup is intricate and is influenced by various factors, including the atmosphere, the geological properties of the surrounding area, and human actions. The ecology is greatly impacted and the usage of river water is also influenced by it. To analyze the chemical composition of river water, it is necessary to carry out a carefully planned sampling and analysis process (Di Matteo *et al*., 2017).

Springs that supply small streams in the headwater regions of rivers are the primary source of water downstream during periods of dry weather and drought. When the river's normal flow is insufficient to meet the year-round water demand, a dam is constructed to impede the water flow. Over time, a man-made lake is created. Building a dam for water extraction is both costly and harmful to the environment, with the potential to cause earthquakes. In addition, adequate rainfall and a broad river basin are necessary for these reservoirs, but over time they will become filled with sediment (Di Matteo *et al*., 2017).

2.2.4 Groundwater

Aquifers serve as a significant source of drinking water globally, providing groundwater. The hydrological recharge of aquifers varies greatly depending on location and is influenced by factors such as climate, geology, soil composition, plant cover, and how land is utilized. Groundwater is replenished through rainfall, which is supplemented by the natural seepage of surface water or through artificial methods of recharge. Around the world, approximately one-fifth of the water used in irrigation and nearly half of the water utilized in industry comes from underground sources. Groundwater in a loosely packed rock only travels a short distance of a few centimeters per day. The rate at which it travels is mainly determined by how steep the slopes of the aquifer are and the ability of the rocks to allow water to pass through them. In rocks that have been consolidated, the speed of water flow can be significantly greater. An example that is commonly seen are the karst formations (Scanlon *et al*., 2002).

Groundwater can be found in aquifers in two distinct situations: the unconfined state and the confined state. In an unconfined aquifer, the water does not completely fill the aquifer and the top surface of the saturated zone can fluctuate in height. In contrast, within a restricted underground water source, the aquifer is entirely filled with water and is covered by a layer that prevents water from escaping. The replenishment of the saturated zone happens when water seeps down from the ground surface through the unsaturated zone. There are numerous benefits to utilizing groundwater as a source for household water supply. In the majority of populated areas around the globe, there exists a significant quantity of underground water. Although the amount being extracted is substantial, it is frequently replenished without difficulty (Scanlon *et al*., 2002).

An additional benefit is that the top layers of soil act as a protective barrier, preventing physical, chemical, and biological damage. This barrier is highly efficient in terms of both quality and cost. In conclusion, the utilization of groundwater tends to provide significant economic advantages for each amount of water compared to surface water. This is due to its abundant availability in local areas, its high reliability during drought periods, and its generally good quality that requires minimal treatment. The hydraulic properties of the soils and rocks determine how pollutants move in an aquifer. When substances are dissolved in water, they typically move with it unless they are either attached to something or delayed by the process of adsorption. So, pollutants typically move through areas that have high permeability. The farther away they are from a groundwater discharge area, the deeper they go into the groundwater system and the greater the extent of the area that gets impacted.

Groundwater exploration involves the use of various techniques such as hydrogeological mapping, hydrogeophysical prospecting, investigation drilling, pumping tests, and observation of groundwater levels. Additionally, methods like remote sensing, isotopic studies, shallow seismic prospecting, and velocity logging are also used in the search for groundwater. Some examples of groundwater comprise springs, boreholes, and wells as stated by Katsanou and Karapanagioti (2019).

2.2.4.1 Springs

Springs are the actual exits of an aquifer. Springs are most common in mountainous or hilly terrains, where the water table meets the ground surface. In the past, community water sources were frequently based on springs, and they continue to be a source of water since spring water has a high natural quality and is reasonably easy to consume. Artesian, gravity, perennial, intermittent, tube, seepage, and thermal springs are the many types of springs (Katsanou and Karapanagioti, 2019).

2.2.4.2 Boreholes and Wells

Drilling a well or a borehole below the water table can be used to acquire groundwater from an aquifer. The water level of boreholes or wells bored into unconfined aquifers is the same as the surrounding aquifer's water table. The water level in boreholes dug into confined aquifers sits at some height above the top of the aquifer but not necessarily above the ground surface. When the water level in a borehole rises above the ground surface, the well exhibits artesian flow, which occurs when water rises to the surface without being pumped. Pumping water from an aquifer causes the water level at the well to fall (Katsanou and Karapanagioti, 2019).

2.3 SOURCES OF WATER POLLUTION

During the past few decades, the need for freshwater has significantly risen as a result of the fast population growth and the rapid industrialization (Ramakrishnaiah *et al*., 2009). Most agricultural development activities, especially the excessive use of fertilizers and unsanitary conditions, pose a significant risk to human health (Okeke and Igboanua, 2003). Human activities, such as urbanization, agriculture, industrialization, and population growth, have caused a decline in water quality in various regions worldwide (Baig *et al*., 2009, Mian *et al*., 2010, Wang *et al*., 2010). Furthermore, the limited availability of water has increasingly hindered efforts to prevent water pollution and enhance water quality. Water pollution has been the subject of extensive research conducted by both government agencies and scientists. Therefore, it is of utmost importance to urgently safeguard the quality of river water due to the grave issue of water pollution and the worldwide scarcity of water resources (Bu *et al*., 2010).

