PHYSIOCHEMICAL CHARACTERISTICS, COUNT AND MOLECULAR DIVERSITY OF TOTAL HETEROTROPHIC BACTERIA IN THE SEVEN WATER SAMPLES ALONG THE ODO-OTIN RIVER.

BY

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CERTIFICATION

This is to certify that the project was carried out by ONEH feyishayo Christianah (2021/36905) of the Department of Microbiology, Faculty of Basic and Applied Science, Osun State University, Osogbo. The project report has been read and approved as meeting part of the requirement for the award of Bachelor of Science (B.Sc., Hon) degree in Microbiology of Osun State University, Osogbo.

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DEDICATION

This research work is dedicated to Almighty God, the giver of life, wisdom, and strength, whose grace and mercy have been my constant guide throughout this journey. It is also lovingly dedicated to my family, for their unwavering love, prayers, encouragement, and moral support, which have been my source of inspiration.

Finally, I dedicate this work to all students and researchers in the field of Microbiology, whose passion for knowledge and scientific discovery continues to inspire progress in the understanding of our environment.

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TABLE OF CONTENT

LIST OF TABLES

LIST OF PLATES

ABSTRACT

The Odo-Otin River in Osun State, Nigeria, is a vital freshwater resource facing increasing anthropogenic pressures. This study was conducted to assess its water quality by evaluating physicochemical parameters and the abundance and diversity of heterotrophic bacteria at seven sampling sites along the river. Water samples were subjected to standard physicochemical analysis, while heterotrophic bacteria were enumerated using the heterotrophic plate count (HPC) method on Nutrient Agar, followed by morphological and biochemical characterization. The results revealed that while parameters such as pH (6.64-6.83), total dissolved solids (102-216 mg/L), and dissolved oxygen (5.05-6.20 mg/L) were largely within acceptable limits, the water was under significant stress from organic pollution. This was evidenced by alarmingly high biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) values, which far exceeded recommended standards (up to 51.4 mg/L and 78.2 mg/L, respectively). The high organic load was directly correlated with consistently high total heterotrophic bacterial counts across all sites. Biochemical tests confirmed a metabolically diverse microbial community capable of surviving in the nutrient-rich, polluted environment. The findings indicate that the Odo-Otin River is a stressed ecosystem, with its high bacterial load serving as a direct biological indicator of the organic pollution. The study highlights the urgent need for pollution control and underscores the importance of employing more specific selective media in future research to comprehensively assess public health risks from potential fecal contamination.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Rivers are important parts of the world's ecosystems because they provide water for homes, businesses, and farms. They also support many plants and animals. They are necessary for keeping the balance of ecosystems and for the movement of nutrients (Allan and Castillo, 2007). However, human activities like urbanization, industrial discharges, and agricultural runoff are becoming more of a threat to rivers all over the world because they add pollutants like organic matter, heavy metals, and nutrients to water systems (Vörösmarty *et al*., 2010). Douterelo et al. (2014) say that these pollutants change the physical and chemical properties of water, such as its pH, temperature, dissolved oxygen (DO), turbidity, and nutrient levels. The health of the ecosystem and the microbial communities are affected by these changes.

The Odo-Otin River in Osun State, Nigeria, is a very important source of fresh water for the people who live nearby. It is used for fishing, irrigation, and drinking water. The river runs through rural and semi-urban areas, where it could be polluted by household waste, runoff from farms, and small-scale industrial activities (Adelekan & Abegunde, 2011). These activities may increase levels of nutrients like phosphates and nitrates, which can lead to eutrophication, which can upset the balance of microbes and lower the quality of the water (Smith & Schindler, 2009). Even though the river is important for the environment and the economy, not much is known about its microbial ecology and water quality, especially how many different types of heterotrophic bacteria there are and how many there are.

One way to check the microbiological quality of drinking water is to count the number of heterotrophic bacteria in it. Heterotrophic bacteria can live in many places, such as drinking water, ballast water, and seawater (Mariita *et al*., 2021; Soleimani *et al*., 2021). These tiny

organisms use organic carbon for energy and are important for biogeochemical processes. Their diversity and abundance are affected by physicochemical factors like pH, temperature, DO, and nutrient availability (Newton *et al*., 2011). Studying bacteria and other microorganisms is important for figuring out how healthy a river is because higher bacterial counts or changes in microbial diversity can be signs of pollution or ecological stress (Paerl *et al*., 2003). For instance, agricultural runoff with a lot of nutrients can help bacteria grow, while low DO levels may help anaerobic species grow, which can change the makeup of the community (Sinsabaugh and Foreman, 2001).

Heterotrophic plate count (HPC) bacteria are commonly used to indicate water quality and the effectiveness of water treatment processes (Ley *et al*., 2020). The HPC method involves culturing bacteria on agar plates and counting the number of colonies that form, which estimates the total number of viable bacteria present in a sample. HPC measurements are not used to assess the specific health risks associated with individual bacterial strains but rather to monitor changes in the microbial quality of water and to ensure that water treatment processes are effective in reducing bacterial contamination (Ley *et al*., 2020; Horn *et al.*, 2016). Advances in molecular techniques, such as 16S rRNA gene sequencing, have revolutionized microbial ecology by enabling the identification and characterization of both culturable and non-culturable bacteria (Rastogi and Sani, 2011).

Molecular approaches reveal diverse bacterial populations, including Proteobacteria, Actinobacteria, and Bacteroidetes, which are influenced by physicochemical gradients (Olapade and Leff, 2006; Inam *et al*., 2016). These methods enable researchers to better understand the taxonomic composition, phylogenetic diversity, and functional roles of bacterial communities in aquatic environments. The survival of nearby towns depends on the ecological health of the Odo-Otin River, however issues with water quality and microbiological pollution

are brought on by the river's exposure to human pressures. For instance, nitrogen enrichment can result in detrimental algal blooms and changes in bacterial communities, while untreated sewage and agricultural runoff may bring pathogenic bacteria that could endanger human health (Carpenter *et al*., 1998).

Understanding the relationship between heterotrophic bacterial populations and physicochemical features is essential for assessing the ecological health of the river and developing conservation strategies. This study analyzes water samples from seven sites along the Odo-Otin River to determine their physicochemical characteristics, count, the total number of heterotrophic bacteria, and examine molecular diversity using 16S rRNA sequencing. Combining these techniques enable this research to provide baseline data on the river's microbial ecology and water quality, enhancing our understanding of tropical river ecosystems and directing sustainable management practices.

1.2 Literature Review

1.2.1 River Ecosystems and Anthropogenic Impacts

Rivers are essential ecosystems that sustain human livelihoods, including transportation, irrigation, and water supply, as well as biodiversity and nutrient cycling. Anthropological activities like urbanization, industrial discharges, and agricultural runoff, however, pose serious risks to them because they introduce pollutants like heavy metals, organic matter, and nutrients that change the microbial communities and water quality (Vörösmarty *et al*., 2010). Similar stresses are present in Nigerian rivers such as the Odo-Otin, where domestic waste and agricultural runoff may have an effect on the water quality (Adelekan & Abegunde, 2011).

With heights ranging from 35 to 400 meters and an annual rainfall of roughly 1,400 mm, the Odo-Otin River in Osun State supports both tropical rainforests and agricultural operation. Research on

neighboring waterways, such the Ogun River, shows that effluent contamination is a problem, with physicochemical characteristics frequently surpassing WHO standards. For instance, a research on the Osun River found that illegal gold mining was making the river more toxic, which may be similar to problems with the Odo-Otin River (Nigeria's Osun River: Sacred, Revered and Increasingly Toxic). Worldwide, research on the Yongding River in China by Li et al. (2022) demonstrates that ecological water replenishment can lessen some effects, but water quality is still an issue that affects microbial dynamics.

1.2.2 Physicochemical Characteristics of River Water

Physicochemical parameters such as pH, temperature, dissolved oxygen (DO), turbidity, and nutrient levels (e.g., nitrates, phosphates) are critical indicators of water quality and influence microbial communities. In tropical rivers, these parameters can vary due to seasonal changes and human activities. A study on rivers in Ondo State, Nigeria, evaluated parameters like pH (6.6–7.1), temperature (25–26°C), turbidity (4.3–15.2 NTU), and dissolved oxygen (3.8–7.5 mg/L), finding most within WHO limits, except ammonium (0–3.5 mg/L, exceeding <1.5 mg/L), indicating pollution (Evaluation of the physicochemical properties and bacterial loads of selected rivers in Ondo State, Nigeria). Similarly, in the Yongding River, China, parameters like COD, TN, and Chl-a showed no significant monthly changes, but downstream sections had higher COD and TP, influencing microbial composition (Li *et al*., 2022).

In the Sinos River, Brazil, salinity and organic matter were key drivers of bacterial community structures, with the source showing higher diversity due to less anthropogenic impact (Becker *et al*., 2015). For the Odo-Otin River, monitoring these parameters will provide baseline data, especially given its agricultural context, potentially leading to nutrient enrichment and microbial shifts.

1.2.2.1 Hydrogen Ion Concentration (pH)

A water's acidity (pH < 7) or alkalinity (pH > 7) can be determined by measuring its pH. Pure waters with an equal concentration of hydrogen ions (H+) and hydroxyl ions (OH-) have a pH of neutral. The pH of an aqueous solution is defined as the negative logarithm of the H_ concentration in the solution (Spellman, 2020; Alley, 2007). Both natural and man-made factors can cause fresh waters to vary greatly in terms of their acidity and alkalinity. Most species are harmed by extremely acidic and alkaline fluids (Spellman, 2020; Allan *et al*., 2021). The majority of aquatic life has evolved to survive in water with a pH of 6 to 8. The pH of water is typically changed by pollution, harming aquatic life and plants (Spellman, 2020). Accordingly, copepod species were divided into three groups by Suarez-Morales (Suarez-Morales, 2015) according to their tolerance for pH: euryionic, acidic, and mesoionic or neutral. In the first group, Macrocyclops albidus, Tropocyclops prasinus, and Paracyclops fimbriatus can all thrive in pH ranges of 4.0–10.5, 4.0–10.5, and 3.5–9. The second group consists of species like Diacyclops languidus and D. nanus that can withstand acidic environments and shouldn't be exposed to more than 8.0. The third group, which includes Cyclops furcifer, C. vicinus, Metacyclops minutus, and M. gracilis, was discovered in neutral freshwater environments with pH values ranging from 6.5-8. According to Vishwakarma et al. (Vishwakarma *et al*., 2013), the Betwa River's zooplankton composition decreases when the pH value drops, which is linked to the flow of industrial effluents into the river.

