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PHYSIO-CHEMICAL AND BIOLOGICAL ASSESSMENT OF STORED WATER SOLD WITHIN OKO, ANAMBRA STATE

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ABSTRACT
Access to portable drinking water is still a far cry in so many communities in Sub-sahara Africa. Storage of water ensures continuity in supply during interruption or draught. Bottled water samples sold in store rooms in Oko were assessed for 21days. Samples were analyzed using Standard laboratory methods and the results compared to WHO standards. pH increased (6.60-6.85) at room temperature. The conductivity was stable at room temperature but increased slightly (5.2-5.4msu/cm) on exposure to sunlight. Hardness, alkalinity and Nitrate content increased slightly in all the samples within the storage period under room temperature and decreased on exposure to sunlight for all the samples. Chloride decreased (36-32mg/l) at room temperature and increased (48-50mg/l) on exposure to sunlight. There was no significant change in lead and cadmium content within the period of storage both under room temperature and on exposure to sunlight. Total Coliform count increased for all the samples at room temperature during the period of storage (0.6 x 10cfu/ml - 4.3 x 10cfu/ml) and decreased on the last day. These values exceeded the WHO standard for coliform bacteria of 0cfu/ml The Physio-chemical properties of all the samples aside Lead and cadmium were within World health Organization (WHO) and Nigeria Drinking Water Regulation Agency (NDWRA) standard. Exposure of water to sunlight alters physio-chemical properties of water. Water is best stored under room temperature for not more than 7 days.

Keywords: Water, Storage, Standard, Physio-chemical, coliform, bacteria

INTRODUCTION
Water is an essential requirement of all life forms. Satisfactory supply of clean, safe, and hygienic drinking water is imperative for health (Khatoo and Pyrzada, 2010). Access to safe drinking water is vital for human existence. Unavailability of good quality drinking water is widespread and this has serious health implications (Khanki *et al.,* 2010). Water related diseases continue to be one of the major health problems globally (Onweluzo and Akugbazie, 2010). The high prevalence of diarrhea among children and infants can be traced to the use of unsafe water and unhygienic practices. In developing countries, 80% of all diseases and over 30% of deaths are related to drinking water (Onifade and Ilori, 2008). Any potable water that is intended for public consumption, bottled, distributed and offered for sale, is regarded as bottled water (Ehlers *et al*., 2004). Bottled water can be obtained from natural springs, wells, boreholes, municipal systems or other sources which are considered to be safe, of sanitary quality and fit for human consumption. This is regarded as natural bottled water. Mineralized bottled water is potable water with added salts (Cabral *et al*., 2002).

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Bottled water is drinking water which has been packaged in plastic bottles ranging in sizes from small single serving polythene terephthalate bottles of 500ml-1.5-litre capacity to large capacity (20 liters) for water coolers (Raynold, 2005). Water in bottles and sachets are readily available and affordable though in Nigeria bottled water is regarded as being safer than water dispensed and sold in sachet but there are concerns about their purity. The integrity of the hygienic environment and conditions where the packaged water (bottled and sachet water) are produced have also been questioned.

Various reasons have been reported for the higher trend of bottled water use in many countries. Some reasons include: Consumer awareness about increasing water pollution; Deficiencies in municipal water supplies in terms of aesthetic, chemical and microbiological water quality; Successful marketing strategies of bottled water by the bottling companies; The easy availability and reasonable pricing has popularized the utilisation of bottled drinking water by a number of people who can afford it; and Bottled water is generally considered safe and is taken for granted by people without question. For example 'spring water' is perceived as a pristine, natural source of water. Bottled mineral water has long been consumed as a safer alternative in countries with reticulated water of uncertain quality (Cabral *et al*., 2002). However, consumers should be aware that bottled water is not necessarily safer than tap water (Ehlers *et al*., 2004).

Safe drinking water is essential for human life. It is generally considered that bottled water is safe for usage by people. For long-distance travelers, it serves as the only source of reliable drinking water but several studies have reported that bottled water does not always meet the acceptability standards. Also, Demand for bottled water has resulted in the spring up of several small-scale entrepreneurs involved in its production and distribution. However, with increasing demand, serious concerns about its quality and safety have arisen subsequently. The chemical and microbiological qualities of packaged water of some manufacturers have been found to be in violation of National standards. Hence, the need to investigate the shelf life of some common bottled water sold in Oko, Anambra state.