Water pollution can occur from two sources.

- Point sources
- Non-point sources

2.3.1 Point Sources

Point sources of pollution are those which have direct identifiable source. Example includes pipe attached to a factory, oil spill from a tanker, effluents coming out from industries. Point sources of pollution include wastewater effluent (both municipal and industrial) and storm sewer discharge and affect mostly the area near it. Whereas non-point sources of pollution are those which arrive from different sources of origin and number of ways by which contaminants enter into groundwater or surface water and arrive in the environment from different non identifiable sources. Examples are runoff from agricultural fields, urban waste etc. Sometimes pollution that enters the environment in one place has an effect hundreds or even thousands of miles away. This is known as trans-boundary pollution. One example is the radioactive waste that travels through the oceans from nuclear reprocessing plants to nearby countries. Water pollutants may be;

i) Organic

ii) Inorganic (Gupta and Singh, 2016).

- **Organic Water Pollutants**

Insecticides and herbicides, organohalides and other chemicals, germs from sewage and animal rearing, food processing wastes, diseases, and volatile organic compounds are among them.

- **Inorganic Water Pollutants**

Heavy metals from acid mine drainage, silt from surface run-off, logging, slash and burn practices, and land infill, fertilizers from agricultural run-off (including nitrates and phosphates, among other things), and chemical waste from industrial effluents can all contribute to them (Gupta and Singh, 2016).

2.3.2 Non-point Sources

Non-point causes of water pollution include agricultural runoff, building sites, and pollutant deposition on the water's surface from the atmosphere (Gupta and Singh, 2016).

2.3.2.1 Urbanization

Phosphorus concentrations in urban catchments are often greater as a result of urbanization. Increased imperviousness, runoff from urbanized surfaces, and municipal discharges all result in increased nutrient loadings to urban streams. As a result, urbanization is a significant driver of stream damage (Paul and Meyer, 2001).

- **Sewage and other Oxygen Demanding Wastes**

The management of solid waste is failing because of the large quantities of organic and non-biodegradable waste that is produced every day. Due to this, waste in many areas of India is disposed of in an unscientific manner, which ultimately results in an increase in pollutants in surface and groundwater sources. The discharge of sewage into the environment can act as a beneficial fertilizer by supplying essential nutrients like nitrogen and phosphorus that promote the growth of plants and animals. The use of chemical fertilizers by farmers also introduces nutrients into the soil, which then flow into rivers and seas and contribute to the fertility of the sewage. When sewage and fertilizers are combined, they have the potential to greatly enhance the growth of algae or plankton. This can lead to the formation of algal blooms, which can cover large portions of oceans, lakes, or rivers. This process decreases the amount of dissolved oxygen in the water, leading to the death of various types of aquatic life (Gupta and Singh, 2016).

- **Industrial Wastes**

A large number of industries are located near rivers, such as steel and paper industries, because they need a significant amount of water for their manufacturing processes. Subsequently, they dispose of their waste, which contains chemicals like acids, alkalis, dyes, and others, by pouring it into the rivers as effluents. Chemical companies involved in the production of aluminum release significant quantities of fluoride into the atmosphere through their air emissions and into water resources through their effluents. The fertilizer industry produces a significant quantity of ammonia, while steel plants produce cyanide. Chromium salts are employed in industrial processes to manufacture sodium dichromate and other compounds that contain chromium. According to Gupta and Singh (2016), these discharges eventually reach water bodies as effluents, which have negative effects on both human health and the organisms residing in the water.

- **Agro-chemical Wastes**

Political motives lead to the provision of subsidies for water and electricity used in irrigation within the agricultural industry. This results in the use of inefficient flood irrigation instead of using more efficient methods like sprinkler and drip irrigation. Cropping methods and agricultural techniques may not always promote the responsible utilization of water. Water losses occur as a result of breaches and seepage, leading to the issues of water logging and salinity. Chemical byproducts used for agricultural purposes, such as fertilizers and various types of pesticides like herbicides and insecticides, are classified as agro-chemical wastes. These substances are commonly utilized in order to increase crop yields. The inappropriate dumping of pesticides from agricultural activities and farms greatly adds pollutants to bodies of water and soil. According to Gupta and Singh (2016), there are several types of pesticides including DDT, Aldrin, Dieldrin, Malathion, and Hexachloro Benzene.

Pesticides contaminate water sources when they are washed off agricultural fields by rainwater or flow into water bodies through surface runoff. Additionally, the spraying and dusting of pesticides in lower areas also contribute to the pollution of water, negatively affecting its quality. A majority of them are not capable of being broken down naturally and remain in the environment for extended periods. These substances have the potential to enter the human body via the food chain, which can result in biomagnification (Gupta and Singh, 2016).

