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COMPARATIVE STUDY OF NITRATE EVALUATION AND BACTERIOLOGICAL CHARACTERISATION OF SHALLOW GROUNDWATER IN ADJOINING TOWNS AROUND OYO AREA, SW NIGERIA

AKANBI O. A., JOSEPH G. D. AND OLAYIWOOLA, J. O
Email: oa.akanbi@acu.edu.ng

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ABSTRACT

The study aimed at assessing the quality of groundwater from forty-five shallow hand-dug wells (HDs) in adjoining towns of Awe, Akinmorin, Ilora and Oyo within the southwestern Nigeria. The amounts of nitrate and bacterial counts and species identification were carried out in water samples in these communities where groundwater is the major reliable water source using spectrophotometric, multiple tube and plate counts techniques.
From the results, nitrate concentration was between 1.48 and 191.8 mg/L in sampled wells and its occurrence in water exceeded the recommended level of 50 mg/L in about 38% of sampled wells across all the towns. Oyo town has the highest nitrate level in water with a range of 5.11 - 191.8 mg/L and average of 71.24 mg/L, compared to 29.87 mg/L in Awe, 41.33 mg/L in Akinmorin, 45.09 mg/L in Ilora. The minimum total bacteria count (TBC) was 26 cfu/100 mL while the total coliform count (TCC) ranged from 14 to 46 cfu/100ml. The presence of coliform bacteria depicts fecal contamination of groundwater in the study area. From the morphological studies, four bacteria species were identified; namely, *Bacillus cereus* (which is the most abundant), *Bacillus species, Pseudomonas aeroginosa* and the *Klebsiella species*. The deepest hand-dug well was 20.8 m in the area and the correlation between the depths of the HDs and nitrate concentrations is negative-indicating that nitrate concentration moderately decreases with increasing well depth. Likewise for bacteria counts, the relationship was indirect in most towns, which depicts that bacterial occurrence will likely fade out as well depth increases in most cases. Also, the cross-plots of nitrate against bacterial counts in groundwater were positive that confirms that high nitrate level in water will favour bacterial activities. From these deductions, deeper wells are recommended and remedial decontamination of functioning wells should be in place in these communities to guarantee safety of human lives.

KEYWORDS: Shallow-groundwater, Nitrate, Bacteria, Hand-dug well, Well-depth

INTRODUCTION

Water is one of the most important natural resources required by living things (Duru *et al*., 2017; Onyango *et al*., 2018) and clean water supply is crucial for human well-being for drinking, domestic, industrial and even for recreational purposes (WHO. 2017). Groundwater is often regarded as a source of clean water found within the zone of saturation. Even then, there are reported cases of groundwater contaminated with enteric microbes such as bacteria, viruses, parasites and fungi (Niyogi 2005, Mora et al., 2017, Akanbi et.al., 2023).

These pathogens can enter water bodies either from a point source like human and animal and discharge of untreated or partially treated effluents from wastewater treatment plants (Donovan et al. 2008, Musyoki et al. 2013). Microbes can also be from non-point sources that is through rainwater surface run-offs, storm sewer spillages or overflow. When there is faecal contamination of water, a wide range of pathogenic microorganisms are transmitted to humans orally or by skin contacts. These groups of microorganisms are known as enteric organisms and examples are; Salmonella, Shigella, Enteroviruses, Pseudomonas aeruginosa, Klebsiella sp., Vibrio spp.

Akanbi O.A., Department of Earth sciences, Ajayi Crowther University, Oyo Nigeria
Joseph G.D., Department of Earth sciences, Ajayi Crowther University, Oyo Nigeria
Olayiwoola, J.O., Department of Biological sciences, Ajayi Crowther University, Oyo, Nigeria

and Aeromonas hydrophila (Edberg, et. al. 2000; Fujioka, 2001; Granum, 2007; Mena, 2009).

Likewise, high concentration of nitrates in drinking water can be injurious and can pose health risks such as methemoglobinemia in infants. This ailment reduces the oxygen- carrying capacity of the blood (WHO, 2017) in infants and pregnant women. The sources of nitrates in water are both natural and human-induced. For instance, atmospheric deposition, industrial emissions, vehicle exhaust and agricultural practices are potential sources of nitrates in the atmosphere which eventually infiltrate groundwater (Sickles and Shadwick, 2002; Seinfeld, and Pandis, 2006). Also, nitrate contamination is often from anthropogenic sources like sewage plants and open defecations and solid waste.

The proximity of water wells to surface contaminants often expedites migration of pollution plume and shallow wells that are typically less than 50 meters deep are more susceptible to nitrate and bacterial contamination (EPA, 2020). This is because effluents from surface sources, such as agricultural runoff, sewage, and fertilizers, can easily infiltrate the shallow groundwater (WHO, 2017). Hence, the aim of this study is to enumerate and characterize the coliforms and other bacteria species as well as nitrate amount in groundwater samples collected from different hand-dug wells in adjoining towns within the southwestern Nigeria. These connected towns including Awe, Akinmorin, Ilora and Oyo are not connected with town water supply and individual households tap from shallow hand-dug wells to meet their water need (Akanbi and Olukowade, 2018). Most of the communities within these towns are not having proper waste disposal mechanism and sanitary conditions of most household wells are poor.

