ANALYSIS OF SACHET WATER, BOTTLED WATER AND BOREHOLE WATER CONSUMED IN AND AROUND ANAMBRA STATE POLYTECHNIC, MGBAKWU

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Abstract
Water is an inorganic, odourless, tasteless, transparent, and nearly colourless chemical substance made up of hydrogen and oxygen existing in gaseous, liquid, and solid states (Zumdahl, 2020). About 1.2 billion people worldwide do not have access to clean, safe water, and most of them are in developing countries (Amaechi, 2016). Unsafe water quality is a global public health issue. There is concern that borehole, sachet water and bottle water produced in developing nations may be sold without adequate monitoring, and that water treatment may be subject to contamination. The purpose of this quantitative study was to examine the level of contamination of drinking water sourced from boreholes, bottle water and sachet-packaged water around Anambra State Polytechnic, Mgbakwu, Southeast Nigeria. Local officials may utilize this study's results to develop a standard that can improve the quality of commercially packaged, bottle water, sachet and borehole water for drinking purposes.
INTRODUCTION

Water is an essential element for the survival of all living organisms. In humans, it is shown to make up about 70% of the body mass (Eldon, 2004). The human physiological activities are thereof dependent on water availability. Many infectious diseases in developing countries are associated with contaminated water (Tar et al., 2019). Thus good drinking water is a luxury but one of the most essential requirements of life (Ajewole, 2005).

Studies have shown that over one billion people in the world lack access to safe drinking water and 2.5 billion people do not have access to adequate sanitation services (Tar et al., 2019). In many developing countries including Nigeria, clean pipe borne water availability is limited and inadequate for the teeming population. Thus, an increasing number of people in semi urban areas in the country depend on dug wells and water vendors for water supply (Idowu, 2011).

Unregulated borehole production, sachet water production and bottle water production are public health issues requiring attention because of their effects on general health and productivity of those affected (Amaechi, 2016; Emenike, Tenebe, Omeje, and Osinubi, 2017). Due to authorities' inability to provide safe water for communities, cities in Nigeria struggle to provide adequate clean water to meet citizens' needs (Akpen, Kpohal and Oparaku, 2018). The need for safe water has led to the escalation of unregulated drilling of boreholes, bottle water production and sachet water production. Accurate and timely information on the quality of water is necessary to shape a sound public policy and to implement the water quality improvement programme efficiently. One of the most effective ways to communicate information on water quality trends is with indices. The water quality index (WQI) is commonly used for the detection and evaluation of water pollution and may be defined as a rating reflecting the composite influence of different quality parameters on the overall quality of water (Mishra, 2005). The indices are broadly characterized into two parts: the physicochemical and biological (bacteriological) indices.

Physicochemical indices are based on the values of various physicochemical qualities in a water sample. These are vital for water quality monitoring (APHA, 1998). A number of scientific procedures and tools have been developed to assess the water contaminants (Dissmeyer, 2000). These procedures include the analyses of different parameters such as pH, turbidity, temperature, dissolved oxygen, alkalinity amongst others. These parameters can affect the drinking water quality, if their values are in higher concentrations than the safe limits set by the World Health Organization (WHO) and other regulatory bodies (WHO, 2011).

Bacteriological indices are derived from the biological information and are determined using the species composition of the sample, the diversity as well as the distribution pattern, the presence or absence of the indicator species or groups (Trivedy, 1984). Bacterial contamination of drinking water is a major public health problem worldwide; because this water can be an important vehicle of diarrheal diseases; thus the need to evaluate the bacterial quality (Suthar, 2009).

Monitoring the bacterial quality of drinking water is done through laboratory testing for the coliform groups. The total coliform refers to a large assemblage of gram-negative, rod shaped bacteria that share several characteristics. These include E. coli, Klebsiella, Enterobacter, Streptococcus, Staphylococcus spp etc. A borehole is a narrow hole drilled down through the earth to reach water (Daniel and Daodu, 2016). Sachet water is commercially packaged water bagged in 250 ml polyethylene bags meant for drinking. Bottle water is also commercially bottled water 250ml plastic bottles meant for drinking. Sachet water came into the Nigerian retail market in 1990 (Stephen, 2015). The borehole, bottled water and sachet water production was meant to bridge the gap in drinking water status/quality and access to clean drinking water. Despite the aim to provide safe drinking water through borehole, bottled water and sachet water production, there are many people without safe drinking water in Nigeria (Wright, Dzodzomenyo and Wardrop, 2016). Before water can be described as potable, it has to comply with certain physical, chemical and microbiological standards which are designed to ensure that the water is potable, safe for drinking thus studies will be conducted to ascertain these parameters in varying drinking water sources, borehole water, sachet water and bottled water. It on these bases that this research is conducted to determine the qualities of drinking water sources around Anambra State Polytechnic, Mgbakwu, Nigeria. This research work proposal provides the study's background, problem statement, the purpose of the study, research questions and hypotheses, nature of the study, theoretical framework, assumptions, limitations of the study, delimitations, significance, and a summary of the research.

