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Physico-Chemical Properties and Microbial Contamination of Sachet Water Produced in Gboko, Benue State-Nigeria

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Abstract This study was carried out to ascertain the physico-chemical properties and microbial contamination of sachet water sold in Gboko town. A total of five different sachet water products labeled A, B, C, D and E were purchased randomly in the Gboko market and analysed using the standard sensory, physico-chemical and microbial techniques. Sensory attribute such as; appearance, odour and taste of the samples were done using the human senses. The microbiological parameters carried out were Total Plate Count, coliform test, Streptococuus faecalis and Clostridium test. The Total Plate Count for each sample was; A (260), B (620), C (380), D (400), and E (360). The entire sample had the total plate count more than the NIS and WHO standard of 100. The entire sample has zero coliforms except 'B' water which had 120coliforms. Streptococcus faecalis and Clostridium species were not isolated in any of the samples. The physico-chemical parameters such as turbidity, pH, conductivity, total hardness etc were within the range recommended by NIS and WHO. Test for undesirable or toxic substance such as cyanide, mercury, iron, zinc, lead, arsenic etc showed the result within the approved NIS and WHO standard. From the analysis, the sensory attribute, physico-chemical, toxic and parameter of all water samples were satisfactory except the microbiological parameters which indicated the need for the secondary treatment of B sachet water.

Keywords Physico-Chemical, Microbial Contamination, Sachet Water Produced

1. Introduction

Ancient civilizations established themselves around water sources. While the importance of water quantity for drinking and other purposes was apparent to our ancestors, an understanding of drinking water quality was not well known or documented. Although historical records have long mentioned aesthetic problems (an unpleasant appearance, taste or odour) with regard to drinking water, it took thousands of years for people to recognize that their senses alone were not accurate judges of water quality. Water treatment originally focused on improving the aesthetic qualities of drinking water. Methods to improve the taste and odor of drinking water were recorded as early as 4000 B.C. Ancient Sanskrit and Greek writings recommended water treatment methods such as filtering through charcoal, exposing to sunlight, boiling, and straining [1].

Visible cloudiness (later termed turbidity) was the driving force behind the earliest water treatments [2], as many source of waters contained particles that had an objectionable taste and appearance. To clarify water, the Egyptians reportedly used the chemical alum as early as 1500 B.C. to cause suspended particles to settle out of water. During the 1700s, filtration was established as an effective means of removing particles from water, although the degree of clarity achieved was not measurable at that time. By the early 1800s, slow sand filtration was beginning to be used regularly in Europe. During the mid to late 1800s, scientists gained a greater understanding of the sources and

effects of drinking water contaminants, especially those that were not visible to the naked eye. In 1855, epidemiologist Dr. John Snow proved that cholera was a waterborne disease by linking an outbreak of illness in London to a public well that was contaminated by sewage. In the late 1880s, Louis Pasteur demonstrated the "germ theory" of disease, which explained how microscopic organisms (microbes) could transmit disease through media like water [3-6].

During the late nineteenth and early twentieth century's, concerns regarding drinking water quality continued to focus mostly on disease-causing microbes (pathogens) in public water supplies. Scientists discovered that turbidity was not only an aesthetic problem; particles in source water, such as feacal matter, could harbor pathogens. As a result, the design of most drinking water treatment systems built in the U.S. during the early 1900s was driven by the need to reduce turbidity, thereby removing microbial contaminants that were causing typhoid, dysentery, and cholera epidemics. To reduce turbidity, some water systems in U.S. cities (such as Philadelphia) began to use slow sand filtration. While filtration was a fairly effective treatment method for reducing turbidity, it was disinfectants like chlorine that played the largest role in reducing the number of waterborne disease outbreaks in the early 1900s. In 1908, chlorine was used for the first time as a primary disinfectant of drinking water in Jersey City, New Jersey. The uses of other disinfectants such as ozone also began in Europe around this time, but were not employed in the U.S. until several decades later [7-8].

Federal Regulation of Drinking Water Quality began in 1914, when the U.S. Public Health Service set standards for the bacteriological quality of drinking water. By the late 1960s it became apparent that the aesthetic problems, pathogens, and chemicals identified by the Public Health Service were not the only drinking water quality concerns. Industrial and agricultural advances and the creation of new man-made chemicals also had negative impacts on the environment and public health. In the 1970s and 1980s, improvements were made in membrane development for reverse osmosis filtration and other treatment techniques such as ozonation [9-11]. Some treatment advancements have been driven by the discovery of chlorine-resistant pathogens in drinking water that can cause illnesses like hepatitis, gastroenteritis, Legionnaire's Disease, and cryptosporidiosis. Other advancements resulted from the need to remove more and more chemicals found in sources of drinking water.

