NISEB Journal Vol. 14, No. 2, June, 2014 1595-6938/2014
Printed in Nigeria (2014) Nigerian Society for Experimental Biology
http://www.nisebjournal.org

Effects of Storage/Biofilm Formation on Physico-chemical and Bacteriological Qualities of Potable Water Supply in Benin City

*Atuanya E.I, Seidu, R.I.and Orjiakor, P.
Department of Microbiology, University of Benin,
PMB 1154 Benin City, Nigeria.

Abstract.
Deterioration of water quality during storage and in distribution system remain one of the major difficulties experienced by the potable water suppliers. This study examined the effects of storage/biofilm formation on physico-chemical and bacteriological qualities of potable water supplies in Benin City. In the course of this study, twelve (12) water samples (three bottled water samples, three borehole water samples, three sachet water samples and three tap-water samples) were collected within Benin City metropolis, Edo state, Nigeria. The water samples were stored for six (6) weeks at ambient temperature and then analyzed weekly for bacteriological and physico-chemical qualities. Nutrient agar and Macconkey agar media were employed in culturing of isolates from water samples using standard methods. Total viable bacterial counts increased with storage in all forms of potable water samples analyzed while total coliform which appeared on first week in borehole and tap-water samples was observed in fourth week in sachet water samples. pH, temperature and dissolved oxygen decreased in all forms of potable water samples within six (06) weeks of storage while phosphate value increased throughout the duration of storage. Total bacterial counts obtained from the various water samples indicated that bottled water, borehole water, tap water and sachet water had counts ranging from 2.8 x 10^2 - 3.1 x 10^3cfu/ml, 1.5 x 10^2 - 4.1 x 10^3 cfu/ml, 1.3 x 10^2 - 3.9 x 10^3cfu/ml and 1.0 x 10^2 – 3.0 x 10^3 cfu/ml respectively. Morphological and biochemical tests performed on isolates from water samples revealed the presence of Klebsiella sp. Bacillus subtilus, Pseudomonas aerugimosa Enterobacter aerogenes, Staphylococcus aureus .

Keyword: Storage/biofilm formations, potable water supplies, water qualities.

Introduction

Rediscovery of a microbiologic phenomenon, first described by van leeuwenhoek, that microorganisms attach to and grow universally on exposed surfaces led to studies that revealed surface – associated microorganisms exhibited a distinct phenotype with respect to gene transcription and growth rate. These particular surface-associated microorganism are termed "Biofilm" [1].

A biofilm is an assemblage of microbial cells that is irreversibly associated (not removed by gently rinsing) with a surface and enclosed in a matrix of primarily polysaccharide material . Non – cellular materials such as mineral crystals, corrosion particles, clay or silt particles, or blood components depending on the environment in which the biofilm has developed, may also be found in the biofilm matrix. Biofilm- associated organisms also differ from their planktonic (freely suspended) counterparts with respect to the genes that are transcribed. Biofims may be formed on a wide variety of surfaces, including living tissues, indwelling medical devices, industrial or portable water system piping, or natural aquatic systems. The water system biofilm is highly complex, containing corrosion product, clay material, fresh water diatoms, and filamentous bacterial. The biofilm on the medical device, on the other hand appears to be composed of a single coccoid organism and the associated extracellular polymeric substance (EPS) matrix[1].

The bacterial growth and activity is substantially enhanced by the incorporation of surfaces to which microorganisms could attach (Bottle effect). Biofilm formation occurs step by step, such as formation of conditioning layer, bacterial adhesion, bacterial growth and biofilm expansion[2]. Biofilm can exist on all types of surfaces such as plastic, metal, glass, soil particles, wood, medical implant materials, water and food products. Bacterial attachment is mediated by timbriae, pilli, flagella EPS (extracellular polymeric substance) that act to form a bridge between bacterial and the conditioning film. Biofilm in nature can have high level of organization and they may exist in single layer or three dimensional structure [3].

