



Physicochemical, Bacteriological and Enzymatic Assessment of Water Reservoirs in Ogwa and Ebelle Communities, Edo State, Nigeria

*¹OKANLAWON, TS; ²OKANLAWON, KP; ⁴EKOZIN, AA; ³UNOGWA, GC;
²FASANYA, OS; ²IGHODALO, AJ; ³AKINSOLA, OS

¹Department of Biological Sciences, Glorious Vision University, Ogwa, Edo State, Nigeria

²Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

³Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

⁴Department of Biochemistry, Glorious Vision University, Ogwa, Edo State, Nigeria

*Corresponding Author Email: stephentaiwo610@gmail.com

*ORCID: <https://orcid.org/0000-0003-2316-6698>

*Tel: +2349055479460

Co-authors Email: okanlawonkehinde100@gmail.com; oludayofasanya1@gmail.com; Anuoluwapojoshua20@gmail.com;
olusolaworld@gmail.com; adrielekozin1@gmail.com; gunogwa24@gmail.com

ABSTRACT: Numerous harmful microorganisms can be found in contaminated water. These pathogens, which are frequently seen in feces, may originate from point sources such as wastewater and drainage effluents. Hence, the objective of this paper was to conduct a physicochemical, bacteriological, and enzymatic assessment of water reservoirs in Ogwa and Ebelle Communities, Edo State, Nigeria using appropriate standard techniques. Results obtained for physicochemical characteristics showed that pH (5.81-6.10), temperature (29.9-31.4 °C), total dissolved solids (16.80- 19.60 mg/l), dissolved oxygen (5.32-5.73mg/l), biological oxygen demand (2.67- 5.73 mg/l) are consistent within the study areas. Bacteriological evaluations revealed a heterotrophic plate count ranging from 1.10×10^4 to 5.40×10^4 cfu/ml. The bacterial species present include *Bacillus* sp., *Pseudomonas* sp. and *Escherichia coli*. The Rhodanese enzyme was extracted from two bacterial species. *Bacillus* sp. show the highest ability to produce the Rhodanese enzyme, which is essential for cyanide reduction. It is advised that reservoir water be enclosed with containers to avoid pollution, which could cause illnesses and, in extreme situations, mortality after ingestion.

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In countries with developing economies, poor water quality is regarded as an example of the signs of hardship. Therefore, lowering illnesses associated with water and improving the amount, purity, and hygiene of drinking water have been key elements of the UN's

2015 Sustainable Development objective (SDG) programs in objective 6. Nevertheless, there have been a number of obstacles in the way of providing the populace with safe drinking water. These include water supply pollution from garbage from households

*Corresponding Author Email: stephentaiwo610@gmail.com

*ORCID: <https://orcid.org/0000-0003-2316-6698>

*Tel: +2349055479460

and waste from industries, growing populations, and insufficient hygiene (Alemu *et al.*, 2015). When freshwater fails to satisfy globally accepted criteria, it is typically said to be contaminated (Lin *et al.*, 2022). The amount and type of wastewater that is released into the river, along with other drainage, determines how polluted the water is (Sharma and Bhattacharya, 2017). In order to determine the purity of water, assessment involves assessing its physical, chemical, bacteriological, and enzymatic properties. (Sharma and Bhattacharya, 2017). In Nigeria, rivers, wells, and more recently boreholes are the main sources of water used for drinking and other household needs, particularly in towns and villages. According to the World Health Organization's 2015 study, microbes have an important effect on the quality of these water resources, with bacteria levels exceeding the globally recognized body's criteria and being relatively unacceptable (Olatunji *et al.* 2019). For instance, the existence of *Escherichia coli* in water is a sign of pollution from feces. Bacteria, in specific, can be used as markers. Coliforms are a class of very similar shaped like rods facultative anaerobes, Gram-negative, non-spore-forming bacteria that digest lactose to create gas and acid in 48 hours at 35 °C. With the exception of a few enteric pathogens including *Salmonellae*, *Shigellae*, and enteropathogenic *E. coli*, they primarily reside in soil, water, and animal guts. (Bhumbla *et al.*, 2020).

