

**COMPARATIVE ANALYSIS OF MICLYN WATER AND TWO  
BOREHOLE WATER FROM SABO AND IBIENAFE**

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**A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF  
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**(MICROBIOLOGY OPTION)**

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## **CERTIFICATION**

This is to certify that this project work on Comparative Analysis of Miclyn Water and Two Borehole Water from Sabo and Ibienafe was carried out by

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**Date**

## **DEDICATION**

This work is dedicated to God Almighty for His infinite mercy, love, grace protection and wisdom over our lives during the period of carryout this project work and throughout our academic pursuit.

**ACKNOWLEDGEMENT**

Firstly, we want to give God all the gratitude to God for the success of our project and our lives and for our stay here in Auchi polytechnic. We want to sincerely thank our project supervisor MrSalami O.J. for putting in efforts,time to guide us through the practical work. We are grateful sir, God bless you.We also want to thank our HOD Mr Anthony Ohimaiand all our lecturers for their efforts towards our study,also to our parents and friends,for their guidance, financial and moral support, we are grateful.

Thank you so much God bless you all (Amen).

### iii.

#### ABSTRACT

The study was based on the Comparative Analysis of Myclyn Water and two Borehole Water from Sabo and Ibienafe. Water samples from three borehole in Auch; Water samples from Myclyn water borehole water from Sabo and from ibienafewere aseptically collected in a clean sample containers and transferred immediately to the Microbiology Laboratory of Auch Polytechnic, Auch for further microbiological analysis. Each samples were carefully label prior to further analysis and transferred immediately to the microbiology laboratory of Auch Polytechnic, Auch for further microbiological analysis. The bacteria isolated were *Staphylococcus sp*, *Bacillussubtilis*, *KlebsiellaspandEscherichia coli*. It is suggested that operators of boreholes sellers and Myclyn water should employ the services of professionals to analyse the quality of their water and the government should enforce the Edo State environmental agency to take measures that will curb indiscriminate waste water discharge.

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## **CHAPTER ONE**

### **1.0 INTRODUCTION**

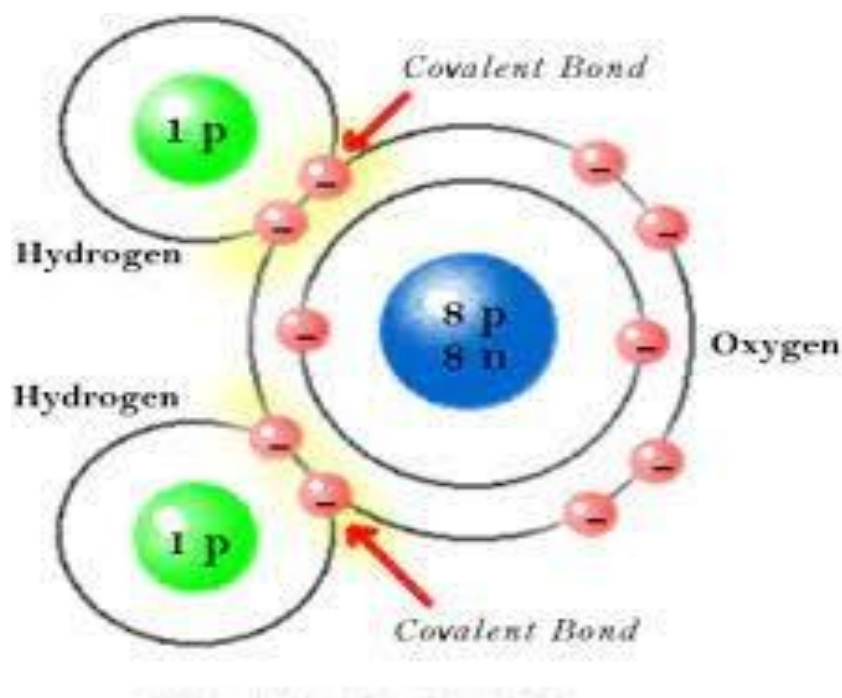
The health of the people depends solely on the quality of water available for consumption. The health aspects of environmental quality were among the first to receive scientific consideration through the recognition of waterborne diseases. Water is a chemical substance with the chemical formula  $H_2O$ . A water molecule contains one oxygen and two hydrogen atoms connected by covalent bonds. Water is a liquid at ambient conditions, but it often co-exists on earth with its solid state, ice, and gaseous state (water vapor or steam). Water also exists in a liquid crystal state near hydrophilic surfaces. Water covers 70.9% of the earth's surface, and is vital for all known forms of life. On earth, 96.5% of the planet's water is found in oceans, 1.7% in groundwater, 1.7% in glaciers and the ice caps of Antarctica and Greenland, a small fraction in other large water bodies, and 0.001% in the air as vapor, clouds (formed of solid and liquid water particles suspended in air), and precipitation. Only 2.5% of the earth's water is freshwater, and 98.8% of that water is in ice and groundwater. Less than 0.3% of all freshwater is in rivers, lakes, and the atmosphere, and an even smaller amount of the earth's freshwater (0.003%) is contained within biological bodies and manufactured products. Water is essential for sustenance of life and determines the overall socioeconomic development of



any nation. In Nigeria, so many programmes to improve water supply and sanitation had been put in place by different administration. Despite this, the hope of meeting the UN millennium development goals (MDGs) target of safe water supply by the year 2015 is still uncertain. The MDGs in supply and sanitation aims to half the proportion of people with portable water supply and basic sanitation. More importantly, a policy/institutional frame work for sustainable rural water supply and sanitation delivery is developed towards achieving the millennium development goal (Nwankwoala, 2018).

