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Evaluation of bacteriological quality and essential elements in commercially bottled/packaged water produced and marketed in Southeastern Nigeria

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The bacteriological quality and levels of essential mineral elements and organic acids were evaluated in 33 randomly selected bottled/packaged water samples produced and marketed in Southeastern Nigeria. The microbiological assay, essential mineral elements and organic acids were determined using American Public Health Association (APHA) standard methods. The distinguishing characteristics of the microorganisms identified were their psychotropic growth capabilities, motility at 37°C, growth in air, negative phenylalanine, deaminase, positive urease, catalase, oxidase and coagulase activities. Data obtained were compared with the accepted standards for safe drinking water as recommended by WHO/UNICEF. Some of the bacteria species recovered from the water samples included *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus*. The results show that only 9% of the samples investigated met the WHO/UNICEF standard of zero microbial load, 73% of the samples fell within 10^3 - 10^4 colony forming units (CFU)/100 ml, while 18% had the poorest with 10^6 CFU/100 ml. Major essential minerals found were calcium, sulphate, chloride, nitrate, bicarbonate and phosphate. Furthermore, the levels of these essential minerals were within the acceptable range in 76% of the samples while in others (24%) they were below the standard range recommended by WHO/UNICEF/APHA. There is need to maintain the approved standard level of chlorination in bottled /packaged water produced and sold in Southeastern Nigeria. Moreover, routine bacteriological quality assay by companies producing bottled/packaged drinking water should be enforced in Nigeria in particular and other developing countries in view of the findings in our study.

Key words: Microorganisms, packaged water, psychotropic growth, bacteriological quality, essential minerals, organic acids.

INTRODUCTION

Commercially bottled/packaged water is mainly produced for human consumption. In Nigeria, it comes in two forms: they are either sealed in plastic containers or in small nylon sachets. The sachet forms are generally called

'pure water' by many Nigerians. 'Pure water' enjoys much better patronage from those in the low socioeconomic class (over half of the population of Nigeria) because they are much cheaper than those in sealed plastic containers. Except for safe fluorides, no other ingredients are added in commercially produced drinking water.

Adherence to quality control standards and international best practices by those who produce packaged water in Nigeria are doubtful for so many reasons. First, most of the producers lack appropriate technology to meet with the acceptable international standards. Second, Oyedeji and co-workers (2010) reported that some producers source their raw water from well water which is not a good source of portable drinking water. Third, findings from local regulatory authorities indicated that some producers of packaged water indulge in very dubious practices by producing under very unhygienic conditions, packaging of untreated water, use of unapproved premises to produce unregistered water, use of non-grade waterproof sachets and marketing of packaged water without production and expiry dates among other sharp practices (Edema and Atayese, 2010).

Unfortunately, the lack of safe municipal and portable water in Nigeria has recently increased the demand for these commercially bottled/sachet water. According to Gardner (2004) this is because of the impression that bottled/sachet water is safer and healthier. Sahota (2005) opined that lack of proper sanitation and unhygienic practices account for the major sources of microbial contamination of any portable water. Nigeria is a country with a population of over 160 million persons. The country is also a market destination for many West Africans and a lot of foreign visitors come to Nigeria for one reason or the other. Therefore, the indiscriminate sale and consumption of sealed water in plastic containers/sachets in Nigeria is of public health significance and this is the major motivation for our study. To the best of our knowledge, there is no study that has comprehensively evaluated the quality of commercially marketed drinking water in all of the Southeast States of Nigeria, an important business hub in West Africa. Southeast Nigeria is made up of five states: Enugu, Anambra, Imo, Abia and Ebonyi states (Figure 1). Furthermore, water-related diseases continue to be a major issue in many developing countries. The high incidence of dysentery, cholera, typhoid and diarrhea has been linked to the consumption of unsafe and non-hygienic drinking water and their production processes (Mead et al., 1999).

Some published studies have reported the detection of heterotrophic and coli form bacteria counts in bottled water (Hobbs, 1962; Craun, 1997; Bhareth et al., 2003). Adelegan (2004) also reported that the increase in the cases of Salmonellosis and typhoid fever in Nigeria is as a result of increased consumption of low quality sachet water. The refusal of some producers of water in plastic containers/sachets to mark production and expiry dates on their products means that the product can also deteriorate before it reaches the consumer (Da Silva et al., 2007).

Examination of water samples for pathogens are usually carried out because many workers have found positive correlation between high density viable counts, total and fecal coli forms and the presence of pathogens such as *Salmonella, E. coli, S. aureus* in drinking water (Hood et al., 1983; LeChevallier, 1990; Payment et al., 1993). Moreover, there have been reported cases of food and waterborne disease outbreaks in both children and adults in Southeastern Nigeria (Blum et al., 1987; Ogan, 1988). For example, it is most probable that the organisms causing these diseases must have been transmitted directly or indirectly to food and water through fecal contamination or by the urine of the carrier animal or man. The study was therefore, conducted to ascertain the quality of commercially marketed drinking water sold in Southeast Nigeria. The levels of microbial contamination and that of essential organic and inorganic ions in the randomly selected water samples were evaluated to determine their conformity with the acceptable reference standards approved by (WHO, 1998; APHA, 1998; UNICEF (2008).

MATERIALS AND METHODS

Sampling

Thirty three (33) water samples collected from thirty three companies producing commercially bottled and/or sachet water in Southeastern Nigeria were analyzed. Samples were collected from fresh stocks supplied to the sellers by the different producers. They were stored in a chilled thermo flask containing ice blocks to regulate the temperature. They were later transported back to the laboratory and processed immediately. The states that make up the Southeastern Nigeria are: Abia, Anambra, Ebonyi, Enugu and Imo. The companies chosen were randomly selected from different locations in the region. Based on the population of companies producing water in plastic containers and sachets in these states, samples were collected as follows: Anambra (8), Abia (7), Enugu/Imo/Ebonyi (6 each). The map of the area covered in the sample collection is shown in Figure 1.

