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Microbial analysis of groundwater sources and streams in Essene, Ikot Abasi Local Government Area, Akwa Ibom state, Nigeria

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ABSTRACT

This study focused on assessing the microbial composition of various water sources in Essene, located within Ikot Abasi Local Government Area, Akwa Ibom State, Nigeria. Water samples were obtained from both groundwater reservoirs and streams and subjected to microbial analysis using standard procedures. The findings revealed that microbial presence was notably higher in stream water compared to groundwater. A total of 17 microbial species were identified, comprising 12 bacterial and 5 fungal species. Among bacterial isolates, Bacillus subtilis exhibited the highest occurrence rate (100%) in groundwater, whereas Proteus sp. and Staphylococcus aureus were the least frequently detected (20%). In stream water, Bacillus cereus was most prevalent (80%), while Enterobacter sp. had the lowest occurrence (20%). Fungal analysis revealed that Penicillium sp. (80%) and Candida sp. (40%) were dominant in groundwater, whereas Candida sp. (80%), Aspergillus flavus (60%), and Aspergillus niger (60%) were the most frequently found in streams. The microbial levels observed in both sources exceeded the WHO standards for potable water, indicating poor microbiological quality in the region. These findings highlight the urgent need for continuous monitoring to mitigate the increasing effects of human activities on microbial contamination in the groundwater and streams of the Essene community.

Keywords: Contamination, Groundwater, Stream and Microbes

INTRODUCTION

Ensuring the availability of safe drinking water for both urban and rural communities is vital in preventing health risks. Water can be sourced from various natural reserves, including streams, lakes, rivers, ponds, rainwater, springs, and wells. However, pure and uncontaminated water remains a temporary state in nature, as environmental conditions and human activities swiftly contribute to pollution (Josiah *et al.,* 2014).

In Nigeria, water pollution is a prevalent issue in both metropolitan and remote areas. The Environmental Protection Agency (EPA, 2016) highlights that microorganisms can occasionally be found in water due to natural pollution sources. These organisms have the potential to cause a range of diseases. Waste materials containing human feces or water exposed to contaminants from industrial or domestic effluents may introduce harmful pathogens, posing a serious health threat when use for drinking or food preparation (UNEP/WHO, 1996).

Research findings from the New York State Department of Health (NYSDH, 2011) indicate that while fecal contamination may contain low concentrations of pathogens, the variety of potential harmful microorganisms is extensive (NYSDH, 2011; UNEP/WHO, 1996). Shittu *et al.* (2008) found that wells located near refuse dumps or septic systems exhibited microbial counts ranging from 1,600 to 1,800 MPN/100ml, significantly higher than wells located farther away from waste sites. Bernard (2013) documented total coliform counts fluctuating between 7 CFU/100ml and 526.7 CFU/100ml, suggesting microbial regrowth, biofilm formation, or the

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infiltration of external substances such as soil and plant debris, all of which compromise water safety.

In South Africa, Lobina and Akoth (2015) observed elevated total coliform levels of 579.4 CFU/100ml in borehole water, attributing this to the close proximity of boreholes to pit latrines. They further noted that some boreholes used electrical pumps, facilitating water distribution through pipelines. According to Ibe *et al.* (2002), rusted pipes contribute to water quality degradation by enabling microbial seepage into boreholes. The World Health Organization (WHO) sets a guideline of zero coliform presence per 100ml, as any detectable coliform organisms indicate a degree of contamination (Bernard *et al*., 2013).

Efforts to improve water accessibility have seen individuals, NGOs, and organizations such as ALSCON facilitating borehole installations for community and personal use. This has made borehole water more available and affordable. Despite having ready access to borehole water for various domestic purposes such as cooking, cleaning, washing, and bathing many residents continue to collect water from streams for drinking and cooking. In these visits to streams, some individuals engage in unhygienic practices like indiscriminate waste disposal, including the wrap-and-throw method of feces disposal. Nevertheless, the belief persists that stream water is more potable than borehole water. Given these perspectives, it is crucial to assess the actual microbiological quality of stream water and verify whether it truly surpasses borehole water in terms of safety.