LITERATURE REVIEW

WATER

Water is the most abundant substance in living system, making up 70% more of the weight of most organisms (Nelson and Cox, 2009). Its unique physical properties which include the ability to solvate a wide range of organic and inorganic molecules, derive from its dipolar structure and exceptional capacity for forming hydrogen bonds. An excellent nucleophile, water is a reactant for product in many metabolic reactions. It has a slight propensity to dissociate into hydroxide ions and protons (Murray *et al.,* 2009).

Properties of Water.

i. Water is a dipole, a molecule with electrical change distributed a symmetrically about its structure. The strongly electronegative oxygen atom pulls electrons away from the hydrogen nuclei; leaving them with a partial positive change, while its two unshared electron pairs, constitute a region of local negative change (Murrey *et a.l,* 2009).
ii. Water, whose two line pairs of sp3 electrons bear a partial negative charge, is an excellent nucleophile, Nucleophilies attack by water generally results in the cleavage of the amide, glycoside, or ester bonds that hold biopolymers together (Kelman *et al.,* 2004).

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iii. Water has higher melting, boiling point, and heatof vaporization than most other common solvents. These properties are a consequence of attractions between adjacent water molecular that give liquid water great internal cohesion.
iv. Water has a higher melting point, boiling point and heat of vaporization than most other common solvents. These properties are consequence of attractions between adjacent water molecules that give liquid water great internal cohesion
v. Water is a good solvent for polar (hydrophilic) solutes with which it forms hydrogen bonds and for charged solutes, with which it interacts electro-statically (Nelson and Cox 2009).
vi. Water is an amphipotic solvent that is, it behaves as an acid, the presence of basic solutes and as a base in the presence of acidic solutes (Skoog *et al.*,2004).
vii. Water is a highly polar molecule, capable of forming hydrogen bonds with itself and with solutes due to possession of very different electro negativity's of hydrogen and oxygen atoms.
viii. Water is both the solvent in which metallic reactions occur and a reactant in many biochemical processes, including hydrolysis, condensation and oxidation-reduction reactions (Nelson and Cox 2009).

Uses of Water

i. Water is used for bathing, washing; cooking, drinking, water is used in all areas of living things.
ii. The high specific heat of water is useful to cells and organisms because it allows water to act as a heat buffer, keeping the temperature of an organism relatively constants as the temperature of the surrounding fluctuates and as heat is generated as a by products of metabolism (Nelson and Cox, 2009).
iii. The high degree of internal cohesion of liquid water due to hydrogen bonding is exploited by plants as a means of transporting dissolved nutrients from the roots to the leaves during the process of transpiration (Nelson and Cox, 2009).
iv. The density of ice is lower than that of liquid water and as a result pond freeze from the top down and the layer of ice at the top insulates the water below from frigid, air, preventing the pond from freezing solid (Nelson and Cox, 2009).
v. Its strong dipole and high dielectric, constant enable water to dissolve large qualities of charged compounds such as salts (Nkanu East- Wikipedia).

Sources of Water

The type of a water resource is usually an indication of its water quality characteristics. The aquatic environment comprise of water in the soil or underground water, surface water and water in the atmosphere (Chukwu, 2009).

2.2.1 Underground Water

Underground water is an aqueous solution in the subsurface to over half the population of the country and provides an even greater percentage of the amount of water need for irrigation and industrial purpose. Water present below the land surface takes on some of the characteristics of that environment. Rainfall and snow melt percolating through the soil zone and unsaturated materials chemically react with the gases, minerals and organic compounds that occur naturally in the subsurface. These reactions continue below the water table as the water flows through the

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aquifer of the many solutes found underground water only a relatively few are present at concentrations greater than 1mg/l under typically natural conditions. These are generally called (Co^{2+}, Magnesium (Mg^{2+}) Sodium (Na^{2+}) and Potassium (K^{+}) and the ions bicarbonate carbonate $HCo3/CO_3^{2+}$), Sulphate ($SO4_2$), Chloride (Cl), and Nitrate (NO^{3-}). An important non-ionic constituent in typical underground water is silicon (Si) (Davis, 2003).

Borehole is a well drilled into subsurface aquifer is a porous and permeable rock hosting water or a water saturated geologic unit or formation that maybe exploiting underground water. It is solely drilled to provide water for drinking domestic and industrial uses.

Surface Water

The use of surface water by man is as old as the existence of human beings, since water, which is a natural resources is indispensable to life (Freeze and Chemy, 2000).

