Microbiological Evaluation and Chemical Analysis of Potable Water in Al-Jabal Al-Akhdar

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ABSTRACT: This study was conducted to evaluate the microbiological and chemical safety of drinking water by using some chemical parameters and microbiological tests such as heterotrophic plate count, total coliforms and fecal coliforms.
In this study 14 samples were obtained from three sources in different locations in Aljabal Alakhdar.
The microbiological examination results were: water purification stations samples showed highest hetrotrophic plate count (total count) among all other sample sources, which were 716 cfu/100ml by ST sample followed by sample SD (668 cfu/100ml) then SS sample (616 cfu/100ml). The other two sources showed large variations in their total counts. The water bottling system samples total counts were 430 cfu/100ml (BO5) and 393 cfu/100ml (BO4), while samples BO3, BO1 and BO2 exhibited lowest counts of 73, 72 and 65 cfu/100ml, respectively. On the other hand the water local purification system samples total counts were in the following order: LB1(503 cfu/100ml), LB2 (397 cfu/100ml), LSH (379 cfu/100ml), LH (295 cfu/100ml), LB3 (257 cfu/100ml) and LJ (87 cfu/100ml).
The total coliform test included the presumptive , confirmative and completed tests. All samples contained total colilforms, eight samples showed positive confirmative test and also confirmed presence of fecal coli form.
The chemical parameters result showed that the highest total dissolved solids (TDS**)** was (138.5 mg/L) by sample LB3, while the lowest was (6 mg/L) by sample LJ. The measured pH ranged between 7.32 and 7.00, except LJ which measured 6.2. The potassium concentrations were 9 – 2 mg /L. The sodium concentrations ranged between 5 and 1 mg/L. The calcium concentration varied from 90 to 2 mg /L. The conductivity showed large variationo ranging between 259.7 and 24.3 µs.
The study revealed that there was fecal coliform pollution in some investigated water sources and this may leads to serious health risk.
Keywords: drinking water, heterotrophic plate count, total coliforms, fecal coliforms, chemical parameters

INTRODUCTION

Potable water is the water that is free from disease-producing microorganisms and chemical substances that are dangerous to health. Many infectious diseases are transmitted by water through the fecal-oral route, mainly due to the fecal contamination

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of raw water, failure in the water treatment process or recontamination of drinking water at source and point of use (1, 2).

The World Health Organization (WHO) reported that 80% of all human diseases in developing countries are caused by polluted water consumption (3)

The presence of pathogens in water for drinking and swimming purposes is of public health significance considering the possibility of the presence of bacteria, protozoa and enteric viruses that are implicated in gastro-intestinal water-borne diseases (4, 5)

The detection and enumeration of disease-causing organisms in surfaces water is difficult, time-consuming, and expensive; and for many of the pathogens, methods for routine monitoring and isolation are nonexistent or the costs for their isolation and enumeration are very prohibitive (6, 7). It is also impossible and impractical to identify all the enteric pathogenic organisms present in the water at any particular time. It is therefore important to identify harmless organisms that could be used as predictors of the presence of pathogenic organisms in groundwater, surface waters, or drinking water after treatment. Some types of bacteria found in the gastrointestinal tracts of humans and other warm-blooded animals have traditionally been used as indicators of the occurrence of some pathogenic organisms in water. These are total coliforms, fecal coliforms, *Escherichia coli*, fecal streptococci, and enterococci. These organisms are always found in the intestines and normally are not present in soil or water; hence, when they are detected in water it can be assumed that the water has been contaminated with fecal material and other kinds of microorganisms capable of causing disease also may be present (8, 9).

Fecal coliforms (FC) are a subgroup of total coliforms consisting mainly of *E. coli*, *Enterobacter*, and some *Klebsiellae*. They inhabit the intestines of warm-blooded animals, because they can grow and ferment lactose at a relatively high temperature (44.5^{O}C) a characteristic that has earned them the name ‘‘thermotolerant coliforms’’ and differentiated them from the other members of total coliforms (10).

