Physicochemical and Bacteriological Study of Hawked Water in Bauchi Local Government, Bauchi

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ABSTRACT

Hawked water usually in a sachet or plastic bottle is considered safe and ready to be taken immediately after purchase. However, not all hawked water in Nigeria can be regarded as safe because not all water manufacturing industries follow the required standard and in most cases scuttle the need to maintain standards due to tax-related issues and sometimes greed. This study presents a systematic assessment of the bacteriological and physicochemical attributes of hawked sachet water collected from five distinct locations within Bauchi Local Government area of Bauchi state, Nigeria. The research was conducted meticulously in a controlled environment to ensure the reliability of the findings. The study sought to determine the levels of physicochemical (pH, hardness, dissolved oxygen, biochemical oxygen demand, total solid, electrical conductivity, nitrogen, chloride, temperature and turbidity) and bacteriological analysis (serial dilution, multiple tube fermentation, total plate count and confirmatory and completed test) of five replicate samples using standard microbiology techniques. The results were compared to World Health Organization (WHO) drinking water quality guidelines. A greater percentage of analyses for physicochemical parameters were below acceptable limits except for the pH value which falls within acceptable limits. The bacteriological analysis showed there is a higher bacterial load in Area C compared to the other five locations while under microscopy, gram-negative rods were observed and recorded under x40 and x100 objective. It can be inferred from the research that certain hawked water within Bauchi, Bauchi state, Nigeria can be considered unsafe because it possesses health risks to consumers and is unsuitable for direct human consumption without proper treatment. The study recommends strict enforcement of laws governing treatment water to protect the people of Bauchi state and other affected locations from further consequences of drinking untreated hawked water.

Keywords: water pollution, water quality, bacteriological analysis, physicochemical analysis

INTRODUCTION

Background of the study

Water is a colourless, odourless highly polar liquid. It has a unique compound in which without it life is impossible. Water is the most abundant and important compound on the earth surface, also over 60 – 69 % of the weight of any cell is composed of water and all chemical reactions associated with life are based on water [11].

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To understand water as a necessity, as a resource and as a factor in pollution, its characteristics, its role in biosphere and its role in living things have to be understood. The properties of water depend on the properties of its constituent atoms [9]. Water acts as a home for a wide variety of micro-organisms, including bacteria, protozoa, algae, worms, and viruses [11]. Water needed for human consumption must be free of pathogenic organisms harmful to the body. In public drinking water sources, the absence of turbidity, color, or any unpleasant taste or smell is of great importance [13].

Water is a solvent that dissolves minerals from rock with which it comes in contact with. Water may contain dissolved mineral and gas that gives it the tangy taste enjoyed by many people. Without these minerals and gases, the water would have a flat taste. Water that contains a lot of calcium and magnesium is said to be hard. Portable water is water that is free of disease-causing organisms and chemical substances that are harmful to living organisms whereas non-portable water is that which is contaminated with domestics, industrial and agricultural waste [1]

Water is a requirement for all life; it is needed to maintain basic health and sanitation. For food production, industrial use, and agricultural use, a sufficient supply of fresh water is required. The natural environment is also critically dependent on water [11]. Water is a major resource that has variety of uses, this include domestic, industrial, recreation and power supply. Microorganisms require water for their biochemical activities [3]. The microbial quality of drinking water may deteriorate during transportation in distribution network, the deterioration may be due to a recontamination by damaged iron pipes which influence the composition, activity disinfection and resistance of biofilm production by bacteria [8].

Taken for example, the cholera epidemic of 1987 in Hamburg and the 1937 outbreak of Croydon typhoid fever in Europe were both triggered by a contaminated town supply system [7]. Waste disposal and wildlife defecation are two main causes of contamination of natural water sources by faeces materials. Because of the strong correlation between these contaminants and the presence of enteric pathogens that endanger water portability, it is important to detect faecal contamination in water [10].

