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Comparative Study of Indicator Organisms in Different Water Sources in North Bank Area of Makurdi

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Abstract

This study compared the presence of indicator organisms in different domestic water sources in the North Bank area of Makurdi. The water sources included stream waters, well waters, and borehole waters. Total bacteria count, total coliform count, microscopic examination, and biochemical tests were the parameters measured. The results obtained showed that all the different water sources had the presence of total bacteria and coliforms except the borehole water along Ter Guma Street (B1) which only showed the presence of total bacteria. The total bacteria count was found to be more (2.91 x 10^7 cfu/ml) in the stream water samples along University of Agriculture Road (ST2) and least in B2 water samples (6.76 x 10^6 cfu/ml) with no significant difference ($p>0.05$). Total coliform counts were found in all the water sources except in B1 water sample, while, the well water sample from Old Lafia Road (W2), had the highest total coliform count (1600 MPN/100 ml). Coliform isolates including Escherichia coli, Streptococcus faecalis, Staphylococcus aureus, Salmonella spp, Pseudomonas aeruginosa, and Klebsiella pneumoniae were found in the water samples, with well water from Old Lafia Road (W2), having more of the isolates. This study reveals that all the water sources studied were contaminated by the presence of microorganisms including indicator organisms, above the recommended by WHO, for a domestic water source. Further study to investigate the physicochemical parameters of these water sources is suggested.

1. Introduction

About 70% of the earth's surface is covered by water (Odo, 2019). Water is an essential component of the biosphere. Life may literary be impossible without water since both plants and animals require water to carry out most of their physiological and biochemical processes. About two-thirds of the human body is made up of water and needs one to seven liters per day to function optimally (Okonkwo *et al.,* 2011). Generally, human beings need water for various purposes majorly categorized as water for agriculture, water for industrial use, and water for domestic uses (Igbeneghu and Lamikanra, 2014). Its availability in the required quality and quantity for these purposes usually brings about enhancement in the health, social and economic sides of human life (Okoro *et al.,* 2017). However, one of the challenges facing mankind is the problem of scarcity of quality water for human consumption, especially in developing countries of the world, including Nigeria (Igbeneghu and Lamikanra, 2014). Water quality is determined by its physical, chemical, and biological characteristics which in turn determines its suitability for the different purposes outlined earlier (Adhikary *et al.,* 2010). Water suitable for human consumption is required to be of safe quality. This implies that such water must not contain any health risk over lifetime consumption. Such water is usually referred to as potable water or drinkable water (WHO, 2006). There are different drinkable water schemes conventionally employed; these are the surface waters (rivers, streams, lakes, etc.) and ground waters (boreholes and wells) (Okoro *et al.,* 2017). Contamination of water is associated with many life-threatening diseases such as diarrhea, cholera, dysentery, typhoid, and polio (WHO, 2016). Sources of water contamination include sewage disposal and open defecation along waterways, industrial effluents, and chemical and agricultural wastes (fertilizers, pesticides, metals, etc.)

(Tank and Chippa, 2013). Ebah *et al,* (2022) reported that groundwater quality can adversely be contaminated by seepage of abattoir effluents and other industrial effluents. Contamination brings about deterioration of water quality and the growth of pathogenic bacteria (*Salmonella typhi*, *Shigella dysentariae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus faecalis,* etc.). Water contamination is major contributors of mortality in developing countries. Annually about 502,000 deaths are attributed to diarrhea emanating from drinking contaminated water (WHO, 2016). Indicator organisms are microorganisms whose presence in water indicates the probable presence of pathogens (disease-causing organisms) (Maal-Bared *et al.,* 2008). Ideally, such microorganisms are non-pathogenic and occur consistently in pathogen-contaminated water. They do not multiply in waters, are reliably detectable even at low concentrations, and are present in greater numbers than and have similar survival times to pathogens (Tallon *et al.,* 2005). The concept of indicator organisms came into effect at the end of the 19th century as a result of breakthroughs recorded in the field of microbiology. The understanding of microorganisms and their relation to disease paved the way for improvements in the quality and safety of drinking water. Since that time, numerous advances have been made in the analysis of drinking water in the efforts to provide safe, clean drinking water to the public. The presence of indicator organisms in drinking water is of great concern. These indicator microorganisms are being used to assess the microbiological quality of environmental water (Ashbolt *et al.* 2001*)*. The aim of this study is to carry out comparative studies of indicator organisms of water in the different water sources in the North Bank area of Makurdi, Benue State.

