Assessment of Nitrate and Escherichia Coli Contamination of Shallow Groundwater of Ajakanga and Environs, Ibadan, Southwestern Nigeria

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Abstract- ***Fourteen shallow groundwater samples were collected from Ajakanga and environs, Ibadan, southwestern Nigeria in order to assess the health implications of the concentration of nitrate andEscherichia coli in the water. The samples were collected into sterile disposable universal bottles and were transported to the laboratory for the coliform detection and isolation of microorganisms within 24–48 hours of collection.The total bacterial in some locations were too numerous to count. However, among those that were within enumeration region, the sample with lowest bacterial load has 12cfu/ml. The most portable numberdetermination for coliform count showed that the water samples contain coliform bacteria within the range of 2–6 coliform counts. The pure culture were all gram negative, rod shape and slender. Generally, nitrate concentration in the shallow groundwater was low ranging from 0.12mg/l to 2.12mg/l compared to the World Health Organization guidelines for portable drinking water of 50mg/l. Soils in unfertilized lands are potential sources of nitrate due to the activity of nitrifying bacteria. The NO_3 concentration in the water can therefore be attributed to natural sources. The Escherichia coligrowth count in the shallow water of Ajakanga and environs, is moderate(4 to10) in most of the locations, scanty (3)and nil.E. coli in the water is attributed to anthropogenic activities.The presence ofE.coli has a significant health risks and can cause diseases such as typhoid, diarrhea and infantile paralysis, when water is used for drinking, personal hygiene, recreation or food processing.***

Indexed Terms- ***Shallow groundwater, Ajakanga, Escherichia coli, Nitrate, Contamination.***

I. INTRODUCTION

Globally, more than 900,000 deaths are reported annually, as a result of exposure to water contaminated with pathogenic bacteria or virus which causes waterborne diseases, such as diarrhea [1]-[3]. Therefore access to clean and reliable water resources is of necessity. This, however, is severely lacking in many areas around the world affecting the health of over 1.1 billion people [4].

The contamination of soil and groundwater with nitrate is a global water quality issue which has been studied by various workers [5]-[9]. Nitrate (NO_3) from human activities such as agriculture, industries and solid waste disposal can result in groundwater nitrate concentrations that exceed drinking water guideline and pose human health risks [10]-[15].

The maximum contamination level (MCL) for nitrate in public drinking water supplies as set by the World Health Organization is 50mg/L as NO_3. Accumulation of nitrate in the body above a certain limit can result in cancer and birth defects.

Escherichia coli, which normally resides in the human large intestine is commonly found in the environment and its presence in drinking water is a sign of contamination and degradation of water quality [16]. Although most *E. coli* strain are harmless, certain strain are pathogenic and cause disease such as watery diarrhea, bloody diarrhea, urinary tract infection, meningitis, and sepsis, which can lead to death[17][18].

Ajakanga, a community in Ibadan, southwestern Nigeria, rely heavily on water obtained from hand dug wells which tap water from shallow groundwater

aquifers that can be easily contaminated as a result of human activities. Consequently, this study is to determine the concentration of nitrate, the population of *Escherichia coli* and establish the relationship between the concentrations of nitrate and *Escherichia coli* population in the shallow groundwater of Ajakanga and environs.

II. DESCRIPTION OF THE STUDY AREA

The study area Ajakanga, Ibadan lies between N7^{0}17'30.5", E3^{0}49'29.15" and 7°19'59.0", 3°50'38.8" on Ibadan sheet No 59 (Figure 1) [19]. The drainage pattern in the area is dendritic, showing irregular branching in all directions, with tributaries joining at all possible angles. However, trellised pattern occur especially in areas where quartzite and gneissic bands are found. Both rock types have suffered periods of diastrophism and are consequently marked by faults, joints, and displacements, which have influenced the drainage pattern, streams have utilized such weak areas, resulting in new features. The major rivers, draining the area are rivers Ajakanga and Ona. They both flow in the North-West to South-East direction following the fracture trend.

