Physicochemical and microbiological assessment of drinking water samples collected from Lagos.

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Submitted: 23rd Sept., 2024; Accepted: 29th Sept., 2024; Published online: 31st October, 2024
DOI: https://doi.org/10.54117/jcbr.v4i5.3
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Abstract

Poor drinking water quality affects people, the economy and the environment. Contaminated water is the primary cause of diseases such as cholera, typhoid fever, diarrhoea and dysentery in Nigeria. These diseases cause death in individuals. This study aimed to investigate the physicochemical parameters and the presence of coliforms in various sachet and bottled water sold in Lagos, Nigeria. A total of 23 processed water samples were used for this study which included 10 sachets of water, 11 bottles of water and 2 dispenser water sold among different vendors in Lagos. The pH, hardness, electrical conductivity, hardness, turbidity, colour, total dissolved solids, total suspended solids, chloride, magnesium and calcium level were analysed. The most probable number method was carried out and the 10^{-3} dilutions of the positive tubes were plated on Eosin Methylene Blue Agar and Mac Conkey Agar. A total of thirteen isolates were identified from the colonies, nine (9) from bottle water and four (4) from sachet water. The isolates were identified using the VITEK method. Among the microbes isolated from the water samples were *Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas fluorescens, and Escherichia coli.* This shows the presence of faecal coliforms in some water samples. This study calls out the need for optimum hygienic practices in water production and packaging companies to eliminate contaminants in water and to ensure the water is properly treated to reduce the chemicals and particles present in sachet and bottled water sold in Lagos.

Keywords : Water quality, VITEK, Coliforms

Introduction

Water is essential for life. It is an indispensable resource needed for the continued existence of all living things including man and adequate supply of fresh

and clean drinking water is a basic need for all human beings (Edema *et al.*, 2011). Improving individuals' access to safe drinking-water can result in tangible health benefits. Although water is one of the fundamental necessities for human daily consumption, a high number of people still lack access to safe drinking-water, the majority of whom live in rural areas, which are commonly served by natural water supplies (Edema *et al.*, 2011)

The lack of accessible, reliable and safe drinking water, together with poor sanitation and hygiene, is estimated to cost Nigeria in access time, loss due to premature death, productive time lost and health care costs reference. Water has been frequently a means of transmission of a wide range of contagious diseases. Diseases such as diarrhoea, typhoid, dysentery, and cholera which continue to pose serious public health risks to the people are transmitted through water. It is therefore necessary that the quality of drinking water must be checked, and the safety ascertained before it is certified good for drinking. According to World Health Organisation (WHO) guidelines for national standards on drinking water quality, safe drinking water is described as having microbiological, chemical, and physical qualities that are within acceptable ranges (WHO, 2011).

From a microbiological perspective, the greatest microbial risks are associated with the ingestion of water that is contaminated with faeces in the form of combined sewage overflow (CSO), non-collective sewage systems, manure spreading, pit stock overflow, domesticated animal contamination, or wildlife contamination with water (Jung *et al.,* 2014). Faeces can be a source of pathogenic bacteria, viruses, protozoa and helminths. Faecally derived pathogens are the principal concerns in setting health-based targets for microbial safety. Microbial water quality often varies rapidly and over a wide range. Short-term peaks in pathogen concentration may increase disease risks considerably and may trigger outbreaks of waterborne disease. Hence, drinking water should be free of all waterborne pathogens and opportunistic microorganisms.

Microbial pathogens including *Salmonella* spp., *Shigella* spp., Coliforms, *Mycobacterium* spp., when consumed in drinking water could be harmful to human health. Conventionally, coliforms are used to assess the microbial quality of water. Coliforms are Gram-negative, rod-shaped bacteria that can grow in the presence of bile salts and ferment lactose at temperatures between 35^{o}C and 37^{o}C, producing acid and gas during a 24–48-hour period. They don't

generate spores and are oxidase-negative. Because they are simple to isolate and count, coliform organisms (such as *E. coli*) are often used as appropriate microbiological indicators of drinking water quality. Other forms of contaminants in water are inorganic contaminants which are based on chemical parameters such as hardness presence of magnesium, mercury, lead, cyanide and fluoride which can be present based on natural or industrial processes or plumbing. Prolonged exposure to such water can result in disease in the long run (Sharma and Bhattacharya, 2016).

In recent times, there has been a shift in consumption of tap water and well water to processed water sold in sachets or bottles. Therefore, this study intends to assess the physicochemical and microbiological properties of sachet and bottled water samples sold in stores in selected tertiary institutions and markets in Lagos.