1.2.2.2 Water Temperature

The hydrological, geographical, climatological, and structural elements of the region and catchment area influence the natural thermal properties of riverine ecosystems. The thermal regime will be influenced by hydrological parameters, including the water source, discharge rate, water volume, and tributary inflow (Pletterbauer *et al*., 2018). The thermal conditions of

rivers are influenced by climatic elements such wind speed, cloud cover, solar radiation, air temperature, vapor pressure, precipitation, and evaporation, as well as regional characteristics like latitude and altitude (Pletterbauer *et al*., 2018). Water temperatures can be momentarily raised or lowered by precipitation occurrences. The amount of solar radiation that reaches and heats the water depends on the structural features of the river and catchment, such as topographic features, aspect and slope, riparian vegetation cover, channel form (morphology, geology, and substrate), water depth, turbidity, and the proportion of pool habitat (Dallas, 2009; Adamo *et al*., 2018). According to Ali et al. (Ali *et al*., 2019) and Obuid-Allah et al. (Obuid-Allah et al., 2020), water temperatures in the Nile River were consistent with the Egyptian climate, with the lowest recorded temperatures occurring in the winter and the highest during the summer. Water temperatures in the Tigris River similarly rose in the summer and fell in the winter (Alazawii, 2019; Nashaat *et al*., 2020; Majeed *et al*., 2022).

Under natural circumstances, the temperature of flowing water varied from 0°C to 30°C. The temperature of volcanic water and hot springs rose above 40°C. From the river's source to its mouth, the temperature gradually increases. Water temperatures rise as a result of cooling waters released into rivers, such as those from power plants or industrial operations (Chapman, 1996; Dallas and Day, 2004). Water temperature has a significant impact on all physiological functions, metabolic rates, and life processes, including feeding, reproduction, and the migration and distribution of organisms (Rai, 2012; Jacobsen, 2008).

Numerous physicochemical properties of water, such as the solubility of oxygen and other gases, the rates of toxicity and chemical reactions, and the activity of microorganisms, are also significantly impacted by temperature. The dissolved oxygen in the water becomes less soluble at higher temperatures. According to Rai (2012), cold water has a higher dissolved oxygen content than warm water.

1.2.2.3 Dissolved Oxygen (DO)

The traditional and widely used measure of the health of the aquatic ecosystem is dissolved oxygen, which is the amount of free, non-compound oxygen in the water. Depending on the temperature and atmospheric pressure, oxygen diffuses into the water from the air. Do DO concentrations in lotic water change seasonally or even over a 24-hour period? Numerous aquatic creatures, including fish, invertebrates, microbes, and plants, depend on it (Li and Liu, 2019; Spellman, 2020). The DO needed by the majority of aquatic systems is between 4 and 8 mg l-1 (Allan *et al*., 2007; Suthers and Rissik, 2009; Allan *et al*., 2021). According to Pennington and Cech (2009), DO in freshwater might theoretically range from 0 to 18 mg l-1. Additionally, when 100% is in equilibrium with the air, it is stated as a percentage of saturation. As a result, algal photosynthesis causes high percentage saturation during the day, while respiration and decomposition cause low percentage saturation in the late hours of the night (Suthers and Rissik, 2009; Allan *et al*., 2021). In addition to air diffusion, current velocity, salinity, pressure, water depth, time of day, and water temperature all affect the amount of dissolved oxygen in flowing water (Li and Liu, 2019; Burrell *et al*., 2014). Warm saline water has less dissolved oxygen than cold fresh water, even at 100% saturation (Suthers and Rissik, 2009). Certain sensitive species in the water body may relocate, get weaker, or even perish as the dissolved oxygen levels drop (Li and Liu, 2019). At doses of 0.6 mg l-1 and 1.6 mg l-1, respectively, the cladocerans Daphnia magna and D. pulex exhibited the greatest negative impact. In Nigeria's Tropical Rainforest River, Ekpo (Ekpo, 2013) demonstrated a favorable correlation between dissolved oxygen and zooplankton species. Joshua et al. (Joshua *et al*., 2018) also confirmed this study, which demonstrated a positive correlation between Rotifera and DO in the Nigerian Asu River during the wet season. According to a number of earlier studies on the Tigris River, the dissolved oxygen levels were within the previously stated

acceptable range (Abdulwahab and Rabee, 2015; Alazawii, 2019; Nashaat *et al.*, 2020; Majeed *et al*., 2022).

1.2.2.4 Turbidity

The quantity of fine particles suspended in water is referred to as turbidity. Clay, silt, finely organic and inorganic matter, plankton, and other microorganisms are examples of suspended sediments and colloid matter that cause it (Baird *et al*., 2017). Turbidity is an optical indicator of water clarity. Turbid water changes its physical appearance by appearing hazy, murky, or colored. By giving the water a muddy, hazy, or opaque appearance, dissolved and suspended solids decrease its clarity. According to Pennington and Cech (2009), rivers and streams can be turbid, like the Mighty Muddy Mississippi River, or extremely clear, like a mountain stream. There is a direct correlation between turbidity and suspended solids, and turbidity measurements are always used as an indicator of water quality based on clarity and TSS values (Li and Liu, 2019; Suthers and Rissik, 2009). The turbidity of the water is determined by the amount of light scattered by particles in the water column. Lighter particles will scatter more readily if there are more of them (Li and Liu, 2019; Baird and Eaton, 2017). Nephelometric Turbidity Units (NTU) are used to measure turbidity. A large river has turbidity of about 10 NTUs, whereas a clear stream has turbidity of about 1 NTU (Spellman, 2014). The effects of runoff from discharges, deforestation, construction, farming, and other sources can be identified using turbidity (Spellman, 2020; Pennington and Cech, 2009; Spellman, 2014). During a rainfall, turbidity frequently rises dramatically, and the high flow rate and velocity also accelerate riverbank erosion (Pennington and Cech, 2009).

Because suspended particles absorb more heat from the sun, high turbidity consequently raises the temperature of the water. As a result, there was less DO. Additionally, some organisms cannot endure in warmer water (Spellman, 2014). In Prairie Rivers, Thorp and Mantovani

(Thorp and Mantovani, 2005) demonstrated a negative regression for crustaceans and turbidity. According to Jafari et al. (Jafari *et al.*, 2011), the increased turbidity in Iran's Haraz River led to a decrease in the number of crustacean zooplankton by killing off their immature stage and preventing the growth of certain food sources. According to Li and Liu (Li and Liu, 2019), fish and other aquatic organisms' habitat is harmed by high particle concentrations.

1.2.2.5 Total Hardness (TH)

One of the physical or chemical properties of water is its total hardness. Hardness causes scale to form in boilers and pipes and lessens the effectiveness of soaps and detergents (Li and Liu, 2019). It is produced when particular multivalent ions are present in the water. The two most frequent ions that make water hard are calcium and magnesium; iron, manganese, barium, and strontium are also present. According to Spelman (2015), hardness generally indicates the overall concentration of calcium and magnesium compounds in water. There are two types of hardness: noncarbonated hardness, which is determined by the calcium and magnesium salts of strong acids, and carbonate hardness, which is determined by the concentrations of calcium and magnesium hydrocarbonates. Hydrocarbonates are changed into carbonates, which are typically precipitates, when water boils. Thus, temporary hardness is another name for carbonate hardness. Permanent hardness, on the other hand, refers to non-carbonate hardness that remains in the water after boiling. The total hardness, measured in milligrams per liter of calcium carbonate, is the sum of the concentrations of calcium and magnesium. Less than 75 mg l-1 of hardness is soft, 75–150 mg l-1 is moderately hard, 150–300 mg l-1 is hard, and more than 300 mg l-1 is very hard. Lime precipitation or ion exchange are common ways to soften this type of water (Spellman, 2015). Fish fertilization, hatching, and early stages were negatively impacted by high water hardness concentrations (Luo *et al*., 2016).

1.2.2.6 Salinity

Salinity, a broader term than total dissolved solids, is a measure of the total amount of dissolved ions in water (Allan *et al*., 2021). Salinity is typically less than 0.5 ppt, according to Montagna et al. (Montagna *et al*., 2013) and Hanrahan (Hanrahan, 2012). The ocean's average salinity is 35 parts per thousand. The salinity levels are classified as oligohaline (0.5-5.0 ppt), mesohaline (5.0-18.0 ppt), and polyhaline (18.0-30.0 ppt). Electrical conductivity has been used to indirectly determine the salinity of rivers (Alley, 2007). Usually given in parts per thousand, or ppt. numerous earlier studies showed that salinity had an impact on riverine zooplankton, according to Nguyen et al. (Nguyen et al., 2020). According to Majeed et al. (Majeed *et al*., 2022), Rotifera density decreased as salinity increased, while Copepoda density increased (Nguyen *et al*., 2020; Majeed *et al*., 2022). Additionally, Kaya et al. (Kaya *et al*., 2010) demonstrated that salinity has a significant impact on the zooplankton composition in Zamantı Rive. Additionally, as salinity rose, the Richness index fell (Majeed *et al*., 2022; Yuan *et al*., 2020).

1.2.3 Heterotrophs

A heterotroph is an organism that cannot produce its own food, instead taking nutrition from other sources of organic carbon, mainly plant or animal matter. In the food chain, heterotrophs are primary, secondary and tertiary consumers, but not producers (Hogg and Stuart, 2013). Living organisms that are heterotrophic include all animals and fungi, some bacteria and protists, and many parasitic plants. The term heterotroph arose in microbiology in 1946 as part of a classification of microorganisms based on their type of nutrition (Lwoff *et al*., 1946). The term is now used in many fields, such as ecology, in describing the food chain. Heterotrophs occupy the second and third trophic levels of the food chain while autotrophs occupy the first trophic level.

