



## Determination of the effect of pollution on the total microbial load and diversity in the surface and groundwater in selected pollution-prone towns in Cross River State, Nigeria

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### Abstract

Groundwater is an important natural resource that majority of the human population depends on for drinking. Potable water is essential for good health and the socio-economic development of man. This research aimed at evaluating the effect of pollution on the total bacterial load and biochemical characteristics of bacteria isolates associated with surface and groundwater samples closed to dumpsites and abattoirs in six Local Government Areas (LGAs) in Cross River State. Borehole water samples were subjected to standard laboratory procedures, and microbiological examination of the borehole water samples was carried out. Pour plate technique was used for plating the samples, 1ml of the sample was transferred into Petri dishes (in triplicate), containing potato dextrose agar (PDA) for fungal enumeration, and nutrient agar (NA) for bacterial enumeration. An aliquot of each sample was transferred into the plate for each sample, thoroughly mixed and allowed to solidify, then incubated at room temperature for 24 hours (for NA) and 72 hours (for PDA). After incubation, the colonies were observed, counted and recorded. Identification of pure cultures was done by studying colony characteristics, and biochemical characteristics. The results revealed that the bacterial population was high in the groundwater samples obtained from Calabar municipality (slaughter house), Ogoja (dumpsite) was next and significantly higher in the bacteria counts than samples obtained from Akamkpa (dumpsite), Calabar municipality (control, dumpsite), Ikom (dumpsite, slaughter house), Ogoja (slaughter house) and Yala (dumpsite and slaughter house) with no significant difference ( $P>0.05$ ) in the mean bacterial counts. The lowest bacterial count was detected in the groundwater samples from Akamkpa (slaughter house), Ikom, Yala and Ogoja (control), and Yakurr (control,

slaughter house, dumpsite) with no significant difference ( $P > 0.05$ ) in the bacteria counts obtained. It was evident from the results, that there was a high level of contamination of groundwater sources within the areas visited, and this calls for urgent health intervention.

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## 1.0 Introduction

Water pollution is a pressing issue on a global scale, resulting from the alteration of the body's physiology by physical, biological, and chemical substances (Daniels *et al.*, 2013). In Nigeria, the rapid urbanization and increased industrial and agricultural activities have significant impacts on the underground water table. The environmental degradation and pollution caused by indiscriminate waste disposal are seen as major threats to water quality, particularly in urban areas (Eni *et al.*, 2011). Contaminated water, which is unfit for consumption and recreational activities, can lead to various illnesses like cholera, dysentery, and typhoid. The intake of polluted water exposes individuals to harmful chemicals that can adversely affect their health. Providing clean drinking water to both rural and urban areas is crucial in preventing health issues. Gomez *et al.* (2002) argued that access to safe drinking water is essential to reducing the spread of waterborne diseases.

Historically, open dumpsites were commonly used for disposing of municipal solid waste in rural and urban areas, offering a straightforward and cost-effective solution. These dumpsites, located in low-lying areas with low value, often give rise to aesthetic and odor issues, attract pests, and pose environmental hazards. To address these challenges, an alternative method

called sanitary landfilling has been developed (Nagendran *et al.*, 2006).

The decomposition of waste in landfills results in the formation of leachate, containing a mix of biological and chemical substances such as inorganic salts, organic matter, and toxic metals. This leachate can percolate through various soil layers, affecting the underground water table. The composition of leachate varies depending on the physical, chemical, and microbiological processes occurring in the waste deposits (Aziz *et al.*, 2010). The presence of leachate in groundwater aquifers poses significant risks to human health and the environment. The movement of leachate from waste sites or landfills, along with the release of pollutants from sediments under specific conditions, can pose a serious threat to groundwater resources if not appropriately managed (Ikem *et al.*, 2002).

Anthropogenic activities, such as the haphazard siting of abattoirs and dumpsites near residential areas in developing nations, serve as additional origins of groundwater contamination. Abattoir operations involve various activities like slaughtering, hide removal, intestine handling, rendering, processing, and cleaning. The waste produced by abattoirs typically consists of blood, oil, minerals, organic solids, salts, and chemicals used during processing (Hassan *et al.*, 2014; Akange *et al.*, 2016). Improper disposal of these

wastes can significantly contribute to water pollution (Enoh *et al.*, 2012). Specifically, abattoir wastewater can elevate the levels of nitrogen, phosphorus, and total solids in nearby water bodies (Akange *et al.*, 2016; FAO, 1997; Okorafor *et al.*, 2012).