The methods for detection, isolation and enumeration of bacteria in the water samples were determined according to the procedures described by American Public Health Association APHA (1998), Chigbu and Sobolev (2007) and Douterelo et al. (2014)

Presumptive total coli forms, fecal coli forms and *E. coli* detection and enumeration in the water samples

In this method, serial dilutions of the water samples were made and

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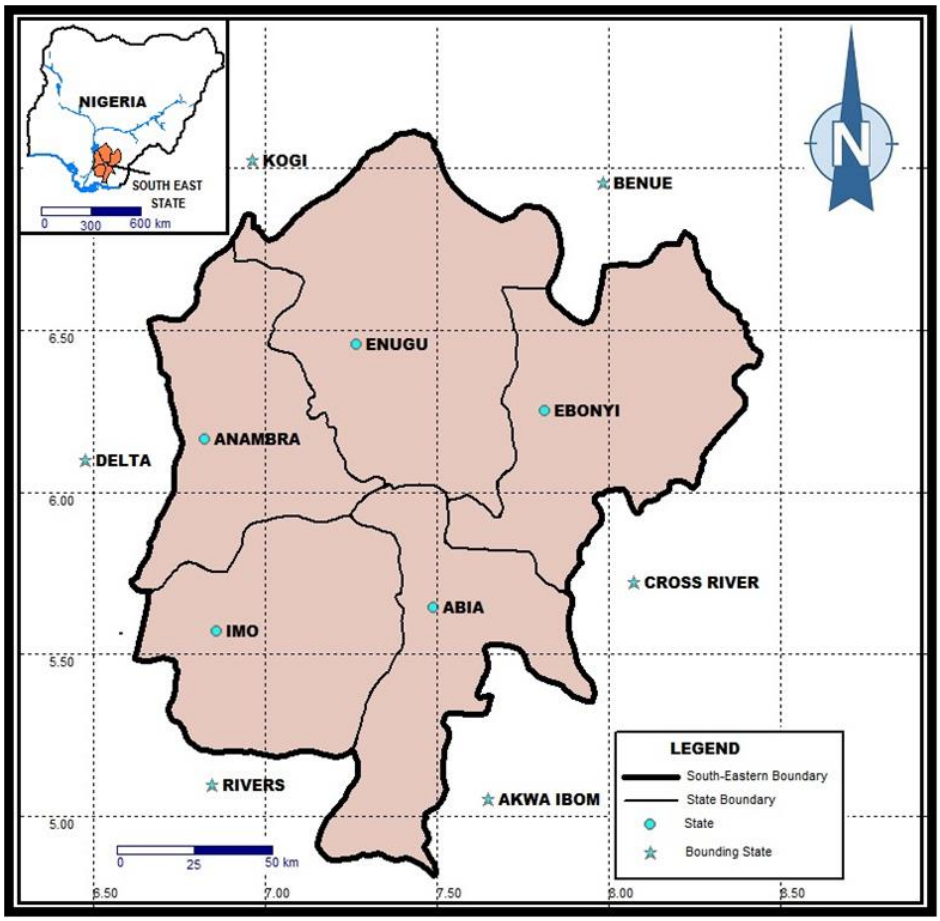

Figure 1. Map of Southeast Nigeria where samples were collected

inoculated into Laury Tryptose broth (LTB) growth media along with fermentation tubes with inverted vials for gas production. Samples were then incubated for 24h at 35°C and for an additional 24 h where there was no growth at the end of the first 24 h. Growth (turbidity), gas bubbles or acid in the tube was due to the fermentation of lactose and indicated the presence of coli form bacteria. A 10- tube most probable number (MPN) method (Multiple Tube technique) was used for the analysis of the water samples.

Confirmation test for the coli form bacteria

The brilliant green lactose bile broth (BGLBB) was used for the confirmation test for the coli form. After preparation, 10 ml of the medium was added into the fermentation tubes such that the media level covered the inverted tubes in the fermentation tubes. The final pH of the broth was 7.2 ± 0.1 after sterilization. All tubes showing growth, gas bubbles or acid reaction in the LTB test were transferred to the BGLBB tubes and incubated for 24-48 h at 35- 37 ± 2°C. Growth or gas production in the tubes showed confirmatory test for the coli form bacteria. The MPN of bacteria present in the sample was calculated using the number of positive BGLBB tubes and the MPN index table (Chigbu and Sobolev, 2007).

Confirmation test for *E. coli* in coli form positive samples

A mixture of the water samples and 4- methylumbelliferyl- b-D-glucoronide (MUG) was added to EC growth medium at a concentration of 50 ml/l. The test is based on the cleavage of MUG to free methylumbelliferyl moiety, which fluoresces in blue color when irradiated with the ultra violet (UV) radiation. EC medium was sterilized before use and the pH after sterilization was 6.9 ± 0.2. The EC medium was tested for fluorescence before use. The EC tube from positive BGLBB tubes were incubated in a water bath at 44.5 ± 2°C for 22-26 h. The inverted Durham tubes were omitted. A positive reaction for *E. coli* was indicated by the presence of blue fluorescence. A tube inoculated with a known positive culture and a negative culture were included for each batch to be tested to serve as a reference in order to eliminate false positives (Chigbu and Sobolev, 2007; Mossel and Vega, 1973).

Mineral and organic acids determination

The essential elements and organic acids in the samples were estimated in the water samples using wet digestion with nitric and perchloric acid to produce complete digestion. The acidified sample was evaporated to the lowest possible volume before precipitation. Nitric acid addition continued until a clear solution was obtained. The values were then read in atomic absorption spectrophotometer and gas chromatography, respectively. The principles of the two methods are based on the formation of colored compounds with appropriate and specific reagents. During the process, the radiant energy of a very narrow wavelength (visible or UV region) is selected from a source, and passed through the sample solution,

Table 1. Range of bacterial contamination of the water samples.

