

The Effects of Prolonged Storage on the Quality of Sachet Water Produced within the Paynesville Municipality of Liberia

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ABSTRACT

The current study underlines the effect of storage on the quality of some sachet water produced in the Paynesville Municipality of Liberia for a period of two months. Ten (10) different brands of sachet water were collected within approximately 24 hours of production and stored at ambient temperature. A portion of the samples was drawn from the stock samples on a weekly basis for further physico-chemical (pH, nitrate, phosphate and dissolved oxygen) and bacteriological (total coliform bacteria, faecal coliform, escherichia coli and faecal streptococci) analyses using APHA and WHO analytical techniques. pH values increased in all of the different brands of sachet water, up to week 6, with about 30% falling outside the Liberian Water Quality Standard limit and steadily decreased just before the end of the study. Dissolved oxygen and nitrate values decreased during the course of the analysis period while phosphate values increased in all the brands tested. The total aerobic heterotrophic bacterial count increased slowly in all brands of the collected sachet water to the undesirable limit after three weeks of storage and progressively decreased between weeks 6 and 8 of the analysis period. Total coliform bacteria were detected in 40% of the brands of sachet water analyzed within the first three weeks and gradually died off towards the end of the experiment. Faecal coliform, Escherichia coli, and faecal Streptococci remained undetected throughout the investigation period. Results of the experiment indicate that all of the brands of sachet water analyzed were in the WHO permissible limit for drinking when stored at ambient temperature within three weeks. However, continuous storage of sachet water outside this period can significantly affect the chemical quality of sachet water to a large extent that exceeds the permissible limit set by the World Health Organization (WHO), thus, leading to a level harmful to human health. Keywords : Paynesville Municipality, Prolonged Storage, APHA, NPHIL, Water Quality STANDARD, Total COLIFORM BACTERIA

I. INTRODUCTION

In Liberia, population growth has overtaken local efforts to expand potable water services, and private sector sale of packaged sachet water has filled an important gap in the household water supply. The last decade has witnessed a massive surge in the number of private vendors of sachet water, but the long-term health implications of this proliferation remain unstudied.

Sachet water is regulated by the Division of Occupational Health and Safety, National Public Health Institution of Liberia (NPHIL). The Institute has a national drinking water quality standard but also makes use of the World Health Organization (WHO) standards for the product regulation, registration, and certification. There has been a tremendous increase in the number of sachet water brands sold on the Liberian markets; however, the question of purity and health concerns need to be carefully investigated.

Sachet water is a machine-sealed water that is prepared and ready for safe drinking(Adiotomre and Agbale, 2015). Different brands of sachet water sold on the Liberian market are consumed daily by poor and middleclass people, simply because it is readily available and cheap relative to the bottled brand. Sachet water is not absolutely germ-free and, as such, it possibly will not be exclusively free of all contagious bacteria. The potential risk associated with sachet water is impurity, which is a function of factors such as packaging, water source, handling, treatment mechanisms and storage conditions (Omalu et al., 2010). Below lengthy storage of packaged water at satisfactory environmental conditions, total aerobic heterotrophic bacteria can reproduce to deleterious to humans intensities that may be (Warburton et al., 1992). Sachet water quality in Liberia may be hindered probably because most of the packaged sachet water is stored in warehouses usually at temperatures that are unfavorable for maintaining product quality. Total aerobic heterotrophic bacterial counts are delicate and practical signs of water treatment efficacy as well as after-growth and biofilm development. There are some of the total aerobic heterotrophic bacteria that have been recognized as unscrupulous pathogens that affect human health (Rusin et al., 1997). The consumption of water containing large numbers of total aerobic heterotrophic bacteria has been reported to cause diseases such as gastroenteritis and mucous membrane infections (Grabow, 1996).

This study sought to examine the effect of extended storage after production on the physico-chemical status and bacteriological quality of certified sachet water brands produced in the Paynesville municipality of Liberia.

