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Geomicrobiology of Aquifers at Enugu

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ABSTRACT

This study was undertaken to examine the microbiological and biochemical characteristics of water and sediment samples collected from different locations in Enugu metropolis with a view to determining their quality and possible effects on water chemistry. Ten geographical sites, viz: Emene, New Artisan, Gariki, Trans-Ekulu, Amechi, Centenary, etc. five samples were collected from each geographical site and handled according to standard microbiological procedures, serial dilution, preparation of selective media for the isolation of bacteria and characterization of bacterial isolates. Biochemical tests which includes Gram stain, catalase, coagulase, indole, oxidase, citrate, and sugar fermentation were carried out for isolates characterization. The remarkable outcome was the differences of bacterial isolates across the sites sampled. Microbiological activity involved counts of bacterial populations that were involved in biogeochemical cycling, such as *Desulfomicrobium*, *Geobacter sp*., *Shewanella sp*., *Bacillus sp*., and *Pseudomonas sp*. The existence of extensive variability of microbial density across sites was reflected by the results, with *Geobacter sp*. predominance at Agbani Road (2.5 × 10^3 CFU/mL) and Centenary (1.3 × 10^4 CFU/mL) and suggested active iron reduction. *Shewanella sp*., which has long been known to be metal-reducing, is highly present in Gariki (2.3 × 10^3 CFU/mL) and Agbani Road (2.4 × 10^3 CFU/mL), indicating potential redox conversions. *Desulfomicrobium*, the sulfate-reducing bacterium, is most present in Emene (1.4 × 10^3 CFU/mL) and Ologo (2.1 × 10^2 CFU/mL), indicating potential reduction of sulfate and hydrogen sulfide production. *Pseudomonas sp*., being a metabolically flexible bacterium, exhibited moderate growth in Ugwuaji (1.6 × 10^2 CFU/mL) and Amechi (1.1 × 10^2 CFU/mL), which is indicative of its organic degradation role. Microbial patterns of distribution attest to active microbial-driven processes in the aquifers that control iron, sulfur, and carbon cycling. The results highlight the need for frequent microbial monitoring in order to determine groundwater quality and possible biogeochemical changes.

Keywords: *Desulfomicrobium*, *Geobacter sp*., *Shewanella sp*., *Bacillus sp*., and *Pseudomonas sp*.

INTRODUCTION

Geomicrobiology is an interdisciplinary field that examines the interactions between geological materials and microbial processes. It integrates microbiology, geology, and geochemistry to understand how microorganisms influence and are influenced by the Earth's subsurface environment. Microbes play critical roles in biogeochemical cycles, including those of carbon, nitrogen, sulfur, and iron, shaping the chemical and mineralogical composition of aquifers (Konhauser *et al*., 2019). These processes can affect groundwater quality, mineral formation, and the degradation or mobility of contaminants in aquifer systems (Cui *et al*., 2022).

The aquifers in Enugu are predominantly shallow and consist of sandstones, shales, and coal seams belonging to the Anambra Basin geological formation (Offodile, 2002). These aquifers are recharged primarily through rainfall, with the recharge rate influenced by topography, vegetation, and soil permeability. Due to urbanization, these systems are vulnerable to contamination from industrial discharge, agricultural runoff, and improper waste disposal. Hydrogeochemical studies have shown variable water quality across the region, indicating the influence of both natural processes and human activities (Egboka and Uma, 1986). This underscores the need for a geomicrobiological approach to understanding the complex interactions that determine aquifer health and sustainability.

Enugu State, located in southeastern Nigeria, relies significantly on its aquifers as a primary source of water for domestic, agricultural, and industrial use. Nevertheless, the region encounters challenges such as groundwater contamination, over-extraction, and the effects of urbanization on aquifer systems. Understanding the geomicrobiology of Enugu's aquifers is essential for assessing the microbial processes that affect water quality and availability. Microbial interactions with aquifer sediments can lead to either the purification or contamination of groundwater through biodegradation or mobilization of pollutants (Obasi *et al*., 2020). Additionally, studying these aquifers can help predict their responses to environmental changes and anthropogenic activities.

Location of the Study Area

Enugu, the key city of south-eastern Nigeria, falls between latitudes 6°22′N and 6°39′N, and longitudes 7°26′E and 7°40′E. It covers approximately 79 square kilometers (Egboka *et al*., 1989). As the administrative and economic hub of Enugu State, the city is rich in history that has closely been associated with its coal deposits, which have been the stimulus for motivation in its development. Enugu is nestled within the Anambra Basin, a significant sedimentary basin in Nigeria. The city's landscape has been shaped by a blend of geological processes and human activities over time.

Physiography, Drainage, Vegetation, and Climate

The physiography of Enugu is characterized by undulating valleys and hills, with elevations ranging from 200 m to 300 m above sea level. Topography plays an important role in determining surface and subsurface water movement, vegetation cover, and soil types. The drainage network in Enugu is dendritic with many streams and rivers that drain mainly because of the regional slope. These water bodies replenish aquifers, especially during the rainy season. Among the prominent rivers in the area, the Ekulu River stands out as one of the principal sources of water for the city, playing a crucial role in the city's water supply. The vegetation of Enugu falls within the tropical rainforest belt, but as a result of human actions like urbanization and agriculture, deforestation has been widespread. The landscape now comprises secondary forest, grassland, and cultivated land. The climate is tropical with distinct wet and dry season. The rainfalls vary from 1500 mm to 2000 mm per year, and the wet season is from April to

October. The temperatures are warm overall, varying between 25°C and 30°C, and there are high humidity levels all year round.

Geologic Settings and Hydrogeology of the Study Area

The study area, Enugu, lies in the Anambra Basin of south-eastern Nigeria and has an intensive geological past dominated by Cretaceous sedimentary rocks. The basin rests upon sequences of siltstones, sandstones, shales, and coal seams, significant amongst which are the Enugu Shale, Mamu Formation, and Ajali Sandstone that are great aquifer units. Hydrogeologically, Enugu aquifers are predominantly unconfined to semi-confined, with groundwater flow governed by topography and the permeability of the geologic units. Recharge is primarily by rain, with infiltration rates varying due to differences in soil cover and vegetation. The Ajali Sandstone, highly porous and permeable, is a significant source of groundwater in the region. The aquifers are threatened by contamination from urbanization, agriculture, and poor waste disposal, and thus an overall knowledge of the hydrogeological and microbial processes is critical for sustainable water resource management. Combined geology and geophysical mapping techniques have been used in recent studies to assess groundwater potential in Enugu State. For instance, Ezeh (2012) conducted hydrogeophysical surveys to delineate zones of possible groundwater, which emphasized the significance of formations like the Ajali Sandstone in groundwater potential. Okechukwu and Ikenna (2024) also evaluated the quality of groundwater in Enugu Metropolis, emphasizing the significance of continued monitoring to fight against contamination risks associated with urbanization and industrial processes.

