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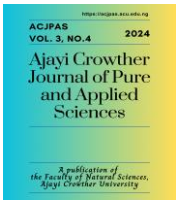

Article

Pollution Assessment of Shallow Groundwater of Urban Area of Part of Southwestern Nigeria

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Abstract

Water samples were collected from thirty hand – dug wells around Oyo township for bacterial and trace elements assessment. Total bacteria and coliform counts were performed using colony count and multiple tube technique and the pure culture was subjected to biochemical and sugar fermentation. Coliform count was estimated using Most Probable Number (MPN) method, while atomic absorption spectrophotometer (AAS) was used for the analyses of iron (Fe), lead (Pb), Zinc (Zn), Copper (Cu) and Cadmium (Cd) in the water samples. The total bacterial count (TBC) was very high as some were too numerous to be estimated. The MPN of the coliform bacteria was between 2 and 6 indicating faecal contamination in all the sampled hand-dug wells. Based on morphological, biochemical and TSI methods, the isolated bacteria from the water samples were identified to belong to two genera; *Escherichia* and *Citrobacter*. For trace element concentration (mg/L), the groundwater is uncontaminated with Zn, Cu and Cd but Fe and Pb occurred above the recommended limits in drinking water in many areas. Therefore, the dependent of rural dwellers on shallow groundwater should be placed under close monitoring so as to forestall outbreak of infection in the society. This is because the presence of coliform bacteria especially *Escherichia coli* is an indication of recent fecal or sewage contamination and presence of iron and lead above recommended levels is most likely from anthropogenic source.

Keywords: *Bacteria, trace element, groundwater, contamination, human input.*

1. Introduction

Human beings require certain trace elements for healthy living and when they are in short fall in diet, the growth and vitality of the body is affected. However, most trace elements are known to cause a wide range of adverse health effects because unlike some organic species and compounds, trace metals and metalloids are not readily assimilated by living organisms. Introduction of trace elements in organisms beyond the tolerable quantity can cause ailments such as dermatitis, cardiovascular diseases, central nervous system (CNS) disorders, lung, kidney and liver damage, birth defects and cancer [1,2,3]. Most human activities in urban areas generate wastes that contain toxic elements and release harmful bacterial to the environment and food chain of man [4,5]. This is more serious in cities lacking efficient waste disposal systems or treatment plants as most Nigerian cities, including the Oyo township in the south western part of Nigeria (Akanbi, et al., 2023). The fact that there is lack of water supply scheme makes many to rely on shallow groundwater system which further complicates and worsen public health of the people in these areas [6,7].

Other factors that can contaminate shallow groundwater are the presence of microbiological organisms that are commonly introduced from direct discharge and percolation of run-off from industrial and sewage effluents. Bacteria considered as indicator organisms for the assessment of the microbiological quality of water are faecal coliforms (FC) which are the major bacterial indicator of faecal pollution. The group of bacteria regarded as FC are bacteria associated with faecal wastes of human and animal origin [8]. Other group of bacteria associated with soil that can contaminate water apart from FC are called Total coliforms (TC) (e.g. bacteria commonly occurring in soil). The presence of coliforms in the water is an indicative of poor general hygienic quality of the water and potential risk factors of infectious diseases from water. High level of FC and TC in water can result in gastrointestinal tract infections which could be fatal and later cause death.

Presently, there is an increasing demand for protection of the quality of the groundwater resource of Nigeria from degradation due to domestic and municipal human activities. The rate of urbanization in Nigeria is alarming and the major cities are growing at the rates of 10 to 15% yearly [9]. Hence, reliance on groundwater due to its acclaimed purity and potability as a result of natural filtration process through subsurface environment is not always tenable. This is as a result of the fact that water has the ability to dissolve most substances along its flow path, including poorly disposed liquid and solid waste products which affects surface and groundwater quality.