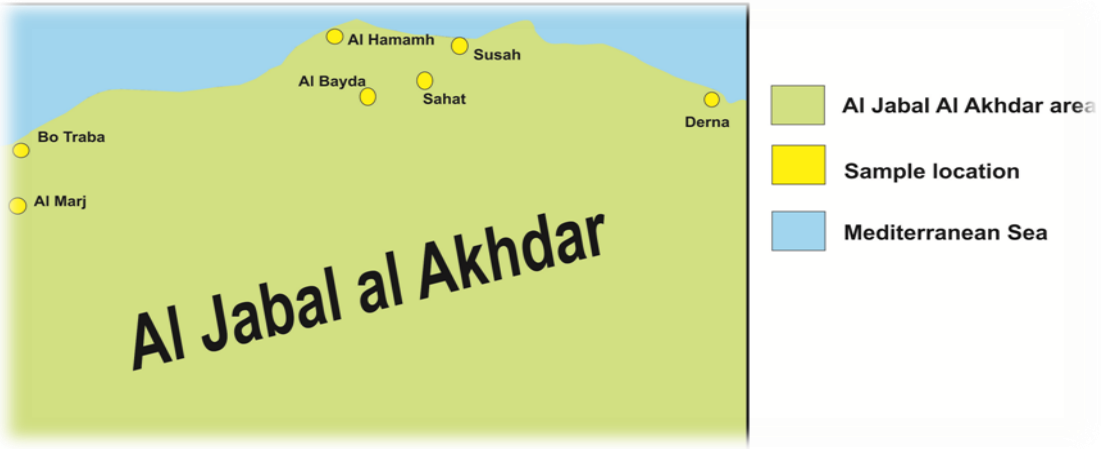
Coliform bacteria are hardier than disease-causing, non-spore-forming bacteria; therefore their absence from water is an indication that the water is bacteriologically safe for human consumption. Their presence, on the other hand is an indication that disease-causing bacteria may be present and that the water is unsafe for drinking.

This study aimed to evaluate the potable water obtained from different sources and locations in Al-Jabal Al-Akhdar by using microbiological methods (Heterotrophic Plate Count, Total Coliforms and Fecal Coliforms) and analysis of some chemical parameters.

MATERIALS AND METHODS

1. Water samples collection:

During this study 14 samples were obtained from three sources (water purification stations (S), water bottling system (BO) and water local purification system (L)) from different locations in Aljabel Alakhdar (Susah (S), Derna (D), Shahat (SH), Albayda (B), BO Traba (T), Alhamama (H) and Almarj (J) by using sterile 6 L plastic bottles. Before collection and were covered tightly labeled with special codes and transported to the laboratory to be examined**.** Each sample was examined by various tests to identify its quality .



2. Bacteriological examinations:

2.1. Heterotrophic Plate Count (Total Plate Count)

Total plate count was conducted by using the membrane filtration method and counting the number of colonies on culture media.

The water samples (100 mL) from each location were filtered through a 0.45 mm sterile filter thereby retaining the organisms on the filter surface. With face upward, the filter was then simply placed on the surface of a culture medium (Plate Count Agar) and incubated for 48 hours. Three replicates of each sample was conducted**.** The results were obtained using colony counter (11, 12).

2.2. Total Coliforms.

Total coliform test included the presumptive , confirmative and completed tests.

2.2.1. Presumptive test for total coliforms.

Membrane filtration method was used. The filter is transferred from the filtration apparatus to the surface of agar plate (M-Endo or LES-Endo). Red colonies with golden (metallic) sheen indicate the presence of coliforms in the samples**.**

However, because some non-coliform bacteria can produce colonies with golden sheen, further tests were conducted to confirm the presence of coliforms in the sample.

2.2. 2. Confirmation test for total coliforms:

The LTB (Lauryl tryptose broth) and BGLBB (Brilliant green lactose bile broth) media were used here to confirm the presence of coliforms in the samples. Growth from each red colony (up to 10 randomly selected colonies) with a golden sheen was inoculated into tubes of LTB and incubated at 35^{O}C for 48 h. Then subcultured full loop from positive LTB (show gas production) into BGLBB medium and incubated at 35^{O}C for 48 h. A confirmed coliform test is one that shows gas production in BGLBB within 48 h (13).