The sources of water determine its inherent quality color of the water is usually an indication of bacterial presence. Water is therefore a valuable resource that needs to be managed on equitable basis to meet the basic need of present and further generations. The consequences of too little water are often fatal. The successful invasion of land involved ways of more efficiently coping with much water [12]. The earth is covered by water, in which only a small portion of the enough water is available to man as fresh water. The oceans hold 97% of the world's water, the remaining 3% being fresh water, of which 2.997% is buried so deep that it cost too much to extract it. At any given time, rivers and lakes hold 0.33% of fresh water, while the atmosphere holds 0.035% [14].

Statement of the problem

The availability and consumption of sachet water, a vital source of potable water in Nigeria, have grown significantly over the years. However, concerns persist regarding the quality and safety of this water source, especially in regions with varying environmental conditions. To address these concerns, this study was undertaken to comprehensively evaluate the bacteriological and physicochemical parameters of sachet water samples obtained from five geographically diverse locations within Bauchi Local Government.

Objectives of the study

The main objective of the study is to comprehensively assess the bacteriological and physicochemical qualities of hawked sachet water from diverse locations within Bauchi Local Government, Bauchi State, Nigeria.

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Scope of the study

The study addresses critical public health concerns related to the consumption of the sachet water and understanding the physicochemical characteristics for assessing its suitability for consumption and identifying potential environmental impacts associated with its production and disposal.

MATERIALS AND METHODS

Study Area

This study was carried out in Bauchi state. Bauchi state lies between latitude 9° 3' north and longitude 8° 50' and 11° east. Rainfall in Bauchi state is between 1300 and 700mm per year in the south and only 700mm in the extreme north. The weather in the south and north is variable. Although it is humidly hot in the south during the first part of the rainy season, the hot, dry, and dusty weather persists up north.

Sample Collection

Eighteen (18) samples were collected from five different areas (Yelwa, Wuntin Dada and Fadaman Mada) from different water sources (Pipe, Well and Stream) within Bauchi metropolis, the samples were collected in sterile sample bottles which was then taken to the Microbiology Laboratory in Abubakar Tafawa Balewa University (ATBU) Bauchi for further analysis.

Preparation of Medium

1. Using an analytical weighing scale, seventy-three grams of lactose broth was weighed and dissolved in 1000ml of sterile distilled water in a conical flask.
2. In 1000ml of sterile distilled water, fifty-two grams of macconkey agar powder was dispersed. The media for the total coliform count.
3. In 1000ml of distilled water, 36g of eosin methylene blue powder was dispersed. The following table shows the media statistics for the faecal coliform count.
4. The media was shaken vigorously and melted in a water bath at 45°c for 40min, before being sterilized in an autoclave at 121°c for 15min for proper dissolution and homogenization. Media was dispersed into oven-sterilized petri-dishes and allowed to solidify under laminar air flow aseptically.

Total Bacterial Count

The total bacterial count was determined by pour plate technique using standard techniques. For the enumeration of bacteria in the samples, a nutritional agar medium was used.

Total Coliform Count

This was determined by the most convenient number (mpn) index method using a 5-5-5 scheme. On incubation at 37oc for 48 hours, macconkey broth was used, and a positive result was seen in acid and gas production [5].

Faecal Coliform Count

Using the pour plate method, the faecal coliform count was determined. E. coli strains appeared to be greenish metallic shine colonies on eosin methylene blue (emb) agar, and this was further confirmed by the organism's ability to ferment lactose [4].

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Source/Identification of Microorganisms

Gram Stain:

The gram stain is the most common and effective staining technique, since it divides bacteria into two groups based on their cell walls' composition. This was done as described by [2]. On a clean slide, a film was made by emulsifying a portion of a colony in a loopful of distilled water. The film was then air dried and fixed by slight flaming and stained as follows:

1. for 1-2 minutes, the smear was stained with crystal violet solution.
2. The smear was quickly washed with water, and the gram's iodine solution was added and left for 1-2 minutes.
3. The 1odine was poured off and the slide was held with 95% ethanol for 5-15 sec.
4. The smear was then treated with tap water and stained with safranin solution for 20 sec.
5. The slide was held with water and allowed to dry. On microscopy, the gram positive organisms appeared purple and the gram negative organisms appeared pink.