MATERIALS AND METHODS

Study Area

Essene originally named Nnung Assang is a rural community in Ikot Abasi Local Government Area. Akwa Ibom State. It received its name from the nearby Essene creek which was known to the Opobo people as Essene Obio River (Figure 3.1). It is located at Latitudes 4°36'N Longitudes 7°48'E and Latitudes 4°29'N and Longitudes 7°54'E.

Description of Groundwater Sources and Streams under Research

Five groundwater sources and five streams in the community were randomly selected for the study using a table of random numbers. The groundwater was labeled as BH1-BH5 and the stream water was labeled as SWA-SWE.

SAMPLE COLLECTION

The water samples were collected using new screw-capped bottles that had been sterilized to avoid contamination. Water samples were collected from boreholes following a sterilization process, where cotton wool was soaked in ethanol, ignited, and then used to sanitize the tap opening before retrieval the tap was opened and allowed to run before water was fetched.

Water was fetched from the stream by rinsing the bottles with the stream water. The water was then fetched and the bottles cocked immediately.

Figure 1: Map of Essene
Source: ALSCON

Table 1: Groundwater samples and their location

Groundwater samples	Location
BH 1	Ikot Osukpong
BH 2	Owok Out
BH 3	Okpot
BH 4	Ute
BH 5	Owok Essen

Table 2: Stream water samples and their location

Groundwater samples	Location
SW A	Ikot Osukpong Stream
SW B	Ayakuk Stream
SW C	Ofon-Ikang Stream
SW D	Okpot Stream
SW E	Ute Stream

All the collected samples were kept in a cooler loaded with ice blocks and then transported to the lab for preservation and analysis.

Microbiological Analysis of Groundwater and Stream Water Samples

Serial Dilution Process

Serial dilution was done according to the protocol of Collins and Lyne (1976). Ten milliliters (10ml) of water sample were measured into a conical flask containing 90ml of distilled water. The mixture was shaken for even distribution and1ml aliquot was aseptically transferred into a test tube containing 9mls of distilled water to give 10^{-1} (tenfold dilution). Further tenfold dilution was carried out to factor 10^{-3} dilution.

Inoculation, Enumeration, Isolation and Identification of Pure Culture

Exactly 1ml of desired diluents of each sample was inoculated into different microbiological media (plates) prepared as instructed by the manufacturers. The total heterotrophic bacteria and fungi were determined by the pour plate method (Harrigan and McCance 1990) using Bacto-nutrient agar and Sabouraud dextrose agar. The total coliform and faecal coliform bacteria were enumerated on MacConkey agar and Eosin Methylene Blue (EMB) agar respectively. Using the same method, the *Salmonella* and *Shigella* count, *Vibrio* count and *Staphylococcus aureus* were enumerated using Salmonella- Shigella agar Thiosulphate – Citrate – Bile salts – Sucrose agar (TCBS) and Manitol salt agar respectively, while Sabouraud Dextrose Agar was used for the estimation of fungal count. The bacterial plates were incubated at 37^0C for 24h according to the method of Collins and Lyne (1976) while fungal plates were incubated at room temperature (28 ± 2 ºC) for 5 days.

Maintenance of Pure Microbial Isolates

To maintain pure microbial isolates, discrete colonies from primary plates were picked with the aid of a sterile wire loop and sub-cultured into fresh agar plates (NA and SDA for bacteria and fungi respectively) and incubated. The process was repeated to obtain monocultures of the microbial isolates. The cultural characteristics of the colonies were observed and pure cultures were stocked in agar slants in McCartney bottles. After 24hrs incubation, the slants were preserved in the refrigerator at 4°C for further use.

Characterization and Identification of Bacterial Isolates

Bacterial isolates were characterized and identified presumptively based on their morphology, cultural and physiological characteristics, confirmatory identification was based on biochemical reactions (Table 2). The following identification tests were carried out: Gram staining test, motility test, catalase test, spore staining test, oxidase test, citrate test, starch hydrolysis test, methyl red-voges proskauer test (MR-VR), lactose test, fructose, sucrose and manitol). The results derived from the test for various isolates were collected and the identification was carried out by comparing the characteristics with known taxa using the scheme of Bargey's Manual of Determinative Bacteriology (Bargey and Holts, 1994). Fungal isolates were identified according to the method of Barnett and Hunter (1987).

