Microbiological and Physiochemical Characterization of Sachet Water Samples Marketed in Nsukka Campus of the University of Nigeria

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Abstract

Physicochemical, biochemical and bacteriological studies of ten (10) brands of sachet water commonly found in the University of Nigeria Nsukka campus were carried out to determine the potability of these sachet water samples for use in the school. Standard conventional methods were employed for the detection of coliforms and other bacteria; physical examination for organoleptic quality such as taste, colour and odour; microscopic examination for sediments and other debris and/or bacteria, protozoa and fungal hyphae as well as chemical and biochemical analyses. Bacteriological examination of samples revealed the presence of the following pathogens: (Log 10 cfu/ml): E. coli (2.0), Streptococcus species (4.95), Micrococcus species, (4.62), Bacillus species (5.04), Staphylococcus species, (5.11), Yeasts (4.06), Actinomycetes (3.11) Klebsiella (2.49) and Pseudomonas species (3.10). Physical examination of samples showed a variable level of taste, turbidity, odour and colour. Chemical analysis on the other hand indicated the presence of metals ranging from lead (0.09 – 1.90 ppm); iron (0.137-0.194 ppm); chromium (0.002 – 0.06 ppm) and aluminum (0.015-0.315 ppm). Absence of copper was apparent from the study. In conclusion, the bacteriological and physicochemical indices of contamination detected from majority of the sachet water samples are indications that the 'pure water' available in the University environment do not meet neither the NAFDAC (2004) nor the WHO (2003) standard and so may not be suitable for drinking purposes.

Keywords: Sachet water, Physicochemical, Biochemical and bacteriological studies, Coliform bacteria

Introduction

The quest for cheap and readily available source of potable water has led to the emergence of sachet water. Packaged water is defined as any potable water processed and offered for sale in sealed food-grade bottles or other appropriate containers for human consumption, (Food Drug Administration, 2002). In Nigeria sachet water is popularly known as 'pure water'.

With the significant increase in sachet or bottled water consumption, there has arisen a growing concern over the microbiological and chemical quality of these products (Anne, 2002). Bottled or sachet water like any other food product, must be processed and packaged under aseptic conditions. Packed water however is generally not sterile, being collected from almost every available water source, ranging from rainwater to tanker-borne water most of which are rusty and unwashed. Contaminants are also introduced during manufacturing and consumer handling, (Warburton and Austin, 1997). Irrespective of their sources, these products are susceptible to microbial contamination. Furthermore the absence of such sterilization procedures such as pasteurization and thermal sterilization for the treatment of pure water increases 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. These further increase the contamination of these products with resultant deleterious public health problems.

Analysis of water samples for presence of microorganisms in relation to human health requires determining principally the pathogenic organisms (Gordon, 1990), the most significant of which are faecal bacteria. The organisms most commonly used as indicator of faecal pollution are the coliform bacteria. Coliform bacteria occur in high numbers in human faeces and can be detected at occurrences as low as one bacterium per 100 ml; therefore they are sensitive indicators of faecal pollution. Organisms found in water are involved in food poisoning, and frequent outbreaks of water-borne infections (cholera, salmonellosis, gastroenteritis, shigellosis etc). Among the heavy metals of medical importance found in water are: aluminum, copper, lead, chromium and iron.

The US Environmental Agency health-based standards (Maximum contaminant levels or MCLS in milligrams per litre (mg/L), approximately equivalent to parts per million (ppm) is as follows: Aluminum: 0.2 mg/l; Copper: 2.0 mg/l; Chromium: 0.05 mg/l; Lead: 0.01 mg/l; Iron: 0.03 mg/l. Minimum standards are generally accepted for the coliform bacteria. However, the stipulated criteria indicate that:

(1) In any year, 90% of sample taken at any point of the system should be coliform free.
(2) Maximum count in any positive test should be 10 coliforms per 100 ml.
(3) No sample should contain more than two *E. coil* per 100 ml.
(4) No sample should contain 1 or 2 E. coil per 100 ml in conjunction with a total count of 3 or more per 100 ml
(5) Coliform should not be detectable in 100 ml of any consecutive samples.