Water is a vital resource in the ecosystem since it supports life of all living organism. Though, it occupies about 70% of the earth's surface, yet a greater percentage of the world's population, most especially in developing countries live without access to safe water [4]. This is due to lack of infrastructure for the treatment of water and its eventual distribution to the populace. In Nigeria, lack of efficient water supply facilities has led to the prospecting of underground water by individual for the provision of drinking water. Because of the problems associated with shallow wells, which include drying-out of water during dry season, seepage and easy contamination, there seems to be increased drilling of boreholes for the search of underground water by

*Corresponding Author: email: atuei _g@yahoo.com.

individuals, educational institutions, industries/commercial outlets and even governmental and non-governmental agencies. Since most of the water obtained from these sources is seldomly not treated because of the perception that they are generally safe to drink, there is need to carryout investigations on the physico- chemical and microbiological attributes of such underground water supplies [4].

Deterioration of the water quality during storage and in distribution system remain one of the major difficulties experienced by the potable water suppliers. Distribution system is one of the vital importance in determining the final quality of potable water. The treated water, when flowing through distribution systems, is adversely affected by the conditions in the distribution system. There are two major factors which contribute heavily in the deterioration of water quality. Bacteria can be introduced into the distribution network from external source by a number of ways such as open reservoirs, breakage due to the new pipeline construction they may disturb the existing distribution system. The bacteria number may increase due to the internal regrowth or after growth of the bacteria and the associated formation of the biofilm. There are various factors which will influence the formation of biofilm in the water distribution system [5] such as type of piping material, temperature type disinfectants, resistance of bacteria to disinfectants etc.

Sachet water is not completely sterile; it may not be entirely free of all infections microorganism. The potential danger associated with sachet water is contamination, which is a factor of the source of water itself, treatment, packaging material, dispensing into packing materials and closure[6].Under prolonged storage of packaged waters at favorable environmental conditions, total aerobic heterotrophic bacteria can grow to levels that may be harmful to human[7]. The study examined the effects of storage/biofilm formation on physico-chemical and bacteriological qualities of potable supplies in Benin City Edo State, Nigeria. The potable water supplies include sachet water, bottle water, borehole water and tap water supplies.

Materials and Methods

Collection of water samples

Twelve potable water sample (three bottled water samples, three sachet water samples, three bore-hole water samples and three tap water samples) were collected within Benin metropolis, Edo State Nigeria. Brand names of bottled water samples collected were UNIBEN table water, Olivia table water and Maria table water. Brand names of sachet water samples collected were Onome sachet water, Uncle-Sam sachet water and UNIBEN sachet water. Borehole water samples were obtained from BDPA housing Estate, Adolor and Isihor quarters. The tap water samples were collected from Iyaro, Ekenwan and UNIBEN staff quarters. Table and sachet water samples were collected within 24hours of production. The four forms of potable water collected were transported to laboratory and stored at ambient temperature for a period six (06) weeks and studies for biofilm formation. Sub-samples were drawn from the stock samples on weekly basis for bacteriological and physico-chemical analyses, using WHO analytical methods. Water samples for analysis of dissolved oxygen (DO) and biochemical oxygen demand (BOD) were collected in pre-sterilized brown bottle and fixed by adding 1.2ml of Winkler solution.

Bacteriological Analysis of Water Samples

The total bacterial counts were determined by pour plate technique using standard methods [8]. Nutrient agar medium was used for the enumeration of viable aerobic bacteria in the water samples and MacConkey agar for coliform bacterial counts. The different potable water samples were serially diluted upto 10^{-3} dilution. Then 0.1ml of the appropriate dilution was plated in MacConkey and Nutrient agar media. Both Nutrient and MacConkey agar plates were incubated at 37^0C for 24hours. After incubation, the number of discrete colonies were counted and recorded in colony forming unit per milliliter (cfu/ml).