MATERIALS AND METHODS

FIELDWORK

A total of forty-five (45) hand-dug wells (HWs) were sampled across four towns; namely Awe where (10 HWs) were sampled; Akinmorin – five (5) samples, Ilora fifteen (15) samples and Oyo township with another fifteen (15) samples. The location coordinates of the HDs were taken using digital GPS meter. Sampled HDs locations are presented in the location map in Figure 1.

Water sampling and well depth measurement

Ground water samples were collected using new one-litre plastic bottles. For precautionary measures, the sample bottles were kept unopened until the point at which it was required for the collection. During sampling process, the stopper and neck of the bottle were protected from contamination by the handler by rinsing the bottles and the lid with the water to be sampled and were quickly closed after the sampling. Samples were well labelled and transported in ice packs to the laboratories for bacteriological and nitrate analyses. Sample labelling is alphanumeric using the first two letters of each town. Well depths were measured with the depth/water level sounder and recorded in metric unit.

LABORATORY ANALYSES

Laboratory analyses followed standard procedures. Nitrate analyses were carried out in the central laboratory of a Federal University in Akure using atomic absorption spectrophotometry (AAS) method. The parameter was selected from the test manager of the spectrophotometer. Two 10ml sample cells were filled with the sample to be tested and 1 pillow (reagent) of test parameter was added to one of the samples in the sample cell and thoroughly shaken. The sample was allowed to stay for some time for reaction to take place. The sample bottle that contains blank was first introduced into the sample chamber to calibrate the machine for the tested parameter, after which the sample cell of investigating sample was introduced. The concentration of nitrate in mg/L was displayed on the spectrophotometer screen.

The microbial loads were analyzed in biological laboratory of Ajayi Crowther University Oyo an follow the specification detailed below.

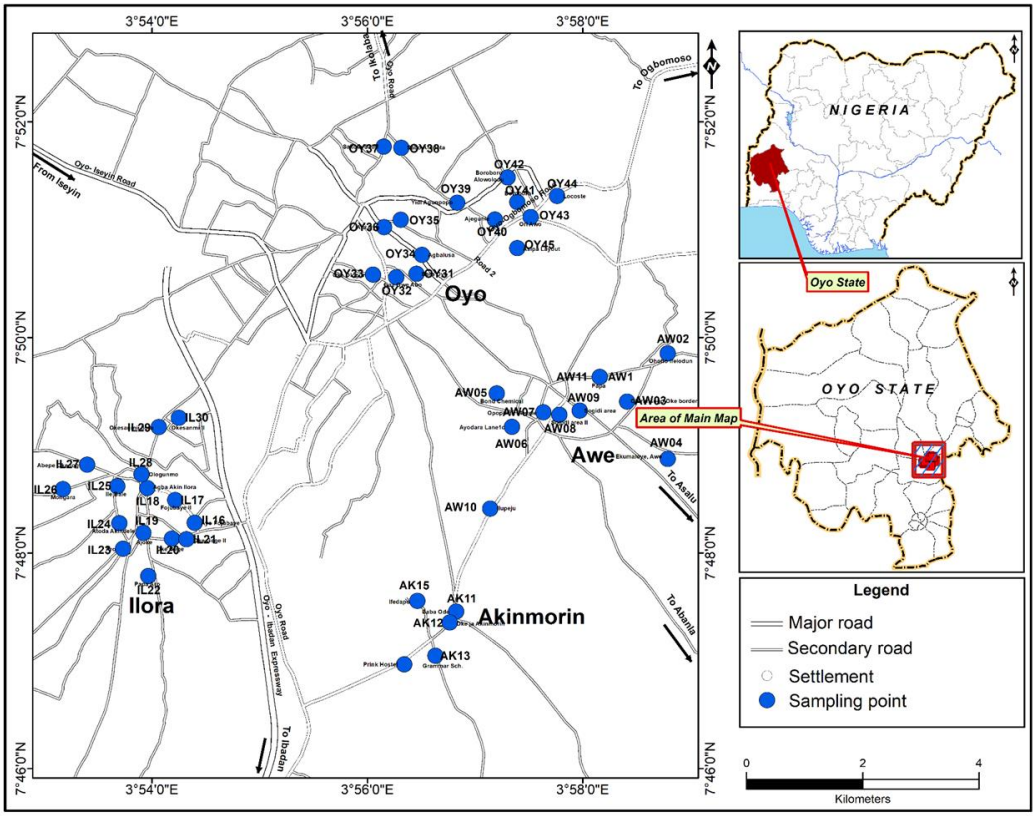

Figure 1: Location map of the study area showing sampled borehole locations

Total Bacteria Count (TBC)

One mL of each of the water samples was inoculated into nutrient agar using pour plating technique and incubated at 37^{O} C for 24 h in an inverted form. The colonies were enumerated by dividing the plate surface into grids and total numbers of the colonies were estimated based the numbers of the grids.

