

Assessment of Microbial Quality of Some Selected Shallow Wells in Ogbomoso, South Western Nigeria

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Abstract The importance of good quality water to the environment has long been realized, hence, the development policy of integrated community. In this research work, the analysis of physical and bacteriological quality of well water was carried out. Thirty (30) samples of the well water were collected and their pH and temperature were determined on the field to avoid interference by changes of environment. The bacteriological analyses were carried out within 24 hours of collection to avoid killing of bacteria present in the water sample. On the average, result shows that six (6) of the wells indicate multiple bacteria, three (3) of the wells indicate single bacteria while no occurrence of bacteria in one of the wells. Shallow well water in ogbomoso is highly contaminated with Salmonella spp, Shigella spp, E.coli spp and it requires disinfection. The entire water sample from the well tested positive to the presence of bacteria which are harmful to human health.

Keywords: Bacteria, Pollution, Modern Low Density (MLD), Modern High Density (MHD), Most Probable Number (MPN)

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1. Introduction

Water is a natural gift to life but there has been the challenge of lack of potable water due to bacteria and fungi contamination by different anthropogenic activities which impair it's quality. Human activities which include soil fertility remediation, indiscriminate refuse and waste disposal, and improper citing of septic and soak away pit are on the increase. The most secure source of safe drinking water is pipe-borne water from municipal water treatment plant for most communities. Often, most water treatment facilities do not deliver or fail to meet the water requirement of the served community due to corruption, lack of maintenance or increased population. In Nigeria, there exist challenges of lack of supply of pipe borne water hence many homes have shallow wells cited around the house at a distance from the septic tank. More than 52% of Nigerians do not have access to improved drinking water supply (Orebiyi et al., 2010). The scarcity of pipe water has made communities to find alternative source of water, ground water sources being readily available. Wells are common groundwater source readily explored to meet community water requirement or make up the short fall. This is the situation in many parts of Nigeria and several other African countries (Adelekan, 2010). These wells serve as major source of water for household uses (drinking, cooking, washing etc). The commonest cause of pollution is attributed to close proximity of septic tanks to wells, unhygienic usage of the wells. Polluted water causes water borne diseases to human and animals which may eventually lead to death if not treated urgently.

In Ogbomoso, potable water supply is a serious problem which results to people searching for water so as to use for domestic purposes. The groundwater in the environment of Ogbomoso is mostly recharged by rainfall, percolation through thin layered soft rock, percolation of surface water in relatively highly weathered and fractured rock and possibly by seepage from streams and rivers around the city. However, there is a growing concern that the use of on-site sanitation facilities may result in groundwater contamination and subsequent diseases outbreak and transmission. Therefore, this study was undertaken for the isolation and identification of bacteria pathogens in water so as to enhance its characterization. This will enhance to know its types, population and probably tracing its source with a view of complete eradication.

1.1. Description of Study Area

The study was carried out in Ogbomoso town which is located in SouthWestern part of Nigeria (West Africa) between latitude 8°06′70″ and 8°06′98.7 North and between longitude 4°14′28.2″E and 4°14′56.9″ East. Ogbomoso North Local Government Area (LGA) is bounded in the North and the East by Surulere Local Government Area (LGA), in the South by Ogbomoso South (LGA) and in the West by Orire (LGA). Ogbomoso North has her headquarter located at Kinnira, Ogbomoso. The principal occupation of the majority of the population is farming and trading although, the populations according to 2006 census figures consist of 103,319 male, 95,401 female and a total population of 198,720 inhabitants who are mostly the Yoruba. Ogbomoso South on the other hand came into existence when it was carved out of the North with a population of 51,249 male, 49,566 female and a total of 100,815 people in her 18 square kilometers land area. It is located in the North-Eastern part of Oyo State and is bounded in the North by Ogbomoso North LGA, in the south by Ogo-Oluwa LGA, in the East by Surulere LGA and in the West by Orire LGA as shown in Figure 1. Ogbomoso South has its headquarters located at Sunsun/Arowomole, Ogbomoso (Adetunde *et al.*, 2009). The Ogbomoso metropolis is located in the savannah zone which makes farming the major occupation of the people of the area.

