Original Research Article

Evaluation of Microbiological Quality of Water, Sediment and Soil Characteristics in Okrika Local Government Area, Rivers State, Nigeria.

ABSTRACT

Water is the most important resource on earth and safe drinking water is essential to sustain life. Most rural communities do not treat their water before consumption. Therefore, the aim of this study was to evaluate the microbiological quality of water, sediment and soil characteristic in Okrika LGA, Nigeria. Samples were collected using sterile bottles from boreholes, hand-dug wells, surface water, sediments and soils using standard methods and analysed accordingly. Results show that Total Heterotrophic Bacterial Count (THBC) ranged from 8.5×10^1 in Kalio-Ama to 2.72×10^5 at Okari-Ama. Total Fecal count (TFC) ranged from 0 to 5.95×10^3 at Isaka Town. Total Coliform Count (TCC) ranged from 0 to 3.95×10^4 at Ogan-Ama. Total Salmonella Shigella Count (TSSC) ranged from 0 to 5.35×10^3 in Ogan-Ama. Total Vibio Count (TVC) ranged from 0 to 2.29×10^4 at Kalio-Ama. The most prominent bacterial isolates from all the stations are of the genera such as *Bacillus, Staphylococcus, Escherichia Coli, Micrococcus, Kiebsiella, Pseudomonas*, *Vibrio*, *Aeromonas, Serratia* and *Alcaligenes* which were isolated across the samples with *Bacillus* as more frequent with 50%. In the dry season, bacterial isolates were 100% susceptible to Gentamycin and Ofloxacin and 100% resistance to *Augumentin*, *Cefuroxime* and Cefixime. In the wet season, isolates had 100% susceptibility to Ciprooflozacin and 100% resistance to Cloxacin, Augmentin and Gentamicine. The result of this study poses a public health risk to consumers who use these sources of water for domestic purposes, recreation and treatments. Diseases like typhoid, cholera, polio, skin and lung infections are eminent.

Keywords: Water quality, Groundwater, Surface water, Water contamination, Sediment, Soil, Antibiotic resistance, Okrika.

1. Introduction

Water is crucial for the survival of living organisms including the functioning of the ecosystems. Preservation of water quality is important for drinking water supply, domestic, ecological and recreational use (LIopis-Gonzalez *et al.*, 2014). Groundwater and surface water are being contaminated by wide range of microbes including faecal coliforms (Sadeghi *et al.*, 2007), through various anthropogenic activities. Majority of these microbes can be carried through urban runoffs, erosion or flooding during heavy rainfalls into rivers. However, contaminated water can cause a range of diseases in the form of gastrointestinal disturbances to life - threatening infections (Ochuko and Thaddeus, 2013; Ogbonna, 2014). Depending on the source, the microbiological quality of water is traditionally assessed by monitoring bacteria of faecal origin like *E.coli* and Enterococcus species (Romper *et al.*, 2002). Their presence in water indicates that such water is not potable.

According to World Health Organisation (WHO, 2019), over 2 billion people use drinking water sources contaminated with faeces. However, 80% of diseases in developing countries are either water borne or sanitation related because majority of the groundwater and surface waters consumed are untreated in rural areas. In Okrika Local Government Area, residents solely depend on groundwater from boreholes and hand-dug wells and are mostly untreated. In Okrika, several companies like Port Harcourt Refinery Company, Notore fertilizer Company generate all manner of wastes. Otokunefor and Obiukwu (2005) observed that the quality of refinery effluent and its impact on the physicochemical quality of Okrika arm of the Bonny River estuary has high oil and grease concentration in the effluent receiving water body in combination with other pollutants. This could have been responsible for the depletion of fish and other aquatic life at the point of impact of the effluent.

However, pathogenic microorganisms present in groundwater are found more along facilities that are discharging sewage effluents or already contaminated surface waters from similar sources (Egboka *et al*.,1989; Ogbonna and Eheriehne, 2017). Shallow wells and deep boreholes are prone to contamination by these pathogens. They can enter from inappropriate waste disposal, discharge of sewage into lagoons, barnyards and landfills. In addition to the presence of *Vibrio cholera* (cholera) and other pathogens such as *Escherichia coli* (gastroenteritis), *Shigella dysenteriae* (dysentery), *Salmonella enteritidis* (gastroenteritis), *Mycobacterium tuberculosis* (tuberculosis) and *Salmonella typhi* (typhoid fever) are of public health concern (Azuonwu *et al.,* 2017). Most of these diseases are transmitted through anthropogenic activities such as improper disposal of wastes which include human, domestic and industrial wastes (Lynch *et al.,* 2006; Odu *et al*; 2010) and are carried in fecal materials discharged into water bodies. (Su and Liu, 2007; Adeleye *et al*., 2010; Edun and Akinrotimi, 2011). Pathogenic bacteria in marine waters are most abundant in the sediments, which can be found on the surface film and in the water column (Bassey *et al;* 2014).

Investigations have shown that sediments can constitute a reservoir of pathogenic microorganisms which can persist in a water body. Subsequently, possible resuspension of faecal indicator bacteria and other pathogens can affect water quality and increase health risks to the population during recreational activities. Therefore, sediment provides complimentary information about the sanitary quality of surface water under tropical conditions (Mwanamoki *et al*., 2014). This study therefore seeks to determine the microbiological quality of groundwater, surface water, sediment and soil in Okrika L.G.A.

2. Materials and Methods

2.1 Sampling Locations

Samples for this study were collected from nine different communities namely; Ogan-Ama,Orupabo-Ama, Kalio-Ama, Geoge-Ama, Abam-Ama, Bulome-biri,Ogoloma,Okari-Ama and Ekerekana in Okrika Local Government Area, Rivers State, Nigeria during dry and rainy seasons. Location of sample points were taken with the use of a Global Positioning System (GPS). A map showing the sampling stations is presented in Figure 1. while a table showing Coordinates of sample points is presented in Table 1.

