# ASSESSMENT OF THE PHYSICOCHEMICAL AND COLIFORM BACTERIA IN IKPOBA RIVER

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**NIGERIA.**

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY,**

**FACULTY OF LIFE SCIENCES,**

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**IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.SC.) DEGREE IN MICROBIOLOGY.**

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## CERTIFICATION

We certify that this project work was carried out by **AMALUM, CHIDERA DEBORAH**

in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.) Degree in Microbiology.

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**PROF. (Mrs). E. E. AKORTHA** **DATE**

**(Project Supervisor)**

_____ _____

**Professor (Mrs). F. I. AKINNIBOSUN** **DATE**

**(Head of Department)**

## DEDICATION

This project is dedicated to the Almighty God, my helper and my source, my parents and also to my lovely husband

## ACKOWLEDGMENTS

I would like to express my profound gratitude and appreciation to all those who stood by me throughout my academic period. First of all, I want to thank God for his grace, his mercy and love Which has brought me this far. And to my Dad, late mr Kenneth Amalum and my mum Mrs Amaka Amalum and my precious husband Ogbuniba Chukwunonso Johnpaul I want to say may God continue to bless you all for your support towards me since the beginning till now. My sincere gratitude to my supervisor, **PROF. (Mrs). E. E. AKORTHA and DR C. AJUZIE** for their generosity, kindness and excellent supervision throughout the duration of my project work.

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# ABSTRACT

Coliform bacteria contamination in river water is a significant public health concern, as it often indicates the presence of fecal pollution, which may harbor pathogenic microorganisms. Coliform bacteria, especially *Escherichia coli*, are commonly used as indicator organisms for water quality assessment due to their presence in the intestines of warm-blooded animals and, therefore, in their waste. This study was aimed at assessing the physicochemical and coliform bacteria in Ikpoba river in Benin City, Edo state. The physicochemical result obtained in this study showed that pH was at 5.0, as against the WHO limit of 6.5-8.5 thus making the water dangerous for consumption by humans and aquatic lives. The electronic conductivity of the water was recorded at 62, against the WHO limit of 1500, this was below the permissible limit. turbidity was at 6.37. Copper, zinc, chromium and cadmium result in this study were 8.73, 26.45, 3.95, 0.042 respectively. The coliform bacteria count obtained in this study showed that the bacteria count ranged at 4.7 X $10^4$± 2.00 cfu/ml -7.7 X $10^4$± 1.00 cfu/ml for all sections of the river. Using the standard microbial method which include cultural, morphological and biochemical characterization, the isolates obtained in this study were *Escherichia coli*, *Citrobacter* sp, *Enterobacter* sp, *Serratia* sp and *Klebsiella* sp. The presence of coliform bacteria such as *Escherichia coli*, *Citrobacter*, *Enterobacter*, *Serratia*, and *Klebsiella* in river water can have serious health and ecological effects. The presence of coliform bacteria, such as *Escherichia coli*, *Citrobacter*, *Enterobacter*, *Serratia*, and *Klebsiella*, along with heavy metals in river water, poses a multifaceted threat to both human health and aquatic ecosystems.

# CHATER ONE

# INTRODUCTION

## 1.1 **Background of the study**

Water, the elixir of life is indispensable in every facet of existence. Apart from quotidian uses, water is also important in many industries for the production of chemicals, cosmetics, and beverages (Lamikanra, 1999; Mishra *et al*., 2012). The importance of rivers, the major source of freshwater, is well evidenced by the historic fact that major civilizations were settled along the banks of a river. However, water's indispensability is handicapped by its pollution which renders it unusable. Water polluted by influx of sewage especially from hospitals, public defecation, slaughter houses, clinics, and animal husbandry contains huge amount of antibiotic resistant bacteria which can ultimately be transferred to humans or livestock (Ahmed *et al*., 2010; Crimmins and Beltrán-Sánchez, 2011; Carlet *et al*., 2012).

