



Physicochemical and Microbiological Assessment of Water Sources in Ilara Mokin

Bada, A.A., Ajayi, J.O, Momoh, A and Enechojo, A.C

Department of Biological Sciences
Elizade University, Ilara-Mokin, Ondo-State, Nigeria

ABSTRACT

The study investigated the physicochemical and microbiological assessment of water sources in Ilara Mokin which were the borehole, stream and well water. Water samples were collected from ten (10) different locations in the community which were ILORO, UBA, HEALTH CENTRE, HOSPITAL ROAD borehole, POJO, UBA, HOSPITAL ROAD stream, TRAVELLER LODGE, UBA ROAD and HOSPITAL ROAD Well. The method used to investigate the study includes the physicochemical and microbiological standard test procedures which includes Gram staining, catalyses, coagulase, Indole, Motility and Sugar fermentation test. The Stream at UBA road and Borehole water at Health centre road contain normal pH of 6.5-7.5. All the water samples were not hard; the Hardness of the water (25-128ppm) was below the WHO standard which was 200ppm. The conductivity level was below (125.67ppm) and above (770.6ppm) the WHO standard of 200ppm. The temperatures were between 24.5-27.4°C. The chlorine levels (0.2-0.9Mg/l) were below the WHO standard of 200ppm and the Alkalinity levels (0.1-0.8Mg/l) were below the WHO standard of (200-600Mg/l). The microbiological results showed that the water from Ilara mokin contained some infectious microorganisms. For borehole water sample, it contained *Staphylococcus epidermidis*, *Escherchia coli*, *Streptococcus mitis*, *Streptococcus pyogenes*, for stream water samples it contained *Staphylococcus aureus*, *Micrococcus varius*, *Micrococcus luteus* and for well water samples it contained *Staphylococcus aureus*, *Proteus vulgaris*, *Enterococcus faecalis*. These microorganisms are causative agents of many water borne diseases. Ilara-mokin should treat their water sources very well before use for domestic and recreational purposes.

Keywords: Physicochemical, microbiological, water, diseases, Ilara-mokin

1.0. INTRODUCTION

Water is a standout amongst the most irreplaceable characteristic assets and is known as the solution of life. Water constitutes around 70% of the body weight of every single living life form. Life is unrealistic on this planet without water. It goes about as a medium for both synthetic and biochemical responses (Rajankar *et al.*, 2009). In creating world, 80% of all maladies are specifically identified with poor drinking water and unsanitary conditions (Olajire and Imeokparia, 2001; Chung *et al.*, 2007). Ground water quality can be influenced by fluctuated contaminations running from natural and inorganic chemicals and organisms. Water contamination (surface and ground water) might be considered as a normally initiated change in water quality or conditions incited specifically or in a roundabout way by man's various exercises which renders it unsatisfactory for sustenance, human wellbeing, industry, agribusiness or recreation interest (Dix, 1981; Cifuentes and Rodriguez, 2005). Groundwater is an important source of drinking water in many nations and may be heavily contaminated in many industrialized nations by industrial waste pits, septic tanks, oil wells, landfills, etc. Aquifers supply drinking water for about 120 million Americans (Kelly *et.al.*, 1997) and supply a quarter of the annual

water demands in the United States. Water pollution is a major global problem which requires ongoing evaluation and revision of water resource policy at all levels (Okonko et al., 2007). It has been estimated that 580 people in India die of water pollution related illness everyday (Tyson and Harrison, 1992).

The aim of this study is to investigate the physicochemical and microbiological analysis of water sources in Ilara-mokin so as to know the water quality of Ilara-mokin in order for the people to have safe water for their uses.

2.0. MATERIALS AND METHODS

Sample Sites

All water samples including surface water (streams) and ground water (well and bore hole water) were collected from selected locations (UBA Road, Pojo Road, Health Centre Near Round about, Iloro Road, Hospital Road) in Ilara mokin, Ondo state,

Sample Collection

The Bore Hole: The water samples were aseptically collected from the source using sterile plastic containers after pumping water sample to waste for one or two minutes, the nozzles of the bore hole were swabbed with cotton wool soaked in 70% ethanol, the water was collected from four different sources which are UBA ROAD, HOSPITAL ROAD, ILORO ROAD, HEALTH CENTRE ROAD.