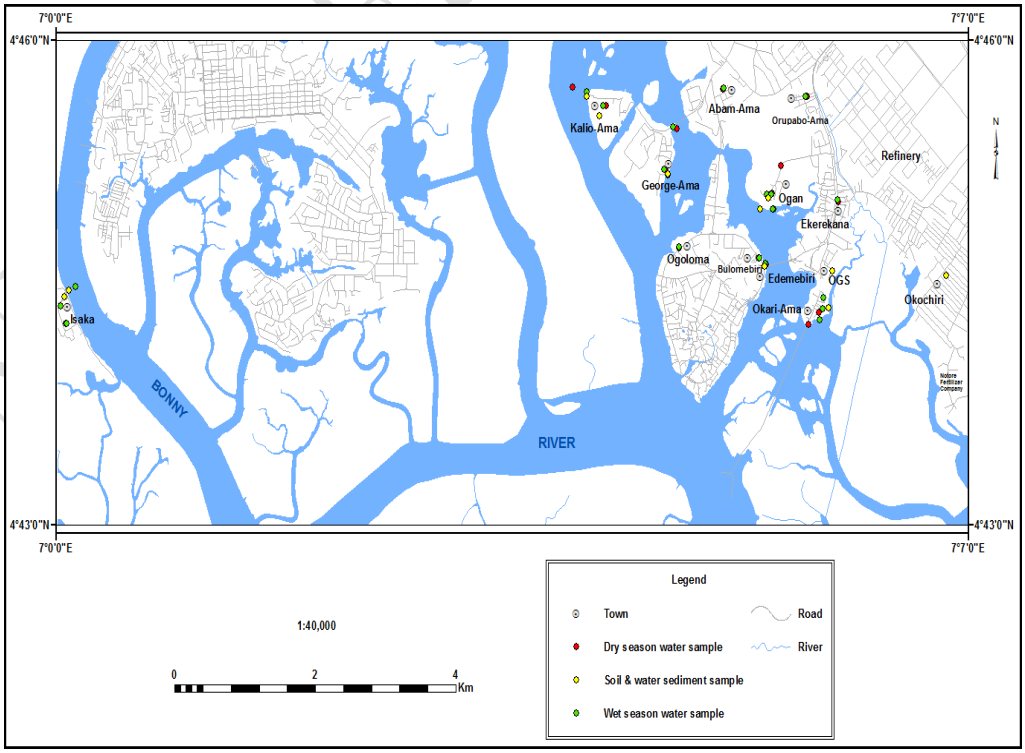

Figure 1: Map showing Okrika Local Government Area with Sampling Locations.

Table 1: Coordinates of the Study Area (Okrika Local Government)

Borehole

S/No.	Location/Sample Stations	Latitude	Longitude Samples
	(Dry Season)		
1.	Ogan-Ama	4°45'13.52"N	7° 5'33.37"E
2.	Orupabo-Ama	4°45'38.98"N	7° 5'45.28"E
3.	Kalio-Ama	4°45'35.74"N	7° 4'11.86"E
4.	Geoge-Ama	4°45'11.93"N	7° 4'39.81"E
5.	Abam-Ama	4°45'42.17"N	7° 5'6.93"E
6.	Bulome-biri	4°44'38.95"N	7° 5'22.83"E
7.	Ogoloma	4°44'42.93"N	7° 4'46.83"E
8.	Okari-Ama	4°44'19.18"N	7° 5'51.01"E
9.	Ekerekana	4°45'0.53"N	7° 5'59.64"E
	(Wet Season)		
1.	Ogan-Ama	4°45'3.10"N	7° 5'27.00"E
2.	Orupabo-Ama	4°45'38.98"N	7° 5'45.28"E
3.	Kalio-Ama	4°45'35.74"N	7° 4'11.86"E
4.	Geoge-Ama	4°45'11.93"N	7° 4'39.81"E
5.	Abam-Ama	4°45'42.17"N	7° 5'6.93"E
6.	Bulome-biri	4°44'38.95"N	7° 5'22.83"E
7.	Ogoloma	4°44'42.93"N	7° 4'46.83"E
8.	Okari-Ama	4°44'19.97"N	7° 5'52.51"E
9.	Ekerekana	4°45'0.53"N	7° 5'59.64"E
10.	Isaka Town	4°44'14.60"N	7° 0'4.43"E
11.	Isaka Town	4°44'22.42"N	6°59'59.86"E

Hand- Dug Well

S/No.	Location/Sample Stations	Latitude	Longitude
	(Dry Season)		
1.	Ogan-Ama	4º45'3.03"N	7º5'29.09"E
2.	Okari-Ama	4º44'24.24"N	7º5'53.12"E
	(Wet Season)		
1.	Ogan-Ama	4°45'3.03"N	7° 5'29.09"E
2.	Okari-Ama	4°44'24.24"N	7° 5'53.12"E
3.	Isaka Town	4°44'14.68"N	7° 0'4.36"E

Surface Water

S/No.	Location/Sample Stations	Latitude	Longitude
	(Dry Season)		
1.	Ogan –Ama	4°44'57.14"N	7° 5'29.43"E
2.	Kalio-Ama	4°45'42.57"N	7° 3'57.51"E
3.	Geoge-Ama	4°45'26.98"N	7° 4'44.35"E
4.	Edeme-biri	4°44'36.33"N	7° 5'26.35"E

5.	Okari-Ama	4°44'14.27"N	7° 5'46.53"E
1.	**(Wet Season)** Ogan-Ama	4°44'57.14"N	7° 5'29.43"E
2.	Kalio-Ama	4°45'40.72"N	7° 4'4.52"E
3.	Geoge-Ama	4°45'26.98"N	7° 4'44.35"E
4.	Edeme-biri	4°44'37.10"N	7° 5'26.15"E
5.	Okari-Ama	4°44'16.42"N	7° 5'51.42"E
6.	Isaka Town	4°44'28.48"N	7° 0'8.70"E

Sediment

S/No.	Location/sample Stations	Latitude	Longitude
1.	Ogan-Ama	4°44'57.09"N	7° 5'23.95"E
2.	Okari-Ama	4°44'20.86"N	7° 5'55.55"E
3.	Edeme-biri	4°44'36.52"N	7° 5'25.71"E
4.	George-Ama	4°45'10.64"N	7° 4'41.47"E
5.	Kalio-Ama	4°45'39.48"N	7° 4'4.14"E
6.	IsakaTown	4°44'27.39"N	7° 0'5.13"E