2. 2 . 3. The completed test

In completed test tubes of lactose broth (for acid and gas production) and a nutrient Agar slants (for gram stain) were inoculated from positive confirmative tubes and incubated for 48 h. Following inoculation and incubation, tubes that showed acid and gas in the lactose broth and the presence of gram-negative bacilli on microscopic examination were further confirmation of the presence of *E. coli*, and they were indicative of a positive completed test (13).

2. 3. Fecal Coliforms

Fecal coliforms (FC) are better indicators of the presence of pathogenic bacteria in water than total coliforms. In this test EC medium was used. EC tubes were inoculated from positive presumptive plates and incubated at 44.5°C for 24–26 h. A positive reaction for fecal coliforms was suggested by gas production in EC tubes (14).

3. Chemical parameters tests.

Some chemical parameters were examined including:

3. 1. Total dissolved solids (TDS)

TDS of water samples can be measured through evaporation and is measured in mg/L.

3. 2. Measuring pH

pH is a measure of the increase of hydrogen ions in water. Using a meter calibrate the probe and meter according to the manufacturer's directions. Two buffers (pH 7 and 10) for calibration were used.

3.3. Conductivity

Conductivity is the measure of water's ability to conduct an electric current. Increased concentration of salts increases the conductivity. The Conductivity Meter is used for testing and units used expressed as microsiemens (µS).

3.4. Concentrations of K, Na and Ca ions

The standard approved method is Absorption Spectrometric (13) was used to measure ions concentration.

RESULTS AND DISCUSSION

The aim of the present study is to evaluate the microbial contamination and quality of the potable water in Aljabel Alakhdar area.

1 Water samples collection:

The sources and locations of the collected samples are shown in table 1.

2. Heterotrophic Plate Count (HPC)

An estimate of the total number of viable microorganisms (yeast, mould, and bacteria) in water is used routinely to assess the water quality, to determine whether changes have occurred in water during storage or distribution due to bacterial regrowth, or to monitor the efficiency of water treatment processes (15)**.** Although it is not enough to determine the safety or hygienic quality of drinking water with regard to the presence of pathogenic organisms**.** and it is difficult to completely remove total organisms from drinking water**.** However heterotrophic organisms cannot manufacture their own food; hence they rely on organic and inorganic materials from other sources for nutrition and so HPC is an indicator of the presence of microorganisms in water.

The total count obtained as shown in figure 1 and plate 1. The HPC of water purification stations samples were 716 cfu/100 ml by ST sample followed by 668 cfu/100ml by isolate SD, while isolate SS showed lowest count (616 cfu/100ml). In case of water bottling system, sample BO5 showed highest count (430 cfu/100ml) followed by sample BO4 (393 cfu/100ml), while samples BO3, BO1 and BO2 have least counts (73, 72 and 65 cfu/100ml, respectively). The highest HPC obtained by the water local

purification system was 503 cfu/100ml by samle LB1 followed by samples LB2, LSH , LH and LB3 whose counts were 397, 379, 295 and 257 cfu/100ml, respectively, whereas sample LJ had least count which was only 87 cfu/100ml.

The highest total count among the samples was shown by sample ST (716 cfu/mL), whereas the lowest total count (65 cfu/mL) was obtained by sample BO2.

In distribution systems, increasing numbers can indicate a deterioration in cleanliness, possibly stagnation and the potential development of biofilms.

According to Bitton (6) HPC bacteria concentrations in drinking water should not be more than 500 CFU/mL.

Heterotrophic plate count is estimated by counting the number of colonies on culture media. This estimate is not accurate, firstly, because the culture media used to enumerate heterotrophic organisms do not support the growth of the different types of heterotrophs equally. Secondly, some of the bacteria occur in viable but non culturable forms. It has been estimated that about 1% of the total bacteria found by direct microscopy are obtained when HPC methods are used (16).

3. **Total Coliforms**

Coliform are a group of non-spore former gram-negative rod-shaped bacteria, identifiable by their ability to ferment lactose to produce acid and gas within 48 h. when incubated at 35.5C. They are recognized as a suitable microbial indicators of drinking-water quality largely because they are easy to detect and enumerate in water.

The presumptive test exhibited that all water samples contained total colilforms which was indicated by presence of red colonies with golden (metallic) sheen as shown in table 2. and plate 2.