Bacteria/samples	Total viable counts (TVC) (CFU/100 ml)	Total coliform counts (TC) (CFU/100 ml)	Fecal coliform counts (FC)(CFU/100 ml)
MW_1	1.2 - 1.3 x 10^6	1.4 - 1.6 x 10^5	1.1 - 1.2 x 10^1
MW_2	1.6 - 1.7 x 10^4	1.8 - 2.0 x 10^3	1.8 - 2.2 x 10^1
MW_3	2.2 - 2.5 x 10^4	2.3 - 2.4 x 10^2	2.2 - 2.3 x 10^2
MW_4	1.5 - 1.6 x 10^4	1.3 - 1.4 x 10^3	2.1 - 2.2 x 10^1
MW_5	1.6 - 1.7 x 10^5	1.8 - 2.0 x 10^3	1.8 - 2.2 x 10^2
MW_6	1.4 - 1.6 x 10^6	1.6 - 2.2 x 10^4	1.7 - 1.9 x 10^2
MW_7	2.1 - 2.2 x 10^3	2.2 - 2.3 x 10^2	1.9 - 2.0 x 10^1
MW_8	2.2 - 2.4 x 10^4	2.2 - 2.4 x 10^2	1.8 - 2.0 x 10^2
MW_9	1.8 - 2.0 x 10^5	1.6 - 1.8 x 10^3	1.6 - 1.70 x 10^2
MW_{10}	1.6 - 1.8 x 10^4	1.86 - 2.2 x 10^2	1.2 - 1.4 x 10^2
MW_{11}	1.8 - 2.0 x 10^4	2.2 - 2.6 x 10^2	2.4 - 2.6 x 10^2
MW_{12}	1.8 - 2.0 x 10^6	2.2 - 2.6 x 10^4	2.4 - 2.6 x 10^2
MW_{13}	1.8 - 2.0 x 10^3	2.2 - 2.6 x 10^2	2.4 - 2.6 x 10^1
MW_{14}	1.8 - 2.0 x 10^5	2.2 - 2.6 x 10^3	2.4 - 2.6 x 10^2
MW_{15}	1.8 - 2.0 x 10^4	2.2 - 2.6 x 10^3	2.4 - 2.6 x 10^1
MW_{16}	ND	ND	ND
MW_{17}	1.8 - 2.0 x 10^6	2.2 - 2.6 x 10^4	2.4 - 2.6 x 10^2
MW_{18}	1.8 - 2.0 x 10^4	2.2 - 2.6 x 10^2	2.4 - 2.6 x 10^2
MW_{19}	1.8 - 2.0 x 10^4	2.2 - 2.6 x 10^2	2.4 - 2.6 x 10^2
MW_{20}	1.8 - 2.0 x 104	2.2 - 2.6 x 102	2.4 - 2.6 x 102
MW_{21}	1.8 - 2.0 x 105	2.2 - 2.6 x 103	2.4 - 2.6 x 102
MW_{22}	1.8 - 2.0 x 106	2.2 - 2.6 x 104	2.4 - 2.6 x 102
MW_{23}	1.8 - 2.0 x 104	2.2 - 2.6 x 103	2.4 - 2.6 x 101
MW_{24}	ND	ND	ND
MW_{25}	1.8 - 2.0 x 106	2.2 - 2.6 x 105	2.4 - 2.6 x 101
MW_{26}	1.8 - 2.0 x 103	2.2 - 2.6 x 102	2.4 - 2.6 x 101
MW_{27}	1.8 - 2.0 x 104	2.2 - 2.6 x 103	2.4 - 2.6 x 101
MW_{28}	ND	ND	ND
MW_{29}	1.8 - 2.0 x 103	2.2 - 2.6 x 102	2.4 - 2.6 x 101
MW_{30}	1.8 - 2.0 x 104	2.2 - 2.6 x 103	2.4 - 2.6 x 101
MW_{31}	1.8 - 2.0 x 103	2.2 - 2.6 x 102	2.4 - 2.6 x 101
MW_{32}	1.8 - 2.0 x 104	2.2 - 2.6 x 103	2.4 - 2.6 x 101
MW_{33}	1.8 - 2.0 x 103	2.2 - 2.6 x 102	2.4 - 2.6 x 101

*Means ± (SD) of 3 determinations. Samples on appropriate media incubated at 35°C - 44.5 ± 2°C as determined by MPN method. MW_1 - MW_{19} = water from different locations in the region. ND = Not detected.

which is contained in the quartz cell. The amount of radiation absorbed at a certain wavelength is proportional to the light absorbing chemical in the sample (Peldszus et al., 1996; Kuo, 1998; Jorge et al., 2007; Nachiyunde et al., 2013).

Statistical analysis

The analysis of variance (ANOVA) and Duncan's New Multiple Range Tests (DNMRT) were used to test the significance of the difference among means. (Steel and Torrie, 1980).

RESULTS

Bacteriological quality of the water samples

Table 1 presents the results obtained for the total viable counts, total coli forms and fecal coli forms. On the basis of total viable counts, it is evident that samples MW_{16}, MW_{24} and MW28 had the highest microbiological quality with no significant detectable bacteria counts ($p>0.05$) compared with the rest of the samples. On the basis of total coli forms and fecal coli forms, MW_1, MW_6, MW_{12}, MW_{17}, MW_{22} and MW_{25} had the poorest sanitary quality with the average counts of 10^6 CFU/100 ml. The values for the other samples ranged from 10^3 to 10^4CFU/100 ml. The overall ranking of the microbiological quality for the entire water samples studied is thus: MW_{16}, MW_{24}, MW_{28}>MW_2, MW_3, MW_4, MW_5, MW_7, MW_8, MW_9 MW_{10}, MW_{11}, MW_{13}, MW_{14}, MW_{15}, MW_{18} , MW_{19}, MW_{20}, MW_{21}, MW_{23}, MW_{26}, MW_{27}, MW_{29}, MW_{30}, MW_{31}, MW_{32}, MW_{33}

Table 2. Population of individual pathogenic organisms in the water samples (CFU/100 ml).