Detection of biofilm in water samples was performed by filtering the stored potable water sample through 45-mm-diameter membrane filters (0.2-mm pore size). The filters were placed in small sterile plastic bags containing 10ml of the original water sample. The bags containing the filters were rubbed manually for a few minutes to remove organism from the filters. One portion of the concentrated sample (0.1ml) was spread directly on the surface of nutrient agar and MacConkey agar plates. The plates were incubated at temperature of 37^0C for 24-48hours. The isolates were sub-cultured to obtain pure cultures. The pure cultures so obtained were transferred to agar slants by streaking and from there further biochemical tests were carried – out to identify the isolates. Detachment of biofilm was also carried –out using sterile swab stick to swab the inner surfaces of the water sample containers weekly for a period of six (06) weeks of storage to collect biofilms. The biofilms were removed from the inner surfaces in an area of approximately $1cm^2$.

Identification of Bacterial Isolates

Aseptically purified representative discrete colonies were obtained by streaking on Nutrient agar plates. These were stored in agar slants for further characterization. All the bacterial isolates were initially examined microscopically for morphological characterization followed by appropriate biochemical test (Gram staining, indole, atalase, motility, citrate utilization, urea production, oxidase, coagulase and oxidative / fermentative utilization of lactose and glucose). The identification of bacterial isolates was done in accordance with the criteria of Bergeys Manual of Determinative Bacteriology [9].

Physico-chemical analysis of potable water samples

Various potable water samples for physico-chemical analyses were collected in ethanol sterilized 2litres plastic containers and were sent to laboratory in ice-packed coolers. Those that could not be analyzed the same day were stored in a refrigerator at a temperature of 4^{o}C. Water sample temperature was determined using mobile-mercury in-glass thermometer immediately after collection and subsequently every seven days for six weeks. This was done by dipping the thermometer into the water sample and recording stable reading. The pH of water samples was determined using Hanna microprocessor pH meter which was standardized with buffer solution of pH range between 4 and 7. he modified Winkler's method [8] was used for determination of dissolved oxygen (DO) and biochemical oxygen demand (BOD_5). Phosphate contents of various potable water samples were determined colorimetrical using Milton Roy spectronic 21D spectrophotometer at wavelength of 660nm. Data collected were subjected to statistical analysis in accordance with Eniola *et al.,*[10]. The correlation coefficient between bacteriological and physico-chemical data were determined.

Results

Morphological and biochemical tests performed on isolates obtained from various potable water samples revealed the presence of *Pseudomonas aerogene, Klebsiella sp., Bacillus subtilis, Enterobacter aerogenes* and *Staphylococcus aureus* as shown on table 1. Result on table 1 indicate the presence of *Pseudomonas aeruginosa* in borehole water sample. *Klebsiella sp* was present in sachet and tap water samples only. *Enterbacter aerogenes* and *Staphylococcus aureus* were detected in borehole and tap water samples. *Bacillus substilis* was detected in all water samples with exception of bottled water samples. No bacterial isolate was detected in bottled water samples analyzed with exception of *Staphylococcus aureus.*

Table 1.: Occurrence of the bacterial isolates from the water samples

Bacterial isolates	Bottled water	Borehole water	Sachet water	Tap water
Pseudomonas aeruginosa	-	+	-	-
Klebsiella sp.	-	-	-	-
Enterobacter aerogenes	+	+	-	+
Staphylococcus aures	-	+	-	-
Bacillus subtilis	-	+	+	+

KEY.
+ = Present
- = Absent

Total viable bacterial counts was seen to increase with storage in all forms of potable water samples while total coliform which appeared in first week in borehole and tap-water samples was observed in the fourth week in sachet water samples as shown on table 2. Interestly, table 2 equally revealed that from week 0 to week 4, there was no occurrence of coliform organisms in bottle water.

The pH values obtained for each of the various water samples analyzed during the six (06) weeks of storage is shown on table 3. pH values were observed to decrease in all forms of potable water samples during the six weeks of storage.

Tables 4,5 and 6 showed the temperature, dissolved oxygen (DO) and biochemical oxygen demand (BOD) values of various forms of potable water samples during six weeks storage period. Dissolved oxygen and biochemical oxygen demand values were observed to decrease in all forms potable water during the six-week storage. Table 7 which showed the phosphate contents of various forms of potable water samples during the six-weeks of storage revealed increase in phosphate contents as the storage lasted.