In the confirmative test coliform is indicated by gas production as shown in plate 2. Eight samples among the 14 samples produced gases, these samples were SD, BO2, BO3, BO4, LJ, LB1, LB2 and LSH. The remaining samples did not contain coliforms as shown in table 2.

Completed test is indicated by acid and gas production and the presence of gram-negative bacilli on microscopic examination are further confirmation of the presence of *E. coli*. The eight samples that showed positive results in confirmative test further confirmed the presence of coliform as shown in table 3.

Total coliform should be absent and the presence of these organisms indicates inadequate treatment. Total coliform should be 0 CFU/100 mL as mentioned by the WHO (1)

4. 4 Fecal Coliforms

Fecal coliforms are a subgroup of total coliforms consisting mainly of *E. coli*, it inhabits the intestines of warm-blooded animals. They can grow and ferment lactose at a relatively high temperature (44.5 °C)**.** *E. coli* is considered the most suitable index of faecal contamination. Fecal coliforms are better indicators of the presence of pathogenic bacteria in water than total coliforms and *E. coli* is far more sensitive to disinfection than are enteric viruses and protozoa. Moreover, regrowth of fecal coliform organisms in the distribution system is unlikely unless sufficient bacterial nutrients are present, unsuitable materials are in contact with the treated water and there is no free residual chlorine. In most circumstances, concentrations of thermotolerant coliforms are directly related to

that of *E. coli*. So their use in assessing water quality is therefore considered acceptable for routine purposes.

The sample sources that showed presence of fecal coliform were SD**,** BO2**,** BO3**,** CB4**,** LJ**,** LB1**,** LB2 and LSH as shown in table 3. which indicated they contained intestinal *E. coli*.

WHO recommended value for fecal coliform is 0 CFU/100 mL (1). So the obtained results have shown that there is fecal contamination.

5. Chemical parameters.

5. 1 Total dissolved solids (TDS)

Total dissolved solids (TDS) in water is a simplified term representing the

solids found in water consist of both inorganic and organic materials, such as soil particles and small pieces of vegetation. They are important water quality concern because they cause turbidity**.** dissolved substances can cause color, odor, and taste problems**.** On the other hand low contents of solids in water correlated with increased mortality and morbidity.

Water containing more than 500 mg/ml of TDS is not considered desirable for drinking water supplies.

The obtained results for TDS of water samples were varied, LB3 sample was 138.5 mg/L contain higher TDS, while sample LJ has lowest TDS contents which was 6 mg/L. As shown in Table 4.

WHO allows a maximum of 500 mg/ml of total solids content (17,18.19).

4. 5. 2 Measuring pH

Hydrogen ions influence many chemical reactions. For effective disinfection with chlorine, the pH should preferably be less than 8 so pH is an important parameter to track. The results of measured pH of water samples ranged between 7.32 (SS) and 7.00 (BO1), except for LJ which measured 6.2 as shown in table 4. The recorded results were in the permissible limit as prescribed under standard values of WHO (17,18.19). (pH range for potability is 6 .5 to 8.5).

4. 5. 3. **Concentrations of K, Na, Ca ions**

The effect of potassium is negligible either to reach minimum or to cause any health problem. Potassium is an essential nutritional element, The potassium concentrations of the water samples ranged between 9 and 2 mg /L as shown in table 4. WHO maximum level is 12 mg/L (17,18.19).

Sodium is not of health concern at levels found in drinking-water, may affect acceptability of drinking water. But sodium is considered harmful at high concentrations to persons suffering from cardiac, renal and circulatory diseases.

The water samples sodium concentrations ranged between 5 and1 mg/L as shown in table 4.

WHO has used 50 mg /L as a maximum acceptable limit and 200 mg /L as an excessive limit(17,18.19).

Calcium has no toxicity concern, waters with high concentrations of calcium and magnesium are called hard waters.

The calcium concentration of the water samples varied from 90 to 2 mg /L as shown in table 4.

WHO has used 75 mg /L as a maximum acceptable limit and 200 mg /L as an excessive limit (17,18.19).

4. 5. 4 Conductivity (EC)

Conductivity is a useful test for quick determination of minerals. Increased concentration of salts increases the conductivity, so absolutely pure water is a poor electrical conductor.