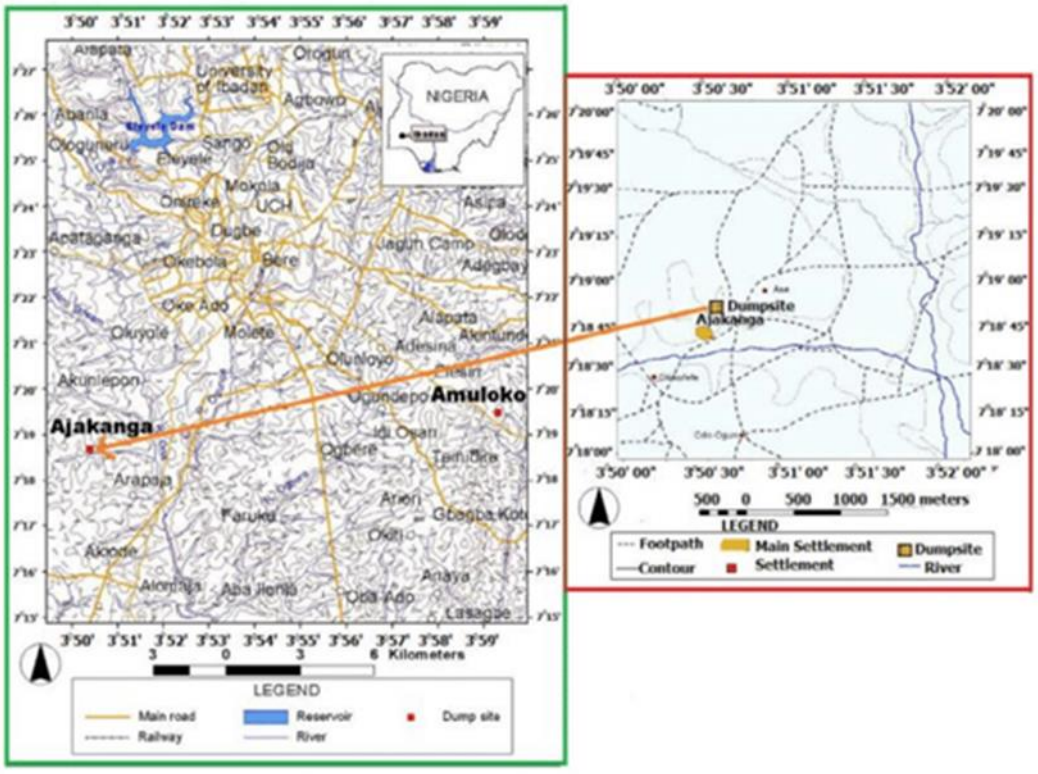

Figure 1: Ajakanga and environs (Extracted from Nigerian Geological Survey Agency, Ibadan Sheet No.59, 1980)

III. GEOLOGY AND HYDROGEOLOGY

The study area, Ajakanga,and its environs in Ibadanlies within the Precambrian Basement Complex of south-western Nigeria which is part of the Pan-African mobile belt lying in the east of the West African craton [20]-[22].

The evolution of the basement rock in Nigeria is associated with the overall evolution of the African continent. Available radiometric dating suggest that the evolution of the basement rocksin Nigeria took place during three main orogenic events which correspond to three major ages that punctuated the Precambrian history of Nigeria; the Liberia, Eburnean and Pan African orogeny. The rock suites of the basement complex include; Migmatite - Gneiss Quartzite Complex Suite; Metasedimentary and metavolcanic rocks (Schist Belts); Pan African Granitoids and the Undeformed acid and basic dykes [20]-[22].

The rock unit found in Ajakanga are; quartzite, banded gneiss, with pegmaties and quartzo-feldspartic intrusions (Figure. 2).