- **Nutrient Enrichment**

The nutrients found in surface water can be categorized as either natural or caused by human activities. The natural system maintains a steady equilibrium between the production and consumption of nutrients, resulting in minimal pollution from natural sources. Contaminants derived from human activities can arise from farming, household, and industrial discharges. The levels of nutrients in streams and rivers have been found to be closely linked to the degree of human land use and disturbance. Both nitrogen and phosphorus enrichment in the watershed can be attributed to agricultural and urban land uses. The level of total nitrogen and nitrate fluxes and concentrations in rivers is also associated with the density of human population (Carpenter *et al.*, 1998).

The primary nitrogen supply in streams and rivers is derived from fertilization, according to a study conducted by Goolsby and Battaglin in 2001. Also, nutrient enrichment of aquatic systems can be caused by both point and nonpoint sources that are influenced by human activities. In comparison to easily trackable and controllable nutrient sources, like point sources, nonpoint sources such as livestock, crop fertilizers, and urban runoff show greater variation in location and time. After the Clean Water Act introduced strict regulation of point source inputs, water pollution in the United States is mostly caused by nutrients from nonpoint sources (Carpenter *et al.*, 1998).

- **Radioactive Wastes**

The presence of radioactive elements in water causes radioactive contamination. They are divided into tiny dosages that temporarily increase metabolism and big amounts that gradually harm the body, producing genetic mutation. Radioactive sand, waters used in nuclear atomic plants, radioactive minerals extraction, nuclear power plants, and the usage of radioisotopes for medicinal and scientific purposes are all potential sources (Gupta and Singh, 2016).

- **Thermal Pollution**

Water temperature changes have a negative impact on water quality and aquatic biota. The majority of thermal pollution in water is produced by human activity. Nuclear power and electric power plants, petroleum refineries, steel melting factories, coal fire power plants, and boilers from industries are some of the major sources of thermal pollution. These sources release large amounts of heat into water bodies, causing changes in the physical, chemical, and biological characteristics of the receiving water bodies. High temperatures reduce the oxygen level of water, disrupt the reproductive cycles, respiratory and digestive processes, and cause other physiological changes that make aquatic existence challenging.

- **Oil Spillage**

Oil discharged into the sea by accident or leakage from cargo tankers transporting petrol, diesel, and their derivatives pollutes marine water significantly. Oil contamination in water is also caused by offshore oil exploration. The leftover oil spreads across the surface of the water, generating a thin coating of water-in-oil emulsion.

- **Disruption of Sediments**

Water pollution that changes the pH level around a plant, such as acid rain, can injure or kill it. Atmospheric sulfur dioxide and nitrogen dioxide, generated by natural and man-made sources such as volcanic activity and the combustion of fossil fuels, react with atmospheric molecules such as hydrogen and oxygen to form sulfuric and nitric acids. These acids are carried to the ground by precipitation in the form of rain or snow. When acid rain falls on the earth, it runs into streams, carrying its acidic chemicals into bodies of water. Acid rain that settles in aquatic habitats reduces water pH and has an impact on aquatic biota.

- **Acid Rain Pollution**

Water pollution that alters a plant's surrounding pH level, such as due to acid rain, can harm or kill the plant. Atmospheric Sulfur dioxide and nitrogen dioxide emitted from natural and human-made sources like volcanic activity and burning fossil fuels\interact with atmospheric chemicals, including hydrogen and oxygen, to form sulfuric and nitric acids in the air. These acids fall down to earth through precipitation in the form of rain or snow. Once acid rain reaches the ground, it flows into waterways that carry its acidic compounds into water bodies. Acid rain that collects in aquatic environments lowers water pH levels and affects the aquatic biota.

2.4 DISEASES FROM WATER

Microbes have a significant impact on the quality of water, and certain types of microorganisms, including *Salmonella* sp., *Shigella* sp., *Escherichia coli*, and *Vibrio cholerae*, are particularly associated with waterborne illnesses (Adetunde and Glover, 2010). These factors are responsible for causing diseases such as typhoid fever, diarrhea, dysentery, gastroenteritis, and cholera. The water supply becomes extremely hazardous when feces contaminate it. Numerous illnesses are kept going through the transmission route of waste to mouth, where the disease-causing agents are only released in human stool (Adetunde and Glover, 2010). The presence of *Escherichia coli* coliforms in feces is used as a signal for the existence of any of these waterborne pathogens (Adetunde and Glover, 2010). There is a suggestion that the primary reason for deaths and illnesses worldwide is the pollution of underground water sources. This issue is responsible for the deaths of over 14,000 individuals every day, with a large portion of those being young children under the age of 5. In the past few years, there has been a growing public worry regarding the state of groundwater due to numerous reports confirming the presence of pollutants. Children are usually at a higher risk of contracting intestinal pathogens, and it has been stated that approximately 1.1 million children succumb to diarrhoeal diseases annually (Steiner and Gurrant, 2006).