The Stream Water: The water samples were collected from three different streams which are UBA ROAD, POJO ROAD, HOSPITAL ROAD, with the use of sterile sample bottles. The sample bottle was lowered into the water and pulled up after observing there were no more bubbles from the bottles.

The Well Water: The water samples were collected from three different wells which are UBA ROAD, HOSPITAL ROAD, TRAVELLER LODGE ROAD, with the use of sterile sample bottles. A strong rope was attached to the neck of each sterile bottle and gently released into the well, the opened bottle was allowed to sink below the water and was pulled up after observing there were no more bubbles from the bottles.

(A) Physicochemical Analysis

The water samples were analyzed for PH, Temperature, Conductivity, Hardness, Acidity, Alkalinity, Chlorine.

pH

pH was obtained by using a meter at the laboratory on the water samples and the readings were recorded using a pH meter, (Gupta, 2009).

Conductivity

An electrode is connected to the meter, immersed into the sample of water so that the water will cover the sensitized electrode and readings were recorded using a HANNA instrument (Navneet et al., 2010).

Temperature

The temperature was obtained by using a HANNA pH meter (H198128) and readings were recorded for each water sample (Diersing, 2009).

Hardness

20ml of the water sample was measured into a 250ml conical flask, added 1ml of ammonia buffer and 1ml EDTA complex solution, added 2 drops of Solochrome black T indicator, titrated the solution against 0.01M EDTA solution to the appearance of a deep blue color which was the end point. (Patil et al., 2012).

Alkalinity

20ml of the water sample was measured into a clean 250ml³ conical flask, three drops of phenolphthalein indicator were added. The samples were titrated with 0.05M H₂SO₄ until the color disappeared and the titre values were recorded. Three drops of methyl orange indicator were added and titrated until the color change from yellow to reddish. (Parihar et al., 2012)

Chlorine

20ml of the water sample were measured into 250cm conical flask, two drops phenolphthalein indicator and five drops of potassium chromate were added respectively. 0.1M AgNO₃ solution was titrated, during the titration a pink colour was seen which mark the end point (Rajini *et al.*, 2010)

Acidity

20ml of the water sample was measured into a 250cm conical flask, added two drops of phenolphthalein indicator, titrated with 0.1m of sodium hydroxide and reading were recorded. (Parihar *et al.*, 2012).

(B) Microbiological Analysis

Nutrient Agar Preparation: 6.72g were weighted and diluted into 240ml of distilled water in 250cm conical flask, the agar was prepared according the manufacturer specification, the medium was autoclave, after autoclaving the medium was allowed to cool at room temperature, 0.5ml of each water sample were pipette into the Petri dish aseptically, the nutrient agar was poured into the petri dish and allow to solidify, the medium was then incubated for 37°C for 24 hours

Eosin Methylene Blue Agar: 8.88g of EMB were weighed and diluted into 240ml of distilled water in 250cm conical flask, the agar was prepared according to the manufacturer specification, the medium was autoclave, after the autoclaving it was allow to cool, 0.5ml of each water sample was pipette into the Petri dish aseptically, the medium was poured into the Petri dish and allow to solidify, the medium was then incubated for 37°C for 24 hours.

CONTROL: Streaking was done on the cultured plate for control

NON CONTROL: Pour plate method was made on the rest plate of nutrient agar and Eosin Methylene Blue.

Gram Staining

Gram staining was done to check if the microorganism present was gram positive or negative.

Clean glass slide was used, a full loop of the microorganism was used to make a smear on the slide then it was heat fixed after was, few drops of methy blue was added to the smear and gently rinsed off after few seconds , few drop of iodine was also added to the smear left it for few second then wash off , few drop of ethanol was added to the smear too and was washed immediately then few drop of safranin was added to the smear and leave for few minute then wash off and the slide was allowed to dry, then it was viewed

Catalyst Test

A smear was made on the glass slide with a drop of water and the microorganism, few drops of hydrogen peroxide was added to the smear, if bubbles were seen the sample was positive and if bubbles were not seen the sample was negative.