Surface water resources consist of the following:

i. Springs
ii. Lakes and reservoirs
iii. Oceans/seas
iv. Rivers/ Streams

2.2.3 Water in the Atmosphere

Atmosphere water sources include all form of precipitation such as snow sheet, rainfall, drizzle, dew, boar, frost, fog, drip, ice pallets and granular snow in rural environments. Where atmosphere pollution is typically collected from roof tops and stored in tanks or cisterns. But because the roof or any collection surface is subject to contamination from nesting and flying birds and air borne dust, no one should assume that this source of water is the most suitable for human consumption (Surface water).

2.2.4 Borehole Water

Physically, ground water is generally clear, colourless, with little or no suspected matter and has relatively constant temperature. It is also generally free from the very minute organism which causes diseases. This is another benefit that results from the slow filtering action provided as the water flow through the ground. Also, the lack of oxygen and nutrients in the ground water makes it an unfavourable environment for disease producing organism to grow and multiply. The chemical quality of ground water is also considerably influence by its relative slow rate of travel through the ground (Kennelly, 2009).

According to Larry, (2006), ground water is known to be purer than surface water, unfortunately, it becomes chemically contaminated by pesticides, herbicides and fertilizers which have been a boom to the world farmers but a bomb to the earth's water. Changes in borehole water may be due to ground water pollution. Water pollution is modification of the physical, chemical and biological properties of water restricting or preventing its application (Chukwu, 2009). The NAFDAC Laboratory, Oshodi during the year 2003 analyzed some borehole water samples for registration purpose and the majority of the sample analyzed showed high microbial load (NAFDAC, 2004).Studies carried out by Ababio, (2001) on selected water source including underground water in Calabar indicated that the physiochemical parameters, biological oxygen

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demand (BOD), Silica and pH were positively correlated with indicator bacteria with count reacting a maximum of 520 faecal coliforms per 100ml. the quality of water sample therefore did not conform to the approved WHO standard for drinking water. Total hydrocarbon, household sources and causes of the ground water pollution (Adekunle *et al* 2007).

Chandra *et al* (2012) asserted that source of pollution of ground water supplied is form highways street through spillages of chemicals and from deciding salt applied during the winter mouths.

Portable Water

Portable water purification devices better defined as point use (POU) water treatment system and field water disinfected techniques are self-contained usually hard carried units used by recreational enthusiast, military personnel survivalists and by people without access to water supply services in developing countries for water purification when they need to obtain drinking water from untreated source e.g rivers, lakes, ground water etc. These personnel device and method aim to render potable (i.e safe and palatable for drinking purpose without diseases causing pathogens).

Techniques includes heat, filtration, activated charcoal absorbing chemical disinfection eg chlorination, iodine, ozonation etc. ultraviolet purification including sodis, distillation and flocculation. Often these are used in combination of many commercial additives available for hiking, camping and other travel in remote areas potable water purification method is also commonly used of household level in developing countries when the source of drinking water is not suitable for drinking

Water Pollution

Water pollution is a big problem in the world. It threatens aquatic life and changes water bodies into unsightly foul smelling scenes. It also affects out health because of the harmful substances that accumulate in aquatic animals, one of our main source of food. The main cause of water pollution is the indiscriminate dumping of solid and liquid waste into water bodies (Ababio, 2001). The public health significance of water quality cannot be over emphasized, many infectious diseases are transmitted by water through the fecal oral route. Diseases contacted by water through drinking water killed about 5million children annually and make 1/6th of the world population sick (WHO, 2004).

A large number of water pollutant maybe broadly classified as

i. Organic pollutants
ii. Inorganic pollutants
iii. Environmental pollutants
iv. Sediments pollutants
v. Radioactive materials
vi. Thermal pollutants

Organic Pollutants

This group includes oxygen demanding wastes, disease carrying agents, plant nutrients, sewage, synthetic organic compounds and oil dissolved oxygen is an essential requirement of aquatic life.