The conductivity of the water samples ranged between 259.7 and 24.3 µs as shown in table 4. Electrical conductivity values for all the investigated samples were found to be at the limit prescribed by the WHO standard (17,18).

Conclusion

Potable water should be safe and acceptable and must be free from all pathogenic organisms. The chemical evaluation indicates that the required minerals are available in reasonable amounts, but the presence of the indicator organism *E.coli* indicates significant level of fecal contamination which demands prompt action for periodic monitoring and disinfection of the water resources.

According to guidelines for drinking water quality, the results indicate that some samples are polluted and cannot be used for drinking purpose. Those water sources that do not conform to National Standard could pose public health problems.

Therefore, the local water authority shall make stronger water exceptional tracking and management systems

Table 1. Sources and locations of the collected samples

No.	Samples code	Sources	Location
1	SS	Water purification stations	Suasah
2	ST	Water purification stations	BoTraba
3	SD	Water purification stations	Derna
4	BO1	water bottling system	Factory1
5	BO2	water bottling system	Factory2
6	BO3	water bottling system	Factory3
7	BO4	water bottling system	Factory4
8	BO5	water bottling system	Factory5
9	LJ	water local purification system	Almarj
10	LB1	water local purification system	Albayda
11	LB2	water local purification system	Albayda
12	LB3	water local purification system	Albayda
13	LH	water local purification system	Alhamama
14	LSH	water local purification system	Shahat

Figure 1. Heterotrophic Plate Count is in (cfu/mL) of the water samle sources

Plate 1. Plate count Agar plates for Heterotrophic Plate Count is in (cfl/mL) of the water samples

Table 2. Total coliform presence in water samples included presumptive test, confirmative test and completed test.

No.	Samples code	Presumptive test	Confirmative test	Completed test
1	SS	+	-	-
2	ST	+	-	-
3	SD	+	+	+
4	BO1	+	-	-
5	BO2	+	+	+
6	BO3	+	+	+
7	BO4	+	+	+
8	BO5	+	-	-
9	LJ	+	+	+
10	LB1	+	+	+
11	LB2	+	+	+
12	LB3	+	-	-
13	LH	+	-	-
14	LSH	+	+	+

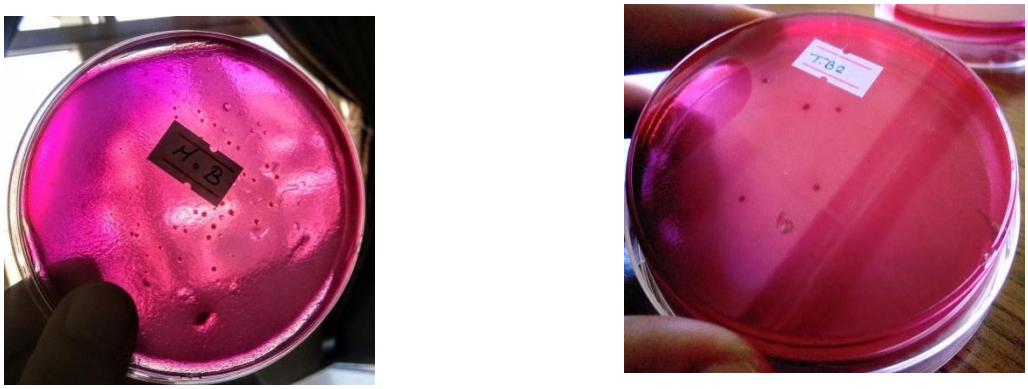


Plate 2. Presence of red colonies with golden metallic sheen

Table 3. Presence of fecal coliform in the water samples

No.	Samples code	Fecal coli form
1	SS	-
2	ST	-
3	SD	+
4	BO1	-
5	BO2	+
6	BO3	+
7	BO4	+
8	BO5	-
9	LJ	+
10	LB1	+
11	LB2	+
12	LB3	-
13	LH	-
14	LSH	+