Dumpsites play a role in groundwater pollution as waste materials within them can seep into the groundwater, potentially leading to microbial contamination (Mor *et al.*, 2006). This contamination can introduce harmful bacteria and viruses into the water supply, posing health risks to humans and animals. Research (Rahman *et al.*, 2019) has linked underground water contamination with *Escherichia coli*, Salmonella, and other pathogenic bacteria to pollutants arising from dumpsites. These microbes can cause gastrointestinal illness and other health problems. Another study (Bello *et al.*, 2020) in Nigeria found that underground water near abattoirs was contaminated with high levels of coliform bacteria and heavy metals, which can affect the health of humans and animals. The impact of pollutants on groundwater continues to raise concern and has become the subject of recent and past investigations (Ahmed and Sulaiman, 2001; Mor *et al.*, 2006).

The significance of safe water sources is underscored by the WHO's report indicating a high number of children (approximately 525,000 children) under 5 succumbing to diarrheal illnesses from unsanitary water (WHO, 2022). The reliance of a significant portion of the global population on groundwater for drinking purposes has made water quality assessment a critical endeavor, particularly given the potential health implications associated with contamination (Mgbemna *et al.*, 2014). Hence, this study was aimed at evaluating the effect of pollution on the total bacterial load and biochemical characteristics of bacteria isolates associated with

surface and groundwater samples in selected pollution-prone towns in Cross River State, Nigeria.

## 2.0 Materials and methods

### 2.1 Study area

The groundwater samples for the research work were collected from different sources in Yakurr, Ikom, Yala, Ogoja, Akamkpa, and Calabar municipality LGA of Cross River State. Yakurr and Ikom are located in the central senatorial district of Cross River State. The sampling sites (dumpsites and abattoirs) are all located at various village settlements within Yakurr and Ikom Local Government Area. Yakurr Local Government Area is located on Latitude 5°45'34" N to 5°48.682' N, and Longitude: 8°5'18" E to 8°5'23" E, with a population of 262,700 according to the last census in 2016. It is a populated place and made up of seven (7) villages. Ikom L.G.A is located on Latitude 5.9667° N to 6.2000° N, and Longitude 8.6000° E to 8.8000° E. The main occupation of the people is farming. There are civil servants, and a few are traders. They make use of this water though not minding whether the water is potable for consumption or not. Akamkpa local government area and Calabar municipality are located in the Southern Senatorial district. Calabar Municipality lies geographically on Latitude 4.9200° N to 4.9900° N, and Longitude 8.3000° E to 8.3800° E. The area has both urban features as well as rural settings in the environs of the metropolis. Two ethnic groups, the Quas and the Efiks form the indigenous population. However, because of its cosmopolitan status there abound people from all parts of the state. By virtue of its location, most of the indigenous populations are engaged in fishing, farming, and trading. Akamkpa lies between Latitude 5.2500° N to 5.4500° N, and Longitude: 8.2500° E to 8.4500° E. The temperatures of both areas range

from 21.0 5°C to 33.15 °C while rainfall ranges from 42.0 to 1401.0 (mm/month). Akamkpa shares a border with Republic of Cameroon. Yala and Ogoja are located in the Northern Senatorial District of the State. Ogoja lies between Latitude 6.5500° N to 6.7500° N, and Longitude: 8.6500° E to 8.8500° E, while Yala lies between Latitude

6.5000° N to 7.0000° N, and Longitude: 8.5000° E to 9.0000° E. Their major occupation is farming. Figure 1 and Plate 1 show the study sample locations, and a waste dumpsite at Yala local government area of Cross River State, respectively.



**Figure 1:** Map of Cross River state showing the study area and the sample locations (Source: Cross River State geographic survey)

## 2.2 Ground water sample collection from boreholes

Water samples were collected from mainly boreholes in six (6) LGAs of the state. Fifty-four (54) groundwater samples were collected in labelled sterile and acidified bottles, covered immediately to prevent contamination, and transported in an ice pack to the Cross River State Water Board laboratory for microbiological examination. Special precautions were observed during sample collection by sterilizing the mouth

of the tap with methylated spirit and then turning on the tap for maximum flow for two minutes before collecting the water samples in the bottles.

## 2.3 Experimental design

The experimental design for the study was a 3x6 factorial in a Completely Randomized Design (CRD). Factor 1: Sources (Control, Dumpsite and Abattoir). Factor 2: Six LGAs (Yakurr, Ikom, Akamkpa, Ogoja, Yala and Calabar Municipality).



**Plate 1:** Dump site at Yala (Photo by Ekpe, 2024)

#### **2.4 Microbiological analysis of samples**

Qualitative microbiological analysis was applied to samples of water that should be representative of the objective under investigation and that should be well conserved to maintain all relevant properties constant until the time of analysis. Bacteriological water analysis was one of the methods of analyzing groundwater to estimate the number of bacteria colonies present.