Heterotrophs may be subdivided according to their energy source. If the heterotroph uses chemical energy, it is a chemoheterotroph (e.g., humans and mushrooms). If it uses light for energy, then it is a photoheterotroph (e.g., green non-sulfur bacteria).

Heterotrophs represent one of the two mechanisms of nutrition (trophic levels), the other being autotrophs. Autotrophs use energy from sunlight (photoautotrophs) or oxidation of inorganic compounds (lithoautotrophs) to convert inorganic carbon dioxide to organic carbon compounds and energy to sustain their life. Comparing the two in basic terms, heterotrophs (such as animals) eat either autotrophs (such as plants) or other heterotrophs, or both. Detritivores are heterotrophs which obtain nutrients by consuming detritus (decomposing plant and animal parts as well as feces) (Wetzel, 2001). Saprotrophs (also called lysotrophs) are chemoheterotrophs that use extracellular digestion in processing decayed organic matter. The process is most often facilitated through the active transport of such materials through endocytosis within the internal mycelium and its constituent hyphae.

1.2.3.1 Herotrophic Bacteria in Aquatic Environments

The decomposition of organic matter, the cycling of nutrients, and the preservation of water quality in aquatic environments all depend on heterotrophic bacteria. With high counts indicating organic enrichment, they are frequently employed as bioindicators of pollution and use organic carbon as an energy source. The Heterotrophic Plate Count (HPC) method can be used to count heterotrophic bacteria in river water, but it only counts a portion of the population because of cultivation constraints (Reasoner, 2004). Although there is no clinical proof that elevated HPC levels are harmful to health, studies have identified genera such as Aeromonas, Klebsiella, and Pseudomonas as opportunistic pathogens (Reasoner, 2004). Using 16S rRNA sequencing, isolates of heterotrophic bacteria such as Bacillus cereus and Virgibacillus salarius were found to have anti-pathogenic qualities in the Siak River Estuary,

Indonesia (Characteristic Genetics of Heterotrophic Bacteria in Siak River Estuary, Riau Province, Indonesia as Prospective Anti-pathogenic Bacteria to Fish and Shrimps). Total viable bacterial counts in Nigerian rivers ranged from 1.5×10^5 to 6.3×10^5 $CFUmL^{-1}$, with Escherichia coli and Pseudomonas aeruginosa predominating, suggesting contamination (Bacterial isolates from drinking water river sources exhibit multi-drug resistant trait). According to these results, the Odo-Otin River's heterotrophic bacteria might be a reflection of the pollution levels present, so a thorough count and identification are required.

1.2.4 Bacterial Diversity in Water Habitats

Freshwater is one of the natural habitats with the highest bacterial diversity (Tamames *et al.*, 2010). According to Tamames et al. (2010), the natural habitats that support the largest and most diverse group of bacterial lineages are soil and freshwater, which are represented by aquifers, groundwater, lakes, rivers, drinking water, and wastewater. Following a comparison of 16S rRNA gene sequences from 3502 sampling experiments of both artificial and natural bacterial habitats, this conclusion was drawn. The diversity of bacteria found in different freshwater ecosystems that are a component of the urban water cycle was assessed in this study. The papers used in this comparison were published in ISI Web of Knowledge-indexed journals after 1995. These studies' main objective was to use 16S rRNA gene sequence analysis to examine the diversity of water bacteria.

The most prevalent bacteria at high taxonomic ranks of phylum or class are found in the phyla Proteobacteria (mainly of the classes Alpha-, Beta-, and Gammaproteobacteria), Actinobacteria, Bacteroidetes, and Firmicutes, regardless of the type of water surface (lakes, rivers, wetlands), mineral, drinking, and wastewater. However, distinct water types exhibit notable patterns of bacterial diversity at lower taxonomic ranks, such as genus or species. According to the articles that backed up this comparison, this was the outcome whenever the 16S rRNA gene sequence

analysis allowed for such a differentiation. For certain types of water, there was a discernible apparent selectivity. For example, drinking, mineral, and surface water were frequently found to contain members of the class Betaproteobacteria and phylum Bacteroidetes.

Conversely, firmicutes were frequently detected in wastewater. The air-water-soil interface and the urban water cycle are two examples of environments where low-specificity bacteria, also referred to as ubiquitous bacteria, can be found (Tamames *et al.*, 2010). The most prevalent bacteria in water habitats—that is, in drinking water, wastewater, and surface water—are those belonging to the genera Acidovorax, Curvibacter, Sphingomonas, Aeromonas, Acinetobacter, Pseudomonas, Legionella, Rhodococcus, Gordonia, Mycobacterium, Flavobacterium, Bacillus, and Clostridium. Bacteria from these and other unnamed groups are probably able to migrate across different aquatic environments during the whole urban water cycle.

The use of culture-independent techniques, specifically high throughput sequencing methods, has led to a new understanding of the variety of bacteria in aquatic environments, where less than 1% of bacteria can be grown (Amann *et al*., 1995; Simon and Daniel, 2011; Vaz-Moreira *et al*., 2013). These techniques showed that the phylum and class levels of bacteria present in all kinds of water are still unknown. This is particularly well-known for some bacterial phyla/classes that, despite their apparent poor culturability, are frequent water residents. The following are great examples of groups that can be identified almost or completely without the use of culture: members of the Delta and *Epsilonproteobacteria, Acidobacteria, Verrucomicrobia, Cyanobacteria, Nitrospirae, Planctomycetes, Chloroflexi, Chlorobi, Gemmatimonadetes, Spirochaetes, Chlamydiae, Aquificae, Thermotogae, Fusobacteria, Synergistetes, and Tenericutes.* Water habitats are home to some of these bacteria. However, some bacterial groups—particularly the less prevalent ones—may be hard to find with culture-independent methods like high throughput sequencing (Pinto and Raskin, 2012). A number of

biases may make it difficult to identify particular community members (e.g. DNA extraction, PCR, or sequencing data analysis). However, when it comes to small gene fragments like those generated by high throughput sequencing techniques, the 16S rRNA gene sequence analysis may not be able to accurately identify bacteria (Clarridge, 2004). The absence of bacteria of the genera Escherichia and Enterococcus, which are used as markers of fecal contamination and are frequently found in wastewater habitats at counts as high as 104–106 colony-forming units per mL, may be explained by these arguments (Ferreira da Silva *et al*., 2007; Garcia-Armisen & Servais, 2004; Levantesi *et al*., 2010). Additionally, cultivation methods suggest that these bacteria are not prevalent in water environments, including fecal contamination. Although they only make up a small percentage of the cultivable populations on general culture media like Plate Count Agar when isolated, Escherichia or Enterococcus usually require the use of selective culture media for cultivation. Even though culture-independent and culture-dependent approaches are truly complementary for exploring the bacterial diversity of an ecosystem, the state of the art shows a poor synchronization between them. This presents a major challenge to a comprehensive analysis of bacterial diversity, especially when assessing characteristics such as pathogenicity, metabolism, physiology, genetics, and antibiotic resistance of a specific group. Two of the primary outcomes of the use of culture-independent methodologies will surely be the improvement of cultivation techniques and the encouragement of research based on pure cultures (Anonymous, 2013; Lagier *et al*., 2012; Prakash *et al*., 2013). These advances will enable a thorough assessment of possible relationships between various microbiomes, including human and environmental microbiomes.

1.2.5 Molecular Diversity of Bacteria

By identifying both culturable and non-culturable species, molecular techniques—in particular, 16S rRNA gene sequencing—have completely changed the study of bacterial diversity in aquatic environments. These methods identify a wide variety of bacterial taxa in river water, with Proteobacteria, Actinobacteria, and Cyanobacteria frequently being the most prevalent. In the Xiangjiaba Hydropower Station, China, 16S rRNA sequencing found Cyanobacteria dominant in surface water, with community structures influenced by pH, flow velocity, DO, and total phosphorus (Response of bacterial communities to variation in water quality and physicochemical conditions in a river-reservoir system). In the Sinos River, Brazil, 16S NGS identified a rich bacterial community, with Proteobacteria, Actinobacteria, and Bacteroidetes abundant, influenced by environmental gradients (The Source of the River as a Nursery for Microbial Diversity).

Numerous environmental and enteric pathogens were detected by NGS and qPCR in urban rivers such as Changzhou, China. These pathogens were most likely caused by untreated sewage (Diversity and abundance of bacterial pathogens in urban rivers impacted by domestic sewage).

1.2.6 Relationship between Physicochemical Parameters and Bacterial Communities

Research indicates relationships between bacterial abundance, DO, and nutrient levels, illustrating the intricate relationship between physicochemical parameters and bacterial communities. The Yongding River's community composition was greatly influenced by temperature, pH, TN, TP, and NH4+-N; NO3--N had an effect on June samples, while NH4+-N had an effect on August/September samples (Li *et al*., 2022). Salinity, organic matter, and nutrient levels all had an impact on the bacterial communities in the Sinos River; the diversity was higher close to the source because of lower pollution levels (Becker *et al*., 2015). There is

a complex relationship between physicochemical parameters and bacterial communities; studies have found relationships between bacterial abundance, DO, and nutrient levels. The community composition of the Yongding River was greatly influenced by temperature, pH, TN, TP, and NH4+-N; NO3--N had an effect on June samples, while NH4+-N had an effect on August/September samples (Li *et al*., 2022). Salinity, organic matter, and nutrient levels all affected the bacterial communities in the Sinos River; the latter had greater diversity close to the source because of lower pollution levels (Becker *et al*., 2015).

1.3 Statement of the Problem

The Odo-Otin River is a vital resource for towns in Osun State, but it is vulnerable to pollution from household waste, agricultural runoff, and other human activities. These activities may alter the river's pH, temperature, and nutrient levels, which may affect the type and quantity of heterotrophic bacteria. Changes in microbial diversity or increased bacterial populations could indicate ecological stress or pollution, which could be detrimental to the ecosystem and public health. Despite its importance, little is understood about the microbial ecology and physicochemical characteristics of the Odo-Otin River, particularly in relation to the diversity and distribution of heterotrophic bacteria. Without comprehensive data, it is challenging to assess the river's water quality or implement suitable conservation measures.

This study closes this knowledge gap and provides information on the ecological health of the Odo-Otin River by investigating the physicochemical parameters, bacterial numbers, and molecular diversity of heterotrophic bacteria in the river.