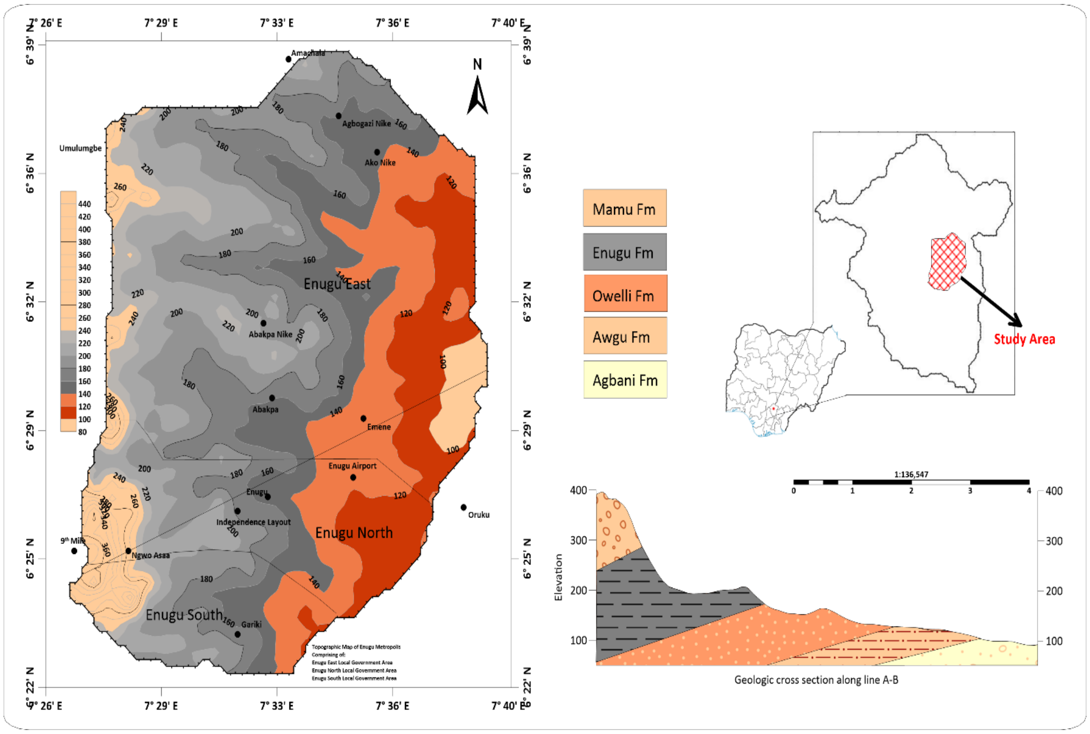

Figure 1: Geologic map of the study area

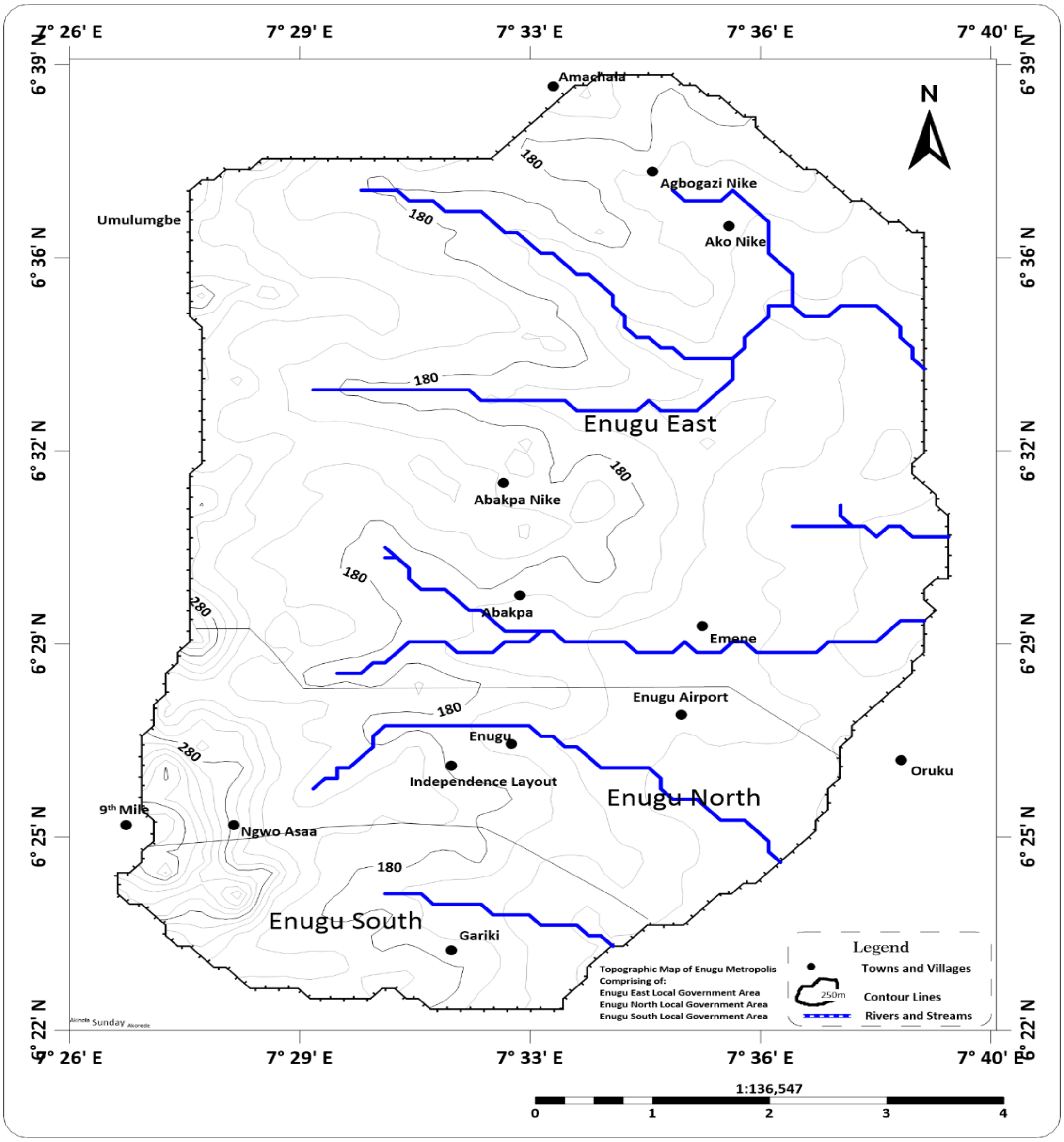

Figure 2: Drainage pattern of the study area

MATERIALS AND METHODS

Sample Collection

Water and sediment samples of about 5 from each locations namely; Emene Water, Ugwuaji Water, New Artesian Water, Garriki Stream, Agbani Road, Ologo Stream, Ekulu River, Well Water Amechi, Ajali River/9th Mile Water and Centenary Water were collected using sterile water bottle. The samples were sent to the laboratory and stored under cool temperature in a refrigerator.

Sample Preparation

The water samples were serially diluted using ten-fold dilution method to decrease the microbial load of the sample. Ten-fold dilutions of the samples were made with sterile normal saline as diluent (Prescott *et al*., 2016).

Media Preparation

The powdered form of Nutrient agar were used and were prepared according to the manufacturer's instruction. The required amount of the media for the analysis were weighed and mixed with distilled water and made to dissolve by heating. It was autoclave at 121^0C for 15minutes. The sterilized medium was allowed to cool down to about 45^0C and was then poured into sterile petri dishes in about 20ml aliquot. The medium was allowed to solidify on the plates and were thereafter used.

Selective Media Preparation for *Geobacter sp.*

Sodium bicarbonate ($NaHCO_3$): 2.5 g, Potassium bicarbonate ($KHCO_3$): 0.1 g, Ammonium chloride (NH_4Cl): 0.25 g, Sodium phosphate (NaH_2PO_4): 0.6 g, Vitamin mix: 10 ml (see standard recipe for trace vitamins used in microbiology), Trace mineral mix: 10 ml (see standard recipe for trace minerals used in microbiology), Cysteine hydrochloride: 0.3 g, Sodium sulfide ($Na_2S{\cdot}9H_2O$): 0.3 g, Acetate (electron donor): 10 mM (e.g., 0.82 g sodium acetate trihydrate), Fumarate (electron acceptor): 20 mM (e.g., 2.32 g sodium fumarate), Agar (for solid media): 15 g. All the components were dissolved in 1 liter of deionized water and the pH was adjusted to 6.8 using NaOH or HCl if necessary. The medium was sterilized by autoclaving at 121°C for 20 minutes. After autoclaving, the medium was cooled to about 50-55°C under an anaerobic atmosphere (e.g., using an anaerobic glove box or by sparging with N_2/CO_2 gas). Reducing agents (cysteine hydrochloride and sodium sulfide) were added to the cooled medium to maintain anaerobic conditions.

Finally, the medium was poured into sterile anaerobic tubes or bottles, and for solid media, it was poured into Petri dishes under anaerobic conditions and allowed to solidify.

Selective Media Preparation for *Shewanella sp*.

The formulation was described as including peptone at 5 g, yeast extract at 1 g, ferric citrate at 0.1 g, sodium chloride (NaCl) at 19.45 g, magnesium chloride ($MgCl_2{\cdot}6H_2O$) at 8.8 g, calcium chloride ($CaCl_2$) at 1.8 g, potassium chloride (KCl) at 0.55 g, and agar at 15 g, with the pH adjusted to 7.6. This mixture was dissolved in one liter of distilled water and autoclaved to sterilize it before use.

Inoculation of Sample

The different dilutions were inoculated into the prepared petri dishes that were labelled based on the dilution and media used. The plates were incubated at 37°C for 24hours for bacteria and 48hours for fungi. After incubation the representative colonies on the plates were counted and recorded (Prescott *et al.*, 2016).

Identification of Bacteria Species

The inoculated plates were examined and different colonies observed using physical and morphological differences were sub cultured to obtain pure cultures. The pure cultures gotten were sub-cultured into an agar slant in bijour bottle and thereafter kept in refrigerator at 4^0C to serve as stock culture. The isolates will be identified and characterized using different biochemical test such as; Gram stain, catalase, coagulase, oxidase, Indole, sugar fermentation test and molecular analysis. (Meena *et al.,* 2015).

Biochemical Characterization

Gram Stain:

One loopful of each of the organisms that were isolated from the different sample were placed and smeared on microscopic slides with a sterile wire loop. The organisms were allowed to air dry and afterwards were heat fixed. Then 5 drops of crystal violet were added on the organisms and were allowed to stand for 1 minute and were washed off with water afterwards. Then 5 drops of iodine solution were added and allowed to stand for 30sec and washed briefly with water. The slides were then tilted and alcohol was used to run on the organisms to decolorize the purple colour. They were immediately washed with water for 5 seconds. 5 drops of safranine were added and were left to stand for 1 minute and were briefly washed off with water. The organisms on the various slides were then examined under the microscope using X100 objective lens (oil immersion). On examination result showed that the organisms retained the purple color of crystal violet proving that the organisms are gram positive.

Catalase Test:

One loop full of the organisms was transferred to a clean glass slides using sterile wire loop. Two drops of 3% hydrogen peroxide (H_2O_2) on the slide and were mixed. Positive result was taken note of by the production of bubbles (Reiner, 2010).