Statistical Analysis

SPSS package (version 10) was used for analyzing the data obtained. Paired t-test was used to analyze the microbiological variations between the stream and groundwater. Correlation between selected parameters ($p<0.05$) in the stream and groundwater was carried out using Pearson's correlation (r).

RESULTS

The microbiological analysis results obtained in the study of the groundwater and stream water in Essene, Akwa Ibom state, Nigeria are presented in Tables below:

Microbial loads of groundwater and stream water samples

Results of the microbial loads of groundwater and stream water from Essene in Ikot Abasi L.G.A. are presented in Tables.3 and.4. The results of the paired t Test are presented in Appendix I. The microbial groups detected included heterotrophic bacteria, *Pseudomonas* sp., coliforms and fecal coliforms, *Salmonella Shigella, Vibrio* sp., *Staphylococcus aureus*, Faecal Streptococci, and fungi.

Table 3 Microbial analysis of water samples from groundwater

	Mean culturable counts (cfu/ml) ± standard deviation				
Microbial groups	BH1	BH2	BH3	BH4	BH5
Total Heterotrophic bacteria	$1.3\times10^3\pm 0.15$	$1.1\times10^4\pm 1.00$	$1.7\times10^4\pm 0.29$	$1.0\times10^4\pm 1.00$	$1.0\times10^4\pm 0.29$
Pseudomonas Count	-	-	-	-	-
Total Coliform Count	-	-	$1.0\times10^3\pm 1.53$	-	$1.2\times10^3\pm 0.15$
Faecal Coliform Count	-	-	-	-	-
Salmonella shigella Count	-	$2.5\times10^2\pm 0.13$	-	-	-
Vibrio count		-	-	-	-
Faecal Streptococci	-	-	-	-	-
Staphylococcus count	-	-	-	-	-
Fungi Count	$1.1\times10^3\pm 0.29$	$1.0\times10^3\pm 1.04$	$1.5\times10^3\pm 0.15$	$1.2\times10^3\pm 1.53$	$1.0\times10^2\pm 0.29$

Table 4 Microbial analysis of water samples from streams

	Mean culturable counts (cfu/ml) ± standard deviation				
Microbial groups	SWA	SWB	SWC	SWD	SWE
Total Heterotrophic bacteria	$2.5\times10^6\pm 0.25$	$2.0\times10^6\pm 0.58$	$2.5\times10^6\pm 0.11$	$1.9\times10^6\pm 0.58$	$1.4\times10^5\pm 0.10$
Pseudomonas Count	$1.0\times10^4\pm1.00$	-	$2.0\times10^5\pm 0.25$	-	-
Total Coliform Count	$5.6\times10^5\pm 0.45$	$5.0\times10^4\pm 0.20$	$9.0\times10^4\pm 0.30$	$1.6\times10^5\pm 0.21$	$1.3\times10^2\pm 0.33$
Faecal Coliform Count	$1.6\times10^4\pm 0.30$	-	-	$2.0\times10^3\pm1.00$	-
Salmonella shigella Count	-	$1.0\times10^4\pm 1.04$	-	-	-
Vibrio count	-	-	-	$2.0\times10^4\pm 0.25$	-
Faecal Streptococci	-	-	$1.6\times10^4\pm 0.30$	-	-
Staphylococcus count	$3.0\times10^4\pm 1.04$	-	-	$2.0\times10^4\pm 0.45$	-
Fungi Count	$3.0\times10^5\pm 0.20$	$1.9\times10^5\pm 0.32$	$1.2\times10^5\pm1.00$	$1.4\times10^5\pm 0.23$	$1.8\times10^3\pm 0.22$

Pseudomonas sp. was not detected in groundwater samples but was present in the streams, with concentrations ranging from $1.0\times10^4\pm1.00$ to $2.0\times10^5\pm0.25$. While all groundwater samples complied with WHO guidelines for drinking water, most stream samples did not meet the required standard, except for SWB, SWD, and SWE.