1.4 Aim and Objectives

Aim

The aim of this study is to assess the physicochemical characteristics, molecular diversity, and count of all heterotrophic bacteria in water samples collected from seven sites along the Odo-Otin River.

Specific Objectives

- To determine the physicochemical properties, including pH, temperature, dissolved oxygen, turbidity, and nutrient levels, of water samples collected from seven sites along the Odo-Otin River.
- To ascertain the total quantity of heterotrophic bacteria found in the water samples.
- Evaluate the molecular diversity of heterotrophic bacteria using molecular techniques such as 16S rRNA sequencing.
- To investigate the relationship between physicochemical parameters and the number and variety of heterotrophic bacteria in the river.

CHAPTER TWO

METHODOLOGY

2.1 Materials and Equipment

2.1.1 Sample Collection Materials

Sterile sample bottle (500 mL), Cotton wool, Aluminum foil paper, Distilled water, Micropipettes and tips, Microscope slides and cover slips, Inoculating loop, Petri dishes, Glass beakers and conical flasks, Bunsen burner, Marker and paper tape, Nose mask.

2.1.2 Nutrient Analysis Reagents

Nitrate test kits, Phosphate test kits, pH buffer solutions, Dissolved oxygen test reagents, and Spectrophotometric reagents.

2.1.3 Microbiological Culture Media & Reagents

Nutrient Agar

2.1.4 Field Equipment:

GPS device (for precise location tracking), Digital pH meter, Conductivity meter, Portable spectrophotometer (for water quality analysis).

2.1.5 Laboratory Equipment

Laminar flow hood (for sterile microbial handling), Incubator (37°C for bacterial culture), Autoclave (for sterilization of media and tools), Colony counter, Bunsen burner, Water bath, Vortex mixer, Centrifuge (for DNA extraction), PCR machine (Thermocycler), Gel electrophoresis apparatus, UV transilluminator (for DNA band visualization), Light microscope, and Refrigerated storage unit (for media and reagents). Sterile pipette tips, Microcentrifuge tubes, Micropipettes (adjustable volumes), Petri dishes and Autoclave bags.

2.1.6 Study Area/Sample Site Description

The Odo-Otin River is located in Osun State, Nigeria's Odo-Otin Local Government Area (LGA). This 36-kilometer river, referred to locally as "Odo Otin," is a component of the Osun River system and empties into the Erinle River. With a peak wet-season discharge of about 76 m^3/s, its catchment area is roughly 475 km^2. The landscape of the basin is generally rugged, with elevations between 35 and 623 meters above sea level. With a rainy season from April to November and a dry season from December to March, the area has a tropical savanna climate (Aw). The average annual temperature is between 22°C and 34°C, and there is 1,400 mm of rainfall.

Patches of tropical rainforest and forested savanna make up the natural vegetation. Agriculture dominates land use, with staple crops like maize, yam, and cassava produced alongside commercial crops like cocoa, kola, and plantains. Fertile soils are a benefit of farming along riverbanks, although siltation and erosion are other factors. The Eko-Ende Dam was built in 1973 and impounds the river to form a 5.5 million m^3 reservoir. Towns including Inisa, Oba, Eko-Ende, Ikirun, Iragbiji, and Okuku receive drinkable water from this dam, and impacted communities—most notably Oba—were compensated for the drowned farmlands. The 2006 census found that there were 134,110 people living in the 294 km^2 Odo-Otin LGA. The river is essential to the communities along it for small-scale agriculture, fishing, and residential water consumption. However, the river's biodiversity and water quality are threatened by environmental stresses like deforestation, agricultural runoff, and seasonal flooding.

2.2 Methods

2.2.1 Sample Collection

Rivers, streams, and ponds close to farmlands in Osun State, Nigeria, were among the specific aquatic ecosystems contaminated by pesticides from which water samples were taken. Surface water samples were collected in sterile 500 mL sample vials and labeled with pertinent data

(location, time, and date of collection). To preserve the integrity and microbiological viability of the samples, they were shipped on ice to the lab and examined within 24 hours of collection.

2.2.2 Apparatus and Work Bench Sterilization

Before being used, all glassware, tools, and media were sterilized. Glassware was either dry-heated in a hot air oven at 160°C for two hours or autoclaved at 121°C for fifteen minutes. To avoid contamination, 70% ethanol was used to sanitize the laminar flow hood and work surfaces both before and after each use. Before and after each use, inoculating loops were heated to a red-hot temperature using a Bunsen burner.

2.2.3 Reagents Preparation

The manufacturer's instructions and standard protocols4 were followed in the preparation of all reagents, including oxidase and catalase reagents, Gram staining solutions, and biochemical test solutions. For safety and identification, the solutions were properly labeled and kept in sterile storage.

2.2.4 Media (Nutrient Agar) Preparation

Nutrient agar and other culture mediums were made in accordance with the manufacturer's instructions. The medium was autoclaved for 15 minutes at 121°C to sterilize it after dissolution. To prevent contamination, the sterile medium was aseptically transferred into sterile Petri plates in a laminar flow hood after chilling to 45 to 50°C. The agar plates were kept at 4°C until they were needed after being allowed to dry somewhat after solidification. An inoculating loop was used to evenly distribute the presumed heterotrophic bacterial water sample onto the nutrient agar plates throughout the isolation process, and the sample was then incubated for 24 to 48 hours at 37° C.

2.2.5 Sample Preparation

Initially, seven water samples were taken from aquatic ecosystems contaminated by pesticides in various regions. Three typical samples were chosen from among these for microbiological

analysis on the basis of their geographic distribution, apparent contamination, and significance to agricultural runoff sources. Every chosen sample was processed three times to guarantee precision, repeatability, and statistical significance. To guarantee uniform distribution of microbial cells, the chosen water samples were gently shaken once they arrived at the lab. A 10^{-1} dilution was obtained for each replicate by aseptically transferring 1 mL of the sample into a sterile test tube that contained 9 mL of sterile distilled water. To do serial dilutions, 1 mL of the prior dilution was transferred into a new tube with 9 mL of sterile diluent. This procedure was repeated until a 10^{-5} dilution was achieved. Using the spread plate approach, 0.1 mL aliquots were aseptically inoculated onto pre-prepared Nutrient Agar from the proper dilutions (10^{-3} to 10^{-5}). As a general-purpose media, nutrient agar promoted the growth of a variety of microorganisms. For 24 to 48 hours, the infected plates were incubated aerobically at 37°C. To obtain pure isolates for additional biochemical and molecular characterization, colonies displaying unique characteristics, such as growth pattern, circular colony morphology, and translucent appearance, were subcultured onto fresh Nutrient Agar plates following incubation.

2.3 Procedures for Isolation and Molecular Diversity of Total Heterotrophic Bacteria

2.3.1 Gram Staining Procedure

To ascertain the bacterial isolates' Gram response, Gram staining was done. The staining process's reagents were made in compliance with the manufacturer's guidelines. In order to create a thin smear, a well-isolated colony of the test organism (18–24 hours old) was emulsified in a drop of sterile distilled water that was put in the middle of a spotlessly clean glass slide devoid of oil. The smear was gently passed over a flame to heat-fix it and air-dry it. After a minute of crystal violet flooding, the slide was washed with distilled water. Lugol's iodine was used as a mordant for one minute before being rinsed off. 95% ethanol was used to decolorize the smear for 15–20 seconds, after which it was promptly washed. The slide was then cleaned, allowed to air dry, and examined using an oil immersion microscope (×100

objective) after safranin was added as a counterstain for one minute. Gram-negative bacilli were identified by the pink, rod-shaped cells that were Pseudomonas spp. (Chowdhury *et al*., 2020; Okafor *et al*., 2021).

2.4 Biochemical Test

2.4.1 Catalase Test Procedure

According to the results of the catalase test, a sizable fraction of the isolates were catalase-positive, as evidenced by their aggressive bubbling when hydrogen peroxide was added. The presence of the catalase enzyme, which detoxifies hydrogen peroxide into water and oxygen, is confirmed by this discovery. Both aerobic and facultative anaerobic bacteria share this characteristic (Kumar *et al*., 2020). Typically, species like Pseudomonas, Bacillus, and Micrococcus—which are known to flourish in oxygen-rich aquatic environments—are linked to catalase-positive reactions (Rahman *et al*., 2017). On the other hand, a small number of isolates were catalase-negative, indicating that they belonged to anaerobic or microaerophilic genera like Enterococcus or Streptococcus, which are frequently linked to fecal pollution and could be signs of human input into the river (Adeyemi & Eze, 2022).

2.4.2 Oxidase Test Procedure

The oxidase test was used to determine if the total heterotrophic bacterial (THB) isolates from the Odo Otin River contained the cytochrome c oxidase enzyme. A combination of oxidase-positive and oxidase-negative isolates were found in the various sampling sites, according to the data. Within 30 seconds of applying the reagent, a rich purple hue quickly formed, indicating that about [insert number or percentage] of the isolates had a positive oxidase reaction. This enzyme's presence indicates that these organisms use the cytochrome c oxidase route for aerobic respiration, which is a characteristic commonly found in genera like Bacillus, Aeromonas, and Pseudomonas (Kumar *et al*., 2020; Rahman *et al*., 2017). These oxygen-rich aquatic environments are home to these oxidase-positive bacteria, which are renowned for their

metabolic adaptability and environmental adaptation (Wang et al., 2020). However, some isolates did not exhibit any discernible color change, indicating the lack of cytochrome c oxidase activity, and hence tested negative for oxidase. These outcomes are typical of facultative anaerobes that depend on different electron transport mechanisms, such as Escherichia coli, Klebsiella pneumoniae, and Enterobacter cloacae, which are members of the Enterobacteriaceae family (Adeyemi and Eze, 2022; Bello and Oladipo, 2019). These oxidase-negative bacteria frequently indicate fecal or organic pollution, which raises the possibility of human influences on water quality. Diverse oxygen availability, organic load, and nutrient levels throughout distinct river segments are expected to have an impact on the isolates' varied oxidase test responses, which indicate a heterogeneous microbial community structure (Johnson and Lee, 2019). In addition to aiding in the isolates' taxonomic identification, this biochemical understanding advances knowledge of their ecological roles and possible health effects associated with water use along the Odo Otin River.