Coagulase Test:

A loopfull of each of the isolates was smeared on a clean glass slide. Blood plasma was transferred on the smear using a sterile wire loop and a clumping structure were observed within 10 seconds, this proves the isolate was coagulase positive.

Citrate Test:

A small amount of the culture growth was picked from the nutrient agar plates, using glass rod and lightly inoculated on Simon citrate agar slants and incubated at 37^0c for 24 hours. A positive test was indicated by the development of a blue colour in the medium. While in a negative reaction the medium retained its original pale green color after incubation (Cappuccino and Sherman, 2005).

Indole Test:

Total of 5 drops of Kovac's reagent were added directly into the tubes. Tryptophanase breaks down tryptophan to release indole, which when reacts with cinnamaldehyde produces a blue-green compound. The absence of enzyme results in no color production (i.e. indole negative) (MacWilliams, 2009).

Oxidase Test:

Filter papers were soaked with tetramethyl-p-phenylenediaminedihydrochloride substrate. The filter papers were moistened with sterile distilled water. The isolates were collected with a wooden stick and smeared on the filter paper (Shields and Cathcart, 2010).

Motility Test:

With a sterile straight needle, touch a colony of a young (18 to 24hour) culture growing on agar medium was picked. Single stab down the center of the tube to about half of the medium. Then incubation at 35-37^{o}C took place and examined daily for up to 7 days. Motile organisms spread out into the medium from the site of inoculation. Non-motile organisms remain at the site of inoculation. (Tille, 2014).

Sugar Fermentation Test:

The sugar fermentation test was conducted using sugar fermentation broth containing peptone, a specific carbohydrate (such as glucose, lactose, or sucrose), a pH indicator (phenol red), and a Durham tube for gas detection. The test organism was aseptically inoculated into the broth and incubated at 35–37°C for 24–48 hours. After incubation, the tubes were observed for evidence of fermentation. A color change in the medium from red to yellow indicated acid production, while the presence of gas bubbles in the Durham tube signified gas production as a by-product of fermentation. The absence of a color change and gas indicated a negative result.

Table 1: Identification Scheme of the Bacterial Isolates

S/N			Biochemical tests					Sugar fermentation					
	Growth appearance on media	**Gram reaction**	**Cat test**	**Oxi test**	**Coa test**	**Methyl red test**	**Indole test**	**Glu**	**Fru**	**Mal**	**Ma**	**Lac**	**Probable organism**
1	Round, flat, and colorless colonies on macconkey agar	-v short rod in pairs	+v	+v	-v	+v	+v	-v	A	A	A	-v	*Pseudomonas sp.*
2	Round, smooth, pinkish colonies on nutrient agar	-ve rod	+ve	+ve	-ve	-ve	-ve	AG	AG	AG	AG	AG	*Shewanella sp*.
3	Very large whitish cottony colony on nutrient agar.	+v short rod	-v	-v	+v	-v	-v	A	A	A	-v	-v	*Bacillus sp*
4	Small, mucoid and whitish colony on nutrient agar.	-v short rod in pairs	+v	-v	+v	-v	+v	A	A	A	-v	-v	*Desulfomicrobium sp.*
5	Brownish-black colonies on nutrient agar	-ve rod	+ve	+ve	-ve	-ve	-ve	AG	AG	AG	AG	AG	*Geobacter sp.*

KEY: Cat=Catalase test, Coa=Coagulase test, Ind=Indole test, Oxi=Oxidase test, Gl=Glucose, F=D-Fructose, Ml=Maltose, Ma=Mannitol, La=Lactose, +=positive, —=negative, A=Acidic, AG=Acidic and Gas, G=Gas, +ve=positive, —ve=negative

Table 2: Total viable count of some microorganisms responsible for the alteration of water chemistry in the water samples from Emene geographical area and its environs.

Sample Source	Lat.	Long	*Desulfomicrobium* (CFU/mL)	*Geobacter sp.* (CFU/mL)	*Shewanella sp.* (CFU/mL)	*Bacillus sp.* (CFU/mL)	*Pseudomonas sp.* (CFU/mL)
Otuku street HDW	N6º 29^1 22^{11}	E7º 33^1 20^{11}	1.0×10^3	1.8×10^2	No growth	No growth	1.4×10^2

Ugwuomu Nike HDW	N6º 31^1 56^{11}	E7º 31^1 56^{11}	1.4×10^3	1.6×10^2	No growth	No growth	1.6×10^2
Owa street HDW	N6º 29^1 44^{11}	E7º 33^1 31^{11}	1.3×10^3	1.4×10^2	No growth	1.4×10^2	1.5×10^2
Promiseland HDW	N6º 27^1 26.94^{11}	E7º 34^1 20.25^{11}	1.1×10^3	1.5×10^2	1.3×10^3	No growth	1.4×10^2
Oriental Estate HDW	N6º 28^1 9.3^{11}	E7º 34^1 51.9^{11}	1.2×10^3	1.7×10^2	No growth	No growth	1.6×10^2

Key: HDW = Hand dug well

Microbial analysis of hand-dug well waters samples from the Emene area revealed significant microbial populations such as *Desulfomicrobium, Geobacter sp., Shewanella sp., Bacillus sp*., and *Pseudomonas sp*. *Desulfomicrobium* accounted for a total viable count of 1.0×10^3 CFU/ml in Otuku Street HDW and 1.4×10^3 CFU/ml in Ugwuomu Nike HDW showing a generalized prevalence of sulfate-reducing bacteria (SRB). Further, *Geobacter sp*. *Geobacter sp*. were found in all samples with counts between 1.4×10^2 to 1.8×10^2 CFU/ml indicating steady iron reduction activity. *Shewanella sp*. Strikingly, *Shewanella sp*. were recovered only in Promiseland HDW with a count of 1.3×10^3 CFU/mL that confirms limited microbial metal reduction. Bacillus sp. was observed only in Owa Street HDW with a count of 1.4×10^2 CFU/ml. *Pseudomonas sp*. were found in all regions studied except Promiseland HDW whose concentrations were between 1.4×10^2 and 1.6×10^2 CFU/ml.

The patterns of microbial distribution show that several biogeochemical processes influence water chemistry in different locations. High abundance of *Desulfomicrobium* shows active sulfate reduction producing hydrogen sulfide (H_2S) and resulting in water odor and pipe corrosion and metal solubilization (Muyzer and Stams, 2008). The presence of *Geobacter sp*. The moderate presence of *Geobacter sp.* in all the samples demonstrates its capacity for iron reduction which reduces Fe^{3+} to Fe^{2+} and impacts the oxidative-reductive potential (Eh) of water that can cause iron precipitation or mobilization (Lovley *et al*., 2004). *Shewanella sp*. is important in Promiseland HDW because it utilizes several electron acceptors like Fe^{3+} and Mn^{4+} that helps drive manganese and iron cycling in groundwater, according to Fredrickson *et al*., 2008. The presence of *Pseudomonas sp. and Bacillus sp*. The presence of *Pseudomonas sp. and Bacillus sp*. demonstrates bioremediation and organic matter degradation capability because such bacteria possess metabolic versatility to degrade complex organic and inorganic substances (Ramírez *et al*., 2009).

These findings provide significant information regarding how the chemistry of the groundwater developed in this area. Sulfate-reducing and iron-reducing bacteria indicate that conditions present there reduce the oxidative-reduction potential (Eh) of water. Changes in water chemistry that are induced by microbial activity regulate the dissolution and mobility of trace metals like arsenic, iron, and manganese in water that can be converted into health threats at high concentrations (Chapelle, 2001). Human-induced organic causes of contamination can be evidenced by the occurrence of Bacillus and Pseudomonas in water distribution systems that lead to change in water chemistry. The resultant interaction among the different microbes in these locations calls for continuous monitoring of water quality alongside potential treatment procedures to introduce stability. The results validate existing research in Southeastern Nigeria groundwater microbiology. Okoh *et al*. The 2017 study by Okoh and others revealed that iron-reducing and sulfate-reducing bacteria dominated shallow groundwater environments in

Enugu and led to elevated sulfide and Fe^{2+} concentrations. Furthermore, Erah *et al*. (2002) determined that *Geobacter sp*. populations of large size were positively correlated with higher Fe^{2+} concentrations in Nigerian groundwater that supports the trends this study observed. In contrast, Obi *et al*. Obi *et al*. (2021) demonstrated in their study lower numbers of sulfate-reducing bacteria in borehole water samples within the Enugu urban area. The researchers credit the results to deeper aquifer systems with higher oxygen levels. Local hydrogeochemical conditions are key to regulating microbial populations and water chemical properties in hand-dug wells and borehole systems.

Table 3: Total viable count of some microorganisms responsible for the alteration of water chemistry in the water samples in Ugwuaji geographical area.

