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COMPARATIVE STUDIES OF BACTERIAL ANALYSIS OF DIFFERENT SOURCES OF WATER IN OKE-GADA EDE OSUN STATE

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Abstract: Water is unarguably one of the most important natural resources necessary for existence of all living things. The major sources of water meant for drinking in most homes in Nigeria, often come from borehole, well, pipe-borne tap water from government's corporation, stream and table water. Five samples from all these sources were collected at Oke-Gada, Ede, Osun state and bacteriological analyses which include presumptive, confirmatory and completed tests were carried out on them at varying time intervals namely; 24 hrs, 15 days and 30 days. A few physicochemical properties including chlorine residual, chemical oxygen demand and biochemical oxygen demand were carried out on the samples. The results obtained were compared with the requirements for portable water by the National Agency for Food and Drug Administration and Control (NAFDAC). Water supply from tap water source gave the finest results that fall within the acceptable range of NAFDAC guidelines, followed by table water, then borehole, and then well water, while stream water source came with the most unacceptable data range. All the sources except tap water showed the presence of *Escherichia coli, Pseudomonas spp, Klebsiella spp, Salmonella shigella*. There was increase in the load or population of microorganisms found in the water as storage day's increase, an indication that shows a direct relationship between storage time and bio-film formation. An irreversible process where microorganisms grow on a surface and produce extracellular polymers that facilitate attachments and matrix formation and build up resistance against disinfectants used to initially treat the waters for the sake of rendering the microorganisms dormant or inactive. COD AND BOD_5 results for all the water samples fell within the NAFDAC range except for stream water where the COD value is higher. An awareness programme to enlighten the locals on the importance of taking water from a source fit for human consumption, and why it is advisable for them to consume their stored water in less than seven (3) days after collection should be carried out in earnest and from time to time.

Keywords: Bio-film, Matrix, Extracellular, *Escherichia coli, Pseudomonas spp, Klebsiella spp, Salmonella shigella*

1.0 Introduction

Life is impossible without water, as it plays a vital role in the proper functioning of the earth's ecosystem (Ajayi, 2011). The unavailability of water both in quality and quantity has been one of the major public health issues in Africa, particularly in Nigeria (Saravanan, 2019). Shortage of portable water supply generally leads to occurrence of many diseases and with its resultant effects on economy; hence provision of portable water is essential in any environment.

In rural areas of most developing countries, there is near extreme water scarcity and lack of access to portable water supply, thus many residents rely mainly on stream, well, and public borehole for survival (Banwo, 2006). In Ede metropolis for instance, the inconsistence nature in the supply of portable pipe-borne water cause many residents to resort to alternative sources of water which may be unfit for human consumption. Reliance on these unsafe sources of water primarily leads to diseases and ultimate death among the people with attendant negative effects on the society. (Adejumo, 2017).

A lot of work has been done on the isolation and identification of bacteria on selected sources of water. However, little or no work has been done in identifying the bacterial loads and types present in different sources of water at varying time interval in a specific location. This study therefore aims to compare bacterial contents of different sources of water aforementioned in Oke-Gada, Ede, Osun State.

2.0 Materials and methods

2.1 Description of Study Area

Ede, a historical and traditional town in southwest Nigeria, has been rapidly experiencing unprecedented growth rate and physical expansion in recent time. This is probably due to its proximate location to Oshogbo, the Osun state capital. The total population of the town, according to the 2006 census, is 159,866. The town houses about three higher institutions of learning, namely; Federal Polytechnic, Ede, Adeleke University, and Redeemers University for all nations. The NYSC Orientation camp of the state is also located in the town among others. Geographically, the study site, Oke-Gada, a popular area in the town, is situated south of Oshogbo on latitude 7^0 40'58.96''N, longitude 4^0 30' 58.99''E. The site is blessed with abundant underground and surface water bodies in both time and space amongst the sub-settlements. Fig1 shows the general map of Ede Township and the location of OkeGada.