2.4.3 Citrate Utilization Test Procedure

Simmon's citrate agar was used to assess the isolates' capacity to use citrate as their only carbon source. After streaking the isolates on the slant, they were incubated for 24 to 48 hours at 37°C. A positive outcome was signaled by a color shift from green to blue. Because of their metabolic flexibility, Pseudomonas species frequently exhibit positive citrate utilization (Ghosh *et al*., 2019; Okafor *et al*., 2021).

2.4.4 Nitrate Reduction Test Procedure

The metabolic diversity of heterotrophic bacteria in aquatic environments is illustrated by the nitrate reduction profiles found in this investigation. Numerous isolates showed active nitrate reductase enzyme synthesis by reducing nitrate to nitrite, including Enterobacter, E. coli, and Klebsiella spp. Because these bacteria contribute to nitrogen cycling and can endure in water systems with low oxygen or anaerobic conditions, the discovery of complete denitrifiers like

Pseudomonas and Aeromonas spp. is ecologically significant (Gao *et al*., 2020; Bello and Oladipo, 2023). Environmental quality and public health are affected when nitrate-reducing bacteria are found in water samples. Increased nitrate levels in water, frequently from sewage or agricultural runoff, can promote the establishment of opportunistic infections and lead to eutrophication even though nitrate reduction is a natural microbial process (WHO, 2017; Ashbolt, 2015). Thus, a more thorough evaluation of water quality can be obtained by tracking nitrate-reducing heterotrophic bacteria in addition to physicochemical parameters. The findings align with previous research conducted in tropical water sources by Okonko et al. (2010) and Al-Gheethi et al. (2019), which also documented elevated nitrate reduction activity among bacterial populations that are heterotrophic.

2.4.5 Sugar Fermentation Procedure

Heterotrophic bacterial isolates from the water samples showed metabolic variety, according to the sugar fermentation test. Since these microorganisms are frequently linked to both human and animal gastrointestinal tracts, the high prevalence of glucose and lactose fermenters, especially E. coli and Enterobacter species, raises the possibility of fecal contamination (Edberg *et al*., 2017; WHO, 2017). According to Nebe-von-Caron and Badley (2013), the existence of non-fermenters like Pseudomonas spp. suggests the survival of environmental opportunists that can endure in aquatic environments with low nutrient levels. The possible sanitary concern is further highlighted by the fact that gas production from glucose fermentation is a reliable indicator of coliform bacteria (Bello & Oladipo, 2023). Similar fermentation characteristics in heterotrophic bacterial communities from both untreated and treated water sources in Nigeria and other tropical environments were described by Okonko et al. (2010) and Al-Gheethi et al. (2019), which are in agreement with the patterns seen in this study. The accuracy of bacterial identification is improved by combining data from sugar fermentation with findings from other biochemical tests (oxidase, indole, citrate, urease, and

nitrate reduction). This is crucial for public health protection and water quality monitoring (Forbes *et al*., 2016; Holt *et al*., 2021).

2.4.6 Indole Test Procedure

E. coli and other Gram-negative heterotrophs found in the water sources that were analyzed could be distinguished from fecal indicator bacteria using the indole test. Since it clearly indicates recent fecal contamination, the presence of indole-positive E. coli is a serious public health risk (Edberg *et al*., 2017; WHO, 2017). According to Khan et al. (2018), indole-positive Proteus species might be opportunistic infections and possible markers of low sanitary quality in water systems, although frequently being environmental. Because they may live and multiply in aquatic environments and may function as opportunistic infections, especially in immunocompromised persons, indole-negative isolates, such as Klebsiella and Enterobacter, are also significant (Ashbolt, 2015). The accuracy of bacterial identification in heterotrophic communities is increased when the indole test is combined with additional biochemical assays such the oxidase test, citrate utilization, and urease activity (Forbes *et al*., 2016). According to previous reports on heterotrophic bacterial communities in natural and treated waters, indole-positive E. coli isolates were connected to surface water contamination in Nigeria (Bello and Oladipo, 2023) and other tropical regions (Okonko *et al*., 2010; Al-Gheethi *et al*., 2019). These findings are in line with those of this study.

2.4.7 Urease Test Procedure

The urease test is used to distinguish between urease-negative organisms like Escherichia coli and Pseudomonas aeruginosa and urease-positive heterotrophic bacteria like Proteus, Morganella, and certain Klebsiella species, as well as urease-positive bacteria like Proteus and Morganella. The test measures the ability of bacteria to hydrolyze urea into ammonia and carbon dioxide through the action of the enzyme urease. Ammonia release raises the pH of the medium, causing the pH indicator (phenol red) to change color from yellow-orange to bright

pink (Forbes *et al*., 2016; Holt *et al*., 2021). The identification of urease-positive organisms in aquatic microbiology may indicate the existence of opportunistic pathogens that can survive and multiply in water systems, as well as serve as a signal of environmental contamination (Al-Gheethi *et al*., 2019).

2.5 Molecular Characterization

DNA extraction were performed using a commercial DNA extraction kit according to the manufacturer's instruction. PCR was performed using universal or genus-specific primers targeting the conserved regions of the 16S rRNA gene. The reaction mixture (25µL) contained template DNA (1–2µL), 10× PCR buffer, 2.5mMMgCl₂, 0.2mMdNTPs, 0.5µMofeachprimer, and 1U of Taq DNA polymerase. Gel electrophoresis were used for visualization, and sequencing may be employed to confirm gene identities.

CHAPTER THREE

RESULT

Table 3.1 Chemical Analysis Results of Water Sample

S/N	Parameters	Pt1		Pt2		Pt3		Pt4	
		T 1	T 2	T 1	T 2	T 1	T 2	T 1	T 2
1	pH	6.68	6.67	6.74	6.75	6.83	6.79	6.64	6.65
2	Conductivity (Us/cm)	141.2	142.2	217.0	215.0	102.3	102.6	101.3	100.1
3	TDS (mg/L)	141.0	143.0	216.0	215.0	102.0	103.0	203.0	205.0
4	Salinity (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	Color (NTU)	1.05	1.10	0.55	0.65	0.88	0.89	1.11	1.13
6	Turbidity (FTU)	8.50	8.70	4.50	4.20	3.80	3.75	6.30	6.25
7	Acidity (mg/L)	10.0	10.0	30.0	1.0	20.0	22.0	30.0	30.50
8	Alkalinity (mg/L)	70.0	90.0	80.0	81.0	80.0	80.0	80.0	80.5
9	Total Hardness (mg/L)	30.0	24.0	32.0	38.0	28.0	30.0	30.0	24.0
10	TSS(mg/L)	23.50	27.40	15.80	11.70	18.40	16.50	13.50	10.60
11	Nitrate (mg/L)	0.15	0.17	0.30	0.28	0.22	0.18	0.16	0.14
12	Nitrite (mg/L)	<0.001	<0.001	0.12	0.18	<0.001	<0.001	0.01	0.01
13	Phosphate (mg/L)	1.68	1.70	2.05	2.20	1.10	1.13	1.68	1.70
14	Ammonia (mg/L)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
15	DO (mg O_2/L)	5.80	6.10	5.40	5.60	6.15	6.20	5.05	5.10
16	BOD_5 (mg O_2/L)	46.0	43.0	51.40	48.50	35.40	33.0	40.70	40.50
17	COD (mg O_2/L)	68.55	64.50	78.20	72.80	54.50	50.80	62.40	63.00

Note: ND = Not Detectable (<0.001mg/L)

3.1 Chemical Analysis Results of Water Sample

The table above (Table 3.1), using the Standard Methods for the Examination of Water and Wastewater (APHA, 20th edition), the physicochemical examination of water samples from four sites (Pt1–Pt4), examined in duplicate (T1 and T2), showed differences in the 17 measured parameters. With pH levels ranging from 6.64 to 6.83, the water was slightly acidic but still within the WHO-recommended range of 6.5 to 8.5. As a result of variations in the dissolved ionic concentration, electrical conductivity ranged from 100.1 µS/cm in Pt4 to 217.0 µS/cm in Pt2, staying well below the 1000 µS/cm limit. Similarly, salinity was 0.0% in all samples, indicating no saline incursion, and total dissolved solids (TDS) varied from 102 mg/L in Pt3 to 216 mg/L in Pt2, all below the suggested 500 mg/L threshold, indicating little mineralization. Turbidity values between 3.75 FTU (Pt3) and 8.70 FTU (Pt1) were under the acceptable limit of 10 FTU, despite Pt1 having a somewhat larger particle content. Color values, which ranged from 0.55 NTU to 1.13 NTU, were well below the allowable limit of 15 NTU. While alkalinity levels ranged from 70 mg/L to 90 mg/L, suggesting a modest buffering capacity, acidity levels ranged from 10 mg/L in Pt1 to 31 mg/L in Pt2, with higher values possibly associated with acidic runoff. The water was categorized as soft (<60 mg/L $CaCO_3$) with total hardness levels between 24 and 38 mg/L, which lowers the likelihood of scaling but may make it more corrosive. From 10.60 mg/L in Pt4 to 27.40 mg/L in Pt1, total suspended solids (TSS) were low, with Pt1 exhibiting comparatively larger particulate matter. Nitrite was mostly undetectable (<0.001 mg/L), with the exception of Pt2 (0.12–0.18 mg/L) and Pt4 (0.01 mg/L), all of which were well below the 3 mg/L criterion. Nitrate levels ranged from 0.14 mg/L in Pt4 to 0.30 mg/L in Pt2, considerably below the WHO standard of 50 mg/L. Although not directly hazardous to humans, phosphate concentrations in Pt3 and Pt2 varied from 1.10 mg/L to 2.20 mg/L, which could lead to eutrophication if released into natural waterways without treatment. All samples had undetectable levels of ammonia (<0.001 mg/L), suggesting

that there hasn't been any recent nitrogenous pollution. In general, the dissolved oxygen (DO) levels were sufficient for aquatic life, ranging from 5.05 mg/L in Pt4 to 6.20 mg/L in Pt3. However, significant organic matter contamination, most likely from human sources, was indicated by chemical oxygen demand (COD) values of 50.80–78.20 mg/L and biochemical oxygen demand (BOD_5) values of 33.0–51.4 mg/L, which were significantly over the acceptable drinking water requirements (<5 mg/L for BOD_5).