The drinking water of most communities is obtained from surface sources: rivers, stream, and lake, such as natural supplies, particularly streams and rivers are likely to be polluted with domestic and industrial waste i.e. the used water of a community (waste water). Many city dwellers (whose water comes from the rivers) are not aware that a considerable portion of their drinking water may have been used for domestic and industrial purpose. Besides drinking and cooking, water is used as solvent in industries and also for cleaning, for cooling (eg power station and stream engines). Till date water has become a scarce commodity. The unavailability of portable water and scarcity of water has hindered the growth and development of plants and caused starvation to man (MICS, 2019). According to Ajayi *et al.*,(2019), water forms the essential medium in which chemical reaction in man's cell proceed. It transport blood, it forms a pool for food digestion, it holds

and helps transport the electrically charged that generate neuron signal and make human brain active. Our physical and biological environment depends on water. The use of water depends on the quality characteristics of water, for uniformity in quality of water for parts of the world. The World Health Organization (WHO) is responsible for the maintenance of the standard which is required of water and all portable water must meet this requirement. Due to this factor, an attempt is made to examine various sources of water in Auchi and environs (Calow *et al.*, 2017).



**STRUCTURE OF WATER H<sub>2</sub>O.**

## **1.2 OBJECTIVE OF THE STUDY**

The main objective of the study is to compare and analyse Miclyn pure water and two borehole water from Sabo and Ibienafe South-Ibie. Specific objective include:

- i. To determine the quality of these water sources.
- ii. To establish which source is of high microbiological quality.

## **1.3 SIGNIFICANCE OF STUDY**

Examination and determination of the presence of chlorine, pH, calcium (Ca), magnesium (Mg), ammonium nitrogen (NH<sub>4</sub>N), nitrate (NO<sub>3</sub>), phosphate (PO<sub>4</sub>), sulphate (SO<sub>4</sub>). Temperature (°C) and coliform count to investigate the harmful chemical residue present in the water to ascertain the standard of the World Health Organization (WHO).

## **1.3 SCOPE OF STUDY**

This study is limited to the determination of the presence of chlorine (Cl), calcium (Ca), magnesium (Mg), ammonium nitrogen (NH<sub>4</sub>N), nitrate (NO<sub>3</sub>), phosphate (PO<sub>4</sub>), sulphate (SO<sub>4</sub>), temperature (°C) and coliform count to ascertain the standard for portable water. Due to substandard production practices/inefficiency of NAFDAC, most commercially sold water in Auchi town is constrained with ill processing. Therefore this research is aimed at the comparative and analysis of Miclyn pure water and two boreholes water from Sabo and Ibienafe South-Ibie.

## **1.4 LIMITATIONS OF THE STUDY**

Getting relevant information and materials for the course of this study was a major limitation encountered. Finance is another problem. Time is another constraint encountered during the course of this study as the researchers have to combine the research work with course work and the time frame for the study is limited.

## **1.5 DEFINITION OF TERMS**

**1.5.1 Water chlorination:** Water chlorination is the process of adding chlorine or chlorine compounds such as sodium hypochlorite to water.

**1.5.2 Contamination:** An impairment of the quality of water by microorganisms, chemicals, sewage or industrial waste which renders water unfit for its intended use.

**1.5.3 Hydrolysis:** The chemical breakdown of a compound due to reaction with water.

**1.5.4 Aquifer:** An underground basin where water is stored after percolating down through many layers of rock and gravel.

## **CHAPTER TWO**

### **2.0 LITERATURE REVIEW**

There are basically 4 (four) sources of water:

Surface water

Rain water

Ground water

Sea water

### **2.1 SURFACE WATER**

These are water from stream, rivers, ponds, lakes, dams and reservoirs, they are rarely portable, direct use is likely to require treatment measures that may be complicated to plan and implement during most refugee emergencies (Azuonwu *et al.*, 2017).

### **2.2 RAIN WATER**

Rain water is reasonably pure when collected from the roof if these are clean and suitable, this method can only be major source of water in areas with adequate and reliable year round rainfall; it requires suitable shelter and individual household storage facilities, it is therefore not a suitable solution in most refugee emergencies. It should also be noted that surface water is particularly likely to be contaminated in the rainy season (Azuonwu *et al.*, 2017).

## **2.3 GROUND WATER**

Ground water is contaminated in aquifer. Aquifers are rocks or group of rocks capable of transmitting, storing and yielding water. They may be formed by loose sediments (silts, sand and gravel) fractured rocks or otherwise porous rocks (fractured leaves granites metamorphic rocks, sandstones etc). The microbiological quantity of ground water is usually very good in view of the filtration undergone by water in its transit rock pores (and to this filtration effects when the size of the fractures in the rocks is large) (Azuonwu *et al.*, 2017).