2. Material and Methods

2.1 Study Area

This study was carried out in the North Bank area of Makurdi Local Government Area. As the name connotes, it is located in the northern part of Makurdi. North Bank may be described as a rural-urban area.

2.1.1 Sample Collection

Water samples were randomly collected from three different sources (boreholes, well, and surface water within the North Bank area of Makurdi, Benue State, Nigeria, comprising; two borehole water samples from different locations (coded B1 and B2 that represent boreholes located at Ter Guma Street and Old Lafia Road respectively), two well water samples (coded W1 and W2, located at Ter Guma Street and Old Lafia Road respectively) and two stream water samples also from different locations (coded ST1 and ST2 representing stream waters located along Ter Guma Street and the University of Agriculture Road respectively). Sterile universal sampling bottles, well labeled, were used and transported immediately after collection in a cellophane bag containing ice blocks to the laboratory for analysis. The samples were examined within two hours of collection.

2.2 Methods

2.2.1 Preparation of Media

Seventy-three grams of lactose broth was weighed using an analytical weighing balance and dissolved in 1000 ml of sterile distilled water inside a conical flask. Fifty-two grams of MacConkey agar powder was dissolved in 1000 ml of sterile distilled water, 36 g of Eosin Methylene Blue powder was prepared in 1000 ml of sterile distilled water, while 28 g of nutrient agar powder was dissolved in 1000ml of distilled water, and 111. 02g mannitol salt agar dissolved in 1000 ml distilled water. For proper dissolution and homogenization, the media were shaken vigorously and melted using a water bath at the temperature of 45°C for 40 min before sterilizing in an autoclave at 121°C for 15 min. Media will be aseptically dispensed into oven-sterilized Petri dishes and allowed to solidify under laminar airflow.

2.2.2 Determination of Total Bacterial Count

The total bacterial count was carried out by pour plate technique using standard methods. The enumeration of bacteria in samples was done using a nutrient agar medium. While mannitol salt agar was used for the isolation of *Staphylococcus aureus* while *Salmonella spp* was isolated on Salmonella-Shigella agar.

2.2.3 Determination of Total Coliform Count

This was determined by the Most Probable Number (MPN) index method using 5-5-5 regimen. MacConkey broth was used and positive results were indicated by acid and gas production on incubated at 37 ℃ for 48 hours (Cheesebrough, 2006).

2.2.4 Determination of Faecal Coliform Count

Faecal coliform count was carried out using Eosin Methylene Blue medium while employing the pour plate technique. On Eosin Methylene Blue (EMB) agar, E. coli strains appear as greenish metallic sheen colonies and this was confirmed by the ability of the organism to ferment the lactose (Burnett and Beauchat, 2011).

2.2.5 Morphological Examination and Identification of Isolates on Media

The cultural characteristics of the isolates on different solid agar were examined. The growth patterns, colony size, edge, and elevation on the plates were noted after 48 hours of incubation at 37°C. Gram staining technique was carried out for the identification and differentiation of each isolated bacteria. (Ryan and Ray, 2008). Microbial identification was performed using the keys provided in Bergey's Manual of Determinative Bacteriology (1994).

2.2.6 Biochemical Tests for Identification of isolates

Biochemical tests will be carried out namely: Catalase, Urease, Indole, gram reaction, acid and gas production, and Citrate following standard procedures (Sule *et al*., 2009).

2.3 Statistical Analysis

Descriptive statistics and analysis of variance were used to analyze data obtained from the study. The level of significance was accepted at $p<0.05$. The results were presented as mean and standard deviation.