Recently, the Centers for Disease Control and Prevention and the National Academy of Engineering named water treatment as one of the most significant public health advancements of the 20th Century. Moreover, the number of treatment techniques and combinations of techniques, developed is expected to increase with time as more complex contaminants are discovered and regulated. It is also expected that the number of systems employing these techniques will increase due to the recent creation of a multi-billion dollar state revolving loan fund that will help water systems, especially those serving small and disadvantaged communities, upgrade or install new treatment facilities [12].

2. Materials and Methods

2.1. Materials

2. 1.1. Study Area

This study was conducted between May and June 2015 in Gboko municipality. Gboko is the capital of Gboko local government in Benue state, North-Central Nigeria. It is also the traditional capital of the Tiv people. The town lies on the latitude and longitudes 7^0 19˙30˝N $9^0$0˙18˝E/ 7.32500^{0}N 9.00500^{0}E. The estimated total population of the municipality during the period of the study was above 500,000. The municipality is located on the savannah region of Nigeria and is characterized by a sub-humid climate with dairy temperature of about 32^0 while the annual rainfall is between 1200-1600mm. Rainy season starts from April to October while dry season starts from November to March. Harmattan is experienced from early December to late January [13].

2.1.2. Sterilization Techniques

All glassware were thoroughly washed with detergent solution and rinsed with distilled water thoroughly then allowed to dry. They were allowed to drain, wrapped with aluminum foil and then sterilized in the oven at 180˚C for 1 hour. The workbench was swabbed with cotton wool soaked with 70% ethanol. All media were sterilized after

homogenizing in the autoclaving at 121˚C for 15minutes [14].All analyses were carried out closed to the naked bunsen burner flame.

2.1.3. Sampling Procedure / size

A total of 5 branded sachet water samples from five (5) different sachet water producing companies were collected in cool boxes and were taken to microbiology laboratory of University of Mkar for bacteriological examination The five brands of sachet water examined.

2.1.4. Media Used /Preparation

The culture media used for the isolation, enumeration and characterization of bacteria include: Nutrient agar (NA), MacConkey agar, KF-*Streptococcus* agar, and Litmus Milk Broth. These media were prepared in accordance with the manufacturer's specifications and sterilized using an autoclave at 121˚C for 15mins.

2.2. Methods

2.2.1. Sensory attributes

The sensory attributes of each water sample was determined using the human senses.

2.2.1.1. Appearance and Odour

Determination of water appearance was carried out using the physical eyes. 100 mL each water sample measured into 250 mL beaker was observed under bright light for the presence of any particulate matter [15].

A 20 ml volume of each water sample was poured into a clean beaker. The beaker was then shaken vigorously to check for any frothing and allowed to settle. The beaker was then observed under bright light for presence of any particulate matter and then brought close to the nose to test for any odor present [16].

2.2.1.2. Taste

Small volumes of each sample was tasted with the tongue and then immediately rinsed with taste free distilled water and the result was recorded accordingly.

2.2.2. Physico-chemical parameters

All the Physico-chemical parameters except total Hardness and Chloride are the ones that required titration method were checked using Multi-parameter Bench Photometer (C 99 & C 200 HI 83000 Series).

2.2.2.1. Determination of Turbidity

Turbidity of each sample was determined using Multi-parameter Bench Photometer (C 99 & C 200 HI 83000 Series). The blank was set to zero with distilled water. Each water sample was added into the photometer and the absorbance reading which displayed on the screen was taken [17].

2.2.3.2. Determination of pH

The pH value of each water sample was measured using an electric pH meter.100mL of the sample were measured and the pH electrode was inserted and stirred a little. The readings were then taken when the meter stabilized and indicated ready [18].

2.2.1.3. Electrical Conductivity

The electrical conductivity of each water sample was determined using conductivity meter. The meter was immersed in the test sample and the readings displayed on the meter were taken [18].

2.2.1.4. Determination of Total Hardness

The hardness of each water sample was determined using titration method. 10 mL of water sample was measured into a small size beaker. Six (6) drops of a buffer solution of approximate pH of 7.0 was added to the water sample and after which, One (1) drop of Maver hardness indicator was added to the mixture and the resulting solution was titrated against EDTA until a colour change of blue was observed. The drops of EDTA additive that caused the colour change above X 20 gave the total hardness of the water sample. (EDTA drops X 20 which is a constant value) [19].

2.2.1.5. Chloride

Test for chloride was done using titration method. One hundred millimeter (100 mL) of water was measured using a measuring cylinder. It was poured in a conical flask that has been properly rinsed with distilled water. Two(2)mL of

5% potassium chromate was added as an indicator and the solution turned yellow. It was titrated with 0.1M silver nitrate until there was a faint pink coloration.

