PHYSICO-CHEMICAL AND BACTERIOLOGICAL QUALITY OF DRINKING WATER SOURCES IN CALABAR MUNICIPALITY, NIGERIA

By

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ABSTRACT

This study evaluated the physico-chemical and the bacteriological quality of five different sources of drinking water in Calabar metropolis, Nigeria, to give a fair geographical representative of the town and to contribute to our understanding of the quality of drinking water in the metropolis. The physico-chemical characteristics such as pH, temperature, turbidity, conductivity, colour, Iron, dissolved oxygen, Calcium, Magnesium, alkalinity, total hardness, Manganese, Sulphate, Chloride, Phosphate, Sodium, Zinc, Copper, total dissolved solid, Nitrate, Nitrite, Ammonia, Ammonium and Potassium were determined following the procedures prescribed by American Public Health Association Standard Method. The bacteriological analysis was carried out using the standard microbiological standard for analysis of water for total and faecal coliform count. The mean temperature of the evaluated waters ranged from 23.03^{o}C-29.3^{o}C, mean pH ranged from 4.37-6.76, while turbidity had a mean range of 0.16NTU-4.13NTU. Conductivity ranged between 39.29µs/cm-120.7µs/cm, dissolved oxygen with 13.30mg/L-4.19mg/L, total dissolved solids ranged from 72.4mg/L-23.5mg/L, while the mean for iron concentration ranged from 0.12mg/L-0.99mg/L.Similarly, the mean for total hardness was 34.2mg/L-17.1mg/L and 7.93mg/L-6.71mg/L for total alkalinity. Others includes Manganese (0.88mg/L-0.02mg/L), Magnesium (16.5mg/L-9.9mg/L), Calcium (9.77mg/L-7.20mg/L, Nitrate (14.6mg/L-3.66mg/L), Nitrite (0.076mg/L-0.009mg/L), Ammonia (0.89mg/L-0.25mg/L), Ammonium (0.52mg/L-0.013mg/L), Zinc (1.01mg/L-0.34mg/L), Chloride (5.73mg/L-0.364mg/L), Fluoride (0.76mg/L-0.277mg/L), Copper (0.61mg/L-0.18mg/L), Sodium (2.73 mg/L - 0.180 mg/L), potassium (5.73 mg/L - 2.0 mg/L), Sulphate (14.8 mg/L - 3.69 mg/L and Phosphate with 4.8 mg/L - 3.69 mg/L. The total coliform count for bottled water ranged between 2.00 cfu/100mL – 19.00cfu/100mL, the total coliform range for sachet water were 6.00cfu/100mLand 15.00cfu/100mLand no faecal coliform was detected. Public water had no growth at all, the stream and borehole bacteriological analysis ranged from 27x10^{1}cfu/mL- 55x10^{1}cfu/mLand12cfu/100mL-33cfu/100mL for total coliform respectively. Faecal coliform ranged from 15x10^{1}cfu/mL-52x10^{1}cfu/ mL for stream and 9.00 cfu100/ mL – 16.00cfu/100 mL for borehole. A total of seven (7) different bacteria species were isolated from the sampled drinking water sources. These included *Proteus* spp., *Streptococcus* spp., *Enterobacter* spp., *Pseudomonas* spp., *E.coli, Chromobacter* spp.,*Salmonella* spp. *and Enterococcus* spp. This study reveals a high

level of poor quality sources of water in the metropolis and makes need for urgent health intervention.

Keywords: Physico-chemical,bacteriological, faecal coliform, hardness and total coliform

INTRODUCTION

Water is essential to sustain life, therefore a satisfactory (adequate, safe and accessible) supply of drinking water should be available to all. Every effort should be made to achieve a good quality of drinking water. Quality is of basic importance to human physiology and man's continued existence depends very much on water availability (Lamikara, 1999*,* Okorafor *et al.,* 2012). Over the years human beings have in adequate access to portable water and the used the ones that are contaminated with disease vectors, pathogens or unacceptable level of toxins or suspended solid. Portable water is the water that is free from disease producing microorganisms and chemicals substances that are dangerous to health(Lamikara, 1999). The provision of portable water to rural and urban population is necessary to prevent health hazard. (Nikoladz*e et al.,* 1989, Lee 1991, Okorafor *et al.,* 2012).

In Nigeria, majority of rural populace do not have access to portable water and therefore, depends on well, streams and river water for domestic use (Shittu *et al.,* 2008), lack of water has become a critical and urgent problem, and it is a matter of great concern to families and communities that depends on non-public water supply system. (Okonko *et al.,* 2008*,* Adegoke *et al.,* 2012*).* Increase in the human population has enacted an enormous pressure on the provision of safe drinking water in the developing countries, (Umeh *et al.,* 2005, Adegoke *et al.,* 2012). Drinking water is one of the oldest public health issues and is associated with multitude of healthrelate concerns. Access to safe drinking water is a prerequisite to poverty reduction and prevention of the spread of water borne and sanitation related diseases (Cosgrove *et al.,* 2000, Gomez *et al.,* 2002, Okorafor *et al.,* 2012, UNICEF2005). Death due to water related diseases add up to more than three million people per year (Opara,2005, WHO 2003a). Infectious diarrhea alone claimed 1.7 million lives in 2002 (Opara, 2005, WHO, 2003b). The relation of disease to water is clearly established and the mechanisms that link different diseases to water have been well described (Feachem, 1975, Opara, 2005*).* It is well established that infectious diseases are transmitted primarily through water supplies contaminated with human and animal excreta (i.e. faeces) (WHO, 1993). Out breaks of water borne diseases continues to occur throughout the world but are especially serious in the developing countries, disease contacted through drinking water kill about 5million children annually and make $1/6^{th}$ of the world population sick (WHO, 2004*,*

Shittu *et al.,*2008*).* The human pathogens that are present in drinking water includes; *Salmonella species*, *Shigella species*, pathogenic *Escherichia coli*, *Vibro cholera*, *Yersinia entercolitica*, C*ampylobacter species*, *Klebsiella* and various viruses such as Hepatitis A, Hepatitis E, Rota virus and parasites such as *Entamoeba histolytica*, and *Giardia* species(Emde *et al.,*1992; Joklik *et al.,*1992 and Agbo*et al.,* 2019). To curb this health problem of unsafe water, bottle water was introduced, but only individuals who have good financial status can afford this product. Low income earners are left with no option but to consume sachet water which is readily available and affordable. (Adegoke *et al.,* 2012). The recognition that microbial infections can be waterborne has led to the development of method of routine examination to ensure safety of drinking water. It is impracticable to monitor drinking water for every possible microbial pathogen. Therefore, normal intestinal organisms are used as indicator of faecal pollution (Lee, 1991, Catwright *et al.,* 1993). These include coliforms group of organisms as a whole. Bacteriological quality of ground water, pipe borne water and other natural water supplies in Nigeria, has been reported to be unsatisfactory, with coliforms counts far exceeding the level recommendation by WHO (Dada *etal.,*1999a, 1999b, Edema *et al.,*2001*).* This happen because of the highly toxic materials and domestic waste that are disposed by dumping them into the earth-water, rivers and streams with total disregard for aquatic life and urban dwellers. Thus, water becomes an important medium for transmission of enteric diseases.

In general, certain requirements must be met for water to be fit for human consumption. These requirements are freedom from organisms and chemicals substances, which might be injurious to health. Drinking water should be of such composition that consumers do not question the safety of the water. This implies that turbidity, colour, taste and odour should be low and micro-organism (e.g. worms, Asellus, aquatic and fly mymphs) should not be present *(*Eja, 2002; Okorafor *et al.,* 2012; Agbo *et al.,* 2019*).* The world Health organization has recommended continuous surveillance of water supplies, which should involve keeping a careful watch at all safety and sustainability of water supplies. This is to be achieved through sanitary inspection and water quality analysis while sanitary inspection identifies potential risk factors of contamination and source of pollution. Water quality analysis confirms whether the water supply is faecally contaminated (WHO, 2004, Cheesbrough 2000, Okorafor *et al.,* 2012). The most preferable method used in analyzing faecal coliforms from water is the membrane filtration technique. Water is the integral part of achieving all the UN Millennium Development Goals. The Millennium Development Goals (MDG) target for water is to halve by 2015 the proportion of people without sustainable

access of safe drinking water and basic sanitation. The WHO (2004) and Okorafor *et al*.,(2012) estimates that these improvements were to be made in sub Saharan African alone, 434,000 child death due to diarrhea would be averted annually. The inhabitants of Calabar municipality, Nigeria have access to boreholes, streams, river and taps as the major source of water supply. The use the water supplied from these sources for drinking and other domestic activities such as cooking, washing bathing, poultry etc. among the inhabitant of Calabar municipality, Nigeria we also see some other drinking water like bottle water and sachet water which are obtained from boreholes and taps that are exposed to microbial contamination through rainfall runoffs, and the fact that they are always or usually constructed very close to pit toilet or sewage tank. Therefore, the determination of the portability and sustainability of such supplies is of serious concerns.

