Abstract: The study investigated the characteristics of packaged water stored under ambient and sunlight conditions. This is with a view to testing the effect of prolonged storage under different storage conditions on its quality. In addition it analyzed the interactions between the parameters. Two packs each of bottled and sachet water was purchased from each factory at the point of production and ready for distribution to wholesalers. Twenty eight pieces of packaged water from each factory were kept at room temperature and the same quantity were kept under sunlight. Physico-chemical and microbiological analyses were carried out on the remaining packaged water within 24hrs. Half of the samples stored at the two storage conditions were removed for analysis at 3 weeks while the remaining half was analyzed after 6 weeks. The physico-chemical characteristics were within the WHO recommended values except for the pH of some samples that have values in the acidic range of 6.2-6.48. All the physico-chemical values increased for samples kept under sunlight. All the water samples showed growth in faecal coliform (4-46 cfu/100 mL) and e.coli (0-13 cfu/100 mL) for samples kept under sunlight at three weeks and this growth increased to the sixth week. The presence of e.coli is an indication that the packaged water is not pure. Displaying packaged water under the sunlight and storing beyond 3 weeks by vendors have effect on the potability of the product. The regulatory bodies should raise awareness and ensure manufacturer have a quality control unit to test on a routine basis.

Keywords: Sachet water, Bottled water, Physico-chemical analysis, Microbiological analysis.

I. Introduction

A reliable and sustainable supply of potable water is highly essential for promoting healthy living and good health [1]. Access to potable drinking water is increasing, but the quality of drinking water has deteriorated due to the presence of toxic elements which pose serious health issues [2]. Majority of people living in rural and urban areas in Nigeria do not have access to potable water, probably due to unavailability or inadequacy where obtainable, thus, bottled and sachet water became a major source of drinking water [3]. Bottled and sachet water are the commercially treated, packaged and distributed water for sale in sealed food-grade containers which are intended for human consumption [4]. Bottled and sachet water has no added ingredients except that it may optimally contain safe and suitable antimicrobial agents [5]. The difference between sachet and bottled water is the material used to package them and it is also believed that bottled water is purer than sachet water [4]. Besides, the ease of availability, convenience to carry, better taste, reasonable cost and freedom from impurities, has made bottled water the choice of drinking water in developing countries [6]. Safety is one of the many reasons for bottled and sachet water consumption. Unfortunately, the problems of its purity and health concerns have begun to manifest in Nigeria [7]. The integrity of the hygienic environment and the conditions where the majority of the water are produced has also been questioned. One of the potential dangers associated with packaged water is contamination and this is a factor of the sources of water itself, treatment, packaging materials, and process of dispensing
into the bottles or sachet and the closure [3].

Prolonged storage of packaged water at favourable environmental condition encourages the growth of total aerobic heterotrophic bacteria to levels that may be harmful to humans [8].

The bacterial contamination of packaged drinking water could be influenced by factors such as their raw water sources, treatment processes which were employed and hygiene practices which were observed in their production. Most sachet water manufacturers utilize well water and contaminated boreholes. Because of the acceptability, consumption and the demand for this packaged water, the business of production and packaging has become a lucrative one without strict adherence to adequate hygiene and good quality. Some unrecorded disease outbreaks associated with bottled water have been recorded [9]. In Lagos, 22% non-compliance with regulatory standards was reported [9] and in Ibadan, bottled water has been reported to comply with standards but 30% of sachet water did not comply [10]. It has also been reported that there is bacterial growth in all the sachet water in Ibadan [11]. The quality of packaged water should not be taken for granted as both total coliform count and faecal coliform count were above the acceptable limits in some water samples [12]. Reports also showed that 60% of the water samples analysed met the WHO limit for drinking when stored at ambient temperature within four weeks period but storage beyond this period led to a diminished aesthetic quality of sachet water and increased proliferation of bacteria to a level deleterious to human health [12]. Findings have shown that there is the presence of *e.coli*, *enterobacteragenes*, *staphylococcus aureus* and *streptococcus faecalis* in packaged water and that some of the water was unsafe for human consumption [13].

Packaged water is generally not sterile; most manufacturers use water from any available water source, ranging from rainwater to tanker services, most of which are rusty and unwashed [8]. In addition, contaminants can be introduced during manufacturing and consumer handling. During processing, most of these packaged waters are done in the absence of sterilization procedures such as pasteurization and thermal sterilization and this increase their susceptibility to contamination by both autochthonous bacterial flora, exogenous contaminating microbes, as well as a variety of other contaminants including mineral salts, organic pollutants, heavy metals and radioactive residues [14]. The objective of the study is to assess the effect of prolonged storage of packaged water in room temperature and under sunlight on the quality.