II. METHODS AND MATERIAL

Geographical Propinquity of the Study

The municipality of Paynesville was chosen for this study because of its geographical and commercial significance. Located on the east of the capital Monrovia, the city is home to the largest commercial area in the country, the Red Light Market District, where most goods sold in the greater Monrovia area are brought in and distributed. Paynesville is geographically larger than the city of Monrovia with a population of 347,000 (LISGIS, 2008).

Sample Collection and Preparation

Ten (10) brands of sachet water with Liberian Ministry of Health certification were randomly collected in different parts of Paynesville, Liberia in bags within 24 hours of production, transported to the Environmental Protection Agency (EPA) Central Laboratory located in Sinkor, Liberia and tested immediately for pH, nitrate, phosphate and dissolved oxygen at ambient temperature. Approximately 50 mL of sachet water was randomly analyzed from three packs of each brand and subsequently mixed thoroughly in separate beakers before analysis. Sachet water samples were tested for the aforementioned physico-chemical parameters and bacteriological assay using APHA analytical method (APHA, 1998).This procedure was repeated weekly for each brand of sachet water for an 8 week period.

Analysis of Physico-Chemical Parameters of Sachet Water Samples

Physico-chemical parameters of the collected brands of sachet water were examined directly after sampling for every other week thereafter. Using a Multi-Parameter Water Quality Portable Meter, the pH, temperature and dissolved oxygen (DO) of the sachet water were determined electrometrically (HI-9828, Hanna Instruments, USA). Before each analysis, the meter was calibrated using buffer standards of pH 4, 6.86, 7 and 10 at room temperature. In addition, nitrate and phosphate in sachet water samples were determined colorimetrically by DR 890 Spectrophotometer (Hach, USA).

Bacteriological Analysis of the Sachet Water Collected

Bacteriological analysis of the selected brands of sachet water was performed straight away after collection for everv other week afterward. The total aerobic heterotrophic bacterial count was obtained using the heterotrophic plate count technique and the spread plate method in accordance with APHA 9215. About 1 ml of each brand of the sample or 0.1ml of final dilution of the sample in sterile Ringer's solution was sterilized onto dry nutrient agar surface in triplicates glass spreader and incubated in an upturned position for 18-24 hours at $35\pm2^{\circ}$ C. Approximately, 30 - 300 on plates were counted up at the culmination of the incubation period. Escherichia coli (E-coli), total coliform, faecal Streptococci and faecal coliform were determined using Membrane Filtration Technique in agreement with the

WHO Guidelines for Drinking Water Quality (2001,Volume 3).

A filtration component constituting a conical flask, vacuum source and porous support was gathered using a flame decontaminated forceps. A 0.45µm millipore germ-free membrane filter was positioned on the porous support. Using the appropriate clamps, the upper funnel was retained at a specified spot and secured. Approximately, 100ml of each brand of the selected sachet water sample was aseptically transferred into the upper funnel and suction used to generate a vacuum. The filtrate was taken separately for each sample after the sample was delivered through the membrane filter. On the pad that had been saturated with McConkey broth for Methylene Blue agar, Eosine, total and faecal coliforms for E-coli as well as Bartley agar and Slanetz for faecal Streptococci, the membrane filter was positioned in the petric dish. Prior to the use of each sample, the upper funnel was then detached and washed with about 200ml of sterilized Ringer's solution. In an plates were upturned position, all incubated approximately at $44 \pm 2^{\circ}$ C (faecal coliforms, E- coli and faecal Streptococci) and 37 $\pm 2^{\circ}$ C (total coliforms) for 18-24 hours.