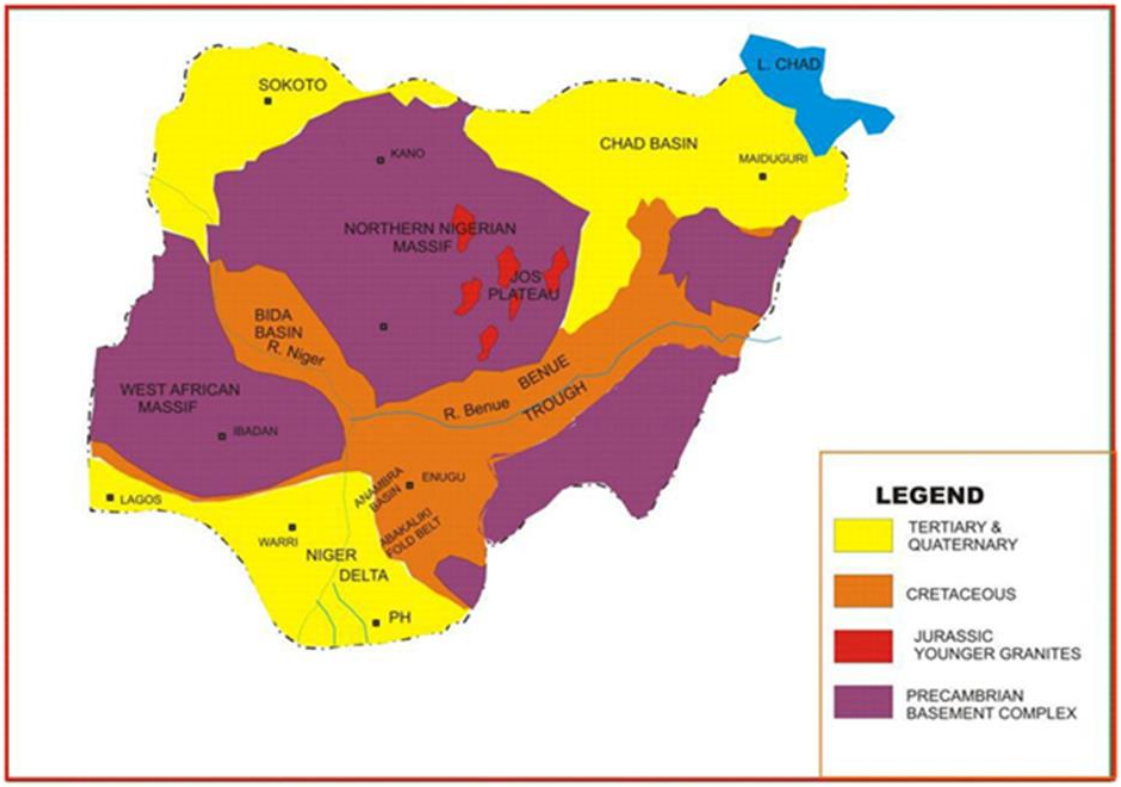

Figure 2: Simplified geological map of Nigeria showing the location of the Nigerian Precambrian Basement Complex

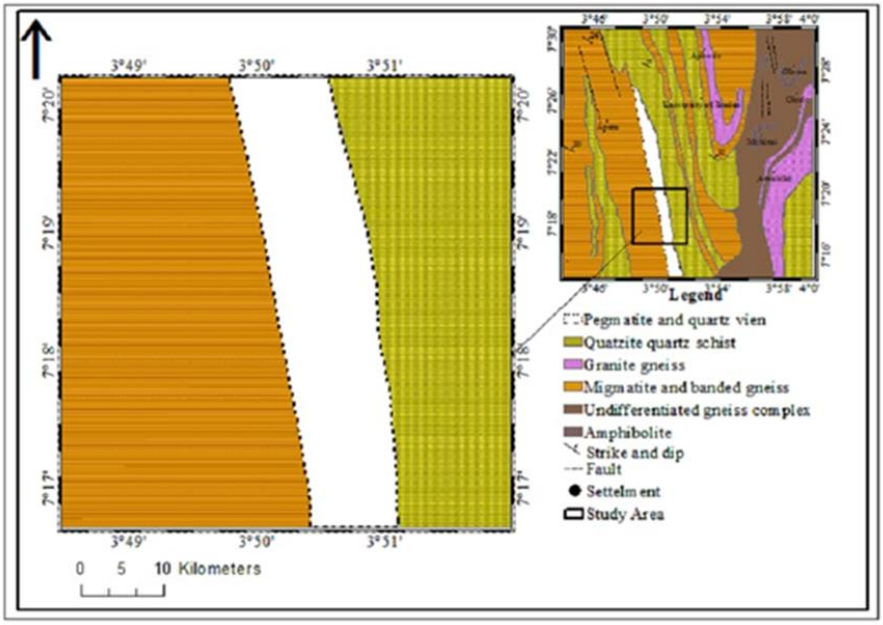

Figure 3: Geological map of Ajakanga and environs, Ibadan. (Source: Nigerian Geological Survey Agency)