It is against this background that this work was carried out with the view of ascertaining the microbiological standards as well as physicochemical compositions of locally available sachet water with resultant safety and potability indices. Despite the large market for bottled water today, there has been relatively few investigations into the public health aspects of these products (Hunter and Burge (1987). Von Wiesenberger (2004) pointed out that some water have very short transit time making them potentially susceptible to contamination. Experimental analysis have reported that some microorganisms are associated with drinking water and that bottled or sachet water cannot undergo any treatment such as pasteurization, and thermal sterilization for the elimination of these microorganisms (Cruickshank, 1968), and when these natural water serve as industrial water for the manufacture of packaged water, there may be the possibility of these packaged water becoming contaminated with these chemicals and heavy metal which may have serious effect on health. Consequently, this study is aimed at examining the physicochemistry and microbiology of sachet water with the view of ascertaining whether these meet the standard to be considered safe for drinking.

Materials and Methods

Collection of samples: The water samples used for this work were sachet or packaged water samples from ten different manufacturers within and outside Nsukka metropolis.

Sample preservation: Sachet water samples from 10 different sources were filled in 100ml amounts into sterile plastic disposable bottles and preserved in aseptic conditions as recommended by the standard methods of Greenberg (1992). Samples were refrigerated at 4°C and analyzed within 24h of collection

pH measurement: This was carried out using a micro-pH meter (pH 600 –Milwaukee) standardized with buffer solutions to about pH 7 and 14 as described by Walter (1981).

Physical examination: Physical examination was aimed at detecting odour, taste, colour, and turbidity in water and the measures of its acceptability or attractiveness to consumers. This involved careful examination of samples for some organoleptic parameters: taste, odour as well as colour, turbidity/presence of sediments.

Odour: Ten 50 ml wide-mouthed glass-stoppered bottles were rinsed with 4M hydrochloric acid until completely odourless, and then re-rinsed with distilled water. The bottles were half-filled with each sachet water sample, stoppered, and shaken vigorously for 2-3 seconds. The stoppers were then removed and bottles observed for odour by putting the nostril near the mouth of the bottle.

Taste: Stoppered bottles were rinsed as described above. Aliquots of each sample were then poured into each bottle and the taste noted immediately.

Colour: Colour profile of each sample was measured by comparing the water samples against a standard prepared from potassium chloro-platinate (K_2PtCl_2) tinted with small amount of cobalt chloride, ($CoCl_2.6H_2O$) which yielded colour very much like water. The standard and test samples were each dispensed into nesler tubes to 50 ml volume and then placed on white surfaces (white tiles) to match the colour of standards against samples. The colour of the sample closest to the standard was selected and the corresponding units recorded.

Acidity Profile: Acidity of samples was measured using the method of Greenberg (1992). Preparation of reagents for the acidity profile was carried out using 100 ml of deionized (Carbon dioxide free) water.

Alkali Standard: Alkali standardization described by Haris and Kratochvil (1981) was carried out by titrating 14.5 ml of $KHC_8H_4O_4$ against 0.02 NaOH. Normality was calculated as follows: Normality = A x B /204.2 x C; where A = $KHC_8H_4O_4$ (g), B = $KHC_8H_4O_4$ (ml) and C = NaOH (ml).

About 100 ml of each sachet water sample was dispensed into 500 ml capacity Erlenmeyer flasks, and to these were added 0.15 ml (3 drops) phenolphthalein indicator and titrated against the standard 0.02N NaOH until faint pink colour development at pH 8.3. Acidity was calculated as follows: Acidity in $CaCO_3$ mg/L = A x N 50000/100 ml sample; where A = NAOH (ml) – titrant and N = NaOH normality.

Microscopy: Microscopic examination of the specimens was carried out to check for ova, cysts, worms, and trophozoites of protozoa. Ten milliliters (10 ml) of each sample was concentrated by centrifugation and a loop of the deposit then viewed under X40 objective of the light microscope.

Viable Bacterial Count: One milliliter (1 ml) of each sample was serially diluted (10-fold) to thin out the microbial population in previously sterilized Ringers solution and 0.1 ml of 10^{-3} and 10^{-4} of each sample was dispensed into nutrient agar and MacConkey plates, spread – inoculated (to ensure even distribution) and then incubated at 37°C for 24 h. Viable bacterial count was carried out using the hand lens, following which discrete colonies were transferred into slants for Gram staining reaction and other biochemical tests according to the method of Cheesbrough (1991).

Presumptive Coliform Test: Coliform test was performed to detect coliform bacteria (using *E. coli* as the indicator organism) in the water samples according to the method described by Cheesbrough (1991).