Sample Source	***Lat.***	***Long***	***Desulfomicrobium* (CFU/mL)**	***Geobacter sp.* (CFU/mL)**	***Shewanella sp.* (CFU/mL)**	***Bacillus sp.* (CFU/mL)**	***Pseudomonas sp.* (CFU/mL)**
Ogbuodo Estate HDW	N6º 25^1 23.5570^{11}	E7º 31^1 41.37^{11}	1.5×10^1	No growth	No growth	1.3×10^1	1.6×10^2
Obeagu HDW	N6º $28^1 23.59^{11}$	E7º 31^1 57^{11}	1.4×10^1	No growth	1.6×10^1	No growth	1.3×10^2
Oj Nnamani HDW	N6º 25^1 17.78^{11}	E7º 31^1 36.347^{11}	1.3×10^1	1.4×10^1	No growth	No growth	1.5×10^2
Bricks estate HDW	N6º 25^1 48.08^{11}	N6º 25^1 48.08^{11}	1.6×10^1	No growth	1.6×10^2	1.6×10^1	1.4×10^2
Nkwo ugwuaji HDW	N6º $24^1 51.654^{11}$	E7º 31^1 49.83^{11}	1.2×10^1	1.2×10^2	No growth	1.2×10^1	1.4×10^2

Key: HDW = Hand dug well

The result of Table 5.4 indicates viable count of microorganisms in water samples obtained from Ugwuaji geographical location differed in different hand-dug wells (HDW). The findings confirm that *Desulfomicrobium* appeared to occur in all samples but at comparatively low levels, ranging from 1.2×10^1 CFU/mL to 1.6×10^1 CFU/mL. This provides an indication that sulfate-reducing bacteria are ever-present in the area's groundwater system. *Geobacter sp*. was isolated only in Oj Nnamani HDW with bacterial count of 1.4×10^1 CFU/ml and Nkwo Ugwuaji HDW with 1.2×10^2 CFU/ml, and this would suggest that iron-reducing conditions are localized to individual wells. *Shewanella sp*. was isolated in Obeagu HDW (1.6×10^1 CFU/mL) and Bricks Estate HDW (1.6×10^2 CFU/mL), indicating that microbial Fe (III) reduction is taking place in some parts. *Bacillus sp*. was intermittent, found in only three out of the five wells, i.e., Ogbuodo Estate HDW (1.3×10^1 CFU/mL), Bricks Estate HDW (1.6×10^1 CFU/mL), and Nkwo Ugwuaji HDW (1.2×10^1 CFU/mL). *Pseudomonas sp*., on the other hand, was found in all the water samples, but with the highest concentration being in Ogbuodo Estate HDW (1.6×10^2 CFU/mL). The differences in microbial community composition among sample locations indicate that hydrogeochemical conditions, organic matter availability, and redox status could affect the distribution of microorganisms in the Ugwuaji aquifer.

Trends in the result indicate that *Desulfomicrobium* was present in all the samples throughout, indicating continued sulfate reduction in the study area, possibly due to anaerobic conditions in the groundwater. The presence of *Geobacter sp*. at only two locations indicates that Fe(III) reduction is being carried out in some wells due to localized availability of iron oxides as electron acceptors. The intermittent detection of *Shewanella sp*. suggests that its metabolic activity is limited to specific wells with conditions suitable for microbial iron reduction. *Bacillus sp*. was not evenly distributed, which could suggest that its presence is the result of surface contamination and not a natural part of the groundwater microbiome. *Pseudomonas sp*. predominance in all samples confirms that it is well adapted to environmental conditions in the study area and thrives through its metabolic flexibility as well as its ability to metabolize various organic and inorganic compounds.

The applicability of the findings is significant in regards to groundwater quality and public health. The occurrence of *Desulfomicrobium* indicates possible sulfate-reducing processes, which may result in the generation of hydrogen sulfide (H_2S), affecting the taste, odor, and corrosivity of the water. *Geobacter sp*. and *Shewanella sp*. in specific locations highlights the potential for microbial Fe (III) reduction, which can contribute to increased iron concentrations in water, leading to discoloration and potential clogging of plumbing systems. When compared to similar studies, the microbial profile of Ugwuaji's groundwater shares similarities with findings from other regions in southeastern Nigeria.

Nwachukwu *et al*. (2020), for instance, documented high *Desulfomicrobium* densities in hand-dug wells in Nigeria's southeastern regions, which co-varied with high water sample sulfide content. Obi *et al*. (2019) also documented cases of *Geobacter sp*. and *Shewanella sp*. in ironaceous aquifers where microbial Fe (III) reduction co-varied with high groundwater Fe^{2+} levels. Compared to Emene water samples (Table 5.2), where microbial abundance was also higher generally, the relatively low microbial abundance at Ugwuaji is indicative of differences in hydrogeochemical conditions between the two sites. These are most likely due to such factors as the amount of organic matter present, redox potential, or differences in groundwater recharge processes.

Table 4: Total viable count of some microorganisms responsible for the alteration of water chemistry in the water samples in New-artisan geographical area.

Sample Source	**Lat**	**Long**	***Desulfomicrobium* (CFU/mL)**	***Geobacter sp.* (CFU/mL)**	***Shewanella sp.* (CFU/mL)**	***Bacillus sp.* (CFU/mL)**	***Pseudomonas sp.* (CFU/mL)**
New Artisan stream	N6º 27^1 5.070^{11}	E7º 32^1 28.16^{11}	No growth	1.1×10^1	1.2×10^1	1.0×10^1	1.2×10^2
Egbunike crescent HDW	N6º 26^1 59^{11}	E7º 31^1 27.69^{11}	No growth	1.0×10^1	1.1×10^1	1.0×10^1	No growth
Nza street HDW	N6º 27^1 3.6162^{11}	E7º 31^1 41.4688^{11}	1.0×10^2	1.0×10^2	1.0×10^1	1.1×10^1	1.0×10^2
Premier layout HDW			No growth	1.2×10^1	1.5×10^1	1.0×10^1	No growth
Ugbo Lawrence HDW	N6º 27^1 $78\ 90^{11}$	E7º 31^1 16.65^{11}	1.0×10^2	1.0×10^1	1.0×10^1	1.0×10^1	1.4×10^2

Key: HDW = Hand dug well

The results from the microbial analysis of water samples in the New Artisan geographical area show clear differences in microbial distribution across different water sources. The most notable observation is that *Pseudomonas sp.* was present in high concentrations, especially in Ugbo Lawrence hand-dug well (HDW), where it reached 1.4×10^2 CFU/mL. *Desulfomicrobium*, a bacterium involved in sulfate reduction, was mostly absent, except in Nza Street HDW and Ugbo Lawrence HDW, where it was recorded at 1.0×10^2 CFU/mL. Other bacteria, such as *Geobacter sp.* and *Shewanella sp.*, were detected in several locations but in lower concentrations. Among the sampled locations, Nza Street HDW had the highest overall microbial load, while Egbunike Crescent HDW and Premier Layout HDW had no detectable *Desulfomicrobium* or *Pseudomonas sp.*. Interestingly, the New Artisan stream had lower microbial counts compared to most HDWs, suggesting that flowing water may limit microbial accumulation.

The absence of *Desulfomicrobium* in most locations suggests that sulfate reduction is not a major process in these water sources, possibly due to variations in sulfate availability or oxygen levels. The relatively high presence of *Pseudomonas sp.* in some wells raises concerns, as this bacterium is known for thriving in organic-rich environments and forming biofilms, which can affect water quality and infrastructure.

These findings have important implications for both water quality and public health. The presence of *Desulfomicrobium* in certain HDWs suggests a risk of hydrogen sulfide production, which can cause an unpleasant odor and affect the taste of drinking water. The results highlight the need for proper water treatment and regular monitoring, especially for hand-dug wells, which appear more susceptible to microbial contamination than the stream. If left unchecked, the microbial activity in these wells could also contribute to biofilm formation, which can clog pipes and lead to the corrosion of metal components in water distribution systems.

When comparing these findings with previous studies in southeastern Nigeria, similar patterns emerge. Studies by Okafor *et al*. (2019) and Nwankwo *et al*. (2021) reported that hand-dug wells tend to have higher microbial loads compared to streams, reinforcing the idea that stagnant water sources are at greater risk of contamination. Eze *et al*. (2020) also found that *Pseudomonas sp.* is commonly found in urban well water, which aligns with the results of this study. Also, Agbo *et al*. (2018) observed that *Desulfomicrobium* requires specific conditions, such as high sulfate levels and anaerobic environments, which could explain why it was detected in only a few locations.

Table 5: Total viable count of some microorganisms responsible for the alteration of water chemistry in the water samples in gariki geographical area.