Discussion

This study was conducted to obtain an insight on bacterial isolates present in biofilms formed in stored potable water samples found in Benin metropolis and also to evaluate their physico-chemical and bacteriological qualities. The stored potable water samples evaluated in this study include the followings- bottled water samples, borehole water samples, sachet water samples and tap – water samples. Occurrence of bacterial isolates obtained from these potable water samples as shown on table 1 include the following- *Pseudomonas aeruginosa, Klebiella sp. Enterobacter*

Table 2: Total viable bacterial and total coliform counts of various forms of potable water samples

	DURATION									
	Week 0		WEEK 1		WEEK2		WEEK 3		WEEK 4	
	TBC (CFU/ml)	TCC (CFU/ml)	TBC (CFU/ml)	TCC (CFU/ml)	TBC (CFU/ml)	TCC (CFU/ml)	TBC (CFU/ml)	TCC (CFU/ml)	TBC (CFU/ml)	TCC (CFU/ml)
BW	-	-	1.1×10^2	-	2.8×10^2	-	2.2×10^3	-	3.1×10^3	-
BHW	1.5×10^2	-	2.4×10^2	1.2×10^2	3.1×10^2	1.6×10^3	3.8×10^3	1.9×10^3	1.4×10^3	2.0×10^3
TW	1.3×10^3	-	4.0×10^2	1.0×10^2	3.8×10^2	1.5×10^2	4.2×10^2	2.3×10^2	3.9×10^3	3.1×10^3
SW	1.0×10^2	-	1.9×10^2	-	2.0×10^2	-	2.7×10^2	3.0×10^3	3.0×10^3	1.5×10^2

KEY: TBC = Total bacterial counts (cfu/ml) TCC = Total Coliform counts(cfu/ml), BW= Bottled water sample, BHW = Bore hole water sample, TW = Tap water sample, SW = Sachet water sample.

aerogenes, Staphylococcus aureus and *Bacillus subtilis.* Results obtained in this research conforms to that of Eniola *et al.* [10] and Adegoke *et al.* [11]. in which their studies detected the presence of *Pseudomonas Spp, Enterobacter spp, Klebiella spp.* and *Staphylococcus aureus.* Biofilms are suspected to be the primary source of microorganism in drinking water distributions systems that are fed with treated water and have no pipeline breaches. Such biofilms and are of particular concern in older drinking water distribution systems. By adopting this sessile mode of life, biofilm-embedded microorganisms enjoy a number of advantages over their planktonic counterparts.

Table 3: pH of water samples in storage for six weeks duration

Period	BW	BHW	TW	SW
Week 0	7.00 ±0.18	7.74±3.23	7.65±2.80	7.10±2.60
Week 1	6.89 ±0.04	7.71±3.26	7.61±2.81	6.95±2.60
Week 2	6.86 ±0.06	7.69±3.25	7.59±2.80	6.92± 2.60
Week3	6.84±0.06	7.67±0.02	7.58±3.40	6.90±2.80
Week 4	6.83±0.06	7.66±3.25	7.55± 1.95	6.89±2.26
Week 5	6.81±0.09	7.65±2.06	7.53±1.85	6.87±2.30
Week 6	6.80±0.12	7.64±3.21	7.51±1.56	6.85±2.41

KEY: BW = Bottle water sample, BHW = Bore – hole water sample, TW=Tap water sample, SW= Sachet water sample

Table 4: Temperature (°C) of water samples in storage for six weeks duration

	Sample code/ Temperature (°C)			
Period	BW	BHW	TW	SW
Week 0	26,20±0.04	27.79± 12.08	27.00± 10.23	26.79±9.57
Week 1	26.75±0.66	28.00± 12.29	27.14± 10.36	26.88±9.67
Week 2	27.18±0.11	28,10± 12,25	27.47± 10.40	26.97±9,71
Week 3	27.22±0.01	28.00±0.68	26.26±11.84	26.80± 10.07
Week 4	27.71±0.42	28.81± 12	27.61±7.15	27.76± 8.62

KEY: BW = Bottled water sample, BHW = Bore-hole water sample, TW = Tap water sample, SW .= Sachet water sample.