One of the major causes of child mortality under five is waterborne illnesses (WHO and UNICEF, 2000; Howard and Bartram, 2003). The group of coliform bacteria from the family Enterobacteriaceae was chosen as water potability indicators as they inherently populate the gut of warm blooded mammals; end up in their feces and indicate the presence of other pathogens. (Berg and Metcalf, 1978; Mishra *et al*., 2012; Mishra *et al*., 2013). These bacteria are very receptive to drug resistance genes and also readily propagate it to the other pathogens prevalent in the vicinity, therefore pose as potent health hazards. Hence, regular monitoring of coliform levels in the environment provides insight into the status of water potability, warns prior incidence of various public health concerns and paves way for designing remedial measures.

Water is a fundamental element of life on Earth, playing a crucial role in the health and well-being of both human and animal populations. Rivers, which serve as major sources of freshwater, are essential for drinking water, agriculture, industrial processes, recreation, and as habitats for diverse ecosystems. However, rivers are increasingly vulnerable to contamination by various pollutants, one of the most concerning of which is the presence of coliform bacteria. Coliform bacteria, particularly fecal coliforms, are commonly found in rivers worldwide and are considered a major indicator of water quality and potential health risks. These bacteria originate from the intestinal tracts of warm-blooded animals, including humans, and their presence in river water signifies potential fecal contamination and the presence of pathogenic microorganisms (Tallon *et al*., 2020; Rijal *et al*., 2021).

Rivers are dynamic systems that play a crucial role in shaping landscapes, supporting ecosystems, and providing water resources for human activities such as agriculture, industry, and drinking. The physicochemical properties of rivers, including temperature, pH, dissolved oxygen, turbidity, conductivity, and nutrient concentrations, are fundamental in determining the health and functionality of these water bodies. These properties directly affect the organisms living within rivers and the broader ecological processes in aquatic environments. Moreover, they influence the river's suitability for various human uses and have far-reaching implications for biodiversity, water quality, and ecosystem services (Giller and Malmqvist, 2020; Dodds and Whiles, 2019). These properties result from a complex interplay of natural processes such as weathering, erosion, precipitation, and biological activity, as well as anthropogenic influences like pollution, deforestation, and dam construction (Allan and Castillo, 2007; Vörösmarty *et al*., 2010).

Access to safe drinking water is one of the primary determining factors toward environmental sustainability and improved public health. Various physicochemical, hydrological, and biological processes as well as human activities may influence the availability and quality of safe water which is accessible to the people. Groundwater is considered to be less prone to pathogenic contamination as compared to surface water because of natural filtration by the aquifer sand (Ravenscroft *et al*., 2017). However, recently groundwater has also been reported to be contaminated mainly due to the absence of proper waste management system, leakage from sewage pipes, open defecation system, extraction of groundwater in excess, drilling of wells in an unsuitable condition, low depth of water table due to human activities, and mostly because of the ignorance toward water conservation (Tripathi and Sharma, 2011; Das, 2019), which can cause a sudden outbreak of waterborne diseases (Wang *et al*., 2014). In the Indian subcontinent, 60% of the rural population use groundwater for drinking purpose (Mukherjee *et al*., 2015).

The consumption of contaminated drinking water is the root cause of 80% of diseases occurring globally, either through chemical imbalances or microbial contamination (World Health Organization, 2004). In developing nations including India, the scenario is particularly concerning as a large number of people do not have access to safe drinking water. Each year, nearly 2 million people die from waterborne diseases in the developing nations, especially children under the age of five (World Health Organization, 2008), mostly from diarrhea caused by coliform bacteria (Shar *et al*., 2008). Among other microorganisms, the presence of coliform bacteria in groundwater is one of the major threats to human health and possesses the risk of an outbreak of waterborne diseases. Transportation of coliform bacteria from surface and subsurface sources is dependent on various factors like the specific hydrogeological settings, depth of tube well, tube well construction, and the amount and intensity of precipitation (Atherholt *et al*.,

2017). Shallow groundwater is more vulnerable than the deeper one because of the shorter vertical distance and the attenuation of pathogenic bacteria depends on retention and die-off in the flow path (van Geen *et al*., 2011). Recent fecal contamination and the potential presence of pathogenic bacteria in water can be detected by the presence of thermotolerant fecal coliform bacteria, generally known as fecal indicator bacteria (FIB) (Kostyla *et al*., 2015), especially Escherichia coli in water because they are always found in untreated waste from humans and other warm-blooded animals (Atherholt *et al*., 2017; Dayanti *et al*., 2018).