Coagulates Test

A smear was made on the glass slide with a drop of water and the microorganisms; few drop of plasma was added to the smear and rock to see if there was agglutination.

Indole Test

Inoculate the tryptophan broth with broth culture and emulsify isolated colony of the test organism in the broth, incubate at for 24 – 48 hours then added 0.5ml of Kovacs reagent to the broth culture there was a color change of pink on top of the medium forming a ring shape if the organism was positive.

Motility Test

0.75g of Nutrient agar and 1.3g nutrient broth was measured and diluted into 100ml of distilled water, dispensed 5.75ml into the test tube and autoclave, after autoclaving after the medium to cool to a state of semi solidify the dip inoculums straight in the test tube and incubated for 24 hours.

Sugar Fermentation

It tested for the presence of acid and/or gas produced from carbohydrate fermentation. Medium containing a single carbohydrate source such as glucose, lactose, sucrose, galactose and maltose were used for this purpose. Indicator Phenol red was also present in the medium; which will detect the lowering of the pH of the medium due to acid production. Small inverted tubes called Durham tube was also immersed in the medium to test for the production of the gas (hydrogen or carbon dioxide). 5g of each sugar was measured into a conical flask, 250 ml of distilled water was added to the sugar sample, a

magnetic stirrer was dropped inside the conical flask for stirring, 10ml of each sugar sample was dispensed into the test tubes which were labeled according to the sugars. Few drops of phenol red indicator was added to the sugars in the test tubes, after which it was autoclave for 120°C for 15 minute, after autoclaving the sample was allow to cool and the microorganisms were inoculated into each samples and incubated for seven days.

RESULTS

Figure 1 showed the result of the pH level of the water samples. The pH level of Borehole at Health Centre road and Stream UBA road were closed to natural and are within the WHO standard which was 6.5-7.5. The pH level in borehole at Iloro road was very low compared to the WHO standard.