Materials and methods

Description of the study area and sample collection

The following locations were surveyed for sachet water and bottled water. They included the University of Lagos (Akoka), Federal College of Education (Akoka), Yaba College of Technology (Yaba), Lagos State University (Ojo), Bariga, College of medicine of University of Lagos (Idi- araba) and Iyana - Ipaja market, Lagos State. The samples were processed at the Department of Microbiology Laboratory and Department of Chemistry, University of Lagos, while the VITEK identification was carried out at Lagos University Teaching Hospital, Idi-Araba Lagos

The samples were collected using random sampling methods without repetition from stores in the mentioned locations. A total of twenty-three water samples which include Sachet water (10), bottled water (11), Dispenser water (2), were obtained. The samples were brought in their packaged state to the laboratory.

Isolation of coliforms:

Most probable number (MPN) Test:

Coliform bacteria in the water sample were determined by the most probable number test. This test was performed sequentially in three stages:

1) Presumptive coliform test: This was used to detect coliforms in a water sample. In this test, MacConkey broths were prepared according to the manufacturer's instructions. Durham tubes were put invertedly into all the tubes and sterilised by autoclaving. They were inoculated with different water volumes.

a) 10 mL of water sample was inoculated in each of 5 tubes containing 10 mL of the double strength of MacConkey broth.
b) 1 mL of water sample was inoculated in each of 5 tubes containing 5 mL of the single strength of MacConkey broth.
c) 0.1 mL of water sample was inoculated in each of 5 tubes containing 5 mL of the strength of MacConkey broth.

All the inoculated tubes were incubated at 37°C for 48 hours. After incubation, the tubes which had produced both acid and gas were counted. The tubes showing gas production and acid formation were further inoculated for confirmatory tests.

2) Confirmed coliforms test: This test is used to confirm the presence of coliforms. In this test, water samples from all the positive presumptive MacConkey broth tubes were inoculated into two sets of tubes of Brilliant Green Lactose Bile salt broth and incubated at 37°C for 48 hours.

3) Completed coliform test: Positive tube from the confirmatory was streaked on the plates of MacConkey and Eosin Methylene Blue Agar and incubated at 37°C and 44.5°C for 24 hours. After 24 hours, the colonies that had growth were transferred to sterile nutrient broth and incubated at 37°C for 24 hours. The presence of total coliform and faecal coliform bacteria was confirmed by Gram Staining.

VITEK identification

VITEK 2 is an automated microbiology system that utilises growth-based technology for identification. The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities. A sterile swab or applicator stick was used to transfer colonies of isolates into 3.0 mL of sterile saline. The identification cards were inoculated with microorganism suspensions with an integrated vacuum apparatus. A test tube containing the microorganism suspension was placed into a special rack (cassette) and the identification card was placed in the neighbouring slot while inserting the transfer tube into the corresponding suspension tube. The filled cassette was placed into the vacuum chamber station. After the vacuum is applied and air was reintroduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells. All card types were incubated at 35.5ºC. Each card was removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator

until the next read time. Data were collected at 15-minute intervals during the 10 hours incubation period.

Determination of physicochemical parameters

1) Colour: The colour was determined using HACH DR 2000 direct reading spectrophotometer, method 8025. Each sample was first filtered and measured against previously filtered deionized water as blank at wavelength of 455 nm.
2) pH: The pH of each water sample was estimated using the Test-2 pH metre. The pH metre was first of all standardised against buffer solutions pH 4, 7, and 9.2 after which samples were tested in turn.
3) Electrical conductivity: The electrical conductivity of each water sample was measured using potable combined Electrical conductivity/TDS/Temperature metre (HM Digital COM-100). The electrical conductivity metre was standardised with 342 ppm sodium-chloride calibration solution after which the different water samples were tested in turn.
4) Turbidity: The turbidity of each water sample was measured using HACH DR 2000 direct reading spectrophotometer method 8237. The turbidity of the sample was estimated against deionized water as a blank at a wavelength of 450nm.
5) Total Solids: The total solids were determined gravimetrically by taking aliquot of each water sample in a clean, dry beaker. The water was then evaporated on a hot plate until all the water was almost dry. The drying process was completed in an oven whose temperature was set at 150˚C. The difference in mass of the empty beaker and the beaker containing the solids was computed. The total suspended solids (TSS) of each water sample was measured using HACH DR 2000 direct reading spectrophotometer, method 8006. The TSS of each sample was estimated against deionized water as blank at a wavelength of 810nm. The Total dissolved solids (TDS) of each water sample was measured using potable combined Electrical conductivity/TDS/Temperature metre (HM Digital COM-100). The electrical conductivity metre was

standardised with 342 ppm sodium chloride calibration solution after which the different samples were tested in turn.

6) Total Hardness: This was determined by measuring 100cm3 of water sample into a 250 cm3 conical flask and 2.0ml buffer solution was added and mixed. Eight drops of Erichrome black T indicator were introduced followed by titration with 0.01M Ethylene Diamine Tetraacetic Acid (EDTA) solution. A colour change from wine red to pure blue indicated the end point. The entire procedure was carried out for each of the water samples. The Total hardness, for each sample, was then computed.