Total coliform count values for the groundwater samples ranged from $1.0\times10^3\pm1$. Total heterotrophic bacteria values for the groundwater samples ranged from $1.0\times10^4\pm1.00$cfu to $1.7\times10^4\pm0.29$cfu while that of the stream ranged from $1.4\times10^3\pm0.10$cfu to $2.5\times10^6\pm0.25$. None of the water samples met the WHO standard for drinking water (zero cfu).There was no significant difference in the groundwater samples and streams (p=0.001). The streams however recorded higher values than the groundwater.

53 to $1.2\times10^3\pm0.15$ while that of the stream ranged from $1.3\times10^2\pm0.33$cfu to $9.0\times10^4\pm0.30$cfu. None of the water samples met the WHO standard for drinking water (zero cfu) except BH1, BH2 and BH4. The streams however recorded higher values than the groundwater.

Feacal coliform was not present in the groundwater samples but was present in the streams. The stream had values that ranged from $1.6\times10^4\pm0.30$cfu to $2.0\times10^3\pm1.00$cfu. All the groundwater samples met the WHO standard for drinking water (zero cfu) but the streams did not meet the standard except SWB, SWC and SWE.

Salmonella shigella was present only in BH2 with a mean value of $2.5\times10^2\pm0.13$ and SWB with a mean value of $1.0\times10^4\pm1.04$. All the other water samples of both the groundwater samples and streams met the WHO standard for drinking water. The groundwater however recorded higher value than the stream water.

Vibrio sp. was absent in all the water samples except SWD with a mean value of $2.0\times10^4\pm0.25$. All the water samples met the WHO standard for drinking water except SWD.

Feacal streptococci was absent in all the groundwater samples but present in the streams. The mean value of the streams was $1.6\times10^4\pm0.30$. All the water samples met the WHO standard for drinking water except SWA and SWD.

Staphylococcus sp. was absent in all the groundwater samples but present in some stream water samples with mean values that ranged from $2.0\times10^4\pm0.45$ to $3.0\times10^4\pm1.04$. All the water samples met the WHO standard for drinking water except SWA and SWD.

Fungi count values of the borehole water ranged from $1.0\times10^2\pm0.29$ to $1.5\times10^3\pm0.15$ while that of the streams ranged from $1.2\times10^5\pm1.00$ to $3.0\times10^5\pm0.20$. None of the water samples met the WHO standard for drinking water. There was no significant difference in the groundwater samples and streams (p=0.004). However, the streams recorded higher values than the groundwater.

Pearson's correlation coefficient analysis indicated a positive strong relationship between total heterotrophic bacteria and total coliforms; feacal coliform and Staphylococcus count at 0.05 level of probability (r = 0.969; r = 0.962 respectively) while a moderate but positive significant relationship was recorded between total coliforms and feacal coliform; total coliforms and Staphylococcus at 0.05 level of probability (r = 0.616; r = 0.606) respectively in the stream water.

Table 5: Distribution of diverse bacterial isolates in groundwater samples

Microbial isolate	BH1	BH2	BH3	BH4	BH5
Bacillus subtilis	+	+	+	+	+
Klebsiella sp	-	+	-	-	+
Lactobacillus sp	-	+	-	+	-
Bacillus cereus	+	-	+	-	-
Micrococcus sp	-	-	+	+	-
Proteus sp	-	-	+	-	-
Staphpylococcus aureus	-	-	-	+	-

Source: Field data (2017)

Table 6: Distribution of diverse bacterial isolates in stream samples

Microbial isolates	SWA	SWB	SWC	SWD	SWE
Bacillus subtilis	+	-	+	-	-
Bacillus cereus	+	-	+	+	+
Micrococcus sp	+	+	-	-	+
Escherichia coli	+	-	-	+	-
Pseudomonas aeruginosa	-	-	-	+	-
Citrobacter sp	-	-	+	-	-
Enterobacter sp	-	-	-	-	+
Staphpylococcus aureus	+	+	+	-	-
Specie Richness	5	2	4	3	3

Source: Field data (2017)