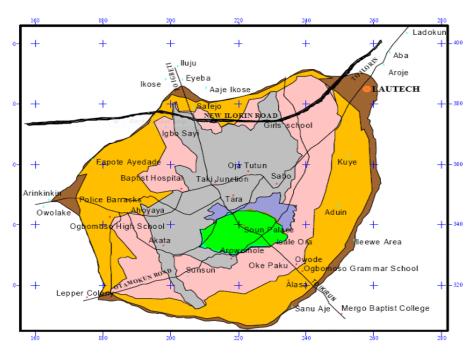


Figure 1. Map of Ogbomoso Town (Source: Modified from [1])

2. Methodology

Visual Inspection of the selected wells and sanitary system of the immediate environment of the well were conducted. Distance between the various sanitary system and the well were measured. Thirty (30) ground water samples were collected from shallow wells (three (3) from each well) for physical and bacteriological analysis. The samples were taken at selected locations in Ogbomoso North and South Local Government Area, comprising of densely populated, sparsely populated and medium populated region. Also, the sample containers of 100ml each were first rinsed thoroughly with the waters to be sampled and samples were collected in a sterilized crystal bottles. The samples were stored in an ice box immediately so as to maintain the microbial population and processed for microbial presence on the same day before transporting to a certified laboratory.

2.1. Laboratory Analyses

The experiments were carried out in National Horticulture Research Institute (NIHORT), Ibadan and Food Science Laboratory, Lautech, Ogbomoso, Oyo State. Physical Analysis (Temperature and pH), Bacteriological Analysis (Isolation and Characterization) were carried out on the water samples. For bacteriological analysis, materials such as petri-dishes, test tubes, measuring cylinder, pipette, erlenmeyer flasks and beaker that are heat stable were sterilized in the hot oven at 180°C for 3hours. The bottles for prepared medium, distilled water and McCartney were sterilized in an autoclave at 121°C for 15minutes at normal atmospheric pressure. Wire loops

and inoculating needles were sterilized by flaming until red-hot over a blue flame from bunsen burner. The surface of the work bench was also sterilized with cotton wool soaked in 70% alcohol to disinfect it by creating an aseptic environment for the bench work. The growth medium used in this study were Nutrient Agar (NA), MacConkey agar, and Ferrous Sulphate prepared. The water samples collected from the different wells at various depth were shaken properly.

Bacteria were isolated from the water sample using serial dilution method by measuring 1ml of the water sample into nine (9) mls of sterile distilled water in a test tube blocked with cotton wool and mixed properly to make a dilution of 10⁻¹. Mixture of 1ml of the sample and sterile distilled water was again introduced into another test tube containing 9ml of sterile distilled water blocked with cotton wool to make serial dilutions of 10^{-2} . This procedure was repeated in turn until a dilution of 10⁻⁷ was obtained. From 10^{-2} to 10^{-7} diluents, 1ml each of the diluents was pipett separately into different sterile petridishes respectively. Sterilized nutrient agar was transferred into the plates using pour-plate method under aseptic conditions. These plates were rocked gently to facilitate even distribution of the diluents. The plates were labelled and allowed to set after which they were incubated at room temperature (25-30)°C for twenty (24) hours. This procedure was repeated for each water sample. After the 24hours incubation the plates were examined. After the process of isolation, the organisms were then sub cultured into sterile plates by transferring tiny portions of the bacteria growth in the culture plates into the sterile plates containing sterile nutrient agar with the aid of a sterile wire loop under aseptic condition and each plate were labeled. These were then incubated for 24hours at room temperature. This procedure was repeated until pure cultures were obtained. The isolates were observed for colour and shape on each plate.

Microscopic examining was adopted. Thin smear of the organisms isolated were made on separate clean glass slides using a broom stick or wire loop and they were well labelled. These were then heat fixed before staining with crystal violet for thirty (30) seconds. This was washed off gently with running tap water. It was then stained with iodine for 1ml and washed off with water before decolorizing with alcohol and washed off with water. It was then counter stained with safranin and wash off in running tap water. It was be allowed to dry before adding little oil emersion and viewed under the microscope. These isolated organisms were transferred into sterile plate containing sterile MacConkey agar with the aid of a sterile wire loop and then labelled plate by plate. These were then incubated for about twenty (24) hours before observing for growth and colour. Colonies which developed after incubation were examined for cultural features such as growth, shape, elevation, size, pigmentation and consistency while different reaction test were used in identifying various bacteria in different isolates. Identification of isolates was carried out by employing macroscopic, microscopic, physiological and biochemical tests. Eighteen (18) hour cultures of each isolates were used for the tests.