In some developing counties like Nigeria where dangerous and highly toxic industrial and domestic wastes are disposed of by dumping them into the earth water, rivers and streams with total disregard for aquatic life and urban dwellers, water becomes an important medium for this transmission of enteric diseases in most communities. In Calabar municipality, Nigeria most of the communities depends in the available stream, borehole during dry season. Streams in Calabar, Nigeria may be polluted by chemical effluent from both industrial and commercial establishments as well as organic and inorganic substances that Itah *et al.,*(1996) observed. This may be responsible for intermittent outbreak of typhoid fever, paratyphoid fever and cholera in recent years in Calabar, Nigeria. Apart from the toxic chemicals in domestic and industrial waste discharged into these water bodies, the underground waters become contaminated by pesticides, herbicides and fertilizer as they are applied by farmers and if this water is been taken in by humans it will lead to serious health hazard. This study will therefore evaluate the Physico-chemical and bacteriological quality of drinking water in Calabar municipality, Nigeria and also seek to identify and characterize bacteria isolates associated with drinking water in Calabar municipality, Nigeria.

MATERIALS AND METHODS

Study Area and Site

This study was carried outin Calabar municipality, Cross River State, Nigeria. Calabar municipality lies between latitude 04^{o} 15’ and 5N and longitude 8^{o}25’E. The municipality is bounded in the north by Odukpani Local Government Area, in the northeast by Great Qua

River (Akpabuyo Local Government Area). Its southern shores are bounded by the Calabar River and Calabar South Local Government Area. It is politically divided into 10 wards. It has a population of one hundred and seventy-nine thousand three hundred and ninety-two at the 2006 census (NPC web) It has an area of 331.551 square kilometers. Calabar municipality is a coastal town lying within the tropical region. The local government has two main seasons, the rainy and dry seasons. Calabar municipality is made up of three tribes namely; Qua's, Efut's and Efik's (Agbo & Mboto, 2012).

The municipality has industries and establishments. E.g. Seaport, Free Trade Zones, Airport, Export Processing Zone (EPZ), Naval and Army Base, Tinapa, NNPC depot, Cement Factory (UNICEM) etc. most dwellers are civil and public servants, some are factory workers. Their sources of drinking water include boreholes, dug wells, pipe borne water, streams, rivers, and packaged waters (sachet and bottled).

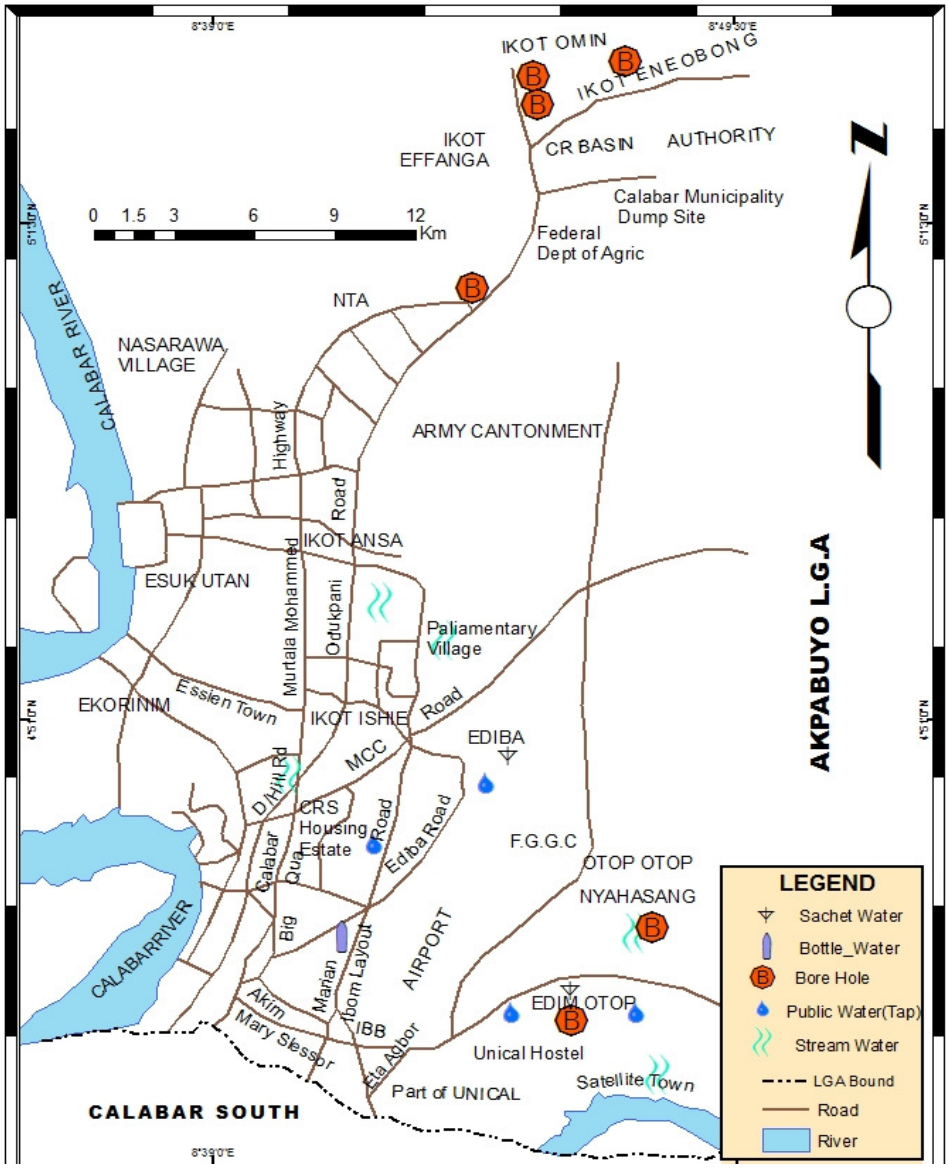

Figure 1: Sample collection sites

Sample collection

Water samples for bacteriological and physico-chemical analysis where collected from five (5) different sources of drinking water in Calabar municipality, Nigeria and at different locations all in Calabar municipality. The sources of drinking water and their designated sites: Stream, Boreholes, Pipe borne water, Sachet water and bottle water were gotten from different water vendor outlet all in Calabar Municipality, Nigeria. The water samples were collected in duplicate in two batches. Samples were analyzed in the laboratory within two hours of collection.

Borehole: five different samples were collected at random at different streets in Calabar municipality, Nigeria namely; Edim Otop Street, Ikot Enebong Street, Ikot Omin, Ekosin Junction as shown in figure1. The samples were collected through convenience sampling into a sterile bottle and put in an ice packed box, the water samples were analyzed for the listed parameters, temperature, conductivity, turbidity, colour, iron, dissolved oxygen, calcium, magnesium, total hardness, alkalinity, manganese, sulphate, chloride, phosphate, sodium, zinc, copper, total suspended solid, nitrate, nitrite, ammonia, potassium.

Bottle water: Five different brands of bottled water were collected in the study area (Calabar municipality, Nigeria): **Ev, Ne, Qu, Cw**, and **Aq**. The following bottled water sample was analyzed for the following parameters. Temperature, conductivity, turbidity, colour, iron, dissolved oxygen, calcium, magnesium, total hardness, alkalinity, manganese, sulphate, chloride, phosphate, sodium, zinc, copper, total suspended solid, nitrate, nitrite, ammonia, potassium.

Pipe borne water: Five samples were collected through convenience sampling from five (5) utility points around Calabar municipality and analyzed for all the above parameters. Temperature, conductivity, turbidity, colour, iron, dissolved oxygen, calcium, magnesium, total hardness, alkalinity, manganese, sulphate, chloride, phosphate, sodium, zinc, copper, total suspended solid, nitrate, nitrite, ammonia, potassium.

Sachet water: Five different brands of sachet water; **UTWa, CW, ATW, ZTW** and **UTW**. Were randomly selected, samples and analyzed for the above parameters.Temperature, conductivity, turbidity, colour, iron, dissolved oxygen, calcium, magnesium, total hardness,

alkalinity, manganese, sulphate, chloride, phosphate, sodium, zinc, copper, total suspended solid, nitrate, nitrite, ammonia, potassium.