PROBLEM STATEMENT

The need to access safe water has forced many developing nations to opt for providing safe drinking water from boreholes, bottle water and sachet packaged water (Atta, 2017). Most of the bottle water sachet-packaged water produced in Nigeria are sold without sufficient treatment and monitoring and may not be free from physical, chemical, and bacteriological contamination (Emenike et al., 2017; Omalu et al., 2010). In some instances, the borehole, bottled water and sachet water production companies' storage tanks and pipes are not well maintained to ensure good quality and safety of water stored in them (Daniel and Daoda, 2016). According to Omalu et al. (2010), the physical assessment of some bottled water and sachet water in Nigeria is necessary. There is an increasing understanding that contaminated water consumption is responsible for several health-related disorders such as waterborne infections like diarrhea and typhoid fever (Aroh et al., 2013; Musa et al., 2018). The proliferation of unregulated boreholes, bottled water and the sachet portable water production in Mgbakwu, Awka North and its surrounding environment has raised concerns about water quality and suitability for safe drinking and other domestic purposes in line with regulatory standards. Waterborne
diseases account for 80% of developing nations' illnesses, killing a child every 8 seconds (Akpen et al., 2018; Ibrahim, Umuru and Akinsorji, 2007). Dada (2009) and Shigut et al. (2017) described the weaken of monitoring and production quality of borehole water, bottle water and sachet water packaging and the 6 treatment process. Dada (2009) recommended the need for more research, as most water sources were inadequately regulated.

OBJECTIVE OF THE STUDY
The purpose of the current quantitative study is to examine the level of physical, chemical, and bacteriological contamination of borehole, sachet and bottle water around Anambra State Polytechnic, Mgbakwu, Awka north local government area of Anambra State, Nigeria to find out if the boreholes, bottle water and sachet water sold, is in conformity or otherwise with the standards set by the regulatory body.

Also, the correlation and significance difference between the physical, chemical, and bacteriological parameters of drinking water sourced from the borehole, bottle water and sachet-packaged water will be examined statistically.

MATERIALS AND METHODS
Study Area
The study area is around Anambra state Polytechnic, Mgbakwu, Awka North Local Government Area, which is made up of nine villages. The town is with a population of over five hundred and also houses. However, the town is characterized by low level of environmental sanitation, no slums but scattered poor housing in and with lack of potable water and improper management of wastes especially in the indigenous core areas characterized by high density and low income populations mostly the Indigenes.

Sampling of Waters
From the borehole about 10 brands of water sold in the town at the time of this study, will be selected by simple random sampling method (here, the selection of units from a population is based on the principle of randomization. Every unit of the population has a calculable (non-zero) probability of being selected.

Random Sampling
A subset of the population in which every member of the population has an equal likelihood of being selected.) from various vendors. The distributions of samples were as follows: bottle water 10 brands, Sachet water 10 brands were picked from the shops around for ease of calculation and also the higher the sample size the better the representation of each brand in the actual population. It the assumption that the bottle water quality will be satisfactory relying on the fact that the quality control of those factories producing bottle water is very high in the large scale industries. Therefore all the water samples from the 10 brands will collected from different sellers in different outlets. These will be purchased directly from water vendors in the shops, Hotels, food serving areas (Bukas) and motor parks in the nine villages around the state polytechnic. The samples will be collected and stored in cool boxes and transported to the laboratory. The number collected on a day will immediately processed for physico-chemical and bacteriological analysis as described in Standard Methods (APHA, 1998). For bacteriological analysis, the bottles and sachets will be opened under aseptic conditions.

Methodology
The physical parameters included pH, temperature, total dissolved solids (TDS), oxidation reduction potential (ORP) and electrical conductivity (EC). Chemical parameters included cation and anion constituents: aluminum, calcium, chromiuim VI, iron, magnesium, zinc, chloride, cyanide, fluoride, nitrite, and nitrate, total alkalinity, total hardness and total and free chlorine. Hanna C-100 spectrophotometer (HI 83099 COD and Multiparameter Photometer made in UK) and chemical reagents supplied along with the kit were used for analyses. Total hardness was determined using EDTA titration method using Eriochrome Blak T indicator. Chloride was determined using the silver nitrate titration method using potassium chromate indicator. Total alkalinity was measured titrimetrically using mixed indicator. For bacteriological analysis (Total and faecal coliforms), multiple tube method was used. The culture media used were MacConkey Broth (MB) and Brilliant Green Bile Broth (BGBB).