There are several different issues with rural pollution of water. Waste products from farming and other operations, as well as poor hygiene, are among the primary contributors of pollutants in water supplies (Tabor *et al.*, 2017).

Additionally, the integrity of the distribution system and the quality of the drinkable water are typically compromised by breaks in the system, aging and poor maintenance, and low chlorine levels. In order to prevent water-related illnesses, it is necessary to properly secure the water supply from contamination and to regularly monitor water sources. One technique for evaluating the sanitary state of water is the ongoing monitoring of water's purity assessment which involves the identification of biological indicators. It is generally acknowledged that physicochemical factors including temperature, turbidity, pH, nitrate, and others are important indicators of drinking water quality. These factors either have a direct impact on microbiological quality or have an impact on the wellness of people and disinfection effectiveness. Enzymes play fundamental roles in various physiological processes within organisms, including fish. The distribution of enzymes across different tissues reflects the specific metabolic demands and functional adaptations of organisms to their respective

environments. Environmental factors, including temperature, pH, dissolved oxygen levels, and pollutant exposure, can significantly influence enzyme distribution in tissues damage ((Fazio *et al.*, 2020). Temperature, in particular, has profound effects on enzyme activity and kinetics, with optimal temperatures varying among different enzymes and species. Ebelle and Ogwa communities are known for cassava processing and the disposal of this effluent in the river and reservoirs might be primarily responsible for cyanide leakage. In a gaseous form, cyanide reacts with hydrogen ions to create hydrocyanic acid, a very toxic substance with an almond-like odor. Sulfurtransferases, like Rhodanese, catalyze the detoxification of cyanide. The enzyme is known as cyanide thiosulphate a common enzyme known as sulfur transferase, uses a twofold displacing method to induce the exchange of a sulfur atom from suitable donors to nucleophilic acceptors *in vitro*. Throughout the process of catalysis, the enzyme alternates amongst a sulfur-free and a persulfide-containing state, forming a persulfide bond through the reduction of a catalytic cysteine molecule.

Industrial practices have considerably increased the amount of cyanide in lakes and rivers. Cyanide-containing liquid has to be carefully handled before being released to rivers and lakes to reduce ecological catastrophes. A number of the difficulties faced by cyanide-based enterprises in meeting their standards and recycling water is the elimination of cyanide from the wastewater (Kuyucak and Akcil 2013). Cyanotrophic microbes like *Pseudomonas* sp. (Oyedjeji *et al.*, 2013), *Bacillus pumilus* (Kandasamy *et al.*, 2015), and *Bacillus cereus* (Itakorode *et al.*, 2019) have been shown to endure cyanide exposure despite its adverse consequences because of how they can manufacture cyanide using gastrointestinal enzymes like rhodanese. Pollutant exposure, including heavy metals, pesticides, and organic pollutants, can disrupt enzyme function and expression in fish tissues, leading to adverse physiological effects. For example, exposure to heavy metals such as mercury and lead can inhibit enzymes involved in antioxidant defense (e.g., superoxide dismutase, catalase) and disrupt cellular redox homeostasis, leading to oxidative stress and tissue damage (Fazio *et al.*, 2020). Therefore, the objective of this paper was to conduct physicochemical, bacteriological and enzymatic assessment of water reservoirs in Ogwa and Ebelle Communities, Edo State, Nigeria.

MATERIALS AND METHODS

Sample Collection: Cassava effluent samples were collected in sterile containers and transported to the laboratory for microbiological evaluation. A sum of

fifteen samples were gathered throughout the research work from different locations the in Ogwa community, Esan West local government area of Edo State, Nigeria. It is about at latitude 6°30' 20.16" North, and longitude 6°12' 30.24" East while are Ebelle is located at latitude 6° 49'77' 0" N and longitude 6° 22'59' 1" E.

Assessment physicochemical parameters: Physiochemical analyses were conducted on the water sample collected, and these included pH, temperature, biological oxygen demand and total dissolved solids

pH: The acidity or alkalinity of the collected wastewater samples was assessed by measuring their pH. A calibrated pH meter, adjusted with a pH 7 buffer solution, was employed for this purpose. The meter's probe was submerged in the water samples, and the readings were taken three times for each sample. The average value of these readings was then calculated and documented (Corwin and Yemota, 2020).