III. RESULTS AND DISCUSSION

pH of the Sachet Water Samples

The pH values of the selected brands of sachet water samples are shown in **Table 1** and **Figure 1**. The pH is one of the parameters that shows the visual quality of water such as taste and odor which may not reflect any serious health consequence (WHO, 1996). Although pH plays a significant part in the determination of bacterial population growth and multiplicity in sachet water, it is essentially a parameter that indicates the presence or absence of certain contaminants. The pH values recorded in this study varied from 6.53 to 8.66. The values of pH obtained from the different brands of sachet water were within the Liberian Water Quality Standard (6.5-8.0) in all the 10 samples analyzed at the onset of the investigation (Week 0), while an increase in pH values were detected in all the selected samples up to week 6 with about 30% falling outside the Liberian National Standard followed by steady decrease between Week 6 and Week 8.

Table 1 The Elevation in pH Levels of the Selected Brands of Sachet Water

Sample	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
SW1	6.71	6.74	6.78	6.79	7.41	7.76	7.32	6.79
SW2	6.53	6.60	6.71	7.28	8.25	8.69	8.61	8.60
SW3	7.22	7.26	7.31	7.40	7.43	7.43	7.42	7.40
SW4	7.71	7.73	7.75	7.77	7.92	7.94	7.91	7.83
SW5	7.61	7.63	7.66	7.66	7.70	7.03	6.92	6.83
SW6	6.60	6.67	6.78	7.38	7.79	8.41	8.40	8.38
SW7	6.56	6.58	6.67	6.69	6.71	6.74	6.71	6.69
SW8	6.77	6.77	6.79	7.12	7.34	7.93	7.22	7.20
SW9	7.80	7.88	7.93	8.11	8.57	8.74	8.66	8.62
SW10	6.72	6.74	6.74	6.99	7.21	7.54	7.11	6.95
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NB: Results in bold are above permissible limits; SW = Sachet Water,

The elevation in pH levels between week 0 and week 6 could be attributed to the production of basic metabolic waste products, thus amassing bacterial population. According to Prescott *et al.* (1999), microorganisms habitually change the pH of their own surroundings by generating acidic or basic metabolic waste products. According to Edbert *et al.* (2017), drinking with such a high pH could lead to a condition called alkalosis, which may cause irregular heartbeats, coma and imbalanced electrolyte levels in consumers.

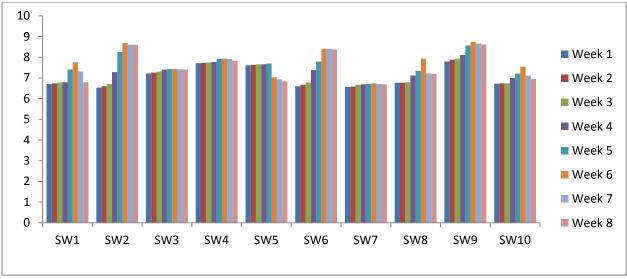


Figure 1: pH values of the selected brands of sachet water collected in 8 weeks period

The Weekly Temperature Variations of the Collected Sachet Water

The temperature readings of the different brands of sachet water samples are depicted in **Table 2** and **Fig. 2**. The temperature values ranged from 25.0 to 28.9° C. The different temperature values obtained during the examination period fell within the ideal growth range (20-45°C) for mesophilic bacteria as well as human pathogens (Prescott *el al.* 1999).

Samples	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	
SW1	25.2	26.5	26.1	25.3	25.8	28.9	26.9	26.5	
SW2	25.1	25.0	27.9	25.6	25.0	27.9	25.1	25.6	
SW3	25.1	25.4	28.9	28.8	28.7	26.2	27.9	27.7	
SW4	25.9	25.6	25.7	25.6	25.0	25.5	25.3	28.1	
SW5	25.7	25.1	25.0	28.2	25.6	28.6	25.5	25.5	
SW6	28.7	27.4	26.9	28.1	27.8	25.9	28.4	26.6	
SW7	25.9	26.3	25.4	25.2	28.9	25.1	26.5	27.8	
SW8	27.7	27.0	27.3	25.6	26.0	25.7	25.0	25.7	
SW9	25.8	25.3	26.4	27.1	25.3	25.1	27.4	27.3	
SW10	25.7	25.1	26.2	27.1	25.6	25.1	25.3	25.2	
SW – Sachet Water									

 Table 2 The temperature (°C) variations for the 8 week period

The microbiological characteristics of drinking water are closely associated with temperature through its special effects on the water- treatment methods and the growth and existence of microorganisms (WHO, 1996). Accordingly, the growth of certain microorganisms may be improved by warm water circumstances and could plausibly lead to the growth of indecorous tastes and odors.