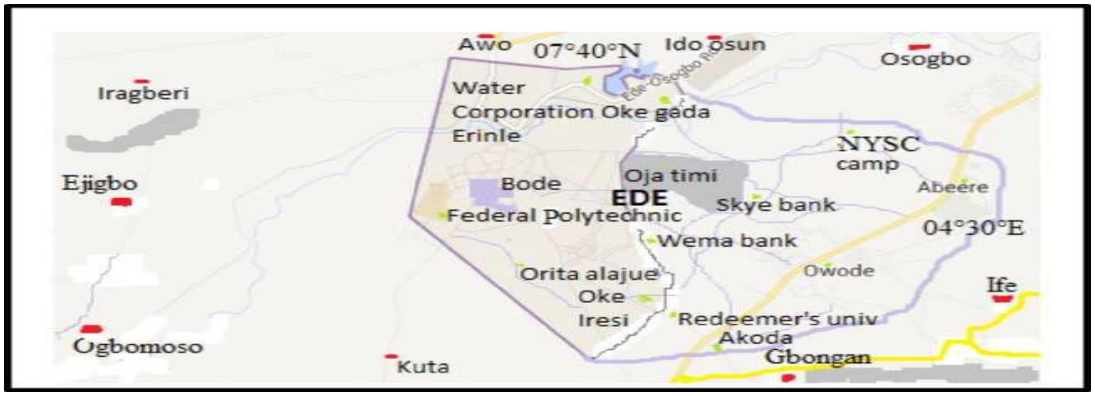

Fig1: Map showing OkeGada in Ede metropolis of Osun State (Ogunsumi, *et al*, 2022)

2.2 Collection of water samples

Five newly bought 5 litre kegs, washed with detergent and properly rinsed with distilled water were used to collect water samples from each of the sources of water i.e. Tap, well, borehole, table and stream, all at Oke-Gada. Figure 2 shows the samples collected taken to the laboratory taken to the laboratory for bacteriological analyses which was carried out at 24 hrs, 15 days and 30 days after sample collections. The tests carried out on each of the water sample include the presumptive, determination of total bacteria counts, confirmatory and complete tests, chemical and biochemical oxygen demands. All the materials used for the study was properly sterilized.

1. 

Fig 2: Water samples from five different sources at Oke-Gada (Ogunsumi *et al*, 2022)

2.3 Bacteriological Examination

The most probable number (MPN) method was used. This is a statistical method which is based on the random dispersion of microorganisms per volume in a given sample. In this process, measured volumes of water are added to a series of tubes containing a liquid indicator growth medium (Aryal, 2018). The media receiving one or more indicator bacteria would show growth and a characteristic colour change. The colour change would be absent in those receiving only an inoculums of water without indicator bacteria. From the number and distribution of negative and positive reactions, the MPN of indicator organisms in the sample may be estimated by reference to statistical tables. The process is completed in three steps:
Presumptive test
Confirmed test
Completed test

2.3.1 Presumptive test

MacConkey purple media of single and double strength was first prepared in test tubes with Durham's tube and then autoclaved. Three sets of test tubes containing five tubes in each set; one set with 10 ml of double strength (DS) other two containing 10 ml of single strength (SS) were picked. With the aid of sterile pipettes, 10 ml of water was transferred to each of the DS broth tubes. 1 ml of water sample was transferred to each of 5 tubes of one set of SS broth and 0.1 ml water was transferred to five tubes of remaining last set of SS broth tubes. The tubes were incubated at 37°C for 24 hours. After incubation, gas production in Durham's tube and the color change of the media was keenly observed. The number of positive results from each set was recorded and compared with the standard chart to give presumptive coliform counts per 100 ml water sample.