Total hardness in mg/l $CaCO_3$

$$= \frac{\text{ml of EDTA x M x 100 x 1000}}{\text{ml of sample}}$$

7) Calcium Hardness: This was determined by measuring 100 cm^3 of the water sample into a 250cm^3 conical flask and 1cm^3 of 4M NaOH and 200g of murexide indicator were added. This was then titrated with 0.01M EDTA to a violet colour end point. The procedure was repeated for each water sample and the hardness computed as given below.

Calcium hardness in mg/l $CaCO_3$

$$= \frac{\text{ml of EDTA x M x 100 x 1000}}{\text{ml of sample}}$$

The Magnesium level was calculated using the formula: 0.243 x (Hardness - Calcium Hardness)

8) Chloride: This was determined by measuring 50 ml of the sample, 1 ml of potassium chromate was added and it was titrated with standardised 0.01M Silver nitrate to a red – pinkish yellow, for sample with expected high conductivity 0.1M standardised silver nitrate was used.

Mg/l chloride =

$$\frac{\text{Titre value x Molarity of Silver nitrate x 35,450}}{\text{ml of sample}}$$

Statistical analysis

The t-test, p values and correlation coefficient were calculated using Statistical Package for Social Science (SPSS).

Results

The presence of coliforms was determined using the most MPN method, revealing that

four water samples tested positive, with Puw C showing the highest count (Table 1). The positive tubes were plated using a 10^3 dilution on the Agars. Growth of colonies with different characteristics confirmed the presence of coliform bacteria, including some faecal coliforms, in the water samples (Table 2). *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas fluorescens*, and *Escherichia coli* (Table 2) were among the isolates identified. The total coliform and faecal coliform counts were also determined (Table 3).

Table 1: Most Probable Number (MPN)

Water samples	10 ml of Double strength	1 ml of Single strength	0.1ml of Single strength	MPN(per 100ml)
Taw A	0	0	0	<2
Taw B	0	0	0	<2
Taw C	0	0	0	<2
Taw D	0	0	0	<2
Taw E	4	2	0	22
Taw F	5	3	2	140
Taw G	1	1	0	4
Taw H	0	0	0	<2
Taw I	0	0	0	<2
Taw J	0	0	0	<2
Taw K	0	0	0	<2
Puw A	4	0	0	13
Puw B	0	0	0	<2
Puw C	5	5	5	>1600
Puw D	0	0	0	<2

Puw E	0	0	0	<2
Puw F	0	0	0	<2
Puw G	0	0	0	<2
Puw H	0	0	0	<2
Puw I	0	0	0	<2
Puw J	0	0	0	<2
Diw A	0	0	0	<2
Diw B	0	0	0	<2

Table 2: Colony characteristics of organisms present in water samples

Isolate code	Medium	Colonial Characteristics	Gram's reaction	Organisms identified by VITEK
Taw EI	EMB Agar	- Pink with dark centre, Opaque, Convex, small, Mucoid, Entire, Circular.	Gram negative rods	- *Klebsiella pneumoniae* subsp. *pneumoniae*
Taw EII	MacConkey Agar	- Colour pink, Opaque, Convex, Large, Mucoid, Entire, Circular.	Gram negative rods	- *Klebsiella pneumoniae* subsp. *pneumoniae*
Taw FI	EMB Agar	- Light pink, small, non-mucoid, translucent, entire.	Gram negative rods	- *Enterobacter cloacae complex*
Taw FII	MacConkey Agar	- Vibrant pink coloured, opaque, large, circular, non-mucoid, Entire, Round, convex,	Gram negative rods	- *Enterobacter cloacae complex*

Taw GI	EMB Agar	- Pink with dark centre, Opaque, Convex, small, Mucoid, Entire, Circular.	Gram negative short rods	- *Klebsiella pneumoniae* subsp. *pneumoniae*
Taw GII	EMB Agar	- Light pink, small, non-mucoid, translucent, entire.	Gram negative rods	- *Enterobacter cloacae complex*
Taw G III	MacConkey Agar	- Vibrant pink coloured, opaque,non-mucoid, Entire, Round	Gram negative rods	- *Enterobacter cloacae complex*
Taw G IV	MacConkey Agar	- Colour pink, Opaque, Convex, Large, slimy, Mucoid, Entire, Circular.	Gram negative rods	- *Klebsiella pneumoniae* subsp. *pneumoniae*
Taw G V	MacConkey Agar	- Small, circular, motile, non-mucoid, faint green	Gram negative rods	- *Pseudomonas flourescens*
Puw CI	EMB Agar	- Green metallic Sheen, Small, medium with entire margin, opaque, slightly raised.	Gram negative rods	- *Escherichia coli*
Puw CII	EMB Agar	- Pink, opaque, non-mucoid, Entire, Round	Gram negative rods	- *Enterobacter cloacae complex*
Puw CIII	MacConkey Agar	- Pink, swarming, opaque, small, medium, raised, entire, round	Gram negative rods	- *Escherichia coli*
Puw CIV	MacConkey Agar	- Vibrant pink coloured, opaque, non-mucoid, entire, round	Gram negative rods	- *Enterobacter cloacae complex*