After inoculation of the media with the samples, the GBBB culture tubes were incubated at 37°C for 2 hours before transferring them to 44°C incubator for 18 hours. The MB cultures were incubated at 37°C for 18 hours. After the incubation period, the cultures were inspected for changes in colour and gas production. Those showing growth with or without gas production were noted. Those showing no changes in colour were re-incubated for additional 24 hours. The tubes showing changes in colour are counted and the MPN count expressed per 100 ml of sample as per the Mac-Grady's Probability Table. The cultures that showed growths were also sub-cultured on Mac Conkey agar plates to obtain discreet colonies to facilitate easy isolation and identification of the predominant organisms. Quality control and Quality Assurance were ascertained appropriately. Standard Methods for water analysis as described by the American Public Health Association (Mara and Oragui 1985, APHA 1998) were employed. The coliform counts were expressed as cfu/ 10 ml.

Sampling of Water
A total of 30 packaged water samples comprising of 10 brands of borehole water sold in the town at the time of this study, 10 brands of bottle water and 10 brands of Sachet water were purchased directly from water vendors in the
markets, food serving areas (Eateries, Bukas), motor parks and retail outlets of some of the producers in the metropolis. However, the choice of 10 to 10 to 10 samples of borehole to sachet to bottle was for ease of calculation and also the higher the sample size the better the representation of each brand in the actual population. The samples were stored in cool boxes and transported to the laboratory without delay. The samples collected were processed within six hours of collection.

**PRESUMPTIVE COLIFORM TEST BY:**  
Multiple Tube Method  
In this method, both single strength and double strength sterile Mac Conkey Broth (MB) were used. Here, 50mls of the Double strength MB was placed in a tube, and 10mls each, was placed into 5 tubes containing inverted Durham tubes for the collection of gas produced. 1ml of the water sample were added into each tube, mixed thoroughly and incubated aerobically at 37°C for 18-24 hours after which Statistical tables was used to derive the concentration of organisms in the original sample.

**Determination Of Viable Bacterial Counts**  
The numbers of tubes with positive presumptive test were sub-cultured on fresh Plate Count Agar (PCA) and the colonies were counted for each dilution, using the formula stated as follows. Plates showing total counts of about 2 colonies were selected and the number of viable bacterial per ml of sample was determined by multiplying the number of colonies counted by the dilution factor and capacity of pipette as expressed mathematically below;

\[ \text{Number of colonies counted} \times \text{dilution factor} \times \text{Volume of pipette} = \text{a orgs/ml.}\]

**Identification of Isolates**  
The isolates from Plate Count Agar were sub-cultured on Mac ConkeyAgar and Nutrient Agar. Pure isolates of resulting growth were identified using morphological and biochemical methods as described by APHA, (1998). The sterility of each batch of test medium was confirmed by incubating one or two un-inoculated tubes or plates along with the inoculated tests. The un-inoculated plates or tubes were always examined to show no evidence of bacterial growth. After inoculation of the media with the samples, the MB cultures were incubated at 37°C for 18 hours. After the incubation period, the cultures were inspected for changes in colour and gas production. Those showing growth with or without gas production were noted.

Those showing no changes in colour were re-incubated for additional 24 hours. The tubes showing changes in colour were counted and the MPN count was expressed per 10 ml of sample as per the Mac-Grady’s Probability Table. The cultures that showed growth were also sub-cultured on MacConkey agar plates to obtain discreet colonies to facilitate easy isolation and identification of the predominant organisms. Quality control and Quality Assurance were ascertained appropriately. Standard Methods for water analysis as described by the American Public Health Association (Mara and Oragu 1985, APHA 1998) were employed. The coliform count is expressed as cfu/10 ml.

**Gram Staining**  
Gram stain was done on each bacterial isolate and examined microscopically using oil immersion objectives. The reaction test was carried out on all the different isolates.

**Motility Test By Hanging Drop Method**  
This was done to determine the presence of motile organisms. A ring of Plasticine of about 2cm in diameter was made on a grease free slide. A loopful of a 24 hour broth culture of gram negative bacilli was placed at the center of a clean cover slip measuring about 22 x 22mm in dimension. The slide was gently pressed on the cover slip such that the drop of the culture was positioned at the center of the Plasticine ring. The slide was inverted and the cover slip seen uppermost. The preparation was examined under x10 and x40 objectives. Motility was indicated by movement of the bacterial cells within the hanging drop.