## **2.4 SEA WATER**

Sea water can be used for almost everything but drinking, thus reducing fresh water requirements. In locations where no adequate sources of water exist but where sea water is near, desalinization is one possible but costly option. Neither of the two basic method distillation using the same heat nor the use of modern desalinization plants is likely to meet immediate fresh water requirements in major refugee emergency and it therefore strongly discourage if no water source are available at a given site, relocation of refugees must be considered as a matter of urgency (Azuonwu *et al.*, 2017).

## **2.5 USES OF WATER**

The uses of water cover a wide range and too numerous to be listed here, it will only suffice to say that without water there cannot be life because practically, all

biological and chemical processes that sustain life are dependent on water. Water is an inevitable ingredient of life not only to man and animals but to plants.

All industrial processes utilize water, in the agriculture industries a lot of water is used for irrigation and planting, in chemical industries such as perfume, lotions, paste etc. In electrical industries, water is used for generation of hydroelectric power. In the transport industries water is used as a medium for conveying heavy duty cargo.

Lastly, in all homes water is used or consumed daily as food for domestic purpose such as cleaning, washing, cooking, bathing (Okeke, 2019).

Owing to the fact that water is very essential in life and its availability is from different sources which have distinguished aesthetic characteristics quality that can affect man who depends on water for his activities, it has become imperative to check and determine empirically the possibility of presence of Chlorine (Cl), Calcium (Ca), Magnesium (Mg), Ammonium nitrate (NH<sub>4</sub>N), Nitro oxide (NO<sub>3</sub>) phosphate (PO<sub>3</sub>), sulphate (SO<sub>4</sub>), Temperature (°C).

Coliform count is studied to know whether harmful chemical residue are present in the water present in borehole water and Miclyn water.

## **2.6 THE CHEMISTRY OF WATER**

Water is clean, colourless liquid and appears blue when viewed through a thickness of 6metre. The colour results not only from the physical properties but also from

the suspended impurities. Water has a chemical formula  $H_2O$ . Oxygen atom has two unpaired electrons needed to pair with hydrogen only one unpaired electron, thus oxygen atom has greater electro-negativity (alteration for electron) than hydrogen atoms. In bonding situation in water molecule, the electron shared by the hydrogen and oxygen molecule are displaced towards the oxygen atom hence the hydrogen atom develop partial charges, the molecule posses an electric dipole. Water is an electrical insulator which may be polarized by the action of an applied electric fluid hence water is called a di-electric. It has di-electric constant of 78 at  $25^{\circ}C$ . Water in one of its three physical state (solid, liquid and gas) is present in greater or lesser quantity in or virtually all the earth. The atmosphere and all things living or dead, water which is important from the start point of water resources development fall into the categories of atmospheric moisture surface water and sustain existence (Ababio, 2016).

## **2.7 QUALITY CHARATERISTICS OF WATER**

The quality characteristics of water can be grouped into physical and chemical characteristic of water. They are further discussed below:

### **2.7.1 Physical Characteristics**

The most important physical characteristics of water is total solid content which is composed of floating matters in solution. Our important physical characteristics include odour, temperature, density and turbidity.



## **Total Solid**

This consist of settled solid, filterable and non-filterable solid volatile suspended solid and fixed suspended solids gives an indication of the level of colloidal of organic origin. Water containing suspended matter which may eventually settle always cause difficulties due to the formation of deposit in pipes and tanks. Suspended solid given a measure of the turbidity of indispensable factors in waste water treatment (Seth *et al.*, 2019).

## **Odour**

Odour in water is caused by gases added to water as treatment.

## **Temperature**

The temperature of water is commonly higher than that of water supplied for household and industrial activities

## **Colour**

Natural water is colourless. Colour of water depends on the amount of material taken up (Uzoigwe and Agwa, 2019)

### **2.7.2 Chemical Characteristics**

These include pH, alkalinity and hardness, total dissolved solid, chlorine, ion, magnesium and copper, it also includes nitrogen, phosphorus is biochemical oxygen demand (BOD) composition (Seth *et al.*, 2019).

## **2.8 CHEMICAL TREATMENT**

The treatment of water to make it fit for our use can be done in three following ways, Firstly, untreated water is passed through settling tanks where chemicals like sodium aluminate ( $\text{NaAlO}_2$ ) are added to cause coagulation. The impurities clump together and settle down. rapidly. Next, the water is passed through a filter bed to remove the remaining fine particles of dirt. Then the water is treated with chemical i.e. chlorine to kill germs. Finally, the treated water which is now clean and safe of germs is stored in a reservoir (Ababio, 2019).

## **2.9 TREATMENT OF WATER**

There are three methods available for treatment of water. Water can be disinfected by boiling, by adding oxidant like chlorine or by exposing it to ultraviolet light.

### **2.9.1 Boiling**

Boiling water is highly effective as a treatment method, vigorous boiling for minutes kills bacteria, including disease causing organism and giardia cysts. Any heat source, such as electric or gas, stove or fire wood can be used to boil water. Even microwave, ovens can be used to heat water to boiling. This makes it the most widely available form treatment Mineral deposit may build up the vessel used for boiling water Soaking the vessel in weak acid solution such as vinegar or lemon juice can help dissolve the mineral scale. Boiled water can taste stale and it

is not usually drawn from the tap. It is offline treatment that requires separate water storage (Oyebande, 2016).