Table 3: Total coliform and faecal coliform counts in water samples

MacConkey Agar

Water Sample	Colony number	Colony Forming Unit CFU/ml
Taw E	60	6.0×10^5
Taw F	72	7.2×10^5
Taw G	32	3.2×10^5
Puw C	99	9.9×10^5

Eosin Methylene Blue Agar

Taw E	37℃ -12	1.2×10^5
	44℃ - 7	7.0×10^4
Taw F	37℃ - 16	1.6×10^5
	44℃ - 7	7.0×10^4
Taw G	37℃- 17	1.7×10^5
	44℃ - 5	5.0×10^4
Puw C	37℃- 19	1.9×10^5
	44℃- 9	9.0×10^4

CFU/ml - Colony Forming Unit

$$\frac{\text{Number of Colonies} \times \text{Dilution factor}}{\text{Volume of Aliquot}}$$

Dilution factor = 10^3 Volume of Aliquot = 0.1ml

Physicochemical Parameters

The physicochemical parameters such as pH, temperature, electrical conductivity, total suspended solids, total dissolved solids, calcium, chloride, magnesium levels, turbidity, hardness, and colour were determined. All the water samples were colourless. Among the bottled and dispenser water samples, 31% were within the standard pH range, while 69% were slightly acidic (Figure 1). The other tested physicochemical parameters were within the standard range (Figures 3 and 5). The sachet water samples had a pH range that was slightly acidic (Figure 2), with the other parameters aligning with the standard limits (Figure 4), except for Puw H, which had higher values of calcium and magnesium, influencing its hardness (Figure 6). The t-test result reveals that there is no significant difference between the sachet water properties with the WHO and NIS standards (Table 4) .

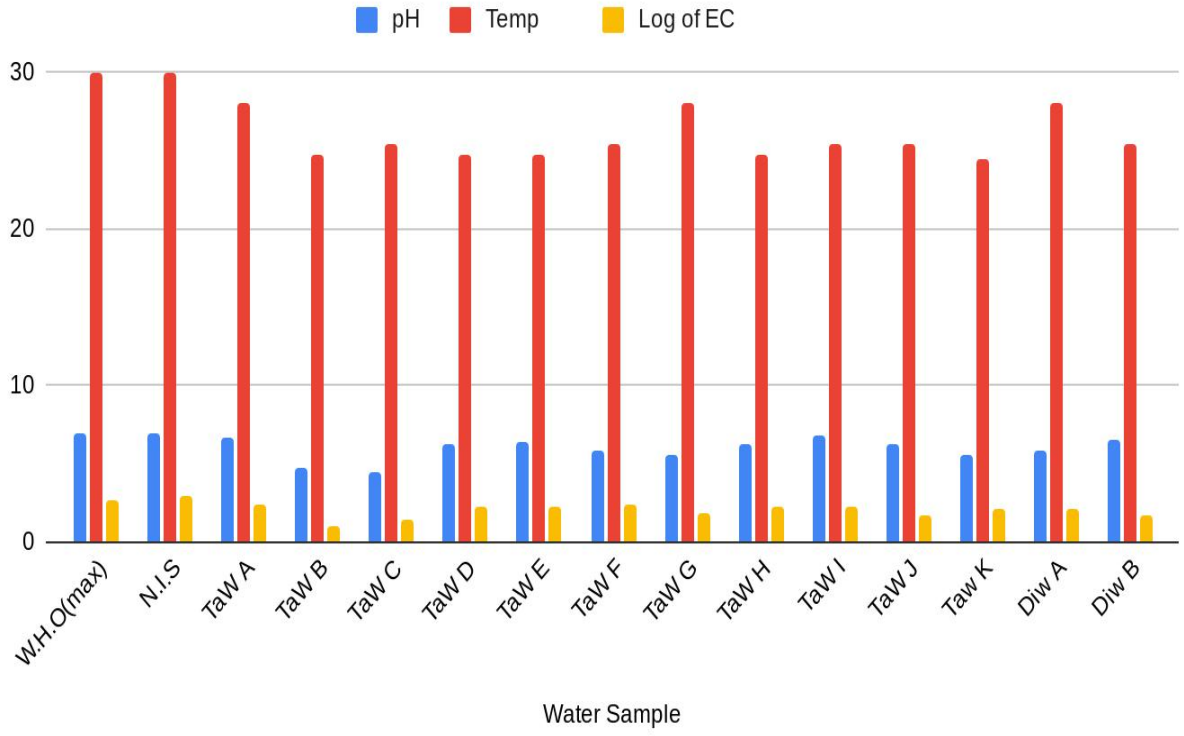


Figure 1: The pH, temperature and log of Electrical conductivity of the bottled and dispenser water samples.
EC= Electrical conductivity. Temp = Temperature