3. Results

Table 1 below represents the mean and standard deviation of the total bacteria count of different domestic water sources from selected locations in the North Bank area of Makurdi. The results indicate the presence of total bacteria counts in all the water samples tested. Stream water from the University of Agriculture Road (ST2) produced the highest count ($2.91\pm4.33x10^7$cfu/ml) while the borehole water from Old Lafia Road (B2), had the lowest total bacteria counts ($6.76\pm1.01x10^6$mg/ml). The results of the total coliform counts are presented in Table 2. The results show the number of positive tubes of McConkey broth containing the different water samples, and their corresponding coliform counts. The well water samples (W1 and W2), Stream water samples (ST1 and ST2), and the borehole water from Old Lafia Road (B2), all had positive tubes along with coliform counts, whereas the borehole water from Ter Guma Street (B1), had no positive tubes, though with < 2 MPN/100ml. The highest total coliform count (1600 MPN per 100l) was recorded in W2 water sample. Table 3 represents the results of growth morphology and microscopic examination of coliform isolates from the different water sources and the probable coliform bacteria present. The results showed that ST1 water sample contained the presence of *Staphylococcus aureus, E. coli, Klebsiella pneumoniae,* and *S. faecalis*, ST2 water sample had the presence of *Staphylococcus aureus, S. faecalis*, *E. Coli* and *Salmonella spp.*W1 water sample, on the other hand, showed the presence of *E. coli* and *Salmonella spp,* while W2 water sample had *E. coli, P. aeruginosa, Klebsiella pneumoniae,* and *S. faecalis.* Also, B2 water sample showed the probable presence of *E. coli* and *Salmonella spp.* Table 4 shows the results of biochemical tests carried out on the coliform isolates of the different domestic water sources of some selected locations of the North Bank area of Makurdi. The results showed that ST1 water sample contained the presence of *Staphylococcus aureus, E. coli, Klebsiella pneumoniae,* and *S. faecalis*, ST2 water sample had the presence of *Staphylococcus aureus, S. faecalis*, *E. coli* and *Salmonella spp.*W1 water sample, on the other hand, showed the presence of *E. coli* and *Salmonella spp,* while W2 water sample had *E. coli, P. aeruginosa, Klebsiella pneumoniae,* and *S. faecalis.* Also, B2 water sample showed the probable presence of *E. coli* and *Salmonella spp.* The percentage frequency distribution of the different bacteria isolates obtained from the different water sources was also evaluated. The results (Table 5 and Table 2) show that *Escherichia coli* had the highest percentage of occurrence, while *Pseudomonas aeruginosa* was the least. Table 1 shows the number of Coliform isolates per water sample studied.

Table 1: Mean values of total bacteria count of different water sources.

Water source	Total Bacteria count (cfu/ml)
ST1	$1.02\pm1.54x10^7$
ST2	$2.91\pm4.33x10^7$
W1	$2.46\pm3.64x10^7$
W2	$1.88\pm2.81x10^7$
B1	$8.67\pm1.40x10^6$
B2	$6.76\pm1.01x10^6$

Keys: *ST1= stream water along Ter Guma Street, ST2 = Stream water along University of Agriculture Makurdi Road, W1 and W2= well water Sources, B1= Borehole water from Ter Guma Street, B2= Borehole water along Old Lafia Road,*

Table 2: Total coliform count of the different domestic water sources

Water Source	No. of positive tubes	MPN/100ml	95% confidence limit	
			Lower	Upper
ST1	5 – 4 – 3	280	120	690
ST2	4 - 1 – 1	21	9.0	55
W1	4 – 4 – 2	34	16	80
W2	5 – 5 – 4	1600	600	5300
B1	0 – 0 – 0	<2	-	-
B2	2 – 1 – 2	9	3.0	24

Keys: *ST1= stream water along Ter Guma Street, ST2 = Stream water along University of Agriculture Makurdi Road, W1 and W2= well water Sources, B2= Borehole water along Old Lafia Road, B1= Borehole water from Ter Guma Street.*