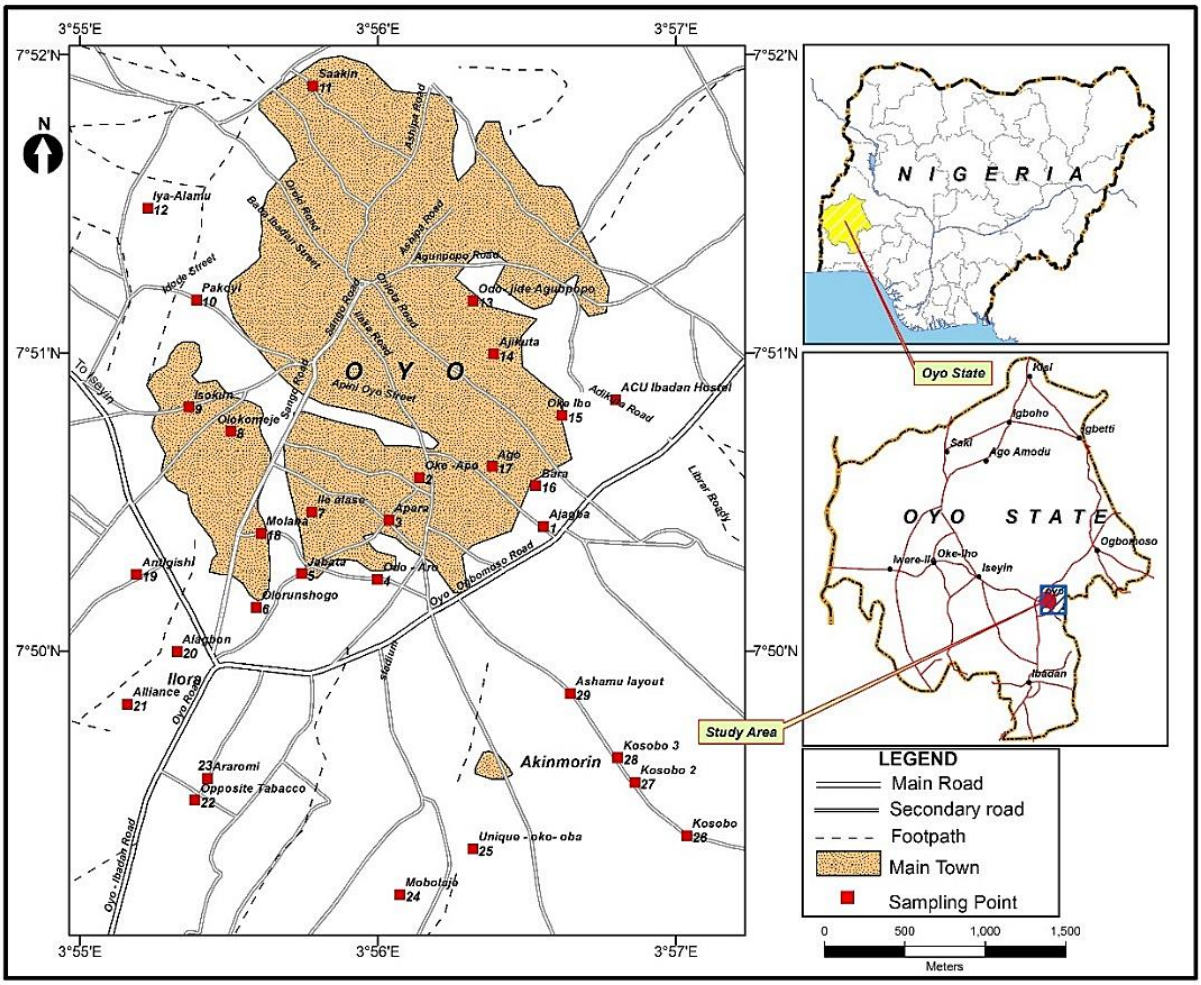

Figure 1: Location of the study area with sampled hand-dug wells

The present study is focused on the sub-urban areas of Oyo Town in southwestern Nigeria (Figure 1). In these areas, groundwater is the only reliable source of water supply. The water is mostly extracted from hand-dug wells at shallow depths mostly less than 15 m. In addition to this, many houses around the town lack proper solid and human waste disposal system, it is expedient to evaluate the microbes and some trace elements in the groundwater samples across Oyo township area.