Groundwater occurrence in much of southwestern Nigeria is essentially semi-confined to unconfined [23]. Due to the crystalline nature of the rock types in this region, the porosity and permeability necessary for the occurrence of large resources is lacking. However, appreciable porosity and permeability may develop through fracturing and weathering processes. Consequently, the weathered and fractured zones are the potential aquifers in basement terrains. Depth to water level rarely exceeds 24m, while most aquifers in this region occur within 40m from the surface under unconfined conditions [23]. An average yield of 0.41/s and borehole depth of 40-80m is estimated for the crystalline basement rocks in Nigeria [24].

IV. MATERIALS AND METHODS

- MULTIPLE TUBE FERMENTATION
- Sample Collection

A total of 14 water samples were collected from hand dug wells within the study area using new polythene bags and collected into sterile disposable universal bottles. The samples were adequately labelled and kept under cool temperature. The coordinates (Table 1) of each well location were adequately determined on the topographical map with the aid of a GPS device (Figure 4).

Table 1: Coordinates of sample location

SAMPLE LOCATION	NORTHING	EASTING	ELEVATION (m)
Sample 1	7° 18' 38.2"	3° 50' 24.9"	154
Sample 2	7° 18' 46.6"	3° 50' 30.4"	139
Sample 3	7° 18' 45.0"	3° 50'29.0"	161
Sample 4	7° 18' 42.2"	3° 50'32.3"	137
Sample 5	7° 18'40.0"	3° 50' 27.1"	144
Sample 6	7° 18' 55.5"	3° 50' 38.8"	157
Sample 7	7° 18' 31.8"	3° 50' 16.9"	154
Sample 8	7° 19' 10.6"	3° 50' 50.5"	177
Sample 9	7° 19' 25.5"	3° 51' 10.9	168
Sample 10	7° 19' 59.0"	3° 51' 20.1"	149
Sample 11	7° 18'14.2"	3° 49' 51.0"	148
Sample 12	7° 18' 01.8"	3° 49' 45.6"	165
Sample 13	7° 17' 30.5"	3° 49' 22.6"	178
Sample 14	7° 17' 48.0"	3° 49' 15.7"	172

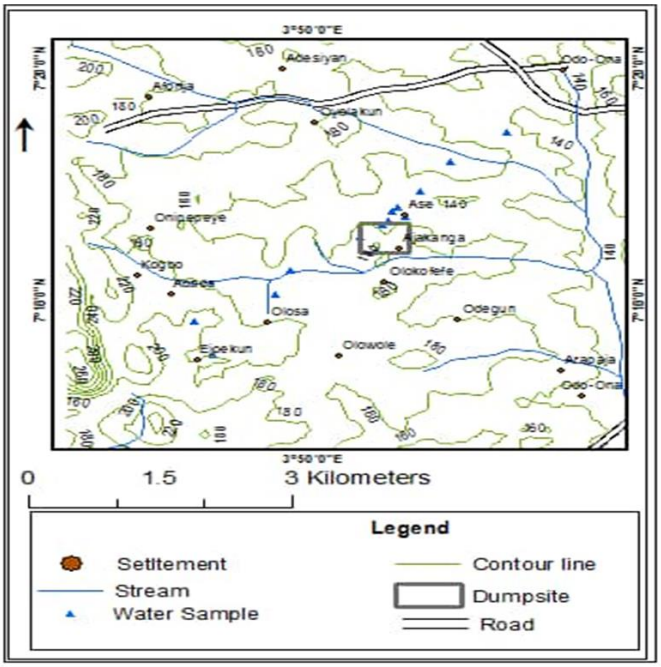

Figure 4: Locations of water samples from Ajakanga and environs

- Sterilization of Materials

Glass wares such as Erlenmeyer flasks, beakers, McCartney bottles, Flavour bottles, etc. were washed and sterilized in a hot-air oven at 160°C for 1hour. The inoculating loop and needles were sterilized by flaming to red hot and allowed to cool. The work bench was disinfected with cotton wool soaked in 70% ethanol before and after analysis.