Determination of Most Probable Number (MPN) of Coliforms: Number of positive tubes with acid (yellow coloration) and gas production were matched with the McCrady's Statistical Table, and the most probable number (MPN) of Coliforms present in 100 ml of each sample was thus determined. For the confirmation test, a loopful of cultures from the presumptive test was inoculated into brilliant green broth containing *Durham tubes* and incubated for 48 hrs at 37^{0}C. Gas production confirmed presence of *E. coli.* Cultures were further inoculated into eosin methylene blue medium and incubated at 37^{0}C for 24 hrs. A positive test was indicated by purple-green metallic sheen on the surfaces of the colonies.

Determination of heavy metals: Heavy metal analysis described by Welcher (1965) was performed to ascertain the level of these in the test water sample as these have been reported to cause serious health problems such as cancer, learning inability, gastric irritation, etc. when found in concentration higher than the tolerant concentration.

Copper: In the determination of copper, diphenylthiocarbazone was added to the water sample which formed dithizonate complex. Citrate and cyanide were then added to the aqueous solution to prevent other metals from forming complexes. The copper was then recovered from the complex by extraction with very dilute nitric acid and estimated using dithizone at pH 9-9.5 and the red colour measured absortiometrically at 520 nm.

Lead: Sodium diethyldithiocarbamate was added to the water sample producing a yellow-brown coloration. Citrate was added next to prevent interference due to iron and precipitation of phosphate after which gum arabic was added to stabilize the colour. The yellow colour carbamate complex was then extracted using carbon tetrachloride and the absorbance measured using a spectrophotometer at 440 nm.

Chromium: Diphenylcarbazide was added to the water sample to yield a red-violet complex. This complex was stable for about an hour and its absorbance measured at 540 nm.

Iron: Ten (10) -phenanthroline in aqueous solution was added to the sample to produce an orange-red complex. Concentrated H_2SO_4 was first added to destroy all organic matter in the sample. Fe (II) was oxidized to Fe (III) and all interfering anions removed and the solution made more acidic using concentrated HCl and Fe (III); then extracted using isopropyl ether. Water was used to re-extract Fe (III) and then reduced to Fe (II) using hydroxylamine. The solution was buffered using ethanoate (acetate) and finally orange-red complex was formed upon the addition of phenanthroline. The absorbance was read at 510 nm.

Aluminum: Aluminum was quantitatively determined by 8-Hydroquinoline from an acetic acid-acetate buffer solution; 5 ml of the digested sample was pipetted into a test tube. 5 ml of acetic acid-acetate buffer was added to the sample to bring the pH to 4.5 and the allowed to stand for 20 mins. The sample was centrifuged at 10, 000 rpm for 20 mins. The supernatant was decanted into another test tube, while 2 ml of 8 hydroquinoline was added and absorbance read at 510 nm.

Results

Results of the microscopic view of analyzed sachet water samples revealed the absence of ova, cysts or trophozoites of protozoa in the test samples.

Table 1 presents the results of physical examination of analyzed samples. Taste of Chlorine and rancidity were apparent in samples A, E, F, and H. Odour was detected in only one sample. Colour however met the acceptable standard which should not exceed 15 TCU. Result of chemical analysis of samples for heavy metals is presented in Table 2. Samples exceeded the NAFDAC, SON (1990) and WHO tolerant concentrations for lead, while sample A, E, F and H exceeded tolerant ratios for chromium. However, all samples met the NAFDAC required standard for aluminum and iron.

Table 1: Physicochemical Analysis of Sachet Water samples

Sample	Colour (TCU)	Turbidity	Odour	Taste	pH at 20°C	Acidity
A	15	NT	+	+	6.00	2.20
B	13	NT	-	-	6.54	23.46
C	12	NT	-	-	6.60	2.50
D	14	NT	-	-	6.50	5.40
E	15	NT	-	+	6.10	2.30
F	13	NT	-	+	6.02	2.40
G	13	NT	-	-	7.10	5.60
H	12	NT	-	+	6.00	2.40
I	14	NT	-	-	7.20	7.20
J	15	NT	-	-	6.50	5.50

Legend: TCU = True colour unit, NT = not tested, - = negative, + = positive

Table 2: Heavy metal analysis of sachet water samples

Sample	Lead (mg/ml)	Iron (mg/ml)	Chromium (mg/ml)	Aluminum (mg/ml)	Copper (mg/ml)
A	0.178	0.147	0.060	0.280	-
B	0.145	0.194	0.002	0.100	-
C	0.048	0.137	0.023	0.110	-
D	0.018	0.120	0.012	0.030	-
E	0.170	0.184	0.063	0.315	-
F	0.162	0.147	0.060	0.251	-
G	0.110	0.190	0.012	0.025	-
H	0.190	0.160	0.065	0.176	-
I	0.09	0.150	0.042	0.015	-
J	0.015	0.146	0.002	0.100	-

In Table 3, results of the viable bacterial counts obtained from bacteriological assay of test sachet waters is shown. Significant bacterial counts (in colony forming units- cfu/ml) was observed (p = 0.05). The result obtained showed significant amount of gas production in four of the samples

tested. In Eosin Methylene blue medium, there was also purple metallic sheen on the colonies, thus showing the presence of *E. coli* in the water samples.