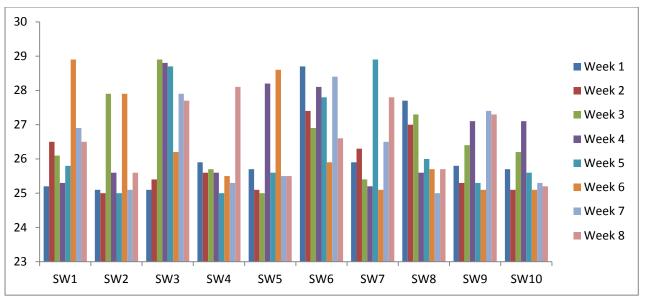


Figure 2: Temperature (°C) of sachet water in 8 weeks period

The Dissolved Oxygen (DO) Values for the Sachet Water Samples

Dissolved oxygen (DO) is a significant aspect of the chemistry and microbiology of water. The DO values obtained for the different brands of sachet water samples over the 8 week period are presented in **Fig. 3**. The DO values ranged from 4.12 to 6.11 mg/l. Shrinkage in DO was basically detected in the different brands of sachet water samples during the course of the study period; a clue of potential bacterial respiration of organic constituents by the bacterial flora of the collected sachet water samples examined, which is similar to result obtained by Michael *et al.* (2015). According to WHO report (1996), the propensity for the level of dissolved oxygen to decrease with time signifies potential microbial respiration of organic materials. On the other hand, the dissolved oxygen over the 8 week period of this study was within the tolerable perimeter of 5-8 mg/L agreed by WHO. Comparative analysis of DO in the different brands of sachet water samples is depicted in Table 3.

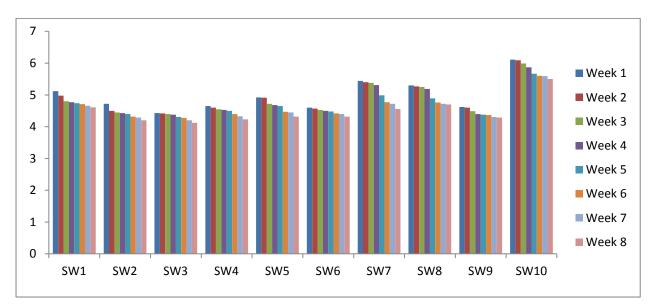


Figure 3: Dissolved Oxygen values (mg/l) of sachet water in 8 weeks period

Samples	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
SW1	5.12	4.98	4.80	4.77	4.74	4.71	4.66	4.61
SW2	4.72	4.50	4.45	4.43	4.40	4.32	4.29	4.20
SW3	4.43	4.42	4.40	4.38	4.31	4.28	4.20	4.12
SW4	4.65	4.60	4.55	4.53	4.50	4.40	4.33	4.23
SW5	4.92	4.91	4.72	4.68	4.65	4.47	4.45	4.32
SW6	4.60	4.57	4.53	4.50	4.48	4.42	4.40	4.32
SW7	5.44	5.40	5.38	5.31	4.99	4.77	4.72	4.56
SW8	5.30	5.27	5.25	5.19	4.89	4.76	4.72	4.70
SW9	4.62	4.60	4.49	4.40	4.38	4.37	4.31	4.29
SW10	6.11	6.09	5.99	5.87	5.67	5.60	5.59	5.50
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Table 3 Comparative analysis of DO in the different brands of sachet water samples