Total chlorine = Titre value of 0.1M $AgNO_3$x 3.55 [20]

2.2.3. Microbiological analysis

2.2.3.1. Total Plate Count

The total plate count method relies on bacteria growing a colony on a nutrient medium so that the colony becomes visible to the naked eye and the number of colonies on the plate can be counted. The laboratory procedure involves making serial dilutions of the samples. One (1) ml of each water sample was serial diluted into 9ml of sterile de-ionized water. After serial dilution, 0.1 ml of the diluents was inoculated into a sterile Petri-dish and a cool molten nutrient agar medium was poured into the Petri-dish and the mixture was agitated gently and allowed to solidify before it was incubated aerobically at 37^{o}C for 24 hours. After overnight incubation, colonies on the nutrient agar plate were observed and recorded as colony forming unit per mL (CFU/mL) [21].

2.2.3.2. Total Coliform Count

A standard coliform count was performed by the membrane filtration technique. 100 mL of the water sample was passed through a 0.45 um membrane filter under suction system. The filter paper was then removed and aseptically placed unto a sterile Petri-dish containing sterile macConkey Agar. The plates were incubated aerobically at 35^o for24 hours after which the plates were examined and the number of coliform colonies were counted and expressed as total coliform per 100 mL of water [22].

2.2.3.3. *Streptococcus faecalis* Test

The test for the *Streptococcus* fecal was carried out by employing KF- *Streptococcus* agar. The agar is a selective medium used for the isolation and enumeration of *fecal enterococci* in water, food stuffs and some minerals. Using Pour Plate method, 0.1 ml of the test water sample was placed into a sterile petri-dish and 15ml of prepare KF-*Streptococcus* medium at 45^{o}C was poured into the petri-dish. The mixture was thoroughly mixed and the agar was allowed to solidify after which, the plates were incubated in an inverted position at 35± 2^{o}C for 45-48 hours. Membrane filter technique can also be employed. The red or pink colonies were counted as *Streptococ*ci *faecalis*, while colonies with orange, yellow, white or other colors were not counted. The number of fecal *enteroccoci* was calculated per 1ooml of water [23].

2.2.3.4. *Clostridium* Species Test

The test was carried using Litmus Milk Broth. The medium is used for maintenance of lactic acid bacteria and for determination of bacteria action on milk. It is especially useful in species differentiation within the genus *Clostridium.* The medium prepared in test tubes was boiled for 2 minutes and then cooled with tightened caps to room temperature. A pure culture of *Clostridium* species grown on cooked meat medium was inoculated into the medium. Immediately after incubation, the medium was over-layered with 1ml mineral oil (Vaspar). The test tubes were incubated aerobically with loosened caps at 35± 2^{o}C. Some test tubes were incubated anaerobically with tightened caps at 35± 2^{o}C and were examined for 7 days for reactions. Fermentation of lactose and/ or dextrose in the milk with production of acid (pink color) including stormy fermentation (strong evolution of gas) indicates the presence of certain *Clostridium* strains (American Type Culture Collection, 2006).

3. Results and Discussion

3.1. Results

Table 1 above shows the sensory attributes of five different sachet water products in Gboko town. All the samples have clear appearance, and for odour there were all unobjectionable. The taste of the water samples was not carried out since the samples had microbial growth.

Table 1: Sensory Attributes of five different sachet water examined in Gboko town

Sample	Sensory Attributes		
	Appearance	**Odour**	**Taste**
A	Clear	Unobjectionable	Not tasted
B	Clear	Unobjectionable	Not tasted
C	Clear	Unobjectionable	Not tasted
E	Clear	Unobjectionable	Not tasted
C	Clear	Unobjectionable	Not tasted
NIS/WHO STANDARD	Clear	Unobjectionable	Not tasted

Table 2 below shows the microbiological index of five sachet water samples examined in Gboko town. The total plate count for each sample was: A (260), C (380), D (400) and E (360). None of the samples had coliform except B Water which had 120 coliforms. *Streptococcus faecalis* and *Clostridium species* were not isolated in any of the samples.