Motility Test

Using a hanging-drop technique, the test was used to distinguish between motile and non-motile bacteria. A little immersion oil was first placed around the slide's edge, and a small loop of the culture was transferred to a clean dry covered slip with a wire loop. After that, the cover slide was inverted over the cover slip, so that the drop will be in the middle of the cavity and the slide was pressed down gently but slimy so that the oil seals the cover slip in place.

Physicochemical ParameterspH Determination

The pH of a 100ml aliquot of each sample was measured in a beaker and determined by a ph meter. This was done in the laboratory at the time of the analysis.

Color Determination

Within 2 hours of sampling, the color was determined. This was done after samples were allowed to rest on a bench to reach room temperature. A 50-ml sample was weighed in a special test tube used for color analysis. The color disc was rotated until a common color match is found for the samples.

Test for Odor

Water sample(s) was collected into a petri-dish, and then carefully perceived to determine the water odor.

Test for Temperature

Water samples were allowed to rest for a few days before being cooled and a thermometer was then inserted into each of the water samples. Afterward, all readings were recorded.

RESULTS

Table I: Physico-Chemical properties for Area BZ

Parameter	Unit	WHO standard	Average
pH	–	6.5-8.5	6.9

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Hardness	mg/L	300-600	98.2
D.O.	mg/L	2	2.08
B.O.D.	mg/L	5	1.44
Turbidity	NTU	5	6.2
Temperature	^{O}C	25	27
T.S.	mg/L	500-2000	258
Nitrogen	mg/L	25-50	16.2
Chloride	mg/L	250-1000	20
E.C.	m^2/m	100	6.2

Key:

D.O = Dissolved Oxygen,

B.O.D = Biological Oxygen demand,

TS = Total Solid,

E.C = Electrical conductivity,

S = Sample,

NTU = Nephlometric turbidity unity, ^{o}C = Celcius

Table II: Physico-Chemical properties for Area C

Parameter	**Unit**	**WHO standard**	**Average**
pH	–	6.5-8.5	7.14
Hardness	mg/L	300-600	96
D.O	mg/L	2	2.04
B.O.D	mg/L	5	1.52
Turbidity	NTU	5	7.8
Temperature	^{O}C	25	23.8
TS	mg/L	500-2000	202
Nitrogen	mg/L	25-50	19.8
Chloride	mg/L	250-1000	69.2
E.C	m^2/m	100	4.2

Key:

D.O = Dissolved Oxygen,

B.O.D = Biological Oxygen demand,

TS = Total Solid,

E.C = Electrical conductivity,

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S = Sample,

NTU = Nephlometric turbidity unity,

^{o}C = Celcius

Table III: Physico-Chemical properties for Area E

Parameter	Unit	WHO standard	Average
pH	–	6.5-8.5	6.74
Hardness	mg/L	300-600	108
D.O	mg/L	2	1.72
B.O.D	mg/L	5	1.12
Turbidity	NTU	5	6.4
Temperature	^{O}C	25	25.4
TS	mg/L	500-2000	476
Nitrogen	mg/L	25-50	15,2
Chloride	mg/L	250-1000	75.2
E.C	m^2/m	100	5.0

Key:

D.O = Dissolved Oxygen,

B.O.D = Biological Oxygen demand,

TS = Total Solid, E.C = Electrical conductivity,

S = Sample, NTU = Nephlometric turbidity unity,

^{o}C = Celcius

Table IV: Physico-Chemical properties for Area F

Parameter	Unit	WHO standard	Average
pH	-	6.5-8.5	7.94
Hardness	mg/L	300-600	230
D.O	mg/L	2	1.68
B.O.D	mg/L	5	1.41
Turbidity	NTU	5	4.6
Temperature	^{O}C	25	25.2
T.S	mg/L	500-2000	684
Nitrogen	mg/L	25-50	12.6
Chloride	mg/L	250-1000	35.6
E.C	m^2/m	100	6