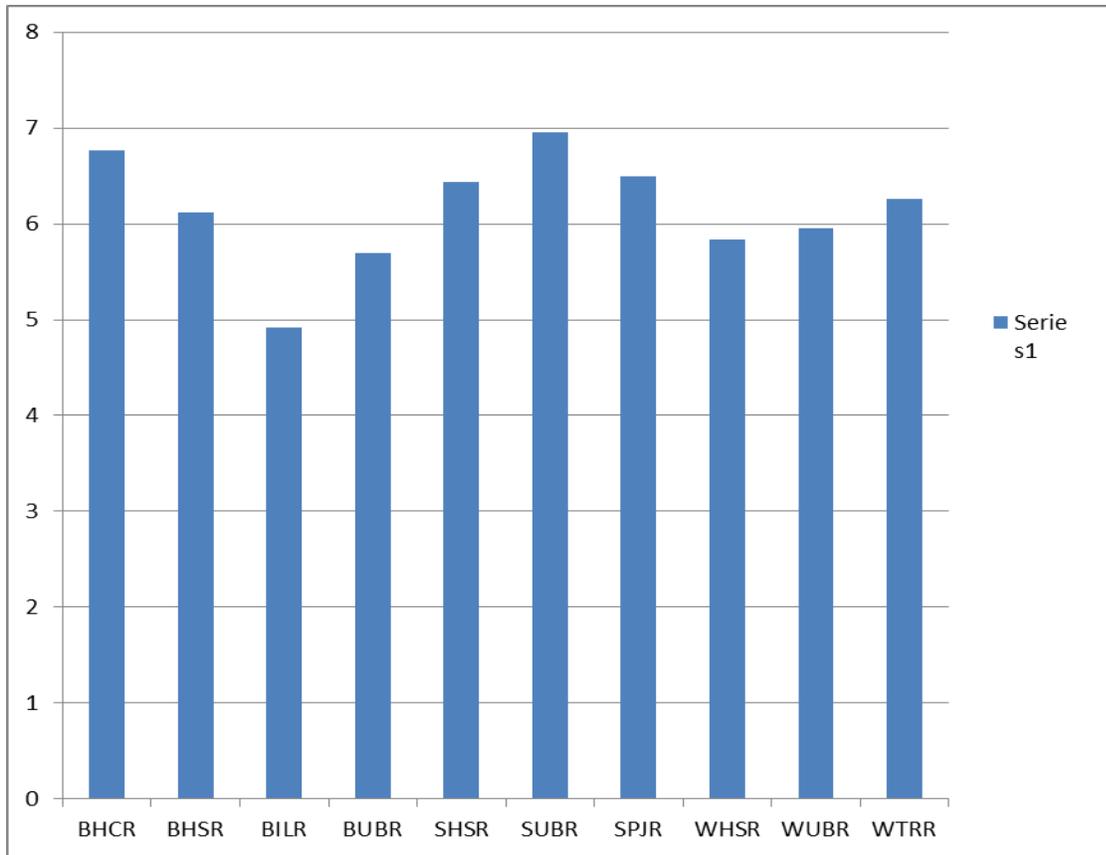


Fig .1: Graphical representation of the pH level of the water samples

Figure 2 showed the results for the temperature of the water samples. For Borehole the temperatures were at the permissible level, while the temperature for stream and well water were below the permissible level.

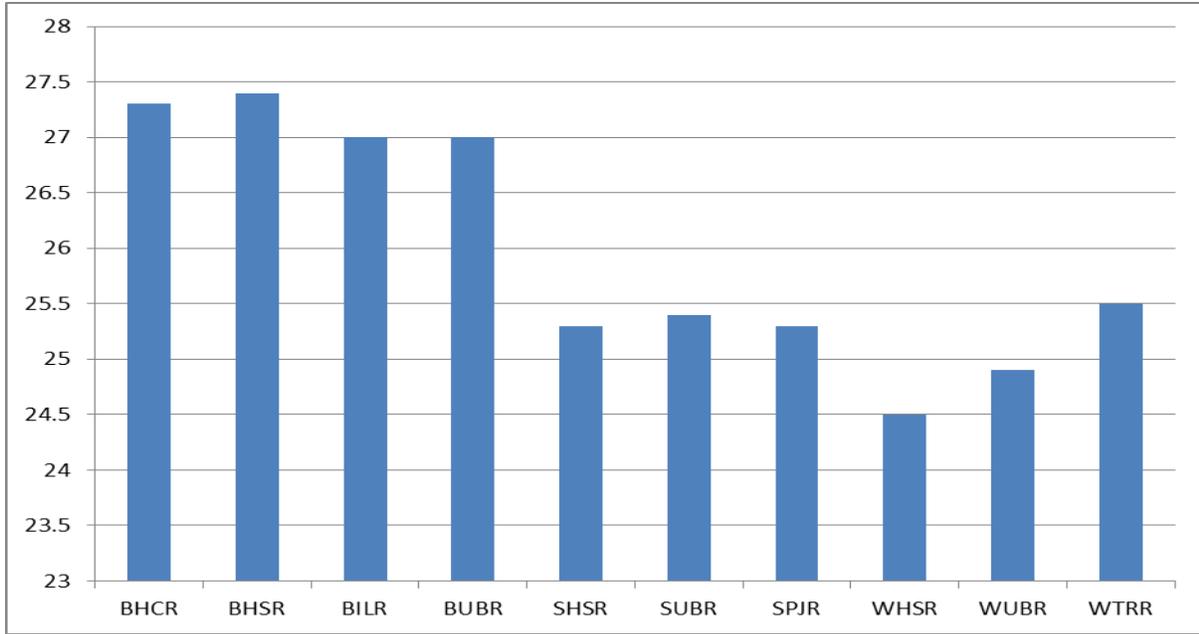


Fig . 2: The graphical representation of the temperature level in all the water samples.