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The optimum dissolved oxygen valve is an index of pollution mainly due to organic matter eg sewage (Domestic and animal). Industrial water food from food processing plants, paper mills and tanneries; water slaughter houses and meat packing centeres, run-off from agricultural lands etc. organic pollutants can further be classified thus;

1. **Refuse and Sewage:** It is a common practice to dump refuse and human waste into river for easy disposal. The waste or sewage is mostly organic matter. It is broken down into simple substance by decomposers, mainly bacteria. In the process, the bacteria use up the dissolved oxygen. Too much sewage in a water body causes an increase in the bacterial population. This reduces the level of oxygen in water. If the oxygen level falls too much, the water body becomes clogged up and foul smelling water polluted by sewage contains many disease causing organisms (Ababio, 2001)
2. **Industrial Water:** Many factories empty their chemical waste directly into rivers and seas without converting them into harmless substance first. These chemicals include fuels, plastics, plasticizers, fibers, elastomes, organic solvents, detergents, paints insecticides food additives and pharmaceuticals. Their presence in water impacts objectionable and offensive taste, colour and odour (Ababio, 2001).
3. **Agricultural Waste:** These include pesticides, insecticides, rodenticides, molluscides, herbicide and fungicides. The negative aspect of these is that their residue has moved through ecosystems and are threatening to destroy the food chain (Chatterjea and Shinda, 2007).
4. **Crude oil Spills:** Accidents and carelessness in oil rigs and takers cause crude oil spills mainly in the coastal waters. The oil floats on water and kills most of the marine life in the affected areas. The oil then becomes washed up on the beach temporarily people from using the water and the beach for recreation (Ababio, 2001).

Inorganic Pollution

These include the heavy metal advancement in technology has led to high level of industrialization leading diverse and complex existing in the metallic or compound forms (Acres, 2001) Heavy metals may also occur naturally as a result of normal geological phenomena such as formation of dunes, weathering of rocks and reaching.

Environmental pollution

Environmental pollution is the contamination of physical and biological components of the eaten/atmospheric system to such an extent that normal environmental process is adversely affected. Perhaps the overriding theme of these definitions is the ability of the environment to absorb and adopt to changes brought about by human activities. In one word, environmental pollution takes place when the environment cannot process and neutralize harmful by products of human activities (for example, poisonous gas emissions) in due course without any structural or functional damage to its systems. Infact “the due course” itself may last many years during which the nature will attempt to decompose the pollutants, in one of the worst cases that of radioactive pollutants. It may take as long as thousands of years for the decomposition of such pollutants to be completed. Pollution occur, on the one hand, because the natural environment does not know how to decompose the unnaturally generated element (i.e anthropogenic pollutants). And on the other, there is a lack of knowledge in the paint of humans on how to decompose these pollutants artificially pollution matter first and foremost because it has negative

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impacts on crucial environment service such as provision of clean water without which life on earth as we know it would not exist.

Sediments pollutants.

The natural process of soil erosion gives rise to sediments in water. It represents the most extensive pollutants of surface water. Bottom sediments are important sources of inorganic metals in streams (Chattarjea and Shinda, 2007).

Radioactive Material

For human activities responsible for radioactive pollution,

- ❖ Mining and processing of ores to produce usable radioactive substances
- ❖ Radioactive material in nuclear power plants
- ❖ Radioactive isotopes in medicine, industry and research.

Exposure of high level of radiation adversely affects human health. It is decidedly connected with the incidence of leukemia and other form of cancel (Chatter Jea, 2007).

Thermal pollutants.

Several industries like oil refineries, steel mills and breweries use water for cooling coal fired or nuclear fuel fired steam power plants-usually water from a nearby rivers or lake is pumped in and used for the cooling process. The resulting worm water is than emptied black into the river or lake. This causes on increase in temperature of water. As a result, less oxygen on dissolves in it leading to a decrease in dissolved oxygen of water and thus affects aquatic life (Ababio, 2001).

Underground water pollutions/ pollutant

An underground water pollutant is any substance that when it reached an aquifer, makes the water unclean or otherwise unsuitable for a particular purpose (groundwater pollution 2009). Various processes some of which maybe man made or anthropogenic, generate pollutants and contaminants that enter underground water flow systems. These processes include physiochemical weathering mass wasting, erosion, agricultural activities/mining oil exploration etc. some pollution sources in rural environments that are usually ignored even though they may be hazardous, include pit latrines, wide scale and indiscriminate uses of the bush for defecation etc. Various environmental problems can arise as a result for underground water pollution. A major consequence of underground water pollution includes the potential contamination of surface water. This can happen if the rivers stream, or lake in the area are recharged by a polluted aquifer-salmonellosis, bacillary, dysentery, schistomiasis, helminthiasis, and viral infections are known to have been transmitted through drinking underground waters polluted by surface waters and sewage (Egboka *et al.,*2002). The industrialized world has accumulated great amounts of pollutants and contaminates with their environments.

