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**Research Article**

# Bacteriological Load Compared Between Sachet Water Samples Collected From Gwale Local Government and That of Kano Municipal, Kano Metropolis

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**Abstract:** Water is a resource that is both invaluable and vital to the existence of all living organisms, but this valued resource is increasingly being threatened as human populations grow and demand more water of high quality for domestic purposes and economic activities. The conducted research was aimed to assessing Bacteriological quality of sachet water samples sealed and sold and consumed in Gwale Local Government and Kano Municipal of Kano metropolis. The bacteriological assessment of sachet water samples collected were determined by Aerobic Plate Count (APC), test for Coliforms and Biochemical Test (IMViC). This test was continued by staining technique and concluded with microscopy were some members of Coliforms were viewed. APHA, (2002).
**Keywords:** organisms, Aerobic Plate Count (APC), (IMViC).

## INTRODUCTION

Water quality assessment is a very complex subject, in part because water is a complex medium intrinsically tied to the ecology of the planet Kozlowsky-Suzuki, B. and Bozelli, L.R. (2004). To determine water quality therefore, several parameters must be examined. The complexity of water quality assessment as a subject is reflected in the many types of measurements of water quality. Among the key parameters listed by WHO (2011) for the determination of water quality for domestic use are Conductivity, dissolved oxygen (DO), pH, color of water, taste and odor, turbidity, total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), micro-organisms such as faecal coliform bacteria (*Escherichia coli*), cryptosporidium and *Giardia lamblia*; nutrients (fertilizers), dissolved metals and metalloids (lead, mercury, arsenic, etc.) and dissolved organics. Water related health problems are a growing human tragedy, and according to WHO (2002), it kills more than 5 million people a year with infants being the most affected. This figure seems to be the highest as compared to wars and disasters (UNESCO, 2003). The objective of this study was to analyze the bacterial load from the collected samples from both local governments.

## MATERIALS AND METHODS

### Sample Size

Yamane (2011) provides a simplified formula to calculate sample sizes. Sample sizes can be collected Equally, Proportionally or by key collection, ere equal sample collection was employed. This formula is used to calculate the sample sizes in scientific researches. A 95% confidence level and P = 0.5 are assumed for the formula.

$$n = \frac{N}{1 + N * e^2}$$

$n = 400/1+400*0.05^2$
$n = 400/1+9$
$n = 400/10 = 40$

Where n is the sample size, N is the population size, and e is the level of precision.

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**Bacteriological Analysis**

**Aerobic Plate Count (APC)**

For the enumeration of Mesophillic *bacteria* in treated water samples, the serial dilution method as described by the American Public Health Association (APHA 1992) was employed. 11ml of water sample was mixed with 99 ml of 1% peptone water. The sample was shaken thoroughly to make a homogenate solution, this give the dilution of $10^{-1}$. 1ml of this prepared solution was transferred in to 9ml of the diluents (0.1% peptone water), this give the dilution of $10^{-2}$. This procedure was repeated up to the fifth dilution which gave the dilution of $10^{-5}$.

The dilution bottles were agitated to respond settled materials. 1ml of each dilution was then pipette into separate corresponding Petri dish in duplicates. About 15ml of nutrient agar (NA) cooled to $45^0$C was poured in to each plate. The sample and the agar medium were mixed by rotating the plate on a flat surface and allowed to solidify. The Petri-dishes were then inverted and incubated at $35^0$C for 48 hours. Plates containing between 30-300 colonies were selected and counted. The number obtained was multiplied by the dilution factor. This gave the number of bacterial colony forming unit per ml of the treated water sample, (CFU/ml). This above procedure was repeated for other treated (package) water samples.

**The following formula was used to calculate the number of bacteria colony forming units per mill of the treated (package) water samples.**

N(ml) = n/vd

Where; N= the number of bacterial colony per ml of treated water sample.

n= Number of colonies counted. v= volume of sample (inoculums) used.

d= dilution factor.

N(ml) = $30/1*10^{-1}$ = 3.00cfu/ml Lower limit

N(ml) = $300/1*10^{-1}$ = 30.00cfu/ml Upper limit

**3.5.4 Enumeration of *Staphylococcus Aureus***

For the enumeration of *Staphylococcus aureus* in the well water samples, serial dilution methods as described by the American Public Health Association (APHA 1992) was employed.

11ml of treated water sample was aseptically measured and transferred in a clean conical flask containing 99ml of 1% Peptone water and stirred to make a homogenate mixture.