The formation of 10% gas or more in the Durham tube within 24 to 48 hours, together with turbidity in the growth medium and the color change in the medium constitutes a positive presumptive test for coliform bacteria, and hence for the possibility of fecal pollution. Meanwhile, No growth or formation of gas in

Durham's tube is an indication of a negative results. The test is presumptive only because under these conditions several other types of bacteria can produce similar results (Aryal, 2018)

2.3.2 Confirmed Test

It is a known fact that some microorganisms other than coliform could also produce acid and gas from lactose fermentation. Thus, to confirm the presence of coliform, a confirmatory test is usually carried out. For this purpose, a loopful of suspension from a positive tube was inoculated into a 3 ml lactose-broth or brilliant green lactose fermentation tube and then to an agar plate (EMB agar or Endo Agar) or slant.

- Inoculation of the lactose-broth

The inoculated lactose-broth fermentation tubes were incubated at 37°C and gas formation was inspected after 24 ± 2 hours. Further incubate up to a maximum of 48 ± 3 hours to check gas production was carried when no gas production was seen.

- Inoculation in media slants

A loopful of suspension from a positive tube was taken and was inoculated on the agar surface. The agar slants was incubated at 37°C for 24 ± 2 hours and colonies was examined macroscopically.

Formation of gas in lactose broth and the demonstration of a coliform-like colony on the EMB agar indicated the presence of a member of the coliform group in the sample examined.

Coliforms produce colonies with a greenish metallic sheen which differentiates them from non-coliform colonies (show no sheen). The presence of typical colonies at high temperatures (44.5 ± 0.2) indicates the presence of thermotolerant *E.coli.* Meanwhile, the absence of gas formation in lactose broth or the failure to demonstrate coliform-like colonies on the EMB agar is an indication of a negative result. (Aryal, 2018)

2.3.3 Completed Test

A typical coliform colony from the agar plate was transformed into a tube of brilliant green bile broth with placed Durham's tube and on the surface of a nutrient agar slant. This was incubated at 35°C for 24 hours, the broth was checked after 24 hours for the production of gas, and Gram staining was performed for organisms on the nutrient agar slant. The presence of gas in the brilliant green bile broth tube and Gram-negative, non-spore-forming rods on NA slant constitutes a positive completed test for the presence of coliform bacteria, which, in turn, infers possible contamination of the water sample with fecal matter, while, absence of growth and gas formation in the broth as well as absence of gram-negative, non-sporing rods on Gram staining is an indication of a negative result (Aryal, 2018).

Figure 3 shows the flow scheme of the most probable number procedures. The different stages of the presumptive, confirmatory and completed tests were shown. The different reagents, agar and technique used were also shown in the chart at every stage of the analyses were properly documented in the diagram.

