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## Bacteriological and physico-chemical analysis of sachet water in North Central Nigeria

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### Abstract:

Fifteen samples, made up of five brands of sachet packaged water samples labelled as A, B, C, D and E in Minna metropolis were examined for bacteriological and physico-chemical properties. The bacteriological quality of the water samples were examined using multiple tube techniques (MPN). All the five brands of packaged sachet water samples contained bacteria. Brand A showed a high average index of 398MPN/100ml, brand B has 49MPN/100ml, brand C has 18.8MPN/100ml and brand D had 7MPN/100ml while brand E had 12MPN/100ml. The isolates were identified as *Escherichia coli*, *Streptococcus faecalis*, *Bacillus subtilis*, *Staphylococcus spp*, *Pseudomonas aeruginosa*, *Klebsiella spp* and *Salmonella typhi*. The physico-chemical parameters were also determined using standard methods; whose values fell below WHO standards for drinking water except manganese whose values exceeds the maximum permitted limit for water quality of the standard organisation of Nigeria (SON).

**Key Words:** Bacteriological quality, Multiple tube techniques, Packaged water.

### Introduction:

Good quality water is odourless, colourless, tasteless, and free from faecal pollution [1]. A reliable supply of clean wholesome water is highly essential in a bid to promoting healthy living among the inhabitants of a defined geographical region [2]. Safe and potable water supplies in urban centres in Nigeria are still inadequate in spite of four decades of independence and several efforts from various governments [3]. The standard industrialized world model for delivering of safe drinking water and sanitation technology is, however, not affordable in most of the developing world [4]. Consequently, given the renewed global commitments towards the Millennium Development Goals (MDG) marked for 2015, the importance and contribution of locally sourced low-cost alternative drinking water schemes to sustainable access in rural and urban settings of developing nations cannot be overemphasized [5]. One of such local intervention in Nigeria where public drinking water supply is unreliable is drinking water sold in polythene sachets. Water in sachets is readily available and affordable, but there are concerns about its purity. The integrity of the hygienic environment and conditions where the majority of the water in sachets are produced has been questioned [6]. Apart from environmental contaminants, contamination from improper vendor handling also poses threats to the health of the ignorant consumers who drink often times without any proper cleaning of the sachets. Previous studies have identified handling as the source of infection in food and water-borne diseases in several countries [4]. Water related diseases continued to be one the major health problems globally [7].

The National Agency for Food and Drug Administration and Control (NAFDAC) is mandated to enforce compliance with internationally defined drinking water guidelines, but regulation of the packaged water industry aimed at good

quality assurance has remained a challenge to the agency [6]. To control this menace of contaminated water in sachets, NAFDAC declared a possible 'gradual' nationwide ban on sachet water to allow manufacturers of sachet water to start winding down or change to bottle packaging [6]. Successful implementation of this ban has remained far from reality as the sachet water market is witnessing tremendous growth, especially among the poor and middle social classes. Few studies conducted in recent years on the quality of packaged water in Nigeria focused primarily on the end-product, leaving out the processes that determine the fate of the packaged water, and the people (various stakeholders involved) in whose hands lie the will and power to effect the desired change. Consequently, practicable recommendations aimed at changing the status quo have not yet emerged. This paper investigated the physicochemical, microbial quality and health impact of sachet water produced in North Central Nigeria.

### Materials and methods:

#### Sample Collection

Five brands of packaged water samples labelled A, B, C, D and E were collected from different locations in Minna metropolis, North Central-Nigeria and taken to the laboratory for analysis.

#### Methodology

##### Physicochemical Analysis

The physical parameters included temperature, pH and electrical conductivity. Chemical parameters included dissolved oxygen was carried out by titration method [7], total alkalinity, total hardness determined using EDTA titration method [8], Calcium, Magnesium, Phosphate (PO<sub>4</sub>), Nitrate (NO<sub>3</sub>) and heavy metals (cu, fe, pb, cr, mn,) were determined [9].

### Culture Media

All apparatus (glass wears) used were thoroughly washed with detergent and rinsed several times with the water, glass wears were then air dried before sterilization in an oven at 160°C for 2 hours. Media used include Nutrient agar, Salmonella/shigella agar, Eosine methylene blue agar and MacConkey agar. All the media used were sterilized by autoclaving at 121°C for 15 minutes and prepared according to the manufacturer's specification.

Presumptive test was performed using the multiple tube method. After inoculation of the media with the samples, the culture tubes were incubated at 37°C for 2 hours before transferring them to 44°C incubator for 18 hours. After incubation the cultures were inspected for changes in colour and gas production. Those showing growth with or without gas production were noted. Those showing no changes in colour were re-incubated for additional 24 hours. The tubes showing changes in colour were counted and the MPN count was expressed per 100 ml of sample as

per the MacGrady's probability Table. The cultures that showed growth were also sub-cultured on the MacConkey agar plates to obtain discrete colonies to facilitate easy isolation and identification of the predominant organisms. Standard methods for water analysis as described by the American Public Health Association [10] were employed. Coliform counts were expressed as cfu/100ml. For gram staining was also performed.