II. Methodology

A. Study Area

The study locations are in Osogbo metropolis; the capital of Osun State (Figure 1). The city is on latitude 7°46' and longitude 4°33'. It has two local government Areas (LGAs); Osogbo LGA with headquarter in Oja-oba and Olorunda LGA with headquarter in Igbonna. The population in 2013 was 499,999 [15].

B. Sampling Procedure

Three major bottled water factories were randomly and purposively selected from Osogbo metropolis. The factories were labelled A, B and C and the sachet and bottled water obtained from them have been labelled AS, BS, CS (Sachet water from factories A, B and C) and AB, BB, CB (Bottled water from factories A, B and C) accordingly.
The selection ensured that the factories are well patronized by residents of the town. Two cartons of bottled water and 2 bags of sachet water (each pack contains 12 bottled water and 20 pieces of sachet water) were purchased from each of the factories at the point of production and ready for distribution to wholesalers. They were labelled accordingly and subsequently transported to water and environmental laboratory of Rural Water Environmental and Sanitation Agency (RUWESA) and microbiological laboratory of Osun State University, Osogbo. Twenty-eight pieces of packaged water from each factory were kept at room temperature and another twenty-eight pieces were kept under sunlight. Storing under the conditions followed the same procedure used by water vendors; the bottled and sachet water were kept in their high-density polyethylene (HDPE) nylon packs and stored in the room. The ones stored under sunlight followed the same procedure of displaying the packaged water for sale. This is to represent the different conditions which these packaged waters are subjected to by water vendors. Physicochemical and microbiological analyses were carried out on the remaining packaged water within 24hrs of purchase. Half of the samples stored at the two storage conditions were removed for analysis at 3 weeks while the remaining half was analyzed after 6 weeks. Physicochemical characteristics analyzed and microbiological analysed were pH, conductivity, temperature, total dissolved solids, (TDS), faecal coliform, e.coli and parameters that are indicators of pollution (chloride, sulphate, nitrate, phosphate).

C. Analysis of Water Samples

Samples were analyzed for sulphate using the turbidimetric method. This method is based on the formation of barium sulphate in colloidal form by sulphate in the presence of (acidified hydrochloric acid) barium chloride. The process is enhanced in the presence of glycerol. The absorbance of the colloidal solution was measured using a colourimeter. Nitrate was determined using brucine method. The method is based on the principle that the reaction between brucine and nitrate produces a yellow colour suitable for colourimetric determination of nitrate. The colour intensity is measured at 420 nm using a colourimeter. Phosphate was analysed using ammonium molybdate and ascorbic acid. The method is based on the principle that orthophosphate reacts with ammonium molybdate to form an acid which can be reduced by ascorbic acid to form a colour change. Absorbance was read at 600 nm using ultraviolet (UV) spectrophotometer. Chloride was determined
using mercury (II) nitrate method. The method is based on the principle that mercury ions combine with chloride ions to form poorly ionized HgCl₂ without precipitation and therefore making the endpoint easier to detect. Conductivity, pH and TDS were measured using HANNA handheld meters. Microbiological analysis was done using the membrane filtration method. The principle is that the nutrient media promotes the growth of coliform while inhibiting the growth of other bacteria. The study used Mac-Conkey agar as the media. All equipment used was sterilized in an autoclave at 121°C. The Petri dish holding the filter and nutrient media were placed in the incubator at 35±0.5°C for 24 hours and the number of colonies found on each filter was determined.

D. Analysis of Result

The results obtained from both sachet and bottled water were subjected to univariate analysis (descriptive) of Excel sheet to show the differences in the temporal variation and storage. The linear correlation of the (Palentological Statistics) PAST 3.0 software was used to get the relationship that existed between the parameters analysed. The correlation is reported using the correlation coefficient (r) to measure the strength of association of the parameters. The study has three categories of r values; close to 1 (0.90-1.00) is very strong, (0.80-0.89) is fair and 0.70-0.79 is weak.