Indicator Organisms

Indicator organisms are microorganism whose presence in water indicates probably presence of pathogens (disease causing organisms). Ideally such micro-organism are non pathogenic, occur constantly in pathogen contaminated water, do not multiply in water are reliably detectable even at low concentration and are present in greater numbers than and have similar survival times to pathogens.y Traditionally, the feacal coliform group has been used as an indicator of bacterial

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pathogen presence and general waste water contamination (U.S Environmental protection Agency, 2004).

In 2006, the USAPA, updates its guidance to recommend that a particular feacal coilform E.coil, be monitored instead because of its stronger relation to the occurrence of swimming associated gastro intestinal illness (Greenberg, E.A, Clesceri, L.S and Eaton, A.D 2000). Coliphage are a class of viruses that infact *E.coli* they have higher resistance to environmental stresses and disinfection than bacteria viruses more closely than their bacteria counterpart (Mara and Horan 2003).

Microbiological Analysis

Microbiological testing in portable (bottled and public supply) waste, marine and desalinated waters. The service includes: microbiological testing including the presence/ absence and enumeration of various microorganism including.

i. Total coliforms: these bacteria are commonly found in the intestines of warm blood animals or the environment, for example, soil water and grain high coliform level in water can serve to indicate poor sanitation and the possibility of other pathogens proliferating since the condition for growth are similar.
ii. Faecal coliforms: bacteria of feacal origin whether animals or human is an indicator of faecal contamination in water. This test can be used to detect any failure in water treatment.
iii. Faecal streptococci: Along with faecal coilforms, faecal streptococci also serve as an indicator of feacal contamination of water.
iv. Enterococci: The presences of entrococci serve as an indicator of feacal contamination of water. This analysis is primarily used to monitor the effectiveness of waste water treatment plants and pollution of marine water.

Water Borne Disease

Drinking water is contaminated with faecal matter from typhoid or dysentery individual can lead to an outbreak of the disease. Transmission through contaminated water supply is by far, the most serious enteric disease such as typhoid fever and cholera. There are different types of water borne disease and their complication which includes locateriosis, cholera, giardiasis salmonellosis, crystromori, Daasi and cydlosporiasis etc. Most of the mortality and morbidity associated with water related diseases are due to directly or indirectly to infectious agents. Untreated water sources such as surface water (stream, rivers, lakes etc or unprotected open wells which are for water born bacterial infections such as cholera, typhoid fever, disinfection of water reduces the incident of this diseases such as hepatitis, gastroenteritis as well as an unknown number of ill defined disease caused by the other enteric viruses (Adenovinises, echoviruses) (Bartrain *et al.,*2003). Although most intestinal microbes are non pathogenic, some cause enteric disease.

However, typhoid fever can be transmitted by contaminated food and by direct contact with an infected person, but the most common way of transmission is through materials from actual cases or from carries of the food contaminated with extra from patients and convalescent carries. It is a disease of antiquity and has been the cause of untold suffering and death. The mild causes it describes gastroenteritis caused paratyphoid organism but severe causes are more typical.

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Enteric viruses causing various clinical aliments, not necessarily diarrhea, but are transmitted by water include poliovirus, rotavirus, hepatitis avirus (Abbaspaur, 2000) and Hepatitis B virus (Shelton and Scibia, 2012).

Protozoa water borne disease dysentery caused by Entamoba histolytica inhibits the intestines and forms a resistant cyst. It the cyst is excreted with faeces, they contaminate water cause disease as Entamoba histolytica giardiasis by diarrhea and general discomfort. (Okonkwo *et al.,* 2008, Kennedy, 2009).

Health Effect of Contaminated Borehole Water.

In many developing countries, microbial contamination of borehole water is causing various diseases. Children, women, immune compromised individuals and rural resident are considered to be at the highest risk of contracting water borne pathogenic microorganism. People can become infected by water borne pathogenic agents, if they either consume contaminated water directly or indirectly through its use in food production, processing, or preparation. Streams are specifically prone to bacterial contamination and a broad ranged pathogens causing human disease are water borne. However, people in rural parts of developing countries, in particular sub sahara African, may not have access to improved water source and are forced to use water from contaminated sources.