Stream: Four different streams and one spring around Calabar municipality; Satellite town, Parliamentary 1 and 2, Nyak Asang stream and Unicem spring were collected using a sterile bottle with about 20cm deep from the surface of the water. The stream was sampled and analyzed for the listed parameters. Temperature, conductivity, turbidity, colour, iron, dissolved oxygen, calcium, magnesium, total hardness, alkalinity, manganese, sulphate, chloride, phosphate, sodium, zinc, copper, total suspended solid, nitrate, nitrite, ammonia, potassium.

Physico-chemical analysis

The conventional parameters used in assessing the quality and portability of water for drinking are level of Suspended Solids, Total Dissolved Solid, Appearance, Hardness, Conductivity, pH, Colour, Odour, etc. AWWA/APHA, 1998 and Okorafor *et al*.,2012.

Temperature: The temperatures of the samples were taken, using a thermometer. The bulb of the thermometer was dipped into the water sample in a beaker and allows standing for some minute before the reading was taken (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Conductivity: Conductivity Meter (**Model: Hanna Instrument H18733**) was used. The conductivity meter probe was rinsed with distilled water and inserted into the sample in a beaker, conductivity reading was displayed(Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

pH: The pH was determined with a pH meter **(Model: Mettler Toledo Mp 220)**. The pH meter probe was inserted into the water sample in a beaker, the READ key was pressedand the pH reading was taken (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Turbidity: A turbidity meter was used. **(Model: Hanna Instrument H193703).**

Procedure: The sample was placed in the turbidimeter bottle and the bottle wiped clean with a cloth to erase any finger print that may affect the reading. The bottle was then placed on the

turbidometer and the **read** key pressed, the turbidity reading was displayed (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Colour: The colour was determined using **Lovibond Comparator**. The test kit was assembled and the water sample was poured into a tube and place in the right hand of the comparator. The disc was place on the comparator and noted as the colour value.

Iron (Method: APHA, 2010)

Procedure: 5 mL of water sample was placed in a test tube and 0.3 mL of iron reagent (Fe) was added, shaken and allowed to stand for 3 minutes the iron concentration was determine at a wave length of 420nm in spectrophotometer.

Dissolved Oxygen

Procedure: The dissolved oxygen was determined using APHA (2010) method. A reaction cell was filled to overflow and 1 glass bead was added into it. Oxygen reagent 02 – 1k was added (5 drops). Another 5 drops of oxygen reagent 02 – 2k were added and mixed for 10 seconds Lastly 10 drops of oxygen reagent 02 – 3k was added mixed and dissolved oxygen value read out in the spectrophotometer at a wave length of 498nm. The summary of the result are presented in table 1 to 5 for the different water samples(Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Calcium

Procedure: 0.1 mL of the sample was placed in a test tube using pipette and 0.5 mL of calcium reagent Ca-1 was added and mixed. 0.4 mL each of calcium reagent Ca-2 and Ca-3 were also added to the test tube and mixed. The sample was allowed to stand for 8 minutes to elicit full colour development and then filled into a reaction cell, placed in spectrophotometer where the calcium concentration was displayed (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Magnesium

Procedure: 1 mL of the sample was placed in a reaction cell and mixed and 1 mL of magnesium reagent Mg-1k added to it. This was allowed to stand for 3 minutes and thereafter, 0.3 mL of magnesium reagent Mg-2k added, mixed and placed in spectrophotometer. Magnesium concentration was read at a wavelength of 568 nm (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Total Hardness

Procedure: 1 mL of the sample was placed in a reaction cell and 1 mL of total hardness reagent H-1k added with a pipette. Three minutes reaction time was allowed before total hardness was determined in spectrophotometer at a wavelength of 450nm (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Alkalinity

Procedure: The alkalinity was determined using titrimetric method. The sample was placed up to the 5 mL mark in the test tube and 1 drop of methyl red indicator was added to it. The sample turns blue and a drop wise titration was carried out using reagent TL AL7 until there was a colour change. The value in the syringe was taken as the alkalinity value for the sample (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Manganese

Procedure: Manganesewasdetermined using spectrometric method. Five (5) mL of the water sample was placed in a test tube and 4 drops of manganese reagent Mn-1 was added and shaken. This was allowed to stand for 2 minutes. Thereafter, 0.2 mL each of manganese reagents Mn-2 and Mn-3 were added, shaken and allowed to stand for another 2 minutes before reading the manganese concentration from the spectrophotometer at a wavelength of 520 nm (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Sulphate

Procedure: 2.5 mL of the water sample was placed in a test tube and 0.2 mL of sulphate reagent SO_4-1A added and mixed. 1 level spoonful of sulphate reagent SO_4-2A powder was added and mixed. The solution was then tempered in a water bath at 40^oC for 5 minutes. 2.5 mL of sulphate reagent SO_4-3A was added, mixed and the solution filtered using **Whatman No. 1** filter paper. 0.4 mL of sulphate reagent SO_4-4A was then added to the filtrate and mixed. The solution was again tempered in a water bath for 7 minutes at 40^oC. This was transferred into a round cell and placed in spectrophotometer to read off the concentration of sulphate in the water sample. A wavelength of 520 nm was used (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Chloride

Procedure: 5 mL of the water sample was placed in a test tube and 2.5 mL of chloride reagent Cl-1 was added and mixed. Chloride reagent Cl-2 was also added, shaken and allowed to stand for 1 minute before reading out the chloride concentration from the spectrophotometer at a wavelength of 460 nm (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Phosphate

Procedure: 5 mL of the water sample was placed in a test tube and 0.5 mL of the phosphate reagent PO_4-1A added to it and mixed. This was followed by the addition of 1 level spoonful of phosphate reagent PO_4-2A. 5 minutes reaction time was allowed before reading out the phosphate concentration at a wavelength of 420 nm (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Sodium

Procedure: 0.5 mL of sodium reagent Na-1k was placed in a reaction cell and 0.5 mL of the water sample added to it and mixed. A reaction time of 1 minute was allowed before reading the concentration of sodium from the spectrophotometer (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Zinc

Procedure: 10 mL of the water sample was placed in a glass vessel and 1 micro-spoonful of zinc reagent Zn-1k was added and shaken to dissolve (this is the pretreated sample). 0.5 mL of zinc reagent Zn-2k was placed in a reaction cell and 2.0 mL of the pretreated water sample added to it and mixed. 0.5 mL of zinc reagent Zn-3k was also added into the reaction cell and mixed. Zinc concentration was then determined in the spectrophotometer (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Copper

Procedure: 5 mL of the water sample was placed in a reaction cell and 0.5 mL of copper reagent Cu-1k was added and mixed. 5 minutes reaction time was allowed before copper

concentration was determined (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Total Dissolved Solids (**TDS):** This was determined by multiplying through the conductivity value. The conductivity of the sample was determined and the value multiplied by 0.6 to get the TDS. TDS = Conductivity x 0.6 (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Nitrate

Procedure: 1 micro-spoonful of nitrate reagent NO_3-1A was placed in a dry test tube and 5 mL of nitrate reagent NO_3-2A added into it and mixed to dissolve. 1.5 mL of the sample was added slowly and shaken. This was allowed to stand for 10minutes and nitrate concentration was read out from the spectrophotometer at a wavelength of 520 nm (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Nitrite

Procedure: 5 mL of the water sample was placed in test tube and 1 micro-spoonful of nitrite reagent NO_2-AN was added and shaken to dissolve. A time of 10 minutes was allowed before reading out the nitrite concentration in the sample (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Ammonia

Procedure: 10 mL of the water sample was placed in a calibrated plastic cup and 2 drops of ammonia reagent 1 as well as 8 drops of ammonia reagent 2 **(Nessler Solution)** were each added to the water sample and mixed. After 5 minutes, the solution was poured into the colorimetry tube and the nearest colour match was used to determine ammonia concentration (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Potassium

Procedure: 2 mL of the water sample was placed in a reaction cell and mixed. 0.6 mL of potassium reagent K-1k was added and mixed, 1 level micro-spoonful of potassium reagent K-2k also added, mixed and allowed to stand for 5 minutes. The concentration of potassium was read out from the spectrophotometer at a wavelength of 690 nm (Okorafor *et al*.,2012; Brooks *et al.,* 2017; Iyakndue*et al.,* 2017).

Bacteriological Analysis

All the media used were prepared based on manufacturer's instruction and sterilized in the autoclave at 121^{o}C for 15mins. These were poured into sterile petri dishes (20 mL each) and allowed to cool before inoculation.The glass wares and the stainless steel filtration unit used were also sterilized in the hot air oven at 150^{o}C for 1hr.