### **Advantages of Boiling Water**

- a. Readily available.
- b. Well suitable for emergencies and temporary disinfection of water.
- c. Can eliminate volatile organic chemicals out of water.
- d. Extremely disinfectant that kills even giardia cyst.

### **Disadvantages of Boiling Water**

- a. Requires a great deal of heat.
- b. Time for water to boil and cool before drinking is high.
- c. It can give water a stale taste.
- d. Typically limited capacity.
- e. Requires special storage for treated water.

### **2.9.2 Chlorine Treatment**

Chlorine kills bacteria, including disease causing organisms and the nuisance organism and bacteria. However low level of chlorine used normally to disinfect water is not infective treatment for giardia cyst. The concentration of the chlorine solution added, the time that the chlorine is in contact with the organism, and the quantity of water These effects can be summarized in the following manners:

- i. As the concentration of the chlorine increases the required content

time to disinfect decreases.

- ii. Chlorination is more effective as water temperature increases.
- iii. Chlorination is less effective as water pH increases (becomes more alkaline)
- iv. Chlorination is less effective in cloudy (turbid) water
- v. When chlorine is added to water, part of it combine with other chemicals in water like manganese, hydrogen sulphide and ammonia and is not available for disinfection. The amount of chlorine that react with the other chemicals plus the amount required to achieve disinfection is chlorine demand of the water (Ajayi *et al.*, 2017).

### **Advantages of Chlorination**

- i. Provide residual disinfection
- ii. Chlorine is readily available at reasonable cost.
- iii. Low electrical requirement.
- iv. Can be used for multiple water problems.
- v. It can treat large volumes of water

### **Disadvantages of Chlorination**

- i. Requires contact time of 30minutes for simple chlorination
- ii. Turbidity (cloudy water) can reduce the effectiveness of chlorine

### **2.9.3 Filtration**

Filtration is the process in which solid particles in a liquid or gaseous fluid are removed by the use of a filter medium that allows the fluid to pass through while retaining the solid particles (Abogan, 2019).

Once the flocs have settled to the bottom of the water, the clear water on top is filtered to separate additional solids from the water. During filtration, the clear water passes through filters that have different pore sizes and are made of different materials (such as sand, gravel, and charcoal). These filters remove dissolved particles and germs, such as dust, chemicals, parasites, bacteria, and viruses. Activated carbon filters also remove any bad odors (Thivya *et al.*, 2018).

Water treatment plants can use a process called ultrafiltration in addition to or instead of traditional filtration. During ultrafiltration, the water goes through a filter membrane with very small pores. This filter only lets through water and other small molecules (such as salts and tiny, charged molecules) (Abogan, 2019).

### **2.9.4 Ozonation**

Ozonation (also referred to as ozonisation) is a chemical water treatment technique based on the infusion of ozone into water. Ozone is a gas composed of three oxygen atoms (O<sub>3</sub>), which is one of the most powerful oxidants. Ozonation is a type of advanced oxidation process, involving the production of very reactive oxygen species able to attack a wide range of organic compounds and all

microorganisms. The treatment of water with ozone has a wide range of applications, as it is efficient for disinfection as well as for the degradation of organic and inorganic pollutants. Ozone is produced with the use of energy by subjecting oxygen (O<sub>2</sub>) to high electric voltage or to UV radiation. The required amounts of ozone can be produced at the point of use but the production requires a lot of energy and is therefore costly (WHO/UNICEF, 2017).

## **2.10 DRINKING WATER INDICATORS**

The following is a list of indicators

- Alkalinity
- Colour of water
- pH
- Taste and odour (geosmin, 2-methylisoborneol (MIB). etc.)
- Dissolved metal and salts (sodium chloride, potassium, calcium, manganese, magnesium)
- Microorganisms such as fecal coliform bacteria (*Escherichia coli*), *cryptosporidium*, and *giardia lamblia*.
- Dissolved metals and metalloid (lead, mercury, arsenic, etc.)
- Dissolved organics: colored dissolved organic matter (CDOM) dissolved organic carbon (DOC)
- Radon

- Heavy metals
- Pharmaceuticals
- Hormone analogs

### **2.10.1 Physical Assessment**

#### **pH**

It is important to measure pH at the same time as chlorine residual since the efficacy of disinfection with chlorine is highly pH dependant where the pH exceeds 8.0, disinfection is less effective. To check that pH is in the optimal range for disinfection with chlorine (less than 8.0), simple test may be conducted in field using comparators such as that used for chlorine residual. With some chlorine comparators, it is possible to measure pH and chlorine simultaneously. Alternatively, portable pH electrodes and meters are available. If these are used in the laboratory, they should be calibrated immediately before each test. Results may be inaccurate if the water has a low buffering capacity (Babalola, 2017).

#### **Turbidity**

Turbidity is important because it affects the acceptability of water to consumers, and the selection and efficiency of disinfection with chlorine since it exerts a chlorine demand and protects microorganisms and may also stimulate the growth of bacteria. In all processes in which disinfection is used, the turbidity must always be low-preferably below 1 NTU or JTU (these units are interchangeable in

practice). It is recommended that, for water to be disinfected, turbidity should be consistently less than 5 NTU or JTU and ideally have median value of less than 1 NTU (Franson, 2021)

## **Temperature**

The most common physical assessment of water quality is the measurement of temperature. Temperature impacts both the chemical and biological characteristics of surface water. It affects the dissolved oxygen level in the water, photosynthesis of aquatic plants, metabolic rates of aquatic organisms, and the sensitivity of these organisms to pollution, parasites and disease (Vaishali and Punita, 2020).