Decimal dilution of the sachet water ($1\text{-}10^{-1}$, to $1\text{-}10^{-5}$, was prepared by successive transfer of 1ml of the treated water homogenate to 9ml sterile 0.1% peptone water in dilution bottles. 0.2ml of a dilution of the homogenate was pipette onto the surface of previously dried duplicate plate of Baird parker medium and a sterile bent glass rod was used to spread the inoculums. The plates were incubated at $37^0$c for 24 hrs. Black and shining colonies were selected and counted. The result was reported as number of Staphylococcal colony forming unit per ml of the water sample (CFU/ml)

**Gram staining**

A colony from the purified subculture was isolated and emulsified in sterile distilled water and a thin preparation was made on the slide. This was evenly spread on the slide to cover an approximated area of about 15-20mm in diameter. After the smear, the slide was left on a rack to dry making sure the slide is protected from dust and sunlight. The smear was then fixed using gentle heat, by rapidly passing the slide with the smear uppermost, three times through the flame of a Bunsen burner. The slide was then placed on the back of the hand just to make sure too much heat is not applied, which can affect or even kill the microorganism. The smear was then allowed to cool before staining. The glass slide containing the smear was placed on the staining rack and covered with crystal violet stain and allowed for 60sec. The stain was then washed off with distilled water. The water was then completely tip off and the smear was covered with Lugol's iodine for 60sec. The iodine was also washed off using distilled water. The smear was then decolorized rapidly with acetone and washed immediately with clean water. The smear was covered with neutral red stain for 2min, and again was washed off using clean water. The back of the slide was wiped clean, and placed on a draining rack for the smear to air-dry.

**Biochemical Tests/IMViC Reactions**

IMViC reactions are a set of four useful reactions that are commonly employed in the identification of members of family enterobacteriaceae. The four reactions are: Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test. The letter "i" is only for rhyming purpose.

i- **Indole test:** Some bacteria produced indole from amino acid tryptophan using the enzyme typtophanase. Production of indole was detected using Kovac's reagent. Indole reacts with the aldehyde in the reagent to give a red color. An alcoholic layer concentrates the red color as a ring at the top. Bacteria tested were inoculated in peptone water, which contains amino acid, tryptophan and incubated overnight at 37°C. Following incubation few drops of Kovac's reagent were added. Kovac's reagent consists of para-dimethyl aminobenzaldehyde, isoamyl alcohol and Concentrated HCl. Formation of a red or pink coloured ring at the top is taken as positive. Example, bacteria: *Escherichia coli*: Positive; *Klebsiella pneumoniae*: Negative

ii- **Methyl Red (MR) test:** This is to detect the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Some bacteria produced large amounts of acids from glucose fermentation that they overcome the buffering action of the system. Methyl Red is a pH indicator, which remains red in color at a pH of 4.4 or less. The bacteria tested were inoculated into glucose phosphate broth, which contains glucose and a phosphate buffer and incubated at 37ºC for 48 hours. Over the 48 hours the mixed-acid producing organisms produced sufficient acid to overcome the phosphate buffer and remain acid. The pH of the medium was tested by the addition of 5 drops of MR reagent. Development of red color is taken as positive. MR negative organisms produce yellow color. Example: *Eschericihia coli*: Positive; *Klebsiella pneumoniae*: Negative.

iii- **Voges Proskauer (VP) Test:** While MR test is useful in detecting mixed acid producers. VP test detects butylene glycol producers. Acetyl-methyl carbinol (acetoin) is an intermediate in the production of butylene glycol. In these test two reagents, 40% KOH and alpha-naphthol were added to test broth after incubation and exposed to atmospheric oxygen. If acetoin is present, it is oxidized in the presence of air and KOH to diacetyl. Diacetyl then reacted with guanidine components of peptone, in the presence of alphanaphthol to produce red color. Role of alpha-naphthol is that of a catalyst and a color intensifier. Bacteria tested was inoculated into glucose phosphate broth and incubated for at least 48 hours. 0.6 ml of alpha-naphthol was added to the test broth and shaken. 0.2 ml of 40% KOH was added to the broth and shaken. The tube was allowed to stand for 15 minutes. Appearance of red color is taken as a positive test. The negative tubes were held for one hour, since maximum color development occurs within one hour after addition of reagents. Examples: *Escherichia coli*: Negative; *Klebsiella pneumoniae*: Positive.

iv- **Citrate Utilization Test:** This test detected the ability of an organism to utilize citrate as the sole source of carbon and energy. Bacteria were inoculated on a medium containing sodium citrate and a pH indicator bromothymol blue. The medium also contains inorganic ammonium salts, which is utilized as sole source of nitrogen. Utilization of citrate involves the enzyme citritase, which breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down to pyruvate and CO2. Production of $Na_2CO_3$ as well as $NH_3$ from utilization of sodium citrate and ammonium salt respectively resulted in alkaline pH. This results in change of medium's color from green to blue. Bacterial colonies are picked up from a straight wire and inoculated into slope of Simmon's citrate agar and incubated overnight at 37ºC. If the organism has the ability to utilize citrate, the medium changes its color from green to blue. Examples: *Escherichia coli*: Negative; *Klebsiella pneumoniae*: Positive.