Inoculation Technique

The samples were shaken to mix and 100 mL measured from it and filtered through membrane filter (0.45µm pore size). This filter allows water particles to pass through but bacteria cells are trapped.

After filtration, the membrane filter was carefully removed using sterile forceps and placed on the molten agar. Each sample had two (2) plates. These plates were incubated for 24 hours at 37^{o}C. Emerging colonies after the period of incubation were enumerated using a colony counter(Okorafor *et al.,* 2012).

Serial Dilution

One milliliters of the water samples from (borehole and surface water) each were transferred into nine (9 mL) of sterile distilled water in a separate test tube. Logarithms dilution ranging from 10^{-1} to 10^{-3} was made for each of the water samples. 1 mL of the desired aliquot is transferred into a sterile petri dishes and viable plate count was determined using pour plate method. Faecal and total coliform counts were performed for each sampleand were inoculated in the appropriate media (i.e. MF-C agar and MacConkey agar). The plates are incubated at 37^{0}C for 24 - 48 hours, and observed for growth, the colony counter is used in counting the colonies, and those with 2- 22*cfu/ mL* (colony forming unit) are counted(Ikpoh *et al.,* 2013).

Maintenance of Pure Culture

The growth from the plates especially those from the MacConkey agar plates had mixed colonies (culture) needed to be isolated in their pure form. The bacteria representatives (i.e. from each colony) was picked and sub-cultured onto a fresh sterile nutrient agar medium. Purity of isolates was enhanced and obtained through repeated streaking. The pure culture

that was obtained now provides the pure culture of that isolates and were maintained on nutrient agar slants as stock culture for characterization (Ikpoh *et al.,* 2013).

Characterization of Bacterial Isolates

The bacteria isolates were characterized based on their cultural morphologyand biochemical test according to (Collins and Lyne, 1976, Cheesbrough, 2006). The identification was done using the manual for identification of medical bacteria (Cowan and Steel, 1985). The biochemical tests used for characterization and identification of bacteria includes; Grams reaction, motility test, catalase test, coagulase test, oxidase test, methyl-red test, Voges Proskaurer test and sugar fermentation test.

Statistical Analysis

Statistical analyses of the Physico-chemical result were carried out to deduce the range of uncertainty to statistical test with the analysis of variance (ANOVA) to assess the drinking water quality. The statistical analysis one way ANOVA was applied to estimate whether it is statistically significant among the group in analysis and the significance reported at ($P<0.05$). The f test analysis was applied to find out the null hypothesis, the statistics were performed within brands and between brands using SPSSver. 20.

RESULTS AND DISCUSSIONS

Mean of Physico-chemical Analysis of Sampled Bottled Water

A total of five bottled water were sampled in duplicate. All the sampled water were clear in appearance, the colour was less than 5.0, temperature ranges from 28.8^{o}C to 27.1^{o}C with the highest from **Ev** water, **Ne** water had the highest pH of 6.89 and 6.68 from **Aq** water, turbidity ranges from 0.69NTU -0.111NTU very low compared to the NIS and WHO standard. Conductivity ranged between 235.6µs/cm -12.8µs/cm while Dissolved Oxygen ranges from 7.25mg/L-3.46mg/L, total Dissolved Solids ranged between 141.4mg/L-7.7mg/L, iron concentration ranges from 0.24mg/L-0.09mg/L, Total Hardness from 17.1mg/L, 7.97mg/L-7.90mg/L for Total Alkalinity, the range of Manganese, Magnesium, Calcium, Nitrate, Nitrite, Ammonia, Ammonium, Zinc, Chloride, Fluoride, Copper, Sodium, Potassium, Sulphate and Phosphate are as follows; 0.045mg/L-0.01mg/L, 20.1mg/L-7.2mg/L,

13.7mg/L-5.0mg/L,11.2mg/L-4.30mg/L, 0.014mg/L-0.004mg/L, 0.07mg/L-0.01mg/L, 0.00mg/L, 0.65mg/L-0.18mg/L, 0.61mg/L-0.105mg/L, 0.60mg/L-0.045mg/L, 0.135mg/L-0.055mg/L, 0.30mg/L-0.08mg/L, 3.12mg/L-2.05mg/L, 3.70mg/L-2.05mg/L and 6.53mg/L-3.30mg/L respectively. A summary of the result is shown in Table 1.

Mean of Physico-Chemical Analysis of Sampled Sachets Water

All the sampled sachets water was clear in appearance. Their colour was less than 5.0 compared to the standards, with temperature range of 27.3^{o}C-26.3^{o}C. The pH for **CW**had the highest pH (6.94), with turbidity ranging from 0.355NTU -0.17NTU, Conductivity ranged from 71.5 µs/cm - 23.15µs/cm. The concentration of dissolved Oxygen ranged between 13.5 mg/L-11.0mg/L with**CW**having the highest and **ZTW** with the least. The lowest concentration of total Dissolved Oxygen was found in **Aq**(13.9mg/L) and the highest in **ZTW**(47.7mg/L). Iron concentration ranged from 0.25mg/L to 0.30mg/L and**ZTW** had the maximum value, Total Hardness concentration observed from sachets water ranged between 34.2 mg/L-17.1mg/L. Total alkalinity varied from 7.98mg/L-6.59mg/L, Manganese concentration ranged from 0.055 mg/L-0.03mg/L, Magnesium concentration varied from 26.3 mg/L-9.0mg/L, Calcium concentration ranges from 9.4 mg/L-5.05mg/L, Nitrate from 16.9 mg/L-7.65mg/L, Nitrite, Ammonia, Ammonium, Zinc, chloride, Fluoride, copper, sodium, potassium, sulphate, and phosphate the values were from 0.0135 mg/L-0.004mg/L, 0.60mg/L-0.45mg/L, 0.07mg/L-0.05mg/L, 0.44mg/L-0.65mg/L, 2.40mg/L-1.25mg/L, 0.70mg/L-0.45mg/L, 0.40mg/L-0.20mg/L, 0.90mg/L-0.55mg/L, 2.1mg/L-4.15mg/L, 2.9mg/L-1.8mg/L and 6.1mg/L-3.75mg/L respectively. A Summary of this result is presented in Table 2.

Mean of Physico-Chemical Analysis of Sampled Public Water

All the sampled public water (tap) was clear in appearance. Their colour was less than 5.0 compared to the standards, with temperature range of 26.0^{o}C-24.75^{0}C. Wb_4(Marian road) had the highest pH (5.88) and the lowest pH (4.94) from Wb_1 (Edim Otop Street), turbidity ranged from 0.245-0.120NTU, Conductivity ranged from 51.1 µs/cm - 45.25µs/cm. The concentration of dissolved Oxygen ranged between 13.75 mg/L-12.50mg/L. Total Dissolved Oxygen ranged from 30.5 mg/L-27.1mg/L Iron concentration ranged from 0.145mg/L to 0.105mg/L, total hardness had from 34.2mg/L in all. Total alkalinity varied from 7.62 mg/L-7.06mg/L, Manganese concentration ranged from 0.035 mg/L-0.015mg/L, Magnesium concentration varied from 22.85 mg/L-10.1mg/L, Calcium concentration ranges from 11.45

mg/L-8.15mg/L, Nitrate from 4.55 mg/L-1.60mg/L, Nitrite, Ammonia, Ammonium, Zinc, chloride, Fluoride, copper, sodium, potassium, sulphate, and phosphate the values were from 0.045 mg/L-0.0045mg/L, 0.81 mg/L-0.61mg/L, 0.35 mg/L-0.25mg/L, 1.31 mg/L-0.76mg/L, 6.81 mg/L-5.11mg/L, 0.87 mg/L-0.66mg/L, 0.81 mg/L-0.31mg/L, 4.41 mg/L-1.81mg/L, 6.81 mg/L-5.10mg/L, 25.2 mg/L-18.1mg/L and 16.0 mg/L-5.65mg/L respectively. A Summary of this result is presented in Table 3.