For detection of pathogenic bacteria in water samples, 1 mL of each water sample was inoculated into MacConkey and Eosin Methylene Blue (EMB) agar incubated at 37° C for 24 hours. Distinct colonies were sub-cultured into nutrient agar to obtained pure culture which were then transferred to slants for the purpose of keeping the pure culture.

Morphological and Biochemical Identification

The bacteria cultures were smeared, gram stained and view with oil immersion under light microscope. Catalase production was carried out using 3% H_2O_2 on freshly grown bacteria culture and watched for gas bubbles or effervescence. Oxidase production were carried out using oxidase reagent according to Fawole and Oso, (2007). Sugar fermentation, H_2S and gas production were performed using Triple Sugar Iodine (TSI) agar according to Abiala *et al*., (2016).

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Total Coliform Count (TCC)

The total coliform count in the water samples were carried out using multiple tube fermentation technique according to the method of Bartram and Pedley ,1996. The coliform counts were then evaluated using Most Probable Number (MPN) technique

DATA EVALUATION

The mean, minimum, and maximum calculations was carried out using Microsoft Excel 2016 program. Degree of correlation is a measure of the strength of the linear relationship between two variables. The range for correlated values were calculated using Microsoft Office Excel (2016). Cartographic works were done with mapping tools and Software including ArcMap and Surfer software.

RESULTS AND DISCUSSION

The results of measured well depth, nitrate concentrations and bacteria count in wells are presented in Table 1. All the sampled wells are regarded as shallow since their depths were all below 50 m (WHO, 2017). At Awe town, the depths were between 6.1 and 17.2 m; for Akinmorin 7 to 19 m; at Ilora - 4.4 to 20.8 m and for Oyo town, the measured wells depths were between 4.6 to 11 m

Table 1: Results of bacterial counts and nitrate concentration in water samples

s/no	Sample No.	Location name	Well Depth (m)	Nitrate (mg/L)	TBC cfu/100mL	TCC (cfu/100mL)
1	AW01	Papa	17.2	10.50	44	18
2	AW02	Ohoho	6.3	16.56	TNTC	22
3	AW03	Olorunsogo	11.7	11.80	50	18
4	AW04	Ekumalooye	6.1	8.99	220	22
5	AW05	Bond Chemical	10.9	17.09	168	22
6	AW06	Ayodara Lane	9.2	37.78	72	46
7	AW07	Opopo	14	25.18	172	22
8	AW08	Sogidi I	10.6	71.10	55	46
9	AW09	Sogidi II	13.7	56.08	72	46
10	AW10	Ilupeju	9.2	43.60	51	18
		Min.	**6.1**	**8.99**	**8.99**	**18**
		Max.	**17.2**	**71.1**	**TNTC**	**46**
		Avg.	**10.89**	**29.87**	**-**	**28**
11	AK11	Baba Ode	7	68.70	80	46
12	AK12	Oke-Oja	7.1	88.13	136	18
13	AK13	Akinmorin Gram. Sch.	8.2	22.29	50	24
14	AK14	Pink Hostel	19	13.02	124	26
15	AK15	Ifedapo	9.2	14.51	132	46
		Min.	**7**	**13.02**	**50**	**18**
		Max.	**19**	**88.13**	**136**	**46**
		Avg.	**10.1**	**41.33**	**104.4**	**32**
16	IL16	Ayo Fojubaye	12.9	1.48	60	24
17	IL17	Fojubaye II	12.3	35.03	TNTC	46
18	IL18	Agba-Akin Ilora	8.4	97.90	52	22
19	IL19	Ajoke	7.3	74.81	41	18
20	IL20	Oke gege I	14.3	13.95	73	18
21	IL21	Oke gege II	11	19.78	85	46
22	IL22	Papa Aro	20.8	35.56	106	24
23	IL23	Aro	6.9	88.58	TNTC	46
24	IL24	Atodah	8.2	76.32	200	18
25	IL25	Gboranja	9.5	85.10	30	46
26	IL26	Mongara	11.1	12.64	89	18
27	IL27	Abepe Okedogi	7.3	7.98	56	18
28	IL28	Ologunmo	4.4	102.80	70	46
29	IL29	Okesanmi I	9.4	3.56	36	24
30	IL30	Okesanmi II	18.9	20.91	68	14
		Min.	**4.4**	**1.48**	**30**	**14**
		Max.	**20.8**	**102.8**	**TNTC**	**46**
		Avg.	**10.85**	**45.09**	**-**	**28.53**
31	OY31	Bara	5.8	123.68	TNTC	46
32	OY32	Oke-Ebo	9.5	40.67	26	14
33	OY33	Oke-Ologun	8.6	69.90	116	24
34	OY34	Agbaluasa	5.8	72.21	59	46
35	OY35	Isale-Oyo	4.6	24.75	68	22
36	OY36	Ara-oyo	7.3	125.55	276	46
37	OY37	Sakoto sabo	4.8	191.8	TNTC	18
38	OY38	Aradota	7.4	112.10	115	46
39	OY39	Yidi agun popo	5.5	147.43	98	24
40	OY40	Ajegunle	5.4	17.86	50	46
41	OY41	Boroboro I	11	22.90	66	46
42	OY42	Boroboro II	9.5	31.15	83	18
43	OY43	Ori-Awo	7	5.11	75	46
44	OY44	Locoste	10	41.53	212	18
45	OY45	Asipa	7.7	42.10	126	24
		Min.	**4.6**	**5.11**	**26**	**14**
		Max.	**11**	**191.8**	**TNTC**	**46**
		Avg.	**7.33**	**71.25**	**-**	**32.27**