Table 4. Chemical analysis of water isolates

No.	Samples	TDS (mg/L)	EC (µS/cm)	K (mg/L)	Ca (mg/L)	Na (mg/L)	pH
1	SS	35.9	66.3	5	21	2	7.32
2	ST	142.3	259.8	7	10	3	7.10
3	SD	40.8	73.2	2	19	2	7.02
4	BO1	34.8	88.4	2	20	2	7.00
5	BO2	72.4	127.7	3	41	3	7.08
6	BO3	108.3	232. 6	7	53	5	7.10
7	BO4	64. 6	123.1	3	31	2	7.50
8	BO5	91.4	224.5	9	66	3	7.35
9	LJ	6.0	24.3	2	2	1	6.20
10	LB1	132.7	262.3	7	54	4	7.10
11	LB2	24.2	50.7	2	18	1	7.20
12	LB3	138.5	263.7	9	56	5	7.29
13	LH	39.6	65.8	2	21	2	7.17
14	LSH	42.8	74.8	3	30	1	7.05

المستخلص: أجريت هذه الدراسة على بعض مصادر مياه الشرب في المنطقة للتاكد من سلامتها وصحة استخدامها للشرب بتقييمها ميكروبيولوجيا و كيميائيا، وذلك باستخدام بعض المعايير الكيميائية والاختبارات الميكروبيولوجية مثل عد المحتوي الميكروبي الكلي ، القولونيات الكلية، القولونية البرازية. اشتملت هذه الدراسة على 14 عينة من ثلاثة مصادر في مواقع مختلفة في الجبل الأخضر.

نتائج الفحص الميكروبيولوجي: أظهرت عينات محطات تنقية المياه أعلى محتوى ميكروبي (العدد الكلي) بين جميع مصادر العينات الأخرى، حيث بلغ 716 وحدة مكونة للمستعمرة/ 100 مل للعينة ST، تليها العينة SD (668 وحدة مكونة للمستعمرة / 100 مل) ثم عينة SS (616 وحدة مكونة للمستعمرة / 100 مل). أظهر المصدران الآخران اختلافات كبيرة في العدد الكلي. حيث كان العدد الكلي لعينات نظام المياه المعلبة (المصانع) 430 وحدة مكونة للمستعمرة / 100 مللي (BO5) و 393 وحدة مكونة للمستعمرة / 100 مللي (BO4)، بينما أظهرت العينات BO3 و BO1 و BO2 أقل عدد وهو 73 و 72 و 65 وحدة مكونة للمستعمرة / 100 مللي ، على التوالي. من ناحية أخرى ، كان العدد الكلي لعينات نظام التنقية المحلية للمياه على الترتيب التالي: LB1 (503 وحدة مكونة للمستعمرة / 100 مللي) ، (397 LB2 وحدة مكونة للمستعمرة / 100 مللي) ، LSH (379 وحدة مكونة للمستعمرة / 100 مللي) ، LH (295 وحدة مكونة للمستعمرة / 100 مللي) ، LB3 (257 وحدة مكونة للمستعمرة / 100 مل) و LJ (87 وحدة مكونة للمستعمرة / 100 مل).

يتضمن اختبار القولونيات الكلي علي: الاختبارات الافتراضية والتأكيدية و التكميلية. اظهرت النتائج ان جميع العينات تحوي القولونيات الكلية ، وأظهرت ثماني نتائج ايجابية للاختبار التأكيدي . وأكدت هذه الثمانية عينات أيضًا وجود القولونية البرازية.

أظهرت نتيجة المعلمات الكيميائية أن أعلى (TDS) المواد الصلبة الذائبة كان (138.5 مجم / لتر) العينة LB3 ، بينما كان أقلها (6 مجم / لتر) العينة LJ. وتراوح قياس الأس الهيدروجيني بين 7.32 و 7.00 ، باستثناء العينة LJ الذي كان قياسه 6.2. كانت تركيزات البوتاسيوم 9 – 2 ملجم / لتر. تراوحت تركيزات الصوديوم بين 5 و 1 مجم / لتر. تراوح تركيز الكالسيوم من 90 إلى 2 مجم / لتر. أظهرت الموصلية تباينًا كبيرًا تتراوح بين 259.7 و 24.3 µs.

كشفت الدراسة عن وجود تلوث بالقولونية البرازية في بعض مصادر المياه التي تم فحصها مما قد يؤدي هذا إلى مخاطر صحية خطيرة.

الكلمات المفتاحية: مياه الشرب ، العدد الكلي الميكروبي ، القولونيات الكلية ، القولونية البرازية ، المعايير الكيميائية.