Temperature: Temperature refers to the degree of coldness or hotness of a body, for water, affects its state (liquid, solid or vapour). The temperatures were measured at the site of collection using the mercury in glass thermometer (Agbo *et al.*, 2019).

Dissolved oxygen: Dissolved oxygen (DO) is a crucial parameter in assessing water quality, as it provides insights into various factors such as microbial activity, photosynthesis, nutrient levels, and water stratification (Vikal, 2009). As summer temperatures rise, DO levels tend to decrease due to increased microbial activity and higher temperatures. After incubating the water sample at 293 K for five days, the DO is measured using Winkler's method. This technique involves determining the oxygen consumed by bacteria during the incubation period by comparing the initial and final DO readings. The procedure requires specialized BOD bottles that prevent the entry of external oxygen (APHA, 2015).

Biochemical oxygen demand: Biological oxygen demand (BOD) refers to the quantity of dissolved oxygen that aerobic microorganisms need to break down organic matter in a water sample over a defined temperature and time period. BOD is frequently used to assess the organic contamination levels in water, serving as an indicator of pollution. It is typically measured in milligrams of oxygen.

Total dissolved solids: Total solids are made up of dissolve solid and suspended solids (TS = DS + SS). Dry clean dish was placed in an oven at a constant speed of 103-105 °C, cool at room temperature in a desiccator. Note the weight. After mixing thoroughly

the effluent sample of 100-250 ml was pipette in to the dish. Dry the residue for 1 hour in an oven at a constant temperature, transfer quickly into a desiccator, cool at room temperature and weigh. Subtract the weight of the dish from the weight of the residue to obtain the weight of the total solid as shown in equation 1.

$$\text{Total solids (mg/l)} = \frac{\text{mg.total solids} \times 1000}{\text{ml samples}} \quad (1)$$

Estimation of bacteriological parameters: Isolation: For the serial dilution, one milliliter of the sample was transferred into tubes containing sterile distilled water. After allowing the molten agar to cool, 15 milliliters were aseptically poured onto the plates to ensure uniform distribution of colonies across the agar surface. The plates were gently rotated in both clockwise and counterclockwise directions before leaving the agar to solidify. MacConkey agar and nutrient agar plates were then incubated upside down at 37°C for 24 hours. (Olutiola *et al.*, 1991; Okanlawon *et al.*, 2024).

Identification and Characterization: Bacterial isolates were identified based on their colony appearance, cellular structure, and biochemical properties. The identification of the bacterial isolates was carried out following the guidelines in Bergey's Manual of Determinative Bacteriology (Okanlawon *et al.*, 2023).

Evaluation of enzymatic activities: Protein assay: Protein concentration was measured using Bradford's method (1976). Absorbance was recorded at 595 nm, and the protein levels were calculated from a standard curve generated with bovine serum albumin (BSA) as the reference standard.

Rhodanese activity: The Enzyme Rhodanese was assayed from the crude sample using modified method of Sorbo (1953) which include the following reagent; Borate buffer, Sodium thiosulfate buffer, KCN, 15 % Formaldehyde and Sorbo reagent. A test and a blank were prepared and readings were taken at the wavelength of 460 nm.

Effect of pH on enzyme activity: The impact of pH on enzyme activity was evaluated following the method outlined by Agboola and Okonji (2004). The enzymes were tested using different buffer systems: 50 mM citrate buffer (pH 3.5), 50 mM phosphate buffer (pH 6.7), 50 mM Tris-HCl buffer (pH 8), and 50 mM borate buffer (pH 9-10). Rhodanese activity was measured by replacing the standard assay buffer with each of these buffers.

Effect of temperature on enzyme activity: Enzyme activity was analyzed across a temperature range of 30°C to 90°C to determine the enzyme's optimal operating temperature. The assay mixture was incubated for 1 minute at each specific temperature, after which the reaction was stopped. Absorbance was measured at 460 nm to assess rhodanese activity.