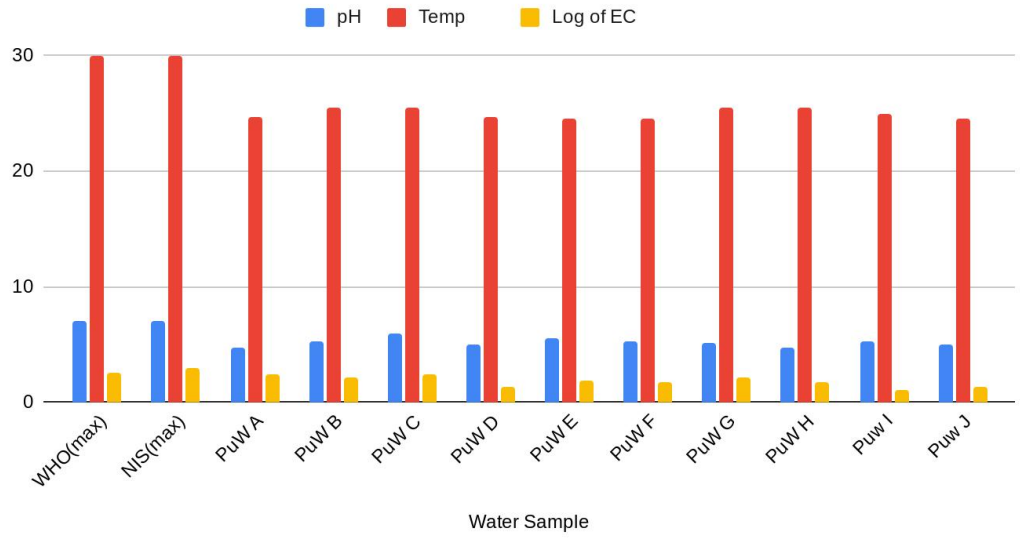


Figure 2: The pH, temperature and log of Electrical conductivity of the sachet water samples. EC= Electrical conductivity. Temp = Temperature

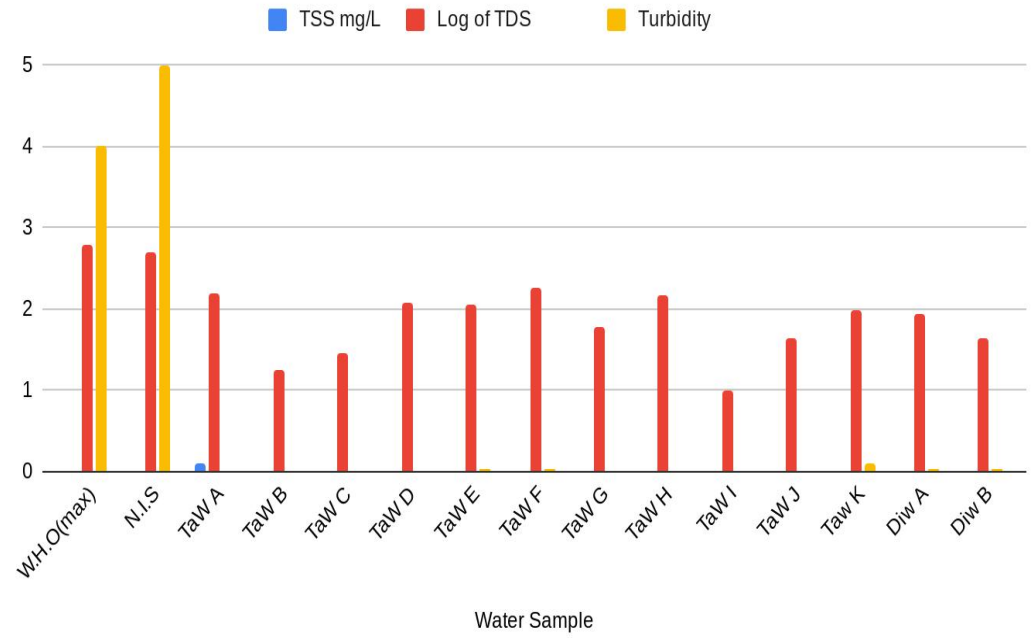


Figure 3: The total Solids and turbidity of the bottled and dispenser water samples. TSS= Total Suspended Solids. TDS=Total Dissolved Solids.