Drinking water quality

Industrial and agricultural chemicals have heavy impact on water quality worldwide. Eutrophication of surface waters and nitrification of ground water from agricultural practice have greatly affected parts of the world. Water takes on various characteristics and properties vary and are dependent on the material encountered, which maybe classified as physical, chemicals or biological according to its nature and means of detecting water of good chemical and physical quality is necessary from the points of views of its acceptability by the people, the protection of the health of the consumer and the conservation of water system (WHO 2003).

MATERIALS AND METHOD

Sample Collection

Five brands of bottle water were bought from open shops in Oko (Lakers, Ivy, Pasachute, Equate and Rocktama) and taken to the laboratory within 24 hours for analysis. The bottled water were kept on the bench and examined for every 7days for 21days (Day0 Day7 Day14 and Day21). All samples will be tested prior to the expiration specified by the manufacturers.

Microbiological analysis

Microbiological tests will be carried out in microbiology Laboratory Federal Polytechnic Oko. The procedures were carried out on the first day of collection then repeated on Day 7, Day14 and Day 10

Enumeration and isolation of total and faecal *coliform*

Total *coliforms* were estimated using the most probable number (MPN) method. MacConkey's lactose bile salt broth with bromocresol purple as indicator was used for the presumptive tests. With a sterile pipette, 50ml of each of the water sample was aseptically dispensed into 50ml double strength broth, another 10ml of the sample into each of the five tubes containing 10ml double strength broth and another one ml of the sample was then inoculated into each of the

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second five culture tubes containing 5ml single strength MacConkey broth with Durham's tubes. Inoculated tubes of MacConkey broth were incubated at 37°C for 24 to 48 hours. After 24-48 hours of incubation, the cultures were observed for the presence of acid production and gas formation. Reference to Mc Crady's table showed the most probable number (MPN) of presumptive *coliform* counts in 100ml of the sample water analyzed. A sterile pipette was used to transfer 1ml of the culture from the positive presumptive fermentation tubes into tubes containing 5ml brilliant green lactose bile broth aseptically and incubated for 24-48 hours at 37°C. Following incubation, culture positive tubes were inoculated into MacConkey agar for total *Coliform* and Eosin Methylene Blue agar for faecal *coliform* and incubated at 37°C and 44°C respectively.

Determination of heterotrophic plate count/ total viable count

Heterotrophic plate count of all water samples were determined using dilution plate method technique and standard plate count agar medium. Serial dilutions were prepared (using peptone water) and 1 ml of the sample or dilution was transferred to a sterile, empty petri dish. Plate count agar was melted by heating in boiling water and then allowed to cool in a water bath to 44 - 46° C. Approximately 15 ml of the agar medium was poured into the petridish containing the sample. The sample and agar were mixed thoroughly by rotating the plate several times. When the media has solidified, the plates were inverted and incubated at 35 °C for 48 to 72 hours. Following the appropriate length of incubation, suitable plates from different dilutions were selected and the visible colonies were counted using a colony counter. Then the average colonies were counted and expressed as colony forming unit per ml of water.
Isolates were further identified using appropriate biochemical test.

3.4 Identification of isolates

Representative isolates from total coliforms and total viable counts were identified. Standard isolation techniques were employed. MacConkey agar was used to isolate lactose fermenting gram negative bacilli and Mannitol salt agar for the isolation of salt resistant bacteria. Pure isolated colonies were Gram differentiated and then biochemically identified using Indole, Catalase, Citrate, Oxidase, Coagulase and Urease tests.

RESULTS

Three of the bottled water samples had PH which lies within the acceptable PH standard for drinking water which is 6.5 to 8.0 recommended by WHO as a guideline value (WHO, 2008) as seen on Table 1 on Day 1 and sample I11 attained the range after 14 days as a result of reduction reaction.

Table 1: PH of Bottled Water Samples

Samples	Day0	Day7	Day14	Day 21
I	6.60	6.60	6.80	7.20
II	7. 40	7.40	7.80	7.60
III	6. 20	6.20	6.50	6.80
IV	6.50	6.50	6.50	6.80
V	5.80	5.80	6.30	6.50

The conductivity values were within range for safe drinking water.

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Table 2: Conductivity of Water Samples(msu/cm)

Sample	Day 0	Day 7	Day 14	Day 21
I	5.50	5.50	5.50	5.50
II	5.80	5.80	5.80	5.80
III	5.20	5.20	5.20	5.20
IV	5.80	5.80	5.80	5.70
V	6.40	6.40	6.40	6.40

All water samples for the period of storage were moderately hard.