Table 3: Growth morphology and microscopic examination results of coliforms isolated from different water sources

Water source	Gram reaction	Morphology	Colour of colonies on EMBA	Colour of colonies on NA	Colour of colonies on MSA	Colour of colonies on SSA	Probable isolate
ST1a	+ve	Cocci	Colourless	Golden yellow	Golden brown	Nil	*Staphylococcus aureus*
ST1b	-ve	Rod	Greenish metallic sheen	Greyish white	Nil	Pink	*Escherichia coli*
ST1c	-ve	Rod	Pink	Greyish opaque	Nil	Pink	*Klebsiella pneumoniae*
ST1d	+ve	Cocci in chain	Colourless	Nil	No growth	Colourless	*Streptococcus faecalis*
ST2a	-ve	Rod	Nil	Colourless	No growth	Colourless with black center	*Salmonella spp*
ST2b	+ve	Cocci	Colourless	Golden yellow	Golden brown	Nil	*Staphylococcus aureus*
ST2c	+ve	Cocci in chain	Colourless	Nil	No growth	Colourless	*Streptococcus faecalis*
ST2d	-ve	Rod	Pink	Greyish white	Nil	Pink	*Escherichia coli*
W1a	-ve	Rod	Pink	Greyish white	Nil	Pink	*Escherichia coli*
W1b	-ve	Rod	Nil	Colourless	No growth	Colourless with black center	*Salmonella spp*
W1c	-ve	Rod	Greenish metallic sheen	Greyish white	Nil	Pink	*Escherichia coli*
W2a	-ve	Rod	Greenish metallic sheen	Greyish white	Nil	Pink	*Escherichia coli*
W2b	-ve	Rod	Nil	Opaque	Nil	Irregular colonies	*Pseudomonas aeruginosa*
W2c	+ve	Cocci in chain	Colourless	Nil	No growth	Colourless	*Streptococcus faecalis*
W2d	-ve	Rod	Pink	Greyish paque	Nil	Pink	*Klebsiella pneumoniae*
W2e	-ve	Rod	Greenish metallic	Greyish white	Nil	Pink	*Escherichia coli*

			sheen				
B2a	-ve	Rod	Greenish metallic sheen	Greyish white	Nil	Pink	*Escherichia coli*
B2b	-ve	Rod	Nil	Colourless	No growth	Colourless with black center	*Salmonella spp*

Keys: *ST1= stream water along Ter Guma Street, ST2 = Stream water along University of Agriculture Makurdi Road, W1 and W2= well water Sources, B2= Borehole water along Old Lafia Road, B1= Borehole water from Ter Guma Street.a,b,c,d. e = replicates*

Table 4: Biochemical test results of coliform isolates of the different domestic water sources

Water source	Catalase	Citrate	Indole	Coagulase	Urease	Gas formation	Probable isolate
ST1a	+ve	+ve	-ve	+ve	+ve	-ve	*Staphylococcus aureus*
ST1b	+ve	-ve	+ve	-ve	-ve	+ve	*Escherichia coli*
ST1c	+ve	+ve	-ve	-ve	+ve	+ve	*Klebsiella pneumoniae*
ST1d	-ve	-ve	-ve	-ve	-ve	-ve	*Streptococcus faecalis*
ST2a	+ve	-ve	-ve	-ve	-ve	-ve	*Salmonella spp*
ST2b	+ve	+ve	-ve	+ve	+ve	-ve	*Staphylococcus aureus*
ST2c	-ve	-ve	-ve	-ve	-ve	-ve	*Streptococcus faecalis*
ST2d	+ve	-ve	+ve	-ve	-ve	+ve	*Escherichia coli*
W1a	+ve	-ve	+ve	-ve	-ve	+ve	*Escherichia coli*
W1b	+ve	-ve	-ve	-ve	-ve	-ve	*Salmonella spp*
W1c	+ve	-ve	+ve	-ve	-ve	+ve	*Escherichia coli*
W2a	+ve	-ve	+ve	-ve	-ve	+ve	*Escherichia coli*
W2b	+ve	+ve	-ve	-ve	-ve	-ve	*Pseudomonas aeruginosa*
W2c	-ve	-ve	-ve	-ve	-ve	-ve	*Streptococcus faecalis*
W2d	+ve	+ve	-ve	-ve	+ve	+ve	*Klebsiella pneumoniae*
W2e	+ve	-ve	+ve	-ve	-ve	+ve	*Escherichia coli*
B2b	+ve	-ve	+ve	-ve	-ve	+ve	*Escherichia coli*
B2c	+ve	-ve	-ve	-ve	-ve	-ve	*Salmonella spp*