Mean of Physico-Chemical Analysis of Stream Water Sampled

All the sampled streams were clear in appearance. Their colour was less than 5.0 compared to the standards, with temperature range of 27.6^{o}C-26.8^{o}C. The pH for Nyak asang (Ny) water had the highest pH (6.11) and the lowest from Unicem spring (Us) with pH (4.37), with turbidity ranging from 5.72 NTU -3.39NTU, Conductivity ranged from 94.1 µs/cm -42.8µs/cm. The concentration of dissolved Oxygen ranged between 5.29 mg/L-3.10mg/L. The lowest concentration of total dissolved oxygen was found in Nyak asang (Ny) (25.5mg/L) and the highest in Parliamentary stream (P_2) 56.6mg/L. Iron concentration ranged from 1.13mg/L to 0.76mg/L. Total Hardness concentration observed from sachets water ranged between 34.2 mg/L-17.1mg/L. Total alkalinity varied from 7.79mg/L-7.07mg/L, Manganese concentration ranged from 1.21mg/L-0.55mg/L, Magnesium concentration varies from 10.7mg/L-9.25mg/L, Calcium concentration ranges from 8.15mg/L-6.35mg/L, Nitrate from 21.1mg/L-9.05mg/L, Nitrite, Ammonia, Ammonium, Zinc, chloride, Fluoride, copper, sodium, potassium, sulphate, and phosphate the values were from 0.014mg/L-0.0043mg/L, 1.05mg/L-0.71mg/L, 0.63mg/L – 041mg/L, 1.21mg/L-0.81mg/L, 6.05mg/L-3.20mg/L, 0.69mg/L-0.42mg/L, 0.81mg/L-0.31mg/L, 2.40mg/L-0.89mg/L, 4.50mg/L-2.10mg/L, 32.5mg/L-18.5mg/L and 19.4mg/L-11.2mg/L respectively. A Summary of this result is presented in Table 4.

Mean of Physico-Chemical Analysis of Sampled Borehole Water

Five samples were picked in duplicate; and the result is as follows; the appearance and colour were clear and less than 5.0 respectively, the range value for temperature, pH, turbidityand conductivity were as follows, 30.3^{o}C-27.9^{0}C, 4.63-4.14, 0.42 NTU -0.17NTU and 79.8 µs/cm -29.0µs/cm respectively. The value for Dissolved Oxygen and Total Dissolved Solid ranged from 14.1 mg/L-11.2mg/L and47.8 mg/L-17.4mg/L, the value for total hardness was 17.1mg/L for all sampled water, total alkalinity ranged from 6.84 mg/L-6.69mg/L, the value for manganese, magnesium and calcium ranges as follows, 0.04 mg/L-0.02mg/L, 10.2 mg/L-

9.25mg/L and 8.15 mg/L-6.55mg/L, the concentration of nitrate and nitrite ranged from, 6.35 mg/L-4.30mg/L and 0.06 mg/L-0.03mg/L respectively. The highest concentration in ammonia was found in BH_3 with 0.71mg/L and the least is from BH_4 with 0.51mg/L, that of ammonium the highest and lowest was from $BH_{3\&5}$ and $BH_{2\&4}$ (0.05mg/L-0.03mg/L). Zinc concentration ranged from 1.07 mg/L-0.71mg/L, Chloride and Fluorides concentration ranged from 5.80mg/L-2.05mg/L and 0.64mg/L-0.17mg/L. copper concentration ranged from 0.42 mg/L-0.11mg/L, sodium ranged from 1.15mg/L-0.78mg/L, the concentration of potassium ranged from 2.75 mg/L-1.35mg/L, sulphate 6.05 mg/L-4.25mg/L while phosphate ranged between 4.75 mg/L-2.65mg/L. A summary of the result is presented in Table 5.

Table 1: Summary of the Result of Physico-Chemical Analysis of Bottled Water Sold in Calabar Municipality

NIS	WHO	Aqu	Qu	Cw	Ev	Ne	Parameters/units
Clear	Clear	Clear	Clear	Clear	Clear	Clear	**Appearance**
15	20	<5.0	<5.0	<5.0	<5.0	<5.0	**Colour (pt Co)**
20-30	20-30	27.5	27.6	27.1	28.8	27.3	**Temperature (^{o}C)**
6.5-8.5	6.5-8.5	6.72	6.68	6.81	6.72	6.89	**pH**
5.0	5.0	0.221	0.457	0.69	0.111	0.111	**Turbidity (NTU)**
500	500	12.8	235.6	107.9	83.6	163.5	**Conductivity**
14	14	3.46	5.40	4.66	4.95	7.25	**Dissolved Oxygen**
1000	1000	64.8	141.4	7.7	50.2	98.1	**Total dissolved**
0.30	0.30	0.14	0.24	0.14	0.11	0.09	**Iron (mg/L) Fe**
150	500	17.1	17.1	17.1	17.1	17.1	**Total hardness**
-	400	7.92	7.90	7.94	7.92	7.97	**Total alkalinity**
0.20	0.05	0.045	0.04	0.03	0.02	0.01	**Manganese (mg/L)**
100	20	7.7	20.1	7.20	11.1	11.85	**Magnesium (mg/L)**
-	50	5.0	13.7	5.75	6.05	8.5	**Calcium (mg/L) Ca**
50	45	10.4	11.2	5.5	4.30	9.45	**nitrate(mg/L) N**
0.2	0.1	0.005	0.014	0.011	0.013	0.004	**Nitrite (mg/L) N**
1.0	1.0	0.025	0.01	0.03	0.07	0.035	**Ammonia (mg/L)**
0.50	0.50	0.00	0.00	0.00	0.00	0.00	**Ammonium (mg/L)**
5.0	3.0	0.45	0.65	0.21	0.215	0.18	**Zinc (mg/L) Zn**
250	250	0.40	0.30	0.40	0.61	0.105	**Chloride (mg/L) Cl**
1.50	1.50	0.60	0.50	0.17	0.045	0.07	**Fluoride (mg/L) F**
1.0	1.0	0.125	0.135	0.055	0.055	0.055	**Copper (mg/L) Cu**
200	200	0.155	0.255	0.30	0.12	0.08	**Sodium (mg/L) Na**
100	200	2.34	3.12	2.50	2.70	2.05	**Potassium (mg/L) K**
100	200	3.35	2.60	3.70	2.05	2.63	**Sulphate (mg/L)**
100	200	3.30	5.04	6.35	5.60	4.30	**Phosphate (mg/L)**

Table 2: Summary of the Result of Physico-Chemical Analysis of Sachet Water Sold in Calabar Municipality

NIS	WHO	ZTW	UTWa	UTW	CW	ATW	Parameters/units
Clear	Clear	Clear	Clear	Clear	Clear	Clear	**Appearance**
15	20	<5.0	<5.0	<5.0	<5.0	<5.0	**Colour (pt Co)**
20-30	20-30	26.95	26.3	27.3	26.4	26.8	**Temperature (^oC)**
6.5-8.5	6.5-8.5	4.82	6.94	5.09	4.2	5.99	**pH**
5.0	5.0	0.355	0.22	0.22	0.17	0.23	**Turbidity (NTU)**
500	500	27.12	65.9	54.4	71.5	23.15	**Conductivity (µS/cm)**
14	14	11	13	8.5	13.5	12	**Dissolved Oxygen**
1000	1000	47.7	32.7	39.51	16.3	13.9	**Total dissolved solids**
0.30	0.30	0.30	0.30	0.25	0.20	0.25	**Iron (mg/L) Fe**
150	500	17.1	34.2	34.2	17.1	17.1	**Total hardness (mg/L)**
-	400	6.59	7.98	7.25	6.67	7.66	**Total alkalinity (mg/L)**
0.20	0.05	0.055	0.055	0.04	0.03	0.03	**Manganese (mg/L) Mn**
100	20	9.0	26.3	24.8	12.05	10.4	**Magnesium (mg/L) Mg**
-	50	8.1	7.95	9.4	5.05	6.75	**Calcium (mg/L) Ca**
50	45	8.25	15.0	9.90	7.65	16.9	**Nitrate (mg/L) N**
0.2	0.1	0.005	0.0135	0.011	0.0125	0.004	**Nitrite (mg/L) N**
1.0	1.0	0.6	0.6	0.45	0.5	0.5	**Ammonia (mg/L) NH_3**
0.50	0.50	0.06	0.055	0.06	0.05	0.07	**Ammonium (mg/L)**
5.0	3.0	0.44	0.55	0.65	0.50	0.55	**Zinc (mg/L) Zn**
250	250	1.25	1.45	1.75	2.10	2.40	**Chloride (mg/L) Cl**
1.50	1.50	0.60	0.55	0.45	0.45	0.7	**Fluoride (mg/L) F**
1.0	1.0	0.35	0.30	0.40	0.20	0.30	**Copper (mg/L) Cu**
200	200	0.55	0.65	0.75	0.90	0.85	**Sodium (mg/L) Na**
100	200	2.1	3.1	2.65	3.25	4.15	**Potassium (mg/L) K**
100	200	2.4	1.8	2.2	2.3	2.9	**Sulphate (mg/L) SO_4**
100	200	3.75	4.2	5.2	6.1	4.55	**Phosphate (mg/L) PO_4**