III. Results and Discussion

Figure 2 shows that sample BB has the lowest pH (6.2) when the sample is tested within 24 hours while samples AS and CS has the highest pH (9.15) at 6 weeks under sunlight. The values of the pH fluctuated in all the packaged water for the different storage conditions but the variation is more evident in samples kept under sunlight than those kept under room temperature (Figure 2). None of the samples maintains a neutral pH (7.0) of treated water but the mean is within the WHO guidelines of 6.5-8.5 except for sample CS, samples BB and CB. The source of water used by the 3 factories is groundwater in form of boreholes, thus, the low pH in some of the packaged water could be associated with this source. In addition, acidity in groundwater is location-dependent; most of the groundwater in the basement complex of Nigeria is acidic [16; 17 and 18]. In order to get the interaction/relationship of the pH with other parameters and to note if the storage condition is affecting the result of the analysis, the Pearson Correlation revealed that, sample AS has a very strong correlation (r = 0.98) with EC and fair correlation (r = 0.85, r = 0.89 and r = 0.78) with TDS, PO₄ and temperature, respectively. The pH of sample BS did not have any correlation with any of the analyzed parameters. The correlation of the pH of samples BB and CB with temperature is fair (r = 0.88 and r = 0.83) which shows that the effect of temperature on the pH is significant. The study expects the pH to decrease with time and temperature for samples subjected to ambient temperature and sunlight but the pH fluctuated (Figure 2). The USEPA set 15 minutes as the window to perform a pH analysis of a liquid because the pH of a liquid can change over time and it can fluctuate due to photosynthesis and respiration in the water and the degrees of change depend on the alkalinity of the water. The prolonged storage of the packaged water might have influenced the pH. However, the result of the study agrees with the report of [14] who also reported cases of acidic bottled and sachet water in Ibadan metropolis. The lowest temperature (22.1°C) is obtained in sample AS at 6 weeks when kept in room temperature while the highest temperature (29.3°C) is obtained in the bottled water at 6 weeks when exposed to sunlight (Figure 3).
The temperature of all the water samples fluctuated for all the periods of analysis but is higher in the samples kept under sunlight (Figure 3). This is expected, but the correlation reveals that the temperature only influenced some values of parameters analyzed.

The temperature has a strong correlation ($r = 0.98$, $r = 0.91$ and $r = 0.91$) with nitrate, chloride and total coliform in samples AS and CS respectively. In bottled water, temperature has strong correlation ($r = 0.98$) with sulphate and weak correlation ($r = 0.75$, $r = 0.71$) with phosphate and *faecal coliform* in sample BB. The correlation of temperature with *e.coli* in sample CB is high ($r = 1.0$) and this shows that the temperature is influencing the result of the *e.coli*. The value of temperature in drinking water recommended by WHO is 25°C, particularly upon transits [19]. High temperatures (27±0.3°C) enhance the growth of microorganisms and may increase problems related to taste, odour, colour and corrosion [20]. In low temperature, the viscosity of water increases, this, in turn, diminishes the efficiency of the settling of the solids that the water quality may contain because of the resistance that the high viscosity offers to the downward motion of the particles as they settle [21]. Higher temperature (27±0.3°C) may also activate the dissolution of metals and cause rapid deterioration of other physicochemical parameters [22]. Figure 4 shows that samples CS and CB have the lowest chloride values (7 mg/L) and (6 mg/L) respectively while samples AS, BB and AB have the highest values of 26 mg/L and 35 mg/L. The concentration at 24 hours is different from when the packaged water is stored under sunlight and room temperature; chloride values increase when the samples were placed inside sunlight (Figure 4). The interaction/ correlation reveals that samples BS and CS have strong correlation ($r = 0.95$, $r = 0.92$) with pH. The correlation is similar to that in the bottled water; thus, it can be concluded that pH is one of the factors controlling the chemistry of the water samples. However, the chloride values are far lesser than the WHO recommended values of 250 mg/L. Excess chloride in water impacts bad tastes and may indicate contamination from urine and sewage [23]. Small quantities of chloride have little effect on the use of water but with sodium ions, the taste is detectable at about 250 mg/L and with calcium or magnesium the taste may be undetectable at 1,000 mg/L [24]. Figure 5 shows that samples CS and CB have the lowest nitrate values (0.07 and 0.08 mg/L) and
samples AS have the highest value (1.60 mg/L). The values of nitrate are below the WHO recommended, but these values increased in the samples put under sunlight (Figure 5).

The result of the study agrees with [25] on sachet and bottled water in Nigeria. Nitrate correlated with pH in almost all the water samples. The sources of high concentrations of nitrate in water are mostly anthropogenic, particularly indiscriminate waste disposal and agricultural practices [26]. However, the possibility that the sources of the packaged water are in the transition zone between natural and human influences cannot be ruled out. When nitrate is high in drinking water, it may be associated with increased risk of bladder and ovarian cancer, genotoxic at the chromosomal level and insulin-dependent diabetes [27]. It has also been suggested that chronic exposure to high levels of nitrate in drinking water may have adverse effects on the cardiovascular system. Increased concentrations of nitrate often cause blood disorders [28].

The result of sulphate (Figure 6) shows that samples CS and CB have the lowest values (0.05 and 0.03 mg/L), while samples AS and AB have the highest values (0.05 and 0.05 mg/L) respectively. The pattern of sulphate of all the packaged water stored under different conditions did not follow any definite trend (Figures 6) but the fluctuations are evident for the packaged water kept for 6 weeks. However, it is lower than the 200 mg/L WHO recommended values. The correlation of sulphate with pH is similar to that of nitrate except in samples AS and CS. When water is having micro-organisms, they can reduce $\text{SO}_4^{2-}$ to $\text{S}^-$. leading to depletion of sulphate [29]. A high concentration of sulphate in the drinking water can cause laxative effects in humans.