3. Results and Discussion

3.1. Physical Chemical Parameters

The samples were collected in February, 2015 during the dry season when there was low recharge to the groundwater aquifer through infiltration. The ten (10) wells at various sites collection serves as the source of water for the people in the environments. Various conditions surrounding the wells are presented in Table 1. Well 1, 2, 3, 6 and 8 are uncovered. Table 2 shows the physical characteristics of the groundwater samples analysed. The pH and Temperature were compared with USEPA and WHO standards (6.5-8.5) and (24.5-39.7)°C, respectively. All the samples were found to be within the permissible limit with value ranges from 5.46-6.65 in pH while temperature ranges from 23.4-26.00°C. Sample 8 has the highest pH value of 6.65 and Sample 5 has the lowest value of 5.46. Sample 4 has the highest temperature with value of 26°C and Sample 6 has the lowest value 23.4°C.

Table 1. Conditions of the Sample Wells							
Sample Label	Location of Sampling Point	Condition of the Well	Observation on Water Surface				
1	Porte	Uncovered and with Rings	Oily and Cloudy				
2	Osaro	Uncovered and without Ring	Oily and Cloudy				
3	Osaro	Uncovered and without Ring	Oily, Nylons, Small Woods, Leaves, Soil particles.				
4	Osaro	Covered and with Rings	Cloudy				
5	High School Area	Covered and with Rings	Oily				
6	Isale High School	Uncovered and with Rings	Oily				
7	Isale High School	Covered and with Rings	Oily and Cloudy				
8	Sabo Garage	Uncovered and without Rings	Oily, Nylons, Small Woods, Leaves, Soil particles.				
9	Stadium Area	Covered and with Rings	Cloudy				
10	Stadium Area	Covered and with Rings	Cloudy				

Table 2. Physical Parameters of the Water Samples								
Samples	pН	Temperature (°C)						
1	6.10	24.90						
2	5.57	25.40						
3	6.12	24.30						
4	5.90	26.00						
5	5.46	24.60						
6	6.40	23.40						
7	5.80	24.60						
8	6.65	25.70						
9	6.06	24.50						
10	6.20	23.60						

The characteristic and description of the identified micro organisms are presented in Table 3 and Table 4, respectively. The presumptive and concentration of indicative organism per 100ml of water samples was used in the study. Salmonella spp, e.coli and Shigella spp are presumptive organisms isolated from Sample 1 with salmonella spp having cellular characteristics of gram negative rod shaped, motility and cultural characteristics of creamy shiny colony as shown in Table 3. E.coli has cellular characteristics of gram negative short rod and cultural characteristic of pink colony. Shigella spp showed cellular characteristics of gram negative rod shaped, no motility and cultural characteristics of small round and creamy colony. Salmonella spp, Shigella spp and E. coli are presumptive organisms isolated from Sample 2 possessing the same characteristics with same organisms isolated in Sample 1 accordingly as described in Table 3. Likewise shigella spp, e.coli, salmonella spp are presumed in Sample 3 with the same characteristics. Furthermore, two (2) presumed organisms (e.coli, salmonella spp and shigella spp, salmonella spp) were probable in Sample 6 and 8, respectively with characteristics of the former as shown in Table 3. Sample 4, 7 and 9 have presumed organism of salmonella spp with the same characteristics as presented in Table 3. No occurrence of organism was noticed in Sample 10. Salmonella spp was only found in Sample 4, 7 and 9 having population of 100 percent as shown in Table 4. Sample 6 and 8 had (e.coli, salmonella spp) and (shigella spp, salmonella spp), with population

of 45, 55 and 45, 55 percentages, respectively. Sample 5 and 10 were found to have no trace of bacteria. Moreover, sample 1, 2 and 3 had three (3) different presumptive organisms. The organisms include (salmonella spp, e.coli, shigella spp) with percentages of 40, 30, 30, respectively, in Sample 1 and 2 had (salmonella spp, shigella spp, e.coli) with percentages of 60, 25, 15, respectively. For Sample 3, (shigella spp, e.coli, salmonella spp) with percentages of 30, 25, 45, respectively were noticed as presented in Table 4. Most Probable Number (MPN) of bacteria is salmonella

spp as it occurs in high percentages in most water samples. For Modern Low Density (MLD) area where samples 5 and 10 were taken, the presumptive existence of no bacteria was noticed. Modern High Density (MHD) where samples 4, 7, and 9 were obtained, salmonella spp dominate the samples with percentages of 100 as indicated in Table 4. There exist cases food poisoning and typhoid in the area where Sample 4 was collected. It was noticed that multiple bacteria is present in six (6) of the wells as shown in Table 4.