Water is crucial for human existence and the well-being of society. Nevertheless, a significant portion of the global population faces a lack of sufficient and secure drinking water. At present, there is a worldwide problem of water scarcity that impacts over 40% of the global population. Moreover, projections indicate that approximately 3 billion individuals will lack access to clean water by the year 2025 and will be residing in an environment where water scarcity is a prominent issue. Consequently, water-related illnesses and fatalities remain a worldwide problem in both industrialized and developing nations. Although the majority of cases occur in underdeveloped nations, waterborne diseases outbreaks are also witnessed in developed countries (Blasi *et al*., 2008).

Waterborne diseases are caused by drinking water mostly contaminated by human or animal excrement which contain pathogenic micro-organisms. Globally, waterborne diarrhea illness is leading among diseases that cause mortality and morbidity, killing 1.8 million people and causing approximately 4 billion cases of illness annually. Moreover, in less developed countries waterborne diarrhea continues to be a leading cause of death and illness among children with 90% of diarrhoeal deaths being borne by children under five years. Poor sanitation, inadequate safe drinking water and poor hygiene practices are major attributable factors to waterborne diseases occurrence (Nyagwencha *et al*., 2017; Njiru *et al*., 2016).

Waterborne diseases are those diseases that are transmitted through the direct drinking of contaminated water with human or animal excreta. Contaminated drinking water when used in the preparation of food can be the source of food borne disease through consumption of the same microorganisms. Most waterborne diseases are characterized by diarrhoea, which involves excessive stooling, often resulting to dehydration and possibly death. Approximately 4 billion cases of diarrhoea reported each year cause at least 1.8 million deaths with 90% of the cases being children under the age of five years. These deaths represent approximately 4% of all deaths, and 18% of children under-five years' deaths in developing countries, diarrheal disease accounts for an estimated 4.1% of the total daily global burden of disease and is responsible for the deaths of 1.8 million people every year and mostly concentrated on children below 5 years in developing countries (Njiru *et al*., 2016).

In rural African regions, fecal contamination of water arises from runoffs from nearby bushes and forest which serve as defecation sites for rural dwellers. Waterborne disease can be caused by protozoa, viruses, bacteria, and intestinal parasites. Some of the organisms remarkable for their role in the outbreak of waterborne disease include Cholera, Amoebic dysentery, Bacillary dysentery, Cryptosporidiosis, Typhoid, Giardiasis, Paratyphoid, Balantidiasis, Salmonellosis, Campylobacter enteritis, Rotavirus diarrhea, E. coli diarrhea, Hepatitis A, Leptospirosis and Poliomyelitis. Most waterborne diseases are often transmitted via the fecal-oral route, and this occurs when human fecal material is ingested by drinking of contaminated water (Nyagwencha *et al*., 2017).

2.5 MUNICIPAL WATER PURIFICATION SYSTEM

The following points highlight the four main processes employed in a municipal water purification system to ensure the supply of non polluted drinking water. The processes are:

- Flocculation
- Sedimentation

- Filtration
- Disinfection

(Karapanagioti, 2016).

2.1.1 Flocculation

Water is collected in big tanks or basins after being pumped from a raw water reservoir (natural source) for a sufficient amount of time to allow large particle debris to settle at the bottom. Following the removal of the debris, the water is treated with flocculants such as aluminum sulphate, alum, colloidal silicate, or calcium, which generate a floe that precipitates and transports microorganisms to the surface (Liu *et al*., 2013).

2.1.2 Sedimentation

Following coagulation, the water is placed in the settling basin for a suitable period of time to allow residual materials to settle. Sedimentation, on the other hand, significantly lowers the microbial population of the water while also eliminating the majority of the suspended particles (Gillespie *et al*., 2014).

2.1.3 Filtration

Following sedimentation, the water is passed through sand filters to remove flocks of live creatures. Filtration is vital because it can remove protozoan cysts as well as about 99% of bacteria from water. Water can also be filtered via activated charcoal to eliminate potentially hazardous chemical molecules that give the water an unpleasant color or taste (Fransisque *et al*., 2009).

2.1.3.1 Slow Sand Filters

The sand filters are made up of multiple layers including fine sand, coarse sand, and fine and coarse gravel. The bottom of the structure consists of tiles with small holes to allow filtered water to enter. The top layer of fine sand acts as a biological filter by trapping particles like colloidal floccules, microorganisms, and algae in its gaps. This layer also holds onto bacteria, effectively removing them from the water. The effectiveness of the filter improves even more since the negatively charged bacteria are stuck to the positively charged colloidal material in the layer. Typically, this filter eliminates about 98% of the bacteria found in water. This method has the potential to purify around 5 to 7 million gallons of water per acre of filter, but it is slow and typically needs multiple acres of land.

2.1.3.2 Rapid Sand Filters

This approach uses the same equipment as slow sand filters, but the sand filter surface is significantly smaller and requires regular backwashing. This allows for the quick filtration of water. Rapid sand filters have the potential to supply up to 150 million gallons of water per day (Fransisque *et al*., 2009).