- Preparation of Media

The media used were Plate Count Agar, Nutrient agar (HiMedia Laboratories Limited, India), Eosin Methylene Blue agar (HiMedia Laboratories Limited,

India) and Triple Sugar Iron (TSI) agar (Lab M Limited, United Kingdom). All these media were prepared according to the manufacturer's instructions and sterilized in the autoclave at 121°C for 15 minutes.

V. CULTIVATION, ISOLATION AND MAINTENANCE OF BACTERIAL ISOLATES

- Total Plate Count

The water sample (1 ml) was inoculated into plate count medium using pour plating technique and then incubated at 37°C for 24 hours. The number of the colonies were estimated by creating a grid for the colony count.

- Multiple Tubes Fermentation for Most Probable Number of coliform:

Water sample were inoculated into sterilized lactose broth with Durham tube inverted to detect the presence of gas production. Different volume of water sample were measured with 0.1 ml and 1 ml as single strength while 10 ml was inoculated as double strength. The extent of sugar fermentation is shown in the Figure 1.The tubes were incubated for 48 hours at 37°C. MPN of the coliform was determine according WHO standard (APHA, 1992).

- Cultivation and Isolation of microorganisms

A loopful from the tube where fermentation and gas production occurred was streaked onto solidified EMB agar. The streaked plates were then incubated at an inverted position aerobically at 37°C for 24 hours. After 24 hours of incubation, distinct colonies were selected randomly and streaked on nutrient agar plates to obtain pure cultures. This is then incubated aerobically in an inverted position at 37°C for 24 hours. The pure culture isolates (24 hours) were transferred to nutrient agar slants and stored at 0-4°C.

VI. CULTURAL IDENTIFICATION

- Colonial Morphology

The colonial morphology was examined to determine the macroscopic/visible characteristics of the colonies.

VII. MORPHOLOGICAL CHARACTERISTICS

- Gram Staining

This method is used to divide bacteria morphologically into two groups which are Gram positive, which appear purple coloured and Gram negative, which appear red or pink coloured. The Gram staining of the bacteria isolates were carried out as follows:

A smear of the organism was prepared on a clean, grease-free glass slide by emulsifying a bacteria colony in a drop of distilled water. The smear was allowed to air-dry and heat fixed by passing through the flame three times. The heat fixed smear was flooded with the primary stain (crystal violet) for 60 seconds and rinsed with clean water, the smear was covered with the mordant (Lugol's iodine) for 60 seconds and rinsed with clean water, the smear was decolorized rapidly with 95% ethanol and washed immediately with water, the smear was counterstained with safranin for 60 seconds and rinsed with water. The reverse side of the slide was wiped with filter paper and the slide was allowed to air-dry. A drop of immersion oil was placed on the smear and viewed under the microscope at 100× objective.

VIII. BIOCHEMICAL TESTS

The biochemical tests carried out prior to the identification of the organisms are: catalase production, oxidase reaction, sugar fermentation (lactose, glucose and sucrose), hydrogen sulphide and gas production using Triple Sugar Iron (TSI) agar.

- Catalase Test

This test is used to differentiate bacteria that produce enzyme catalase (mostly aerobic microorganisms) from those that are catalase negative (obligate anaerobes). This test indicates the presence of the enzyme catalase which catalyses the release of oxygen from hydrogen peroxide. A drop of 3% hydrogen peroxide solution was placed on a clean glass slide and a sterile inoculating loop was used to pick colonies of a 24 hours old bacteria culture and was emulsified in the hydrogen peroxide solution. The slide is then observed for the presence of bubbles or effervescence. The presence of bubbles indicates a catalase-positive

reaction while the absence of bubbles indicates a catalase-negative reaction.

- Triple Sugar Iron Assay

This test is used to determine the utilization of different sugars by bacteria. Triple Sugar Iron (TSI) agar was used; this medium contains 1% lactose, 1% sucrose, 0.1% glucose and ferrous ammonium sulphate and sodium thiosulphate. It also contains a pH indicator; phenol red to indicate acid production. TSI agar is used for the identification of enteric organisms due to their ability to ferment lactose, glucose or sucrose and to liberate sulphides from ammonium sulphate or sodium thiosulphate. The medium (15ml) was dispensed into MacCartney bottles and sterilized in the autoclave at 121^{o}C for 15minutes. The bottles were then slanted in a way which resulted in a butt and slant and then allowed to solidify. An inoculating needle was used to pick small quantity from the 24hours bacterial isolates and stabbed at the bottom of the medium. The bottles were incubated at 37^{o}C for 24hours.