Samples	Isolate Code	Cultural Characteristics	Cellular Characteristics	Probable Identification	
	1.1	Creamy and shiny colony	Gram negative, rod shaped and motile	Salmonella spp	
1	1.2	Pink colony	Gram negative short rod	E.coli	
	1.3	Small round and creamy colony	Gram negative rod -shaped, non motile	Shigella spp	
2	2.1	Creamy and shiny colony	Gram negative and motile	Salmonella spp	
	2.2	Creamy rod colony	Gram negative rod and non motile	Shigella spp	
	2.3	Pink colony	Gram negative	E. coli	
3	3.1	Creamy round Colony	Gram negative, rod non- motile	Shigella spp	
	3.2	Pink colony round shaped	Gram negative rod	E.coli	
	3.3	Creamy and shiny	Gram negative rod	Salmonella Spp	
4	4.1	Creamy and shiny colony	Gram negative rod	Salmonella spp	
5	Nil	Nil	Nil	Nil	
6	6.1	Pink round colony	Gram negative rod	E.coli	
	6.2	Creamy and shiny colony	Gram negative rod	Salmonella spp	
7	7.1	Creamy and shiny colony	Gram negative rod shaped	Salmonella spp	
8	8.1	Creamy and round colony	Gram negative rod	Shigella spp	
	8.2	Shiny and creamy colony	Gram negative rod	Salmonella spp	
9	9.1	Creamy and shiny colony	Gram negative rod shaped	Salmonella spp	
10	Nil	Nil	Nil	Nil	

Table 2 Characteristics and	Description of Identified Organism

Table 4. Presumptive and Concentration of Indicator Organism per 100ml of the Water Samples

Samples	Isolate Code No.	Triple gas	Sucrose	Lactose	H_2S	Starch	Gelatinase	Ornithine decarboxylase	Nitrate red.	Probable Isolate	Population of Isolates per Samples
	1.1	+	+	+	+	_	_	+	+	Salmonella spp	40
1	1.2	+	D	+	_	+	_	D	+	E.coli	30
	1.3	+	_	_	_	_	_	_	+	Shigella spp	30
	2.1	+	+	+	+	_	_	+	+	Salmonella spp	60
2	2.2	+	_	_	_	_	-	_	+	Shigella spp	25
	2.3	+	D	+	_	+	-	D	+	E. coli	15
	3.1	+	_	_	_	_	-	_	+	Shigella spp	30
3	3.2	+	D	+	_	+	-	D	+	E.coli	25
	3.3	+	+	+	+	_	_	+	+	Salmonella Spp	45
4	4.1	+	+	+	+	_	_	+	+	Salmonella spp	100
5	Nil	Nil	Nil							Nil	NIL
6	6.1	+	D	+	_	+	-	d	+	E.coli	45
	6.2	+	+	+	+	_	_	+	+	Salmonella spp	55
7	7.1	+	+	+	+			+		Salmonella spp	100
	8.1	+	_	_	_	_	-	_	+	Shigella spp	45
8	8.2	+	+	+	+	_	_	+	+	Salmonella	55
9	9.1	+	+	+	+	_	_	+	+	Salmonella spp	100
10	Nil	Nil	Nil							Nil	NIL

represent presence of organisms

D represent no reaction

represent absence of organisms

From the present investigation, the following conclusions and recommendation were drawn:

Bacteriological analyses results show high concentration of microorganisms which are pathogen causative diseases due to improper disposal of organic waste within the environment. The presence of high isolate in the water samples is concluded to be responsible for the rising incidence of water borne diseases in the area. Therefore, the water is unsafe for drinking for the occupants of the studied areas. The study from identification, isolation, biochemical test and frequency distribution of the bacteria isolated from the water samples such as *salmonella spp*, *shigella spp* and *e.coli* are responsible for chronic diseases and should be avoided in water for consumption purposes.

4.1. Recommendations

The following recommendations were drawn based on the study:

Shallow water wells are very susceptible to contaminations as infiltration transport contaminants to the groundwater. Most of the examined wells are shallow, for this reason, monitoring groundwater quality in study areas is important. Top level of hand dug wells should be elevated at most 3fts above the ground level as most of the wells from the study areas have the same top level with ground level which will enhance easy contamination of the wells.

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