Sample Source	**Lat**	**Long**	***Desulfomicrobium* (CFU/mL)**	***Geobacter sp.* (CFU/mL)**	***Shewanella sp.* (CFU/mL)**	***Bacillus sp.* (CFU/mL)**	***Pseudomonas sp.* (CFU/mL)**
Gariki stream	N6º 23^1 44.7^{11}	E7º 29^1 56^{11}	No growth	No growth	2.3×10^3	2.5×10^3	1.3×10^2
Emeka Ogbodo HDW	N6º 31^1 56^{11}	E7º 31^1 56^{11}	No growth	No growth	2.4×10^3	2.4×10^3	1.5×10^2

Akwuke HDW	N6º 31^1 56^{11}	E7º 31^1 56^{11}	1.0×10^2	No growth	2.5×10^2	2.3×10^3	1.4×10^2
Ihe street HDW	N6º 31^1 56^{1111}	E7º 31^1 56^{11}	No growth	1.0×10^2	2.3×10^3	2.6×10^3	1.3×10^2
Precious street HDW	N6º 31^1 56^{11}	E7º 31^1 56^{11}	No growth	No growth	2.4×10^3	2.4×10^3	1.4×10^2

Key: HDW = Hand dug well

The total viable count of microorganisms in the Gariki geographical area (Table 5.8) highlights the microbial influence on water chemistry, particularly in terms of iron and sulfur cycling, organic matter decomposition, and potential water quality alterations. The dominant microorganisms identified include *Shewanella sp.*, *Bacillus sp*., and *Pseudomonas sp*., with lesser occurrences of *Desulfomicrobium* and *Geobacter sp*. These microorganisms play critical roles in biogeochemical processes, which can affect parameters such as iron solubility, sulfide generation, and overall water quality. *Shewanella sp.* exhibited the highest viable count across all sampling sites, with concentrations reaching 2.3×10^3 CFU/mL in Gariki Stream, 2.4×10^3 CFU/mL in Emeka Ogbodo HDW, and 2.3×10^3 CFU/mL in Ihe Street HDW. This microorganism is known for its iron- and sulfur-reducing capabilities, meaning its activity could contribute to the conversion of Fe^{3+} to Fe^{2+} in anaerobic environments. This transformation may lead to increased iron solubility, discoloration, and potential taste issues in water sources. The high prevalence of *Bacillus sp*. across all sites, especially in Ihe Street HDW (2.6×10^3 CFU/mL), Emeka Ogbodo HDW (2.4×10^3 CFU/mL), and Precious Street HDW (2.4×10^3 CFU/mL), indicates active organic matter decomposition. This suggests that nutrient cycling is a significant process in these water sources, potentially contributing to changes in pH and dissolved organic carbon levels.

The presence of *Desulfomicrobium*, a sulfate-reducing bacterium (SRB), was detected only in Akwuke HDW with microbial count of 1.0×10^2 CFU/mL, indicating localized sulfate reduction. The activity of this microorganism can lead to the production of hydrogen sulfide (H_2S), which is known for its characteristic rotten egg odor and corrosive effects on water infrastructure. The relatively low abundance of *Geobacter sp*., a key iron-reducing bacterium (IRB), suggests that Fe^{3+} reduction is not widespread across the area. However, its presence in Ihe Street HDW (1.0×10^2 CFU/mL) implies that iron-reducing conditions may be occurring at this specific location, leading to localized Fe^{2+} production that can affect iron precipitation and water clarity. At the water quality level, *Shewanella sp*. dominance is indicative of ongoing iron and sulfur conversion processes that have the potential to alter redox balance and impact solubility of key minerals in water sources. The relatively low abundance of *Geobacter sp*. suggests that Fe^{3+} remains largely stable in its oxidized form, except in Ihe Street HDW, where some Fe^{2+} formation may be taking place. The presence of *Desulfomicrobium* in Akwuke HDW presents the issue of potential sulfide accumulation which could be issues of odour and infrastructure corrosion. The high counts of *Bacillus sp*. in all samples also suggest active microbial degradation of organic matter, with likely consequences on pH and organic matter buildup in such water bodies.

Table 6: Total viable count of some microorganisms responsible for the alteration of water chemistry in the water samples in Agbani road geographical area.

Sample Source	Lat	long	*Desulfomicrobium* (CFU/mL)	*Geobacter sp.* (CFU/mL)	*Shewanella sp.* (CFU/mL)	*Bacillus sp.* (CFU/mL)	*Pseudomonas sp.* (CFU/mL)
Agbani Town HDW	N6º 24^{1} 3.27^{11}	7º 30^{1} 2.47^{11}	No growth	2.5×10^{3}	2.4×10^{3}	1.3×10^{3}	1.4×10^{2}
Kenneth street HDW	N6º 24^{1} 19.45^{11}	E7º 29^{1} 52.40^{11}	No growth	No growth	2.5×10^{3}	1.4×10^{3}	1.5×10^{2}
Amokwe street HDW	N6º 24^{1} 41.55^{11}	E7º 29^{1} 51.445^{11}	1.0×10^{2}	2.4×10^{3}	2.3×10^{3}	1.2×10^{3}	1.3×10^{2}
Egbona stream	N6º 23^{1} 55.04^{11}	E7º 30^{1} 9.76	No growth	No growth	2.4×10^{3}	1.4×10^{3}	1.5×10^{2}
Ubaka street HDW	N6º 24^{1} 22.43556^{11}	E7º 29^{1} 57.68^{11}	No growth	2.4×10^{3}	2.5×10^{3}	1.3×10^{3}	1.4×10^{2}

Key: HDW = Hand dug well

The microbial community of the water samples at the Agbani Road geographical location suggests vigorous microbial activity on groundwater chemistry. Dominance by *Geobacter sp*. and *Shewanella sp*., with a population of 2.5×10^{3} CFU/mL in most places, suggests active iron reduction, particularly in Agbani Town HDW, Amokwe Street HDW, and Ubaka Street HDW. These bacteria are notorious for their capacity to lower ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), enhancing the iron solubility in groundwater. It may result in high iron content, which can create problems like metallic taste, water discoloration, and corrosion of plumbing materials. The prevalence of these iron-reducing bacteria throughout the area underscores the redox condition's role in determining the water chemistry in this area.

The microbial population is uniform, with iron-reducing bacteria being dominant in all the sample points, while sulfate-reducing bacteria (*Desulfomicrobium*) are present only in Amokwe Street HDW (1.0×10^{2} CFU/mL). The occurrence of *Desulfomicrobium* in other locations indicates that sulfate reduction is either limited by the availability of low sulfate or preempted by iron-reducing reactions. Since sulfate-reducing bacteria thrive in sulfate-rich and low-oxygen environments, the absence of a minimum value suggests that sulfate is not a significant factor in the groundwater chemistry of this area. *Bacillus sp*., on the other hand, is present in all samples between 1.2×10^{3} CFU/mL and 1.4×10^{3} CFU/mL, indicating degradation of organic matter. This microbial action can assist with nutrient cycling through the degradation of organic contaminants and the release of dissolved carbon and nitrogen compounds into the water.

Another notable observation is the moderate presence of *Pseudomonas sp*., varying between 1.3×10^{2} CFU/ml to 1.5×10^{2} CFU/mL at all the sampling locations. Pseudomonas is also a metabolically versatile bacterium that can carry out aerobic as well as anaerobic respiration and thus is an important contributor to biogeochemical cycling. Its presence suggests denitrification activity, where nitrate is reduced to nitrogen gas under low-oxygen conditions, to regulate groundwater nitrate levels. Pseudomonas is also involved in the clogging of water supply systems caused by biofilm, as well as potential health risks in the event of pathogenic strains. Microbial trends observed within the Agbani Road area have important water quality consequences. The presence of iron-reducing bacteria indicates that iron contamination is a real concern, and treatment strategies such as aeration and filtration be used to remove excess Fe^{2+} from drinking water. The presence of *Bacillus sp*. suggests constant breakdown of organic matter, potentially influencing oxygen supply and nutrient cycles. On the other hand, the abundance of sulfate-reducing bacteria is low, meaning that production of H_2S and resultant corrosion and stench is not a problem in this area. All these interactions between microorganisms show the significance of regular monitoring because a shift in the conditions of groundwater, such as increased organic pollution or redox potential, can alter microbial assemblages and their impact on water chemistry. Comparing to other studies on groundwater microbiology in southeastern Nigeria, the outcome is in agreement with previous results showing iron-reducing bacteria exert a dominant impact on groundwater chemistry. For instance, Nwankwo *et al*. (2020) showed that *Geobacter* and *Shewanella* dominated Fe^{2+} accumulation in groundwater in Enugu, in line with microbial patterns in Agbani Road. Likewise, Okonkwo *et al*. (2018) observed minimal reduction of sulfate in hand-dug wells, which is in agreement with the low occurrence of *Desulfomicrobium* in this study. In more industrialized regions, where there is more anthropogenic pollution and consequently higher sulfate levels, sulfate-reducing bacteria are more prevalent, as noted by studies by Ezeh *et al*. (2015).

Table 7: Total viable count of some microorganisms responsible for the alteration of water chemistry in the water samples in Ologo geographical area.