Water temperature is critical because it is an important quality in environmental parameters. It is important to measure water temperature. By doing so, we can see the characteristics of the water such as the chemical, biological, and physical properties of the water, as well as the possible health effects. Water temperature is an important factor in determining whether a body of water is acceptable for human consumption and use (Agbabiaka and Sule, 2019).

## **Total Suspended Solids (TSS)**

Total suspended solids (TSS) are defined as solids in water that can be trapped by a filter. To measure TSS, the water sample is filtered through a pre-weighed filter. The residue retained on the filter is dried in an oven at 103–105°C until the weight



of the filter no longer changes. The increase in weight of the filter represents the TSS (Bello *et al.*, 2019).

### **Total Dissolve Solids (TDS)**

Dissolved solids" refer to any minerals, salts, metals, cations or anions dissolved in water. Total dissolved solids (TDS) comprise inorganic salts, principally calcium, magnesium, potassium, sodium, bicarbonates, chlorides, and sulfates and some small amounts of organic matter that are dissolved in water (Bello *et al.*, 2019).

## **2.11 STANDARDS AND REPORTS**

The water policy of the European Union is primary codified in three directives:

- Directive on urban water treatment (91/271EEC) of 21 may 1991 concerning discharging of municipal and some industrial waste water.
- The drinking water directive (98/83/EC) of 3 November1998 concerning potable water quality.
- Water frame work directive (2000/60EC) of 23 October 2000 concerning water resources management.

## **2.12 BACTERIOLOGIAL ANALYSIS**

The principal risk associated with water in small community supplies is that of infectious disease related to faecal concentration. The microbiological examination of drinking water emphasizes assessment of the hygienic quality of supply This

require the isolation and enumeration of organisms that indicate the presence of faecal contamination (Callow *et al.*, 2020).

### **2.11.1 Thermotolerant Coliform Bacteria**

Thermotolerant coliform bacteria are the coliform organisms that are able to ferment lactose at 44-45°C; the group includes the genus *Escherichia* and some species of *klebsiella*, and *citrobacter*. Thermo tolerant coliforms other than *E. coli* may also originate from organically enriched water such as industrial effluents or from decaying plant materials and soils. For this reason the term "faecal" coliform, although frequently employed, is not current, and its use should be discontinued. Regrowth of thermotolerant coliform organisms in the distribution system is unlikely unless sufficient bacteria nutrients are present, unsuitable materials are in contact with the treated water, the watertemperature is above 13°C, and there is no free residual chlorine (Nicol, 2020)

### **2.11.2 Coliform Organisms (Total Coliform)**

Coliform organisms have long been recognized as a microbial indicator of drinking water quality, largely because they are easy to direct and enumerate in water. The term "coliform organism" refers to gram-negative, rod-shaped bacteria capable of growth in the presence of bile salts or other surface active agents with similar growth inhibiting properties and able to ferment lactose at 35-37°C with the production of acid, gas and aldehyde within 24-28 hours. They are also oxidase

negative and non-spore forming and display B-galactosidase activity (McGuigan *et al.*, 2018).

## **2.12 PHYSICOCHEMICAL ANALYSIS**

### **2.12.1 Chlorine Residual**

The disinfection of drinking water supplies constitutes an important barrier against waterborne diseases. Although various different disinfectants may be used, chlorine in one form or the other is the principal disinfectant agent employed in small communities in most country (Babalola, 2019).

Three types of chlorine residual may be measured:

- i. Free chlorine (the most reactive species, i.e. hypochlorous acid and hypochlorine ion)
- ii. Combined chlorine (less reactive and more persistent specie formed by the reaction of free chlorine specie with organic material and ammonia)
- iii. Total chlorine (the sum of the free and combined chlorine residuals) water sample should therefore be analyzed for free chlorine immediately on sampling and not stored for later testing The method recommended for the analysis for chlorine residual in drinking water employs N,N-diethyl-p phenylenediamine, more commonly referred to as DPD Methods in which O-tolidine is employed were formally recommended, but the substance is a carcinogen, and the method is

inaccurate and should not be used Analysis using starch-potassium iodide to obtain or prepare DPD (Harvey and Reed, 2019).

### **2.12.2 Aesthetical Parameters**

Aesthetical parameters are those detectable by the sense, namely turbidity, colour, taste and odour. They are important in the monitoring community water supplies because they may cause the water supply to be rejected and alternative (possibly poorer quantity)sources to be adopt, and they are simple and expensive to monitor quantitatively in the field (Oteze, 2016).