#### **2.5 Enumeration and identification of microorganisms**

Determination of viable plate count of isolates was done following the method of Iyakndue *et al.* (2017) and Rao *et al.* (2017). The pour plate technique was used for plating the samples, 1ml of the sample was transferred into Petri dishes (in triplicate) and the media used was potato dextrose agar (PDA) for fungal enumeration and nutrient agar (NA) for bacterial enumeration. An aliquot of each sample was transferred into the plate for each sample and then thoroughly mixed and allowed to solidify, then incubated at room temperature for 24 hours (for NA) and 72 hours (for PDA). After incubation, the colonies were observed, counted and recorded. Identification of pure cultures from each sample was done by studying colony characteristics, microscopy, and

biochemical characteristics. Identification was accomplished by using binocular to view the isolate.

#### **2.6 Isolation and purification of the isolates**

The bacterial and fungal colonies were purified by repeated subculturing into nutrient agar using the pour plate method and potatoes dextrose agar using the spread plate method, respectively, which were incubated at room temperature for 24 hours (for NA) and 72 hours (for PDA). From these pure cultures, stock cultures were prepared on agar slants in stock bottles (using NA for bacterial isolates, and PDA for fungal isolates) for subsequent characterization and identification.

### **3.0 Results**

#### **3.1 Microbial populations in groundwater**

##### **3.1.1 Total heterotrophic bacterial counts (THBC) of groundwater samples**

Bacteria are ubiquitous in nature; most of the bacteria are harmful to human health. Water source meant for human consumption, containing disease causing bacteria could be detrimental to the well-being of humans. The bacterial counts observed in the groundwater samples obtained from Calabar municipality (abattoir) were higher

than the counts obtained in other sources and locations. Though the bacterial population was high in the groundwater samples obtained from Calabar municipality (abattoir) indicating contamination of the water source, Ogoja (dumpsite) was next and significantly higher in the bacteria counts than that obtained from Akamkpa (dumpsite), Calabar municipality (control, dumpsite), Ikom (dumpsite, abattoir), Ogoja (abattoir) and Yala (dumpsite and abattoir) with no significant difference ( $P > 0.05$ ) in the mean bacterial counts. The lowest bacterial count was detected in the groundwater samples from Akamkpa (abattoir), Ikom, Yala and Ogoja (control), and Yakurr (control, abattoir, dumpsite), with no significant difference ( $P > 0.05$ ) in the bacteria counts obtained. Akamkpa control had no bacteria growth in the borehole water samples (Table 1). However, among the different local government areas in which borehole water sampling was done, Ogoja water samples had the highest bacteria counts, followed by Ikom and Yala borehole water samples while Akamkpa, Calabar municipality, and Yakurr had the lowest bacterial counts with no significant variation in mean values.

### 3.1.2 Total fungal counts (TFC) of groundwater samples

It was observed from the result as presented on Table 1 that the fungal counts in groundwater samples from Ikom (dumpsite) were the highest, followed by the groundwater samples from Yala (dumpsite and abattoir), This was also followed by the water sample from the Calabar municipality dumpsites area. It was also observed that the controls in all the different locations had the lowest fungal counts in the drinking water source. Table 2 shows that the groundwater from Yala had the highest fungal counts, followed by Ikom, also followed by the borehole water sample in Calabar municipality and Ogoja while

Akamkpa and Yakurr had the lowest fungal population in their drinking source.

### 3.1.3 Distinct Fungal isolates from sampling locations

Fungal isolates from Akamkpa were yeast and *Aspergillus* sp. The yeast had a percentage occurrence of 18.18%, while the *Aspergillus* sp had a percentage occurrence of 9.09%. The fungal isolates from Ikom LGA were *Fusarium* sp., *Aspergillus* sp., *Pencillium* sp. and *Trichophyton* sp with percentage occurrences of 18.18%, 18.18%, 9.09%, and 9.09%, respectively. Calabar Municipality fungal isolates were *Aspergillus* sp with 18.18% percentage occurrence. Yala LGA fungal isolate was *Fusarium* sp. with 9.09% percentage occurrence. Yakurr LGA fungal isolates were *Candida albicans* and yeast with 9.09% percentage occurrence each. The results for the percentage occurrence of fungi and distinct fungal isolates from sampling locations are summarized in Table 3.