SW-Sachet water

The Values of Nitrate in the Sachet Water Samples

Nitrate values obtained for the different brands of sachet water samples collected are shown in **Fig. 4.** The values of nitrate ranged from 0.32 to 2.14 mg/L, falling below both the Liberian Water Quality standard and WHO limit, respectively. Nitrate values decreased during the course of the 8 week study period. The decrease in nitrate values could plausibly be ascribed to their consumption by microorganisms for growth and reproduction (Prescott et al., 1999). For the duration of the research, the nitrate levels in the water samples tested remained within both the Liberian Water Quality Standard (40 mg/L) and the WHO limit (50 mg/L) for drinking water.

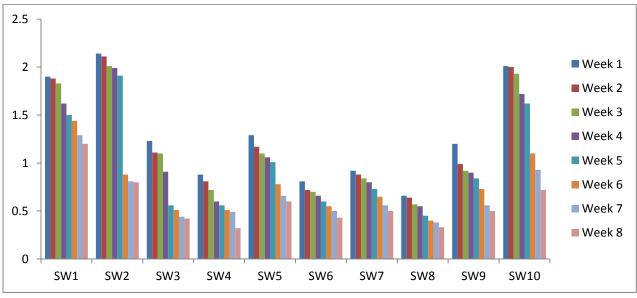


Figure 4: Nitrate values (mg/L) of sachet water in 8 weeks period

The Values of Phosphate in Sachet Water Samples

Phosphate values are presented in **Fig. 5.** The values of phosphate ranged from 0.31-2.11 mg/L. Phosphate values increased all the way through the 8 week examination period; a condition that could be ascribed to microbial death and accretion of metabolic waste (Prescott *et al.*, 1999).

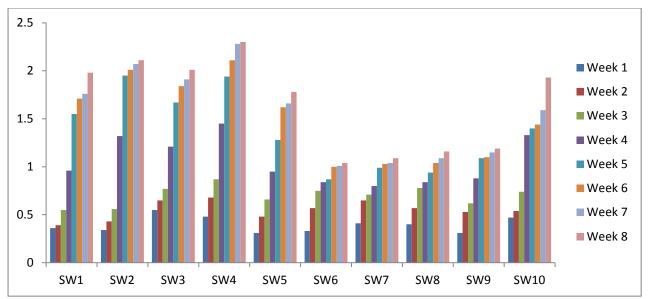


Figure 5: Phosphate values (mg/l) of sachet water in 8 weeks period

Total aerobic heterotrophic bacterial (THB) counts obtained from different brands of sachet water samples collected are presented in **Table 4.** The counts of total aerobic heterotrophic bacteria ranged from 0.0 to 2.067×10^{3} cfu/ml of the different brands of sachet water samples examined. This is in accordance with Akinde *et al.* (2011) who detected the variation in the levels of microbial contamination in samples of different brands of sachet water. A steady increase in total aerobic heterotrophic bacterial counts was detected in all the different brands of sachet water samples tested up to week 6 followed by a decrease in counts up to the end of the testing period, a growth arrangement characteristic of microorganisms growing in a closed system (Brock and Madigan, 1988). WHO drinking water quality specifications tolerate total aerobic heterotrophic bacterial counts of 100 cfu/ml (Allen *et al.*, 2002). Though, the aforementioned limit was topped by all the different brands of sachet water samples analyzed after three weeks of storage.

Total coliform bacteria (TCB) identified in 40% of the different brands of sachet water samples during the first three weeks are shown in **Table 4** while faecal coliform (FC) were unobserved in the different brands of sachet water examined during the course of the investigation. Total coliforms are usually used as signs of the general sterile quality of treated drinking water whereas faecal coliforms give a much nearer clue of faecal pollution (Ashbolt *et al*, 2001). According to World Health Organization limit, not a single faecal coliform should be identified in drinking water. Not like total aerobic heterotrophic bacteria, total coliform counts decreased in different brands of sachet water samples tested. Entirely, the total coliforms recorded in the study appeared to die off after week 5 of the investigation. Prescott *et al.* (1999) indicated that indicator bacterium should not reproduce in the contaminated water and produce an overestimated value in the midst of the criteria for indicator organisms. This is acceptable to the choice of coliform bacteria by the World Health Organization as indicator organisms (WHO, 2001).