### **2.12.3 Colour**

Colour in drinking water may be due to the presence of coloured organic matter, eg humic substances, metal such as iron and manganese, highly coloured industrial waste. Drinking water should be colourless. For the purpose of surveillance of community water supplies, it useful to note the presence or absence of observable colour at the time of sampling. Changes in the colour of the water and the appearance of new colours serves as indicators that further investigation is needed (Offodile, 2016)

### **2.12.4 Taste and Odour**

Odour in water is mainly caused by the presence of organic substance. Some odours are indicative of increased biological activity, others may result from industrial pollution Sanitary inspection should always include the investigation of

possible or existing sources of odour, and attempts should be made to correct an odour problem. Taste problem(which are sometimes group with odour problems) usually account for the largest single category of consumer complaints. Generally, the taste buds in the oral cavity detect the inorganic compounds of metals such as magnesium, calcium, sodium, copper, iron and zinc. As water should be free of objectionable taste and odour, it should not be offensive to the majority of the consumers. It is advisable to avoid direct tasting and swallowing of water. Under these circumstances, a sample should be taken for investigation to the laboratory (World Bank, 2021).

#### **2.12.5 Chlorinated Drinking Water and the Risk of Bladder Cancer**

Findings have showed that chemicals can be harmful when they are inhaled or absorbed through the skin, as well as when they are ingested (Cristiana *et al.*, 2017).

Chemicals, most commonly chlorine, used to disinfect water can produce by-products that have been tied to increased cancer risk, Villanueva and her team point out. The most prevalent chlorination by-products, chemicals called the trihalomethanes (THM), can be absorbed into the body through the skin or by inhalation. Disinfection of water by chlorination or, to a lesser extent, by Chloramination, can produce a number of by-products due to the interaction of chlorine with naturally occurring organic substances in water. Included in these by-

products are the trihalomethanes (THM). Those which have been of greatest interest are: chloroform, bromodichloromethane, Dibromochloromethane, tribromomethane. Study participants who drank chlorinated water were at 35% greater risk of bladder cancer than those who didn't, while use of swimming pools boosted bladder cancer risk by 57%. And those who took longer showers or baths and lived in municipalities with higher THM levels were at increased cancer risk. When THM is absorbed through the skin or lungs, it may have a more powerful carcinogenic effect because it does not undergo detoxification via the liver (Nwankwoala, 2019).

#### **2.12.6 Chlorination of Drinking Water can with a False Sense of Security**

Protozoan parasite such as cryptosporidia and Giardia lamblia and viruses like hepatitis A and E, rotaviruses, Noroviruse, Poliovirus and echovirus can be present in drinking water even when the water is chlorinated People become extremely sick by swallowing a few protozoa since they rapidly reproduce once inside a host organism (WHO 2013).

## **CHAPTER THREE**

### **3.0 MATERIALS AND METHOD**

#### **3.1 Materials**

- Water samples from Myclyn water and two borehole water one from Sabo and the other from ibienafe.
- Nutrient agar
- Sabouraud dextrose agar
- Measuring cylinder
- Petri dishes
- Beaker
- 10ml pipette
- Flat bottom flask
- Weighing balance
- Test tubes
- Test tube rack
- Autoclave
- Incubator
- Slide
- Microscope
- Cotton wool

- Distilled water
- Spatula
- Wire loop
- Crystal violet
- Lugos iodine
- Acetone alcohol
- Safranine
- Phenol red
- Kovac's reagent
- Glucose
- Sucrose
- Lactose
- Maltose
- Durham tube
- Spirit lamp
- Ethanol
- Lactophenol in cotton blue stain

### **3.2 Sample Collection**

Water samples from three borehole in Auch; Water samples from Myclyn water (A) borehole water from Sabo (B) and from ibienafe (C) were aseptically collected



in a clean sample containers and transferred immediately to the Microbiology Laboratory of Auchi Polytechnic, Auchi for further microbiological analysis.

### **3.3 Sterilization of Materials**

All glass wares were first washed with detergent and rinsed with distilled water, wrapped with aluminum foil after drying and sterilized by dry heat method in the oven at a temperature of 160<sup>o</sup>c for 2-3hrs.

### **3.4 Disinfection of Working Area**

The working area were disinfected thoroughly before and after use with ethanol (75% v.) cotton wool was soaked in ethanol and used to clean the working bench; a Bunsen burner was put on and the flame was allowed to burn, this helped in sterilizing the air in the laboratory.

### **3.5 Culture Media**

The media used in this study is Nutrient Agar and Sabouraud Dextrose Agar; the media were prepared according to manufacturer's specification.

#### **3.5.1 Preparation of Nutrient Agar**

28g of nutrient agar powder was weighed using a weighing balance and dispensed into a beaker; 1000mls of distilled water was measured using a measuring cylinder and dispensed into the beaker containing the agar powder; it

was stirred to dissolve for 10mins. The mixture was transferred into a conical flask and the neck of the flask was corked with cotton wool wrapped in aluminum foil. It was autoclaved at a temperature of 121°C and pressure of 15psi for 15-20minutes. the sterilized agar was allowed to cool to about 45°C and then aseptically poured into Petri dishes and allowed to set (Cheesbrough, 2006).

### **3.5.2 Preparation of Sabouraud Dextrose Agar**

65g of Sabouraud dextrose agar powder was weighed using a weighing balance and dispensed into a beaker; 1000mls of distilled water was measured using a measuring cylinder and dispensed into the beaker containing the agar powder; it was stirred to dissolve for 10mins. The mixture was transferred into a conical flask and the neck of the flask was corked with cotton wool wrapped in aluminum foil. It was autoclaved at a temperature of 121°C and pressure of 15psi for 15-20minutes. the sterilized agar was allowed to cool to about 45°C and then aseptically poured into Petri dishes and allowed to set (Cheesbrough, 2006).