IX. RESULTS AND DISCUSSION

- ANALYTICAL RESULTS

The total bacterial count in some locations were observed to be high beyond what could be counted and therefore regarded as too numerous to count. However, among those that were within enumeration region, the sample with lowest bacterial load was observed to be 12 cfu/ml as observed in sample S5 (Table 2).

The most portable number (MPN) determination for coliform count showed that the water samples contain coliform bacteria and the count observed was within the range of (2 – 6) coliform counts. This result revealed that there is presence of coliform which is an evidence of recent faecal contamination of the water source (Table 3).

The pure culture were identified based on their morphological and biochemical characteristics of the isolates. They are all Gram negative, rod shape and slender that is they are not robust. They exhibited the properties of fermenting all the three sugar and gas production. A few of them were able to produce H_2S (Table 4). The evidence of sugar fermentation and gas production is shown in figure 5, while H_2S production is shown in figure 6.

Table 2: Total Bacterial Count of the Water Sample

S/N	SAMPLE	PLATE COUNT
	S1	*TNTC
	S2	156
	S3	*TNTC
	S4	38
	S5	12
	S6	*TNTC
	S7	124
	S8	*TNTC
	S9	148
	S10	36
	S11	256
	S12	108
	S13	TNTC
	S14	TNTC

*TNTC – Too Numerous to Count

Table 3: Coliform Count of the Water Sample

Sample	10 ml		1 ml		0.1 ml		*MPN	
CODE	Acid	Gas	Acid	Gas	Acid	Gas		
S1	+	+	-	-	+	+	4	101
S2	+	-	+	+	+	+	4	011
S3	+	+	+	+	+	+	6	111
S4	-	-	-	-	-	-		000
S5	+	-	+	+	+	+	4	011
S6	+	-	+	+	+	+	4	011
S7	+	-	+	+	+	+	4	011
S8	+	-	+	+	+	+	4	011

S9	+	+	+	+	+	+	6	111
S10	+	+	+	+	-	-	4	110
S11	+	+	+	+	-	-	4	110
S12	+	-	+	+	-	-	2	010
S13	+	-	+	+	+	+	4	011
S14	+	+	+	+	-	-	4	110

*MPN – Most Probable Number

Table 4: Morphological and Biochemical Characteristics of the Isolates from Water Samples

Isolate Code	Gram React.	Shape	Arrangement	Catalase	Appearance on EMB	Oxidase	Glucose	Lactose	Sucrose	H_2S	Gas	Probable Identity
S 1		Rod	Singly	+	Mucoid	+	+	+	+	+	+	*Aeromona* ssp.
S 2	-	Rod	Singly	+	Mucoid	+	+	-	+	+	+	*P. miralis*
S 3	-	Rod	Singly	+	Mucoid	+	+	+	+	+	+	*Aeromona* ssp.
S 4	-	Rod	Singly	-	Greenish	-	+	+	+	-	+	*E. coli*
S 5	-	Rod	Singly	+	Mucoid	+	+	+	+	+	+	*Aeromona* ssp.
S 6	-	Rod	Singly	-	Mucoid	-	+	+	+	+	+	*C. freundii*
S 7	-	Rod	Singly	+	Mucoid	+	+	+	+	+	+	*Aeromona* ssp.
S 8	-	Rod	Singly	+	Mucoid	+	+	+	+	+	+	*Aeromona* ssp.
S 9	-	Rod	Singly	+	Mucoid	+	+	+	+	+	+	*Aeromona* ssp.
S 10	-	Rod	Singly	+	Mucoid	+	+	+	+	+	+	*Aeromona* ssp.
S 11	-	Rod	Singly	-	Greenish	+	+	+	+	-	+	*E. coli*
S 12	-	Rod	Singly	+	Mucoid	+	+	+	+	+	+	*Aeromona* ssp.
S 13	-	Rod	Singly	+	Greenish	-	+	+	+	-	+	*E. coli*
S 14	-	Rod	Singly	+	Mucoid	+	+	+	+	+	+	*Aeromona* ssp.

Figure 5: Acid formation and gas production by bacterial isolate.