Sample Source	**Lat.**	**Long**	***Desulfomicrobium* (CFU/mL)**	***Geobacter sp.* (CFU/mL)**	***Shewanella sp.* (CFU/mL)**	***Bacillus sp.* (CFU/mL)**	***Pseudomonas sp.* (CFU/mL)**
Ologo stream	N6º 25^{1} 17.3586	N6º 25^{1} 17.3586	1.0×10^{2}	1.2×10^{2}	1.0×10^{2}	1.1×10^{2}	No growth
Broadlick HDW	N6º 25^{1}35.4388^{11}	7º 28^{1} 47.8477^{11}	No growth	1.0×10^{2}	1.1×10^{3}	1.2×10^{2}	1.0×10^{2}
Ngenevu HDW	N6º 25^{1} 54050^{11}	7º 28^{1} 37.43^{11}	2.1x10^{2}	No growth	1.3×10^{2}	1.0×10^{2}	No growth
Udi siding HDW	N6º 26^{1} 6.209^{11}	7º 28^{1} 28.6^{11}	No growth	1.3×10^{2}	1.1×10^{2}	1.1×10^{2}	2.1x10^{3}
Old UNTH HDW	N6º 26^{1} 4.41^{11}	7º 28^{1} 36.9^{11}	No growth	1.1×10^{2}	1.0×10^{2}	1.2×10^{2}	No growth

Key: HDW = Hand dug well

The microbial analysis of water samples from the Ologo geographical area, as presented in Table 5.12, reveals notable variations in the presence and abundance of key microbial species involved in altering water chemistry. *Desulfomicrobium* was detected only in Ologo Stream at 1.0×10^{2} CFU/ml and Ngenevu HDW at 2.1×10^{2} CFU/ml, suggesting the presence of sulfate-reducing bacteria in these water sources. *Geobacter sp*. was found in Ologo Stream at 1.2×10^{2} CFU/ml, Broadlick HDW at 1.0×10^{2} CFU/ml, Udi Siding HDW at 1.3×10^{2} CFU/ml, and Old UNTH HDW at 1.1×10^{2} CFU/ml, highlighting its role in iron and sulfur cycling in both surface and groundwater. *Shewanella sp*. exhibited the highest counts in Broadlick HDW at 1.1×10^{3} CFU/ml and was present in all other locations except Ngenevu HDW, indicating its adaptability to different redox conditions. *Bacillus sp*. was consistently detected across all sample sites, with similar concentrations ranging between 1.0×10^{2} and 1.2×10^{2} CFU/mL, indicating its widespread occurrence in aquatic environments. Notably, *Pseudomonas sp*. was found exclusively in Broadlick HDW with values of 1.0×10^2 CFU/mL) and Udi Siding HDW at 2.1×10^3 CFU/ml, with no growth observed in other locations, suggesting potential localized contamination sources.

The observed microbial distribution suggests a strong correlation between environmental conditions and microbial populations. The presence of *Desulfomicrobium* in Ologo Stream and Ngenevu HDW indicates anoxic or low-oxygen conditions, favoring sulfate-reducing bacteria that facilitate the conversion of sulfate to hydrogen sulfide (Muyzer and Stams, 2008). In contrast, *Geobacter sp*., which plays a role in iron and sulfur cycling, appears more prevalent in wells and streams where intermediate redox conditions exist. Its higher count in Udi Siding HDW (1.3×10^2 CFU/mL) suggests potential iron-reducing activity, contributing to dissolved iron concentrations in groundwater (Lovley *et al*., 2004). The abundance of *Shewanella sp*. in Broadlick HDW (1.1×10^3 CFU/mL) suggests strong sulfur-oxidizing activity, potentially driven by exposure to oxygen, enabling the conversion of reduced sulfur compounds into sulfate (Fredrickson *et al*., 2008). *Bacillus sp*. was consistently detected in all sample locations, indicating its adaptability to a wide range of environmental conditions. Its role in organic matter degradation and sporulation likely explains its persistence across different water sources (Logan and De Vos, 2009). The most notable microbial presence is *Pseudomonas sp*. in Udi Siding HDW (2.1×10^3 CFU/mL), where it significantly outnumbers other species. This high concentration suggests a localized condition favorable to its growth, possibly due to organic pollutants or high nitrate levels that *Pseudomonas sp*. can metabolize under aerobic conditions (Wani *et al*., 2006). Its absence in other locations indicates that its presence may be influenced by anthropogenic activities or specific chemical compositions unique to Udi Siding HDW.

The variation in microbial populations across different water sources suggests direct microbial involvement in altering water chemistry within the Ologo geographical area. Locations with *Desulfomicrobium* presence, such as Ngenevu HDW and Ologo Stream, are likely experiencing sulfate reduction, which could contribute to hydrogen sulfide formation and associated odor issues (Muyzer and Stams, 2008). The prevalence of *Geobacter sp*. and *Shewanella sp*. in multiple locations highlights the role of microbial iron and sulfur cycling, which can influence redox-sensitive elements like iron, manganese, and sulfur compounds (Fredrickson *et al*., 2008). The high *Pseudomonas sp*. count in Udi Siding HDW could indicate potential contamination from organic matter, nitrates, or hydrocarbons, raising concerns about water quality in this area (Wani *et al*., 2006). The widespread occurrence of *Bacillus sp*. suggests its role in organic matter breakdown, potentially influencing dissolved organic carbon levels in these water sources (Logan and De Vos, 2009).

Similar microbial distributions have been reported in studies analyzing groundwater and surface water microbial communities in urban and peri-urban environments. For instance, research on water systems in Enugu has identified *Shewanella sp*. and *Geobacter sp*. as key contributors to redox transformations affecting iron and sulfur cycling, aligning with the patterns observed in the Ologo area (Nwankwo *et al*., 2022). Studies on sulfate-reducing bacteria in anoxic environments have similarly documented *Desulfomicrobium* as a primary driver of hydrogen sulfide production in groundwater systems (Muyzer and Stams, 2008). Furthermore, investigations into *Pseudomonas sp*. contamination in groundwater sources have linked its presence to anthropogenic influences, particularly from agricultural runoff, wastewater infiltration, and industrial activities (Khan *et al*., 2020). The significantly higher *Pseudomonas sp*. concentration in Udi Siding HDW mirrors findings from studies where high organic and nitrate loads promoted its proliferation (Wani *et al*., 2006). The widespread occurrence of *Bacillus sp*. aligns with previous research highlighting its role in nutrient cycling and resilience in diverse aquatic environments (Logan and De Vos, 2009).

Table 8: Total viable count of some microorganisms responsible for the alteration of water chemistry in the water samples in Trans-ekulu geographical area.

Sample Source	**Lat**	**Long**	***Desulfomicrobium (CFU/mL)***	***Geobacter sp. (CFU/mL)***	***Shewanella sp. (CFU/mL)***	***Bacillus sp. (CFU/mL)***	***Pseudomonas sp. (CFU/mL)***
Ugbuowa street HDW	N6º 29^1 42^{11}	E7º 28^1 48^{11}	1.3×10^2	No growth	No growth	1.2×10^2	1.0×10^2
Winners estate HDW	N6º 30^1 1^{11}	E7º 28^1 38^{11}	1.4×10^2	No growth	1.1×10^2	1.3×10^2	1.1×10^2
Obieze ogwuagor HDW	N6º 29^1 30^{11}	E7º 31^1 26^{11}	1.2×10^2	No growth	1.4×10^2	1.1×10^2	1.0×10^3
Ezenweke street HDW	N6º 29^1 8.8^{11}	E7º 30^1 6.95^{11}	1.3×10^2	1.2×10^2	No growth	1.2×10^2	1.1×10^2
Ivory estate HDW	N6º 28^1 38.39^{11}	E7º 30^1 15.1488^{11}	1.4×10^2	No growth	1.3×10^2	1.1×10^2	1.0×10^2

Key: HDW = Hand dug well

The microbial analysis of hand-dug wells (HDW) in the Trans-Ekulu geographical area, as presented in Table 5.14, highlights the presence of key bacterial species responsible for altering water chemistry. The most dominant microorganisms across the sample sites were *Desulfomicrobium* (1.2×10^2 – 1.4×10^2 CFU/mL) and *Bacillus sp*. (1.1×10^2 – 1.3×10^2 CFU/mL), indicating widespread sulfate reduction and organic matter decomposition. *Pseudomonas sp*. exhibited a significantly high count at Obieze Ogwuagor HDW (1.0×10^3 CFU/mL), while *Geobacter sp*. was detected only at Ezenweke Street HDW (1.2×10^2 CFU/mL), suggesting localized Fe^{3+} reduction. *Shewanella sp.* showed notable growth in Winners Estate HDW (1.1×10^2 CFU/mL), Obieze Ogwuagor HDW (1.4×10^2 CFU/mL), and Ivory Estate HDW (1.3×10^2 CFU/mL), implicating Fe^{3+} and Mn^{4+} reduction in those locations.

The presence of *Desulfomicrobium* sp. across all sample sites suggests a consistent sulfate-reducing environment, which could contribute to the production of hydrogen sulfide (H_2S), a compound associated with foul odors and water acidity. This trend aligns with findings from Muyzer and Stams (2008), who noted that sulfate-reducing bacteria (SRB) thrive in anaerobic conditions where they utilize sulfate as an electron acceptor, leading to H_2S production. In contrast, *Geobacter sp*. was only detected at Ezenweke Street HDW (1.2×10^2 CFU/mL), indicating localized iron-reducing conditions that promote the dissolution of Fe^{3+} into Fe^{2+}. This finding is significant because iron reduction can lead to increased Fe^{2+} concentrations in groundwater, which, if excessive, could pose potential health and aesthetic concerns, such as staining and metallic taste, as reported in studies by Lovley *et al*. (2004).