Comparison of Nitrate Concentration in Groundwater Samples

In the study area, nitrate concentrations range from 1.48 to 191.8 mg/l with the average of 50.01 mg/l. The highest amount of nitrate was obtained at OY37 within Oyo Town characterised with improper sanitation and shallower wells. Ranges of nitrate concentrations by town is presented in Table 2.

Table 2: Statistical of nitrate concentration

Towns	Min.	Max.	Mean	WHO (2017) Guideline limit (mg/L)	NIS/SON (2007) Guideline limit (mg/L)	Samples with nitrate conc. exceeding 50 mg/L (%)
Awe	8.99	71.10	29.86	50	50	20
Akinmorin	13.02	88.13	41.33			40
Ilora	1.48	102.80	45.09			40
Oyo	5.11	191.8	71.24			47

In Awe, nitrate concentration was 8.99 – 71.1 mg/L with an average of 29.86 mg/L (Table 2). Generally, nitrate levels in shallow groundwater in Awe are within acceptable drinking water standards, except at Sogidi area with sample No. AW08 and AW09, where concentrations exceeded the limits. In contrast, samples from Akinmorin have more amount of nitrate with a range of 13.02 – 88.13 mg/L and average of 41.33. For Ilora, the range is 1.48 – 102.8. the average amount at Ilora is greater than what we have in Awe and Akinmorin towns and amount of nitrate in six samples exceed the safety limits. Based on average concentration, Oyo town has the largest level of nitrate in groundwater with 71.24 mg/L and range of 5.11 - 191.8 mg//L (Table 2).

Comparing the nitrate results with guideline limit of 50 mg/L, from the nitrate concentration map presented in Figure 2, it was observed that there is high nitrate concentration in most towns with the exception of Awe main town. However, nitrate concentration at Sogidi area in Awe town is above the recommended limit of 50 mg/L. Nitrate occurrence is higher in Oyo town and most high concentrations that exceeded 100 mg/L are found in HDs with well depth below 10 m. The highest concentration of nitrate in the study area which is 191.8 mg/L occurred at Sabo (OY37).