Potable water should contain <10 CFU of total coliform (TC) and nearly no fecal coliform per 100 ml of water (World Health Organization, 2004). The Indo-Gangetic-Brahmaputra basin is one of the largest freshwater resources in the world, which is already threatened by geogenic arsenic contamination in inland aquifers (Mukherjee *et al*., 2015) and saltwater intrusion in coastal aquifers (Das and Mukherjee, 2019). Against the backdrop of the geogenic groundwater contamination and industrial pollution, improper sanitation practices add another threat to the available freshwater resources, which directly has an acute impact on human health (Verhougstraetea *et al*., 2015). In India, more than 500 million of its population are still practicing open defecation, resulting in the unhealthy disposal of fecal waste in the vicinity of the drinking water sources. The dependency on groundwater for drinking purposes needs attention toward the necessity of groundwater quality monitoring at regular intervals. The Nadia district is known to have actively involved in surface water and groundwater interaction process in terms of meteoric recharge and base flow to river in the favorable aquifer-aquitard framework, which consists of medium-to-coarse grain sand to silts (Mukherjee *et al*., 2008; Hussain *et al*., 2017).

## 1.2 AIM of Study:

This project aimed to evaluate the coliforms presents in Ikpoba River and the physicochemical analysis of the river.

## 1.3. OBJECTIVES

The respective objectives were to:

1 isolation and identification of coliform bacteria from Ikpoba river samples

2. antibiotic profiling of the isolated bacteria

3. physicochemical evaluation of the samples

# CHATER TWO

# LITERATURE REVIEW

## 2.1 **Coliform Bacteria**

Coliform bacteria are a diverse group of Gram-negative, rod-shaped bacteria that are widely used as indicators of fecal contamination in water bodies. The term "coliform" is not a specific taxonomic classification but rather a collective name for a group of bacteria that share certain biochemical characteristics, such as their ability to ferment lactose with the production of gas within 48 hours at 35-37°C (Chambers *et al*., 2018). Coliform bacteria are categorized into three groups: total coliforms, fecal coliforms, and *Escherichia coli* (*E. coli*) (Edberg *et al*., 2000).

Total coliforms are a broad group that includes both fecal and non-fecal species. They are naturally present in the environment, including soil, water, and vegetation, and are generally not harmful. However, their presence in drinking water or recreational water bodies indicates potential contamination and warrants further investigation. Fecal coliforms are a subset of total coliforms that are specifically associated with fecal material from warm-blooded animals. They are more closely related to human and animal fecal waste and are considered a better indicator of fecal pollution in water than total coliforms (Leclerc *et al*., 2001; Araujo *et al*., 2020).

*E. coli*, a specific species within the fecal coliform group, is the most reliable indicator of fecal contamination. While most strains of *E. coli* are harmless and part of the normal flora of the human intestine, certain strains, such as *E. coli O157*

, are pathogenic and can cause severe gastrointestinal illness and even death (Nataro and Kaper, 1998; Rhoads *et al*., 2017). The detection of *E. coli* in water, therefore, signifies recent fecal contamination and the potential presence of pathogenic microorganisms.

## 2.2 SOURCES OF COLIFORM CONTAMINATION IN RIVER

Coliform bacteria can enter river systems through multiple sources and pathways. The primary sources of coliform contamination include untreated or inadequately treated sewage discharges, agricultural runoff, and stormwater runoff. Human activities such as defecation near water bodies, improper disposal of wastewater, and leakage from septic systems also contribute significantly to the contamination (Kistemann *et al*., 2002; Beattie *et al*., 2022).