Soil

S/No.	Location/Sample Stations	Latitude	Longitude
1.	Okochiri Community	4°44'36.01"N	7° 7'13.03"E
2.	Okrika Grammar School	4°44'34.92"N	7° 5'57.42"E
3.	George-Ama	4°45'10.29"N	7° 4'40.90"E
4.	Ogan-Ama	4°45'1.38"N	7° 5'27.67"E
5.	Isaka Town	4°44'25.46"N	7° 0'3.76"E

2.2 Area of Study

Okrika local Government Area of Rivers State is located within the Niger Delta region, one of the zones in the coastal part of Southern Nigeria. The area lies between latitudes 4°43'0"N and 4°46'0"N and longtitudes 7° 0'0"E and 7° 7'0"E. It is a mangrove environment characterized by consistent salt water inundation as a result of tidal action and flooding, extensive sandy bottom and mudflat. The climate in Okrika is characterized majorly by two seasons, the dry season which begins in November and ends in February, while the wet season begins in March and ends in October. Annual rainfall is about 3000m and distinct relative humidity and evaporation. It is ecologically endowed with vast biodiversity like, fish,crustaceans,mollusc, crabs and others (Ogunola and Falaye (2019)

The area plays host to the Port Harcourt Refinery Company (PHRC) and the Pipeline Product Marketing Company (PPMC),all subsidiaries of the Nigerian Petroleum Corporation (NNPC). The area is also host to the Okrika Refinery jetty and terminal used for loading and unloading of oil and gas products and other oil and gas related activities. Okrika creeks are used for effluent/wastewater drainage outlets from the Port Harcourt Refinery and former National Fertilizer Company of Nigeria (NAFCON), now Notore Fertilizer Company, leading to its vulnerability to ecological degradation and damage. Other major activities carried out along the creeks are fishing, recreation, dredging, transportation and discharge of wastes (Marcus *et al*.,2013).

2.3. Samples Collection

A total of ten samples each of groundwater and surface water samples were collected each locations using sterile sample bottles, sediment and soil samples were collected using sterile spatula to scoop sediment and soil matter

into sterile sample bottles. All samples were kept in ice-packed coolers and transported immediately to the microbiology laboratory. Samples were collected from February, 2020 to November, 2020.

2.4. Microbiological Analyses

2.4.1:Serial Dilution

One millilitre each of the water samples were separately added to 9 ml of normal saline (diluents). After thorough shaking, further 10-fold (v/v) serial dilutions were made by transferring 1 ml of the original solution to freshly prepared normal saline diluents to a range of 10^{-4} dilutions (Prescott *et al.,* 2005).

2.4.2: Inoculation, Incubation and Enumeration

2.4.2.1:Total Heterotrophic Bacteria (THB)

Total Heterotrophic Bacteria was enumerated as described by Prescott *et al* (2005). Bacterial Colonies that appeared on the nutrient agar plates which were inoculated in duplicate with an aliquot of 0.1ml. The inocula were then spread evenly on the surface of the media using a sterile spreader.and incubated for 24 hours at 37.2°C were counted and the mean expressed as cfu/ml/g for the samples (Ogbonna *et al.,* 2019). The colony forming unit per millilitre/gram was calculated using the formula below;

$$\text{CFU/ml} = \frac{\text{number of colonies}}{\text{Dilution x volume plated}}$$

2.4.2.2:Total Coliform Counts (TCC)

Total Coliform Counts was enumerated as described by Prescott *et al* (2005). Bacterial Colonies that appeared on the MacConkey agar plates which were inoculated in duplicate with an aliquot of 0.1ml and incubated for 24 hours at 37.2°C were counted and the mean expressed as cfu/ml/g for the samples (Ogbonna *et al.,* 2019).

2.4.2.3: Feacal Coliform Counts

Feacal Coliform Counts was enumerated as described by Prescott *et al*, (2005). Bacterial Colonies that appeared on the Erosin Methylene Blue (EMB) agar plates which were inoculated in duplicate with an aliquot of 0.1ml and incubated for 24 hours at 44.2°C were counted and the mean expressed as cfu/ml/g for the samples (Ogbonna *et al.,* 2019).

2.4.2.4:Total *Salmonella-Shigella* Counts (SSC)

This was determined with the *Salmonella-Shigella* agar using the spread plate method as described by Prescott *et al*. (2005). Bacterial Colonies that appeared on the *Salmonella-Shigella* aga plates which were inoculated in duplicate with an aliquot of 0.1ml and incubated for 24 hours at 37.2°C were counted and the mean expressed as cfu/ml/g for the samples (Ogbonna *et al.,* 2019).

2.4.2.5:Total *Vibrio species* Count

Total *Vibrio* count was determined with the Thiosulphate Citrate Bile Salt (TCBS) agar using the spread plate technique as described by Prescott *et al.,* (2005). Inoculate 0.1m of the serially diluted samples onto sterile pre-dried TCBS agar plates in duplicates. The inocula were then spread evenly on the surface of the media using a sterile spreader. The plates were then incubated at 37^{o}C for 24 hours, after which the colonies that developed were counted and the mean total *Vibrio* counts were recorded.

2.4.3: Purification of Isolates

After incubation, pure isolates were obtained by picking (with sterile inoculating loop) distinct culturally and morphologically different colonies from the various plates. These were subjected to streaking on sterile nutrient agar in plates until pure distinct colonies were formed described by Prescott *et al*. (2005).

2.4.4:Identification of Bacterial Isolates by Cultural Methods

Pure bacterial isolates were identified by the method described by Collins *et al.,* (1989), and Cheesbrough (2006). Pure bacterial isolates were subjected to Biochemical tests. Bacterial isolates were identified with reference to the Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994)

2.4.5: Antibiotics Susceptibility Testing

The antibiotic susceptibility patterns of the isolates to common antibiotics were evaluated using the Kirby Bauer disc diffusion technique and 0.5 MacFarland's ($1.5x10^{8}$ cfu/ml) was employed in inoculum suspensions preparation according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) and Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2006; Okonko *et al*., 2009).

3. Results and Discussion

The result of the microbiological analysis are presented in Tables 2-6. The microbial counts for Heterotrophic bacteria were observed to be high in wet season for borehole water, hand-dug well, sediment and soil samples.