Table 3: Total Hardness of Water Samples (mg/l)

Sample	Day 0	Day 7	Day 14	Day 21
I	116	122	122	118
II	130	134	134	127
III	124	139	139	120
IV	102	102	102	108
V	127	127	127	118

The alkalinity of the water samples increased during the period of storage.

Table 4: Alkalinity of Water Samples (mg/l)

Sample	Day 0	Day 7	Day 14	Day 21
I	16	16	20	18
II	24	24	28	26
III	32	32	38	34
IV	12	12	12	12
V	18	18	18	18

Nitrate content of the water increased during the period of storage. Nitrate content level was safe for consumption.

Table 5: Nitrate of Water Samples (mg/l)

Sample	Day 0	Day 7	Day 14	Day 21
I	4.315	4.732	4.732	4.326
II	6.473	7.647	7.647	6.264
III	3.746	3.972	3.972	3.872
IV	3.542	3.754	3.852	3.852
V	6.234	6.542	6.638	6.638

The chloride decreased under storage as shown on Table 6.

Table 6: Chloride of Water Samples (mg/l)

Sample	Day 0	Day 7	Day 14	Day 21
I	36	36	32	32
II	46	46	44	44
III	42	42	40	40
IV	46	43	42	42
V	55	52	50	50

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There was no significant change in the lead and cadmium content of the water for the period of storage.

Table 7: Lead Content of Water Samples (ppm)

Sample	Day 0	Day 7	Day 14	Day 21
I	0.034	0.034	0.034	0.034
II	0.043	0.043	0.043	0.043
III	0.068	0.068	0.068	0.068
IV	0.034	0.034	0.034	0.034
V	0.043	0.043	0.043	0.043

Table 8: Cadmium Content of Water Samples (ppm)

Sample	Day 0	Day 7	Day 14	Day 21
I	0.014	0.014	0.014	0.014
II	0.117	0.117	0.117	0.117
III	0.216	0.216	0.216	0.216
IV	0.014	0.014	0.014	0.014
V	0.117	0.117	0.117	0.117

The five (5) bottled water samples examined in this study were all positive for the presumptive, confirmatory and complete test. The contaminants were found to be *Staphylococcus aureus* and *Escherichia coli* from the biochemical test.

The lowest Total bacteria count was for sample IV with 2.5 x 10Cfu/ml on Day 1 as shown on Table 9. The Total bacteria count for all the water samples increased after day 10 on the shelve. The lowest coliform count was 0.6 x 10Cfu/ml for sample IV and highest was 4.3 x 10Cfu/ml for Sample II as shown on Table 10. The total coliform count increased steadily on Day 5 and day 10 as shown on Table 10. Sample IV had the least Total Bacteria count and Total Coliform count after 10 days on the shelve so can be said to be the best bottled water from this study.

Table 9: Total Bacteria Count (cfu/ml)

Sample	Day0	Day7	Day14	Day 21
I	4.6 x 10	8.4 x 10	4 .9 x 10	2.5 x 10
II	3.3 x 10	3.5 x 10	7.8 x 10	4.7 x 10
III	3.5 x 10	4.7 x 10	8.2 x 10	4.6 x 10
IV	1.4 x 10	2.2 x 10	2.3 x 10^2	1.6 x 10
V	4.4 x 10	6.8 x 10	7.8 x 10	5.2 x 10

Table 10: Total Coliform Count (cfu/ml)

Sample	Day 0	Day 7	Day 14	Day 21
I	2.2 x 10	2.4 x 10	3.6 x 10	2.3 x 10
II	2.6 x 10	4.3 x 10	2.5 x 10	1.4 x 10
III	1.4 x 10	3.4 x 10	2.8 x 10	1.5 x 10
IV	0.6 x 10	1.3 x 10	1.6 x 10	1.2 x 10
V	3.3 x 10	3.7 x 10	3.9 x 10	2.2 x 10

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Table 11: Total Staphylococcus Count (cfu/ml)

Sample	Day 0	Day 7	Day 14	Day 21
I	5.2 x 10	4.5 x 10	3.4 x 10	4.6 x 10
II	6.8 x 10	5.2 x 10	8.4×10^2	6.3×10^2
III	7.4 x 10	3.6 x 10	5.6×10^2	3.5 x 10
IV	4.6 x 10	4.4 x 10	3.8×10^2	4.6×10^2
V	4.7 x 10	2.5×10^2	7.2×10^2	8.4 x 10

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Discussion

The physical and bacteriological state of packaged water is a very important aspect that should be observed by all the packaging companies. The result from this study showed that all the bottled water samples were contaminated with *Staphylococcus aureus* and some *Escherichia coli*. *Staphylococcus aureus* is seen in water due to poor hygiene or poor handling because *Staphylococcus aureus* is a normal flora on human palms or skin while *E. coli* is recovered from the water as a result of faecal contamination or the water is faecally populated but no contamination with *Salmonella spp*. according to the national industrial standard and WHO standard (NIS, 2007, WHO, 2008). Most of the bottled water samples were within the acceptable PH range for drinking water.