Agricultural runoff, particularly from livestock farms, is a major source of coliform contamination in rivers. Manure from cattle, pigs, and poultry contains high levels of fecal coliforms, which can be washed into nearby water bodies during rainfall events. Furthermore, the application of manure as fertilizer in agricultural fields can result in runoff during rain, further contributing to contamination. Wildlife, including birds and mammals, can also be a source of coliform bacteria, though their contribution is generally smaller compared to human and livestock sources (Pachepsky and Shelton, 2011; Staley *et al*., 2021).

Stormwater runoff is another significant pathway for coliform contamination. During rainfall, stormwater can collect fecal material from various surfaces, such as streets, sidewalks, and roofs, and transport it to rivers. Urban areas with combined sewer systems are particularly vulnerable to coliform contamination during heavy rainfall events, as the systems may overflow and discharge untreated sewage directly into water bodies (Haile *et al*., 1999; Dhiman *et al*., 2023).

## 2.3 POLLUTION OF RIVER WATER AND COLIFORM AS SANITARY STANDARDS

The United Nations confirms that almost 2 million tons of waste finds its way to the water bodies globally (UNWAP, 2003; Ross, 2010). About 1.2 billion people defecate along the banks of the rivers in developing and under-developed nations (WHO and UNICEF, 2008). Seventy percentage of the industrial wastes have been estimated to be disposed untreated into the water supplies (UN-WATER, 2009). Although the treated sewage allowed to be discharged into the rivers should not exceed the Biochemical Oxygen Demand (BOD) limit of 30 mg/L, Total Suspended Solids (TSS) limit of 50 mg/L (Tyagi *et al*., 2013), a mere 18% of the 33,000 mld sewage spawned daily is treated. Several investigations have been undertaken over time to monitor water quality and more precisely its potability; among all the parameters available, the microbiological ones largely influence water usability, as has been established by many studies reporting high levels of contamination in the freshwater bodies (WHO, 2006; Mishra *et al*., 2012, 2013; Harnisz *et al*., 2015; Parvez *et al*., 2016; Gomes Freitas *et al*., 2017).

## 2.4 Fecal and Non-fecal Coliform

The group of coliform is further subdivided into total and fecal coliforms while total includes both soil intermediates and fecal forms, fecal coliforms confines to those from fecal origin, used as standard microbial indicators of water quality since 1920 (Ashbolt *et al*., 2001). The fecal coliform group, i.e., *Escherichia coli (E. coli), Klebsiella*, and *Enterobacter* inhabit the intestinal tract of warm blooded animals (Dufour, 1977). The use of coliform bacteriological analysis for water potability testing dates back to 1880 when Von Fritsch found *Klebsiella pneumoniae* and K. rhinoscleromatis in human feces (Geldreich, 1978). Even the USEPA recommends the use of E. coli as efficient indicator for evaluating freshwater quality (United States Environmental Protection Agency [USEPA], 1986).

## 2.5 Methods of Detection- Past and Present

Percy and Grace counted bacteria from water on Robert Koch's solid gelatin media (Hutchinson and Ridgway, 1977), then Theodor Escherich discovered *Bacillus coli* (Escherich, 1885); later renamed as *E. coli* by Castellani and Chambers (1919). By 1893, the 'Wurtz method' of enumerating B. coli by directly plating water samples on litmus lactose agar employing the idea of acid formation from lactose fermentation was used by sanitary bacteriologists (Ashbolt *et al*., 2001). In 1893 Durham's tubes were used for detecting gas formation from fermentation (Durham, 1898). In 1905 MacConkey's broth was used as a diagnostic for bile tolerant lactose-fermenting bacteria (MacConkey, 1905).

Till date the most preferred and costeffective method for elaborate detection of coliforms is the Most Probable Number (MPN) comprising of the presumptive, confirmed and completed test. A set of double and single strength lactose broth with inverted durham tubes are inoculated with measured amounts of water to be tested, coliform ferment the lactose present in the broth producing acid and gas. The MPN of coliforms present in 100 ml of water is estimated by the number of positive tubes against the MPN table. Samples from the positive tubes are streaked on EMB agar plates in the confirmed test, discrete black nucleated colonies with green metallic sheen are E. coli. Single colonies are re-inoculated into the Brilliant Green Bile Lactose Broth, while bile inhibits the growth of gram-positive microbes; brilliant green bars the propagation of certain gram-negative bacilli allowing the growth of lactose-fermenting coliforms (Mishra *et al*., 2012, 2013). To test for thermo-tolerant coliform modified Eijkman test has been proposed by the WHO based on the principle that only fecal coliforms are able to ferment lactose at 44.5◦C, hence MacConkey broth with inverted durham's tubes are inoculated with measured amounts of water samples and are incubated at 44.5◦C for the occurrence of acid and/or gas (WHO, 2011).