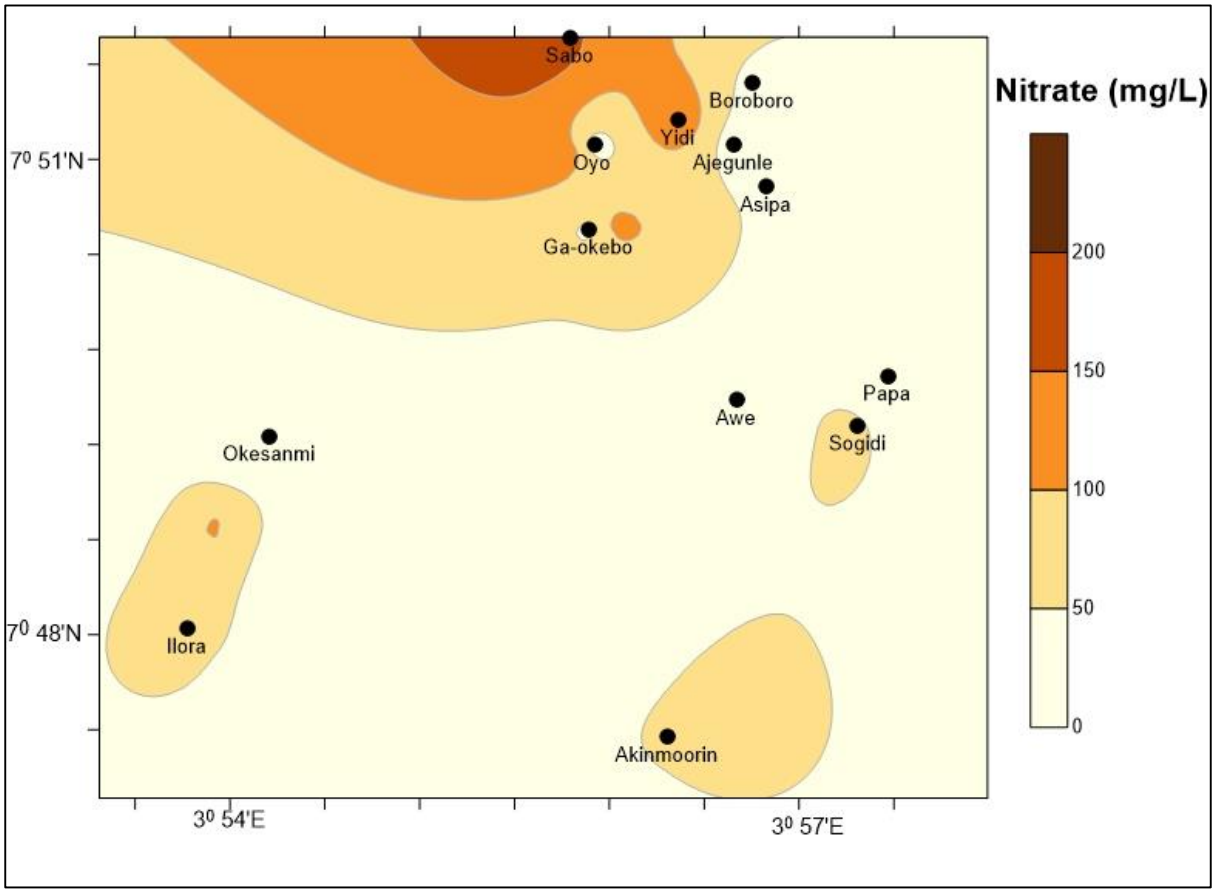

Figure 2: Nitrate occurrence spread in the study area

Nitrate concentration exceeded recommended level of 50 mg/L in seventeen (17) HDs (representing about 38%) out of the total sampled wells in all towns. The frequencies of water samples with nitrate concentration exceeding 50 mg/L in each town is presented in Table 2. Oyo town has the highest with 47 %, Akinmorin while the frequencies in Ilora and Awe are 40% each and Awe having just 2 wells (representing 20%) with concentration above guideline limit. This implies that Oyo town's wells have the highest nitrate levels compared to the other three locations. The higher nitrate concentration in the wells in Oyo town is adjudged to be due to the shallowness of most HDs in this town. The average depth of HDs in Oyo town is 7.3 m. Other reasons are due to improper disposal of human wastes evidenced by open defecation within township area, discharge of untreated wastewater from homes and inappropriate HDs design that allow direct discharge.

Well depth versus nitrate occurrence in water

The plots of HDs depths and the respective nitrate concentrations in water are presented in Figure 3. By observation, the relationship is mostly indirect in towns and the degrees of association is moderate, pointing to the fact that nitrate concentration is not favoured with increasing well depth. The peak negative relationship between nitrate concentrations and well depths is at Akinmorin with R = - 0.59, while for Ilora R = - 0.51 and at Oyo R = -0.44 (Fig. 3).