Figure 6: Production of Hydrogen Sulphide by bacterial isolate

(Indicated by the dark colour of the TSI agar).

- RESULT OF THE WELL WATER ANALYSIS

The sampled waters were analyzed for NO_3 anion and the results are presented along with the *E. coli* count in Table 5.

Table 5: Concentration of Nitrate and Escherichia coli in the shallow groundwater of Ajakangaand environs

Sample Code	NO_3 (mg/l)	Escherichia coli	
		Count	Growth
S1	2.12	4	Moderate
S2	0.16	4	Moderate
S3	0.12	6	Moderate
S4	0.18	0	NIL
S5	0.16	4	Moderate
S6	0.19	4	Moderate
S7	0.15	4	Moderate
S8	0.16	4	Moderate
S9	0.12	6	Moderate
S10	0.11	4	Moderate
S11	0.16	4	Moderate
S12	0.14	2	Scanty
S13	0.12	4	Moderate
S14	0.15	4	Moderate

- NITRATE (NO_3)

Nitrate (NO_3) is a naturally occurring form of nitrogen found in soils. Nitrogen is essential to all life. Most plants requires large quantities to sustain high yields. Natural sources of nitrate in ground water includes igneous rocks, plant and animal debris. However, nitrate can also be contributed to natural waters anthropogenically from human faeces. The concentration of nitrate observe in the sampled waters fall below the maximum permissible concentration of 50mg/l set by Nigeria Industrial Standard [25] and World Health Organization [26] for water intended for human consumption. Generally, nitrate concentration in the shallow ground water of the study area is low ranging from 0.12mg/l to 2.12mg/l (Table 5 and Figure 7) compared to the World Health Organization guidelines for portable drinking water which is 50mg/l. Nitrate leaching from unfertilized grassland and natural vegetation is normally minimal, although soils in such areas contain sufficient organic matter to be a large potential sources of nitrate due to the activity of nitrifying bacteria in the soil. The NO_3 concentration in the area can therefore be attributed to natural source.

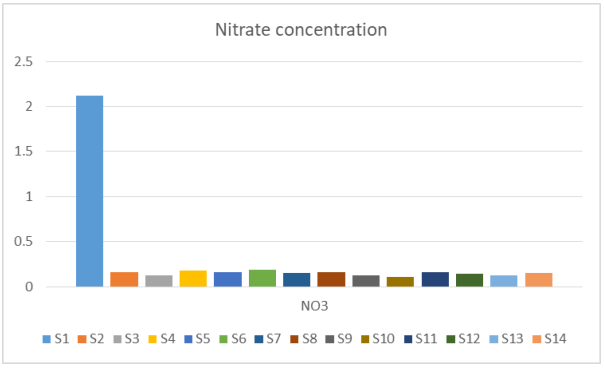

Figure 7: Concentration of Nitrate in the shallow groundwater of Ajakanga and environs

X. MICROBIAL EVALUATION

Ideally, drinking water should not contain any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution. Most risk to human health associated with water stems from the presence of disease-causing micro-organisms. Many of these micro-organisms originated from human waste [26]. Human feaces can contain a variety of intestinal pathogens which cause diseases ranging from typhoid, gastroenteries, dysentery, enteritis, and tuberculosis. Escherichia coli is used as an indicator of the presence of feacal matter. Therefore, the presence of Escherichia coli in the shallow groundwater is an indication of contamination from feacal matter which showed poor sanitary management.

The *Escherichia coli*growth count in the shallow water of Ajakanga and environs, showed moderate count between 4 to10 in most of the location (1,2,3,5,6,7,8,9,10,11,13 and 14), scanty count of 3 was observed in location 12, while *Escherichia coli* was totally absent in location 4 (Figures 7 and 8).The presence of*E.coli* has a significant health risks when water is used for drinking, personal hygiene, contact recreation or food processing [26] which can result to infections such as typhoid, dysentery and infantile paralysis.