2. Materials and Methods

2.1 Bacteria Contamination Analyses

For bacterial contamination analyses, the glass wares used 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. The 30 samples collected in disposable bottles were sterilized and transported to the laboratory for coliform detection and isolation of microorganisms within 24 - 48hours of collection. Multiple tubes techniques were used for detection of coliform in the water sample. Lactose broth was prepared to industrial standard specification with phenol red as indicator. The lactose broth (10 ml) was dispensed into test tubes as single strength while double concentration of the lactose was used as double strength for the lactose fermentation. 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.

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. Water sample were inoculated into sterilized lactose broth with Durham tube inverted to detect the presence of gas production. Different volume of water samples was 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 plate 1. The tubes were incubated for 48 hours at 37°C. MPN of the coliform was determined according to world health organization [10] standard.

2.2 Cultivation and Isolation of microorganisms

A loopful from the tube where fermentation and gas production occurred was streaked onto solidified Eosin Methylene Blue (EMB) agar which is a selective and differential media used for the identification of Gram-negative bacteria, specifically the Enterobacteriaceae. 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.

2.3 Cultural Identification and Colonial Morphology

The colonial morphology was examined to determine the macroscopic/visible characteristics of the colonies by gram staining. Gram staining is used to divide bacteria morphologically into two groups which are Gram positive (which appear purple colour) and Gram negative (which appear red or pink colour). The Gram staining of the bacteria isolates was 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 [11].

2.4 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 sulfide and gas production using Triple Sugar Iron (TSI) agar.

2.5 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 [12].

2.6 Sugar Fermentation

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, 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 sulfide from ammonium sulphate or sodium thiosulphate. The medium (15 ml) 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 24 hours bacterial isolates and stabbed at the bottom of the medium. The bottles were incubated at 37^{o}C for 24 hours [12].

2.7 Trace elements analyses

Minor and trace elements analysed for included iron (Fe), lead (Pb), Zinc (Zn), copper (Cu) and cadmium (Cd) using atomic absorption spectrophotometer (AAS) with air-acetylene gas mixture as oxidant. The samples were aspirated after calibrating the equipment with relevant standard solutions for each element (Fe, Pb, Zn, Cu and Cd). The results were recorded in mg/L.

3. Results and Discussion

3.1 Bacterial coliform count

The result of bacterial coliform count is presented in Table 1 and typical bacterial species identified in water from the morphological and biochemical characterization is presented in Table 2, while the measured and analysed physicochemical and trace elements concentrations are presented in Table 3. The statistical summary along with guideline limits of world health organization [10] and standard organization of Nigeria, [13] were presented in Table 4.

The lowest count of 3 and 4 cfu/ml (Estimates) was observed in locations 5 and 12. The bacterial load that was observed too high to be counted therefore regarded as too Numerous to Count (TNTC) in some of the water samples. There was no growth in the control sample (Table 1). The MPN determination for coliform count showed that the water samples contain coliform bacteria as the count observed was within the range of 2 – 6 coliform counts per 100 ml of water. Majority of the water samples were observed to have slightly higher estimate of coliform bacteria (Table 2).

The pure culture was identified based on their morphological and biochemical characteristics of the isolates. They are all Gram negative (that is bacteria with thin cell wall), rod shape and slender that is they are not robust. They exhibited the properties of fermenting all the three sugar and gas production. Few of them was able to produce H_2S (Table 3).