Table 3: Summary of the Result of Physico-Chemical Analysis of Public Water Supply in Calabar Municipality

Parameters/units	Wb1	Wb2	Wb3	Wb4	Wb5	WHO	NIS
Appearance	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Colour (pt Co)	<5.0	<5.0	<5.0	<5.0	<5.0	20	15
Temperature (^oC)	25.05	26.10	24.85	25.90	24.75	20-30	20-30
pH	5.47	5.47	4.94	5.88	5.75	6.5-	6.5-
Turbidity (NTU)	0.120	0.120	0.123	0.245	0.236	5.0	5.0
Conductivity (µS/cm)	51.1	51.1	50.4	45.75	45.25	500	500
Dissolved Oxygen	12.50	12.50	13.75	13.50	12.65	14	14
Total dissolved solids	30.3	27.50	30.35	27.41	27.18	1000	1000
Iron (mg/L) Fe	0.125	0.120	0.105	0.145	0.125	0.30	0.30
Total hardness (mg/L)	34.2	34.2	34.2	34.2	34.2	500	150
Total alkalinity (mg/L)	7.44	7.06	7.18	7.62	7.55	400	-
Manganese (mg/L) Mn	0.035	0.025	0.030	0.025	0.015	0.05	0.20
Magnesium (mg/L) Mg	22.85	13.50	10.15	10.15	11.9	20	100
Calcium (mg/L) Ca	11.45	10.55	8.15	8.25	10.45	50	-
Nitrate (mg/L) N	1.60	4.55	4.05	4.25	3.85	45	50
Nitrite (mg/L) N	0.004	0.013	0.0105	0.012	0.045	0.1	0.2
Ammonia (mg/L) NH_3	0.71	0.81	0.71	0.61	0.71	1.0	1.0
Ammonium (mg/L)	0.25	0.35	0.31	0.35	0.31	0.50	0.50
Zinc (mg/L) Zn	1.31	0.81	0.76	1.03	0.85	3.0	5.0
Chloride (mg/L) Cl	6.31	5.11	6.81	5.31	5.11	250	250
Fluoride (mg/L) F	0.87	0.71	0.85	0.66	0.71	1.50	1.50
Copper (mg/L) Cu	0.57	0.32	0.31	0.81	0.43	1.0	1.0
Sodium (mg/L) Na	4.41	2.11	3.20	1.81	2.11	200	200
Potassium (mg/L) K	6.30	5.10	6.81	5.31	5.11	200	100
Sulphate (mg/L) SO_4	25.2	18.1	10.6	8.70	11.35	200	100
Phosphate (mg/L) PO_4	16.0	9.35	8.10	5.65	6.35	200	100

KEY: Wb_1= Edim Otop Street, Wb2= Orok Street off Ediba, Wb3= Edim Otop Close West, Wb_4= Marian Road Wb5=Ikot Efa Street.

Table 4: Summary of the Result of Physico-Chemical Analysis of Stream Water in Calabar Municipality

Parameters/units	P_1	P_2	Ny	Us	St	WHO	NIS
Appearance	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Colour (pt Co)	<5.0	<5.0	<5.0	<5.0	<5.0	20	15
Temperature (^oC)	27.5	27.6	27.2	27.1	26.8	20-30	20-
pH	5.15	5.28	6.11	4.37	4.81	6.5-	6.5-
Turbidity (NTU)	2.41	3.67	5.72	5.49	3.39	5.0	5.0
Conductivity (µS/cm)	94.1	93.4	42.8	91.6	69.4	500	500
Dissolved Oxygen (mg/L)	5.29	4.39	5.05	3.10	3.25	14	14
Total dissolved solids (mg/L)	55.4	56.6	25.5	53.6	45.8	1000	1000
Iron (mg/L) Fe	1.06	1.13	0.81	1.21	0.76	0.30	0.30
Total hardness (mg/L)	17.1	17.1	17.1	17.1	17.1	500	150
Total alkalinity (mg/L)	7.77	7.33	7.73	7.79	7.07	400	-
Manganese (mg/L) Mn	0.95	0.99	0.55	1.21	0.76	0.05	0.20
Magnesium (mg/L) Mg	10.7	9.20	9.85	9.65	10.3	20	100
Calcium (mg/L) Ca	6.35	8.15	7.30	7.35	6.85	50	-
Nitrate (mg/L) N	12.6	27.1	13.5	9.05	11.65	45	50
Nitrite (mg/L) N	0.004	0.009	0.008	0.014	0.003	0.1	0.2
Ammonia (mg/L) NH_3	0.81	0.91	0.71	0.99	1.05	1.0	1.0
Ammonium (mg/L) NH_4	0.42	0.53	0.41	0.63	0.61	0.50	0.50
Zinc (mg/L) Zn	0.91	1.21	1.11	1.00	0.81	3.0	5.0
Chloride (mg/L) Cl	6.05	5.55	3.20	5.75	3.85	250	250
Fluoride (mg/L) F	0.69	0.67	0.46	0.59	0.42	1.50	1.50
Copper (mg/L) Cu	0.61	0.81	0.81	0.51	0.31	1.0	1.0
Sodium (mg/L) Na	2.40	2.11	0.89	1.15	1.07	200	200
Potassium (mg/L) K	4.50	3.45	2.50	3.15	2.10	200	100
Sulphate (mg/L) SO_4	28.1	32.5	22.1	18.4	20.8	200	100
Phosphate (mg/L) PO_4	11.4	14.6	19.4	15.3	13.5	200	100

Key: P_1= Parliamentary Stream before the Bridge,P_2= Parliamentary after the SEMATEC, Ny=Nyak Asang Stream, Us=Unicem Spring, St= Satellite Town Stream

Table 5: Summary of the Result of Physico-Chemical Analysis of Borehole Water in Calabar Municipality

Parameters/units	BH_1	BH_2	BH_3	BH_4	BH_5	WHO	NIS
Appearance	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Colour (pt Co)	<5.0	<5.0	<5.0	<5.0	<5.0	20	15
Temperature (^oC)	29.3	27.9	30.3	29.5	29.8	20-30	20-30
pH	4.37	4.13	4.44	4.30	4.63	6.5-	6.5-
Turbidity (NTU)	0.19	0.17	0.27	0.20	0.42	5.0	5.0
Conductivity (µS/cm)	22.4	79.8	29.0	36.2	29.1	500	500
Dissolved Oxygen	11.2	14.1	14.1	13.1	14.1	14	14
Total dissolved solids (mg/L)	13.4	47.8	17.4	21.7	17.4	1000	1000
Iron (mg/L) Fe	0.19	0.23	0.70	0.14	0.21	0.30	0.30
Total hardness (mg/L)	17.1	17.1	17.1	17.1	17.1	500	150
Total alkalinity (mg/L)	6.89	6.74	6.84	6.82	6.69	400	-
Manganese (mg/L) Mn	0.03	0.04	0.03	0.02	0.03	0.05	0.20
Magnesium (mg/L) Mg	9.25	9.80	10.3	10.6	10.4	20	100
Calcium (mg/L) Ca	8.15	7.30	7.15	6.55	6.85	50	-
Nitrate (mg/L) N	6.35	5.55	4.40	5.25	4.30	45	50
Nitrite (mg/L) N	0.06	0.03	0.03	0.05	0.04	0.1	0.2
Ammonia (mg/L) NH_3	0.61	0.60	0.71	051	0.61	1.0	1.0
Ammonium (mg/L)	0.04	0.03	0.05	0.03	0.05	0.50	0.50
Zinc (mg/L) Zn	1.07	0.81	0.75	0.71	0.82	3.0	5.0
Chloride (mg/L) Cl	5.80	4.25	2.05	2.30	2.35	250	250
Fluoride (mg/L) F	0.35	0.64	0.28	0.17	0.56	1.50	1.50
Copper (mg/L) Cu	0.42	0.13	0.11	0.13	0.12	1.0	1.0
Sodium (mg/L) Na	1.15	1.03	0.84	0.98	0.78	200	200
Potassium (mg/L) K	2.75	1.45	2.70	1.80	1.35	200	100
Sulphate (mg/L) SO_4	5.65	4.25	6.05	4.85	5.25	200	100
Phosphate (mg/L) PO_4	4.30	3.70	4.75	2.65	3.05	200	100

Key: Bh_1=Edim Otop, Bh_2= Ikot Omin, Bh_3=Ikot Enebong, Bh_4= Ekosin Junction, Bh_5=Nyak Asang

Mean Bacteria Count for Sampled Bottled and Sachet Water

The mean faecal count and total coliform bacteria count per 100 mL of sampled water were obtained from five different brands of bottle and sachets water collected in duplicate and the mean result is presented in Table 6. The mean ranged for total coliform bacteria for sampled bottled water ranged from 2 cfu/100ml to 19cfu/100ml and no coliform was detected. The total coliform count for sachets water ranged from 6cfu/100mlto 15cfu/100mland zero for faecal coliform count. The zero faecal coliform shows that they have met the World Health Organization standard for drinking water *(≤ zero*cfu/100ml). The summary of this result is presented in Table 6.