### **3.6 preparations of inoculums and Inoculation**

Each water samples were aseptically transferred into plates of the nutrient media using the streak plate techniques. A sterile wire loop was used to take an aliquot of the inoculums and aseptically transferred to the plates and streaked.

### **3.7 Purification and Storage of Isolates**

Bacterial colonies that appeared on the primary isolation plates were subcultured onto fresh nutrient agar plates to obtain pure cultures of the different isolates. The final cultures containing discrete colonies were transferred onto slants made with McCartney bottles and test tubes containing nutrient agar. The slants were stored in the refrigerator at 40°C for further studies.

### **3.8 Characterization and Identification of Isolates**

The bacteria isolates from different samples were grouped on the basis of colonial morphology. The criteria used, were the size of the colony, color, surface, edge, slope and elevation as described by Cheesbrough, 2006.

## **3.9 BIOCHEMICAL METHODS USED IN THE IDENTIFICATION OF BACTERIA ISOLATES**

### **3.9.1 Gram's Staining Techniques**

A thin smear each of the pure 24hours old culture was prepared on clean grease-free slides, allowed to air-dry then fixed by passing swiftly over a burning flame. The slides containing the smears were flooded with crystal violet solution for 60sec. rinsed with water and blot to remove water residue. The smears were again flooded with Lugol's iodine which acts as a mordant for 60sec. rinsed with water and blot. They were then decolourized with acetone alcohol for a few

seconds and rinsed after which they were counter stained with safranin for 30-60secs washed with water and kept to air dry before they were observed under the oil immersion objective of the microscope. Gram negative cells appeared pink or red while the gram positive organisms appeared purple.

### **3.9.2 Catalase Test**

The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs.

A loopful of 24hrs old culture was placed on a clean slide and a drop of 3% hydrogen peroxide was placed on it. Gas seen as white bubbles indicate the presence of catalase enzyme which is a positive result.

### **3.9.3 Coagulase Test**

Coagulase is an enzyme-like protein and causes plasma to clot by converting fibrinogen to fibrin. Coagulase test is used to differentiate *Staphylococcus aureus* (positive) which produce the enzyme coagulase, from *S. epidermis* and *S. saprophyticus* (negative) which do not produce coagulase.

Place a drop of physiological saline on each end of a slide, or on two separate slides. With the loop, straight wire or wooden stick, emulsify a portion of the isolated colony in each drop to make two thick suspensions.

Add a drop of human or rabbit plasma to one of the suspensions, and mix gently. Look for clumping of the organisms within 10 seconds. No plasma is added to the second suspension to differentiate any granular appearance of the organism from true coagulase clumping.

#### **3.9.4 Indole Test**

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole. Isolated cultures were inoculated into peptone broth medium, incubated at 37<sup>0</sup> C for 48 hours after which 5 drops of Kovac's reagent was added to the culture. A red ring indicates indole production.

#### **3.9.5 Oxidase Test**

The oxidase test detects the presence of a cytochrome oxidase system that will catalyse the transport of electrons between electron donors in the bacteria and a redox dye- tetramethyl-*p*-phenylene-diamine. The dye is reduced to deep purple color. This test is used to assist in the identification of *Pseudomonas*, *Neisseria*, *Alcaligenes*, *Aeromonas*, *Campylobacter*, *Vibrio*, *Brucella* and *Pasteurella*, all of which produce the enzyme cytochrome oxidase.

- ✓ Strip of Whatman's No. 1 filter paper was soaked in a freshly prepared 1% solution of tetramethyl-p-phenylene-diamine dihydrochloride.
- ✓ After draining for about 30 seconds, the strips were freeze dried and stored in a dark bottle tightly sealed with a screw cap.
- ✓ For use, a strip was removed, laid in a petri dish and moistened with distilled water.
- ✓ The colony to be tested was picked up with a platinum loop and smeared over the moist area.
- ✓ A positive reaction is indicated by an intense deep-purple hue, appearing within 5-10 seconds, a "delayed positive" reaction by colouration in 10-60 seconds, and a negative reaction by absence of colouration or by colouration later than 60 seconds.

#### Methyl Red (MR) Test

- ✓ Using pure cultures of organisms taken from an 18-24 hour pure culture, inoculate the broths
- ✓ Incubate aerobically at 37 degrees C. for 24 hours.
- ✓ Following 24 hours of incubation,
- ✓ Add 2 to 3 drops of methyl red indicator.
- ✓ Observe for red color immediately.

### **3.11 identification of fungi isolates**

The Sabouraud dextrose agar plates were cultured at room temperature for 4-5days after which the colonial characteristics was observed and lactophenol in cotton blue stain was used to stain the growth and observed under the 10x objective.

## CHAPTER FOUR

### 4.0 RESULTS AND DISCUSSION

#### 4.1 RESULTS

The results of the comparative analysis of the microbial load of water in Auchimetropolis are presented in tables 4.1 and 4.2 showing the total plate count, morphological, cultural and biochemical characteristics of bacteria isolated from the water samples as well as the cultural and morphological characteristics of fungal isolates.