Keys: *ST1= stream water along Ter Guma Street, ST2 = Stream water along University of Agriculture Makurdi Road, W1 and W2= well water Sources, B2= Borehole water along Old Lafia Road, B1= Borehole water from Ter Guma Street.a,b,c,d. e = replicates*

Figure 1: Number of bacterial isolates in the different water sources

Keys: *ST1= stream water along Ter Guma Street, ST2 = Stream water along University of Agriculture Makurdi Road, W1 and W2= well water Sources, B2= Borehole water along Old Lafia Road, B1= Borehole water from Ter Guma Street.*

Table 5: Frequency occurrence of bacteria isolates of the different domestic water sources

Bacteria Isolates	Percentage (%) Frequency Distribution
Escherichia coli	38.9
Streptococcus faecalis	16.7
Pseudomonas aeruginosa	5.6
Klebsiella pneumoniae	11.1
Salmonella spp	16.7
Staphylococcus aureus	11.1

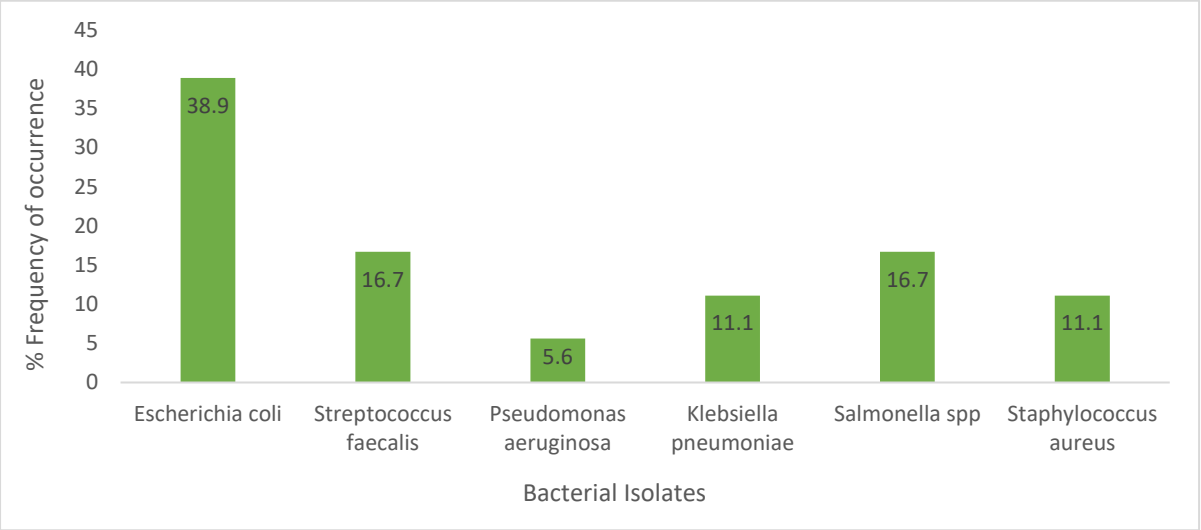

Figure 2: Percentage frequency of occurrence of bacterial isolates of the different water samples