Mean Bacteria Count for Sampled Public Water

Table 7 shows the bacterial count for sampled public water (tap), there was no growth on the faecal and total coliform plates after 48 hours of incubation at appropriate temperature (35^oC and 44 ± 0.5^oC) respectively. This result is satisfactory and it complies with the international standard for drinking water set by the World Health Organization.

Mean Bacteria Count for Sampled Stream Waters

Table 8 shows the mean plate count for stream waters. The total and faecal coliform count per millimeter (mL) of water obtained from serial dilution of sample to power 1 (10^{-1}). The samples taken in duplicate from five different location in Calabar Municipality. The result ranged from $27x10^1$cfu/ mLto $55x10^1$cfu/ mLfor total coliform and $15x10^1$cfu/ mLto $52x10^1$cfu/ mL for faecal coliform (Satellite town) stream had the highest faecal coliform count, Nyak Asang stream had the least faecal coliform count. The highest total coliform count was from Unicem stream and the least from Nyak Asang stream.

Mean Bacteria Count for Borehole Water

Borehole water sampled from five different boreholes in different location in the study site. The result ranged between 12 cfu/100 mL to 33cfu/100 mL for total coliform and 9cfu/100 mL to 16cfu/100 mL for faecal coliform. Ekosin junction (BH_4) had the highest value of total coliform and Ikot Omin (BH_2) with the least, while Nyak asang borehole (BH_5)and Edim Otop

Street(BH_1) had the lowest and the highest value respectively for faecal coliform. A summary of this is presented in Table 9

Characteristic and identification of bacterial isolates

Based on the morphological and biochemical characteristics of bacteria isolated from the water sources as presented in Table 10. The following bacteria were isolated, *Escherichia coli, Enterobacter* spp.*, Chromobacters* spp.*, Proteus* spp.*, Pseudomonas* spp. and *Streptococcus* spp.

Percentage Occurrence of Bacteria Isolate from Drinking Water

The organism with the highest occurrence in the studied water sample *Pseudomonas* spp.23.5%, followed by the *Proteus* spp.and *Escherichia coli* with 20.5%, *Streptococcus* spp.14.7%, *Enterobacter* spp.8.8%, *Chromobacter* spp.2.9%, *Salmonella* spp.2.9% and *Enterococcus* spp.2.9%, stream water had the highest bacteria and bottled water with the least bacteria (2) presented in Table 11.

Table 6: Mean Bacterial Count for Bottled and Sachets Water Sample

Samples	Total Coliform Counts (cfu/100 mL)	Feacal Coliform Counts (cfu/100 mL)
Ev bottled water	4	_
Cw bottled water	3	_
Ne bottle water	2	_
Aq bottled water	8	_
Qu bottled water	19	_
Sachets		
UTWa	-	-
UTW	-	-
ATW	15	-
CW	10	-
ZTW	5	-

Table 7: Mean Bacterial Count for Public (tap) Water Sample

Samples	Total Coliform Counts (cfu/100 mL)	Feacal Coliform Counts (cfu/100 mL)
Wb1	_	_
Wb2	_	_
Wb3	_	_
Wb4	_	_
Wb5	_	_

KEY: Wb_1=Edim Otop Street, Wb_2= Orok Street off Ediba, Wb_3= Edim Otop Close West, Wb_4=Marian Road, Wb_5= Ikot Efa Street, NG=No Growth

Table 8: Mean Bacterial Count for Stream Water Sample

Samples	Total Coliform Counts($x10^1$cfu/ mL)	Feacal Coliform Counts ($x10^1$cfu/ mL)
P_1	49	25
P_2	53	30
Ny	27	15
Us	55	28
St	43	52

Key: P_1=Parliamentary Stream before the Bridge, P_2= Parliamentary after the SEMATEC, Ny=Nyak Asang Steam, Us= Unicem Spring, St= Satellite Town Stream

Table 9: Mean Bacterial Count for Borehole Water Sample

Samples	**Total Coliform Counts** (cfu/100 mL)	**Feacal Coliform Counts**(cfu/100 mL)
BH_1	30	16
BH_2	12	10
BH_3	23	14
BH_4	33	14
BH_5	21	9

Key: Bh_1= Edim Otop, Bh_2= Ikot Omin, Bh_3= Ikot Enebong, Bh_4= Ekosin Junction, Bh_5= Nyak Asang

Table 10: Summary of Morphological and Biochemical Characteristic of Bacteria Isolated from Drinking Water

Cultural Characteristics	Gram's Reaction	Shape	Motility	Citrate	Indole	Oxidase	Methyl Red	Voges Paskauer	Catalase	Lactose	Glucose	H_2S	Probable Organisms
Irregular, swarming and colourless	_	R	+	+	+	_	+	_	+	_	AG	_	*Proteus* spp.
Creamy, circular, convex, smooth and moist	+	C	_	+	NR	NR	NR	NR	+	_	A	_	*Streptococcus* spp.
Pale yellow, circular, convex and smooth	_	R	+	_	_	_	_	+	+	+	AG	+	*Enterobacter* spp.
Translucent, moist and spreading	_	R	+	+	+	_	+	_	+	_	A	+	*Pseudomonas* spp.
Pink, irregular, raised, moist, shinny	_	R	+	_	+	_	+	_	+	+	AG	_	*Escherichia coli*
Blue,round, circular, smooth	_	R	_	_	+	+	_	_	+	_	G	+	*Chromobacter* spp.
Colourless and transparent	_	R	+	+	_	_	+	_	+	_	AG	+	*Salmonella spp*
Tiny pink and wrinkled	+	C	+	_	_	_	_	+	+	+	AG	_	*Enterococcus* spp.

Key: (+) positive, (-) negative, (NR) not relevant, (R) rod, (C) cocci, (A) acid fermentation, (G) gas production,

Table 11:Summary ofFrequency of Occurrence of Bacteria Isolated From Drinking Water

Probable Organism	Bottled Water	Borehole Water	Public Water	Sachets	Streams	% Occurrence
Proteus spp.	+	+	_	+	+	20.5
Streptococcus spp.	_	+	_	+	+	14.7
Enterobacter spp.	_	_	_	+	+	8.8
Pseudomonas spp.	+	+	_	+	+	23.5
E. coli	_	+	_	_	+	20.5
Chromobacter spp.	_	_	_	_	+	2.9
Salmonella spp.	_	_	_	_	+	2.9
Enterococcus spp.	_	+	_	_	_	2.9

Key: - (not present), + (present)

Qualitative drinking water supply is often a major challenge to many developing countries including Nigeria. In this study, the physico-chemical and bacteriological quality of drinking water sources in Calabar metropolis, Nigeria was investigated by collecting five different sources, they include; Borehole, Streams, Public water (tap), bottled and Sachets waters.

The findings in the study of all the evaluated water sources have temperature within the normal range that is in conformity with WHO (2011) standard and NIS. In a study reported by Itah *et al.,*(2005), the temperature range of all the public water supplies investigated was within the normal range.

Temperature is one of the most essential parameters in water. It has significant impact on growth and acidity of ecological life and greatly affects the solubility of oxygen in water. When the temperature is high the pH of the water will change thereby favouring the growth of some organisms. The pH of the water sampled compared to the standards was with the specific range. The turbidity of the water was within the standard except that of stream which was above the standard. The ability of the sampled water to be able to conduct electricity has no health implication on humans or organisms whether it high or low like it is observed in this study. Dissolved oxygen and Total dissolved solid of the five sampled water compared to the standard were within the acceptable limit prescribed. Similarly, the Iron concentrations of the studied sample were within the normal limit and are in conformity with the findings of Okorafor *et al.,* 2012.

Total Hardness is caused by the presence of calcium and magnesium salt, all the water samples have their values within the permissible limits. The quantitative capacity of an aqueous media to react with H^+ ions (*i.e.* Total Alkalinity) for all sampled water were all within the limits. The health implication for manganese if high causes neurological disorder and the study reveals that they are all within the standard acceptable limit. Magnesium concentration of the water sampled, as one of the causes of water hardness were within the Nigerian Standard and few where within the World Health Organization Standard. Calcium is one of the compounds that cause hardness of water if present. Their concentrations compared to the standards were all within ranges for all the sampled water. The concentration of the different forms of nitrogen (nitrate, nitrite, ammonia and ammonium) gives a useful indication of the level of micro-nutrients in the water and hence the ability to support plant growths. The results obtained were compared with the standard.