Effect of metal ions on enzyme activity: The influence of various metal ions on rhodanese activity was studied according to the method of Lee *et al.* (1995). The enzyme was exposed to three concentrations (0.1 mM, 1 mM, and 10 mM) of HgCl₂, NaCl, FeCl₃, and MnCl₂. The reaction mixture without added salts served as a control, representing 100% activity.

RESULT AND DISCUSSION

Water plays a crucial role in sustaining life, but ensuring its availability and safety for human consumption is vital. It is essential to ensure water is free from harmful bacteria, toxic chemicals, and physical contaminants (Anani and Olomukoro, 2021; Olatunji *et al.*, 2020). The counts of *Escherichia coli*, *Bacillus* sp., and *Pseudomonas* sp. were measured, with Ebelle exhibiting higher counts across all three bacterial species compared to Ogwa as presented in table 1. For instance, *E. coli* in Ebelle reached 5.4 x 10⁴ CFU/ml, significantly exceeding the acceptable limit of 100 CFU/ml for drinking water as set by Nigerian standards and WHO guidelines. This suggests a critical level of contamination, possibly due to fecal pollution or inadequate sanitation facilities. Similar studies by Olatunji *et al.* (2020) found elevated levels of pathogenic bacteria in water sources, correlating with unsanitary conditions in rural areas. The presence of these organisms indicates potential health risks associated with water consumption from pipes, and underground sewage seepage. The consumption of water tainted with these microorganisms poses serious health risks, including urinary tract infections, cholera, dysentery, typhoid, and gastroenteritis. These findings align with those of Islam *et al.* (2020), highlighting the urgent need for public health interventions. Pathogenic bacteria like *Enterobacter* and *Escherichia* species were also found at alarmingly high levels in the water from these communities, necessitating immediate community health attention.

Table 1: Result for heterotrophic bacterial count

	<i>Escherichia Coli</i>	<i>Bacillus Sp.</i>	<i>Pseudomonas sp.</i>
Ogwa	1.5x10 ⁴	1.1x10 ⁴	1.8x10 ⁴
Ebelle	5.4x10 ⁴	1.9x10 ⁴	3.6x10 ⁴

The presence of all three bacterial isolates in both communities indicates widespread microbial

contamination. The results demonstrate that *E. coli*, *Bacillus* sp., and *Pseudomonas* sp. are prevalent in both Ogwa and Ebelle as presented in table 2. Studies conducted by Islam *et al.* (2020) also reported similar prevalence of these pathogens in various water bodies, underscoring the public health implications of consuming contaminated water.

Table 2: Prevalence of bacteria isolate

SOURCE	<i>Escherichia Coli</i>	<i>Bacillus Sp.</i>	<i>Pseudomonas sp.</i>
Ogwa	+	+	+
Ebelle	+	+	+

Legend: + = Present of organism

The physicochemical properties, including temperature, pH, TDS, dissolved oxygen (DO), and biochemical oxygen demand (BOD), were analyzed for both Ogwa and Ebelle as presented in table 3 and 4 respectively. Temperature ranged from 29.9 °C to 31.4 °C, which is typical for tropical climates. Both locations had pH values below the neutral range (6.0-8.5), indicating acidic conditions (e.g., 5.81 in Ogwa and 5.90 in Ebelle). Acidic pH levels can enhance the solubility of heavy metals, posing additional risks to water quality. Values were low, with maximum TDS recorded at 19.60 ppm, well within the acceptable limits (≤500 mg/L as per WHO). Dissolved oxygen levels were relatively low, with values around 5.51 mg/L to 5.73 mg/L, which may impact aquatic life and indicate organic pollution. BOD levels ranged from 2.67 mg/L to 5.20 mg/L, suggesting some organic matter presence, which could further influence microbial growth. Egbueri *et al.* (2020) noted similar pH levels in contaminated water bodies, linking acidic conditions to organic waste decomposition. The BOD and DO findings correlate with studies indicating that high BOD levels often coincide with lower dissolved oxygen levels due to microbial consumption of oxygen during organic matter decomposition. While the pH of the sachet water samples fell within the acceptable range for drinking water, the water quality was compromised by organic waste contamination. This promoted microbial decomposition activity, resulting in increased acidity. In contrast, the presence of inorganic pollutants, largely due to human activities, raised the alkalinity of the water, further degrading the aquatic ecosystem. These observations are consistent with findings from previous studies by Amadi *et al.* (2012) and Olatunji and Anani (2020), which reported similar issues in other water bodies.