An efficient sensor based presence/absence method has also been established for confirming the presence of *E. coli*. The chromogenic substrate β-galactosidase enzyme assay detects the color change through a live webcam with Wi-Fi connected sensor node, in the water sample when mixed with X-gal containing reagent (Kim and Myung, 2015). Biosensors have also been developed to check the presence of waterborne pathogens in food (Thakur and Ragavan, 2013).

Although several other methods such as ELISA and PCR are also existent, however, they are laboratory based methods which necessitate insolation, pure culture of the organism, also require expert personnel, expensive instruments, multiple steps and do not offer real-time data (Yoon and Kim, 2012). With relatively fast generation times but inability to survive longer in limited water samples the duration from field-to-lab massively affects the coliform numbers often leading to inaccurate quantification. Paper based microfluidic diagnostic devices though relatively inexpensive are largely single test based (Yetisen *et al*., 2013). Nonetheless, when it comes to large water bodies being polluted mere detection of coliform presence is not enough, it needs complete quantitation of the degree of pollution for remedial steps to be devised; hence inexpensive traditional counting methods which give real-time numbers are preferred. So far only one study has professed the use of sensor for detecting and enumerating E. coli in water (Hesari *et al*., 2016).

## 2.6 EFFECTS OF COLIFORM BACTERIA ON HUMAN HEALTH

The presence of coliform bacteria in river water poses significant health risks to humans, primarily due to the potential presence of pathogenic microorganisms. Pathogens such as *Salmonella, Shigella, Vibrio cholerae*, and various enteric viruses are often associated with fecal contamination and can cause a range of waterborne diseases. The ingestion of contaminated

water, either directly through drinking or indirectly through recreational activities, can lead to gastrointestinal illnesses, skin infections, and other health issues (Craun *et al*., 2006; Gil *et al*., 2018).

Gastrointestinal illnesses, such as diarrhea, dysentery, and gastroenteritis, are the most common health effects associated with coliform contamination. These illnesses are typically caused by pathogenic strains of *E. coli, Salmonella*, and *Shigella*, which infect the intestinal tract and cause symptoms such as abdominal pain, fever, and vomiting. In severe cases, particularly in vulnerable populations such as children, the elderly, and immunocompromised individuals, these infections can lead to dehydration, kidney failure, and even death (Ashbolt, 2004; Aiken *et al*., 2020).

Exposure to coliform-contaminated water can also cause skin, ear, eye, and respiratory infections. These infections are often contracted through recreational activities such as swimming, fishing, or boating in contaminated rivers. Pathogens such as *Pseudomonas aeruginosa*, which thrive in fecal-contaminated water, can cause ear and skin infections, particularly in individuals with open wounds or compromised immune systems (Fleisher *et al*., 1996; Cimenti *et al*., 2022).

## 2.7 EFFECTS OF COLIFORM BACTERIA ON ANIMAL HEALTH

The impact of coliform bacteria on animal health is profound, particularly in aquatic ecosystems where water quality directly affects the survival and health of aquatic organisms. Fish, amphibians, and other aquatic animals can be exposed to coliform bacteria and associated pathogens through ingestion, skin contact, or respiratory uptake of contaminated water. High levels of fecal coliforms in water can lead to outbreaks of fish diseases, such as furunculosis and

columnaris, which can result in significant fish kills and disrupt local aquatic ecosystems (Austin and Austin, 2012; da Silva *et al*., 2021).