## RESULTS

**Aerobic Plate Count (APC)** values obtained from various sachet water samples Collected from Gwale and Kano Municipal of Kano Metropolis

| Colony Forming Unit (CFU)/100ml of Sachet Water Samples | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | GWALE | | | | MUNICIPAL | | | |
| S/No. | A | B | n | $10^{-1}$ | A | B | N | $10^{-1}$ |
| 1 | 76 | 69 | 72.5 | 7.25 | 66 | 80 | 73 | 7.3 |
| 2 | 100 | 96 | 98 | 9.8 | 89 | 96 | 92.5 | 9.25 |
| 3 | 88 | 72 | 80 | 8 | 101 | 87 | 94 | 9.4 |
| 4 | 93 | 82 | 87.5 | 8.75 | 98 | 100 | 99 | 9.9 |
| 5 | 72 | 80 | 76 | 7.6 | 82 | 96 | 89 | 8.9 |
| 6 | 67 | 74 | 70.5 | 7.05 | 79 | 84 | 81.5 | 8.15 |
| 7 | 90 | 87 | 88.5 | 8.85 | 87 | 79 | 83 | 8.3 |
| 8 | 95 | 102 | 98.5 | 9.85 | 72 | 88 | 80 | 8 |
| 9 | 58 | 63 | 60.5 | 6.05 | 99 | 86 | 92.5 | 9.25 |
| 10 | 83 | 67 | 75 | 7.5 | 102 | 106 | 104 | 10.4 |

Source: Laboratory Assessment, 2016

**Morphology, Gram staining and Biochemical properties of bacterial isolates in sachet Water samples sold in Kano metropolis.**

| Colonial Morphology | Microscopic Examination | Indole test | Methyl red | Voges Proskauer | Citrate test | Gram Staining | Suspected Organisms |
|---|---|---|---|---|---|---|---|
| Small Circular Colonies | Short Rod In Singles | - | - | + | + | - | *Salmonella* spp |
| Opaque cream yellow growth | Gram positive Cocci in Clusters | - | - | + | + | + | *Staphylococcus aureus* |
| Shiny viscous Colonies | Gram negative Short Rod | - | - | + | + | - | *Klebsiella spp* |
| Green metallic sheen colonies | Gram negative rods | - | - | + | + | - | *Pseudomonas spp* |
| Green metallic sheen colonies | | + | + | - | - | - | *E. coli* |

Source: Laboratory Analysis, 2016

**Table 2 Shows water quality in terms of percentage of the bacterial load between the sampling Areas from the most excellent to the most unsatisfactory.**

| SAMPLE GRADE | | | | | | |
|---|---|---|---|---|---|---|
| Locations | Excellent | Satisfactory | Suspicious | Unsatisfactory | Good samples in percent (%) | Bad samples in percent (%) |
| Municipal | 7 | 13 | 8 | 2 | 93 | 7 |
| Gwale | 7 | 14 | 6 | 3 | 90 | 10 |
| Total | 14 | 27 | 14 | 5 | 183 | 17 |

**Complict of Interest**

There was no complict of interest as far the authors concerned

**DISCUSSION**

This study has presented the bacteriological analysis of some sachet water samples taken from different residential area and locations in Kano metropolis at random from August, 2015 to March, 2016. Almost all the sachet water were registered with appropriate regulatory agency (NAFDAC) and very few of the sachet water producers indicated manufacturing date, expiring date and batch number on the sachet, therefore not complying with the labeling compliance as stipulated by the WHO, (2011). Bacteriological analyses were based on Coliform count using the Most Probable Number (MPN) technique. The results of coliform count using the Most Probable Number (MPN) is shown in Table 1 to 12 which defined the degree of contamination and the bacteriological quality of the collected sachet drinking water sample brands in Kano metropolis. Going by the zero tolerance levels stipulated by regulatory agency for coliforms in drinking water, a cumulative figure of 36% meets the standards of quality water and a cumulative figure of 64% (n = 100) of all the identified packaged water did not meet the existing standards as shown in Table 1 to 12 respectively. Previous studies in other parts of the country and Kano itself reported similar bacterial load indicative of poor water quality (Olayemi, 1999; Itah and Akpan, 2005). Relatively high aerobic colony counts are indicative of poor, unhygienic handling and processing. Bacterial growth in water may be unnoticed even in transparent packaged water and the presence of some of these microorganisms may pose a potential risk to consumer (Geldriech, 1996). This indicates that the consumer would not have any clear information of knowing if the water is within the standard limit for drinking water. It can be seen that most samples of the water brands were found to contain coliform species, which is about 64% of the total number of the sachet water brands examined. Therefore, the presence of species as well as *E. coli* which is also a member of the *coliform* group found in the water sample brands, suggests that these sample of water brands have been contaminated with feces either of human or animal origin (Okonko *et al.*, 2008).