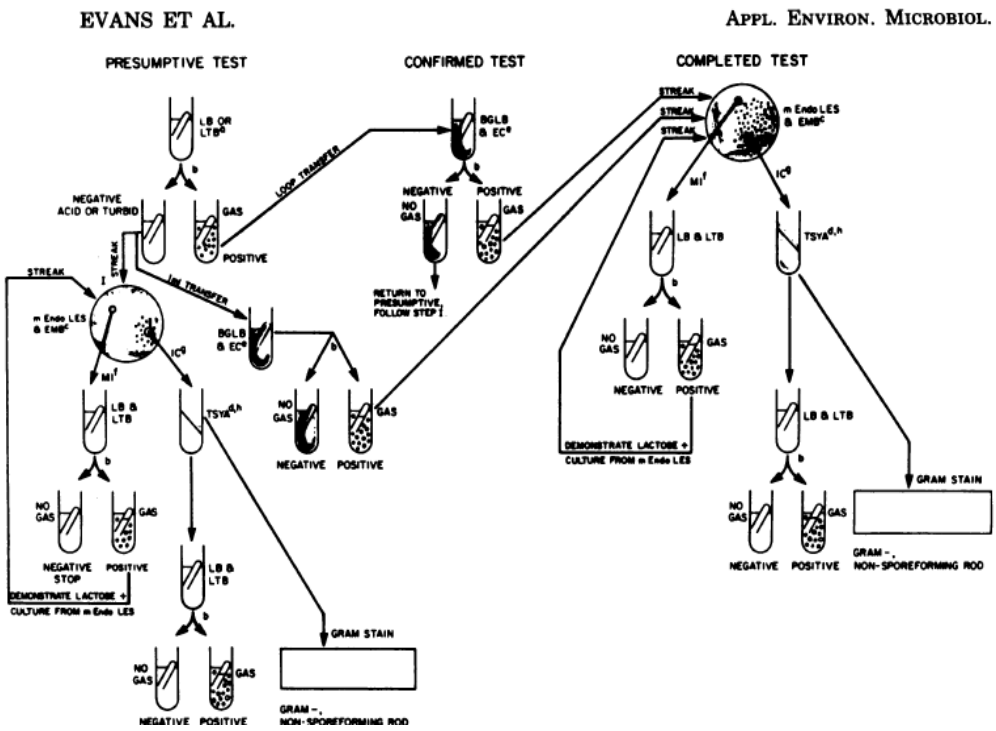

Fig 3: Flow scheme for M-MPN procedure. Abbreviations: (a) lactose broth (LB) and lauryl tryptose broth (LTB); (b) tubes examined after 24 and 48 h at 35°C; (c) m-Endo agar LES and eosin methylene blue (EMB) agar; (d) incubated for 24 h at 35°C; (e) brilliant green lactose bile (BGLB) broth and EC broth; (f) multiple inoculation (MI) technique; (g) inoculation of an isolated colony (IC); (h) tryptic soy yeast extract agar (TSYA) – Evans *et al*, 1981.

2.3.4 Chlorine Residual

10ml of water sample was poured into a residual cuvette. DPD tablet was grinded and poured into the sample and mixed thoroughly. Chlorine residual comparator was inserted to record the most matched value.

2.3.5. Biochemical Oxygen Demand

The BOD_5 test was also carried out on each of the samples of water. 300m/s of each of the water samples was measured and poured into BOD bottle. The initial value for the dissolved oxygen was obtained through a metre. Then the samples were then incubated for 5days at 20°c and the final dissolved oxygen was also determined. The values of the final dissolved oxygen were subtracted from the initial one to get the BOD value.

Chemical oxygen demand (COD) is the amount of dissolved oxygen that must be present in water to oxidize chemical organic materials. The COD test uses a chemical (potassium dichromate in a 50% sulfuric acid solution) that "oxidizes" both organic (predominate) and inorganic substances in a wastewater sample. The test was carried out by taking 10ml of water sample into a round bottom reflex flask. Some glass beads were added to prevent the solution from bumping into the flask while heating. 1 ml of mercury sulfate solution was added to the flask and mixed properly by swirling the flask. 5ml of potassium dichromate solution was then added before adding slowly and carefully 15 ml silver sulfate – sulfuric acid solution. The reflex condenser was connected and the content was digested using a hot plate for 2 hours. After digestion, the flask was cooled and the condenser was rinsed with 25ml of distilled water collecting in the same flask. 2-4 drops of ferroin indicator was added to the flask and this was titrated with 0.025M ferrous ammonium sulfate solution to the end point. The blank preparation should be made in the same manner as sample using distilled water instead of the sample.

2. Then the chemical oxygen demand is calculated using the formula.

$$COD = \frac{8 \times 1000 \times DF \times M \times (V_B - V_S)}{\text{Volume of sample in ml}}$$

Where DF – Dilution factor, M- Molarity of standardized Ferrous Ammonium Sulfate Solution
V_B – Volume consumed in titration with blank preparation, V_S – Volume consumed in titration with sample preparation.