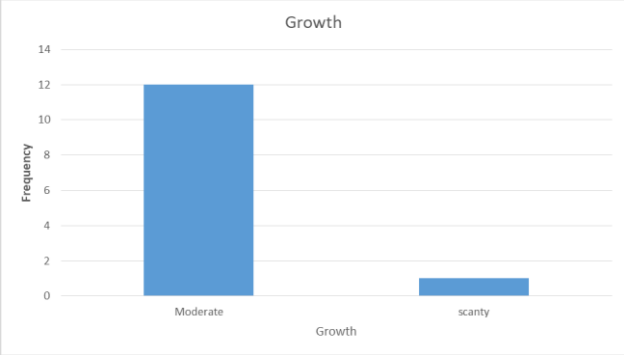

Figure 8: Growth of Escherichia coli in the shallow groundwater of Ajakanga and environs

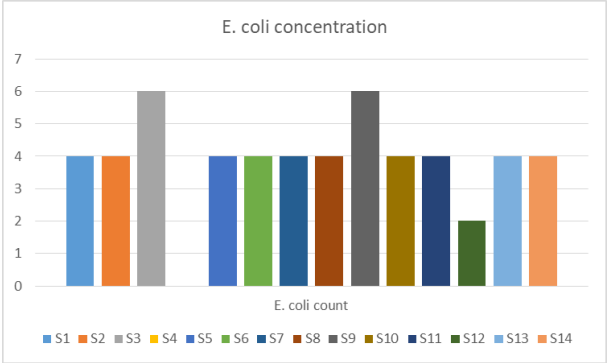

Figure 9: Concentration of *Escherichia coli* in the shallow groundwater of Ajakanga and environs

XI. DESCRIPTIVE STATISTICS

The descriptive statistics for the parameters tested in the shallow groundwater is shown in Table 6 NO_3 concentration range from 0.11 to 2.12 with a mean of 0.2886, while *E. coli* has a minimum value of 0.00 and a maximum value of 6.00.

Table 6: Descriptive statistics of the tested parameters in the shallow groundwater of Ajakanga and environs

	N	Minimum	Maximum	Sum	Mean	Std. Deviation	Coefficient of Variation
NO_3	14	.11	2.12	4.04	.2886	.52765	182.8
E.coli	14	.00	6.00	54.00	3.8571	1.46009	37.85
Valid N (listwise)	14						

- Pearson's Correlation Coefficient

Although only two variables, E. coli and nitrate, were tested, the Pearson's correlation coefficient was applied in order to determine the relationship between the two parameters. The interpretation of Pearson's correlation coefficient is presented in Table 7 [27].

Table 7: Interpretation of Pearson's Correlation Coefficient [27]

Correlation Value	Strength and Direction of Correlation
(-0.8) – (-1.0)	Strongly negative
(-0.5) – (-0.8)	Moderately negative
(-0.2) – (-0.5)	Weakly negative
(+0.2) – (-0.2)	No association
(+0.2) – (+0.5)	Weakly positive
(+0.5) – (+0.8)	Moderately positive
(+0.8) – (+1.0)	Strongly positive

The Pearson's correlation coefficient matrix (Table 8) plotted between NO_3 and *E. coli* in the shallow groundwater showed that there is no association between their sources. The low concentration of NO_3 in the shallow groundwater suggest a natural source for the NO_3, while *E.coli* is contributed from anthropogenic activities.

Table 8: Pearson Correlation coefficient matrix between NO_3 and *E.coli* of the shallow groundwater of Ajakanga and environs

		NO_3	E.coli
NO_3	Pearson Correlation	1	.006
E.coli	Pearson Correlation	.006	1

a. Listwise N=14

CONCLUSION

The concentration of nitrate in the shallow groundwater of Ajakanga and environs was generally low. Its value range from 0.12mg/l to 2.12mg/l which is below the maximum permissible concentration of 50mg/l set by both the Nigeria Industrial Standard (NIS), and World Health Organization (WHO) for water intended for human consumption, while the population of E. coli in the water is low to moderate as shown by the growth count which was within the range of 2-6. Soils in unfertilized lands commonly contain sufficient organic matter to be a large potential sources of nitrate due to the activity of nitrifying bacteria in the soil. The NO_3 concentration in the area can therefore be attributed to natural source.

The presence of E.coli in the shallow groundwater has implications to man and the ecosystem. The use of these water can cause infections such as typhoid, diarrhea, and infantile paralysis.

Pearson's coefficient of correlation showed that there is no association between NO_3 and E.coli indicating that the NO_3 and E.coli in the shallow groundwater originated from different sources.

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