Samples/bacteria	MW_1	MW_2	MW_3	MW_4	MW_5	MW_6	MW_7	MW_8	MW_9	MW_{10}	MW_{11}
Escherichia coli	-	-	-	-	-	-	-	-	$5.4x10^3$	$2.1x10^4$	-
S. aureus	-	-	-	-	-	-	-	$3.5x10^3$	-	$4.1x10^3$	-
B. subtilis	-	-	-	-	$4.0x10^3$	$3.0x10^3$	$9.4x10^5$	$4.1x10^3$	-		$1.4x10^4$
Acinetobacter sp.	$4.7x10^3$	-	-	-	-	-	-		-		-
Salmonella sp.	-	-	-	-	-	-	-	-	-		-
Alcaligenes sp.	-	-	-	$1.3x10^4$	-	-	-	-	-		-
Pseudomonas sp.	-	-	-	-	$4.0x10^3$	-	-	-	-		-
Klebsiella sp.	-	-	$1.0x10^3$	-	-	-	-	-	-		-
Aerobacter sp.	-	-	-	-	-	-	-	-	-	$1.2x10^3$	$1.3x10^4$
Lactobacillus sp.	-	-	$1.0x10^3$	-	-	-	-	-	-	$1.3x10^3$	-
Leuconostoc sp.	-	-	-	$1.0x10^3$	-	-	-	-	$1.0x10^3$		-
Clostridumperfrigenes	-	-	-	-	-	-	-	-	-	-	-

*Means ± (SD) of 3 determinations. Samples on appropriate media incubated at 35°C - 44.5 ± 2°C as determined by MPN method. MW_{20} - MW_{33} = water from different locations in the region; ND = Not detected.

Table 2. Contd.

Samples/bacteria	MW_{12}	MW_{13}	MW_{14}	MW_{15}	MW_{16}	MW_{17}	MW_{18}	MW_{19}	MW_{20}	MW_{21}	MW_{22}
Escherichia coli	-	-	-	-	NS	-	-	-	$5.4x10^3$	$2.1x10^4$	-
S.aureus	$4.7x10^3$	-	-	-	NS	-	-	$3.5x10^3$	-		-
B. subtilis	-	-	-	-	NS	$3.0x10^3$	$9.4x10^5$	$4.1x10^3$	-		$1.4x10^4$
Acinetobacter	$4.7x10^3$	-	-	-	NS	-	-		-		-
Salmonella sp.	-	-	-	-	NS	-	-	-	-		-
Alcaligenes sp.	-	-	-	$1.3x10^4$	NS	-	-	-	-		-
Pseudomonas sp.	-	$1.0x10^3$	-	-	NS	-	-	-	-		-
Klebsiella sp.	-	-	$1.0x10^3$	-	NS	-	-	-	-		-
Aerobacter sp.	-	-	-	-	NS	-	-	-	-	$1.2x10^3$	$1.3x10^4$
Lactobacillus sp.	-	-	$1.0x10^3$	-	NS-	-	-	-	-	$1.3x10^3$	-
Leuconostoc sp.	-	-	-	$1.0x10^3$	NS-	-	-	-	$1.0x10^3$		-
Clostridumperfrigenes	-		-	-	NS-	-	-	-	-	-	-

*Means ± (SD) of 3 determinations. Samples on appropriate media incubated at 35°C - 44.5 ± 2°C as determined by MPN method MW_{12} - MW_{22} = water from different locations in the region. ND = Not detected.

>MW_1 , MW_6, MW_{12}, MW_{17}, MW_{22} and MW_{25}. The results were further subjected to Duncan's new multiple range test. On the basis of the total viable counts, all the samples were found to be significantly ($p<0.05$) different from each other. Similarly, on the basis of total coli forms and fecal coli forms, the samples were found to be statistically different at 5% level of significance. Furthermore, the results obtained on the basis of individual microorganisms (Table 2), indicated that some samples had pathogenic organisms notably, *E. coli, B. subtilis* and *S. aureus* with the average contamination of 10^3CFU/100 ml.

Table 3 presents the essential elements and pH of the water samples. The calcium ion contents of the samples varied. The values ranged from 6.00 mg/ml in MW_7 to 24.00 mg/ml in MW_{33} and the difference was significant ($p<0.05$). Sample MW_{10} had the highest sulphate concentration of 0.039 mg/ml compared with MW_1 with the least value (0.010 mg/ml). There were significant ($p<0.05$) differences in the chloride concentrations among the samples (Table 3). However, MW_{14} and MW_{28} had similar values (17.54 mg/ml). The nitrate ion levels of the samples ranged from 0.100 to 2.34 mg/ml with MW_4 having the highest value and MW_1 the least. Samples MW_2 and MW_6 had similar value (1.34 mg/ml). The bicarbonate ion concentrations of the samples varied. Sample MW_{22} had the highest value (20.24 mg/ml) while MW_4 had the least (7.45 mg/ml). The phosphate ion levels ranged from 5.56 mg/ml in MW_{21} to 20.26 mg/ml in MW_{33}. The logarithm of hydrogen ion concentration (pH) of the entire samples were comparable.

Table 4 presents the organic acid concentrations of the water samples. The oxalic acid ion ($HOOCCOO^{-}$) contents of the samples were of the order of 0.98 to 4.66

Table 2. Contd.