The total bacterial count result of the bottled water were high, exceeding the acceptable range of 1.0×10^2 CFU/ml recommended by E.P.A and W.H.O which is the standard limit of total bacteria count for drinking water (E.P.A, 2002; WHO, 2008). Bottled water sample IV had the least TBC and TCC after ten days on the shelve, hence can be said to have the best microbiological quality.

According to the Nigeria industrial standard, the standard microbiological limit for drinking water for total *coliform* and faecal coliform is 100cfu/ml and 0cfu/ml respectively (NIS, 2007) but *E. coli* which is as a result of faecal comtamination was found to be present in some bottled water samples. The presence of coliform is an indication of poor treatment methods or contact with ground water surfaces (Isikwue and Chikezie, 2014).

Three of the bottled samples showed pH values within the range (6.5 – 8.5) recommended by WHO and NAFDAC on the first day, Pasachute bottled water attained the Ph limit after two weeks of storage and Rocktama after three weeks of storage. Intake of high level oof water with low Ph can lead to acidosis. The chemical characteristics of all the brands of water evaluated were within the range of standard values recommended for chloride, total hardness, phosphate, nitrate, sulphate, iron, potassium, sodium and calcium by WHO and NAFDAC this agrees with the report of Ojekunle and Adeleke (2017) for sachet and bottle water sold in Ibadan. The implication of this result is that the processors of these brands of water obtain raw water from chemically good sources and adopt standard operating procedures for chemical water treatment. Oyeku et al, (2001) and Nwosu and Ogueke (2004) also reported good chemical characteristics of sachet water sold in Lagos and Owerri metropolis respectively. However, the level of total hardness in all the brands of water evaluated was moderately hard. This can be explained by Oko been in a rainforest region with water bodies bodering the community. Storage after 21 days did not affect the water hardness significantly. There was slight increase in the water alkalinity which could be as a result of microbial activity within the sample during the period. Sodim was within the recommended limit (< 20 mg/L) high level of sodium can lead to a high Na/K and

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Na/total cation ratio which may be of great concern from the perspective of human pathology (NRC, 1989; APHA, 1998). According to NRC (1989), a Na/K ratio of 1 or less than 1 is preferably recommended. According to Kleiner (1999) drinking water can contribute up to 10% of our sodium intake, it may be pertinent to advocate that levels of sodium in drinking water be declared on labels so as to alert consumers on sodium restricted diets.

Donato et al (1990) reported that soft water that is water containing small amount of magnesium and Calcium is associated with increased morbidity and mortality from Cardiovascular diseases. Studies also suggest that increase intake of soft water is also linked to bone Fracture in children Nitrate and Chloride were within the recommended limit but increased slightly during the period of storage this can be associated with the production of secondary metabolites from microbial activity. Cadmiun and Lead content exceeded the recommended level for all the bottle water assayed this can be related to the soil type and erosion. Oko is part of the geographical region in the south east heavily threatened by erosion and is about losing almost all of its land mass to it.

Conclusion

Based on the result *Staphylococcus aureus* were detected in the bottled water samples as a result of poor handling and faecal contamination, therefore it is advised that the bottled water handlers should observe proper hygiene when handling bottled water; especially washing of their hands before working on bottled water and also proper treatment of the water before being bottled.

5.3 Recommendation

It is recommended that there is need for continuous water quality monitory and steady sanitation in water production plants. There is also need for NAFDAC to intensify in the routine monitorimg of activities in the packaged drinking water industries. The safety of bottled water should be ensured through comprehensive regulatory programs at both federal and state level. NAFDAC regulations for bottled water should be protective of public health and there should be continuous adoption of packaged water quality standards. The used appropriate treatment processes should therefore be utilized for production of quality and safe bottled drinking water. It is also recommended that there is great need for further research studies on the quality of bottled water.

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