### 3.1.4 Distinct bacterial isolates from sampling locations

The bacterial isolates from Akamkpa LGA were *Enterobacter* sp., *Klebsiella* sp., *E. coli* and *Streptococcus pyogenes*, with a percentage occurrence of 5.56% each. Those from Ikom LGA were *Klebsiella* sp., *Bacillus* sp., *Staphylococcus aureus*, *E. coli*, *Citrobacter* sp, *Enterobacter* sp with a percentage occurrence of 11.11%, 11.11%, 5.56%, 5.56%, 5.56%, and 5.56% respectively. Bacterial isolates in Ogoja LGA were *S. aureus* and *Klebsiella* sp with percentage occurrence of 5.56%, each, while those in Yala LGA were *S. pyogenes* and *Enterococcus faecalis* with a percentage occurrence of 5.56% also. Bacterial isolates from Calabar Municipality were *Pseudomonas aeruginosa* and *E. coli* with a percentage occurrence of 5.56%, each (Table 4).

**Table 1:** Microbial populations of underground water in pollution-prone areas of Cross River State

Location	Source	THBC (cfu/ml)	TFC (cfu/ml)
<b>Akamkpa</b>	Control	ND	0.10 <sup>f</sup> ±1.0
	Dumpsite	0.33 <sup>c</sup> ±1.29	0.67 <sup>d</sup> ±0.94
	Slaughter	0.07 <sup>d</sup> ±0.6	0.22 <sup>f</sup> ±0.60
<b>Calabar municipal</b>	Control	0.27 <sup>c</sup> ±0.03	0.012 <sup>f</sup> ±0.10
	Dumpsite	0.31 <sup>c</sup> ±0.87	0.82 <sup>c</sup> ±0.10
	Slaughter	0.90 <sup>a</sup> ±0.17	0.41 <sup>c</sup> ±0.36
<b>Ikom</b>	Control	0.01 <sup>d</sup> ±0.00	0.08 <sup>f</sup> ±0.01
	Dumpsite	0.39 <sup>c</sup> ±0.06	1.20 <sup>a</sup> ±2.00
	Slaughter	0.27 <sup>c</sup> ±0.05	0.52 <sup>c</sup> ±0.07
<b>Ogoja</b>	Control	0.05 <sup>d</sup> ±0.03	0.17 <sup>f</sup> ±0.03
	Dumpsite	0.74 <sup>b</sup> ±0.10	0.69 <sup>d</sup> ±0.09
	Slaughter	0.20 <sup>c</sup> ±0.03	0.51 <sup>c</sup> ±0.01
<b>Yakurr</b>	Control	0.02 <sup>d</sup> ±0.03	0.07 <sup>f</sup> ±0.03
	Dumpsite	0.14 <sup>d</sup> ±0.01	0.68 <sup>d</sup> ±0.01
	Slaughter	0.10 <sup>d</sup> ±0.01	0.09 <sup>f</sup> ±0.01
<b>Yala</b>	Control	0.04 <sup>d</sup> ±0.01	0.18 <sup>f</sup> ±0.01
	Dumpsite	0.32 <sup>c</sup> ±0.02	1.04 <sup>b</sup> ±0.06
	Slaughter	0.27 <sup>c</sup> ±0.01	1.02 <sup>b</sup> ±0.05
	<b>Least Significant Difference (LSD)</b>	0.05	0.10

Means with the same superscript along the vertical arrays indicates no significant difference (P>0.05)

**Table 2:** Microbial populations of ground water samples collected from different local government area in Cross River State

Location	THBC (cfu/ml)	TFC(cfu/ml)
Akamkpa	0.11 <sup>c</sup> ±0.09	0.33 <sup>d</sup> ±0.09
Calabar municipal	0.14 <sup>c</sup> ±0.01	0.45 <sup>c</sup> ±0.02
Ikom	0.22 <sup>b</sup> ±0.07	0.60 <sup>b</sup> ±0.05
Ogoja	0.33 <sup>a</sup> ±0.03	0.46 <sup>c</sup> ±0.02
Yakurr	0.09 <sup>c</sup> ±0.02	0.28 <sup>d</sup> ±0.03
Yala	0.21 <sup>b</sup> ±0.14	0.75 <sup>a</sup> ±0.04
LSD	0.06	0.08

Mean with the same superscript along the vertical arrays indicate no significant difference (P>0.05).