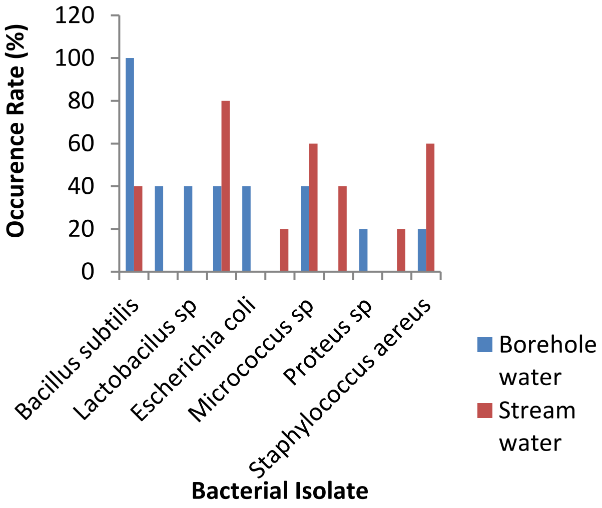

Figure 2: Occurrence Rate (%) of diverse bacterial isolates in groundwater and stream water samples

Fungal diversity of groundwater and stream water samples

Identified organisms were *Aspergillus niger*, *Geotrichum* sp, *Penicilluim* sp, *Candida* sp., and *Aspergillus niger* in borehole water and stream water. Among the fungi isolated from the groundwater sample, *Penicillium* sp (80%), and *Candida* sp. (40%) were the most predominant while *Candida* sp (80%), *Aspergillus flavus* (60%), and *Aspergillus niger* (60%) were the most predominate in the stream water. The result also revealed that *Candida* sp had the highest occurrence rate in both groundwater and stream water samples (Figure.6).

Table 7: Cultural and morphological characteristics of the fungal isolates

Isolate	Colony	Type of Soma	Nature of hyphae	Special vegetative structure	Asexual spores	Special reproductive structure	Conidial head	Vesicle shape	Probable organism
1	Dense felt yellow green	Filamentous	Septate	Foot cell	Globose conidia	Phialides borne directly on the vesicle sclerotia	Radiate	Sub-glubose	*Aspergillus flavus*
2	White smooth membranous colony	Filamentous	Septate	-	Cylindrical conidia	Apothecium	Arthrosporous	-	*Geotrichum* sp
3	Creamy milky colony	Pseudo-hyphae at 28±2°C	Septate	Conida	Blastoconidia	Apothecium	Radiate	Globose	*Candida* sp
4	Compact white or yellow basal dark colony	Filamentous	Septate	Foot cell	Globose conidia	Smooth wall erect conidiophores	Globose	Globose	*Aspergillus niger*
5	Blue-green colony	Filamentous	Septate	Broom-like appearance	Subglobose conidia	Highly 3-stage branched conidiophores	-	-	*Penicillum* sp

Table 8: Distribution of fungal isolates in Groundwater samples

Microbial groups	BH1	BH2	BH3	BH4	BH5
Aspergillus flavus	-	-	-	-	-
Gestrichum sp.	-	+	-	+	-
Candida sp.	+	+	-	+	+
Penicillium sp.	-	-	+	-	-
Aspergillus niger	-	-	-	-	-
Specie Richness	1	2	1	2	1

Source: Field data (2017)

Table 9: Distribution of fungal isolates in stream water samples

Microbial groups	SWA	SWB	SWC	SWD	SWE
Aspergillus flavus	+	-	+	-	+
Gestrichum sp.		-	+	+	-
Candida sp.	+	+	+	-	+
Penicillium sp.	+	-	-	+	-
Aspergillus niger	-	+	+	-	+
Specie Richness	3	2	3	3	3

Source: Field data (2017)