Nitrate concentration for all sampled water was within the acceptable limits that of Nitrite also but, that of Unicem spring was slightly high above the limit. Thus, high concentrations of nitrite can cause cyanosis in infants less than 3months (NIS, 2007). Ammonia and Ammonium as the derivatives of Nitrogen enters the water body through the organisms that survives or by sewage effluent and runoff from land were manure has been applied or stored in the case of surface water like stream that shows little slight vibration in the studies but still within the range of acceptable limit. Zinc and copper are also a trace element that is needed in our system, but excess amount of copper will cause gastrointestinal disorder (i.e. when it exceeds the limited standards). Chloride are common constituents of all natural waters, higher value of it impacts a salty taste to the water, making it unacceptable for human consumption.

The chloride value in this study is within the acceptable range. Fluoride is essential for human beings as a trace element, higher concentration of this element causes toxic effect (Dental Fluorosis), but a small amount of fluoride protects tooth decay and enhances bone development (Kundu *et al.,* 2001). Sodium, Potassium and Sulphate were all within the acceptable range given by the International and National standard. A high concentration of sulphate may induce diarrhea and intestinal disorder.

Phosphate in water occurs in the form of orthophosphate, polyphosphate (Kataria *et al.,* 2011). The present study reveals that all the sampled water was all within the acceptable limits that was given by the standard and also fit for human consumption.

The result of the bacteriological analysis of the five sources of water sampled revealed the unsanitary condition of most drinking water with some contaminated with coliforms and pathogenic bacteria. This finding is similar to that reported byAdekunle *et al*., (2004) in Ibadan,Itah *et al.*, (2005), Ezeugwune *et al*., (2009) in Nnewi, Oladipo *et al.*, (2009) in Ogbomoso, Adegoke *et al*, (2012). Similarly,Ademoroti(1996), which showed the presence of some bacteria in sachet water, borehole water, stream water respectively while Zvidzai *et al.*, (2001) in the study of microbial community analysis of drinking water sources from rural areas of Zimbabwe detected faecal coliform and *Escherichia coli* which were attributed to poor treatment handling of water. Some studies however have revealed that coliform bacteria are widely found in nature and do not necessarily indicate faecal pollution (Binnie 2002, Griffith *et al.,* 2003).

Adegoke *et al.,*(2012) observed that the presence of bacteria in some branded water like bottle and sachet water could be as a result of poor environmental condition, poor treatment and handling methods in processing industries. Other sources of possible contamination enumerated includes; poor handling by distributors and seller, insufficient sterilization of the sachets and bottles used in packaging the water, contamination with bacteria of the vending machine use in packaging and the duration of the packaged (branded) water.

The study reveals that out of the five different brands of bottle water randomly selected none had faecal coliforms but all the five brands had total coliform, despite all having NAFDAC approved numbers. NAFDAC standard states that total and faecal coliform levels must be *zerocfu/100 mL.* This means that the contaminants might not be of faecal origin but, it may be due to some environmental factor (production, machine, staff or the container (Kolawole, 2009).

Adekunle, (2004) stated that theconsumption of bottled water is increasing rapidly in developing countries especially among the middle and high incomes earners as it is generally perceived to be pure, clean and of good quality. This has led to the sales of different brands of bottled water in the study area. Although disease outbreaks due to contaminated bottled water are rare, bottled water has been found to cause traveler's diarrhea.

In this study all the samples investigated had zero faecal coliform count. Only 40% (2/5)out of the 5 brands had zero total coliform count. As useful as sachets water is to the society, the results of the analysis raised doubts as to it quality says Adekunle *et al.,* (2004).

The findings of no faecal and total coliforms in all the sampled public water is in line with the international and Nigeria standard of drinking water *(*≤ zerocfu/100 mL). Kolawole, (2009) in the chemical and bacteriological quality of drinking water in Calabar municipality reported no feacal and total coliform in public water.

The analyses of borehole water samples show that none of the samples were portable for drinking with the level of faecal and total coliforms which are observed from this study. This is pathetic because borehole water is the most accessible source of drinking water in the study area. The presence of faecal coliform suggests faecal contamination and the possible presence of pathogenic bacteria like *Salmonellatyphi*(Itah*et al.*,2005). The result agreed with the earlier reports by Itah *et al.,* (1996) in the study of bacteriological characteristics of rural water supply in Calabar and that of Agbu *et al*., (1998) in Samaru, Zaria as well as Adesiyen in Katsina in terms of high density of coliforms obtained.

The faecal coliform and total coliform of streams water samples around the study area was very high above the standard set for untreated water samples (10cfu/100 mL). This is probably due to contamination as this water flows from the source down to the fetching point and the various activities (washing and bathing) being carried out by residents of the area. The analysis also revealed the presence of *Proteus* species, *Streptococcus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Escherichia coli*, *Chromobacter* spp., S*almonella*spp. and *Enterococcus* spp. These organisms are of public health importance. Most organisms obtained from the drinking water sources in Calabar have earlier been reported in drinking water sources in Calabar. Edema *et al.,*(2001) reported that the presence of *Enterobacter* and *Proteus* species in water samples suggest that these organisms could originate from burst pipes along distribution lines of drinking water plant used in production of such water *Pseudomonas* species whose presence is of significant value in determining the extent of water sample implies faecal pollution dating to remote period (Itah *et al.,* 2005).

The physical condition of an environment is one of the factors that can contribute to the state of our drinking water supplies. In this environment of study (especially the stream surrounding in satellite town) was dirty and bushy. Some people use it as dumping site which may have contributed to the highest number of coliformsrecorded in stream water sample. The zero faecal coliform from public water sample and some brands of packaged water maybe due to the routine treatment of their sources. This study reveal that public water supply meets the WHO standard for drinking water ($\leq$zero cfu/100 mL).Schlegel, (2002) reported that*Enterobacter* spp. isolated from the water samples are example of non faecal coliforms and can be found in vegetation and soil which serves as sources by which pathogens enter the water The British Standard Institute specified that counts greater than 10^4 are considered unsatisfactory for *Enterobacter* spp.

The presence of total coliforms and faecal coliform, *E.coli*, *Salmonella* spp., *Shigella* spp. and *Vibro* spp. have been documented as criteria for evaluating drinking water and as a standard for protectingthe public by limiting the levels of contaminants in drinking water (EPA, 2002).

The result of statistical analysis of each analyzed parameter and the value of F test and significance are presented in the tables above. The calculated F value were observed in the range of 0.000-0.956, the F critical is 5.19 for stated level of confidence (typically 95%) which mean

that the difference being tested are statistically significant (*) and non-statistically significant (**) at 95% confidence level.

CONCLUSION

This study has revealed the unsanitary state of some drinking water consumed in this part of the country under study. As most samples contain bacterial indicators of faecal pollution as expected in streams. However, the presences of bacteria in some brand of bottled, sachet and borehole water sampled were not expected. This is pointer to the poorquality standard of some waters that are being consumed by the inhabitant of Calabar Municipality. Tap water regulation makes it mandatory that the public water supply is tested daily and that findings are freely available for scrutiny. While most bottled and sachets water are safe, their bacteria contents mean that they are not as safe as tap water. The boreholes in the study area are not regulated or monitored by any regulatory body, based on these findings. It has been known from this research that the quality of drinking water sources in Calabar Municipality is poor and the majority of the residents do not treat water. There should be awareness on effective household water treatment on how to treat and maintain the microbial quality of water at the household level.

RECOMMENDATIONS

i. The Cross River State Water Board (CRSWB) Management should ensure periodic check on their pipe line to avoid leakages and prevent future contamination of this source of drinking water being the best in this study.

ii. They should also carryout proper enumeration, registration and regulation of boreholes in the study area to enable periodic examination of this source of drinking water.

iii. CRSWB should make pipe borne water available and affordable for all residents of Calabar Municipality, Nigeria.

iv. Due to poor quality of packaged drinking water (National Agency for Food Drugs Administration and Control) NAFDAC should

a) Undergo periodic and regular visit to the packaged water factories for re-assessment of their GMP not less than 4 times yearly.

b) Undergo periodic re-testing by randomly sampling and analyzing packaged water being produced to ascertain if the quality still meet.

v. Water treatment by individual or household should be encouraged by government through intensive campaign by workshops and seminar on the importance of water treatment before use.

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