**Table 4.1: Total Plate Count for Comparative Analysis of the Microbial Load of Borehole Water in Auchimetropolis.**

| Sample | Dilution factor | Mean Number of colonies per plate | Mean Total number of bacterial count in colony forming unit (cfu/ml) |
|--------|-----------------|-----------------------------------|--|
| A      | $10^{-2}$       | 06                                | $0.6 \times 10^{-2}$   |
| B      | $10^{-2}$       | 16                                | $1.6 \times 10^{-2}$   |
| C      | $10^{-2}$       | 15                                | $1.5 \times 10^{-2}$   |





## 4.2 DISCUSSION

Table 4.1 showed the range and mean values of total bacterial counts of water samples collected and analysed. Total viable bacterial counts of the samples were relatively low except for all samples. The lowest counts of total bacterial were recorded in sample A, myclyn water with a mean plate count of  $0.6 \times 10^{-2}$  while the highest counts of total bacterial were recorded in the sample taking from the borehole in sabo (b), and ibienafe (C) with their mean values of  $1.6 \times 10^{-2}$  and  $1.5 \times 10^{-2}$  cfu respectively.

The Nigerian Standard for Drinking Water Quality (NSDWQ 2007) recommends that drinking water may contain up to 10CFU/100ml total Coliform CFU/100ml, but WHO (2003) recommended  $<10$ CFU/100ml. Similarly, both WHO and NSDWQ recommended that no faecal coliform should be found in any water meant for drinking.

Four genera of microorganisms (or bacteria) were identified (Table 4.2) from the isolates. These genera include: *Klebiella*, *Escherichia*, *Staphylococcus* and *Bacillus*. These Isolates are important human pathogens associated with a variety of infectious diseases such as gastroenteritis, typhoid fever, dysentery, cholera, urinary tract infection, etc. The high number of these pathogens in the water samples from study areas needs public health attention. The high prevalence pathogens in this study are in agreement with the findings of Obi and Okocha,

(2007) in selected borehole waters in World Bank Housing Estate, Umuahia and of Amajor *etal.*, (2012) on enumeration and identification of pathogenic pollution indicators in different water sources used in processing root and tuber crops in Umudike, Umuahia, Abia State, Nigeria. The presence of *Esherichia*, *Staphylococcus* and *Bacillus* in most of the borehole water samples are unacceptable from the public health point of view. These organisms could be pathogenic. Therefore, there is need for caution when using these contaminated borehole water sources for any purposes. Most of the samples were contaminated with both non-faecal and faecal coliform bacteria. The samples with low bacteria counts could be considered to be of better quality for domestic use than the ones with the highest counts of bacteria counts WHO, (1985) specified that potable drinking water should be devoid of total coliform in any given sample. Also, according to US EPA standards, water samples in which coliforms are detected should be considered unacceptable for drinking water as they are regarded as the principal indicators of water pollution.

Water Samples from this source are unfit for drinking and domestic purposes because they had coliform bacteria which conformed to the set standard of WHO, which says that no water sample should contain faecal coliform in any 100ml of water sample, while other samples analysed were fit for consumption without purification.

*Aspergillus specie* was the only fungi isolated from all the occurrence of this fungus may cause diverse effects on human health as they have the potential of producing mycotoxins daily intake of such water containing mycotoxin could result in bioaccumulation in the body which could be hazardous to human health. *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger* are known to produce aflatoxins, ochratoxins and fumitremogin which are carcinogenic and are capable of causing kidney and liver disorders as well as invasive and non-invasive aspergillosis.

## **CHAPTER FIVE**

### **5.0 CONCLUSION AND RECOMMENDATION**

#### **5.1 Conclusion**

This investigation suggests that not all borehole waters are safe for consumption though the microbiological studies showed that some of the borehole water were microbiologically better than others as their bacteriological qualities indicative is of health risk to the inhabitants of the geographical location. The sites of boreholes are very important as clean and hygienic environment promote safety of water. The geologist drilling boreholes have to be educated on the importance of ensuring that dump sites are not used for drilling of boreholes. Moreover, the populace needs to be educated on the importance of maintaining clean and hygienic environment around the borehole to ensure the safety of water from such sources. The building of borehole requires underground pipe laying which supposedly need to meet up with its minimum requirement of Health and Safety rules; also, periodic maintenance, dumpsites, pit latrines, suck-away and drainages ought to be far from borehole water pipes to avoid cross contamination, this was likely not the case in some of these areas thus, making the quality of borehole water and its portability in-doubt.

This will provide information on the actual health problems on ground as well as contribute to the use of untreated groundwater in schools. This will lead to

recommendation of realistic remediation methods for each specific health problem. Information obtained would be valuable in the design and implementation of intervention strategies if required. Hence, this will enable the provision of data available to indicate that groundwater in the study areas does not meet the national guidelines of water for human consumption unless treated before use. Interventions such as the implementation of point of use water treatment could be advocated.

## **5.2 RECOMMENDATION**

It is therefore recommended that before drilling of boreholes, the site should be properly surveyed to ensure that it is not close to possible contaminants like waste dumps, toilets and other sinkable materials that can be detrimental to health.

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