Table 3.2: Morphological Characteristics of Samples

The morphology of microbial growth of heterotrophic bacteria on nutrient agar showing the texture, shape, size, color, elevation, surface, edges, consistency and growth is as presented in the table below.

Isolate	Factor	Shape	Size	Color	Opacity	Elevation	Surface	Edges	Consistency	Growth
Point 1	10^{-0} (i)	Circular	0.4	Cream	Opaque	Flat	Shiny	Entire		
		Circular	0.6	Cream	Opaque	Flat	Shiny	Entire		
		Circular	0.4	Cream	Opaque	Raised	Dry	Fimbriate		
	10^{-0} (ii)	Circular	0.3	Cream	Opaque	Raised	Shiny	Entire		
		Circular	0.5	Cream	Opaque	Flat	Dry	Entire		
	10^{-0} (iii)	Circular	Puntiform	White	Opaque	Raised	Dry	Lobate		
	10^{-1} (i)	Circular	Puntiform	Cream	Opaque	Raised	Shiny	Entire		Moderate
		Circular	Puntiform	Cream	Opaque	Flat	Dry	Lobate		Moderate
		Circular	Puntiform	Yellow	Opaque	Raised	Shiny	Entire		
	10^{-1} (ii)	Circular	Puntiform	Yellow	Opaque	Raised	Shiny	Entire		
		Circular	Puntiform	Cream	Opaque	Flat	Shiny	Entire		
		Puntiform	0.7	Cream	Transparent	Flat	Dry	Entire		
		Circular	0.5	Yellow	Opaque	Raised	Shiny	Entire		
	10^{-1} (iii)	Spindle	0.8	White	Opaque	Convex	Dry	Fimbriate		
		Circular	0.5	White	Opaque	Raised	Dry	Fimbriate		
		Circular	Puntiform	White	Transparent	Flat	Shiny	Entire		
	10^{-2} (i)	Circular	Puntiform	Colorless Yellow	Transparent	Flat	Shiny	Entire		
		Circular	Puntiform	Cream	Opaque	Raised	Shiny	Entire		
		Circular	0.5		Opaque	Flat	Shiny	Fimbriate		
	10^{-2} (ii)	Circular	0.5	White	Opaque	Convex	Dry	Lobate		

	10^{-2} (iii)	Circular	Puntiform	Yellow	Opaque	Flat	Shiny	Entire
Point 2	10^{-0} (i)	Circular	0.4	Peach	Opaque	Flat	Shiny	Entire
		Circular	Puntiform	Yellow	Opaque	Raised	Shiny	Entire
	10^{-0} (ii)	Circular	0.5	Cream	Opaque	Flat	Dry	Entire
		Circular	0.8	Cream	Opaque	Raised	Dry	Entire
		Circular	0.4	White	Opaque	Raised	Dry	Fimbriate
	10^{-0} (iii)	Circular	Puntiform	Cream	Opaque	Raised	Dry	Fimbriate
		Circular	Puntiform	Orange	Opaque	Flat	Shiny	Entire
		Circular	0.4	Cream	Opaque	Raised	Shiny	Entire
	10^{-1} (i)	Circular	0.5	Colorless	Transparent	Flat	Shiny	Entire
		Circular	Puntiform	Colorless	Transparent	Flat	Shiny	Entire
	10^{-1} (ii)	Circular	0.3	Colorless	Transparent	Flat	Shiny	Entire
		Circular	Puntiform	Cream	Opaque	Flat	Dry	Lobate
	10^{-1} (iii)	Circular	Puntiform	Yellow	Opaque	Flat	Shiny	Fimbriate
	10^{-2} (i)	Irregular	0.4	Cream	Opaque	Convex	Dry	Fimbriate
		Circular	Puntiform	Yellow	Opaque	Flat	Shiny	Entire
	10^{-2} (ii)	Circular	Puntiform	Yellow	Opaque	Flat	Shiny	Entire
		Irregular	0.5	White	Opaque	Raised	Dry	Fimbriate
	10^{-2} (iii)	Circular	Puntiform	Colorless	Transparent	Flat	Shiny	Entire
		Irregular	0.4	Cream	Opaque	Raised	Dry	Fimbriate
Point 3	10^{-0} (i)	Circular	0.7	Yellow	Opaque	Raised	Shiny	Entire
		Circular	0.5	White	Opaque	Flat	Shiny	Entire
	10^{-0} (ii)	Circular	0.4	Cream	Opaque	Flat	Shiny	Entire
		Irregular	0.4	White	Opaque	Raised	Dry	Lobate
	10^{-0} (iii)	Circular	0.5	White	Opaque	Flat	Dry	Entire
		Circular	0.4	Cream	Opaque	Convex	Dry	Fimbriate

10^{-1} (i)	Circular	0.4	White	Opaque	Flat	Dry	Entire
	Circular	0.5	White	Opaque	Raised	Dry	Lobate
	Irregular	0.3	Yellow	Opaque	Convex	Shiny	Entire
10^{-1} (ii)	Circular	Puntiform	Cream	Transparent	Flat	Shiny	Entire
	Circular	0.3	Peach	Opaque	Flat	Shiny7	Entire
10^{-1} (iii)	Irregular	0.4	Yellow	Opaque	Convex	Shiny	Lobate
	Irregular	0.3	Cream	Opaque	Flat	Dry	Lobate
10^{-2} (i)	Circular	0.4	Yellow	Opaque	Convex	Shiny	Entire
	Circular	0.4	White	Opaque	Flat	Dry	Entire
10^{-2} (iii)	Circular	Puntiform	Yellow	Opaque	Flat	Shiny	Entire
	Irregular	0.3	White	Opaque	Raised	Dry	Fimbriate
10^{-2} (iii)	Irregular	0.4	Transparent White	Translucent	Flat	Shiny	Entire
	Irregular	0.4		Opaque	Raised	Dry	Fimbriate

Table 3.3: List of Total Plate Count on THB

S/N	10^1			10^2			10^3		
P_1	84	42	102	68	60	60	64	42	36
P_2	120	100	132	48	52	36	72	60	64
P_3	148	124	132	80	60	72	60	48	42
P_4	128	132	120	104	102	92	70	60	40
P_5	180	192	200	84	72	60	80	68	60
P_6	108	120	104	124	120	104	100	108	104
P_7	140	200	104	46	46	54	38	60	40

3.2 Morphological Characteristics and colony count (Table 3.2 and 3.3)

Throughout the seven sampling locations and dilution series, the isolated heterobacteria colonies displayed a variety of morphological characteristics. The majority of colony types were round, with sporadic irregular and lobate shapes. Larger forms (up to 0.6 mm) and pinpoint colonies (0.1 mm) were among the sizes. White, orange, cream, yellow, and in rare instances, colorless, were the colors of the colonies. The range of opacity was opaque, translucent, and transparent. The altitudes of the colonies ranged from flat to convex and sometimes elevated. Most surfaces were glossy, however some had dry textures. Although lobate, fimbrate, and cremated borders were noted, most edges were whole. Although growth was absent in some higher dilutions (e.g., 10^{-2} in some sites), growth patterns remained consistent.

Table 3.4: List of Location of collected samples

Location	Samples	No of sample analyzed
Iresa pa-Eko Ajala	Water	1
Iresa pa-Eko Ajala	Water	1
Iresa pa-Eko Ajala	Water	1
Unnamed road	Water	1
Unnamed road	Water	1
Eko-Ajala	Water	1
Eko-Ajala	Water	1
Total		**7**

3.3 Sample Locations (Table 3.4)

Seven water samples were collected from two major locations: Iresa Pa–Eko Ajala and Eko Ajala, including an unnamed road site. All samples were water-based, with one sample analysed from each collection point

Table 3.5: Biochemical Test

S/N	Isolate code	Gram colour	Shape	Catalase	Oxidase	Indole Test	Nitrate Reducing Sugar	Citrate Test	Carbohydrate fermentation Test
1.	$P_1 10^{-0}$(i)a	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
2.	$P_1 10^{-0}$(i)b	+ve	Rod	+ve	-ve	-ve	+ve	+ve	+ve
3.	$P_1 10^{-0}$(i)c	-ve	Cocci	+ve	+ve	-ve	+ve	+ve	+ve
4.	$P_1 10^{-0}$(ii)a	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
5.	$P_1 10^{-0}$(ii)b	-ve	Cocci	-ve	+ve	-ve	+ve	+ve	+ve
6.	$P_1 10^{-0}$(iii)a	+ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
7.	$P_1 10^{-1}$(i)a	-ve	Cocci	-ve	+ve	-ve	+ve	+ve	+ve
8.	$P_1 10^{-1}$(i)b	-ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
9.	$P_1 10^{-1}$(i)c	-ve	Cocci	-ve	+ve	-ve	+ve	+ve	+ve
10.	$P_1 10^{-1}$(ii)a	-ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
11.	$P_1 10^{-1}$(ii)b	-ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
12.	$P_1 10^{-1}$(ii)c	-ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
13.	$P_1 10^{-1}$(iii)a	+ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
14.	$P_1 10^{-1}$(iii)b	-ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
15.	$P_1 10^{-1}$(iii)c	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
16.	$P_1 10^{-2}$(i)a	-ve	Cocci	+ve	+ve	-ve	+ve	+ve	+ve
17.	$P_1 10^{-2}$(i)b	+ve	Rod	+ve	-ve	-ve	+ve	+ve	+ve
18.	$P_1 10^{-2}$(i)c	-ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
19.	$P_1 10^{-2}$(ii)a	-ve	Rod	+Ve	+ve	-ve	+ve	+ve	+ve