The total dissolved solids (TDS) levels observed in this study were within the WHO (2015) guideline of 500 mg/L for potable water. This may be attributed to the presence of dissolved ions like sodium and calcium,

along with organic matter. Additionally, other physicochemical parameters, including conductivity, dissolved oxygen, and biochemical oxygen demand, were found to comply with WHO (2015) standards for drinking water, in agreement with findings from Egbueri (2020) and Egbueri *et al.* (2020).

Table 3: Result of the physicochemical analysis for water reservoirs in Ogwa

Sample	Temperature, (°C)	TDS (ppm)	DO, (mg/L)	BOD (mg/L)
O1	30.5	5.81	16.80	5.51
O2	31.4	6.00	19.60	5.73

Table 4: Result of the physicochemical analysis for water reservoirs in Ebelle

Physicochemical parameters					
Sample	Temperature, (°C)	pH	TDS (ppm)	DO, (mg/L)	BOD (mg/L)
E1	29.9	5.90	17.00	5.32	3.10
E2	31.2	6.10	18.10	5.64	5.20

The total cyanide concentration measured at 3081.67±57.75 µg/ml indicates significant contamination, as cyanide is a toxic compound that can pose severe health risks to humans and aquatic life. High levels of cyanide have been reported in water bodies affected by industrial discharge, highlighting the need for stringent monitoring and remediation efforts. The protein concentrations of the bacterial isolates were notably higher for *Bacillus* sp. (147.05 mg/ml) compared to *E. coli* (98.23 mg/ml) and *Pseudomonas* sp. (67 mg/ml) as shown in table 5. This suggests that *Bacillus* species may have a higher metabolic activity or biomass in the studied environments. Similar findings have been reported by Amadi *et al.* (2012), indicating that *Bacillus* species often thrive in nutrient-rich environments, which may relate to organic contamination. The specific activity of rhodanese in table 6 was higher in *Bacillus* sp. (6.311 U/ml) than in *Pseudomonas* sp. (5.34 U/ml), suggesting that *Bacillus* species may possess a greater enzymatic capacity for detoxifying compounds like cyanide. Research by Lee *et al.* (1995) also highlighted the significance of rhodanese activity in various bacterial species, emphasizing its role in bioremediation processes.

Table 5: Protein concentration

Bacteria Sample	Specific Activity(U/ml)
<i>Bacillus</i> sp.	147.05±0.54
<i>E. coli</i>	98.23±0.04
<i>Pseudomonas</i> sp.	67±0.02

Table 6: Specific activities of rhodanese enzyme from the bacterial species

Bacteria Sample	Specific Activity(U/ml)
<i>Bacillus</i> sp.	6.311±0.01
<i>Pseudomonas</i> sp.	5.34±0.02

Saidu (2005) and Chae *et al* (2006) investigated the parameters associated with kinetics (Km and Vmax. The results of the kinetic analysis in figure 1 show that *Bacillus* sp. and *Pseudomonas* sp. have a high affinity for these substrates, indicating that the enzyme is catalytically effective. It was discovered that 70 °C was the ideal temperature for the synthesis of rhodanese.

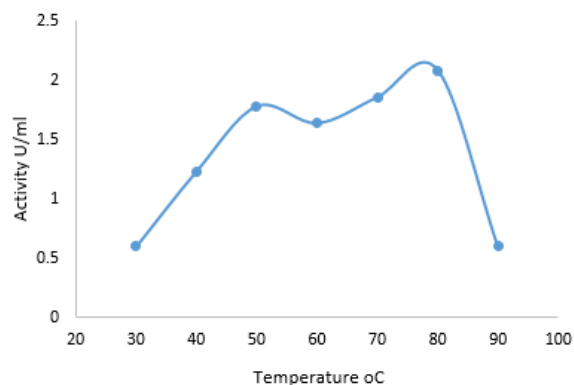


Fig 1: Effect of temperature on rhodanese activities from *Bacillus* sp. (1) and *Pseudomonas* sp. (2).