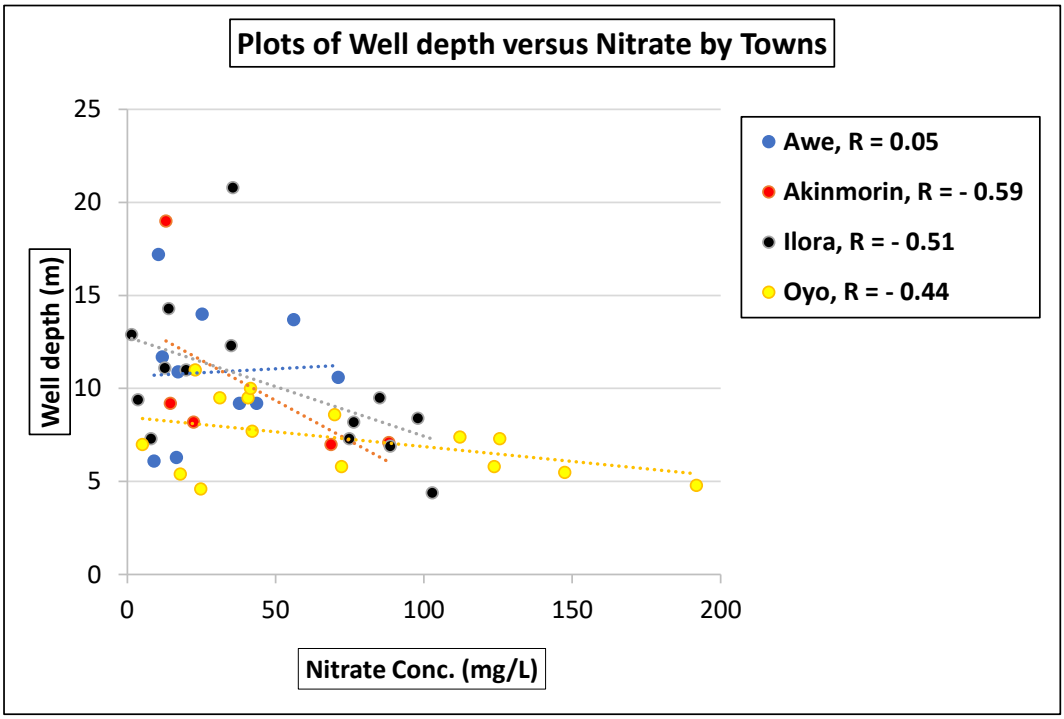

Figure 3: Plots of well depths versus nitrate conc. by Towns

Bacteria Counts in Water Samples

There is presence of bacteria in all the wells as shown in Table 1. The total bacteria count in the sample collected from this location had very high bacteria count across all the samples in this work. The counts ranges from 44 cfu/mL to 220 cfu/mL of the bacterial colonies. Similarly, some samples were observed to have too numerous bacteria and are too numerous to count (TNTC) which reflected the level of the bacterial load (Table 1).

The total coliform count (TCC) in all the sampled wells was between 14 and 46 cfu/mL. TCC in all water samples was more than 2 colonies as standard for drinking water. Hand-dug wells with 46 cfu/mL were seventeen spreading across the study area including AW06, AW08, AW09, AK11, AK15, IL17, IL21, IL23, IL25, IL28, OY31, OY34, OY36, OY38, OY40, OY41, OY43. Samples IL30 and OY32 had the lowest TCC with 14 coliform bacteria.

Bacterial species in water

Water samples were randomly isolated for the morphological and biochemical identification of bacterial in water samples. Form the results, four bacteria species were recognised and they are namely; *Bacillus cereus*, *Bacillus* species, *Pseudomonas aeroginosa* and *Klebsiella* species (Table 3). The most abundant bacteria in sampled well were the *Bacillus cereus* which is Gram positive and rod shape organism (Table 3) with a 61% abundance distribution. It was also observed that there were lower proportion of the Gram-negative bacteria in the water samples (Table 4) that composed of *Pseudomonas aeroginosa Klebsiella* specie.

The *Bacillus* species and *Pseudomonas aeroginosa* distribution were similar each having 16% distribution and lastly the *Klebsiella* species that amounts to 7% (Figure 4).

Table 3: Morphological and Biochemical of the Gram-Positive Bacteria Isolated from groundwater

Sample no	Gram	Cell Shape	Catalase	Starch Hydrolysis	V.P	Probable Organisms
AW3	+	Rod	+	+	+	*Bacillus cereus*
AW4	+	Rod	+	+	+	*Bacillus cereus*
AW7	+	Rod	+	-	+	*Bacillus* sp
AW9	+	Rod	+	+	+	*Bacillus cereus*
AW10	+	Rod	+	+	+	*Bacillus cereus*
AK11	+	Rod	+	+	+	*Bacillus cereus*
AK13	+	Rod	+	+	+	*Bacillus cereus*
IL16	+	Rod	+	+	+	*Bacillus cereus*
IL18	+	Rod	+	+	+	*Bacillus cereus*
IL19	+	Rod	+	+	+	*Bacillus cereus*
IL20	+	Rod	+	+	+	*Bacillus cereus*
IL21	+	Rod	+	+	+	*Bacillus cereus*
IL22	+	Rod	+	+	+	*Bacillus cereus*
IL24	+	Rod	+	-	+	*Bacillus* sp
IL26	+	Rod	+	+	+	*Bacillus cereus*
IL27	+	Rod	+	+	+	*Bacillus cereus*
IL28	+	Rod	+	+	+	*Bacillus cereus*
IL29	+	Rod	+	+	+	*Bacillus cereus*
IL30	+	Rod	+	-	+	*Bacillus* sp
OY32	+	Rod	+	+	+	*Bacillus cereus*
OY34	+	Rod	+	-	+	*Bacillus* sp
OY35	+	Rod	+	+	+	*Bacillus cereus*
OY36	+	Rod	+	+	+	*Bacillus cereus*
OY37	+	Rod	+	-	+	*Bacillus* sp