Figure 3 showed the result for conductivity levels of water samples. The conductivity level for some of the water samples were above (for bore holes at Health center, Hospital road, Iloro road and well), within (for stream at UBA road) and below (for Bore hole at UBA road and stream at Hospital road) the WHO standard which was 200ppm

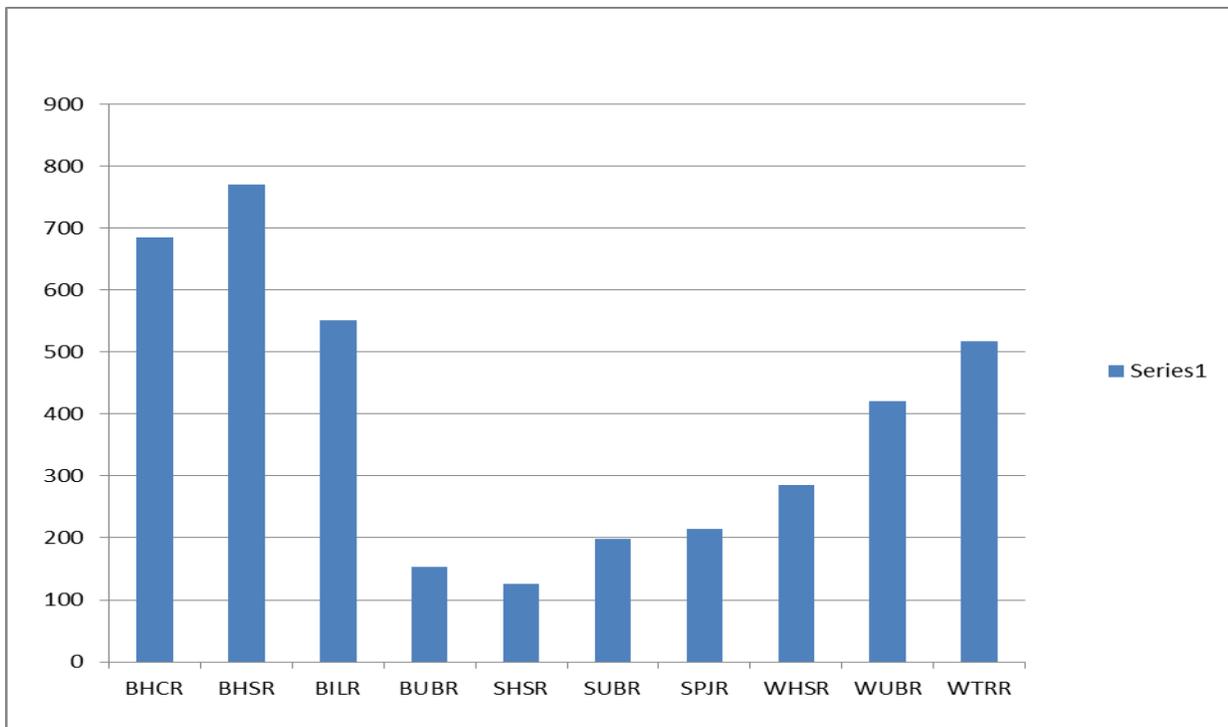


Fig 3: The graphical representation of the conductivity level of the water samples

The results for the table 1 showed that the alkalinity value of the borehole water ranged between 0.05-0.8 mg/l, for the stream the water ranges between 0.05-0.075mg/l, while for well water was between the range of 0.075- 0.1 mg/l. The alkalinity value of borehole, stream and well water sample were very low compared to the WHO standard of 200- 600Mg/l. The acidity of water from borehole ranged between 0.05-0.43 mg/l, for the stream water the acidity value ranged between 0.1- 0.5 mg/l while for well water ranges between 0.15-2.5 mg/l, The hardness of water for borehole water sample ranges between 25-128 mg/l, for stream water it ranges between 33-51 mg/l, while for the well water it ranged between 38- 63 mg/l. The hardness of the water was lower compared to the WHO standard of 200pm. The chlorine content of the borehole water ranged between 0.2- 0.8 mg/l, for stream water sample ranges between 0.3- 0.9 mg/l, while for the well water the chlorine content ranged between 0.6-0.7 mg/l The chlorine content of the water samples of borehole, stream and well were very low compared to the WHO standard of 250mg/l