2.1.4 Disinfection

Disinfection is the last stage of purifying water in cities, and its purpose is to prevent any harmful microorganisms from being present in the water. Sodium or calcium hypochlorite can be used to disinfect water in small towns and local areas, while larger cities typically rely on chlorination as the traditional disinfection method. The presence of chlorine in water can effectively eliminate bacteria as it reacts with the water molecules, producing hypochlorous and hypochloric acids. These acids have strong antimicrobial properties. Chlorine is acquired in a liquid state when subjected to pressure, but it converts into a gas when it enters water. It readily mixes with water and apart from eliminating microorganisms, it also efficiently breaks down organic substances through oxidation (Liu *et al*., 2013).

However, the chlorine requirement for a certain volume of water increases as the amount of organic matter in the water increases. In other words, the chlorine demand varies depending on the organic content of the water. In any situation, it is important that the remaining level of chlorine in drinking water should not go beyond 0.2ppm after disinfection because there is a negative consequence of producing small quantities of organochlorine compounds, specifically trihalomethane (THM), which is known to cause cancer. Luckily, research has shown that chloroamination is an inexpensive method for reducing the production of trihalomethane (THM). As a result, it is becoming increasingly popular as a substitute for chlorination.

This process involves the direct generation of monochloramine in water for disinfection purposes by adding ammonium either before or at the same time as chlorine or hypochlorite. Mono-chloramine is highly efficient and generates significantly reduced quantities of trihalomethanes (THMs). Once the water has been disinfected, it is then stored in expansive reservoirs before being distributed for consumption (Van der Wielen *et al*., 2016).

2.6 PURIFICATION PROCESS OF WATER PRODUCTION UNIT A

The purification process used by the company falls in line with the standard municipal water purification process. Water is purified by filtration using carbon filters and ultraviolet (UV) sterilization.

The purification process takes place in four stages.

- **Collection**

Water is collected from an underground source and stored in a storage tank

- **Treatment plant**

The water is then moved to the treatment plant which consists of three cylinders, the first one containing granite stone and sand, the second one containing hydrolite and adolite and the third one containing the activated charcoal.

- **Filtration**

The water is then moved to the carbon filter and white membrane filter for filtration. The carbon filter removes the colour, odour and makes the water clean.

- **Disinfection**

Disinfection is the final purification treatment. This is carried out using ultraviolet (UV) radiation to reduce the concentration of pathogenic microorganisms and most viruses that would have evaded the filtration process.

- **Packaging**

The water follows a pipe to a storage tank where it is packaged as sachet water or is stored in a tank if it is to be packaged as bottle water.

2.7 PURIFICATION PROCESS OF WATER PRODUCTION UNIT B

- Water is first collected in a borehole from underground water sources and Dosing of the water follows by addition of soda ash (Na_2CO_3) to purify the water. Soda ash is added to reduce the acidity of the untreated water. It also has an added benefit of softening the water.

- The water is then transported through pipes to a composite tank where further purification occurs to remove smell, taste and odour from the water. The composite also contains carbon, marble and granite which also filter the water.
- The water goes through membrane filters with size range from 0.5 µm to 5 µm. The use of membrane filters purifies the water. This is called filter sterilization.
- The purified water finally goes through ultraviolet (UV) light for further sterilization after which the water is packaged and stored.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 COLLECTION OF SAMPLES

The water samples used for this study were obtained directly from the reservoirs where water is stored before packaging and taken to the laboratory for analysis.

3.2 STERIZATION OF MATERIALS

Measuring cylinders, glass rods, beakers and conical flasks required for this research work were soaked and washed in detergent and rinsed with distilled water. They were covered with cotton wool, wrapped with aluminum foil paper and heat sterilized in an autoclave in an inverted position at 170^{o}C for 5 minutes.

3.3 PREPARATION OF NUTRIENT AGAR

Nutrient agar (NA) was used as the medium for the antibacterial assay. The medium was prepared according to manufacturer's specification. 28 g of nutrient agar powder was dissolved completely in 1000mL of distilled water according to manufacturer's instructions. The resulting mixture was then poured into a conical flask, covered with an aluminum foil paper and sterilized in an autoclave at 121◦C for 15 minutes. Fulchin was also added to the prepared nutrient agar to inhibit the growth of any chanced fungi present.

3.4 PREPARATION OF POTATO DEXTROSE AGAR

Potato dextrose agar (PDA) was prepared by completely dissolving 39 g of potato dextrose agar powder in 1000 ml of distilled water according to the manufacturer's instructions. The resulting mixture was then poured into a conical flask, covered with an aluminum foil paper and sterilized in an autoclave at 121^{o}C for 15 minutes. Chloramphenicol was added to the prepared nutrient agar to inhibit the growth of any chanced fungi present.

3.5 PREPARATION OF EOSIN METHYLENE BLUE AGAR

Eosin methylene blue agar was prepared by dissolving 36 g of agar powder in 1000 ml of distilled water under heat and continuous stirring. The mixture was then immersed in an autoclave and sterilized at 121^{o}C for 15 minutes. The agar was then poured into Petri dishes after cooling to 50^{o}C and the allowed to solidify.