3.2 Bacterial contamination assessment

The total bacterial and coliform counts were evaluated in this study. The total bacterial count was found to be very high in some locations within the study are which agreed with the report of Belghiti *et al.* [14]. It was found that the total bacterial count in two of the locations were very low which is in line

with earlier report of Said Lotfi *et al.,* (2020) [15]. The total coliform counts of all the water samples were found to be less than 10 coliform counts. These findings were in agreement with standard requirement of 10 total coliform count per 100 ml for NSDWQ but in deviant from the standard of WHO and NSDWQ that emphasize zero total coliform count per 100 ml [16,10]. Bacteria isolated from the water samples were mainly *Citrobacter freundii* and *Escherichia coli* which are indicators of feacal or sewage contamination of the groundwater. These bacteria are part of the coliform that serve as indicator of feacal or sewage contamination.

Table 1: TBC and MPN of Coliform

S/N	Sample No	Location name	CFU/ml*	10 ml		1 ml		0.1 ml		MPN
				Lac	Gas	Lac	Gas	Lac	Gas	
1	OY01	Ajagba	15	+	+	+	+	-	-	4
2	OY02	Oke-Apo	TNTC	+	+	-	-	-	-	2
3	OY03	Apara	TNTC	+	+	+	-	-	-	2
4	OY04	Odo-Aro	TNTC	+	+	+	+	+	+	6
5	OY05	Jabata	3	+	+	+	+	+	+	6
6	OY06	Olorunshogo	TNTC	+	+	+	+	+	+	6
7	OY07	Ile-Alase	26	+	+	+	+	+	+	6
8	OY08	Olokomeje	11	+	+	+	+	+	+	6
9	OY09	Isokun	67	+	+	+	+	-	-	4
10	OY10	Pakoyi	53	+	+	+	+	+	+	6
11	OY11	Saakin	112	+	+	+	+	+	+	6
12	OY12	Iyalamu	4	+	+	+	+	-	-	4
13	OY13	Agunpapo	TNTC	+	+	+	+	+	+	6
14	OY14	Adikuta	TNTC	+	+	+	+	+	-	4
15	OY15	Oke Ebo	12	+	+	-	-	-	-	2
16	OY16	Bara	144	+	+	+	+	+	+	6
17	OY17	Ago	52	+	+	-	-	-	-	2
18	OY18	Molaba	30	+	+	+	+	+	+	6
19	OY19	Antigishi	18	+	+	+	+	-	-	4
20	OY20	Alagbon	72	+	+	+	+	+	+	6
21	OY21	Alliance	100	+	-	+	+	+	-	1
22	OY22	Tobacco	92	+	+	+	+	-	-	4
23	OY23	Araromi	128	+	+	+	+	+	+	6
24	OY24	Mabolaje	48	+	-	+	+	-	-	1
25	OY25	Oko-Oba	7	+	+	+	+	+	+	6
26	OY26	Kosobo	TNTC	+	+	+	+	+	+	6
27	OY27	Kosobo-2	60	+	+	+	+	+	+	6
28	OY28	Kosobo-3	20	+	+	-	-	-	-	2
29	OY29	Ashamu	38	+	+	+	+	+	+	6
30	OY30	ACU	12	+	+	-	-	-	-	2

*CFU/ml – Colony Forming Unit per milliliter of water sample

Table 3: Values of pH, water conductivity, TDS, anions with minor and trace elements in groundwater samples collected within the study area