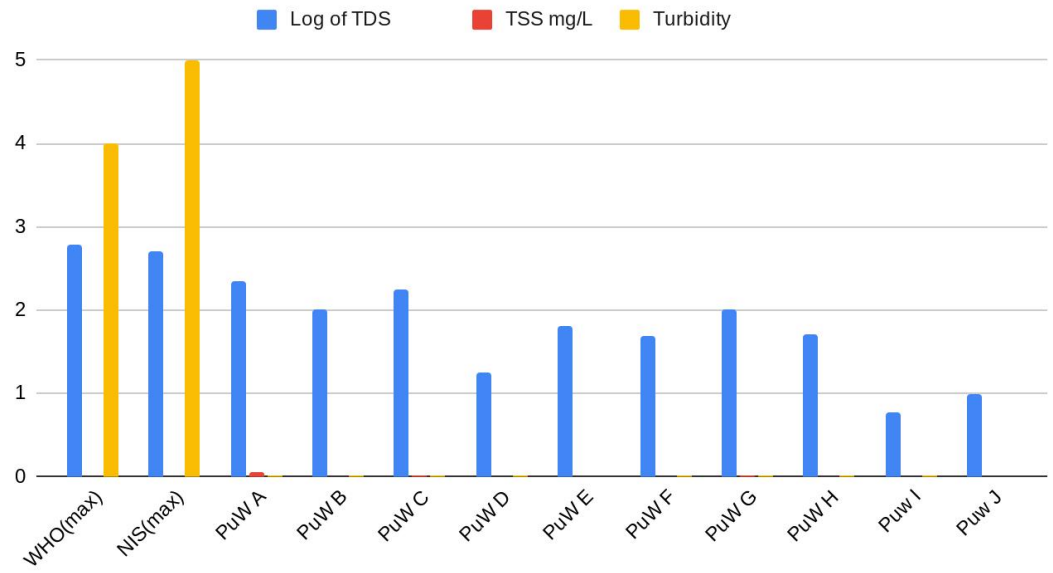


Figure 4: The Total Solids and Turbidity of the sachet water samples.
TSS= Total Suspended Solids. TDS=Total Dissolved Solids.

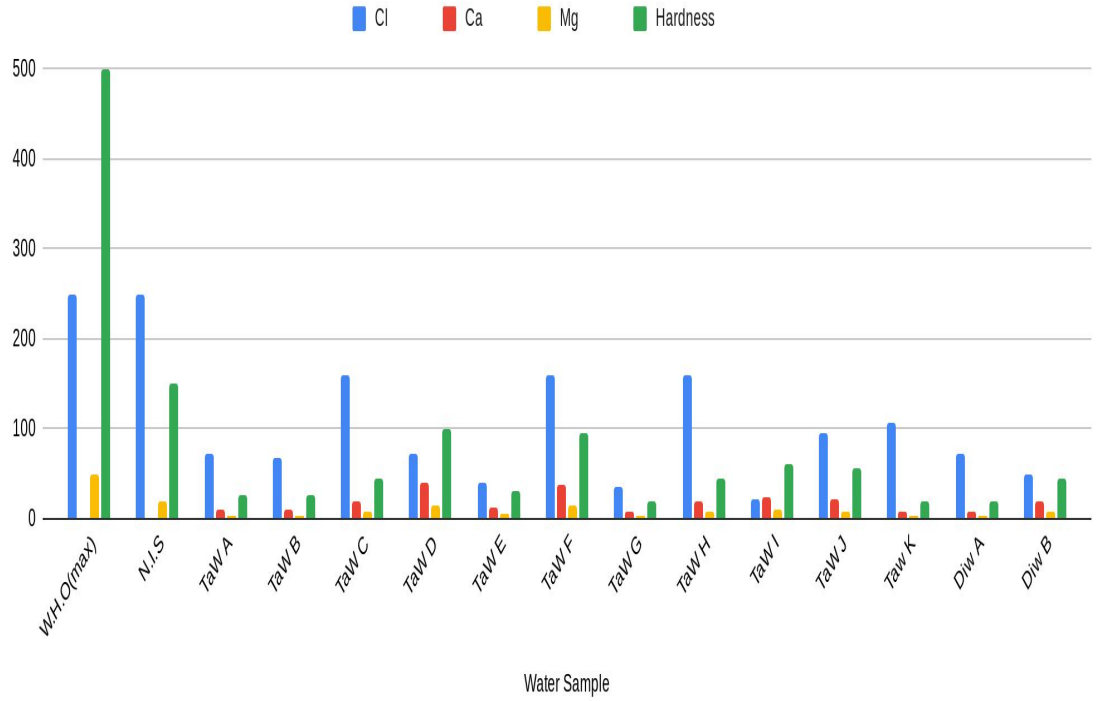


Figure 5: The Chloride, Calcium, Magnesium and hardness level of the bottled and dispenser water samples.
Cl = Chloride Ca = Calcium Mg = Magnesium