3.6 SERIAL DILUTION AND INOCULATION

From each of the water samples, 10 ml was measured using a volumetric flask to prepare a stock solution. Six test tubes were filled with 9mL of sterile distilled water to make dilutions from 10^{-1}-10^{-6}. Using a micropipette, 1ml was taken from the stock solution and added to 9mL of distilled water to make the first (10^{-1}) dilution, then 1mL was taken from the first dilution and mixed with 9mL of distilled water to make the second dilution (10^{-2}). The procedure was repeated until a dilution of (10^{-6}) was achieved.

3.7 INOCULATION

The samples were inoculated using the pour plate technique. 0.1mL was transferred from each test tube into Petri dishes using a micropipette and molten agar was added and mixed with the inoculants and the medium was allowed to cool and solidify. The plates were incubated at room temperature, placing them in an inverted position to prevent water of condensation from trickling back into the medium.

3.8 VIABLE COUNT

Total number of colonies on each plate was counted with the help of colony counter and Colony Forming Unit (CFU) per mL of sample was deduced using formula:

CFU = No. of colonies x Dilution factor / Sample volume (mL)

3.9 IDENTIFICATION OF BACTERIAL ISOLATES

Bacteria identification was done by Gram staining and microscopy and colonial morphological examination. Biochemical tests were further used to identify the bacteria isolates.

3.9.1 Gram Staining

The gram staining technique was used to differentiate between Gram-positive and Gram-negative bacteria. A drop of distilled water was placed on a clean, grease-free glass slide, and a single isolate of 24 hours old culture was mixed in it. The smear was made by spreading the culture on the slide.

The smear was fixed by passing the glass slide through a flame. The slide was flooded with crystal violet for 1 minute and then washed off with distilled water. Iodine solution was added to the smear and left for 1 minute and then washed off with distilled water. Ethanol (a decolorizing agent) was added for 30 seconds and washed off with distilled water. The smear was then counter-stained with saffranine for 1 minute and then washed off and left to air dry. The slide was observed under the microscope. Gram-positive bacteria appeared dark purple and Gram-negative appeared red.

3.9.2 Citrate Test

A slant of Simmons citrate agar was prepared according to the manufacturer's description. Then using an inoculating needle, the test organism was stabbed into the medium and then spread on the surface of the medium using an inoculating loop. The test tube was then incubated for 48hours. The production of a blue colour from the breakdown if citrate indicates a positive result.

3.9.3 Indole Test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole. Tryptophan broth was prepared in test tubes, sterilized in an autoclave and incubated aseptically for 48 hours. 0.5 mL of Kovac's reagent was then added to the broth culture. The formation of a red colour in the reagent layer on top of the agar indicated a positive result.

3.9.4 Oxidase Test

The oxidase test detects the presence of a cytochrome oxidase system that will catalyse the transport of electrons between donors in the bacteria and a redox dye tetramthyl-p-phenylene-diamine. A small piece if filter paper was soaked in 1% Kovacs oxidase reagent. Using a sterile loop, a well isolated colony is picked and smeared on the paper. A positive test was indicated by a deep purple colour change.

3.9.5 KOH test

Glass slides were cleaned with alcohol and left to air dry. Potassium hydroxide was then added unto the slide and a loopful of the bacterial isolate to be used was smeared in the slide using a sterile wire loop. The appearance of a slimy consistency on the slide differentiates a Gram positive bacterium from a Gram negative one.

3.10 IDENTIFICATION OF FUNGAL ISOLATES

Identification of fungal isolates was carried out using colonial morphological characteristics, and staining using lacto phenol cotton blue stain followed by microscopy. Morphological characteristics such as colony size, colour, texture and margination were recorded as well as hyphae structure using microscopy.

CHAPTER FOUR

RESULTS

The total heterotrophic bacterial counts, coliform counts and fungal counts obtained from water samples from unites A and B were recorded and represented in CFU/ml. The results are shown in Table 1.

Cultural examination was carried out on the bacterial and fungal isolates. Cultural characteristics such as colony shape, size, elevation, optical activity, margination, pigmentation, spore type and hyphae structure were evaluated and recorded in Tables 2 and 3. Biochemical tests were carried out to further identify the bacterial isolates and Gram staining was done observe the Gram reaction of the bacteria cells. The bacteria that were isolated are *Proteus* sp., *Salmonella* sp, *Shigella* sp. *Enterobacter* sp. *Pseudomonas* sp and *Escherichia coli,* while the fungi isolates were *Penicillium citrinum*, *Penicilim oxalicum*, *Mucor* sp and *Aspergillus fumigatus*.