Samples/bacteria	MW_{23}	MW_{24}	MW_{25}	MW_{26}	MW_{27}	MW_{28}	MW_{29}	MW_{30}	MW_{31}	MW_{32}	MW_{33}
Escherichia coli	-	NS	-	-	-	NS	-	-	$5.4x10^3$	$2.1x10^4$	-
S.aureus	$4.7x10^3$	NS	-	-	-	NS	-	$3.5x10^3$	-		-
B. subtilis	-	NS	-	-	$4.0x10^3$	NS	$9.4x10^5$	$4.1x10^3$	-		$1.4x10^4$
Acinetobacter sp.	$4.7x10^3$	NS	-	-	-	NS	-		-		-
Salmonella sp.	-	NS	-	-	-	NS	-	-	-		-
Alcaligenes sp.	-	NS	-	$1.3x10^4$	-	NS	-	-	-		-
Pseudomonas sp.	-	NS	-	-	$4.0x10^3$	NS	-	-	-		-
Klebsiella sp.	-	NS	$1.0x10^3$	-	-	NS	-	-	-		-
Aerobacter sp.	-	NS	-	-	-	NS	-	-	-	$1.2x10^3$	$1.3x10^4$
Lactobacillus sp.	-	NS	$1.0x10^3$	-	-	NS	-	-	-	$1.3x10^3$	-
Leuconostoc sp.	-	NS	-	$1.0x10^3$	-	NS-	-	-	$1.0x10^3$		-
Clostridumperfrigenes	-	-	-	-	-	NS	-	-	-	-	-

*Means ± (SD) of 3 determinations. Samples on appropriate media incubated at 35°C - 44.5 ± 2°C as determined by MPN method. MW_{23} - MW_1 = water from different locations in the region; ND = Not detected.

Table 3. Essential mineral elements and pH of the water samples.

Parameters/samples	MW_1	MW_2	MW_3	MW_4	MW_5	MW_6	MW_7
Calcium (mg/l)	11.10^a±1.0	16.0^c±1.0	15.0^c±1.2	11.0^a±1.30	22.0^d±1.00	$11.0±1^a.20$	6.00^e±1.30
Sulphate (mg/ml)	0.010^d±0.001	0.013^d±0.001	0.037^c±0.002	0.010^d±0.001	0.110^d±0.001	0.015^d±0.002	0.07^e±0.001
Chloride (mg/l)	14.27^a±1.2	11.38^b±1.3	2.84^c±1.00	14.20^a±1.20	11.36^b±1.30	19.88^d±2.00	17.04^e±1.20
Nitrate (mg/ml)	0.100^a±0.001	1.34^b±0.002	0.105^a±0.001	2.34^c±0.010	0.31^d±0.002	1.34^b±0.001	0.664^e±0.020
Biocarbonate (mg/l)	19.00^a±2.00	7.77^b±1.20	20.00^a±2.00	7.45^b±1.20	17.00^c±1.20	16.00^c±1.20	15.90^c±1.50
Phosphate (mg/l)	11.00^a±1.20	13.60^c±1.50	14.00^c±1.00	10.00^a±2.0	20.20^d±2.00	12.50^b±1.00	6.00^e±0.20
pH	6.20^b	6.10^b	6.10^b	6.20^b	6.10^b	6.00^b	6.00^b

*Means ± (SD) of 3 determinations. Values on the same row with different superscripts are significantly different ($p<0.05$). MW_1 - MW_7 = municipal water from different locations in the region. WHO/UNICEF recommended values: pH (6.5-8.5), Phosphate (<20 mg/l), Bicarbonate (<20 mg/l), Nitrate (0.02mg/l, Chloride (250 mg/l), Sulphate (100 mg/l), Ca^{2+} (< 20 mg/l).

Table 3. Contd.

Parameters/samples	MW_8	MW_9	MW_{10}	MW_{11}	MW_{12}	MW_{13}	MW_{14}
Calcium (mg/l)	11.10^a±1.0	15.0^c±1.0	14.0^c±1.2	10.0^a±1.30	23^d±1.00	$11±1^a.20$	7.0^e±1.30
Sulphate (mg/ml)	0.012^d±0.001	0.013^d±0.001	0.039^c±0.002	0.011^d±0.001	0.110^d±0.001	0.014^d±0.002	0.007^e±0.001
Chloride (mg/l)	14.26^a±1.2	11.38^b±1.3	2.84^c±1.00	14.22^a±1.20	11.36^a±1.30	19.87^d±2.00	17.54^e±1.20
Nitrate (mg/ml)	0.100^a±0.001	1.32^b±0.002	0.106^a±0.001	2.43^c±0.010	0.33^d±0.002	1.34^b±0.001	0.664^e±0.020
Biocarbonate (mg/l)	18.66^a±2.00	8.22^b±1.20	20.00^a±2.00	8.20^b±1.20	17.00^c±1.20	15.00^c±1.20	16.10^c±1.50
Phosphate (mg/l)	10.00^a±1.20	11.50^b±1.50	14.20^c±1.00	10.00^a±2.0	20.10^d±2.00	12.20^b±1.00	6.22^e±0.20
pH	6.10^b	6.11^b	6.10^b	6.10^b	6.11^b	6.01^b	6.00^b

*Means ± (SD) of 3 determinations Values on the same row with different superscripts are significantly different ($p<0.05$). MW_8 - MW_{14} = municipal water from different locations in the region. WHO/UNICEF recommended values: pH (6.5-8.5), Phosphate (<20 mg/l), Bicarbonate (<20 mg/l), Nitrate (0.02 mg/l, Chloride (250 mg/l), Sulphate (100 mg/l), Ca^{2+} (< 20 mg/l).

mg/ml. MW_{33} had the highest value (4.66 mg/ml), while MW_{23} had the least (0.98mg/ml). There were significant($p<0.05$) differences amongst the samples. The formic acid ion ($HCOO^-$) levels of the various samples varied. The values ranged from 1.12 mg/ml in MW_{13} to 4.24mg/ml in MW_{33} and the differences were significant ($p<0.05$). The acetic acid ion ($CH3COO^-$) values ranged from 0.687 to 6.682 mg/ml. The acetic acid contents of MW_8, MW_9, MW_{20} and MW_{30} samples were similar (6.682 mg/ml) ($p>0.05$). Similarly, samples MW_{13} and MW_{24} had the same value (0.687 mg/ml).