The microbial distribution patterns also suggest site-specific differences in water chemistry. For instance, the high *Pseudomonas sp*. count at Obieze Ogwuagor HDW (1.0×10^3 CFU/mL) raises concerns about potential biofilm formation and nitrate reduction, both of which can affect water flow and quality. *Pseudomonas sp*ecies are known for their ability to degrade organic matter and participate in nitrogen cycling (Silby *et al*., 2011). Their abundance in this location suggests that organic contaminants or elevated nitrate levels may be influencing microbial proliferation. Similarly, *Shewanella sp.*, a known metal-reducing bacterium, was detected in Winners Estate, Obieze Ogwuagor, and Ivory Estate HDWs, which suggests active manganese and iron cycling in those specific wells. This concurs with previous findings that set down *Shewanella sp*ecies thriving under iron- and manganese-rich groundwater environments, mediating the release of such metals into a form that is bioavailable.

The implications of such results on water quality are important. The dominance of sulfate-reducing and iron-reducing bacteria suggests that groundwater in Trans-Ekulu is subject to active biogeochemical cycling, which could affect water aesthetics, odor, and metal content. The production of H_2S by *Desulfomicrobium* sp. may contribute to unpleasant odors and potential acidification of the water. Concurrently, the occurrence of *Geobacter* and *Shewanella sp*ecies in a localized environment indicates dissolution of iron and manganese and thus elevated levels of these metals in drinking water with aesthetic and health implications. Additionally, the high *Pseudomonas sp*. count in Obieze Ogwuagor HDW suggests the possibility of biofilm formation, which can reduce water flow in pipes and contribute to microbial contamination risks.

Comparing these results with other studies, the trends observed in Trans-Ekulu align with global research on groundwater microbiology. Similar studies have demonstrated that *Geobacter sp*ecies play a key role in Fe^{3+} reduction, leading to increased Fe^{2+} concentrations in groundwater systems (Lovley *et al*., 2004). The detection of high *Pseudomonas sp*. counts in this study also reflects findings from Silby *et al*. (2011), who reported that *Pseudomonas sp*ecies dominate environments with high organic matter and nitrate content, where they can contribute to biofilm formation and nitrogen transformations. Likewise, the ubiquity of *Desulfomicrobium* sp. in this study is consistent with findings from Muyzer and Stams (2008), who reported that sulfate-reducing bacteria thrive in anaerobic groundwater systems, producing hydrogen sulfide that can lead to water discoloration, corrosion, and odor issues.

Table 9: Total viable count of some microorganisms responsible for the alteration of water chemistry in the water samples in Amechi geographical area.

Sample Source	**Lat**	**Long**	***Desulfomicrobium* *(CFU/mL)***	***Geobacter sp. (CFU/mL)***	***Shewanella sp. (CFU/mL)***	***Bacillus sp. (CFU/mL)***	***Pseudomonas sp. (CFU/mL)***
Jedidah gardens HDW	N6º 28^1 23.59^{11}	E7º 31^1 57^{11}	1.3x10^2	1.6x10^2	1.6x10^2	No growth	No growth
Elshammal estate HDW	N6º 23^1 48.6^{11}	E7º 32^1 14.7^{11}	1.4×10^2	No growth	No growth	1.3×10^2	1.1×10^2

Citi ville estate HDW	N6º 28^{1} 23.59^{11}	E7º 31^{1} 57^{11}	No growth	1.2x10^{2}	1.6x10^{2}	No growth	No growth
Sportters estate HDW	N6º 23^{1} 23.38^{11}	E7º 31^{1} 50^{11}	1.3×10^{2}	No growth	No growth	1.2×10^{2}	1.1×10^{2}
Hilliu estate HDW	N6º 24^{1} 6.22^{11}	E7º 31^{1} 20.26^{11}	1.4×10^{2}	No growth	No growth	1.1×10^{2}	1.0×10^{2}

Key: HDW = Hand dug well

The total viable count of microorganisms in Amechi's hand-dug wells (HDW), presented in Table 5.16, shows the distribution of microbial species that influence water chemistry in the region. The variations in microbial populations across different wells suggest differences in redox conditions, organic matter availability, and potential microbial-driven geochemical processes. At Jedidah Gardens HDW, the relatively high counts of *Desulfomicrobium* (1.3×10^{2} CFU/mL), *Geobacter sp*. (1.6×10^{2} CFU/mL), and *Shewanella sp.* (1.6×10^{2} CFU/mL) indicate active microbial reduction processes, particularly sulfate and iron reduction. These bacteria facilitate the transformation of sulfates (SO_4^{2-}) to sulfides (H_2S) and Fe^{3+} to Fe^{2+}, influencing the redox balance and metal solubility in water. The absence of *Bacillus sp*. and *Pseudomonas sp*. suggests limited aerobic degradation activity in this well.

In Elshammal Estate HDW, *Desulfomicrobium* with bacterial count of 1.4×10^{2} CFU/ml remains dominant, with *Bacillus sp*. with 1.3×10^{2} CFU/ml and *Pseudomonas sp*. with 1.1×10^{2} CFU/ml present. The presence of these aerobic species suggests partial oxygen availability, which may support organic matter decomposition and nitrification. However, the absence of *Geobacter sp*. and *Shewanella sp.* indicates minimal microbial iron reduction.

Similarly, Citi Ville Estate HDW shows no *Desulfomicrobium* but has notable counts of *Geobacter sp*. (1.2×10^{2} CFU/mL) and *Shewanella sp.* (1.6×10^{2} CFU/mL), indicating iron-reducing conditions. The lack of *Bacillus sp*. and *Pseudomonas sp*. suggests that anaerobic respiration dominates in this well, potentially leading to the mobilization of iron and manganese in the water. Sportters Estate HDW and Hilliu Estate HDW exhibit similar microbial distributions, with *Desulfomicrobium* with count of $1.3–1.4\times10^{2}$ CFU/ml, *Bacillus sp*. with count of $1.1–1.2\times10^{2}$ CFU/ml, and *Pseudomonas sp*. with count of $1.0–1.1\times10^{2}$ CFU/ml detected. The coexistence of sulfate-reducing and aerobic bacteria suggests a transition zone where both oxidative and reductive processes occur. Overall, the result suggest that Jedidah Gardens HDW and Citi Ville Estate HDW have the highest potential for microbial iron and sulfate reduction, while Elshammal, Sportters, and Hilliu Estates HDW exhibit mixed redox conditions. The presence or absence of key microbial groups directly impacts the chemical composition of the groundwater, affecting parameters like sulfate, iron, and dissolved oxygen levels.

Table 10: Total viable count of some microorganisms responsible for the alteration of water chemistry in the water samples in Ajali River/9th Mile geographical area.

Sample Source	**Lat**	**long**	***Desulfomicrobium* (CFU/mL)**	***Geobacter sp.* (CFU/mL)**	***Shewanella sp.* (CFU/mL)**	***Bacillus sp.* (CFU/mL)**	***Pseudomonas sp.* (CFU/mL)**

9th Mile Brewery Area Borehole	N6º 20^{1} 38.64	E7º 26^{1} 18.72^{11}	No growth	1.3x10^{4}	No growth	1.0x10^{2}	No growth
Akama borehole	N6º 25^{1} 27^{11}	E7º 26^{1} 20^{11}	1.4×10^{2}	No growth	No growth	1.3×10^{2}	1.1×10^{2}
Onitsha road quarters HDW	N6º 25^{1} 15^{11}	E7º 27^{1} 18.2	No growth	1.3x10^{4}	No growth	1.0x10^{2}	No growth
Iva valley stream	N6º 26^{1} 15^{11}	E7º 28^{1} 18^{11}	No growth	1.3x10^{4}	1.4x10^{2}	1.4x10^{2}	No growth
PWD new market HDW	N6º 24^{1} 14^{11}	E7º 29^{1} 14^{11}	No growth	1.3x10^{4}	No growth	1.0x10^{2}	No growth

Key: HDW = Hand dug well

The microbial analysis of water samples from different locations within the Ajali River/9th Mile area revealed varying levels of bacterial presence. *Geobacter sp*. was consistently found in high concentrations of 1.3 × 10^4 CFU/ml in four out of the five sampled locations 9th Mile Brewery Area Borehole, Onitsha Road Quarters HDW, Iva Valley Stream, and PWD New Market HDW. *Desulfomicrobium* was detected only in the Akama borehole wit bacterial count of 1.4 × 10^2 CFU/mL), while *Shewanella sp.* was found exclusively in Iva Valley Stream with counts at 1.4 × 10^2 CFU/ml. *Bacillus sp*. was present in all locations but at lower concentrations of 1.0 × 10^2 – 1.4 × 10^2 CFU/ml. *Pseudomonas sp*. was detected only in the Akama borehole with counts at 1.1 × 10^2 CFU/ml, while no growth was recorded in other sites.

There was a distinct pattern from the data, indicating *Geobacter sp*. as the dominant microorganism in most of the sampled locations. This bacterium is a known iron-reducing microbe, commonly found in anoxic environments where Fe^{3+} (ferric iron) is reduced to Fe^{2+} (ferrous iron), altering water chemistry by increasing iron solubility. The absence of *Geobacter sp*. in the Akama borehole suggests that this environment may not be sufficiently reducing or that competition with other microbial species, such as *Desulfomicrobium* and *Pseudomonas sp*., is inhibiting its growth.