**Table 3:** Percentage of Occurrence of Fungal species in groundwater samples

Location	Fungal Isolates	Percentage (%)
Akamkpa	Yeast,	18.18
	<i>Aspergillus</i> sp.	9.09
	<i>Fusarium</i> sp.	18.1
Ikom	<i>Aspergillus</i> sp.	8
	<i>Penicillium</i> sp.	18.18
	<i>Trichophyton</i> sp.	9.09
Calabar Municipality	<i>Aspergillus</i> sp.	9.09
	Yeast	18.18
Yala	<i>Fusarium</i> sp.	9.09
Yakurr	<i>Candida albicans</i>	9.09
	Yeast	9.09
Ogoja	Nil	0.00

**Table 4:** Percentage of occurrence of bacterial isolates in groundwater samples

Location	Bacterial Isolates	Percentage (%)
Akamkpa	<i>Enterobacter</i> sp	5.56
	<i>Klebsiella</i> sp.	5.56
	<i>E. coli</i>	5.56
	<i>Streptococcus</i>	5.56
Ikom	<i>Klebsiella</i> sp	11.11
	<i>Bacillus</i> sp.	11.11
	<i>S. aureus</i>	5.56
	<i>E. coli</i>	5.56
	<i>Citrobacter</i>	5.56
	<i>Enterobacter</i>	5.56
Calabar Municipality	<i>P. aeruginosa</i>	5.56
	<i>E. coli</i>	5.56
Yala	<i>S. pyogenes</i>	5.56
	<i>E. faecalis</i>	5.56
Yakurr	<i>Enterobacter</i>	5.56
	<i>E. coli</i>	5.56
Ogoja	<i>Staphylococcus</i>	5.56
	<i>Klebsiella</i> sp	5.56



#### 4.0 Discussion

The presence of microbiological contamination in groundwater poses significant and serious implications for public health, especially in small communities and developing nations where groundwater is commonly used for drinking (Jonnalagadda and Mhere, 2001). While natural groundwater is typically of good quality, findings show its deterioration can occur rapidly due to inadequate protection measures and ineffective resource management. This agrees with Awofeso *et al.* (2022) in a review of the challenges and some best practices for mitigating groundwater quality deterioration in developing countries. Contaminated groundwater can result in increased rates of illnesses and deaths from diarrheal diseases, and in some cases, outbreaks. The proper disposal of waste through land-based systems plays a crucial role in safeguarding groundwater quality and public health. In semi-urban areas, the use of improper water and sanitation technologies poses long-lasting and severe health risks. Inadequately designed sewage treatment facilities and land application of sewage can lead to groundwater pollution near water sources. The survival of microorganisms, particularly viruses, in such conditions is not well comprehended, but there are indications of prolonged pathogen survival, thereby escalating public health hazards (Jonnalagadda and Mhere, 2001).

The bacterial strains identified include *Enterobacter* sp., *Klebsiella* sp., *S. pyogenes*, *Bacillus* sp., *S. aureus*, *E. coli*, and *Citrobacter* sp. These findings are consistent with the study by Rahman *et al.* (2019), which linked *E. coli*, *Salmonella*, and other pathogenic bacteria pollutants from garbage dumps to groundwater contamination. The contamination of groundwater as observed in this study can lead to poor drinking water quality, water supply

shortages, degradation of surface water systems, expensive clean-up operations, high costs for alternative water sources, and potential health issues. The repercussions of contaminated groundwater or deteriorated surface water are often severe. Bello *et al.* (2020) also supported these findings, stating that groundwater near slaughterhouses was tainted with high levels of coliform bacteria and heavy metals, posing risks to human and animal health. Contaminated groundwater can pose a significant public health threat, potentially causing illness or fatalities if ingested. In extreme cases, as corroborated by EPA, (2024) and USGS (2018), the groundwater may become so contaminated that it is no longer suitable for drinking.

In some instances, contaminated groundwater can be remediated and reused, provided the contamination is not too severe and the municipality is willing to allocate significant resources. The present study indicates that numerous microorganisms have the capacity to contaminate groundwater. Consumption of water containing bacteria and viruses can lead to diseases like hepatitis, cholera, or giardiasis. The most effective approach to mitigate health risks associated with poor drinking water quality is by preventing contaminants from infiltrating groundwater (Jonnalagadda and Mhere, 2001). Consistent with WHO (2006), the findings of this study are crucial, as the detection of fecal coliform bacteria in groundwater, signifies a potential health hazard for individuals exposed to the water.

#### 5.0 Conclusion

The study aimed to assess the impact of pollution on the bacterial load and biochemical characteristics of bacterial isolates in groundwater samples near dumpsites and abattoirs across six LGAs in Cross River State. The results demonstrated a significantly higher

bacterial population in groundwater samples from locations like Calabar municipality (slaughterhouse) and Ogoja (dumpsite) compared to other sites. The significant bacterial counts in these areas underscore the urgent need for health interventions to mitigate pollution and ensure safe drinking water for the local population. It further emphasizes the importance of continuous monitoring and implementation of stringent waste management practices to protect public health and promote sustainable socio-economic development.

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