DISCUSSION

Bacteriological analyses of the water samples revealed

Table 3. Contd.

Parameters/samples	MW_{15}	MW_{16}	MW_{17}	MW_{18}	MW_{19}	MW_{20}	MW_{21}
Calcium (mg/l)	10.10^{a}±1.0	16.20^{c}±1.0	16.10^{c}±1.2	11.0^{a}±1.30	21.60^{d}±1.00	$12.22±1^{a}$.20	6.10^{e}±1.30
Sulphate (mg/ml)	0.011^{d}±0.001	0.014^{d}±0.001	0.036^{c}±0.002	0.011^{d}±0.001	0.110^{d}±0.001	0.014^{d}±0.002	0.007^{e}±0.01
Chloride (mg/l)	14.24^{a}±1.2	11.34^{b}±1.3	2.86^{c}±1.00	14.22^{a}±1.20	11.32^{b}±1.30	19.78^{d}±2.00	17.24^{e}±1.20
Nitrate (mg/ml)	0.101^{a}±0.001	1.32^{b}±0.002	0.103^{a}±0.001	2.23^{c}±0.010	0.34^{d}±0.002	1.35^{b}±0.001	0.662^{e}±0.020
Biocarbonate (mg/l)	20.22^{a}±2.00	8.32^{b}±1.20	20.00^{a}±2.00	8.10^{b}±1.20	16.00^{c}±1.20	16.20^{c}±1.20	16.24^{c}±1.50
Phosphate (mg/l)	9.50^{a}±1.20	12.06^{b}±1.50	14.00^{c}±1.00	10.00^{a}±2.0	20.00^{d}±2.00	12.00^{b}±1.00	5.56^{e}±0.20
pH	6.00^{b}	6.10^{b}	6.10^{b}	6.00^{b}	6.10^{b}	6.00^{b}	6.00^{b}

*Means ± (SD) of 3 determinations.Values on the same row with different superscripts are significantly different ($p<0.05$). MW_{15} - MW_{21} = municipal water from different locations in the region. WHO/UNICEF recommended values: pH (6.5-8.5), Phosphate (<20 mg/l), Bicarbonate (<20 mg/l), Nitrate (0.02 mg/l, Chloride (250 mg/l), Sulphate (100 mg/l), Ca^{2+} (< 20 mg/l).

Table 3. Contd.

Parameters/samples	MW_{22}	MW_{23}	MW_{24}	MW_{25}	MW_{26}	MW_{27}	MW_{28}
Calcium (mg/l)	12.10^{a}±1.0	15.20^{c}±1.0	16.0^{c}±1.2	11.50^{a}±1.30	21.50^{d}±1.00	$10.10±1^{a}$.20	5.10^{e}±1.30
Sulphate (mg/ml)	0.011^{d}±0.001	0.012^{d}±0.001	0.038^{c}±0.002	0.010^{d}±0.001	0.111^{d}±0.001	0.015^{d}±0.002	0.066^{e}±0.001
Chloride (mg/l)	14.24^{a}±1.2	11.33^{b}±1.3	2.82^{c}±1.00	14.20^{a}±1.20	11.36^{b}±1.30	19.86^{d}±2.00	17.54^{e}±1.20
Nitrate (mg/ml)	0.100^{a}±0.001	1.32^{b}±0.002	0.106^{a}±0.001	2.31^{c}±0.010	0.32^{d}±0.002	1.32^{b}±0.001	0.664^{e}±0.020
Biocarbonate (mg/l)	20.24^{a}±2.00	8.00^{b}±1.20	20.24^{a}±2.00	8.00^{b}±1.20	19.47^{a}±2.00	15.50^{c}±1.20	16.20^{c}±1.50
Phosphate (mg/l)	10.11^{a}±1.20	12.40^{b}±1.50	14.23^{c}±1.00	10.10^{a}±2.0	20.20^{d}±2.00	12.24^{b}±1.00	6.10^{e}±0.20
pH	6.10^{b}	6.10^{b}	6.10^{b}	6.20^{b}	6.00^{b}	6.00^{b}	6.00^{b}

*Means ± (SD) of 3 determinations. Values on the same row with different superscripts are significantly different ($p<0.05$). MW_{22} - MW_{28} = municipal water from different locations in the region WHO/UNICEF recommended values: pH (6.5-8.5), Phosphate (<20 mg/l), Bicarbonate (<20 mg/l), Nitrate (0.02 mg/l, Chloride (250 mg/l), Sulphate (100 mg/l), Ca^{2+} (< 20 mg/l).

Table 3. cont'd.