REFERENCES:

1. Abera, S.; Zeyinuidin, A.; Kebede, B.; Deribew, A.; Ali, S.; Zemene, E., (2011). Bacteriological analysis of drinking water source. Afr. J. Microbiol. Res., 5 (18), 2638-2641.
2. American Public Health Association (APHA) (1998). Standard Methods for the Examination of Water and Wastewater, 20th ed., APHA, Washington, DC.
3. APHA , AWWA, WEF, 1995. Standard Methods for the Examination of Water and Waste Water {19th edition }. Washington , D.C:. American Public Health Association .
4. Bitton, G. (2005). Microbial indicators of fecal contamination: application to microbial source tracking. Report Submitted to the Florida Stormwater Association, 71pp
5. Borrego, J.J., Cornax, R., Morinigo, M.A., Martinez-Manzares, E., and Romero, P. (1990). Coliphages as an indicator of fecal pollution in water. Their survival and productive infectivity in natural aquatic environments. Water Res. 24: 111–116.
6. Chigbu, P., Gordon, S., and Strange, T. (2005). Fecal coliform bacteria disappearance rates in a north-central Gulf of Mexico estuary. Estuar. Coast. Shelf Sci. 65: 309–318
7. ISO 1990 Water Quality C Detection and Enumeration of Coliform Organisms , Thermotolerant Coliform Organisms and Presumptive E. coli. Part 1:Membrane filtration Method. International Standard ISO 9308-1, International Organization for Standardization, Geneva.

8. Koster, W., Egli, T., Ashbolt, N., Botzenhart, K., Burlion, N., Endo, T., Grimont, P., Guillot, E., Mabilat, C., Newport, L., Niemi, M., Payment, P., Prescott, A., Renaud, P., and Rust, A. (2003). Analytical methods for microbiological water quality testing. In: A. Dufour, M. Snozzi, W. Koster, J. Bartram, E. Ronchi, and L. Fewtrell (Eds.), Assessing Microbial Safety of Drinking Water-Improving Approaches and Methods. IWA Publishing, London, 304pp.
9. Leonard, D.L. (2001). National indicator study: is an international approach feasible? J. Shellfish Res. 20: 1293–1298.
10. Michiels, C.W. and Moyson, E.L.D. (2000). Bacteriological analysis. In: L.M.L. Nollet (Ed.), Handbook of Water Analysis. Marcel Dekker, New York, pp. 115–141
11. Miner CA, Dakhin AP, Zoakah Al, Zaman M, Bimba J (2016). Physical and Microbiological Quality of Drinking Water Sources in Gwafan Community, Plateau State, Nigeria. Pyrex J. Res. Environ. Stud. 3(1):001-006
12. Neufeld, N. (1984). Procedures for the bacteriological examination of seawater and shellfish. In: A.E. Greenberg and D.A. Hunt (Eds.), Laboratory Procedures for the Examination of Seawater and Shellfish, 5th ed. American Public Health Association, Washington, DC
13. Reasoner, D.J. (2004). Heterotrophic plate count methodology in the United States. Int. J. Food Microbiol. 92: 307–315.
14. Stevens, M., Ashbolt, N., and Cunliffe, D. (2003). Recommendations to change the use of coliforms as microbial indicators of drinking water quality. National Health and Medical Research Council, Australian Government. 42pp
15. Wagner, M., Amann, R., Lemmer, H., and Schleifer, K.H. (1993). Probing activated sludge with oligonucleotide specific for proteobacteria: inadequacy of culture-dependent methods for describing microbial community structure. Appl. Environ. Microbiol. 59: 1520–1525.
16. WHO (1996). Guidelines for Drinking Water Quality (Health Criteria and Other Supporting Information, Vol. 2), 2nd ed., World Health Organization, Geneva
17. WHO (World Health Organization) (2003). Guidelines for Drinking Water Quality, Geneva, Switzerland, 3rd edition, pp. 81-87.
18. WHO, (2003), "Guidelines for drinking water quality", Geneva, Report No: WHO/SDE/WSH 03.04.
19. WHO, (2006), "Guidelines for drinking water quality" Geneva, Report No: WHO/SDE/WSH 06.07.