4. Discussion

This study compared indicator organisms' presence among different water sources from selected locations of the North Bank Area of Makurdi. The total bacteria count, total coliform counts (Most Probable Number), microscopic examinations, and biochemical tests were performed. The results obtained showed the presence of indicator organisms in all the water sources except in borehole water (B1) located along Ter Guma Street (B1). The mean total bacteria count was between 6.76 x 10^6 cfu/ml in borehole water located at Old Lafia Road(B2) and 2.91 x 10^7cfu/ml stream water at Ter Guma Street (ST1). No significant difference ($p>0.05$) was observed among the different water samples. Though, these counts are higher than the acceptable count of 0 cfu/ml for drinking water (NIS, 2007). The high total bacteria count in all the water sources is an indication of a heavy presence of organic matter in the water. The main source of this contamination may be attributed to both human and animal activities (Scott *et al*., 2003)). These sources of bacteria may include surface run-off agricultural farms, animal waste deposition, and pasture. Human activities such as waste disposal and faecal discharge are also possible ways of contamination (Idibie *et al*., 2018). The result of total coliform count showed that the values obtained were all above the WHO standard for coliform bacteria in drinking water, which is zero total coliform per 100ml (Welch *et al*., 1993), for all the water sources except borehole water from Ter Guma Street (B1). The highest total coliform count (1600 MPN/100ml) was recorded in well water from Ter Gumawater (W2). Presence of total coliform counts in water sources is an indication of faecal contamination. None of the water sources examined met the WHO standard and this is in agreement with previous works by Benka-Coker Ohimain (1995) and Idibie *et al.* (2018) who reported high microbial load in water with higher organic matter. The result also showed that six bacterial isolates were obtained from the different water samples, including *Escherichia coli, Streptococcus faecalis, Staphylococcus aureus, Salmonella spp, Pseudomonas aeruginosa, Klebsiella pneumoniae.* Table 5 shows the frequency of occurrence of the isolates. The result indicates that *E. coli*had the highest occurrence (38.9%) followed by *Salmonella and S. faecalis* with 16.7% frequency of occurrence each. *Pseudomonas aeruginosa* had the least occurrence. The high presence of *E. coli* is an indication that majority of the water samples may be unsafe for drinking as there is high likelihood of pathogenic organisms in the water. However, borehole water samples obtained along Lafia Road did not contain *E. coli.*

5. Conclusion

The results of this investigation show that all the water sources studied were contaminated with the presence of microorganisms including indicator organisms, above the recommended value by WHO. This suggests the possible presence of pathogenic microorganisms in these water sources and makes them unsafe for human consumption untreated. Comparatively, the water samples from the two Wells and the two Streams studied, seem more contaminated, while those of Borehole water samples were least contaminated. The presence of these indicator microorganisms poses the danger of potential waterborne disease.

6. Recommendation

Based on the results of this study, we recommend that:

1. The water from these sources is unfit for drinking and domestic purposes.
2. Proper awareness about the unsafe nature of these water should be made to the communities that depend on them and the need to properly treat the water before use.
3. The government should as a matter of urgency provide pipe-borne water to these areas.
4. Further studies should be carried out to investigate the physicochemical parameters of these water sources.

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Appendix

Plate 1: Most Probable Number Result ofW2 (0.1ml)
Keys:ST1= stream water along Ter Guma Street, ST2 = Stream water along Uni-Agric. Road, W1 and W2= well water Sources, B1= Borehole water along Old Lafia Road, B2= Borehole water from Ter Guma Street.

Plate 2: Most Probable Number Result ofW2 (10ml)
Keys:ST1= stream water along Ter Guma Street, ST2 = Stream water along Uni-Agric. Road, W1 and W2= well water Sources, B1= Borehole water along Old Lafia Road, B2= Borehole water from Ter Guma Street.

Plate 3: Most Probable Number Result ofST2 (0.1ml)
Keys:ST1= stream water along Ter Guma Street, ST2 = Stream water along Uni-Agric. Road, W1 and W2= well water Sources, B1= Borehole water along Old Lafia Road, B2= Borehole water from Ter Guma Street.

Plate 4: Most Probable Number Result ofST2 (1ml)
Keys:ST1= stream water along Ter Guma Street, ST2 = Stream water along Uni-Agric. Road, W1 and W2= well water Sources, B1= Borehole water along Old Lafia Road, B2= Borehole water from Ter Guma Street.