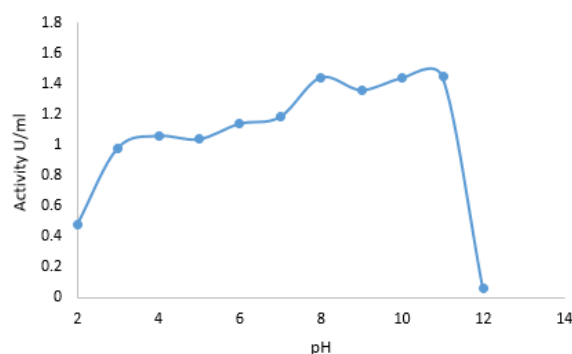


Fig 2: Effect of pH on rhodanese activities from *Bacillus* sp. (1) and *Pseudomonas* sp. (2).

For rhodanese, several comparable or remarkably comparable findings from different bacteria have been published. The figure 2 demonstrated the influence of pH on rhodanese activities from *Bacillus* sp. and *Pseudomonas* sp. which could provide insights into optimizing conditions for enzyme activity in bioremediation efforts. Previous studies have shown that temperature and pH can significantly affect enzyme activity, with optimal conditions often varying by species and environmental factors (Olatunji and Anani, 2020). The results of the investigation on the suppression of enzyme activity by metal ions in figures 3 and 4 showed that the metal ions tested did not significantly reduce the activity of enzymes. According to the study, these ions may be present in the organism's environment, causing it to develop a tolerance for or resistance to the ions. The outcomes

are comparable to those of the efforts of similar to the works of Okonji *et al.* (2011).

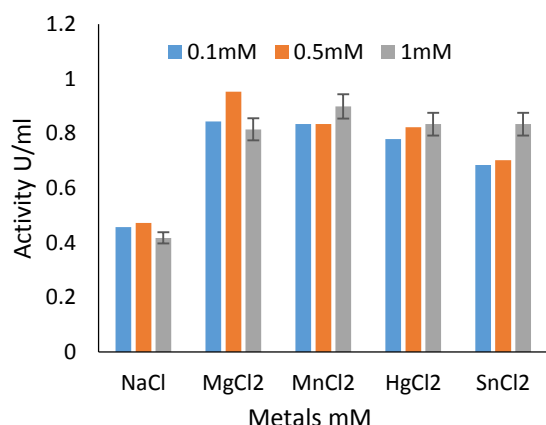


Fig 3: Effect of metals on rhodanese activity from *Bacillus sp.*

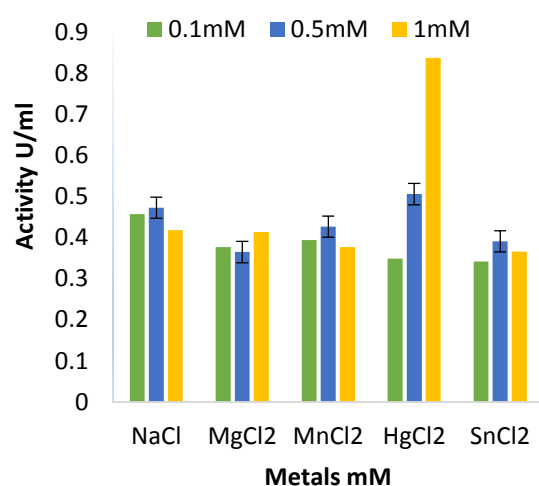


Fig 4: Effect of metals on rhodanese activity from *Pseudomonas sp.*

Conclusion: From the research work carried out, it was obvious that bacteriologically and physicochemically, the quality of water reservoirs is slightly compromised, because of several factors. The substantial amount of rhodanese expression in the isolated bacteria species raises the possibility that this research could serve as a foundation for the development of treating cyanide contaminated water in these communities.

Conflict of Interest: The author reports no potential conflict of interest

Data availability statement: Data utilized for analysis in this study are accessible from the corresponding author upon request.

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