3.0 Results

Table1: Bacterial examination of the different sources of water at 24 hours after collection (Ogunsumi *et al*, 2022)

Sample no	Sample description	PH	Colonies growing on Nutrients Agar (NA) at 37 ^{0}C in 24 hrs	Results of the Presumptive Test for 50ml, 10ml and 1.5ml	Most probable number of coliforms per 100ml of water sample
A	Sachet	7.6	95	1.1.3	09
B	Tap	7.6	0	0.0,0	00
C	Well	6.8	64	1,3,1	11
D	Borehole	7.6	102	1,4,5	40
E	Stream	7.6	cluster	1,5,5	180^+

Table 2: Bacterial examination of the different sources of water 15 days after collection (Ogunsumi *et al*, 2022)

Sample no	Sample description	PH	Colonies growing on Nutrients Agar (NA) at 37 ^{0}C in 24 hrs	Results of the Presumptive Test for 50ml, 10ml and 1.5ml	Most probable number of coliforms per 100ml of water sample
A	Sachet	7.2	108	0.5.5	27
B	Tap	7.4	35	1,5,5	180^+
C	Well	6.6	88	1,5,5	180^+
D	Borehole	7.2	Cluster	1,5,5	180^+
E	Stream	7.2	Cluster	1,5,5	180^+

Table3: Bacterial examination of the different sources of water 30 days after collection (Ogunsumi *et al*, 2022)

Sample no	Sample description	PH	Colonies growing on Nutrients Agar (NA) at 37 ^{0}C in 24 hrs	Results of the Presumptive Test for 50ml, 10ml and 1.5ml	Most probable number of coliforms per 100ml of water sample
A	Sachet	6.8	Cluster	1.5.5	180^+
B	Tap	7.0	298	1,5,5	180^+
C	Well	7.1	Cluster	1,5,5	180^+
D	Borehole	7.2	356	1,5,5	180^+
E	Stream	7.4	Cluster	1,5,5	180^+

Table 4: Bacteria isolates from sample of different water sources in OkeGada.

Isolates sampled	Stream	Sachet	Borehole	Well	Tap
Salmonella spp	+	+			
E.coli	+	+	+	+	
Klebsiella spp	+	_	_	+	_
Pseudomonas spp	+	_	+	+	_

+ Present - Absent

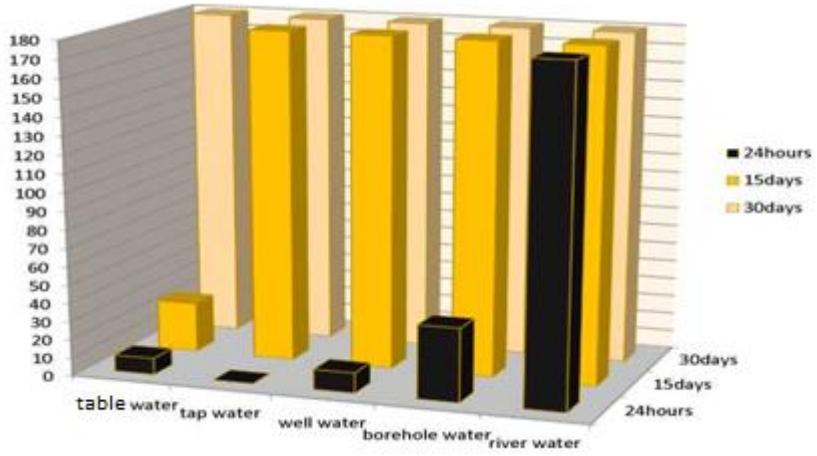

Fig 4: Comparison of MPN of bacteria coliform per 100ml of water sample of different sources of water at different time interval

Table 5: Chlorine residual, COD and BOD values for the various sources of water.