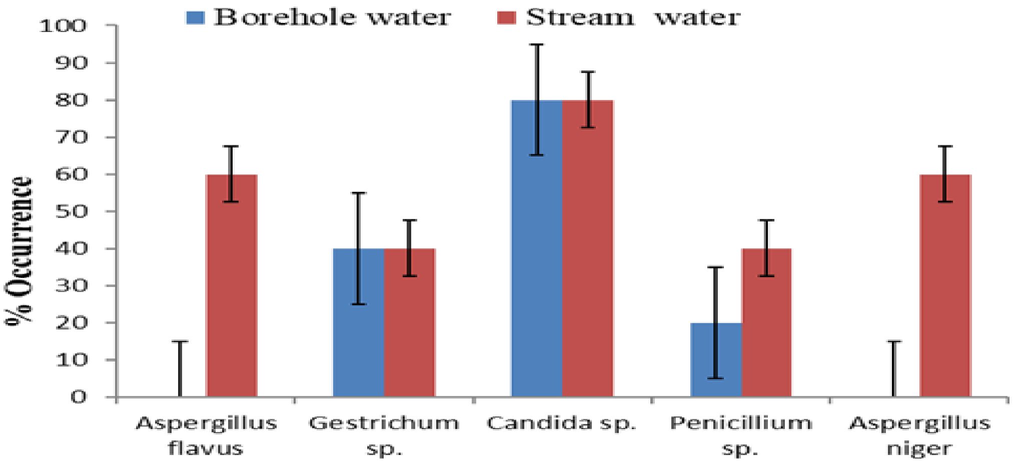

Figure 3: Occurrence rate (%) of diverse fungal isolates in groundwater and stream water
Source: Field data (2017)

DISCUSSION

The analysis of microbial communities in groundwater and stream water demonstrated notable variations in microbial distribution and load across different groups. Findings from this study indicate that stream water exhibited a significantly higher microbial concentration compared to groundwater. Pseudomonas sp. was prevalent in stream water but was absent from borehole samples. Similarly, fungal populations showed substantial differences across various microhabitats, with streams harboring a greater density of fungi than groundwater sources.

Furthermore, bacterial indicators of pollution, including coliforms, fecal coliforms, Salmonella, Shigella, Vibrio sp., and Staphylococcus aureus, were present in higher concentrations in streams than in groundwater. Their presence signifies environmental contamination, with the high occurrence of fecal coliforms suggesting significant human fecal pollution in stream water. Consequently, some stream water samples did not meet the permissible drinking water standards set by the World Health Organization (WHO). According to WHO guidelines (2011), total microbial counts should remain below 1.0×10^2 cfu/ml, with a mandated zero MPN count per 100 ml of water. The results from this study indicate that most of the streams sampled were bacteriologically substandard, carrying potential pathogens harmful to human and animal health.

The high prevalence of heterotrophic bacteria in sampled water sources slightly exceeded previously reported values from Eboh *et al.* (2017) in Delta State, Nigeria, where bacterial counts ranged between 8.0×10^1 and 2.5×10^4 cfu/ml in borehole and well water. This increase could be attributed to contamination from surface runoff carrying environmental residues from human, animal, and plant sources. Research has documented that waste-laden water infiltrates soil layers, leading to underground water contamination (Environmental Protection Agency, EPA, 2016).

A total of 17 microbial species were identified in this study, consisting of 12 bacterial and 5 fungal species, with variations in their distribution across microhabitats. Streams exhibited greater microbial diversity than groundwater. The predominant bacterial species included Gram-positive and Gram-negative rods. Notably, several bacterial strains found in groundwater were also present in stream water. Some of these microorganisms had been previously documented by Eboh *et al.* (2017). Identified bacterial species included *Bacillus cereus*, *Bacillus subtilis*, *Citrobacter sp.*, *Enterobacter sp.*, *Escherichia coli*, *Klebsiella sp.*, *Lactobacillus sp.*, *Micrococcus sp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella sp.*, and *Proteus sp.*. The detected fungal species comprised *Aspergillus flavus*, *Geotrichum sp.*, *Penicillium sp.*, *Candida sp.*, and *Aspergillus niger* in both groundwater and stream water sources. Similar isolates have been reported by previous studies on well and borehole water in Nigerian communities (Eboh *et al.,* 2017; Bello *et al.*, 2013; Ehiowemwenguan *et al*., 2014; Okereke et al., 2014; Adogo *et al*., 2015; Adebawore et al., 2016).

Salmonella belongs to the *Enterobacteriaceae* family, closely related to *Escherichia coli*, and is a Gram-negative, facultative rod-shaped bacterium. In humans, *Salmonella typhi* and *Salmonella paratyphi A* are responsible for two significant diseases—salmonellosis and enteric fever (typhoid) (Kipkemboi, 2011). Its detection in water sources is a serious concern.