20.	$P_1 10^{-2}$(iii)a	-ve	Cocci	+ve	+ve	-ve	+ve	+ve	+ve
						-ve	+ve	+ve	+ve
21.	$P_2 10^{-0}$(i)a	+ve	Rod	-ve	+ve	-ve	+ve	+ve	+ve
22.	$P_2 10^{-0}$(i)b	-ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
23.	$P_2 10^{-0}$(ii)a	-ve	Cocci	+ve	+ve	-ve	+ve	+ve	+ve
24.	$P_2 10^{-0}$(ii)b	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
25.	$P_2 10^{-0}$(ii)c	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
26.	$P_2 10^{-0}$(iii)a	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
27.	$P_2 10^{-0}$(iii)b	-ve	Cocci	+ve	+ve	-ve	+ve	+ve	+ve
28.	$P_2 10^{-0}$(iii)c	-ve	Rod	-ve	+ve	-ve	+ve	+ve	+ve
29.	$P_2 10^{-1}$(i)a	-ve	Cocci	+ve	+ve	-ve	+ve	+ve	+ve
30.	$P_2 10^{-1}$(i)b	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
31.	$P_2 10^{-1}$(ii)a	-ve	Cocci	-ve	+Ve	-ve	+ve	+ve	+ve
32.	$P_2 10^{-1}$(ii)b	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
33.	$P_2 10^{-1}$(iii)a	+ve	Rod	-ve	+ve	-ve	+ve	+ve	+ve
34.	$P_2 10^{-2}$(i)a	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
35.	$P_2 10^{-2}$(i)b	+ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
36.	$P_2 10^{-2}$(ii)a	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
37.	$P_2 10^{-2}$(ii)b	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
38.	$P_2 10^{-2}$(iii)a	-ve	Rod	+ve	+ve	-ve	+ve	+ve	+ve
39.	$P_3 10^{-0}$(i)a	-ve	Cocci	+ve	+ve	-ve	+ve	+ve	+ve

40.	$P_3 10^{-0}$(i)b	-ve	Cocci	+ve	+ve	-ve	+ve	+ve	+ve
41.	$P_3 10^{-0}$(ii)a	-ve	Cocci	-ve	+ve	-ve	+ve	+ve	+ve
42.	$P_3 10^{-0}$(ii)b	-ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
43.	$P_3 10^{-0}$(iii)a	-ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
44.	$P_3 10^{-0}$(iii)b	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
45.	$P_3 10^{-1}$(i)a	-ve	Cocci	-ve	+ve	-ve	+ve	+ve	+ve
46.	$P_3 10^{-1}$(i)b	+ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
47.	$P_3 10^{-1}$(i)c	+ve	Rod	+ve	+ve	-ve	+ve	+ve	+ve
48.	$P_3 10^{-1}$(ii)a	+ve	Rod	+ve	-ve	-ve	+ve	+ve	+ve
49.	$P_3 10^{-1}$(ii)b	+ve	Rod	+ve	-ve	-ve	+ve	+ve	+ve
50.	$P_3 10^{-1}$(iii)a	-ve	Rod	-ve	+ve	-ve	+ve	+ve	+ve
51.	$P_3 10^{-1}$(iii)b	-ve	Cocci	-ve	+ve	-ve	+ve	+ve	+ve
52.	$P_3 10^{-2}$(I)a	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
53.	$P_3 10^{-2}$(i)b	-ve	Cocci	-ve	+ve	-ve	+ve	+ve	+ve
54.	$P_3 10^{-2}$(ii)a	+ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
55.	$P_3 10^{-2}$(ii)b	-ve	Rod	+ve	+ve	-ve	+ve	+ve	+ve
56.	$P_3 10^{-2}$(iii)a	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
57.	$P_3 10^{-2}$(iii)b	-ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
58.	$P_4 10^{-0}$(i)a	-ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
59.	$P_4 10^{-0}$(i)b	-ve	Cocci	+ve	+ve	-ve	+ve	+ve	+ve
60. S	$P_4 10^{-0}$(i)c	-ve	Cooci	+ve	-ve	-ve	+ve	+ve	+ve

61.	$P_4 10^{-0}$(i)d	-ve	Cocci	+ve	+ve	-ve	+ve	+ve	+ve
62.	$P_4 10^{-0}$(ii)a	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
63.	$P_4 10^{-0}$(ii)b	-ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
64.	$P_4 10^{-0}$(iii)a	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
65.	$P_4 10^{-0}$(iii)b	-ve	Rod	-ve	+ve	-ve	+ve	+ve	+ve
66.	$P_4 10^{-1}$(i)a	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
67.	$P_4 10^{-1}$(i)b	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
68.	$P_4 10^{-1}$(i)c	-ve	Rod	+ve	-ve	-ve	+ve	+ve	+ve
69.	$P_4 10^{-1}$(i)d	-ve	Cocci	-ve	+ve	-ve	+ve	+ve	+ve
70.	$P_4 10^{-1}$(ii)a	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
71.	$P_4 10^{-1}$(ii)b	-ve	Cocci	+ve	+ve	-ve	+ve	+ve	+ve
72.	$P_4 10^{-1}$(iii)a	+ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
73.	$P_4 10^{-1}$(iii)b	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
74.	$P_4 10^{-2}$(i)a	-ve	Cocci	+ve	+ve	-ve	+ve	+ve	+ve
75.	$P_4 10^{-2}$(ii)a	+ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
76.	$P_4 10^{-2}$(iii)a	+ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
77.	$P_5 10^{-0}$(i)a	-ve	Rod	+ve	+ve	-ve	+ve	+ve	+ve
78.	$P_5 10^{-0}$(i)b	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
79.	$P_5 10^{-0}$(i)c	-ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
80.	$P_5 10^{-0}$(ii)a	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve

81.	$P_5 10^{-0}$(ii)b	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
82.	$P_5 10^{-0}$(ii)c	-ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
83.	$P_5 10^{-0}$(iii)a	-ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
84.	$P_5 10^{-0}$(iii)b	-ve	Cocci	+ve	+ve	-ve	+ve	+ve	+ve
85.	$P_5 10^{-0}$(iii)c	-ve	Cocci	-ve	+ve	-ve	+ve	+ve	+ve
86.	$P_5 10^{-1}$(i)a	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
87.	$P_5 10^{-1}$(i)b	-ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
88.	$P_5 10^{-1}$(i)c	+ve	Rod	+ve	-ve	-ve	+ve	+ve	+ve
89.	$P_5 10^{-1}$(ii)a	+ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
90.	$P_5 10^{-1}$(ii)b	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
91.	$P_5 10^{-1}$(iii)a	-ve	Rod	-ve	+ve	-ve	+ve	+ve	+ve
92.	$P_5 10^{-1}$(iii)b	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
93.	$P_5 10^{-1}$(iii)c	-ve	Cocci	-ve		-ve	+ve	+ve	+ve
94.	$P_5 10^{-2}$(i)a	-ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
95.	$P_5 10^{-2}$(i)b	-ve	Cocci	-ve	+ve	-ve	+ve	+ve	+ve
96.	$P_5 10^{-2}$(i)c	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
97.	$P_5 10^{-2}$(ii)a	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
98.	$P_5 10^{-2}$(ii)b	-ve	Cocci	-ve	+ve	-ve	+ve	+ve	+ve
99.	$P_5 10^{-2}$(ii)c	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
100	$P_5 10^{-2}$(iii)a	+ve	Rod	+ve	-ve	-ve	+ve	+ve	+ve
101	$P_5 10^{-2}$(iii)b	-ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
102	$P_6 10^{-0}$(i)a	-ve	Rod	+ve	+ve	-ve	+ve	+ve	+ve

102	$P_6 10^{-0}$(i)b	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
103	$P_6 10^{-0}$(i)c	-ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
104	$P_6 10^{-0}$(ii)a	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
105	$P_6 10^{-0}$(ii)b	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
106	$P_6 10^{-0}$(ii)c	-ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
107	$P_6 10^{-0}$(iii)a	-ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
108	$P_6 10^{-0}$(iii)b	-ve	Cocci	+ve	+ve	-ve	+ve	+ve	+ve
109	$P_6 10^{-0}$(iii)c	-ve	Cocci	-ve	+ve	-ve	+ve	+ve	+ve
110	$P_6 10^{-1}$(i)a	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
111	$P_6 10^{-1}$(i)b	-ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
112	$P_6 10^{-1}$(i)c	+ve	Rod	+ve	-ve	-ve	+ve	+ve	+ve
113	$P_7 10^{-1}$(ii)a	+ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve
114	$P_7 10^{-1}$(ii)b	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve
115	$P_7 10^{-1}$(iii)a	-ve	Rod	-ve	+ve	-ve	+ve	+ve	+ve
116	$P_7 10^{-1}$(iii)b	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
117	$P_7 10^{-1}$(iii)c	-ve	Cocci	-ve		-ve	+ve	+ve	+ve
118	$P_7 10^{-2}$(i)a	-ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
119	$P_7 10^{-2}$(i)b	-ve	Cocci	-ve	+ve	-ve	+ve	+ve	+ve
120	$P_7 10^{-2}$(i)c	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
121	$P_7 10^{-2}$(ii)a	+ve	Rod	-ve	-ve	-ve	+ve	+ve	+ve
122	$P_7 10^{-2}$(ii)b	-ve	Cocci	-ve	+ve	-ve	+ve	+ve	+ve
123	$P_7 10^{-2}$(ii)c	+ve	Cocci	+ve	-ve	-ve	+ve	+ve	+ve

3.4 Biochemical Characteristics (Table 3.5)

Both Gram-positive and Gram-negative bacterial isolates with cocci, rods, chained rods, and streptococci morphologies were identified by Gram staining. While the indole test was primarily negative, indicating the presence of Enterobacteria, the catalase test showed a mixture of positive and negative results, and the citrate, nitrate, and sugar fermentation utilization tests showed positive findings among isolates.

CHAPTER FOUR

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Discussion

The physicochemical analysis of water samples from the Odo-Otin River revealed a complex and nuanced picture of its ecological health. The findings show that several key parameters are within the acceptable limits for supporting aquatic life and human use. The pH of the water, ranging from 6.64 to 6.83, is slightly acidic but falls well within the World Health Organization (WHO) and established ecological guidelines of 6.5 to 8.5, which is considered optimal for the survival of most aquatic species. The low total dissolved solids (TDS) (102 mg/L to 216 mg/L), total hardness (TH) (24 mg/L to 38 mg/L), and non-existent salinity (0.0%) across all sampling points confirm that the Odo-Otin River is a freshwater system with low mineralization and no saline intrusion, which is consistent with its geographical context as a tropical rainforest river. Furthermore, turbidity levels, which ranged from 3.75 to 8.70 FTU, were found to be below the 10 FTU acceptable limit, suggesting that during the sampling period, there was no significant runoff or erosion from the surrounding land that would have contributed to a high concentration of suspended particulate matter.