Table 3: Total viable bacterial count of isolates from test samples

Isolate	Counts (Log 10 cfu/ml)
Staphylococcus aureus	5.11
Bacillus species	5.04
Streptococcus species	4.95
Micrococcus species	4.62
Yeasts	4.06
Actinomycetes	3.11
E. coli	2.0
***Klebsiella* spp**	2.49
***Pseudomonas* spp**	3.10

Discussion

Results of the physicochemical and bacteriological assay on the sachet water of common use within the University of Nigeria environment indicates that some of the sachet waters did not meet the required NAFDAC or WHO standard for potable water. From the physicochemical analysis, objectionable odour and taste were observed in some samples. This could be attributed to several factors such as the presence of both inorganic compounds (ammonia, sulphides, chlorides and cyanides); organic substances including unsaturated hydrocarbons as well as bacterial contaminants which could have been introduced during the processing, packaging or distribution stages. The Standard Organization of Nigeria (SON) and NAFDAC maximum allowed limits (TCU) are 100 mg/L; while the WHO maximum permissible limit is 250 mg/L. These permissible values do not impart Chlorine taste or odour to potable water. Result of the analysis showed that Chlorine taste was apparent, suggesting that the value was above the permissible range, and thus the possibility of residual Chlorine, defined as the amount of chlorine in excess of the required level for the treatment of water.

The Institute of Public Analysts of Nigeria (IPAN), indicated that pH of water is one of the most important water parameters. It is a measure of the acidity or alkalinity of water (IPAN, 2005). An optimal pH range is of immense necessity for the clarification and disinfection of potable water, while a range outside the acceptable could enhance the rancidity and subsequently presence of malodour and objectionable taste as observed in the results. Four of the samples (pH - 6.00, 6.40, 6.02, and 6.00) deviated from the WHO permissible pH range (6.5- 8.0). From visual observation, the tested samples were however free of particles.

Result of the chemical analysis indicated the presence of chromium, lead, iron and aluminum. Samples A, E, F and H had higher values of the above than others. This result conforms to earlier report by Pocock *et al.* (1984), that water found in nature generally contains a variety of contaminants such as mineral salts, heavy metals, organic chemicals, and radioactive residues. Therefore when these natural waters serve as industrial water for the manufacture of packaged water, there may be the possibility of these packaged water becoming contaminated with these chemicals and heavy metals which may have deleterious effects on health. The findings in this work are further confirmed by the reports of Goyer (1991), who showed that drinking water containing heavy metals like chromium, aluminum and lead in concentrations higher than the tolerant concentrations have detrimental effect on health resulting to cancer, learning inability and behavioral problem in children.

The isolation of pathogens including *Streptococcus species, Staphylococcus aureus, Micrococcus species, Klebsiella species, Bacillus species, Pseudomonas species, E. coli,* Yeasts and Actinomycetes is indication of serious contamination of samples. The presence of contaminating bacteria such as *Staph aureus, E. coli* could account for the incidence of diarrhea, food poisoning and gastroenteritis common in the University environment especially among the undergraduate students. Results of this study conforms to the finding of Hunter and Burge (1987), who reported that food intoxication could occur due to the presence of bacterial pathogens such as *Staphylococcus* and *Bacillus* in drinking water. Faecal coliforms such as *E. coli,* are the classical indicators of coliform contamination of water. These occur in high numbers in human faeces and can be detected at occurrences as low as one bacterium per 100 ml. US Environmental Agency standard (Maximum contaminant levels or MCLS) for coliform bacteria states that maximum count in any positive test should be 10 coliforms per 100 ml. However, results of the study showed the presence of the Coliforms: *E. coli, Klebsiella* and atypical *E. coli* at values above the maximum accepted standards, an indication of high faecal contamination and consequently the danger of food poisoning and other related gastrointestinal disorders arising from consumption of these sachet waters. The presence of pathogenic organisms including bacteria, yeasts and actinomycetes in the sachet water samples analyzed are further indications that these waters do not undergo appropriate sterilization techniques, and so do not conform to either the NAFDAC or WHO standards for potable water. These views are supported by the report of Cruickshank (1968) indicating that some microorganisms are associated with drinking water; and since bottled water cannot undergo any treatment such as pasteurization, and thermal sterilization for the elimination of these microorganisms, they are never free from bacteria (Guilot and Leclers, 1993). This is because the sources of most sachet water are shallow wells or tanker-delivered water (sources of which remain questionable, just as the tankers are never washed or sterilized).