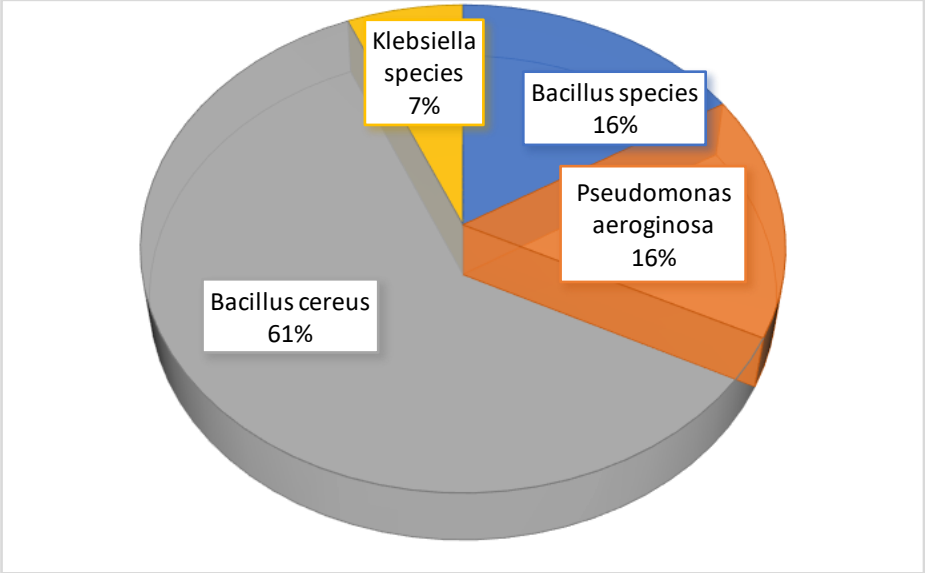

Figure 4: Bacterial species distribution in isolated water samples

Bacterial counts and well depth

Factors that control the proliferation of bacterial in water wells are somewhat diverse in nature, and include; well construction and design, depth of water table, nearness to contamination source and the geology, structural and textural nature of the aquifer and the vadose zones.

The cross plots of the depths of wells against respective bacteria counts and their corresponding relationship coefficients (R) are presented in Figure 5. The depth of wells and water table define well shallowness and it is known that shallower wells tend to have higher bacteria counts due to proximity to surface contaminant sources such as landfills and septic tanks (Chapelle, 2001; Madsen, et. al.2015; Foppen, et. al. 2008; Schafer, et. al. 2010). This is corroborated in the plots obtained at Awe, Ilora and Oyo towns whereby the relationships are indirect and from the R values- the relationships are fairly strong in Awe HDs (R = - 0.58), low in Oyo (R = - 0.21) and almost insignificant at Ilora (R = - 0.05). However, the relationship was direct but low for HDs at Akinmorin (R = 0.31) and the aforementioned insinuation does not hold. This is adjudged to be from better housing layouts and cleaner communities typical of Awe area.

Table 4: Morphological and Biochemical of the Gram-Negative Bacteria Isolated from groundwater

Isolate Code	Gram	Cell Shape	Oxidase	Catalase	Citrate	Glucose	Lactose	Sucrose	H_2S	Gas	Probable Organisms
AW5	-	Rod	+	+	+	-	+	+	+	+	*Pseudomonas aeroginosa*
AK12	-	Rod	-	+	+	+	+	+	-	+	*Klebsiella* sp.
AK14	-	Rod	+	+	+	-	+	+	+	+	*Pseudomonas aeruginosa*
AK15	-	Rod	-	+	+	+	+	+	-	+	*Klebsiella* sp
IL17	-	Rod	+	+	+	-	+	+	+	+	*Pseudomonas aeruginosa*
IL23	-	Rod	+	+	+	-	+	+	+	+	*Pseudomonas aeruginosa*
OY31	-	Rod	+	+	+	-	+	+	+	+	*Pseudomonas aeruginosa*

Cross-plots of nitrate and bacterial counts in groundwater as presented in Figure 6 is positive in three towns aside Awe. These direct relationships that R values ranging from high to low associations indicate that bacterial activities can lead to high nitrate concentration in water. It also shows that nitrate and bacterial can enter into groundwater from common sources and in this case from contaminated surface water infiltration. The relationships between nitrate and bacterial counts at Awe did not follow similar trend as obtained for other towns. The disparity of data evaluation obtained in Awe in comparison to other towns indicate a probable different prevailing environmental conditions and hydrogeological situations at Awe.