Table 2: Microbiological Index of five sachet water examined in Gboko town

Sample	Microbial Index (CFU/mL)			
	Total Plate Count	**Coliforms**	**Streptococcus *Faecalis***	**Clostridium spp**
A	260	0	0	0
B	620	120	0	0
C	380	0	0	0
D	400	0	0	0
E	360	0	0	0
NIS/WHO STANDARD	100	0	0	0

Table 3: Physico-chemical parameters of selected sachet water products in Gboko Town, Nigeria

Parameter	Sample					
	A	**B**	**C**	**D**	**E**	**NIS/WHO Standard**
Settleable solids	Absent	Absent	Absent	Absent	Absent	-
Turbidity (NTU)	1.1	1.1	1.1	1.1	1.2	≤ 5.0
pH	6.88	7.08	6.56	6.63	6.51	6.5-8.5
Conductivity (ms/cm)	47	11	58	52	80	1000
TDS (MG/L)	25	4	30	27	42	500
Total Hardness	11.3	2.5	14.6	12	20	100
Chloride (mg/L)	3.6	0.5	4.2	2.8	2.5	100
Sulphates (mg/L)	2.4	0.2	1.6	1.1	1.3	100
Nitrates (mg/L)	0.8	0.1	0.7	0.4	0.3	10
Total Alkalinity (mg/L)	32	35.6	23.8	26.5	22.6	100
Oxidizability (mg $KMnO_4$)	0.6	1.7	1.5	1.2	0.8	5.0
Nitrates (mg/L)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.1
Free chloride (mg/L)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.1

Table 4: Toxic parameters of selected sachet water samples in Gboko Town, Nigeria

Parameter	Sample					
(Mg/L)	**A**	**B**	**C**	**D**	**E**	**NIS/WHO**
Arsenic	0	0	0	0	0	0.1
Sulphide	0	0	0	0	0	0.01
Iron	0.18	0.11	0.03	0.14	0.05	0.3
Cadmium	0	0	0	0	0	0.003
Copper	0	0	0	0	0	1.0
Cyanide	0	0	0	0	0	0.05
Lead	0	0	0	0	0	0.05
Mercury	0	0	0	0	0	0.001
Zinc	0.4	0.8	0.1	0.4	0.6	5.0

The above table 3 shows the physico-chemical properties of selected sachet water samples in Gboko town. The physico-chemical parameters for all samples were within the range given by National Industrial Standard (NIS)/ World Health Organization (WHO).

Table 4 shows the amount of the undesirable or toxic substance of selected sachet water samples in Gboko town. The parameters were within the range given by NIS/WHO for all samples examined.

3.2. Discussion

A study on the physico-chemical and Microbial contamination of selected sachet water produced in Gboko town was carried out. Results obtained from the sensory attributes, physico-chemical parameters and undesirable or toxic substances confirm (corroborate) with the set standard by National Industrial Standard (NIS) and World Health Organization (WHO) for all the samples examined. For the sensory attributes, all the samples tested were clear in their appearance and all the samples tested were of unobjectionable taste and odour. The microbiological parameters carried out on all the samples did not meet the set standard by NIS and WHO.

The microbiological parameters carried out were: Total Plate Count, Total Coliform Count, *Streptococcus faecalis* and *Clostridium* species test. The Total Plate Count for each sample was; A (260), B (620), C (380), D (400) and E (360). All the samples had Total Plate Count more than the NIS and WHO standard which is 100. All the examined samples had zero (0) number of coliforms except for Sample B water which had 120coliforms. *Streptococcus faecalis* and *Clostridium* species were not isolated in any of the samples. The physico-chemical parameters such as turbidity, pH, conductivity, Total Dissolved Solids, Total Hardness among others were within the standard recommended by NIS and WHO. Test for the Undesirable or Toxic such as Cyanide, Mercury, Iron, Lead, Arsenic, and Zinc among others were also within the approved NIS and WHO standard. The less in the availability of undesirable or toxic substances is attributed to the good source of raw water used for treatment. Borehole which is the source of raw water in Gboko town is proven unequivocally by water Chemists to have less toxic substances than the open source of water such as rivers, streams, ponds, lakes, oceans, seas which are contaminated by domestic and industrial effluents.

Based on the analysis and the results obtained, the sensory attributes, physico-chemical and undesirable or toxic parameters of all the sachet water samples examined were satisfactory except the microbiological parameters. The presence of Total Plate Count more than 100 colonies in all the samples examined is an indication of either of the reasons below; poor treatment (inadequate treatment method), contaminated packaging machine, poor packaging and storage methods. The availability of coliforms in one of the samples indicates that, the raw or treated (processed) water was faecally contaminated.

Some of the results obtained from physico-chemical parameters are similar with studies conducted [2, 25, 26] on the Quality of Sachet Water and Bottled Water in Bolgatanga Municipality of Ghana.

4. Conclusion

Water is the essence of basic survival. Without it, life on Earth would cease to exist. When the quality of drinking water is good, human health is also good. The results obtained from this study are supportive with the conclusions that, the qualities of the sachet water samples with respect to the determined sensory attributes, physico-chemical and undesirable or toxic parameters are within the National Industrial Standard (NIS)/ World Health Organization (WHO) permissible limit. But in terms of the microbiological quality, the samples did not meet the set standards by the above regulatory agencies.

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