Similarly, *Shigella* is a well-recognized pathogen responsible for gastrointestinal infections, particularly in regions with inadequate sanitation. Its low infectious dose facilitates transmission through contaminated food or water. Pathogenic strains of *Shigella* include *S. flexneri*, *S. dysenteriae*, *S. sonnei*, and *S. boydii* (Kipkemboi, 2011), underscoring the health risks associated with its presence in water sources.

The elevated fungal concentrations observed in this study may pose minimal health risks to healthy individuals but could present serious threats to immunocompromised individuals (Makut *et al.,* 2014). Continuous exposure to high fungal spore concentrations, particularly from *Aspergillus spp.*, can lead to pulmonary aspergillosis when inhaled. Most cases occur in individuals with underlying health conditions and weakened immune systems (Shiaka and Yakubu, 2013; Bhatia and Vishwakarama, 2010). Additionally, *A. niger* and other fungal species have been implicated in causing mycotic infections (Makut *et al.,* 2014). Exposure to *Candida albicans* can result in dermatological infections. Moreover, fungi have been linked to respiratory ailments such as asthma, affecting both infants (Jaakkola *et al*., 2010) and adults (Karvala *et al.,* 2010).

CONCLUSION

Results obtained from this study have shown that most of the water sources studied is laden with diverse species of microorganisms including groups of pathogens of both human and animal significance. The presence of pathogenic organisms in some of the water samples rendered it unfit for drinking due to contamination. The quality of water needs to be controlled in order to minimize water borne diseases in the area. Therefore, the groundwater and the streams analyzed may not be wholesome for drinking since they did not meet the different quality specifications and limit of WHO. Hence, the villagers should be educated on the possible risks associated with consumption of borehole and stream water that is not potable. This education should include possible means of treatment of water in the home such as boiling, filtering chlorination so as to prevent health hazards.

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We gratefully recognise the contributions made by the study team and the entire laboratory staff at the University of Uyo, Department of Biochemistry.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

Data produced from this study are available from the corresponding author upon reasonable request.

ETHICAL ISSUES

To ensure that this study was conducted in an ethical manner; a consent letter was gotten from the Ministry of Environment. The researcher visited the community prior to the period of sampling to present the letter and to seek the consent of the host community with reference to the proposed objectives. The community leader assigned one youth to accompany the researcher to all the sampling sites to avoid interrogation and harassment from the indigenes of the community.

REFERENCES

1. Josiah,J.S., Nwangwu, C. O. Omage, K., Akpanyung, O. E. and Dike, D. A. (2014). Physico-chemical and microbiological properties of water samples used for domestic purposes in Okada town, Edo State, Nigeria. *International Journal of Current Microbiology and Applied Sciences* 3(6): 886-894.
2. Environmental Protection Agency, EPA (2016) Human Health and Contaminated Water. http:www.epa.gov/privatewells/human-health-and-contaminated water (Accessed on 7th December, 2016).
3. UNEP/WHO (1996) Water Quality Monitoring- A Practical Guide to the Design and Implementation of Fresh Water Quality Studies and Monitoring Programmes www.who.int/water/Wqmchap10.pdf(Retrieved on 18th December, 2016).
4. New York State Department of Health, NYSDH (2011) Coliform Bacteria in Drinking Water Supplies. www.health.ny.gov/environmental/water/drinking/coliform_bacteria.html(Retrieved on 13th December, 2016).
5. Bernard, N. K. (2013) Physico-chemical and Bacteriological Assessment of selected boreholes and Hand-dug wells in New Edubaise, Ashanti Region. http:/ir.knust.edu.gh/handle/123456789/5323 (Retrieved on 19th December, 2016)
6. Lobina Palamuleni and Mercy Akoth (2015). Physico-chemical and microbiological analysis of selected borehole water in Mahikeng, South Africa. *International Journal of Environmental Research and Public Health*12: 8619-8630.
7. Ibe, S.R., Egereonu, U.U. and Sowa, A.H. (2002) The Impact of hand pump corrosion on water quality in rural areas of West African sub-region.

Environmental Monitoring Assessment Journal. 78(6):31-43.