### Results:

The most probable number (MPN) for the presumptive total coliform count of the water samples ranges from 7 to 398 MPN/100 ml (Table 1). It indicates that water from brand A had the highest total coliform counts of 398 MPN/100ml followed by water from brand B having 49 MPN/100ml while the least was that of brand D. showing that the total coliform count of these water samples were grossly contaminated.

Table 1. Presumptive test values obtained from various packaged water samples using MPN techniques.

Samples	1				2				3				Average MPN index per 100ml
	10ml	1.0ml	0.1ml	MPN/index per 100ml	10ml	1.0ml	0.1ml	MPN/index per 100ml	10ml	1.0ml	0.1ml	MPN/index per 100ml	
Brand A	3	3	2	1100	3	1	2	30	3	0	2	64	398
Brand B	3	1	1	75	3	2	1	30	3	1	0	43	49
Brand C	1	1	0	7	2	2	1	28	2	2	0	43	18.77
Brand D	1	0	0	4	1	0	0	4	2	0	0	9	7
Brand E	2	0	0	9	3	0	0	7	2	1	1	20	12

Table 2. Types of bacteria isolated from various brand of packaged water from different locations in Minna metropolis North Central Nigeria.

SAMPLES	LOCATION	ORGANISM ISOLATED
Brand A	Bosso	<i>Escherichia coli</i> , <i>Streptococci feacalis</i> , <i>Bacillus subtilis</i> , and <i>Staphylococcus aureus</i>
Brand B	Kpakungu	<i>Salmonella typhi</i> , <i>Klebsilla spp</i> , <i>Bacillus subtilis</i> and <i>Streptococcus feacalis</i> ,
Brand C	Tunga	<i>Salmonella typhi</i> and <i>Pseudomonas aeruginosa</i>
Brand D	Maitumbi	<i>Staphylococcus spp</i> , <i>Klebsilla spp</i> , <i>Bacillus subtilis</i> and <i>Staphylococcus aureus</i> .
Brand E	Chanchaga	<i>Staphylococcus aureus</i> and <i>Klebsilla spp</i> .

Table 3. Means  $\pm$  SD values of the physicochemical parameters of sachet water samples in Minna metropolis North Central Nigeria.

Name of Sample	A	B	C	D	E
Ph	7.11 $\pm$ 0.04 <sup>a</sup>	7.21 <sup>a</sup> $\pm$ 0.04	7.57 $\pm$ 0.04 <sup>b</sup>	7.25 $\pm$ 0.04 <sup>a</sup>	7.1461 $\pm$ 0.0407 <sup>a</sup>
Electric. Conductivity ( $\mu$ s/cm)	0.67 $\pm$ 10.09 <sup>a</sup>	77.00 $\pm$ 10.09 <sup>a</sup>	91.00 $\pm$ 10.09 <sup>a</sup>	119.00 $\pm$ 10.09 <sup>a</sup>	97.33 $\pm$ 10.09 <sup>a</sup>
Total Alkalinity (mg/L)	14.33 $\pm$ 3.95 <sup>a</sup>	14.70 $\pm$ 3.95 <sup>a</sup>	14.0 $\pm$ 3.95 <sup>a</sup>	19.57 $\pm$ 3.95 <sup>a</sup>	15.33 $\pm$ 3.96 <sup>a</sup>
Total hardness (mg/L)	24.67 $\pm$ 3.60 <sup>a</sup>	24.00 $\pm$ 3.60 <sup>a</sup>	22.00 $\pm$ 3.60 <sup>a</sup>	37.67 $\pm$ 3.60 <sup>a</sup>	25.00 $\pm$ 3.61 <sup>a</sup>
Calcium Hardness (mg/L)	9.87 $\pm$ 1.34 <sup>a</sup>	8.27 $\pm$ 1.34 <sup>a</sup>	8.80 $\pm$ 1.34 <sup>a</sup>	15.07 $\pm$ 1.34 <sup>a</sup>	10.00 <sup>a</sup> $\pm$ 1.34
Magnesium (mg/L)	9.62 $\pm$ 1.34 <sup>a</sup>	8.03 $\pm$ 1.30 <sup>a</sup>	8.58 $\pm$ 1.30 <sup>a</sup>	14.69 $\pm$ 1.30 <sup>a</sup>	9.75 $\pm$ 1.30 <sup>a</sup>
Nitrate (mg/L)	0.07 $\pm$ 0.09 <sup>a</sup>	1.37 $\pm$ 0.09 <sup>b</sup>	1.81 <sup>a</sup> $\pm$ 0.09	2.23 <sup>a</sup> $\pm$ 0.09	1.74 $\pm$ 0.09 <sup>a</sup>
Phosphate (mg/L)	0.02 $\pm$ 0.00 <sup>a</sup>	0.013 $\pm$ 0.01 <sup>a</sup>	0.02 $\pm$ 0.01 <sup>a</sup>	0.01 $\pm$ 0.01 <sup>b</sup>	0.01 $\pm$ 0.01 <sup>b</sup>
Disolve oxygen (mg/L)	4.00 $\pm$ 0.61 <sup>a</sup>	5.47 $\pm$ 0.61 <sup>a</sup>	4.67 $\pm$ 0.61	5.33 $\pm$ 0.61 <sup>a</sup>	5.17 $\pm$ 0.61 <sup>a</sup>
Chromium (mg/L)	0.01 $\pm$ 3.20 <sup>a</sup>	0.01 $\pm$ 3.20 <sup>a</sup>	0.01 $\pm$ 3.20 <sup>a</sup>	0.01 $\pm$ 3.20 <sup>a</sup>	7.15 $\pm$ 3.20 <sup>a</sup>
Manganese (mg/L)	0.24 $\pm$ 0.04 <sup>a</sup>	0.17 $\pm$ 0.04 <sup>a</sup>	0.15 <sup>a</sup> $\pm$ 0.04	0.23 $\pm$ 0.04 <sup>a</sup>	0.17 $\pm$ 0.04 <sup>a</sup>
Iron (mg/L)	0.16 $\pm$ 0.03 <sup>a</sup>	0.13 $\pm$ 0.03 <sup>a</sup>	0.21 $\pm$ 0.03 <sup>a</sup>	0.22 $\pm$ 0.03 <sup>a</sup>	0.21 $\pm$ 0.03 <sup>a</sup>
Coer (mg/L)	0.09 $\pm$ 0.08 <sup>a</sup>	0.08 $\pm$ 0.08 <sup>a</sup>	0.07 $\pm$ 0.08 <sup>a</sup>	0.23 $\pm$ 0.08 <sup>a</sup>	0.09 $\pm$ 0.08 <sup>a</sup>
Lead (mg/L)	0.007	Nd	nd	Nd	0.007