Parameters/Samples	MW_{29}	MW_{30}	MW_{31}	MW_{32}	MW_{33}
Calcium (mg/l)	11.12^{a}±1.0	15.20^{c}±1.0	14.20^{c}±1.2	11.10^{a}±1.30	24.00^{d}±1.00
Sulphate (mg/ml)	0.011^{d}±0.001	0.013^{d}±0.001	0.032^{c}±0.002	0.011^{d}±0.001	0.110^{e}±0.001
Chloride (mg/l)	14.24^{a}±1.2	11.31^{b}±1.3	2.84^{c}±1.00	13.82^{a}±1.20	11.34^{b}±1.30
Nitrate (mg/ml)	0.101^{a}±0.001	1.23^{b}±0.002	0.106^{a}±0.001	2.32^{c}±0.010	0.35^{d}±0.002
Biocarbonate (mg/l)	20.23^{a}±2.00	7.55^{b}±1.20	7.82^{b}±1.20	8.20^{b}±1.20	7.92^{b}±1.20
Phosphate (mg/l)	10.01^{a}±1.20	12.22^{b}±1.50	14.20^{c}±1.00	10.22^{a}±2.0	20.26^{d}±2.00
pH	6.20^{b}	6.10^{b}	6.20^{b}	6.20^{b}	6.10^{b}

*Means ± (SD) of 3 determinations. Values on the same row with different superscripts are significantly different ($p<0.05$). MW_{29} - MW_{33} = municipal water from different locations in the region. WHO/UNICEF recommended values: pH (6.5-8.5), Phosphate (<20 mg/l), Bicarbonate (<20 mg/l), Nitrate (0.02 mg/l, Chloride (250 mg/l), Sulphate (100 mg/l), Ca^{2+} (< 20 mg/l).

the presence of pathogens and this is in agreement with the reports of LeChevallier (1990); Michiels and Moyson (2000); Chigbu and Sobolev (2007) that implicated these organisms in different waterborne disease outbreaks across the world. The public health significance of these organisms cannot be over-emphasized. A higher load over MID had been implicated in foodborne enteritis (Chigbu and Sobolov, 2007; Onoja et al., 2011), traveller's diarrhea (Gorbach et al., 1975) and water borne diseases (Mackenzie et al., 1994; APHA, 1998; Hunter and Fewtrell, 2001; Feng et al., 2002; Onoja et al., 2011). *E. coli* is a heat sensitive organism that cannot withstand the processing temperature hence, it is evident that its mode of entry must have been through handling and post process re-contamination and cross - contamination (LeChevallier, 1990). Furthermore, *E. coli* is solely an organism of intestinal origin, hence its presence in the water samples is an indication of

Table 4. Essential organic acids content of the water samples*

Samples/organic acids	Oxalic acid (mg/l)	Formic acid (mg/l)	Acetic acid (mg/l)
MW_1	$1.50^{a} \pm 0.020$	$1.22^{a} \pm 0.001$	$2.097^{a} \pm 0.020$
MW_2	$2.10^{a} \pm 0.10$	$1.22^{a} \pm 0.002$	$0.776^{b} \pm 0.020$
MW_3	$2.54^{b} \pm 0.20$	$2.20^{b} \pm 0.100$	$1.043^{c} \pm 0.020$
MW_4	$1.55^{a} \pm 0.10$	$1.42^{a} \pm 0.010$	$1.045^{c} \pm 0.010$
MW_5	$3.64^{c} \pm 0.20$	$2.21^{b} \pm 0.020$	$1.314^{c} \pm 0.010$
MW_6	$3.04^{d} \pm 0.30$	$1.23^{a} \pm 0.010$	$1.084^{c} \pm 0.020$
MW_7	$2.44^{b} \pm 0.20$	$2.20^{b} \pm 0.010$	$2.623^{d} \pm 0.020$
MW_8	$3.22^{d} \pm 0.40$	$3.20^{c} \pm 0.002$	$6.824^{e} \pm 0.400$
MW_9	$2.44^{b} \pm 0.20$	$2.44^{b} \pm 0.001$	$6.824^{e} \pm 0.500$
MW_{10}	$2.42^{b} \pm 0.10$	$1.66^{a} \pm 0.002$	$3.159^{f} \pm 0.100$
MW_{11}	$4.64^{e} \pm 0.20$	4.22 ± 0.002	$3.675^{f} \pm 0.020$
MW_{12}	$1.55^{a} \pm 0.020$	$1.20^{a} \pm 0.001$	$2.094^{a} \pm 0.020$
MW_{13}	$1.96^{a} \pm 0.10$	$1.12^{a} \pm 0.002$	$0.687^{b} \pm 0.020$
MW_{14}	$2.52^{b} \pm 0.20$	$1.77^{b} \pm 0.100$	$1.045^{c} \pm 0.020$
MW_{15}	$1.64^{a} \pm 0.10$	$1.43^{a} \pm 0.010$	$1.039^{c} \pm 0.010$
MW_{16}	$3.62^{c} \pm 0.20$	$2.22^{b} \pm 0.020$	$1.313^{c} \pm 0.010$
MW_{17}	$3.22^{d} \pm 0.30$	$1.23^{a} \pm 0.010$	$1.084^{c} \pm 0.020$
MW_{18}	$2.42^{b} \pm 0.20$	$2.11^{b} \pm 0.010$	$2.524^{d} \pm 0.020$
MW_{19}	$2.66^{b} \pm 0.40$	$3.23^{c} \pm 0.002$	$5.823^{e} \pm 0.400$
MW_{20}	$2.40^{b} \pm 0.20$	$2.44^{b} \pm 0.001$	$6.820^{e} \pm 0.500$
MW_{21}	$2.46^{b} \pm 0.10$	$1.64^{a} \pm 0.002$	$3.145^{f} \pm 0.100$
MW_{22}	$4.64^{b} \pm 0.20$	$4.22^{d} \pm 0.002$	$3.574^{f} \pm 0.020$
MW_{23}	$0.98^{a} \pm 0.020$	$1.22^{a} \pm 0.001$	$2.092^{a} \pm 0.020$
MW_{24}	$1.66^{a} \pm 0.10$	$1.22^{a} \pm 0.002$	$0.687^{b} \pm 0.020$
MW_{25}	$2.56^{b} \pm 0.20$	$2.44^{b} \pm 0.100$	$1.045^{c} \pm 0.020$
MW_{26}	$1.66^{a} \pm 0.10$	$1.44^{a} \pm 0.010$	$1.048^{c} \pm 0.010$
MW_{27}	$3.66^{c} \pm 0.20$	$2.24^{b} \pm 0.020$	$1.314^{c} \pm 0.010$
MW_{28}	$2.64^{d} \pm 0.30$	$1.24^{a} \pm 0.010$	$1.073^{c} \pm 0.020$
MW_{29}	$2.44^{b} \pm 0.20$	$2.34^{b} \pm 0.010$	$2.524^{d} \pm 0.020$
MW_{30}	$3.44^{d} \pm 0.40$	$3.22^{c} \pm 0.002$	$6.682^{e} \pm 0.400$
MW_{31}	$2.44^{b} \pm 0.20$	$2.46^{b} \pm 0.001$	$6.622^{e} \pm 0.500$
MW_{32}	$2.44^{b} \pm 0.10$	$1.66^{a} \pm 0.002$	$3.146^{f} \pm 0.100$
MW_{33}	$4.66^{b} \pm 0.20$	$4.24^{d} \pm 0.002$	$3.664^{f} \pm 0.020$