8. Collins,C. H. and Lyne P. M. (1976). *Microbiological Methods*. 4th ed., London: Butterworth's Publishers, pp. 353-384.
9. Harrigan, W. F and McCane, M. E (1990). *Laboratory Methods in Food and Dairy Microbiology* 8th ed., London: Academic press Inc, 210p.
10. Bergey, D.H. and Holts, J.G. (1994) *Bergey's Manual of Determinative Bacteriology,* 9th Edition. Philadelphia: Lipincott Williams and Wilkins Publication, 107p.
11. Barnett, H.L. and Hunter, B.B. (1987) *Illustrated Genera of Imperfect Fungi.* New York: Macmillan Publishing Company, pp. 70-80.
12. Etok, C.A., Udo, S.M., and Eja, M.E. (2004) *General Microbiology Practical Manual.* Calabar: Abison Printing Press, 37p.
13. Cowan, S.T., Steel, K.J., Barrow, G.I. and Feltham, R. K. (2003) *Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd Edition*Cambridge New York: Cambridge University Press.
14. WHO (2011) Guidelines for drinking water quality, fourth edition. http://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en. (Retrieved on 18th December, 2016).
15. Eboh, J. O., Ogu, G. I. and Idara, M. U. (2017). Microbiological Quality of Borehole And Well Water Sources In Amai Kingdom, Ukwuani Local Government Area Of Delta State, Nigeria. *International Journal of Advanced Academic Research/Sciences, Technology & Engineering,* 3(7): 17-28
16. Bello, O. O. Osho, A. Bankole, S.A. and Bello, T. K. (2013). Bacteriological and Physicochemical Analyses of Borehole and Well Water Sources in Ijebu-Ode, Southwestern Nigeria. *Journal of Pharmacy and Biological Sciences*8(2):18-25
17. Ehiowemwenguan, G., Iloma, A.O. and Adetuwo, J.O. (2014) Physico-chemical and Bacteriological Quality of Borehole Water in Eyaen Community Area of Edo State, Nigeria. *International Journal of Basics and Applied Sciences.* 3(2): 60-68.
18. Okereke, H. C., Ogbonnaya, E. S. and Eze, S. O. (2014). Bacteriological and Physicochemical Qualities of Some Borehole Waters in Aba South Metropolis, Abia State Nigeria. *Asian Journal of Natural Applied Science* 3(3):83-91.
19. Adogo, L. Y. Ajiji, M. A., Anyanwu, N. C. J. and Ajide, B. (2016). Bacteriological and Physico-chemical Analysis of Borehole Water in Auta Balefi Community, Nasarawa State, Nigeria. *Britain Microbiology Research Journal* 11(4):1-7.
20. Adebawore, A. A., Awokunmi, E. E., Akinyeye, R. O. and Olanipekun, E. O. (2016). Physicochemical and bacteriological assessment of Hand-Dug Wells Water fom Ile Oluji, Nigeria. *American Journal of Innovative Research and Applied Science*. 3(1):433-440.
21. Makut, M., Nyam, M., Shehu, A. and Anzaku, S. (2014). A Survey of the Microflora in Outdoor Air Environment of Keffi Metropolis, Nasarawa state, Nigeria. *Academic Journal*, 8(27): 2650 – 2655.
22. Kipkemboi, C. (2011). *Bacteriological and Physico-Chemical Quality Of Water From Various Sources In Samburu District And Efficacy Of Selected Plant Products In Water Purification.* MSc Dissertation. Kenyatta University, Kenya, 109p.
23. Shiaka, G. P. and Yakubu, S. E. (2013). Comparative Analysis of Airborne Microbial Concentrations in the Indoor Environment of Two Selected Clinical Laboratories. *Journal of Pharmacy and Biological Sciences,* 8(4): 13 – 19
24. Jaakkola, J. J., Hwang, B. F. and Jaakkola, M. S. (2010). Home Dampnesss and Molds as Determinants

of Allergic Rhinitis in Childhood: A 6 year population base-cohort studies. *American Journal of Epidemiology*, 17 :451 – 459.

25. Karvala, K, Toskala, E., Laukkonen, R., Lappalainen, S. Utiti, and Nordman, H. (2010). New Onset Adult Asthma in Relation to Dump and Molidy in Workplaces. *International Archives of Occupational and Environmental Health,* 83: 855-865.