S/N	Sample No	Location name	pH	EC µs/cm	TDS	NO_3	Cl^-	SO^{2-}_4	Fe	Pb	Zn	Cu
					mg/L							
1	OY01	Ajagba	6.89	25.2	61.73	0.1	9.88	10.58	0.61	0.11	0.084	0.107
2	OY02	Oke-Apo	6.74	55.5	202.78	2.14	12.48	26.61	ND	0.29	0.021	0.049
3	OY03	Apara	6.83	75.4	119.91	1.89	8.88	19.35	ND	0.54	0.182	0.093
4	OY04	Odo-Aro	6.81	74.2	175.05	7.08	10.94	20.94	ND	0.15	0.308	0.184
5	OY05	Jabata	6.67	45.5	102.14	6.94	8.16	9.30	ND	ND	0.011	0.098
6	OY06	Olorunshogo	7.22	48.8	96.79	2.11	2.75	15.20	ND	0.04	ND	0.187
7	OY07	Ile-Alase	6.51	64.6	106.72	2.32	11.86	5.97	ND	0.22	ND	0.12
8	OY08	Olokomeje	6.73	57.1	97.91	8.96	10.75	1.00	ND	0.41	0.241	0.191
9	OY09	Isokun	6.69	74	162.27	9.04	12.14	19.58	ND	0.36	ND	0.144
10	OY10	Pakoyi	6.56	55.5	75.24	2.02	13.01	9.60	0.04	0.30	ND	0.103
11	OY11	Saakin	6.94	89.8	124.79	2.17	10.14	18.30	ND	0.12	0.417	0.164
12	OY12	Iyalamu	7.38	79.7	276.27	3.28	6.82	31.45	ND	0.70	0.506	0.186
13	OY13	Agunpapo	7.09	99.7	225.61	3.45	7.85	27.37	ND	ND	0.198	0.166
14	OY14	Adikuta	7.27	77.8	156.63	1.85	11.36	14.97	0.17	0.09	0.056	0.056
15	OY15	Oke Ebo	7.19	51.5	71.22	2.79	13.85	7.33	0.22	ND	ND	0.078
16	OY16	Bara	7.20	60.7	89.58	7.99	5.61	12.40	0.16	0.15	ND	0.047
17	OY17	Ago	6.77	46.3	97.35	7.82	10.02	11.23	0.06	0.08	ND	0.036
18	OY18	Molaba	6.88	64.6	146.36	8.71	8.14	21.62	ND	0.27	ND	0.093
19	OY19	Antigishi	6.99	65.6	100.32	8.58	7.66	20.38	ND	0.28	0.094	0.105
20	OY20	Alagbon	6.98	59.4	86.36	3.17	3.61	18.90	0.45	0.43	ND	ND
21	OY21	Alliance	7.35	80.4	139.01	2.32	5.08	21.38	0.02	0.05	ND	0.035
22	OY22	Tobacco	6.80	43.8	65.68	1.71	6.99	5.89	ND	0.15	ND	0.068
23	OY23	Araromi	7.17	27.18	99.66	0.10	18.91	4.82	ND	ND	ND	0.030
24	OY24	Mabolaje	6.15	20.3	73.65	0.65	21.08	1.08	0.42	ND	ND	0.05
25	OY25	Oko-Oba	6.58	39.1	49.93	1.41	17.56	BDL	0.67	0.01	0.241	0.123
26	OY26	Kosobo	7.00	18.48	31.23	2.79	7.84	3.64	ND	0.51	ND	0.106
27	OY27	Kosobo-2	6.58	12.78	32.3	0.10	1.01	BDL	0.18	0.59	ND	0.104
28	OY28	Kosobo-3	6.28	6.89	12.84	0.10	2.18	BDL	ND	0.38	ND	0.144
29	OY29	Ashamu	6.07	19.31	43.34	0.89	6.19	2.79	ND	0.26	0.197	0.172
30	OY30	ACU	6.63	66.2	89.3	0.10	8.46	12.32	ND	0.15	0.481	0.161

Table 4: Statistical summary of parameters with world health standards, WHO (2017)