Going by the zero tolerance levels stipulated by regulatory agencies for coliforms in drinking water, a cumulative figure of twenty five percent (25%) meets the standards of drinking water quality and is subsequent percentages were satisfactory, suspicious as well as unsatisfactory as shown in table 3, which were in conformity with that of (NSDWQ, 2007; WHO, 2010). Moreover, the presence of *Pseudomonas aeroginosa* in the sachet sample water brands suggested that the contamination of the water was either through

decay of wastes, improper sanitization and sterilization of the factory equipment or instrument used in the production processes. It can also result from the use of unsterile polythene which was used for the packaging of the water meant for human consumption. The presence of *Salmonella typhi* in the sachet water samples suggested that there was serious pathogenic water borne threat, capable of causing disease to consumers.

The aerobic plate count (APC) result indicated that most of the pure water samples are in conformity with WHO and SON set values for drinking water quality. The contaminated sample based on aerobic plate count (APC) results were pure water samples number 3, 6 and 10 with too numerous colony to count from Dala. The contaminated Samples from Gwale were number 2, 5 and 8 with too numerous colony to count. The contaminated samples with too numerous colony to count from Fagge were samples number 3 and 10. Kano Municipal presented samples number 6 and 9 with too numerous colony to count. Sample number 8 was the only contaminated sample from Tarauni with too numerous colony to count. All the samples collected from Nassarawa presented various values minimal bacterial load. The total percentage of safe sachet water samples Based on this research was 83% and that of unsafe samples was 17%.

**REFERENCES**


1. Abimbola, A. F., Tijjani, M. N., & Nurudeen, S. I. (2006). Some Aspects of Ground Water Qualilty in Abeokuta and its Environs, South Western Nigeria *Abstracts of Research work in Nigerian University*, 2, 178
2. Admasu, M., Kumie, A., & Fentahun, M. (2003). Sustainability of Drinking Water Supply Projects in Rural of North Gondar, Ethiopia, *Ethiopian. Journal of Health Development* (3), 221-229.
3. Akinbile, C. O., & Yussof, M. S. (2011). Environmental impact of leachate pollution of ground Nigeria. *International Journal of Environmental Science and Development,* **2**(1):81-89
4. American Public Health Association. (2005). *Standard Methods for Examination of Water and Wastewate*r, 20th ed; 2002, pp. 213- 256. 3.
5. APHA (American Public Health Association). (2002). *Standard Methods for Examination of Water and Wastewate*r, 20th ed, pp. 213- 256.
6. Aquachem. (2009). http://www.aquachem.ie/ser_analytical.html (Date Accessed: 20th October, 2009)
7. Arab J. G. (2014). Giuliano, Venice, Italy) by principal component analysis and kriging. Environ Pollution **122**(2), 235–244 Frost F, Franke D, Pierson K,Woodruff L, Raasina
8. Aris, A. Z., Abdullah, M. H., Ahmed, A., & Woong, K. K. (2007). Controlling factors of groundwater hydrochemistry in a small island's aquifer. Int J Environ Sci. Technol. **4**(4), 441–450.
9. Assembly of Life Sciences U.S. (1977). "Drinking water and health" National Academy of Science.
10. Ashamanjari, K. G., & Sangeetha, G. B. (2005). Seasonal Physicochemical Studies and Geochemical Parameter of Arkavati River, *Indian Mineralogist Spl.* **2**(1), 36-146. [15].
11. Kozlowsky-Suzuki, B., & Bozelli, L.R. (2004). Resilience of a zooplankton community subjected to marine intrusion in a tropical coastal lagoon. *Hydrobiologia, 522*: 165-177.
12. Yamane, T. (2011). Formulae for determining sample size
13. WHO. (2011). Guidelines for Drinking-Water Quality. World Health Organization, Geneva, Switzerland.