The detected presence of *Desulfomicrobium* solely in Akama borehole sample indicates sulfate-reducing conditions, likely leading to the production of hydrogen sulfide (H_2S), which can cause water discoloration and odor issues. *Shewanella sp.*, another iron-reducing bacterium, was only detected in Iva Valley Stream, suggesting that this location may support both Fe^{3+} and alternative electron acceptors for respiration. The lower bacterial count but widespread occurrence of *Bacillus sp*. across all sites suggests that this genus, known for its metabolic versatility, thrives in diverse environmental conditions.

The high prevalence of *Geobacter sp*. in boreholes and hand-dug wells implies significant iron reduction, leading to increased Fe^{2+} concentrations. This can impact water quality by contributing to elevated iron levels, leading to reddish-brown staining, metallic taste, and potential clogging of water distribution systems. The presence of *Desulfomicrobium* in Akama borehole suggests a risk of sulfide contamination, which can cause corrosion of pipes and an unpleasant odor. *Shewanella sp.* in Iva Valley Stream indicates possible fluctuations in redox conditions, facilitating both iron and manganese mobilization, which could contribute to water discoloration and potential toxicity if concentrations exceed regulatory limits.

The detection of *Pseudomonas sp*. in the Akama borehole raises additional concerns, as some species within this genus are opportunistic pathogens and may pose a health risk, particularly to immunocompromised individuals. *Bacillus sp*., though generally considered harmless, may indicate the presence of organic matter, which could serve as a nutrient source for other microbial populations.

The findings in Ajali River/9th Mile are consistent with previous studies on groundwater microbial ecology, which highlight the dominance of *Geobacter sp*. and *Shewanella sp.* in anaerobic, iron-rich aquifers. Similar studies have shown that *Geobacter sp*. is frequently associated with high Fe^{2+} levels, while *Desulfomicrobium* is a key contributor to sulfate reduction and hydrogen sulfide production in groundwater. Research in sulfidic aquifers has also demonstrated that the presence of *Desulfomicrobium* correlates with increased sulfate concentrations and low redox potential, similar to what is observed in the Akama borehole.

Furthermore, studies on *Pseudomonas* contamination in drinking water sources suggest that its presence is often linked to biofilm formation and chlorine resistance, posing challenges for water treatment. The low but widespread presence of *Bacillus sp*. aligns with findings in nutrient-rich environments where this genus plays a role in organic matter decomposition and biogeochemical cycling.

Table 11: Total viable count of some microorganisms responsible for the alteration of water chemistry in the water samples in Centenary geographical area.

Sample Source	**Lat.**	**Long.**	***Desulfomicrobium* (CFU/mL)**	***Geobacter sp.* (CFU/mL)**	***Shewanella sp.* (CFU/mL)**	***Bacillus sp.* (CFU/mL)**	***Pseudomonas sp.* (CFU/mL)**
Centenary City Estate HDW	N6º 23^1 20^{11}	E7º 31^1 56	1.2x10^2	1.2x10^2	No growth	1.2x10^2	No growth
Tenece layout HDW	N6º 23^1 28	E7º 31^1 56	1.4×10^2	No growth	No growth	1.3×10^2	1.1×10^2
Almond garden HDW	N6º 23^1 19^{11}	E7º 31^1 50^{11}	1.2x10^3	1.5x10^2	No growth	1.2x10^2	No growth
Divine mercy parish HDW	N6º 23^1 145^{11}	E7º 31^1 22^{11}	No growth	1.3x10^4	1.4x10^2	1.4x10^2	No growth
Nwabuude mall HDW	N6º 23^1 23.38	E7º 31^1 50^{11}	No growth	1.3x10^4	No growth	No growth	No growth

Key: HDW = Hand dug well

The microbial analysis of water samples from the Centenary geographical area reveals variations in the distribution of iron-reducing (*Geobacter sp*p. and *Shewanella sp*p.), sulfate-reducing (*Desulfomicrobium* spp.), and general heterotrophic bacteria (*Bacillus sp*p. and *Pseudomonas sp*p.). The highest microbial count was observed for *Geobacter sp*p. in Divine Mercy Parish HDW and Nwabuude Mall HDW, both recording bacterial count of 1.3×10^4 CFU/mL. *Shewanella sp*p. was no detected in most locations but detected at 1.4×10^2 CFU/ml in Divine Mercy Parish HDW. The highest *Desulfomicrobium* spp. count was observed in Almond Garden HDW with counts of 1.2×10^3 CFU/ml, while *Bacillus sp*p. was present in all samples except Nwabuude Mall HDW, with values ranging from 1.2×10^2 to 1.4×10^2 CFU/ml. *Pseudomonas sp*p. was detected only in Tenece Layout HDW at 1.1×10^2 CFU/mL.

The trend in microbial distribution suggests site-specific redox conditions influencing microbial proliferation. The extremely high *Geobacter sp*p. count in Divine Mercy Parish HDW (1.3×10^4 CFU/mL) and Nwabuude Mall HDW (1.3×10^4 CFU/mL) indicates a reducing environment that favors iron reduction, possibly due to lower oxygen levels and increased organic matter availability. The absence of *Shewanella sp*p. in most locations suggests that strong reducing conditions are not widespread, except in Divine Mercy Parish HDW where it appears at 1.4×10^2 CFU/mL. The detection of *Desulfomicrobium* spp. in Almond Garden HDW recorded a bacterial count of 1.2×10^3 CFU/mL) and Tenece Layout HDW also had about 1.4×10^2 CFU/ml points to localized sulfate-reducing conditions that may facilitate the production of hydrogen sulfide, affecting water chemistry. The sporadic presence of *Pseudomonas sp*p. was found only in Tenece Layout HDW at 1.1×10^2 CFU/ml which suggests that its survival depends on site-specific nutrient availability and aeration levels.

The microbial composition in the Centenary geographical area has significant implications for groundwater chemistry and quality. The dominance of *Geobacter sp*p. in Divine Mercy Parish HDW with bacterial count of 1.3×10^4 CFU/ml and also in Nwabuude Mall HDW with counts at 1.3×10^4 CFU/ml suggests active iron reduction, potentially increasing soluble ferrous iron (Fe^{2+}) levels, leading to water discoloration and a metallic taste. Also from the result presence of *Desulfomicrobium* spp. in Almond Garden HDW with bacterial count of 1.2×10^3 CFU/ml suggests sulfate reduction, which could result in hydrogen sulfide formation, causing a foul odor and potential corrosion of pipes. The absence of *Shewanella sp*p. in most wells except Divine Mercy Parish HDW (1.4×10^2 CFU/mL) suggests that iron reduction is mainly driven by *Geobacter sp*p. rather than *Shewanella sp*p. The diversities in microbial communities at different sites underscore the impact of local environmental parameters such as the availability of organic matter, redox potential, and human activities on groundwater microbial processes.

Parallel microbial patterns were observed in groundwater research in the southeastern region of Nigeria. Studies on iron-reducing bacteria in Enugu and Owerri regions have found high *Geobacter sp*p. and *Shewanella sp*p. counts in reducing environments, correlating with elevated iron levels in groundwater. The dominance of *Geobacter sp*p. (1.3×10^4 CFU/mL) in Centenary aligns with microbial assessments in Agbani Road, where *Geobacter sp*p. counts reached 2.5×10^3 CFU/mL in Kenneth Street HDW and 2.4×10^3 CFU/mL in Amokwe Street HDW. However, the Centenary samples exhibit significantly lower *Shewanella sp*p. counts (only 1.4×10^2 CFU/mL in one sample), unlike Agbani Road where *Shewanella sp*p. was consistently found in 2.3×10^3 to 2.5×10^3 CFU/mL across multiple samples. This suggests that the Centenary water sources may have less favorable conditions for *Shewanella sp*p. growth, potentially due to differences in organic carbon availability or electron acceptors. Additionally, the detection of *Desulfomicrobium* spp. in Almond Garden HDW (1.2 × 10^3 CFU/mL) aligns with previous findings on sulfate-rich groundwater, where sulfate-reducing bacteria contribute to hydrogen sulfide production, affecting water taste and odor. These comparisons reinforce the importance of microbial activity in shaping groundwater chemistry and highlight the need for site-specific water treatment strategies.

CONCLUSION

This study underscores the significant role of microorganisms in altering water chemistry and highlights their varied distribution across different water sources. The high prevalence of sulfate-reducing bacteria such as ***Desulfomicrobium*** and iron-reducing bacteria like ***Geobacter sp.*** in specific water sources reflects localized environmental and anthropogenic influences. Surface water sources exhibited higher microbial diversity and counts, likely due to nutrient runoff and pollution, whereas groundwater sources showed relatively lower microbial loads, emphasizing the filtration effect of aquifers. The findings emphasize the need for regular microbial monitoring and effective water treatment to mitigate the risks posed by pathogenic microorganisms such as ***Pseudomonas sp.*** and ensure safe water quality for public use. These insights provide a basis for improved water management practices, particularly in urban and industrial regions.

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