Table 1: Physicochemical parameters (Alkalinity, Acidity, Hardness and Chlorine) for borehole, stream and well water samples

SAMPLE BOREHOLE	ALKALINITY (Mg/l)	ACIDITY (Mg/l)	HARDNESS (Mg/l)	CHLORINE (Mg/l)
HCR	0.8	0.43	128	0.4
HSR	0.1	0.18	95	0.8
ILR	0.05	0.05	45	0.2
UBR	0.5	0.10	25	0.5
STREAM	ALKALINITY (Mg/l)	ACIDITY (Mg/l)	HARDNESS (Mg/l)	CHLORINE (Mg/l)
HSR	0.05	0.1	33	0.9
UBR	0.075	0.28	51	0.3
PJR	0.075	0.5	46	0.4
WELL	ALKALINITY (Mg/l)	ACIDITY (Mg/l)	HARDNESS (Mg/l)	CHLORINE(Mg/l)
HSR	0.075	0.15	38	0.6
UBR	0.1	0.15	57	0.7
TRR	0.075	2.5	63	0.7

Table 2 showed the results of the colony count of the microorganisms, for borehole water colonies count ranged between 3×10^0 - $19. \times 10^2$ cfu/ml for nutrient agar while EMB agar it ranged between 1×10^0 - 13×10^2 cfu/ml for the stream water it ranged between 2.5×10^1 - 19.0×10^1 cfu/ml for nutrient agar while EMB agar ranged between 2×10^0 - 5.5×10^1 cfu/ml while for well water the colonies ranged between 2.8×10^1 - 21.0×10^2 cfu/ml for nutrient agar while EMB agar ranged between 2.1×10^1 - 21.0×10^2 cfu/ml colonies.

Table 2: Colony Count

SAMPLE BOREHOLE	NUTRIENT AGAR MICROBIAL LOAD (cfu/ml)	EMB AGAR (cfu/ml) MICROBIAL LOAD
HCR	10 ¹	
1	3.5x 10 ¹	NIL
2	3.0 x 10 ¹	5x10 ⁰
3	3.7 x 10 ¹	11x10 ⁰
CONTROL	19.0 x 10 ²	NIL
UBR		
1	1.2x 10 ¹	Nil
2	3.1x 10 ¹	2x10 ⁰
3	1.7x 10 ¹	5x10 ⁰
CONTROL	2.1x10 ¹	1.4x10 ¹
HSR		
1	3. 8x 10 ¹	NIL
2	1.6 x 10 ¹	9x10 ⁰
3	2.7 x 10 ¹	8.7x10 ¹
CONTROL	17.1 x 10 ²	13.0x10 ²
ILR		
1	4.6x 10 ¹	1x10 ⁰
2	6.7 x 10 ¹	NIL
3	18.3 x 10 ²	310 ⁰
CONTROL	3 x 10 ⁰	NIL
SAMPLE STREAM	NUTRIENT AGAR	EMB AGAR
HSR		
1	3.5 x 10 ¹	1.5x10 ¹
2	3. 0 x 10 ¹	2.5x10 ¹
3	3.7 x 10 ¹	9x10 ⁰
CONTROL	19.0 x 10 ²	Nil
UBR		
1	8.8 x 10 ¹	1.1x10 ¹
2	7.0 x 10 ¹	8x10 ⁰
3	7.7 x 10 ¹	2x10 ⁰
CONTROL	3.5 x10 ¹	1.5x10 ¹
PJR		
1	11.1x10 ²	3.8x10 ¹
2	2.5x10 ¹	5.0x10 ¹
3	8.8x10 ¹	5.5x10 ¹
CONTROL	4.5x10 ¹	4.7x10 ¹
SAMPLE WELL	NUTRIENT AGAR	EMB AGAR
TRR		
1	3.0x10 ¹	2.5x10 ¹
2	9.0x10 ¹	2.8x10 ¹
3	15.0x10 ²	5.0x10 ¹
CONTROL	2.8x10 ¹	4.8x10 ¹
HSR		
1	21.0x10 ²	13.0x10 ²
2	17.7x10 ²	10.2x10 ²
3	10.5x10 ²	10.0x10 ²
CONTROL	10.0x10 ²	19.0x10 ²
UBR		
1	8.5x10 ¹	9.1x10 ¹
2	7.5x10 ¹	21.0x10 ²
3	4.5x10 ¹	11.5x10 ²
CONTROL	8.2x10 ¹	2.1x10 ¹