Table 2: Seasonal Variations for Total Heterotrophic Bacteria Count (THBC) Across Five Locations

S/N	Locations	Borehole		Hand-dug well		Surface Water		Sediment	Soil
		Dry Season	Wet Season	Dry Season	Wet season	Dry Season	Wet season		
1	Ogan Ama	4.55×10^{3}	2.0×10^{3}	8.0×10^{3}	3.9×10^{4}	1.27×10^{4}	2.45×10^{3}	4.45×10^{2}	4.3×10^{4}
2	Orupabo Ama	7.10×10^{3}	1.02×10^{5}	-	-	-	-	-	-
3	George Ama	8.15×10^{3}	1.0×10^{4}	-	-	2.9×10^{3}	5.1×10^{4}	1.0×10^{3}	4.1×10^{4}
4	Kalio Ama	3.95×10^{3}	4.85×10^{4}	-	-	7.92×10^{4}	1.31×10^{5}	8.5×10^{1}	-
5	Abam Ama	1.22×10^{5}	1.0×10^{4}	-	-	-	-	-	-
6	Ogoloma	3.80×10^{3}	1.5×10^{4}	-	-	-	-	-	-
7	Bulomebiri	4.0×10^{3}	4.15×10^{4}	-	-	-	-	-	-
8	Ekerekana	7.0×10^{3}	7.50×10^{3}	-	-		-	-	
9	Okochiri	-	-	-	-	-	-	-	3.1×10^{4}
10	Okrika Grammar School	-	-	-	-	-	-	-	2.1×10^{4}
11	Okari-Ama	6.15×10^{3}	2.72×10^{5}	7.25×10^{3}	2.75×10^{4}	3.08×10^{4}	2.56×10^{5}	1.25×10^{3}	-
12	Edeme-biri	-	-	-	-	9.99×10^{4}	7.9×10^{4}	6.45×10^{2}	-
13	Isaka Town 1	-	1.02×10^{5}	-		-	-	-	-
	Isaka Town 2	-	2.07×10^{5}	-	1.70×10^{5}	-	1.88×10^{5}	9.65×10^{2}	2.01×10^{4}

(-): Not determined

Table 3: Seasonal Variations for Total Fecal Count (TFC) Across Five Locations

S/N	Locations	Borehole		Hand-dug well		Surface Water		Sediment	Soil
		Dry Season	Wet Season	Dry Season	Wet season	Dry Season	Wet season		
1	Ogan Ama	0	0	0	0	0.45×10^2	3.5×10^2	4.40×10^2	5.15×10^4
2	Orupabo Ama	0	0	—	—	—	—	-	-
3	George Ama	0	0	—	—	0	0	5.5×10^3	5.15×10^4
4	Kalio Ama	0	0	—	—	0	2.45×10^3	1.25×10^3	-
5	Abam Ama	0	0	—	—	—	—	-	-
6	Ogoloma	0	0	—	—	—	—	-	-
7	Bulomebiri	0	6.0×10^2	—	—	—	—	-	-
8	Ekerekana	0	0	—	—	—	—	-	-
9	Okochiri	-	-	-	-	-	-	-	1.75×10^4
10	Okrika Grammar School	—	—	—	—	-	-	-	7.5×10^3
11	Okari-Ama	1.27×10^3	4.50×10^2	0.1×10^2	6.0×10^2	9.70×10^2	4.50×10^2	7.0×10^2	-
12	Edeme-biri	-	-	-	-	2.95×10^2	0	4.1×10^3	-
13	Isaka Town 1	—	26.50×10^2	—		—		—	-
	Isaka Town 2	—	5.95×10^3		5.35×10^3		4.7×10^3	2.35×10^3	3.1×10^4

(-): Not Determined
(0): Not Determined

Table 4: Seasonal Variations for Total Coliform Count (TCC) Across Five Locations

S/N	Locations	Borehole		Hand-dug well		Surface Water		Sediment	Soil
		Dry Season	Wet Season	Dry Season	Wet season	Dry Season	Wet season		
1	Ogan Ama	1.25×10^{2}	0	8.05×10^{2}	5.50×10^{2}	0.10×10^{2}	7.0×10^{2}	0	3.95×10^{4}
2	Orupabo Ama	7.30×10^{2}	2.0×10^{2}	-	-	-	—	-	-
3	George Ama	4.20×10^{3}	0.50×10^{2}	-	-	0.30×10^{2}	1.13×10^{4}	0	1.95×10^{4}
4	Kalio Ama	7.95×10^{2}	1.20×10^{3}	-	-	5.05×10^{2}	1.80×10^{4}	5.0×10^{3}	-
5	Abam Ama	0	1.0×10^{2}	-	-	-	-	-	-
6	Ogoloma	9.05×10^{2}	5.0×10^{2}	-	-	-	-	-	-
7	Bulomebiri	1.31×10^{3}	6.1×10^{3}	-	-	-	-	-	-
8	Ekerekana	4.05×10^{2}	0	-	-	-	-	-	-
9	Okochiri	-	-	-	-	-	-	-	4.5×10^{3}
10	Okrika Grammar School	-	-	-	-	-	-	-	6.0×10^{3}
11	Okari-Ama	5.05×10^{2}	11.0×10^{2}	1.01×10^{3}	3.4×10^{3}	1.77×10^{3}	1.1×10^{3}	$5.5.\times 10^{3}$	-
12	Edeme-biri	-	-	-	-	1.26×10^{2}	3.1×10^{3}	0.7×10^{4}	-
13	Isaka Town 1	-	0	-		-		-	-
	Isaka Town 2	-	2.1×10^{3}		7.0×10^{2}		1.35×10^{3}	0	1.4×10^{4}

(-): Not Determined
(0): Not Determined

Table 5: Seasonal Variations for Total *Salmonella Shigella* Count (TSSC) Across Five Locations