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Key:

D.O = Dissolved Oxygen,

B.O.D = Biological Oxygen demand,

TS = Total Solid, E.C = Electrical conductivity,

S = Sample,

NTU = Nephlometric turbidity unity,

^{o}C = Celcius

Table V: Physico-Chemical properties for Area G

Parameter	**Unit**	**WHO standard**	**Average**
pH	–	6.5-8.5	**6.74**
Hardness	mg/L	300-600	210
D.O	mg/L	2	2.16
B.O.D	mg/L	5	1.24
Turbidity	NTU	5	6.8
Temperature	^{O}C	25	27
T.S	mg/L	500-2000	306
Nitrogen	mg/L	25-50	17.8
Chloride	mg/L	250-1000	20.2
E.C	m^2/m	100	6.38

Key:

D.O = Dissolved Oxygen,

B.O.D = Biological Oxygen demand,

TS = Total Solid,

E.C = Electrical conductivity,

S = Sample, NTU = Nephlometric turbidity unity,

^{o}C = Celcius

Table VI: Bacteriological Result for Area BZ

Sample	**10ml**	**1ml**	**0.1ml**	**mpn/100ml**	**10^{-1} (cfu/ml)**	**10^{-3} (cfu/ml)**
S1	2	1	1	9	4.8×10^2	2.8×10^4
S2	2	1	0	7	3.7×10^2	2.3×10^4
S3	1	0	0	2	$4.4x\ 10^2$	2.6×10^4

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S4	2	0	0	5	4.2×10^2	2.4×10^4
S5	1	1	0	4	3.3×10^2	2.2×10^4
Average				**5.4**	**4.8×10^2**	**2.6×10^4**

Key:

MPN = Most probable Number,

CFU = Colony forming unit, S = Sample

Table VII: Bacteriological Result for Area C

Sample	**10ml**	**1ml**	**0.1ml**	**mpn/100ml**	**10^{-1} (cfu/ml)**	**10^{-3} (cfu/ml)**
S1	3	1	1	14	5.6×10^2	4.2×10^4
S2	2	1	0	7	4.8×10^2	3.4×10^4
S3	2	1	1	9	$6.5x\ 10^2$	4.4×10^4
S4	2	2	1	12	TNTC	5.7×10^4
S5	3	1	0	11	6.2×10^2	4.8×10^4
Average				**10.6**	**5.7×10^2**	**4.5×10^4**

Key:

MPN = Most probable Number,

CFU = Colony forming unit,

S = Sample

Table VIII: Bacteriological Result for Area E

Sample	**10ml**	**1ml**	**0.1ml**	**mpn/100ml**	**10^{-1} (cfu/ml)**	**10^{-3} (cfu/ml)**
S1	1	0	0	2	3.6×10^2	2.2×10^4
S2	0	0	0	22	2.4×10^2	2.0×10^4
S3	0	1	0	2	$2.6x\ 10^2$	2.8×10^4
S4	1	1	0	4	1.2×10^2	1.0×10^4
S5	1	0	0	2	1.4×10^2	1.8×10^4
Average				**2.4**	**2.2×10^2**	**1.9×10^4**

Key:

MPN = Most probable Number,

CFU = Colony forming unit,

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S = Sample

Table IX: Bacteriological Result for Area F

Sample	10ml	1ml	0.1ml	mpn/100ml	10^{-1} (cfu/ml)	10^{-3} (cfu/ml)
S1	2	0	0	5	4.2×10^2	3.6×10^4
S2	1	0	2	6	3.8×10^2	2.6×10^4
S3	1	2	0	6	$2.8x\ 10^2$	3.4×10^4
S4	2	1	0	7	$4.6\ x10^2$	4.0×10^4
S5	1	3	0	8	3.6×10^2	2.2×10^4
Average				**6.4**	**3.8×10^2**	**3.1×10^4**