*Means ± (SD) of 3 determinations. Values on the same column with different superscripts are significantly different ($p<0.05$). MW_1 - MW_{11} = water from different locations in the region. WHO/UNICEF recommended values: oxalic acid (<5 mg/l), formic acid (<4 mg/l), acetic acid (<6 mg/l).

contamination by fecal matter of human and animal origin. Also there is copious evidence that poor handling and sanitation would result in the increased proliferation of microorganisms and this will exacerbate the bacterial populations including pathogens. For example, it has been reported that water used for drinking and domestic uses in Nigeria have been found to be heavily contaminated with fecal matter (Blum et al., 1987; Ogan, 1988).

The possible explanation for the absence of microorganisms including pathogens in some samples could be that proper hygienic processing and handling conditions were maintained thereby, preventing post process re-contamination and cross-contamination. This observation is in agreement with the report of ICMSF (1980), Jay (1986) reported that proper handling after processing could prevent bacteria re- entry into the processed products. The potential health risks associated with these pathogens is that of healthy carriers, especially individuals with nasal carriers and boils. When such persons are involved in handling food items including water, both before and after processing, such individuals would constitute transmission vectors. The above observations are in agreement with the reports of WHO (2011); Hunter and Fewtrell (2001). Hence, people hand-

ling foods including drinking water should be made to pass microbiological tests in order to detect such healthy carriers.

Although *Salmonella* species was not detected in the samples, it could have been due to possible limitations in the analytical procedures since other associated organisms such as *E. coli, S. aureus, Alcaligenes, Acinetobacter, B. subtilis* and *Pseudomonas* were present. Moreover, the frequent case of reported typhoid fever in the region is a proof of the above assertion (Blum et al., 1987; Ogan, 1988). The presence of *S. aureus* in some samples could be due to re- contamination.

The low level or absence of *S. aureus* in some samples is because *S. aureus* is not usually of fecal origin and that its presence in the samples could have been associated with post processing contamination due to bottle/sachet leakage. The public health significance of these microorganisms can hardly be over-emphasized. They have been shown to cause cholera, typhoid fever, hepatitis, diarrhea, gastroenteritis and some emerging strains of *E. coli* have been reported to cause septicemia and urinary tract infections especially, in immuno-compromized individuals (Todar, 2002; Bik et al., 2010).
Table 3 presents the inorganic ions and pH values of the samples. It is evident that their values were within the acceptable levels. The robust Ca: P ratio in the samples is of nutritional importance as low Ca: P ratio has been shown to cause osteoporosis in animals (Orish et al., 2006; Jorge et al., 2007; Nachiyunde et al., 2013).

Although, the in-organic ion concentrations were within the standard limits, the low level or absence of some ions in some samples could be that some must have been used by the organisms to obtain necessary growth nutrients for their proliferation (Barrell et al., 2000).

Table 4 gives the essential organic acids in the water samples. Although organic acids are not regulated in drinking water, short-chain organic acids are formed as by-products during ozonation from natural organic matter present in the water (Chigbu and Sobolev, 2007). Ozonation has been used effectively as drinking water treatment technique for disinfection, destruction of taste and odor compounds and color removal amongst others (WHO, 1993; 1998, WHO/UNISEF, 1996; White, 1999). However, ozonation process should be followed by biological filtration to remove biodegradable organic compound such as organic acids so as to provide biological stable water and prevent bacterial re-growth and water borne disease outbreaks (LeChevallier, 1990; Stenstrom, 1994). The pH values of the water samples show that the levels are within the permissible acceptable standards by NAFDAC, the local regulator of food, water and drug marketed in Nigeria.

Conclusion and recommendations

The results show that although the levels of organisms in most of the water samples were within the acceptable limits, it should however, be a source of concern considering the limitations in the identification, type of organisms identified, and risk of post- treatment contamination and cross - contamination. The essential elements present in the samples were within the acceptable limits. Calcium ion was present in the highest concentration compared to other ions and the Ca/P ratio was good. Based on the findings it is, therefore, recommended that the mandatory standard level of chlorination should be adhered to, and the need for public enlightenment on the frequent routine microbiological checks due to cross- contamination and re-contamination through bottles/sachets leakage be encouraged. Moreover, further studies that should provide insights into the new emerging pathogens such as *E.Coli* 015:H7, *Helicobacter sp*, and *Caliciviruses* should employ the use of molecular methods coupled with throughput parallel processing, bio-informatics and *Cryptosporidium oocysts* tests in the analysis of drinking water. Finally, there is need to correlate bottled/packaged water micro flora with the source flora.

Conflict of interests

The authors did not declare any conflict of interest.

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