Livestock that rely on river water for drinking are also at risk. Cattle, for example, can suffer from diarrhea, mastitis, and reproductive disorders due to exposure to fecal coliforms, particularly pathogenic strains like *E. coli O157*. These infections can lead to reduced milk production, weight loss, and, in severe cases, death. Moreover, wildlife, including birds and mammals that come into contact with contaminated water, can become carriers of coliform bacteria, further perpetuating the cycle of contamination (Geldreich, 1996; Oliveira *et al*., 2023).

## 2.8 Key Physicochemical Properties of Rivers

### 2.8.1 Temperature

Water temperature is a critical physicochemical property of rivers that influences the metabolic rates of aquatic organisms, dissolved oxygen levels, and the overall chemical composition of the water (Caissie, 2006). Temperature affects the solubility of gases; warmer water holds less oxygen, which can limit the availability of this vital gas to aquatic life (Dell *et al*., 2014). Seasonal variations, geographic location, altitude, and the presence of thermal discharges from industrial sources can all contribute to temperature changes in river systems (Webb *et al*., 2008).

Temperature also plays a crucial role in defining the distribution and abundance of aquatic species. For instance, many fish species, such as salmonids, require specific temperature ranges for spawning and growth. In contrast, temperature changes can disrupt these processes, leading to shifts in population dynamics and community structures (Elliott, 2010). Moreover, elevated temperatures can exacerbate the effects of other pollutants, such as ammonia and heavy metals, by increasing their toxicity to aquatic organisms (Cairns *et al*., 2019).

2.8.2 pH

The pH of river water, which measures its acidity or alkalinity, is another vital physicochemical property that affects the solubility and availability of nutrients and toxins (Davies-Colley *et al*., 2000). The pH scale ranges from 0 to 14, with values below 7 indicating acidic conditions and values above 7 indicating alkaline conditions. Most freshwater organisms thrive within a relatively narrow pH range (typically 6.5 to 8.5); deviations outside this range can stress or kill sensitive species (Wetzel, 2001).

Acidification of river water, often due to acid rain or industrial discharges, can lead to the leaching of toxic metals like aluminum into the water, posing a severe threat to aquatic life (Driscoll *et al*., 2001). Conversely, highly alkaline conditions can affect the availability of essential nutrients, such as phosphorus, reducing their uptake by aquatic plants and affecting primary productivity (Jackson *et al*., 2014).

**2.8.3 Dissolved Oxygen**

Dissolved oxygen (DO) is a critical parameter that determines the health and sustainability of river ecosystems. Oxygen enters river water through diffusion from the atmosphere, aeration as water flows over rocks and vegetation, and photosynthesis by aquatic plants and algae (Cole *et al*., 2014). Adequate DO levels are crucial for the respiration of aquatic organisms, including fish, invertebrates, and microorganisms.

Low DO levels, often caused by organic pollution, eutrophication, or thermal discharges, can lead to hypoxic conditions that threaten aquatic life (Diaz and Rosenberg, 2008). Hypoxia can cause massive die-offs of fish and other species, disrupt food webs, and lead to biodiversity loss. Additionally, low oxygen levels can alter the cycling of nutrients and the breakdown of organic matter, affecting the overall productivity and functioning of the river ecosystem (Rabalais *et al*., 2010).

### 2.8.4 Turbidity and Suspended Solids

Turbidity, a measure of the cloudiness or haziness of water, is primarily caused by suspended solids such as silt, clay, organic matter, and microscopic organisms (Bilotta and Brazier, 2008). High turbidity levels can reduce light penetration, affecting photosynthesis in aquatic plants and algae and thereby reducing primary productivity (Davies-Colley and Smith, 2001).

Furthermore, high turbidity can clog fish gills, reduce feeding efficiency, and smother benthic habitats, impacting the survival and reproduction of aquatic organisms (Henley *et al*., 2000). Suspended solids can also adsorb and transport pollutants, such as heavy metals and pesticides, posing additional risks to aquatic life and human health (Pandey *et al*., 2022).

### 2.8.5 Conductivity and Salinity

Conductivity is a measure of water's ability to conduct electricity, which is directly related to the concentration of dissolved ions, such as sodium, chloride, calcium, and magnesium (Sánchez-Rodríguez *et al*., 2021). While some ions are essential for aquatic organisms, elevated conductivity levels can indicate pollution from sources such as agricultural runoff, wastewater discharge, or industrial effluents (Cañedo-Argüelles *et al*., 2013).