Table 4: Total Microbial count (cfu/ml) of sachet water in 8 week period

Sample	Bacterial	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
	THB	16	30	41	196	453	1276	703	656
SW1	TCB	3	3	2	2	0	0	0	0
	FC/EC/ Strep.	0	0	0	0	0	0	0	0
	THB	12	17	22	147	533	1082	620	527
SW2	TCB	2	2	2	0	0	0	0	0

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	FC/EC/ Strep.	0	0	0	0	0	0	0	0
	THB	10	21	26	164	453	1053	639	498
SW3	TCB	0	0	0	0	0	0	0	0
	FC/EC/ Strep.	0	0	0	0	0	0	0	0
	THB	12	29	34	200	540	1210	580	491
SW4	TCB	3	3	2	2	1	0	0	0
	FC/EC/ Strep.	0	0	0	0	0	0	0	0
	THB	4	17	25	182	563	1296	657	597
SW5	TCB	0	0	0	0	0	0	0	0
	FC/EC/ Strep.	0	0	0	0	0	0	0	0
	THB	12	20	22	127	378	739	590	470
SW6	TCB	0	0	0	0	0	0	0	0
	FC/EC/ Strep.	0	0	0	0	0	0	0	0
	THB	10	28	37	192	267	420	710	623
SW7	TCB	2	2	1	1	1	0	0	0
	FC/EC/ Strep.	0	0	0	0	0	0	0	0
	THB	13	17	28	133	345	1270	630	431
SW8	TCB	0	0	0	0	0	0	0	0
	FC/EC/ Strep.	0	0	0	0	0	0	0	0
	THB	0	0	0	0	0	0	0	0
SW9	TCB	0	0	0	0	0	0	0	0
	FC/EC/ Strep.	0	0	0	0	0	0	0	0
	THB	5	13	15	192	417	2067	1016	722
SW10	TCB	0	0	0	0	0	0	0	0
	FC/EC/ Strep.	0	0	0	0	0	0	0	0

SW-Sachet Water

The counts of faecal Streptococci and Escherichia coli as depicted in Table 4 remained 0.0 cfu/ml in all of the different brands of sachet water samples tested during the course of the study period. Faecal Streptococci in potable water is a secondary indicator of faecal pollution while the presence of Escherichia coli confirms faecal pollution of potable water, which is unacceptable (WHO, 1996).

IV. CONCLUSION

This research sought to evaluate the effect of prolonged storage on the microbial and physico-chemical quality of sachet water samples produced within the Paynesville municipality of Liberia. The analytical results showed that prolonged storage caused an increase in pH up to week 6 followed by a decrease up to the end of the • experiment in all sachet water samples tested. The presence of dissolved oxygen coupled with the availability of nutrients aided continuous and rapid proliferation of microbes in the sachet water tested over time.

All sachet water samples analyzed exceeded WHO limit of 100 cfu/ml for total aerobic heterotrophic bacteria within weeks 3 and 8. Total coliform appeared in 40% of sachet water samples analyzed within first three weeks and died off. Faecal Streptococci and E-coli were undetected throughout the investigation period. The present study has revealed that sachet water when stored at room temperature for a long period can increase total aerobic heterotrophic bacteria to a level that may be harmful to human health.

V. RECOMMENDATIONS

The following recommendations are made based on the findings of this research:

• The expiry date of sachet water produced in the Paynesville Municipality of Liberia should not exceed three weeks from the date of production and the public should be sensitized not to drink sachet water that exceeds three weeks from the date of manufacture.