The distribution pattern of the bacteria and fungi isolated were noted and are represented in Tables 5 and 6. Antibiotic susceptibility test was carried out to evaluate the susceptibility of the bacterial isolates to antibiotics. The *in vitro* antimicrobial assay was carried out using the Kirby Bauer disk diffusion technique. The zones of inhibition were measured in millimeters and compared with standard tables to determine the susceptibility and resistance of the bacterial isolates. The antibiotics used were; Septrin, Chloramphenicol, Saprifloxacin, Ciprofloxacin, Amoxacillin, Augmentin, Gentamycin, Pefloxacin, Tarivid and Streptomycin. The results are represented in Table 7.

Table 1: Colony count for the water samples in CFU/ml.

SAMPLE	Heterotrophic bacteria count (NA)	Heterotrophic bacteria count (MCA)	Heterotrophic fungal count (PDA)
A	$4.70x10^4 \pm 5.60$	$1.20\ x10^3 \pm 5.60$	$2.24x10^4 \pm 2.82$
B	$7.85\ x10^4 \pm 2.80$	$1.36\ x10^3 \pm 2.80$	$3.00x10^4 \pm 1.41$

Values represented as mean ± standard error of replicates

KEY:

NA: Nutrient agar

MCA: MacConkey agar

PDA: Potato dextrose agar

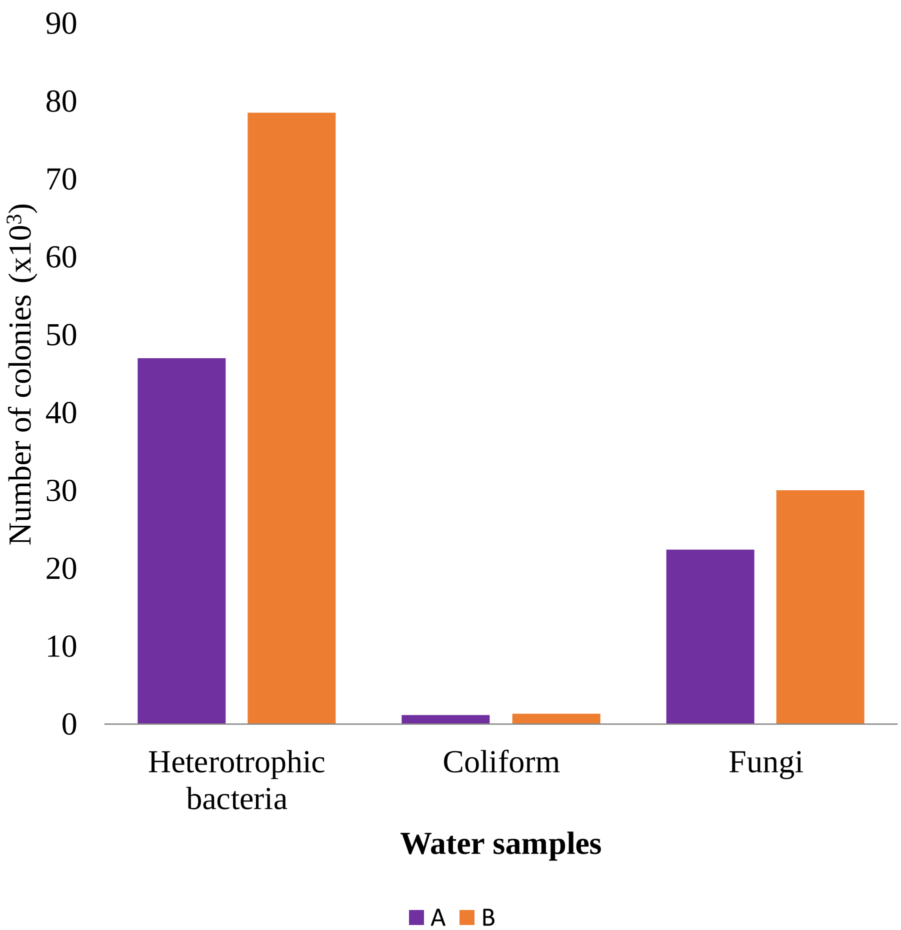

Figure 1: Total microbial load then for the water samples in CFU/ml.

Table 2: Cultural characteristics of the bacterial isolates

Organism	**Shape**	**Size**	**Elevation**	**Transparency**	**Margin**	**Colour (NA)**	**Colour (MCA)**
Proteus sp	Irregular	Large	Flat	Opaque	Entire	Dark	-
Salmonella sp	Filament	Large	Flat	Opaque	Filiform	Cream	Pale
Shigella sonnei	Regular	Medium	Flat	Opaque	Undulate	Cream	Pink
Enterobacter sp	Circular	Small	Flat	Opaque	Entire	Cream	Cream
Pseudomonas sp	Rhizoid	Medium	Low convex	Opaque	Undulate	Light cream	-
Escherichia coli	Irregular	Large	Flat	Opaque	Entire	Cream	Pink

Table 3: Results of the biochemical and morphological tests on the bacterial isolates