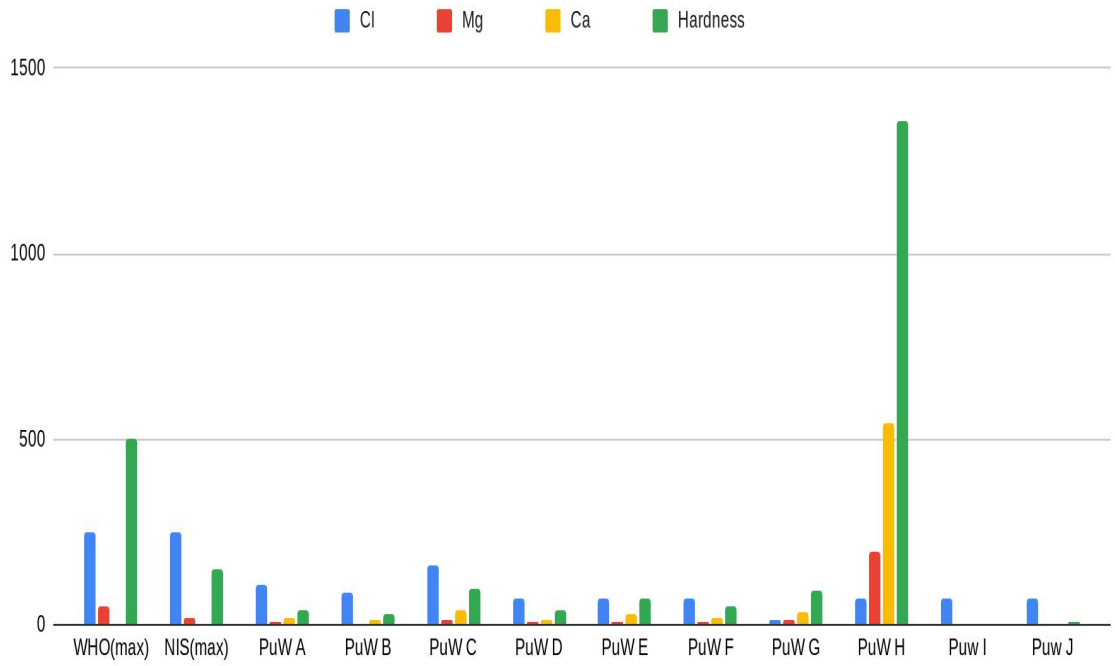


Figure 6: The Chloride, Calcium, Magnesium and hardness level of the sachet water samples. Cl = Chloride Ca = Calcium Mg = Magnesium

Table 4: The t-test for each parameter comparing the different types of water

Parameters	Taw vs Puw (t values)	Taw vs Diw (t values)	Puw vs Diw (t values)
pH	2.12	1.23	1.92
Temperature	0.56	0.45	0.23
Total Suspended Solids	3.78	2.12	1.92
Total Dissolved Solids	2.56	1.89	1.51
Electrical Conductivity	2.45	1.82	1.43
Calcium	3.14	2.34	1.92
Magnesium	2.89	2.01	1.63
Chloride	1.92	1.51	1.23
Turbidity	3.45	2.56	1.92

Parameters	Taw vs Puw (t values)	Taw vs Diw (t values)	Puw vs Diw (t values)
pH	2.12	1.23	1.92
Temperature	0.56	0.45	0.23
Total Suspended Solids	3.78	2.12	1.92
Total Dissolved Solids	2.56	1.89	1.51
Electrical Conductivity	2.45	1.82	1.43
Calcium	3.14	2.34	1.92
Hardness	3.78	2.12	1.92

t-values are a measure of the significant difference between the mean of the two groups in relation to the variability of the data.

Level of significance = 0.05 (95% confidence level)

Table 5: Correlation Coefficient of the bottled and dispenser water

Parameters	Correlation coefficients (r)
pH vs Temperature	0.234
pH vs Total Dissolved Solids	-0.351
pH vs Electrical conductivity	0.567
Calcium vs Magnesium	0.853
Calcium vs Hardness	0.984
Magnesium vs Hardness	0.959
Chloride vs Turbidity	-0.463
Hardness vs Total Dissolved Solids	0.734

Table 6: Correlation Coefficient of sachet water samples

Parameters	Correlation coefficients (r)
pH vs Temperature	0.234
pH vs Total Dissolved Solids	-0.463
pH vs Total Suspended Solids	-0.351
pH vs Electrical conductivity	0.567
Temperature vs Electric conductivity	0.346
Calcium vs Magnesium	0.853
Calcium vs Hardness	0.967
Magnesium vs Hardness	0.923
Chloride vs Turbidity	-0.463
Hardness vs Total Dissolved Solids	0.734

Discussion

Based on the Most Probable Number estimate of the number of coliform bacteria in each water sample, Taw E, F and Puw C showed high high presence of coliform bacteria which is against the WHO standard of <2. The bottled water compared to the sachet water had more positive tubes for coliforms, which contrasts with the work of Okoye and his colleagues who reported that bottled water had no coliforms (Okoye *et al.*, 2022). The presence of coliforms is of great concern as coliforms are responsible for gastrointestinal illness. The presence of coliforms can be due to a potential contamination during the production process such as during water treatment, storing or packaging. Conversely, 9 out of 10 of the sachet water samples tested had MPN values less than 2. The sachet water result did not agree with study carried out in Kebbi which had a great number of coliforms in sachet water (Kalpana *et al*., 2011). Lagos is located in a different geographical location from Kebbi and the water samples were most likely from different manufacturers. So the variation in the results is not out of place.