Table 3 showed the results for the morphology, Gram staining, Catalyze, Coagulase, Indole, Motility and Sugar fermentation test which showed the presence of some microorganisms which are *staphylococcus epidermidis*, *staphylococcus aureus*, *Streptococcus pyogenes*, *streptococcus mitis*, *Escherichia coli*, *proteus vulgaris*, *micrococcus varius*, *Enterococcus faecalis*, *Micrococcus luteus* in the water samples collected from the different locations in Ilara mokin

Table 3: The Biochemical characterization of bacteria in water samples which include morphology, Gram staining, Catalyze, Coagulase, Indole, Motility and Sugar fermentation test of the microorganisms in the water sample

S/N	ISO 1 BILR	ISO 2 BHSR	ISO 3 BHCR	ISO4 BUBR	ISO 5 SHSR	ISO 6 SUBR	ISO 7 WUBR	ISO 8 WTRR	ISO 9 WPJR
SHAPE	Circular	Circular	Circular	circular	Circular	Circular	Circular	Circular	Circular
EDGE	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
COLOUR	Cream	Cream	Cream	Pink	Cream	Cream	Cream	Cream	Cream
ELEVATION	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised
OPACITY	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent
SIZE	Small	Large	Small	Small	Medium	Small	Small	Small	Small
CONSISTENCY	Butyrous	Friable	Butyrous	Friable	Friable	Friable	Butyrous	Butyrous	Butyrous
GRAM STAIN	-	-	+	+	-	-	+	-	+
CELL SHAPE	Cocci	Cocci	Cocci	Cocci	Rod	Rod	cocci	Cocci	Cocci
CATALYSE	-	-	+	+	+	-	+	-	+
COAGULASE	-	+	-	-	-	-	+	-	-
INDOLE	+	+	-	-	-	-	+	-	+
MOTILITY	-	-	-	+	+	-	-	-	+
GLUCOSE	+	+	+	-	+	+	+	-	+
SURCOSE	-	+	+	+	+		+	+	+
FRUCTOSE	+	-	+	+	-		+	+	+
GALATOSE	+	+	-	-	+		+	+	+
MALTOSE	-	-	+	+	-		-	-	+
PROBABLE	<i>Staphylococcus epidermidis</i> ,	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Streptococcus mitis</i>	<i>Escherichia coli</i>	<i>Proteus vulgaris</i>	<i>Micrococcus varius</i>	<i>Enterococcus faecalis</i>	<i>Micrococcus luteus</i>

Table 4 showed the result of the Microbiological analysis test for borehole water showed the presence of some microorganisms which were *Escherichia coli*, *Streptococcus mitis*, *staphylococcus epidermidis*, *streptococcus pyogenis*

Table 4: Distribution of isolates for borehole water sample

S/N	SAMPLE	BACTERIAL ISOLATED
1	ILR	<i>Staphylococcus epidermidis</i>
2	HSR	<i>Escherichia coli</i>
3	UBR	<i>Streptococcus mitis</i>
4	HCR	<i>Streptococcus pyogenes</i>

Table 5 showed the results of some presence of microorganisms which were *Micrococcus luteus*, *Micrococcus varius*, *Staphylococcus aureus* in stream water sample from Ilara mokin.

Table 5: Distribution of isolates for stream water sample

S/N	SAMPLE	BACTERIAL ISOLATED
1	PJR	<i>Micrococcus luteus</i>
2	UBR	<i>Micrococcus varius</i>
3	HSR	<i>Staphylococcus aureus</i>

The results for table 6 showed the presences of some microorganisms which were *staphylococcus aureus*, *proteus vulgaris*, *Enterococcus faecalis*, in well water sample from Ilara mokin.