Parameters	Table water	Tap water	Well water	Borehole	Stream water
Chlorine residual mg/L	Nil	0.02	Nil	Nil	Nil
Chemical Oxygen Demand (COD) g/L	0.00	0.00	30.00	0.00	680.00
Biochemical Oxygen Demand (BOD mg/L	1.64	1.22	0.62	0.02	0.84

Table 6: Standard Microbiological Limits for Water

Parameter	Unit	Maximum permitted	Health Impacts	Notes
• Total Coliform count	• cfu/ml	• 10	• Indication of contamination.	
• Thermotolerant • Coliform or E.coli	• cfu/lOOMl	• 0	• Urinary tract infections, bacteraemia, meningitis, diarrhea, (one of the main cause of morbidity and mortality among children), acute renal failure and haemolytic anaemia.	
• Faecal streptococcus	• cfu/lOOmL	• 0	• Indication of recent faecal contamination.	
• Clostridium perfringens spore	• cfu/lOOmL	• 0	• Index of intermittent faecal contamination.	

4.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1: Discussion of results.

From the results, it was realized that at 24 hrs after collection of the water samples, it was only the tap water that was free from bacteria loads. The source of water that gave the most unacceptable results, was the stream water with cluster loads of bacteria, which might be from human and other anthropogenic activities. The sources of these bacterial contaminations in the stream water may include surface runoff, animal waste deposition and fecal discharge. Fecal discharge of course introduces foreign microbes into the water bodies, making more nutrients readily available to the microorganisms in the water and thereby enhancing their growth rate (Solate *et al* 2012).

On the other hand, the results at 15 and 30 days after collection of the water samples showed that all the sources including tap, had bacteria loads than what was recorded at 24 hours after collection. This definitely came from bio-films formation, an indication that shows a direct relationship between storage time and bio-film formation. An irreversible process where microorganisms grow on a surface and produce extracellular polymers that facilitate attachments and matrix formation and build up resistance against disinfectants used to initially treat the water. The disinfectants don't kill the bacteria but merely render them inactive and dormant for a period, after which the microorganisms could develop immunity against the chemicals leading to the multiplicity in the loads of various bacterial types. It was observed that after three days of sample collection, the tap source, which did not show presence of any microbial contamination abinitio, began to show the presence of microorganisms, which suggests that storing water beyond three days after collection may not be bacteriologically safe for consumption.

Aside this, the results also showed some of the microbial types that were isolated from the various sources of water which include *Eschericai Colt, pseudomonas spp, klebsiella spp, and salmonella shigella.* The presence of these isolates in water is an indication that drinking from these sources may cause the consumers various forms of water borne diseases such as diarrhea, giardiasis, dysentery, and other gastrointestinal diseases. (Oyedeji *et al,* 2010).

The highest and lowest pH values of 7.6 and 6.8 were recorded for all the sources of water which is still within the range of NAFDAC standard (6.5-8.5). The COD and BOD values were found to be appropriate and in the range of NAFDAC standard except for stream water which has a very high COD value.

4.2: Conclusion

The study revealed that tap water samples have the least bacterial contaminants within three days of collection, which makes it suitable for human consumption for that time being. This is followed by table water, borehole and then well water respectively. The stream water source had the highest amount of bacterial contaminants. However, the bacteriological values from total coliform count of each sources of water samples except Tap water did not conform to the stipulated coliform count by NAFDAC as they were higher than the values given in table 6. The high microbial load particularly in the stream, well and borehole water sources make them highly unsuitable for drinking purposes, although they can be used for domestic purposes.

4.3: Recommendations

Constant awareness and sensitization programme is therefore recommended to be carried out from time to time for the residents of Okegada in Ede, with the primary focus of making them aware that of looming dangers in consuming other sources of water aside the tap water sources. It should also be reiterated that stored water be consumed within three (3) days of collection. Personal hygiene should be paramount to the resident of Oke-Gada, Water collected from every other sources apart from the tap water should be boiled, chlorinated or treated before drinking. A water purification method that provides safe drinking water should be made available by government especially for the government boreholes, in order to avoid outbreak of water-borne diseases. The government should ensure that adequate treatment facilities that purify sewage prior to discharge or disposal to save the water from continuous pollution be put in place.

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