The quest for quick money has resulted in "pure water" business and the associated inability to pass through treatment processes to remove all pathogenic organisms and heavy metals that have caused serious health problems. Sachet water, in spite of being sealed is thus observed to constitute health risk to consumers due to the presence in the water of pathogenic microorganisms often associated with food poisoning and intoxication as

well as hazardous heavy metals and their associated risk factors.

In conclusion, it is suggested that the pure water business should be critically reviewed by NAFDAC to ensure that producers comply with standards at every stage of the production and distribution processes. Defaulters should be banned, and fake NAFDAC numbers removed. Follow-up strategies by NAFDAC will help obliterate the menace of production and sale of untreated sachet water (which incidentally bears NAFDAC numbers). However it is observed from this study that every effort is made by an intending pure water producer to acquire the NAFDAC number as a license for production, and once this is got, no further attempt is made to ensure that subsequent productions comply with NAFDAC standards. NAFDAC should also educate both the producers as well as consumers on the health hazards of untreated or contaminated sachet waters.

References

Anne, C. B. (2002). Chemical and Microbiological Quality of sachet water. www.fda.gov/fdac/featrues/402/h20.html 20k.

Cheesbrough, M. (1991). *Medical Laboratory Manual for tropical Countries.* Vol. 2. Cambridge Press: Cambridge, pp. 9-70.

Cruickshank, R. (1968). Medical Microbiology: A Guide to the Laboratory Diagnosis and Control of Infection. E. And S. Living Stone Ltd. London, England. Pp 963-978

Food Drug Administration. (2003). http://fdaus.net/english/html/2003/oct/contact US.html.

Guilot, E. and Leclers, H. (1993). Biological specificity of bottled mineral water. *Journal of Applied Bacteriology.* 74: 345-352.

Goyer, R. A. (1991). Toxic effects of metal. In: *Casarett and Doull's toxicology: The basic of poisons.* (4th ed.). Permagon Press, London. Pp. 662-663.

Gordon, A. M. (1990). *Drinking Water Microbiology.* Spinger-Verlag New York. Pp. 429-437.

Greenberg, A. (1992). Standard Methods for the Examination of Water and Waste Water. 18th Edition. APHA-AWWA-WEF. Washington D.C.

Hunter, P. R. and Burge, S. H. (1987). The bacteriological quality of bottled waters. Epidemiology and Infection. 99: 439-443.

The Institute of Public Analysts of Nigeria (IPAN) (2005). Pre-admission Training Workshop on Foods, Drugs, Cosmetics, Water, Environment and Petroleum. Proceedings.

National Food and Drug Agency (NAFDAC). (2004). NAFDAC To Ban Sachet Pure Water – 97% Samples Contaminated. In: *Consumerlink* Vol. 1, No. 1, pp. 1-3, July.

Pocock, S. J., Shaper, A. G., Ashby, O., Delves, T., and Whitehead, T. P. (1984). Blood lead concentration, blood pressure, and renal function. *Bio. Med. J.* 289: 872-874.

Standard Organization of Nigeria (SON). (1990). Standard Organization of Nigeria Act. *Nigerian Industrial Standards* Chp. 41 No. 8.

Von Wiesenberger, (2004). The Hidden Messages in Water. www.bottledwaterweb.com./booksandvideos.jsp

Walter, L. (1981). Handbook of Water Purification. McGraw-Hill Book Company, U.K., England

Warbuton, D. W. and Austin, D. W. (1997). Bottled water. In: *Microbiology of Food.* Chapman and Hall press, London. Pp. 694-895.

Welcher, F. J. (1965). Standard Methods of Chemical Analysis. Sixth Edition. D. Van Nostrand Company Inc. New York.

World Health Organization (WHO). (2003). MCPA in Drinking Water. Background Document for Preparation of WHO Guidelines for Drinking Water. Geneva, World Health Organization (WHO/SDE/WSH/03,04/38).