S/N	Locations	Borehole		Hand-dug well		Surface Water		Sediment	Soil
		Dry Season	Wet Season	Dry Season	Wet season	Dry Season	Wet season		
1	Ogan Ama	0	0	0	0	0	0	0	5.35×10^3
2	Orupabo Ama	0	0	-	-	-	-	-	-
3	George Ama	0	0	-	-	0	0	1.05×10^3	4.5×10^3
4	Kalio Ama	0	8.0×10^2	-	-	0	8.50×10^2	2.25×10^3	-
5	Abam Ama	0	0	-	-	-	-	-	-
6	Ogoloma	0	0	-	-	-	-	-	-
7	Bulomebiri	0	4.0×10^2	-	-	-	-	-	-
8	Ekerekana	0	0	-	-	-	-	-	-
9	Okochiri	-	-	-	-	-	-	-	0
10	Okrika Grammar School	-	-	-	-	-	-	-	0
11	Okari-Ama	0	8.50×10^2	0	2.0×10^3	0	8.50×10^2	1.2×10^3	-
12	Edeme-biri	-	-	-	-	0	9.50×10^2	1.3×10^3	-
13	Isaka Town 1	-	3.50×10^2	-	-	-	-	-	
	Isaka Town 2	-	4.95×10^3	-	0	-	0	4.6×10^3	4.5×10^3

(-): Not Determined
(0): Not Determined

Table 6: Seasonal Variations for Total Vibrio Count (TVC) Across Five Locations

S/N	Locations	Borehole		Hand-dug well		Surface water		Sediment	Soil
		Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season		
1	Ogan Ama	0	7.50×10^2	0	7.50×10^2	0	0	0	1.9×10^3

2	Orupabo Ama	0	0	-	-	-	-	-	-
3	George Ama	0	0	-	-	0	8.70×10^3	2.50×10^3	5.50×10^3
4	Kalio Ama	0	4.0×10^2	-	-	0	2.29×10^4	6.0×10^2	-
5	Abam Ama	0	0	-	-	-	-	-	-
6	Ogoloma	0	0	-	-	-	-	-	-
7	Bulome-biri	0	0	-	-	-	-	-	-
8	Ekerekana	0	0	-	-	-	-	-	-
9	Okochiri	-	-	-	-	-	-	-	0
10	Okrika Grammar School	-	-	-	-	-	-	-	0
11	Okari-Ama	0	0.50×10^2	0	3.50×10^2	0	0.50×10^2	7.25×10^3	-
12	Edeme-biri	-	-	-	-	0	3.95×10^3	2.95×10^3	-
	Isaka Town 1	-	0	-	-	-	-	-	-
13	Isaka Town 2	-	0	-	0	-	5.00×10^2	6.2×10^3	0

(-): Not Determined
(0): Not Determined

Table 7: Biochemical Characteristics of Bacterial Isolates from Various Samples

S/N	Appearance on Media Plates	Gram RXN	Cell Morphology	Biochemical Tests								Sugar Fermentation Test				Probable Identity of Isolates
				CAT	OXI	CIT	MOT	IND	MR	VP	STH	GLU	LAC	SUC	MAN	

	Morphology														
1	Milky	+ve	Rods	+	-	+	+	-	-	+	+	A	-	-	-	*Bacillus sp*
2	Creamy	+ve	Rods	+	+	+	+	-	-	-	+	-	-	A	-	*Bacillus sp*
3	Pink	-ve	Rods	+	-	+	-	-	+	+	+	A	-	-	-	*Klebsiella sp*
4	Creamy	+ve	Rods	+	+	+	+	-	+	-	+	A	-	-	-	*Bacillus sp*
5	Creamy	+ve	Rods	+	-	-	+	-	+	-	+	-	-	-	-	*Bacillus sp*
6	Yellow gold	+ve	Cocci	+	-	-	+	-	+	+	+	A	-	-	-	*Staphylococcus sp.*
7	Milky	+ve	Rods	+	+	+	+	-	+	-	+	A	-	A	-	*Bacillus sp*
8	Creamy	+ve	Rods	+	+	+	+	-	+	-	+	A	-	-	-	*Bacillus sp*
9	Creamy	-ve	Rods	+	-	+	+	-	-	-	+	-	-	-	-	*Pseudomonas sp*
10	Milky	+ve	Rods	+	+	+	+	-	-	-	+	A	-	A	-	*Bacillus sp*
11	Creamy	+ve	Rods	+	+	-	+	-	-	+	+	A	A	-	A	*Bacillus sp*
12	Creamy	+ve	Rods	+	-	-	+	-	+	-	+	A	-	-	-	*Bacillus sp*
13	Milky	+ve	Rods	+	+	-	-	-	+	-	+	A	A	-	-	*Bacillus sp*
14	Red	-ve	Rods	+	-	-	+	-	+	+	+	A	-	A	A	*Serratia sp*
15	Creamy	+ve	Rods	+	+	+	+	-	+	-	+	A	-	A	-	*Bacillus sp*
16	Creamy	+ve	Rods	+	-	+	+	-	+	+	+	A	-	A	A	*Bacillus sp*
17	Pink	-ve	Rods	+	+	+	+	+	+	-	+	A	A	A	A	*Escherichia coli*
18	Red	-ve	Rods	+	-	+	+	+	+	+	+	A	-	A	A	*Serratia sp*
19	Milky	+ve	Cocci	+	-	+	+	-	+	+	+	A	-	-	-	*Micrococcus sp.*
20	Creamy	-ve	Rods	+	-	+	+	-	+	-	+	A	-	A	A	*Aeromonas sp.*
21	Yellow gold	+ve	Cocci	+	+	+	-	-	+	+	+	A	-	A	A	*Staphylococcus sp.*
22	Dark Green	-ve	Rods	+	+	+	+	-	+	-	+	A	-	-	-	*Vibrio sp*
23	Light Green	-ve	Rods	+	+	+	-	-	-	+	+	-	-	-	-	*Pseudomonas sp*
24	Creamy	-ve	Rods	+	+	-	-	-	-	+	-	-	-	-	-	*Alcaligene sp*

KEY: RXN: Reaction. CAT; Catalase test. OXI; Oxidase test. CIT; Citrate test. IND; Indole test. MR; Methyl Red Test. VP; Voges Proskauer Test. STH; Starch Hydrolysis Test. GLU; Glucose test, LAC; Lactose. SUC; Sucrose. MAN; Mannitol.