High salinity levels can create osmotic stress for freshwater organisms, leading to physiological impairment or death (Herbert *et al*., 2015). For example, freshwater fish may struggle to regulate their internal salt balance in high-conductivity environments, affecting their growth, reproduction, and survival (Griffith, 2017).

### 2.8.6 Nutrient Concentrations

Nutrients, particularly nitrogen and phosphorus, are essential for aquatic plant growth but can become pollutants at high concentrations (Schindler *et al*., 2016). Elevated nutrient levels, often resulting from agricultural runoff, wastewater discharge, and urban stormwater, can lead to eutrophication, characterized by excessive algal blooms, reduced DO levels, and the degradation of water quality (Smith and Schindler, 2009).

Eutrophication can cause significant ecological disruptions, including fish kills, loss of biodiversity, and shifts in community composition (Paerl *et al*., 2018). Moreover, some algal blooms produce toxins harmful to both aquatic organisms and humans, posing additional public health risks (O'Neil *et al*., 2012).

## 2.9 Natural and Anthropogenic Influences on Physicochemical Properties

The physicochemical properties of rivers are shaped by a combination of natural processes and human activities. Natural factors such as geology, climate, and vegetation influence the baseline conditions of river systems. For instance, rivers flowing through limestone regions often have higher pH levels due to the buffering capacity of carbonate rocks, while those in areas with high organic content may have lower DO levels due to microbial respiration (Meybeck, 2003).

Human activities, including agriculture, urbanization, deforestation, and industrialization, significantly alter the physicochemical properties of rivers. Agricultural runoff contributes to nutrient enrichment and increased turbidity, while urbanization and industrial activities introduce various pollutants, including heavy metals, organic contaminants, and thermal discharges (Meybeck and Helmer, 1989). Additionally, dam construction and water extraction can alter flow regimes, temperature, and sediment transport, further impacting river ecosystems (Nilsson *et al*., 2005; Grill *et al*., 2019).

### 2.10. Effects of Physicochemical Properties on Aquatic Life

The physicochemical properties of rivers play a pivotal role in determining the health and diversity of aquatic ecosystems. Aquatic organisms are highly sensitive to changes in their environment, and even small alterations in temperature, pH, DO, or nutrient levels can have significant impacts on species composition, abundance, and ecosystem functioning (Dudgeon *et al*., 2006).

For example, fish species such as trout and salmon are particularly sensitive to temperature and DO changes; they require cool, well-oxygenated waters for spawning and growth. Increased temperatures or reduced DO levels can lead to shifts in species distributions, reduce reproductive success, and increase mortality rates (Isaak *et al*., 2015). Similarly, changes in pH can affect the availability of nutrients and toxins, influencing primary production and the survival of sensitive species (Evans *et al*., 2016).

### 2.11 Effects of Physicochemical Properties on Human Health

Physicochemical properties also have direct and indirect impacts on human health. Rivers with high turbidity or elevated nutrient levels may be unsuitable for drinking, recreation, or irrigation, requiring costly treatment to ensure safety (Flörke *et al*., 2018). Contaminated water can carry pathogens, toxic substances, or harmful algal blooms, posing significant health risks, including gastrointestinal diseases, skin infections, and even cancer (Richardson *et al*., 2007; Kihara *et al*., 2021).

Moreover, changes in river conditions due to climate change and human activities can exacerbate the spread of waterborne diseases and the proliferation of disease vectors, such as mosquitoes, affecting public health outcomes in many regions (Semenza and Paz, 2021). Rivers are essential freshwater resources that support a wide range of ecological functions and provide vital services to human societies, including water for drinking, irrigation, industry, recreation, and other uses. However, the physicochemical properties of river water such as temperature, pH, dissolved oxygen, turbidity, electrical conductivity, and the concentrations of various nutrients and contaminants are critical determinants of water quality and human health (Flörke *et al*., 2018; van Vliet *et al*., 2021). The quality of river water directly impacts human health, as alterations in these physicochemical properties can lead to the proliferation of waterborne pathogens, the accumulation of toxic chemicals, and other adverse health effects.