Values followed by the same superscript alphabet along column are not significant at  $P > 0.05$

The organisms isolated were *Escherichia coli*, *Streptococci faecalis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsilla spp*, and *Pseudomonas aeruginosa* (Table 2). The isolates were initially differentiated on the basis of the cultural and morphological studies after which they were subjected to various biochemical tests.

The result of pH for the five samples (A - E) ranges from 7.11 to 7.57, Manganese values ranges from 0.15mg/l to 0.24mg/l, while total alkalinity which ranged from 10.0 – 19.57mg/l, Calcium, Magnesium, total hardness, Nitrate and Phosphate concentrations falls below WHO standards for drinking water quality (Table 3). The physico-chemical analysis of sample obtained shows that mean of electrical conductivity, alkalinity, total hardness,  $Ca^{2+}$  Hardness  $mg^{2+}$  Hardness, Chromium, Manganese, iron and  $Cu^{2+}$  were not significantly different ( $P > 0.05$ ) for all the samples, while that of pH, Nitrate and Phosphate shows a significant ( $p < 0.05$ ) difference between the samples.

### Discussion:

The presence of coliform groups in this water samples generally suggest that the water may have been contaminated with faeces either of human or animal origin or contaminants enters the production cycle. Other more dangerous micro-organisms could be present [11]. The presence of *Pseudomonas aeruginosa* in drinking water sample, a pathogenic organism renowned for its high resistance to antibiotics, could be due to poor production or processing procedures, which calls for concern [12]. The presence of coliform especially *Escherichia coli* in water simply indicated faecal contamination and the possible presence of bacterial pathogens [13]. The presence of these organisms in drinking water could cause a number of diseases such as gastroenteritis, diarrhoea, typhoid fever and cholera which are indicative of poor drinking water quality.

The physico-chemical properties of sachet water samples indicated that the pH for the five samples were neutral. These values fall below WHO standards and are therefore not suitable for human consumption [14]. The values of Calcium, Magnesium and total hardness were lower than the approved standards of water quality of the World Health Organisation (WHO). Total alkalinity of the water samples falls below WHO standards for drinking water quality. Dissolved oxygen value recorded is lower when compared to the approved standards of water quality of the World Health Organisation (WHO). Lead was not detected in the water samples analysed. Manganese values exceeded the maximum permissible limit for water quality of the Standard Organization of Nigeria (SON). The concentration of iron and manganese in water may be due to dissolution of polyvalent metallic ions from sedimentary rocks, seepage and run off from soil [15].

Nitrate and phosphate concentrations falls below WHO standards for drinking water quality. The sources of these elements in water include magmatic rocks, fertilizers, human excrement and air [16].

The presence of bacteria in this study may be due to improper handling, processing and purification procedures, unhygienic handling after production. About

99.8% of death in developing countries is due to unhygienic water and sanitation, besides, the sources of untreated drinking water could be a veritable reservoir of several other opportunistic pathogens of human and chemical poisoning [17].

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