Test	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Gram stain	-	-	-	-	-	-
Cell type	Rod	Rod	Rod	Rod	Rod	Rod
Arrangement	Disperse	Chains	Disperse	Disperse	Disperse	Disperse
Urease	+	-	-	-	+	-
Indole	-	-	-	-	-	+
Citrate	+	-	-	+	-	-
Catalase	+	+	+	+	+	+
KOH	-	-	-	-	-	-
H_2S	+	+	-	-	-	-
Oxidase	-	-	-	-	+	-
Lactose	-	-	-	-	-	+
Sucrose	-	-	-	-	-	+
Glucose	+	+	+	+	-	+
Suspected organisms	*Proteus mirabilis*	*Salmonella enterica*	*Shigella sonnei*	*Enterobacter cloacae*	*Pseudomonas aeruginosa*	*Escherichia Coli*

Table 4: Cultural characteristics of the fungal isolates

Cultural characteristics	Morphological characteristics		
Nature of colony	**Nature of hyphae**	**Spore type**	**Organism**
Brownish colonies with cream reverse side	Non-septate	Chlamydospore	*Mucor* sp.
Green fluffy colonies with cream reverse side	Septate	Ascospore	*Penicillium citrinum*
Cream fluffy colonies with brown reverse side	Septate	Ascospore	*Penicillium oxalicum*
White fluffy colonies with pale reverse side	Septate	Conidiospore	*Aspergillus fumigatus*

Table 5: Distribution pattern of the bacterial isolates

BACTERIA	SAMPLE	
	A	B
Proteus sp	+	-
Salmonella sp	-	+
Shigella sonnei	+	+
Enterobacter sp	-	+
Pseudomonas sp	+	+
Escherichia coli	+	+

Table 6: Distribution pattern of the fungal isolates

FUNGI	SAMPLE	
	A	B
Mucor sp	+	+
Penicillium citrinum	+	+
Penicillium oxalicum	+	+
Aspergillus fumigatus	+	-

Table 7: Antibiotic sensitivity test on the bacteria isolates

Isolates	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
Proteus sp	0	1	0	2	1	2	3	0	6	6
Salmonella sp	8	0	0	12	0	0	20	22	18	6
Shigella sonnei	0	9	20	16	5	4	10	9	10	0
Enterobacter sp	0	14	15	5	9	0	0	15	0	11
Pseudomonas sp	0	6	1	2	0	0	0	6	1	1
Escherichia coli	3	7	0	1	2	0	9	0	3	0

KEY:

SXT: Septrin

CH: Chloramphenicol

SP: Saprifloxacin

CPX: Ciprofloxacin

AM: Amoxacillin

AU: Augmentin

CN: Gentamycin

PEF: Pefloxacin

OFX: Tarivid

S: Streptomycin

CHAPTER FIVE

DISCUSSION

The current study showed that the water provided to the public from both distribution units was contaminated with harmful bacteria, including *Proteus*, *Salmonella*, *Shigella sonnei*, *Enterobacter*, *Pseudomonas*, and *Escherichia coli*. The distribution companies obtained a range of heterotrophic bacteria counts from $4.70 \times 10^4 \pm 5.60$ CFU/ml to $7.85 \times 10^4 \pm 2.80$ CFU/ml, as shown in Table 1's overall bacterial count. The fungal counts obtained varied between $2.24 \times 10^4 \pm 2.82$ CFU/ml and $3.00 \times 10^4 \pm 1.41$ CFU/ml.

Mucor spp., *Penicillium oxalicum*, *Penicillium citrinum*, and *Aspergillus fumigatus* were detected in both water samples. These findings are consistent with those of a recent study done by Idible *et al*. (2018), who collected bacteria such as *E. coli*, *Staphylococcus epidermidis*, *Enterobacter* sp., and other isolates from borehole water samples in Edo State, Nigeria.

The presence of *Salmonella*, *Shigella*, and other bacteria from the Enterobactericiae family suggested that the packing and handling techniques were unclean and might have been contaminated by ill people. When consumed through food and water, these bacteria produce specific toxic components that cause poor health effects in humans (Arnesen *et al*., 2008). The presence of *Aspergillus* species in the samples shows that humans may be at risk of aflatoxicosis. Toxin production is possible in *Aspergillus* species. Because of their capacity to accumulate in the body over time, they have the potential to induce adverse effects on human health, including birth abnormalities, cancer, liver damage, and genetic alterations when eaten (Pleadin *et al*., 2019).

During antibiotic susceptibility testing, the majority of the bacteria isolates exhibited resistance to several of the antibiotics utilized. This arises because bacteria have the potential to evolve drug resistance mechanisms, such as changing their target locations, undergoing mutation, and reducing drug intake. More study is needed to determine the efficacy of alternative antibacterial agents in preventing growth of these dangerous bacteria and preventing the development of antibiotic-resistant genes in bacterial populations.

5.1 CONCLUSION

The study's findings indicate that the numerous water sources under consideration are polluted with biological and human and animal pollutants. To avoid potential epidemics, adequate water purifying methods should be implemented prior to provision of water to city areas.

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