Table 8: Susceptibility Pattern of Gram Positive Bacteria Isolated during the study period (Dry Season)

Antibiotics	Concentration(µg)	Susceptibility n(%)	Intermediate n(%)	Resistance n(%)
CAZ	30	3 (18.75)	1 (6.25)	12 (75)
CRX	30	5 (31.25)	2 (12.5)	9 (56.25)
GEN	10	16 (100)	0	0
CTR	30	14 (87.5)	0	2 (12.5)
ERY	5	14 (87.5)	0	2 (12.5)
CXC	5	12 (75)	0	4 (25)
OFL	5	15 (93.75)	0	1 (6.25)
AUG	30	14 (87.5)	0	2 (12.5)

* CAZ- Ceflazidine, CRX -Cefuroxime, GEN- Gentamycin, CTR -Ceftriaxone, ERY- Erythromycin, CXC – Cloxacin, OFL – Ofloxacin, AUG - Augmentin

Table 9: Susceptibility Pattern of Gram Negative Bacteria Isolated during the study period (Dry Season)

Antibiotics	Concentration(µg)	Susceptibility n(%)	Intermediate n(%)	Resistance n(%)
OFL	5	4 (100)	0	0
AUG	30	0	0	4 (100)
NIT	300	1 (25)	0	3 (75)
CPR	5	3 (75)	0	1 (25)
CAZ	30	1 (25)	0	3 (75)
CRX	30	0	0	4 (100)
GEN	10	4 (100)	0	0
CXM	5	0	0	4 (100)

* CAZ -Ceftrazidine, CRX -Cefuroxime, GEN – Gentamicine, CXM – Cefixime, OFL – Ofloxacin, AUG - Augmentin, NIT – Nitrofurantin, CPR – Ciprofloxacin

Table 10 Susceptibility Pattern of Gram Positive Bacteria Isolated during the study period (wet season).

Antibiotics	Concentration(µg)	Susceptibility n(%)	Intermediate n(%)	Resistance n(%)
CAZ	30	1 (7.69)	0	12 (92.31)
CRX	30	2 (15.38)	1 (7.69)	10 (76.9))
GEN	10	9 (69.23)	1 (7.69)	3 (23.07)
CTR	30	8 (61.538)	0	5 (38.46)
ERY	5	3 (23.079)	0	10 (76.9)
CXC	5	0	0	13 (100)
OFL	5	5 (38.46)	0	8 (61.538)
AUG	30	2 (15.38)	0	11 (84.615)

* CAZ- Ceflazidine, CRX -Cefuroxime, GEN- Gentamycin, CTR -Ceftriaxone, ERY- Erythromycin, CXC – Cloxacin, OFL – Ofloxacin, AUG - Augmentin

Table 11 Susceptibility Pattern of Gram Negative Bacteria Isolated during the study period (wet season).

Antibiotics	Concentration(µg)	Susceptibility n(%)	Intermediate n(%)	Resistance n(%)
OFL	5	0	1 (50)	1 (50)
AUG	30	0	0	2 (100)
NIT	300	1 (50)	0	1 (50)
CPR	5	2 (100)	0	0
CAZ	30	0	1 (50)	1 (50)
CRX	30	0	1 (50)	1 (50)
GEN	10	0	0	2 (100)
CXM	5	0	1 (50)	1 (50)

* CAZ -Ceftrazidine, CRX -Cefuroxime, GEN – Gentamicine, CXM – Cefixime, OFL – Ofloxacin, AUG - Augmentin, NIT – Nitrofurantin, CPR – Ciprofloxacin

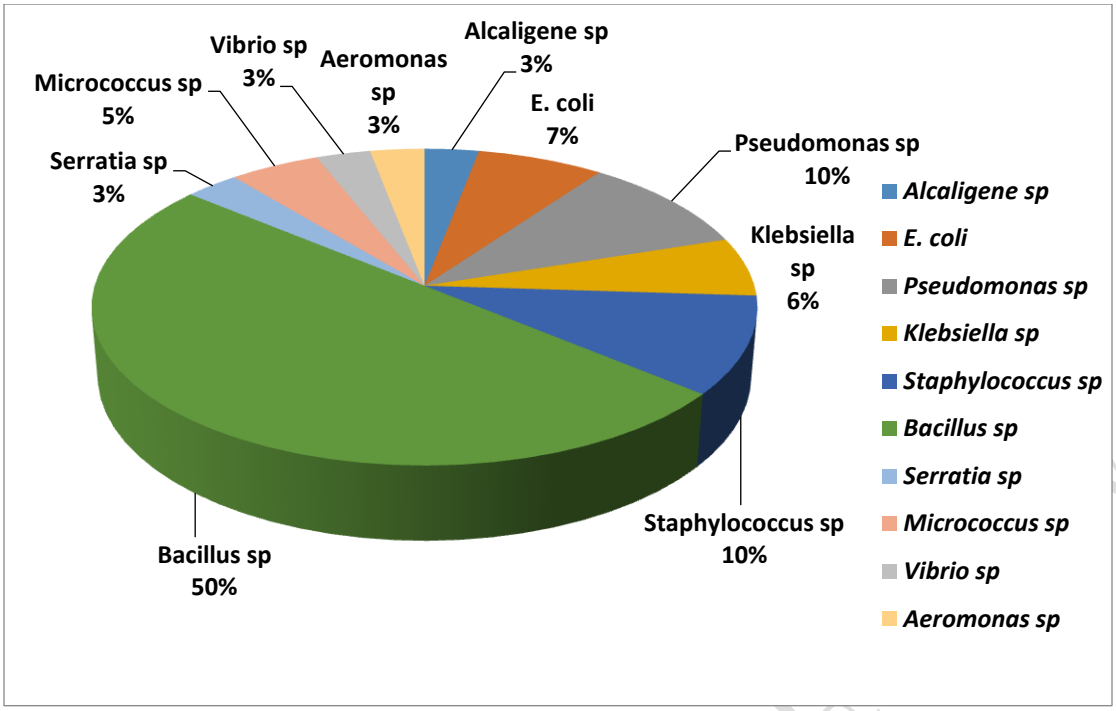

Figure 2. Percentage Frequency of Bacterial Isolates obtained from water, sediment and soil samples during wet and dry seasons in the study area (Okrika L.G.A)

The study provides evidence for microbiological contamination in boreholes, hand-dug wells, surface water sediment and soil in Okrika Local Government Area of Rivers State.

Results of Total Heterotrophic Bacterial Count (THBC) values for boreholes and hand-dug wells for dry and rainy seasons as presented in Table 2 ranged from $2.0x10^3$ cfu/ml in Ogan-Ama to 2.72×10^5 cfu/ml at Okari-Ama respectively. The results showed that all boreholes and hand-dug wells sampled were contaminated with bacteria.