Plate 5: Most Probable Number Result ofST2 (10ml)
Keys:ST1= stream water along Ter Guma Street, ST2 = Stream water along Uni-Agric. Road, W1 and W2= well water Sources, B1= Borehole water along Old Lafia Road, B2= Borehole water from Ter Guma Street.

Plate 6: Most Probable Number Result ofST1 (0.1ml)
Keys:ST1= stream water along Ter Guma Street, ST2 = Stream water along Uni-Agric. Road, W1 and W2= well water Sources, B1= Borehole water along Old Lafia Road, B2= Borehole water from Ter Guma Street.

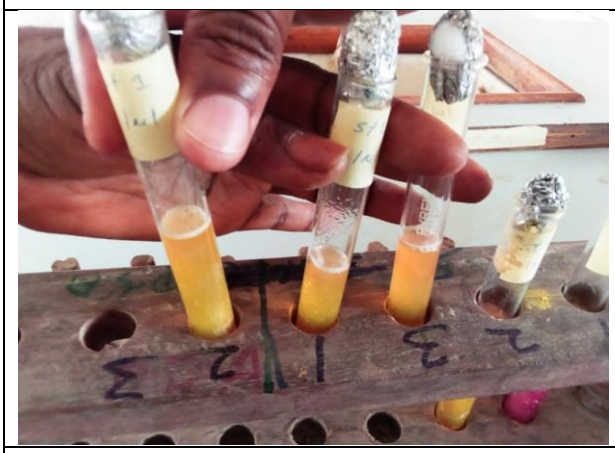

Plate 7: Most Probable Number Result ofST1 (1ml)
Keys:ST1= stream water along Ter Guma Street, ST2 = Stream water along Uni-Agric. Road, W1 and W2= well water Sources, B1= Borehole water along Old Lafia Road, B2= Borehole water from Ter Guma Street.

Plate 8: Most Probable Number Result ofST1 (10ml)
Keys:ST1= stream water along Ter Guma Street, ST2 = Stream water along Uni-Agric. Road, W1 and W2= well water Sources, B1= Borehole water along Old Lafia Road, B2= Borehole water from Ter Guma Street.

Plate 9: Most Probable Number Result ofB1 (10ml)
Keys:ST1= stream water along Ter Guma Street, ST2 = Stream water along Uni-Agric. Road, W1 and W2= well water Sources, B1= Borehole water along Old Lafia Road, B2= Borehole water from Ter Guma Street.

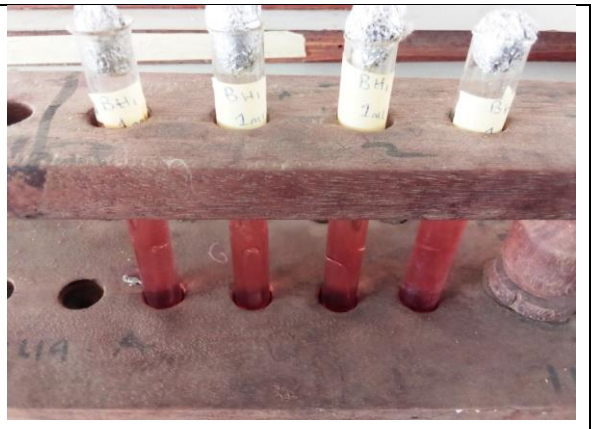

Plate 10: Most Probable Number Result ofB1 (1ml)
Keys:ST1= stream water along Ter Guma Street, ST2 = Stream water along Uni-Agric. Road, W1 and W2= well water Sources, B1= Borehole water along Old Lafia Road, B2= Borehole water from Ter Guma Street.

Plate 11: Most Probable Number Result ofB1(0.1ml)
Keys:ST1= stream water along Ter Guma Street, ST2 = Stream water along Uni-Agric. Road, W1 and W2= well water Sources, B1= Borehole water along Old Lafia Road, B2= Borehole water from Ter Guma Street.