Table 5: Dissolved Oxygen (DO) of water samples in storage for six weeks duration

	Sample code/ Dissolved Oxygen (mg/1)			
Period	BW	BHW	TW	SW
Week0	7.15±0.07	6.95±3.12	9.45±3.19	7.25±2.87
Week 1	7.10±0.00	6.80±2,98	9.35±3.12	7.20±2.82
Week 2	6.95±0.21	6.82±3.00	9.32±3.12	7.10±2.81
Week 3	6.85±0.07	6.78±0.11	9.18±3.72	6.95±3.00
Week4	6.83±0.11	6.66±3.00	9.15±2.52	6.95±2.46
Week 5	6.82±0.09	6.64±1.5	9.13±2.91	6.93±2.71
Week 6	6.80±0.13	6.62±2.1	9.11±3.02	6.92±2.87

KEY: BW = Bottled water sample, BHW = Bore-hole water sample, TW = Tap water sample S W = Sachet water sample.

One advantage is the ability of the extracellular polymeric matrix, they secrete, to capture and concentrate a number of environmental nutrients, such as carbon, nitrogen and phosphate [12].

Total viable bacterial counts was seen to increase with storage in all forms of potable water samples while total coliform which appeared in first weeks in borehole and tap-water samples was observed in the fourth week in sachet water samples (Table 2). The result obtained for total viable bacterial counts of water samples revealed that from 0 to week 4, there was no occurrence of coliform organism in bottled water. This could be due to the proper sterilization and treatment of bottled water before they were packaged. However, in the case of total viable bacterial counts, a value of 1.1 x 10^2cfu/ml was obtained at week 1 for bottled water samples. Total bacterial counts for bottled water samples at weeks 2,3 and 4 were found to be 2.8 x 10^2cfu/ml 2.2 x 10^3 cfu/ml and 3.1 x 10^3cful/ml respectively (table 2).

Table 6: Biological Oxygen Demand (BOD) of water samples in storage for six weeks duration

	Sample code/ Biological Oxygen Demand (mg/L)			
Period	BW	BHW	TW	SW
Week0	3.8±0.85	3.5±1.40	3.4±1.24	2.95±0.14
Week 1	3.5±0.57	3.3±1.57	3.3±1.30	2.7±0.14
Week 2	3.2±0.50	3.3±1.37	3.1±0.95	2.5±0.14
Week 3	2.7±0.07	3.1±0.07	2.7±0.18	2.3±0.1
Week4	2.5±0.07	2.8±1.37	2.4±0.51	2.1±0.14
Week 5	2.4±0.06	2.6±0.90	2.4±0.42	2.1±0.13
Week 6	2.3±0.06	2.4±0.21	2.3±0.12	2.0±0.1

KEY: BW = Bottled water sample, BHW = Bore-hole water sample, TW = Tap water sample, S W = Sachet water sample.

Table 7: Phosphate content of water samples in storage for six weeks duration

	Sample code/ Phosphate (mg/ml)			
Period	BW	BHW	TW	SW
Week0	0.33±0.02	0.46±0.18	0.40±0.15	0.43±0.15
Week 1	3.6±0.02	0.46±0.22	0.42±0.18	0.45±0.22
Week 2	0.41±0.03	0.49±0.23	0.42±0.17	0.46±0.15
Week 3	0.42±0.07	0.50±0.01	0.44±0.02	0.44±0.01
Week4	0.43±0.04	0.51±0.23	0.48±0.08	0.47±0.18
Week 5	0.45±0.03	0.51±0.20	0.50±0.11	0.47±0.11
Week 6	0.46±0.05	0.52±0.23	0.51±0.09	0.48±0.05

KEY: BW = Bottled water sample, BHW = Bore-hole water sample, TW = Tap water sample, S W = Sachet water sample.