It was observed that microbial load in boreholes and hand-dug wells were higher during the wet season than dry season, this could be as a result of high levels of precipitation during the period which may raise the groundwater table and increased susceptibility to pollution (Sasakova *et al*., 2018**).** Comparing with international guideline levels for drinking water, it were poor and unsuitable for drinking. Microbiological contamination according to World Health Organization (WHO, 2003) states that Heterotrophic plate count levels in potable water should be < 500 colony forming unit per milliliter. Count consistently > 500 cfu/ml would indicate a general decrease in water quality. However, evidence of heterotrophic bacteria are dangerous to human health (Edberg and Allen, 2004; Pavlov *et al*., 2004) and may contribute to acute gastrointestinal illness which can result in fever, nausea and diarrhea and vomiting (Macler and Merkle, 2000). Also, knowledge of microbial contamination in aquatic environments is important for maintaining healthy water body for economic purposes and recreational activities. These can be detected by the changes in abundance of bacteria population (Kavka and Poetsch, 2002). From the findings all surface waters during the dry and wet seasons were contaminated with heterotrophic bacteria with the highest of 2.56×10^5 cfu/ml at Okari Ama during the wet season. Subsequently, all sampled sediment were contaminated with the highest of value at 1.25×10^3 cfu/g at Okari Ama. This could be as a result of industrial waste flow and house hold garbage dispose into the Okrika River either direct or through drains, erosion, flooding or run off. Haque *et al*., (2018) recorded maximum THB value of $20.60\times10\pm1.41$ cfu/g which is higher. Soil

sample were also contaminated with the highest value of 4.3×10^4 at Ogan-Ama. This could be as a result of indiscriminate waste disposed which had seeped into the groundwater system.

Total Fecal Count (TFC) for boreholes and hand-dug wells for dry and wet seasons ranged from 0 to 5.95×10^3 at Isaka Town. During the dry season, only Okrika -Ama borehole and hand-dug well recorded fecal contamination while Isaka and Okari-Ama hand-dug wells recorded fecal contamination. Fecal coliforms cause fecal pollution of water which is a serious problem due to the potential for contracting diseases from pathogens. *E. coli* (0157: H7) causes abdominal pain, bloody diarrhea and hemolytic uremic syndrome. This can result in acute renal failure (WHO, 2010). Fecal bacteria pollution in surface water and sediment could be as a result of discharge of wastewater, sewage or direct disposal of human and animal excreta into the river. TFC values for surface water samples ranged from 0 to 4.7×10^3 cfu/ml in Isaka and sediment ranged from 4.40×10^2 cfu/g at Ogan-Ama to 4.1×10^3cfu/g in Edeme-biri. This could pose potential health risks to humans and the environment through the consumption of contaminated fish and sea foods. However, fecal coliforms remain the standard indicator of choice for shellfish and shellfish harvest waters (Akinrotimi, 2015). These observations corroborates with the results of Haque *et al.,* (2018)

TFC values for sampled soils ranged from 7.5×10^3 cfu/g in Okrika Grammar School to 5.1×10^4 cfu/g at Ogan-Ama. The findings showed that the soils sampled from all locations were contaminated with fecal bacteria. This may lead to pollution of water resources in the area through leaching and storm water during heavy rainfall. The higher the level of fecal contamination, the greater the risk of water borne diseases (Keesari *et al.*, 2015).

Total Coliform Count (TCC) values of borehole and hand-dug well for dry and wet seasons ranged from 0 to 6.1×10^3 cfu/ml in Bulome-biri and 5.50×10^2cfu/ml at Ogan-Ama to 3.4×10^3cfu/ml in Okari-Ama. All hand-dug wells recorded values of coliform bacteria during the dry and the wet season indicating coliform contamination of water. Coliform bacteria are also indicators to measure the degree of the pollution and sanitary quality of water. Contamination may be as a result of a layer of bacteria (biofilm) within the well or plumbing system. Surface water may be getting into the well or well water from aquifer which contains bacteria. Most types of coliform bacteria are harmless to humans but some can cause mild illness and a few can lead to serious water borne diseases. The most common symptoms are gastrointestinal diseases and general flu-like symptoms such as fever, abdominal cramps and diarrhea symptoms are most likely in children or elderly members (Penn state, 2016). TCC values for surface water samples during the dry and wet season range from 0.10×10^2cfu/ml in Ogan-Ama to 1.80×10^4cfu/ml at Kalio-Ama. While for sediment, values ranged from 0 to 0.7×10^4cfu/g (Edemebiri). These resuls are in consonance with reports of other works who obtained similar values of 51.67×10^3 to 138.33×10^3MPN/100ml and 140.00×10^4cfu/g (Karbasdehi *et al*., 2017 and Haque *et al.,* 2018 respectively). TCC values for sampled soils ranged from 4.5×10^3cfu/g in Okochiri to 3.95×10^4 cfu/g at Ogan-Ama. The findings showed that soils sampled from all locations were contaminated with coliform bacteria.

Total Salmonella Shigella Count (TSSC) values for boreholes and hand-dug wells during dry and wet season ranged from 0 to 2.0×10^3 in Okari-Ama. During the dry season, there was no Salmonella Shigella contamination recorded in borehole and hand-dug well. This could be due to runoff from the environment as a result of rainfall which can increase the microbial load washed in from the soil. But during the wet season, boreholes at Bulome-biri, Isaka, Kalio-Ama and Okari-Ama recorded contamination while only Okari hand-dug well recorded contamination. TSSC values for sampled surface water during the dry and wet season ranged from 0 to 9.5×10^2cfu/ml at Edeme-biri. During the dry season, all surface water samples recorded 0, that means, no detection of salmonella Shigella bacteria. During the wet season, only Edeme-biri, Ogan-Ama and Isaka surface water recorded contamination. TSSC values for sampled sediment ranged from 0 to 4.6×10^3cfu/g at George-Ama. From the findings, five sampled locations recorded Salmonella Shigella bacteria except Ogan-Ama

which recorded no detection. TSSC for sampled soil ranged from 0 to 5.35×10^3 at Ogan-Ama. The findings showed that TSSC were not recorded at Okochiri and Okrika Gramnar School.