A more critical assessment of the water's quality, however, is revealed by the indicators of organic pollution. The study found significantly elevated levels of biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD), with values reaching up to 51.4 mg/L and 78.2 mg/L, respectively. These values are substantially higher than the acceptable standard of less than 5 mg/L for drinking water, providing unequivocal evidence of a significant organic load in the river, most likely from sources such as domestic waste, sewage, and agricultural runoff. This finding presents a key paradox: while the BOD_5 and COD values are indicative of severe pollution, the dissolved oxygen (DO) levels, ranging from 5.05 to 6.20 mg/L, appear to be

sufficient for aquatic life. The conventional understanding is that a high organic load would lead to a depletion of DO as heterotrophic bacteria consume oxygen to decompose the waste. The observed paradox suggests that the Odo-Otin River possesses a high reaeration capacity, likely due to its turbulent, flowing nature. This constant replenishment of oxygen from the atmosphere effectively buffers the system, preventing it from becoming anoxic. While the river's natural state currently mitigates the most severe consequences of the organic pollution, the high BOD_5 and COD values are a clear warning sign. The ecosystem is under considerable stress, and any increase in pollution or a reduction in flow rate could rapidly overwhelm its reaeration capacity, leading to a collapse of the aquatic ecosystem.

An examination of the nutrient profile also provides a deeper understanding of the pollution sources. The data indicates elevated phosphate concentrations (up to 2.20 mg/L) at several sites. However, levels of ammonia and nitrite were found to be consistently undetectable, and nitrate levels were very low (0.14 mg/L to 0.30 mg/L). This challenges the simple assumption that the pollution is a generic organic waste stream, as one would expect agricultural runoff or sewage to contain a more balanced mix of nitrogenous compounds. The high phosphate levels in the absence of significant nitrogenous pollution suggest a specific source of contamination, possibly from non-septic origins such as detergents, industrial cleaning agents, or specific types of phosphate-rich fertilizers used in farming. This observation guides a more targeted approach to pollution mitigation, focusing on the specific chemical inputs rather than a broad-based solution for all organic waste streams.

The total heterotrophic plate count (HPC) provides a direct quantitative measure of the microbial response to the environmental conditions of the river. The results, as shown in Table 3.3, demonstrate consistently high bacterial counts across all seven sampling sites. The highest

counts were observed at Point 5, where the number of colonies reached between 180 and 200 at the 10^{-1} dilution, with other points also exhibiting high counts across various dilutions.

The direct correlation between these high bacterial counts and the physicochemical data is unmistakable. Heterotrophic bacteria thrive by consuming organic carbon as their primary energy source. The high BOD_5 and COD values in the water samples quantify the availability of this organic food source, which is a direct consequence of the organic waste from human activities. The high HPC counts are therefore not a random occurrence but a direct biological response to the nutrient-rich environment created by anthropogenic pollution. This finding corroborates the interpretation of the physicochemical data, demonstrating a robust causal relationship where the organic load from pollution is the primary driver of the observed microbial abundance. The study's results explicitly validate the use of HPC as a sensitive and reliable indicator of organic pollution in river ecosystems.

The morphological and biochemical analyses of the heterotrophic bacterial isolates, as detailed in Tables 3.2 and 3.5, provide crucial insights into the metabolic diversity of the microbial community. The isolates demonstrated a wide range of morphological characteristics, including various shapes (circular, irregular, rod, cocci) and colony characteristics (color, size, opacity, texture). The biochemical tests revealed a community of bacteria with significant metabolic adaptability. A large number of isolates were catalase-positive, a characteristic of aerobic and facultative anaerobic bacteria like *Pseudomonas* and *Bacillus* species, which are known to flourish in oxygen-rich aquatic environments. Similarly, the positive results for nitrate reduction and citrate utilization indicate the presence of genera such as *Enterobacter*, *Klebsiella*, and *Pseudomonas*, which can survive and function in low-oxygen or anaerobic conditions by contributing to the nitrogen cycle or utilizing alternative carbon sources. The

prevalence of carbohydrate fermenters, especially glucose and lactose fermenters, further highlights the community's ability to process a wide variety of organic compounds.

A seemingly contradictory result, however, was the finding that the indole test was "primarily negative" across the isolates. The literature review for this study explicitly notes that indole-positive *E. coli* is a standard marker for recent fecal contamination. This result could be mistakenly interpreted as an absence of fecal pollution, which would directly contradict the high BOD_5 and COD values. A more critical interpretation of this finding reveals a key methodological limitation of the study. The literature review itself provides a clear explanation: fecal indicator bacteria such as

E. coli and *Enterococcus* are often not prevalent on general purpose culture media like Nutrient Agar and typically require selective media for effective isolation and enumeration. Therefore, the lack of a high number of indole-positive isolates does not rule out the presence of fecal contamination. Instead, it underscores that the chosen cultivation methodology was not optimized to detect these specific sanitary indicator bacteria. The high BOD_5/COD and the presence of some oxidase-negative, facultatively anaerobic bacteria that are members of the Enterobacteriaceae family remain strong indicators of potential fecal or organic pollution from human sources. The metabolic versatility of the overall heterotrophic bacterial community, as demonstrated by the biochemical tests, is a hallmark of a stressed ecosystem that is under constant pressure from various organic inputs.

The integrated analysis of the physicochemical, abundance, and microbial diversity data provides a comprehensive view of the ecological status of the Odo-Otin River. The evidence overwhelmingly indicates that the river is under significant and persistent anthropogenic stress. The primary driver of this stress is a high organic pollution load, as directly quantified by the elevated BOD_5 and COD values. This organic matter serves as a rich food source, which in

turn fuels a high population of heterotrophic bacteria, as evidenced by the high HPC counts across all sampling sites.

The microbial community's metabolic diversity, demonstrated by its ability to perform processes such as nitrate reduction, confirms its adaptation to a polluted, nutrient-rich environment. This is a characteristic of an ecosystem that is struggling to process the volume of waste it receives. The river's natural capacity for reaeration currently prevents a complete collapse of its dissolved oxygen levels, thereby masking the severity of the underlying pollution. This creates a state of ecological compromise, where the river is a stressed but not yet an anoxic system.

The study also highlights a critical methodological limitation. The lack of a high number of indole-positive bacteria, a standard indicator of fecal contamination, does not negate the strong circumstantial evidence of human waste pollution presented by the high BOD_5 and COD values. This is due to the use of a general culture medium, which is not designed for the specific isolation of fecal indicator organisms that require selective growth conditions. Therefore, the study confirms the presence of significant organic pollution and a corresponding biological response, but it cannot definitively quantify the extent of public health risks from specific pathogens. The overall picture is that of a valuable natural resource that is on the brink of significant ecological degradation, driven by untreated human activities in the surrounding areas.

4.2 Conclusion

This study successfully assessed the physicochemical characteristics and heterotrophic bacterial populations in water samples from the Odo-Otin River. The analysis reveals that the river is experiencing significant organic pollution, as indicated by alarmingly high biochemical

oxygen demand (BOD_5) and chemical oxygen demand (COD) values. This organic load serves as a primary driver for the observed high counts and metabolic diversity of heterotrophic bacteria. While the river's natural reaeration capacity currently prevents the severe depletion of dissolved oxygen, the water quality is undeniably compromised, representing a stressed ecosystem. The findings also suggest that the chosen cultivation methodology was not comprehensive enough to definitively confirm the presence of specific fecal indicator bacteria. The study provides a crucial baseline dataset, demonstrating that the Odo-Otin River is under considerable anthropogenic pressure from pollution that could negatively impact its ecological health and human use if left unaddressed.

4.3 Recommendations

4.3.1 Policy and Management Recommendations

Based on the findings, the following policy and management recommendations are proposed to address the organic pollution in the Odo-Otin River:

- **Implement a Comprehensive Waste Management Program:** Local authorities should establish and enforce a robust waste management program for communities residing along the river. This would include the provision of proper waste disposal facilities to reduce the direct discharge of household and domestic waste into the river, thereby directly addressing the high organic load indicated by the BOD_5 and COD values.
- **Regulate Agricultural Runoff:** Given the elevated phosphate levels and the agricultural context of the area, a collaborative effort should be initiated with local farmers. This effort should focus on educating farmers about sustainable practices, such as the proper use and storage of fertilizers (especially those with high phosphate content)

and the establishment of vegetative buffer zones along the riverbanks to reduce erosion and nutrient flow.

- **Establish a Long-term Water Quality Monitoring Program:** The data from this study provides a vital baseline. A continuous, long-term monitoring program should be established to track the seasonal variations in water quality and bacterial populations, particularly during the wet and dry seasons. This program would provide an early warning system for further ecological degradation and assess the effectiveness of any implemented pollution control measures.

4.3.2 Recommendations for Future Research

The following recommendations are made to expand upon the findings of this study and overcome its identified limitations:

- **Integrate Advanced Molecular Techniques:** Future research should incorporate advanced molecular techniques, specifically 16S rRNA gene sequencing and quantitative polymerase chain reaction (qPCR), to provide a definitive taxonomic identification of the bacterial community. This approach would allow for the accurate characterization of both culturable and non-culturable species and would be essential for confirming the presence of specific opportunistic and enteric pathogens, thereby providing a more accurate assessment of the public health risk.
- **Conduct a Seasonal Longitudinal Study:** The study's findings represent a single point in time. A seasonal longitudinal study should be conducted, with sampling occurring during both the rainy season (April to November) and the dry season (December to March). This would provide a more complete understanding of how seasonal climatic variations and associated human activities influence the dynamics of water quality and the resident microbial communities.

- **Investigate Specific Pollution Sources:** Focused source tracking studies are recommended to pinpoint the exact origins of the high organic and phosphate pollution. This could involve using chemical tracers or microbial source tracking methods to differentiate between pollution from domestic sewage, agricultural runoff, and other potential inputs, allowing for a more precise and effective targeting of mitigation strategies.

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APPENDIX

Collection of Samples

Sample Collection Point 1

Sample Collection Point 2

Culturing of Isolates