**BIOCHEMICAL CHARACTERIZATION OF THE ISOLATES**  
Biochemical tests were carried out and all results obtained with reference to (Ibemesim, 2014) were noted.

**Coagulase Test**  
This test is used to determine the ability of an organism to coagulate plasma by the action of the enzyme coagulase thereby converting fibrinogen to fibrin. It is used to differentiate between Staphylococcus aureus and non-coagulase Staphylococci. 0.5ml of 1:10 diluted human plasma was placed in a clean khan tube. 0.1ml of overnight broth culture of the organisms was added. This was mixed, incubated at 370C for 30mins to 6hours and observed for clot formation.(observation was made at 1hr interval)

**Indole Test**  
This determines the ability of an organism to breakdown tryptophan by the activity of an enzyme tryptophanase to release indole as a by-product. In a test tube containing a 24-hour broth culture of the test organism, 0.5ml of kovac’s
reagent was added and shaken. This was observed for colour change at the top layer (ring). A rose pink-red colour ring was taken for positive result and no colour change for negative result.

**Oxidase Test**
This is carried out to determine the oxidase producing ability of some organisms. The tetramethyl paraphenylenediamine dihydrochloride solution is oxidized to a deep purple colour by oxidase enzyme produced by some organisms. A piece of filter paper was soaked in oxidase reagent, a smear of the suspected colony was made on the soaked filter paper. Purple colour indicated positive result within 10 seconds while negative showed no colour change.

**Citrate Utilization Test**
This is based on the ability of an organism to utilize citrate as its only source of carbon and ammonium as its only source of nitrogen. The citrate is metabolized to acetoin and carbon dioxide. A broth culture of the organism to be tested was inoculated into Simmon’s Citrate Agar slope with a straight wire loop and incubated at 37°C for 24 hours. A change in colour from green to deep blue is considered positive.

**Urease Test**
This test is used to determine the ability of an organism to produce the enzyme urease. The urease is able to decompose urea by hydrolysis to give ammonia and carbon dioxide. The ammonia produced makes the medium alkaline so that the colour of the indicator becomes pink. The test organism was stabbed into the medium using a sterile straight wire, and then a streak was made on the slope. This was incubated at 37°C overnight with the covers loosely capped. Colour change to red-pink for positive results while negative showed no colour change.

**DISCUSSION OF RESULTS**
This study showed that 1 out of 10 brands of sachet water were contaminated by different organisms such as Klebsiella species Streptococcus faecalis, Pseudomonas aeruginosa and Escherichia coli. This finding agreed with that of Ibemesim A.O (2009), Umeh et al (2005) in which these organisms were isolated in addition to other organisms. Although physical examination of the water samples analyzed did not show any particulate object or discoulouration of any type yet the presence of pathogenic bacteria in them calls for a serious concern. According to Umeh et al (2005), bacterial growth in water may be unnoticed even in transparent packaged water and the presence of some of these organisms may pose a potential health risk to consumers.

The coliform count range of 0.32 x 102 to 1.95 x 102 recorded in this work is above the value recommended by the WHO. This might be unconnected to the improper sterilization, poor handling of the products in the course of production, transportation and sales of the products. This supports the earlier views of Osibanjo (1999) and Umeh et al (2005) that the sachet water being produced is of questionable quality. The implication therefore was that all the 30 water samples investigated carried NAFDAC (Registration) approval numbers and the products are popularly and freely served at open parties and social functions. Also, the possible contamination of sachet water at the point of production has been confirmed by Chaidez et al (1999) and Dan Rutz (1996) in which they reported that pure water vending machine may not be so pure, after all, because investigations found bacteria like Escherichia coli in the machine. However, it was gratifying to note that all the bottle water analyzed in this study were free from bacterial contamination which possibly showed that the manufacturers adhered strictly to the guidelines set up by NAFDAC and SON.

**CONCLUSION**
The results obtained so far highlights the fact that communities in urban areas suffer from acute portable water shortages. To augment this situation, many entrepreneurs took to packaged water business – production and vending. There is a rush to get into business and as a result quality control has been compromised. Therefore, packaged water other than those in company sealed bottles could pose as a source of waterborne infection as this study has shown that the bottle water is obviously of better quality than the popular sachet water. Even though Nigeria has national guidelines and regulations, and the regulatory agencies, the monitoring of the packaged water quality is poor as shown in this study where a product that has NAFDAC certification still fail to meet standard for portable water. There is, therefore, a need to monitor all those involved in water business to comply with the guidelines to avert possible outbreak of water-borne diseases as a result of consumption of contaminated water.

**REFERENCES**