Total bacterial count in bore-hole water biofilm detachedness during the four weeks duration ranged from 1.5 x 10^2 to 4.1 x 10^3cfu/ml, while total coliform count for bore-hole water samples were found to range from 1.2 x 10^2 to 2.0 x 10^3cfu/ml. Tap water samples analyzed from biofilm detachments indicated that total bacterial count with four weeks storage duration ranged from 1.3 x 10^2 to 3.9 x 10^3cfu/ml. the bacterial count reach its peak on week 4 (3.9 x 10^3cfu/ml) but decrease on the sixth week due to nutrient limitations. (not shown on table 2). The total coliform count for tap water samples between week 2 and week 4 was found to range from 1.0 x 10^2 to 3.1 x 10^3cfu/m, with no coliform detected at week 1 (table 2). Sachet water samples analyzed during the six week storage indicated that total bacterial counts ranged from 1.0 x 10^2 to 3.0 x 10^3cfu/ml, total coliform count in sachet water samples was observed at week 4 with a value 1.5 x 10^3cfu/ml table 2.

The poor bacterial quality of drinking water samples as revealed in through occurrence of unacceptable viable bacterial load and coliform counts in different drinking water sources is a matter of public health concern. Higher occurrence of coliforms in borehole and tap water samples indicate poor handlings and unhygienic conditions. The significance of total coliform count in sachet water samples observed only at week four suggest that expiry date for sachet and bottled water produced in Nigeria should not exceed four weeks from the date of production. Coliforms in water samples indicate an important treatment or distribution system deficiency and therefore a potential public health treat. If potable water samples are positive for total coliforms, the possibility of a treatment system breakdown or cross- connection should be investigated [13].

As shown on table 3, pH was observed to decrease in all forms of potable water samples during the six-weeks storage. Similar results were obtained by Akinde *et al.,* [14] and Agbaje *et al.* [15].for stored sachet and borehole water samples respectively. pH of all forms of potable water samples analysed met with WHO pH standard for drink water which is 6.8 – 8.5. Acidity increases the capacity of water to attack geological materials and leach toxic trace metals into the water, making it potentially harmful for human consumption. Equally, acidity may give a sour taste to water [16]..

Temperature values as shown on table 4 were found to increase with storage in all forms of potable water samples. Temperature for bore-hole water, tap – water, sachet water and bottled water were found to ranged from 27.79 – 28.81^{0}C, 26.26 – 27.61^{0}C, 26.79 – 27.76^{0}C and 26.20 – 27.71 respectively. Similar results were obtained by Agbaje *et al.,* (2012) who revealed the temperature in bore-hole water samples ranged from 31.5 to 35.4^{0}C during storage. Higher temperature is known to attract the growth of pathogen bacteria in drinking water supplies [17].

As shown on table, 5 and 6, the dissolved oxygen and biochemical oxygen demand (BOD) were observed to decrease in all forms of potable water samples during storage. In case of dissolved oxygen (DO), bottled water, borehole water, tap-water and sachet water samples obtained DO values which ranged from 6.80 – 7.15mg/l, 6.62 – 6.95mg/l, 9.11-9.45mg/l and 6.92 – 7.25mg/l respectively table 5. In case of biochemical oxygen demand (BOD), bottled water, borehole water, tap-water and sachet water samples obtained BOD values of 2.3 -3.8mg/l, 2.4 –3.5mg/l, 2.3 – 3.4mg/l, and 2.0 – 2.9mg/l respectively (table 6). Similar results were obtained by Agbaje *et al.* [15].whose BOD values for stored bore-hole water samples ranged from 30 to 117mg/L. As shown on table 7, phosphate content of various forms of potable water samples were observed to increase during storage. Phosphate content of bottled water, borehole water, tap-water and sachet water samples ranged from 0.33-0.46mg/l, 0.46 – 0.52mg/l, 0.40 – 0.15mg/l, and 0.43 – 0.48mg/l respectively. Similar results were obtained by Akinde *et al.* [14] who examined the storage effects on quality of sachet water produced in Port-Harcourt.