Parameters	Minimum	Maximum	Mean	WHO 2017 Guidelines (mg/L)	SON, (2007) Guidelines (mg/L)
pH	6.07	7.38	6.83	-	6.5 – 8.5
EC	6.89	99.70	53.51	1500	1000
TDS	12.84	276.27	107.07	600	500
NO_3	0.10	9.04	3.42	50	50
Fe	0.02	0.67	0.082	0.3	0.3
Pb	0.01	0.7	0.225	0.01	0.01
Zn	0.011	0.506	0.101	3	3
Cu	0.030	0.191	0.107	2	1
Cd	ND	ND	ND	0.003	0.003
TBC	3	TNTC	-	-	-
TCC	1	6	0.182	0	0

3.3 Physico-chemical parameters

The pH which is the measure of acidity/alkalinity of water was between 6.07 to 7.38 and the average is 6.83. The acceptable range for drinking water was between 6.5 and 8.5 [10] (WHO, 2017). With this limit, the pH of three wells at Mabolaje, Kosobo 3 and Ashamu were below 6.5 and are classified as slightly acidic. The total dissolved solids (TDS) and electrical conductivity (EC) were quite low and are not of health concern by the values obtained in all wells. TDS of not more than 500 mg/L are generally acceptable as fresh water for drinking purpose [13]. The TDS of the groundwater samples were between 12.84 and 276.27 mg/L. TDS is an indicator of the degree of mineral/rock dissolution in water. The TDS values obtained for the water samples are quite low and this indicated that the water is shallow and of meteoric source and that the groundwater has not been involved in much mineral dissolution.

Knowing the concentrations of Nitrate in water is important since high concentration can trigger diseases such as Methemoglobinemia in infants. Abundance of nitrate in water also promotes bacterial growth. Nitrate concentration in water spread from 0.1 to 9.04 mg/L. These concentrations are very low compared with the guideline limits of 50 mg/L (Table 4).