Figure 7 shows that the EC values are lowest in the samples CS and CB (0.26 mg/L) and highest in the samples AS and AB (0.24 mg/L). The EC values for all the packaged water samples are not definite, but it increased in samples under sunlight and the highest value is obtained for samples kept for 3 weeks (Figure 7). These values did not agree with the values of [30],[25] and, [14] but the interactions with other parameters show that the EC correlated ($r = 0.96$, $r = 0.85$, $r = 0.87$) with pH, chloride and nitrate, respectively in the samples BS and BB. Increase in the
concentration of dissolved solids, increases the ionic strength of the water but conductivity increases with temperature; this is significant in this study as reflected in samples BS, AB and BB.

The effects of high EC may include disturbances of salt and water balance and high salt concentrations in water. Some of the adverse medical effects of high salt concentrations include heart problem, high blood pressure and renal disease.

Figure 8 shows that the values of TDS decreased on storage in samples AS but the pattern is not regular in both sachet and bottled water (Figure 8). The values are lower than studies of [12]; [30] and [25]. It has been reported that the major elements contributing to dissolved constituents in groundwater include sodium, potassium, calcium, magnesium, silica, the anions, bicarbonate, sulphate, chloride, and nitrate. These constituents come from the dissolution of rocks such as limestone and dolomite, the weathering of less-soluble rocks and man-made contaminants. The attention of the regulatory bodies has been drawn to the demineralised nature (removal or reduction of the constituents) of most packaged water, and prolonged consumption of demineralized water can result in micronutrient deficiencies [30]. When the body lacks the adequate intakes of calcium, it can lead to nephrolithiasis (kidney stones), osteoporosis, hypertension and stroke, colorectal cancer, insulin resistance, coronary artery disease, and obesity. Medically, some of these disorders have treatments but no cures. Inadequate intake of magnesium can cause decreased insulin sensitivity endothelial dysfunction, increased vascular reactions and elevated circulating levels of C-reactive protein. When magnesium is low in the body, there is a risk of hypertension, coronary heart disease, type 2 diabetes mellitus and the metabolic syndrome.

The result of phosphate in all the water samples after 24 hours of production is higher than the 0.03 mg/L (Figure 9) recommended by [20] and it remains the same in sachet water after three weeks. The result fluctuated when kept at different conditions and it became zero in Sample CB after 24 hours. The correlation between sachet and bottled water is different. The reduction in phosphate was not investigated but the temperature has been reported to be one of the parameters that affect the reaction kinetics and
performance of biological nutrient removal in wastewater but there are conflicting reports [31].

All the packaged water contains total coliform for samples analysed within 24 hours (Figure 10). This is an indication that the treatment methods employed may be defective. Although, the results of the total coliform and e.coli are more in the sachet than in the bottled water, the values increase with storage and under the sunlight (Figures 10 and 11). There is the possibility that the treatment may be adequate, and the contamination is from the capping of bottles or sealing of the HDPE for sachet water. Samples CS and CB are not affected by storage until after 3 weeks when the total coliform increased but there are no changes in the e.coli results. The result agreed with most of the past literature [32; 14; 33 and 3] but disagree with [30]. The correlation shows the association of faecal coliform and e.coli with pH and temperature. It has been reported that total coliform counts in water samples are affected by temperature and packaging materials used [14]; polythene sachet is more permeable to air than glass and plastic bottles. The permeability to gases such as water vapour, oxygen and carbon (IV) oxide has been reported to affect the growth and survival of microorganisms. Some studies also reported that bottled water may become contaminated before bottling or during processing. Ineffectiveness or malfunctioning of the treatment process employed could also result in the presence of coliform bacteria in the samples of water [33]. The presence of coliform in ozone-treated bottled water was attributed to defective disinfection. Growth in bacteria count in treated water can also be attributed to uncontrolled disinfection. If the water contains particles that can be a hiding place for the bacterial; rather than killing the bacterial in water, disinfection can render them inactive and at favourable temperature, they can become active [26].
IV. Conclusion and Recommendations.

The water samples in all the three locations are fit for drinking as they met the requirement of WHO but the growth of total coliform and e.coli in both the sachet and bottled water calls for concern. Water stored at room temperature complies with acceptable values of NIS but those kept under sunlight have increased a number of parameters. Vendors of this product should be enlightened on how best to store these packaged water before selling. Regulatory bodies should ensure each manufacturer have a quality control unit where testing could be conducted on a routine basis.

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