Salmonellosis cycle in the environment can involve shellfish. Salmonella Shigella causes well characterized spectrum of diseases in humans that range from asymptomatic carriage to hemorrhagic colitis and fatal typhoidal fever (Obi *et al*., 2003). If effluent from sewage plant passes into a coastal area, edible shellfish (mussels, oysters) can become contaminated. Shellfish can concentrate bacteria as they filter several litres of water per hour. Ingestion by humans of these sea foods (uncooked or superficially cooked) may cause typhoid fever or other salmonellosis. Shigellosis disease begins with fever, anorexia, fatigue and malaise with frequent bloody stools and abdominal cramps. (Popoff and Le Minor, 2005).

Total Vibrio Count (TVC) values for borehole and hand-dug well during the dry and wet seasons ranged from 0 to 7.5×10^2cfu/ml at Ogan-Ama. During the dry season, there were no vibrio contamination in boreholes and hand-dug wells. Contamination during the wet season could be as a result of storm water seepage into the groundwater system or surface contamination of open wells. TVC values of surface water samples during the dry and wet season ranged from 0 to 2.29×10^4cfu/ml at Kalio-Ama, from the records, during the dry season, there was no contamination in the surface water samples, but during the wet season, only Ogan-Ama was free from contamination. Vibrio bacteria species worldwide stems from history and epidemiology of cholera. Vibrios are primarily aquatic bacteria. Species distribution depends on sodium concentrate and water temperature.

V. cholerae, the most important of the vibrio species causes acute and very intense diarrhea with general weakness, muscular pains, potassium in blood drops to very low levels, finally cirulary collapse and dehydration (Farmer and Hickam-Brennas, 2003). Seafoods and water are the major sources of infection. Also, vibrio bacteria can cause skin infection when an open wound is exposed to saltwater or brackish water. Recent outbreaks include multistate outbreak gastrointestinal diseases linked to oysters imported from Mexico in 2019 (US Dept. of Health and Human Services, 2019). Also, outbreak of vibrio parahaemolyticus infections linked to fresh crab meat imported from Venezuela in 2018.TVC values for sampled sediment ranged from 0 - 7.25×10^3cfu/g at Okari-Ama. Five locations recorded vibrio bacteria except Ogan-Ama. TVC values for sampled soil ranged from 0 to 5.50×10^3cfu/g at George-Ama. The findings showed that TVC was not recorded at Isaka, Okochiri and Okrika Grammar School. TVC was recorded only at Ogan-Ama and George-Ama communities.

Bacteria isolated from the study area during the sampling period, showed that *Bacillus* generally dominated with 50%. The least were *Alcaligene*, *Vibrio*, *Serratia* and *Aeromonas* with 3% respectively.

100% of gram positive isolates were susceptible to Gemtamycin (GEN), majority of the organisms were sensitive to all the drugs except Cefuroxime (CRX) resistance, while 75% of the organisms were resistant to Ceflazidine (CAZ).

Subsequently, gram negative isolates in dry season, showed susceptibility to Gentamicine (GEN) and Ofloxacin (OFL) to 100% sensitive to the isolates, while 69.23% and 61.5% of gram negative isolates in wet season were susceptible to Gentamicine (GEN) and Ceftriaxone (CTR).

75% of organisms were also sensitive to Ciprofloxacin (CPR).

Meanwhile, 100% of organisms were resistant to Augmentin (AUG), Cefuroxime (CRX) and Cefixime (CRM). 75% of organisms were resistant to Nitrofurantin (NIT) and Ceftrazidine (CAZ).

Majority of the organisms were sensitive to all drugs except Cloxacin (CXC) which had 0% sensitivity.

For resistance, 100% of organisms were resistant to CXC. The least resistant by organisms was GEN at 23.07%.

Gram negative isolates in wet season showed that 100% were sensitive to CPR and there were 100% resistance to AUG and GEN. However, majority of the organisms were resistant to the drugs except CPR.

Antibiotic Resistance profile of Bacteria were isolated from the samples and evaluated. The results showed the occurrence of microorganisms that indicated fecal contamination above the limit legally allowed for drinking water. The antibiotic resistance profile revealed the existence of antibiotic resistant bacteria. There were higher levels of antibiotic-resistant microorganisms during the wet season than the dry season. Regarding antibiotic drugs, there were high percentage of microorganisms that were not susceptible to Ceftazidime, Cefuroxime, Augmentin, Cloxacillin and Nitrofurantin during the dry season. However, during the wet season, about 70% of microbes were not susceptible to all the antibiotics including Gentamicin, Cefixime, Ofloxacin, Ciprofloxacin, Ceftriaxone and Erythromycin. The high percentage of resistance to commonly uesd antibiotics recorded in this study may be caused by mechanisms such as the synthesis of low affrinity-β-lactams binding proteins, the production of penicillinase, and transferable genetic elements which included plasmids that may contain different resistant genes [Ogbonna and Azuonwu 2019]. The high percentage susceptibility of the tested isolates to Ofloxacin is in agreement with reports by other authors, on multiple-drug resistance bacteria, Ogbonna and Azuonwu (2019), not only that, but also the misuse and overuse of antibiotics. Also, lack of access to clean water, sanitation and hygiene (WASH) for both humans and animals; poor infection and disease prevention and control in health-care facilities and farms and poor access to quality affordable medicines.The results of antibiotic profile obtained in this study revealed the need to improve the water system in Okrika communities and to educate the population on hygiene and sanitation.

Conclusion and Recommendation

This study shows that groundwater and surface water in Okrika Local Government Area are vulnerable to microbial contamination due to human activities, lack of well protection and the hydrological characteristics of the area. For human health issues, recognizing vulnerability is crucial where groundwater is the only source of drinking water in the area. The high number of indicator bacteria observed indicates the poor quality of water being used by these communities for drinking and the seafoods being consumed.

It is therefore necessary to apply strong prevention measures immediately to save groundwater and surface water system in the location. Adequate monitoring and surveillance of these water sources should be carried out and treatment of water should be mandatory.

Prevention of diseases by boiling of water before consumption should be taken seriously by residents.

Also, efforts by all stakeholders and government should be geared towards prevention of outbreak of an epidemic in such areas.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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