- Sachet water factories should be mandated by regulatory authorities to print the production and expiry dates of their water on the sachets, so as to appropriately guide consumers and prevent water borne diseases.
- The regulatory authorities should put in place standardized storage mechanisms that could help increase shelf life
- Regulatory authorities should conduct periodic sanitary inspections of sachet water factories to ensure conformity.

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VII. REFERENCES

- Adiotomre KO and Agbale NR (2015). Comparative analysis of sachet water samples sold in Benin City, Edo State, Nigeria. International Journal of Innovative Environmental Studies Research 3(3):22-35.
- [2]. Akinde SB, Nwachukwu MI and Ogamba AS (2011). Storage effects on the quality of sachet water produced within Port Harcourt metropolis, Nigeria. Journal of Biological Sciences 4: 157-164
- [3]. Allen M J, Edberg S C. and Reasoner D J. 2002. Heterotrophic plate count (HPC) bacteria - what is their significance in drinking water? Presented at the NSF International / WHO symposium on HPC bacteria in drinking water, April 22-24, 2002, Geneva, Switzerland. pp. 29-45.
- [4]. APHA 1998. Standard Methods for the Examination Of Water And Wastewater. 20th Edition, American Public Health Association, American Water Works Association, Water Environment Federation. United Book Press, Inc., USA.

- [5]. Ashbolt N J, Grabow W K. and Snozzi M. 2001. Indicators of microbial water quality. In: Water Quality Guidelines: Guidelines, Standards, and Health. Fewtrell L. and Bartram J. (Ed). World Health Organization Water Series. IWA Publishing, London. pp 289-315.
- [6]. Brock T D. and Madigan M T. 1988. Biology of Microorganisms. 5th Edition. Prentice-Hall International, London. pp. 835.
- [7]. Edbert DC, Sandra AU, and Ebere EC. 2017. Storage and its Effect on Chemical Quality Indicators in Sachet Water Brands Sold in Owerri Municipal, Imo State, Nigeria. World News of Natural Sciences. WNOFNS 12:73-81.
- [8]. Grabow W O. 1996. Waterborne Diseases: Update on Water Quality Assessment and Control. Water SA 22: 193-202.
- [9]. Liberia Institute of Statistics and Geo-Information Services (LISGIS), 2008. The enumeration of the 2008 population and housing census.
- [10]. Michael B.F., Ashley R.W., Mohammed F.J., George S., Robert E.S.B., and Jamie K.B. 2015. Microbiological and chemical quality of packaged sachet water and household stored drinking water in Freetown, Sierra Leone. PLoS One 10(7) 1-17.
- [11]. Omalu ICJ, Eze GC, Olayemi IK, Gbesi S, Adeniran LA, Ayanwale AV, Mohammed AZ. and Chukwuemeka V. (2010) Contamination of Sachet Water in Nigeria: Assessment and Health Impact. Online J of Health and Allied Sci., 9 (1): 1 3.
- [12]. Prescott L M, Harley J P. and Klein D. A. 1999. The influence of environmental factors on growth. Microbiology. 4th Edition. McGraw-Hill Companies, Inc., USA, pp. 123-132.
- [13]. Rusin, P.A., Rose, J.B., Haas, C.N. and Gerba, C.P. (1997) Risk assessment of opportunistic bacterial pathogens in drinking water. Rev. Environ. Contam. Toxicol. 152, 57–83.
- [14]. Warburton D W, Dodds K L, Burke R, Johnston M A. and Laffey P J. 1992. A review of the microbiological quality of bottled water sold in Canada between 1981 and 1989. Can. J. Microbiol., 38:12-19.
- [15]. WHO 1996. Guidelines for Drinking Water Quality: Health Criteria and Other Supporting Information.2nd Edition, Vol. 2 World Health Organization, Geneva.
- [16]. WHO 2001. Guidelines for Drinking Water Quality: Microbiological Methods. 2nd Edition, Vol. 1 World Health Organization, Geneva.