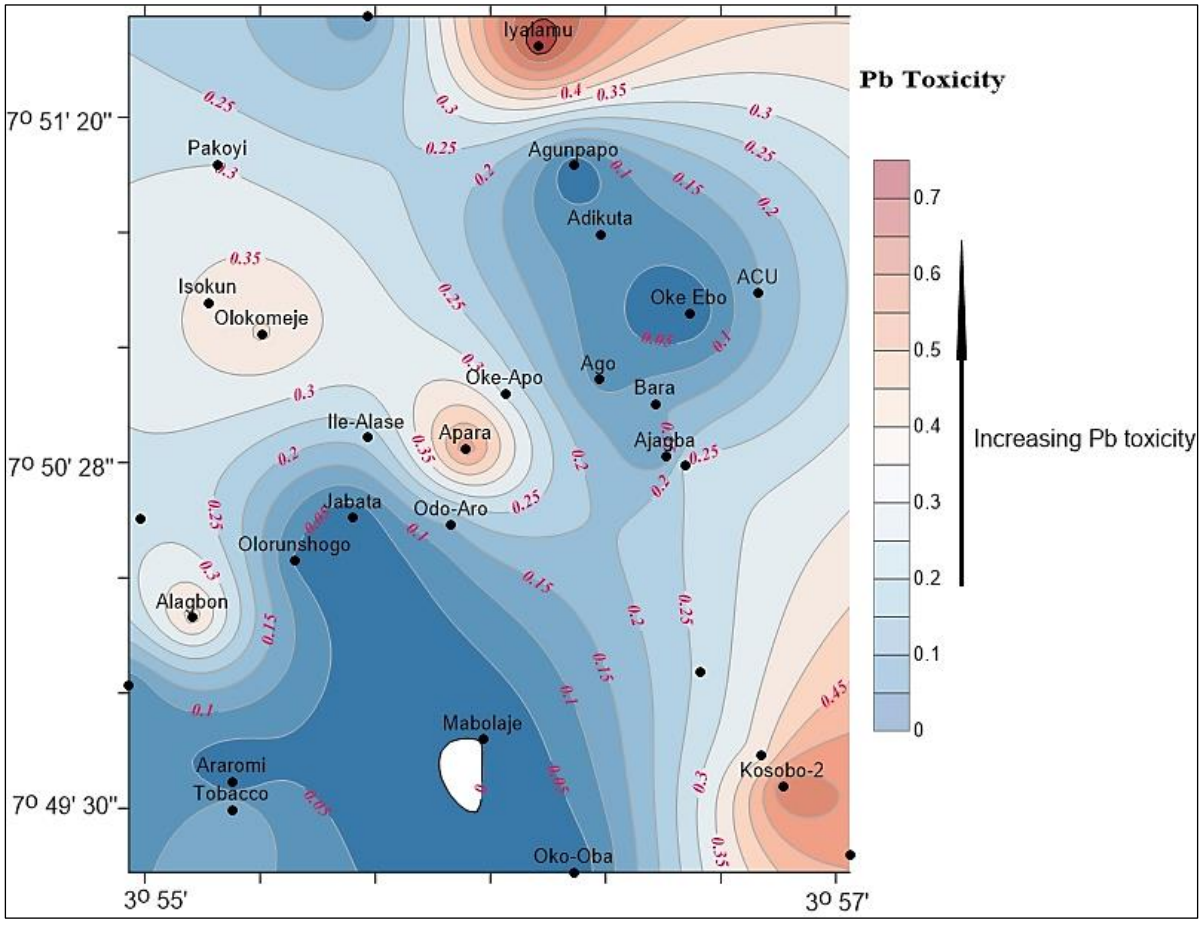

Figure 2: Spatial distribution of Pb toxicity in wells across the study area

3.4 Minor and Trace elements concentrations

Fe was detected in ten (10) locations with the highest concentration occurring at Oko-Oba in sample (OY25) with concentration of 0.67mg/L (Table 4). Fe occurrence in natural water is not of health concern but when the concentrations exceed 0.3 mg/L it gives offensive taste and odour (WHO, 2017). Other hand-dug wells with sensitive Fe concentrations are found at Ajagba OY01, Alagbon (OY20) and at Mabolaje (OY24). Lead (Pb) occurred at significant level in most of the hand-dug wells. The concentrations in twenty-five wells exceeded the guideline limits of 0.01 mg/L. Pb occurred below

detection limit in five wells while its occurrence was very significant in most other wells particularly at Apara, Olokomeje, Iyalamu and Kosobo. The spread of lead toxicity is presented in Figure 2. Zinc being an essential trace element was found in eleven wells but the concentrations were all far below hazardous level of 3 mg/L in the wells. zinc is an essential element required by human for efficient body metabolism. Copper is also found in the samples, except at Alagbon in sample (OY20). Cu concentrations in hand-dug wells were between 0.03 and 0.19 mg/L. Cu is also an essential trace element having a tolerable limit of 2 mg/L in drinking water. Cadmium was not detected at all in any of the well.

4. Conclusions

The presence of coliform bacteria in water is an outright indication of fecal contamination. So, the groundwater of the study area is not potable as a result of bacterial contamination. Nitrate concentration in water is far from being at a threat level; however, from the results of trace elements concentrations; Pb occurred beyond tolerance levels in most of the wells. Likewise, Fe concentrations in three wells were above the recommended limits. The concentrations of other trace elements in the wells are not at contamination levels, even then; occurrence of trace elements at non-toxic levels in drinking water is still a health concern to human since most trace elements are able to bioaccumulate in living organic cells and built- up to toxic levels once there is continual ingestion.

It is therefore highly recommended that the groundwater of the study area be treated for lead and bacterial contamination prior to drinking. Furthermore, a better alternative is for reticulation of these wells and establishment of communal water treatment plants by appropriate government agencies in addition to ensuring that groundwater wells are sited in vicinity that is far from human waste disposal systems such as latrines, soak-aways and dumpsites.

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The author declared no conflict of interest in the manuscript.

Authors' Declaration
The author(s) hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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