Key:

MPN = Most probable Number,

CFU = Colony forming unit,

S = Sample

Table X: Bacteriological Result for Area G

Sample	10ml	1ml	0.1ml	mpn/100ml	10^{-1} (cfu/ml)	10^{-3} (cfu/ml)
S1	1	1	0	4	2.10^1	6×10^3
S2	0	3	0	6	1.4×10^2	6×10^3
S3	0	0	2	4	$4x\ 10^1$	2.0×10^4
S4	2	0	0	5	1.0×10^2	1.0×10^4
S5	0	1	1	4	14×10^2	NVC
Average				**4.6**	**7.6 `x 10^2**	**10.5×10^4**

Key:

MPN = Most probable Number,

CFU = Colony forming unit,

S = Sample

DISCUSSION

From the five tables of physico-chemical analysis the flowing can be discussed. For Area BZ all the parameters met the standard for WHO except the turbidity and acidity which are above WHO standard of 5 NTU and 4mg/l. It could be due to vegetable fibres and micro-organisms all of which may come from the source of the water. The acidity could be due to the presence of CO2, H2 CO3 and H CO3 that were not neutralised fully. It may also be due to industrial waste.

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For Area C also all the parameters met the requirement for WHO drinking water except turbidity and acidity which are above the WHO standard. It could be due to vegetable fibres and micro-organisms all of which may be due to the source of the water. The acidity could be due to the presence of CO2, H2 CO3 and H CO3 that were not neutralised fully. It may also be due to industrial waste.

For Area E also only the turbidity and acidity that did not met the WHO standard. It could be due to vegetable fibres and micro-organisms all of which may be due to the source of the water. The acidity could be due to the presence of CO2, H2CO3 and HCO3 that were not neutralised fully. It may also be due to industrial waste all of which could be due to environmental factors.

While for Area F the water is hard as it has hardness value of 230mg/l and the pH is slightly Alkaline. This is due to the presence of metallic ions such as Ca2+ and Mg2+. It could also be due to the presence of Sr2+ and Fe2+

For Area G the water is also hard as it has hardness value of 210mg/l, this is due to the presence of metallic ions such as Ca2+ and Mg2+. It could also be due to the presence of Sr2+ and Fe2+. Also, the water is slightly turbid as it has turbidity value of 6.8 NTU. It could be due to vegetable fibres and micro-organisms all of which may be due to the source of the water.

From the five tables of Bacteriological Analysis Area C has the highest bacterial load (10mpn/100ml) and x 102 for 10-1 dilution and 4.5 x 104 for 10-3 dilution.

Area F is following Area C with 6mpn/100ml and 3.8 x 102 for 10-1 dilution and 3.1 x 104 for 10-3 dilution.

Area BZ has 5mpn/100ml and 4.0 x 102 for 10-1dilution and 2.6 x 104 for 10-3 dilution.

While for Area E and G the mpn/100ml value for Area G (4mpn/100ml) is higher than 2mpn/100ml of Area E and for the total plate count Area E has 2.2 x 102 for 10-1 dilution and 1.9 x 104 for 10-3 dilution which is higher than that of Area G with 7.6 x 102 for 10-1 dilution and 1.0 x 104 for 10-3 dilution.

For microscopy, Gram negative rods were observed and recorded under x40 and x100 objectives.

The variation in the bacterial load could be due to the point at which the water was source. It could also be due to environmental factors such as rainfall, pH, Hardness D.O, electrical conductivity and Turbidity etc. The standard for coliform count is zero per 100ml.

The results of the bacteriological analysis revealed the unsanitary state of all the samples. The presence of coliform in the samples indicates faecal contamination of such samples, and it suggests the presence of enteric photogenes such as *salmonella typhi*, *vibrio cholerae*, *aeromonas hydrophila*, and *yersinia enterocolitica* [6]. Samples containing indicator bacteria with a high coliform density and a high viable bacterial count are not suitable for human consumption.