Table 6: Distribution of isolates for well water sample

S/N	SAMPLE	BACTERIAL ISOLATED
1	HSR	<i>Staphylococcus aureus</i>
2	UBR	<i>Proteus vulgaris</i>
3	TRR	<i>Enterococcus faecalis</i>

4.0 DISCUSSION

Physicochemical parameters showed that the pH of some of the samples collected from the water sources were below the WHO standard of 6.5 - 7.5 (in Hospital road, Iloro road, UBA road in borehole, Hospital road and Pojo for stream and Hospital road, UBA road and Travelers lodge for well water), while some were in between (in UBA road for stream and Health centre road for borehole (WHO, 2003, 2006). Those below the WHO standard indicate that the water sources were acidic while those that fall within the WHO standard had normal pH level. These indicate that the Stream at UBA road and Borehole at Health centre road had normal pH level while the well water was acidic. This increased acidity could be attributed to the presence of acidic metabolites. Hardness may be defined as the concentration of all multivalent metallic cations in solution. The principal ions causing hardness in natural water are calcium and magnesium. Others, which may be present though in much smaller quantities, are iron, manganese, strontium and aluminum. The result showed that the borehole, stream and well water were very low in hardness because the level of hardness were below the WHO standard of 200ppm (Patil et,al, 2012). Conductivity which is the measure of the capacity of an aqueous solution to pass electric current was found to be below and above the WHO permissible limit of 200ppm (Patil et,al, 2012). The low conductivity values of the samples show that the dissolved salts were not minimal. The temperature of the water was 27°C, the chlorine content of the water were below WHO standard and this indicate that the

water had a low amount of chlorine, The acidity level of the water was acidic. The alkalinity levels of the different sources of water were below the WHO permissible limit which showed that the water sources were not alkaline.

The analysis of the colony count in the water samples revealed the presence of heterotrophic bacteria in all the water sources. The WHO standard for heterotrophic bacteria in potable water states that the total heterotrophic bacteria count should not be more than 100cfu/ml (WHO, 2003, 2006). For borehole, the water sample at Iloro road had higher colony count for nutrient agar but lower colony count for EMB agar when compared to the WHO standard. For stream, the water sample at Pojo had higher colony count for nutrient agar but lower in EMB agar when compared to the WHO standard while for well, the water samples at Traveler lodge and Hospital road had higher colony count for nutrient agar but for EMB agar it was high in Hospital road and some UBA road when compared to the WHO standard. The presence of counts exceeding the WHO limits indicates that the water samples contain high concentration of bacteria that could make the water unsafe for drinking.

The total coliform and faecal coliform obtained from the water samples include: For Borehole water sample at Iloro road contain *Staphylococcus epidermidis*, Hospital road contain *Escherichia coli*, UBA road contain *streptococcus mitis* and Health centre contain *Streptococcus pyogenes*. For stream water sample at pojo contain *Micrococcus luteus*, UBA contain *Micrococcus varius*, Hospital road contain *Staphylococcus aureus*. For well water sample at hospital road contain *Staphylococcus aureus*, UBA road contain *Proteus vulgaris*, Travelers lodge contain *Enterococcus faecalis*.

These organisms are important human pathogens associated with a variety of infectious diseases such as gastroenteritis, typhoid fever, dysentery, cholera, urinary tract infections and others (Orji, et al. 2006; Nwido et al., 2008). They are known as causative agents of many water borne diseases and may indicate that these water sources are not advisable for domestic uses such as drinking, bathing and cooking. Their entry into water sources could be attributed to seepages from nearby septic tanks, as opined by (Nguendo-Tongsi 2011) or through deliberate and indiscriminate deposition of animal waste and human faeces into streams as commonly observed in some riverine areas. Patridis et al (2002) opined that the presence of *Escherichia coli* which is the most common indicator of faecal pollution in a water sample is an indication of the presence of other enteric pathogens. This confirms the result of an earlier study by Okoli et al. (2005), indicating that most boreholes at Hospital road are heavily contaminated with faecal matter.

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