Conclusion

The results obtained in this study are of significance as the presence of unacceptable level of bacterial load and coliform bacteria which developed and became culturable due to storage could be devastating to human health when drunk. Expiry date of sachet and bottled water produced in Nigeria should not exceed four weeks from the date of production. The public should be sensitized not to drink sachet water that has exceeded four weeks from the date of manufacture if the sachet water is not refrigerated. Federal and State governments should ensure proper monitoring of bottled and sachet water producers to reduce incompetent and unsanitary water factories. Storage tanks of borehole and other potable water supply factories should be cleaned / washed on a regular basis as often as possible to reduce the accumulation of biofilms in such tank. Borehole/pipe-bornewater storage in containers at homes should be also discouraged to avoid development of biofilms which harbor pathogenic microorganism.

References

1. Dolan R M; Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases* **8** (9): 882-890, 2002.
2. Kumar C G; Significance of microbial biofilm in food industry: A review. *International Journal of Food Microbiology* **43:**9-27, 1998.
3. Deibel V; Biofilms. *Journal of Food Safety* **1**:6-7, 2001.
4. Lateef O, Oloke, J K and Guegum, E B The prevalence of bacterial resistance in clinical, food, water and some environmental samples in southwest Nigeria. *Environmental Monitoring and Assessment.* **100:**59-69, 2012.
5. Bishop P I; Biofilm structures and Kinetics. *Water Science Technology.* **36:**287-294, 1997.
6. Omalu I C, Eze, G C, Olayemi, I K, Gbesi, S, Adeniran, L A, Ayanwale, A V, Mohammed, A Z and Chukwuemeka V; Contamination of sachet water in Nigeria, Assessment and Health impact. *Online Journal of Health and Allied Sciences* **9**(1): 1-3, 2010.
7. Warburtion D W, Dodds, K L, Burke, R, Johnson, M A and Laffey, P J; A review of microbiological quality of bottled water sold in Canada between 1981 and 1989. *Canadian Journal of Microbiology* **38:**12-19, 1992.
8. American Public Health Associateion (APHA) (1998). *Standard Methods for Examination of Water and Wastewater* 19th edition. American public Health Association, Washington D.C. 1193pp, 1998.
9. Holt J G, Kragy, H R, Sneathe, R H A and Williams, S T; *Bergey's Manual of Determinative Bacteriology.* 9th edition. Williams and Wilkens company Baltimore USA. 84-240pp, 1994.
10. Eniola K I, Obafemi, D Y, Awe, S F, Yusuf, I I, Falaiye, O A and Olowe, A O Effects of containers and storage conditions on bacteriological quality of borehole water. *Nature and Science* **5** (4) 1-6, 2007.

11. Adegoke O, Adebayo B, Olugbenga E, Oni E S, Ugbaja, K, and Nahedo A, Microbiological examination of sachet water sold in Aba, Abia state, Nigeria. *Global Research Journal of Microbiology* **2** (1): 62-66.
12. Simoes L C, Simoes, M and Vieira, M J Microbial interactions in drinking water biofilms. *Biofilms* **5:** 43-53, 2006.
13. World Health Organization (WHO); *Guidelines for Drinking water quality: Microbiological Methods.* 2nd edition. Vol 1. World Health Organization, Geneva, 2001.
14. Akinde S B, Michael, I N and Adindu, S O, Storage effects on the quality of sachet water produced with Port-Harcourt metropolis, Nigeir1a. *Jordan Journal of Biological Sciences* **4** (3): 157-164.
15. Agbaje L. Lateef, K and Semawon, O A; Quality assessment of some groundwater sample in Ogbomoso metropolis, Southwest Nigeria. *Journal of Environment and Earth Science* 2 (6): 39-48, 2012.
16. Koro B, Dibal, J M and Ndakawa, I I; Elemental analysis of tap and borehole water samples in Maiduguri, Nigeria. *European Journal of Applied Sciences*. **1** (2): 26-29, 2009.
17. Bello O O; Oshe, A, Bankole, S A and Bello, T K; Bacteriological and physico-chemical analyses of borehole water well-water sources in Ijebu – Ode, Southwestern Nigeria. *Journal of Pharmacy and Biological Sciences* 8 (2):18-25, 2013.