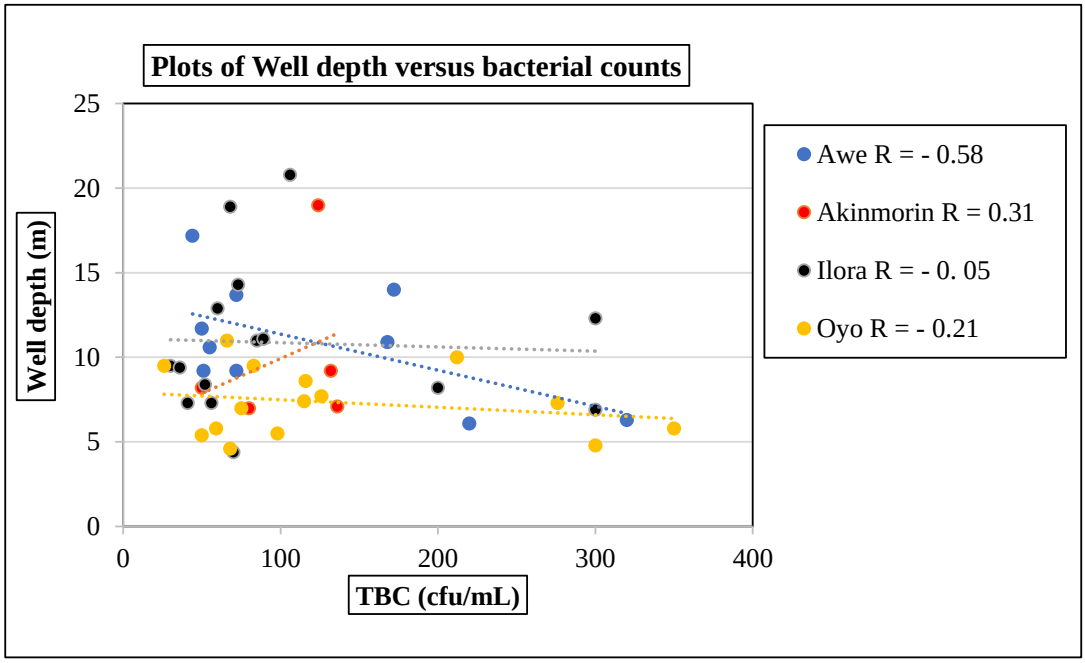

Figure 5: Cross-plots of well depth and Bacteria counts in Hand-dug wells

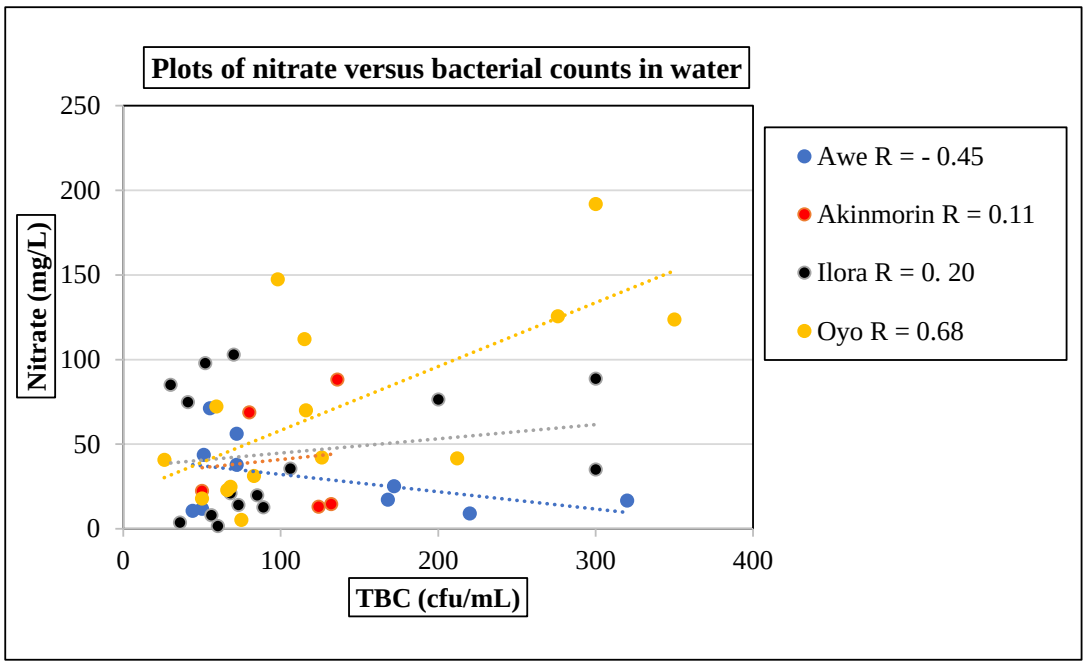

Figure 6: Cross-plots of nitrate and bacteria counts in water

CONCLUSION

In the present study, areas with nitrate levels exceeding permissible limits have been identified, notably in Sogidi area of Awe (AW08 and AW09), Baba Ode and Oke-Oja of Akinmorin (AK11 and AK12) and many locations in Ilora and Oyo communities. In fact, nitrate enrichment is higher than recommended limit of 50 mg/L in thirty-eight percent of all sampled hand-dug wells exceeded 50 mg/L recommended limits. Bacterial is found in all the sampled wells. This pathogen even occurred in very large amount that is too many to counts in five wells at Ohoho (AW02), Fojubale (IL17), Aro (IL23), Bara (OY31) and Sakoto (OY37). The identified bacterial species are *Bacillus cereus*, *Bacillus* species, *Pseudomonas aeroginosa* and *Klebsiella* species. From the present studies it was also seen that shallow well is most likely to be infested with bacteria and nitrate reduces at higher depth which implies that water from deeper wells will be more hygienic than shallow hand-dug wells.

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