The sachet water samples with coliforms had *Klebsiella pneumoniae* subsp. *pneumoniae, Enterobacter cloacae* complex and *Escherichia coli.* This result correlates with the result obtained from the study conducted by Maduka and his colleagues in Owerri who reported the presence of *E. coli* in the water (sachet or bottled or both) samples (Maduka *et al.,* 2014). The organisms identified to be present in the bottled water sample were *Klebsiella pneumoniae* subsp. *pneumoniae, Enterobacter cloacae complex, Pseudomonas flourescens.* This correlates with the report of the study carried out by Ugochukwu and her colleagues in Kaduna who reported the presence of *Klebsiella* and *Pseudomonas* in the water samples (Ugochukwu *et al.*, 2015). The presence of these organisms in the drinking water samples may be traced to the natural source of water or may have been introduced during processing and or handling. The presence of *E. coli* in the water sample is an indication of faecal contamination. The presence of *E. coli* is most often accompanied by the presence of dangerous enteric pathogens like *Shigella*, *Salmonella* and *Campylobacter* species which are associated with various diseases. This microbial contamination can also be as a result of ineffectiveness or malfunctioning of the water treatment process employed which then calls to question the effectiveness of NAFDAC oversight on water production.

Although an MPN value less than 2 is a requirement from WHO for water safety, water samples can only be guaranteed safe only when other parameters such as the physicochemical parameters (pH, Hardness, DS) are within the normal range recommended by WHO. For instance, the acidity of water samples affects the taste of the water and may not be pleasant for consumption. It is therefore necessary that the acidity is within the recommended range. The pH of 8 out of 23 water samples were between 6.5 - 7 which fell within the WHO recommended range while the rest were observed to be outside the WHO and NIS standards. The remaining 15 samples cannot be certified safe even if their MPN is less than 2. This result is consistent with the pH result of water samples tested by Uduma in Kano (Uduma, 2014).

A positive correlation was observed between the pH and the electrical conductivity as the electrical conductivity expectedly increased with increase in pH. The electrical conductivity ranged from 10.0 $\mu S/cm^3$ to 240.0 $\mu S/cm^3$. This is consistent with the findings of Uduma (2014). High values of

conductance usually indicate high dissolved gases and other chemicals present in the water. There is no recommended value for conductivity however, values above 400 $\mu S/cm^3$ may affect the chemical quality of drinking water.

Most of the water samples contained no dissolved solids(TDS) while a very few show a very minimal amount of dissolved solids present. This is in agreement with WHO standards that drinking water with a total dissolved solids (TDS) level of less than about 600 mg/l is generally considered to be good and it can be said that this water is safe for consumption on account of TDS.

The levels of magnesium and chloride and hardness tested for the bottled water were within the standard limit for WHO (magnesium - 50 mg/L, chloride - 250 mg/L, hardness - 500 mg/L) and NIS (magnesium - 20 mg/L, chloride - 250 mg/L, hardness -150 mg/L) . This is consistent with the findings of Taiwo and his colleagues whose results were within the WHO and NIS standard range (Taiwo *et al.* 2010). Results showed a positive correlation between the calcium, chloride, magnesium levels and hardness. Also, a strong positive correlation existed between the hardness of the samples and total dissolved solids suggesting that the water hardness is largely driven by the presence of dissolved minerals. A negative correlation between the chloride and turbidity across the samples was observed. An increase in the chloride level resulted in a decrease in the turbidity level. This suggests that the decrease in turbidity was a result of a proper treatment process thereby making the water clearer and free of contaminants. A high turbidity may indicate contamination with potentially harmful organisms and other pollutants that enabled their growth.

A comparison of the MPN results with the physico-chemical results showed that Taw F and Puw had high MPN values (140 and >1600 respectively) as well as high calcium content. This suggests a link between the bacterial contaminations with some of the parameters. It may be that the activity of the microorganisms in the water sample aided the increase of calcium in the water sample. Further investigation may be necessary to understand the relationship.

Conclusion

The results from this study indicated that there were coliforms in some of the samples tested. The physicochemical parameters in the various sachets and bottles of water sold in tertiary institutions in Lagos were also outside

the acceptable ranges in some of the samples. This calls for improvement in the quality assurance process of drinking water production in Lagos state. The bottles and packaging materials should be properly sterilised before use. It is also advised that this water is stored properly by the vendors. Quality assurance and control systems should be put in place in the water production units and should be strictly adhered to. It is also advised that there should be periodic visits by regulatory agencies to the site of production to inspect the water production processes.

Acknowledgement

The authors are grateful to the Department of Microbiology and Department of Chemistry, Faculty of Science University of Lagos, Nigeria and the Department of Medical Microbiology, College of Medicine, University of Lagos for providing appropriate support in terms of equipment and laboratory used for the study.

Conflict of Interest Statement:

The authors declare that no competing financial or non-financial interests exist.

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