Although most of the samples met the physico-chemical requirements, the turbidity and acidity of most of the samples failed to meet the bacteriological criteria because the turbidity and acidity were high. Also Area F and G water was hard as the value of hardness reached 230mg/L respectively.

CONCLUSION

The results of this study have revealed the unsanitary state of water consuming in the residential areas such as areas bz, c e, f, and g., as all the samples contained bacterial and physico-chemical characteristics, some

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of which have been found to be above the recommended [13] requirements for drinking water. They have therefore been declared unsuitable for human consumption.

RECOMMENDATION

1. Water samples should be examined at regular intervals throughout the year.
2. There should be constant surveillance of the distribution system for leakages of pipes and urgent repairs should be done.
3. Flushing of the distribution system should be done at intervals to decontaminate and reduce biofilm formation.
4. Construction of boreholes should be based on WHO guideline for its location.
5. A proper disposal of sewage and waste should be emphasized to reduce contamination of subsurface water.
6. The need to boil water for consumptions should be emphasized to prevent outbreaks of gastrointestinal illnesses.
7. More comparative studies should be carried out to gather more data to enable us advice authority more appropriately.
8. The water co-operation should be well funded so as to prevent outbreak of disease. Water being universal food stuff should be protected jealously.
9. More efforts should be place on maintenance of pipes and storage tanks.

REFERENCES

1. Aladi, E. H. (1994): Bacteriological Quality of Potable water in Ahmadu Bello University, Zaria B. Sc (microbiology) Project, ABU Zaria. Pp 4.
2. Arnold, E.G. Rhodes, R.T., Lemore, S.C. (1985): Standard Method for Examination of water and Waste Water. Port City Press. Baltimore, Marylland. pp. 391-393.
3. Black, J.G. (1999): Microbiology: Principles and Explorations (4th ed) Prentice Hall Inc. Simon and Secluster Viacom Company, Jew Jersey. p. 31.
4. Botkin, D., Kaller, E.A. (1998): Environmental Science. John Wiley and Sons, Inc, pp 415-420.
5. Cheesbrough, M., (2004): District Laboratory Practice in Tropical Countries. 2nd Edn.,
6. Itah, A.Y., Etukubo, S.M and Enomfon, A. (1996): Bacteriological and Chemical Analysis of some Rural Water Supplies in Calarba, Nigeria; West African Journal of Biological and Applied Chemistry 41-1-10.
7. Kim, N.K and Stone, D.W (2010): Organic Chemicals in Drinking Water, New York State Department of Health, New York.
8. Lechevallier, M.W. Norton, W.O., Siegel's J.E and Mpartezd, A. (1995): Evaluation of the Immuno-Flourescent Procedure for Detection of Giardiacyst and Cryptosporidium Occyst in Water. Journal of Applied and Environmental Microbiology, 61, 690-697.
9. Nester, E. W., Roberts, C. E., Pearall, N. N., Anderson, D. G. and Nester, M. T. (1998): Microbiology: A Human Perspective (2nd ed), McGraw Hill Publishers. USA. Pp 27
10. Ogunseitan, O.A (1996); Removal of Cafflienein Sewage by Pseudomonas Putida Implication for Water Pollution Index. World Journal of Microbiology and Biotechnology, pp 1225-256.
11. Okafor, N. (1985): Aquatic and Waste Microbiology, Fourth Dimension Publishes U.K pp 4-12.
12. Priuitt N.L. Underworld, L.S and Survey, W. (2000): Bioenquiry; Making Connections in Biology John Wiley and Sons, Inc. New York. P98.
13. WHO (2000): Guidelines of Drinking Water Quality; Health Citeria and other Supporting Information pp 4-6.
14. Zanipinin, M., Canesi, L., Bett, M., Ciacei, C., Tarsi R., Gaoo, G and Pruzo, C (2003): Role for Mannote Sensitive Haemagglutinin in Promoting Interaction between Vibrio cholera, Ether and mussel Hemolymph. Applied General Microbiology 69(9):5711.