The physicochemical properties of river water are influenced by a complex interplay of natural processes and anthropogenic activities. Natural factors such as geology, climate, and hydrology shape the baseline conditions of river water, while human activities including urbanization, industrialization, agricultural practices, and waste disposal introduce various pollutants and stressors that alter these properties (Vörösmarty *et al*., 2010; Damania *et al*., 2019).

Physicochemical properties of river water encompass a wide range of characteristics that define its quality and suitability for various uses. Key properties include temperature, pH, dissolved oxygen (DO), turbidity, electrical conductivity, and the concentrations of nutrients and pollutants. These properties are interrelated and can influence each other, thereby affecting water quality and human health in multiple ways (Davies-Colley and Smith, 2001; Allan and Castillo, 2007).

Temperature is a fundamental physical property of river water that affects its chemical characteristics and biological processes. Water temperature influences the solubility of gases (such as oxygen), the rate of chemical reactions, and the metabolic rates of aquatic organisms (Caissie, 2006; Webb *et al*., 2008). Elevated temperatures, often resulting from industrial discharges, urban runoff, or climate change, can reduce dissolved oxygen levels, promote the growth of harmful algal blooms, and increase the proliferation of pathogenic microorganisms (Dell *et al*., 2014; O'Neil *et al*., 2012). High water temperatures can also enhance the toxicity of certain pollutants, such as heavy metals and ammonia, thereby increasing their harmful effects on human health (Cairns *et al*., 2019). For instance, warm temperatures can promote the conversion of less toxic forms of ammonia to more toxic forms, exacerbating the risk of poisoning for humans who come into contact with or consume contaminated water (Glibert *et al*., 2014). Additionally, increased temperatures can facilitate the growth and spread of vector-borne diseases, such as those transmitted by mosquitoes, which thrive in warmer water conditions (Semenza and Paz, 2021). The pH of river water, which measures its acidity or alkalinity, plays a crucial role in determining the solubility and availability of nutrients and contaminants. Most aquatic organisms, including those that directly affect human health (such as bacteria and algae), have specific pH ranges in which they thrive (Wetzel, 2001; Jackson *et al*., 2014). Deviations from these ranges can significantly impact water quality and health risks. Acidic conditions (low

pH) can increase the solubility of toxic metals like lead, mercury, and aluminum, which can then enter the human body through drinking water, leading to various health issues, including neurological and developmental disorders (Driscoll *et al*., 2001; Reimann and de Caritat, 2019). Alkaline conditions (high pH) can also affect human health by altering the bioavailability of nutrients and potentially increasing the concentrations of certain pollutants (Evans *et al*., 2016).

Dissolved oxygen (DO) is a critical parameter for the health of aquatic ecosystems and is also essential for maintaining water quality suitable for human use. Low DO levels, often resulting from organic pollution, nutrient enrichment, and thermal pollution, can create hypoxic conditions that are detrimental to aquatic life (Diaz and Rosenberg, 2008; Rabalais *et al*., 2010). These conditions can promote the growth of anaerobic bacteria that produce harmful substances such as hydrogen sulfide and methane, which can cause adverse health effects in humans (Bricker *et al*., 2020). Hypoxia in river water can also promote the growth of pathogenic bacteria, such as Vibrio cholerae, which can cause severe waterborne diseases like cholera (Colwell, 2019). Moreover, low DO levels can impair the natural purification processes of rivers, leading to the accumulation of organic matter and other contaminants that pose risks to human health (Flörke *et al*., 2018).

High water temperatures can promote the growth of pathogenic microorganisms, including bacteria, viruses, and protozoa, that cause waterborne diseases (Lipp *et al*., 2002; Semenza and Paz, 2021). Elevated temperatures can also exacerbate the effects of other pollutants, such as heavy metals and organic chemicals, by increasing their toxicity or enhancing their bioavailability (Cairns *et al*., 2019; Peng *et al*., 2021). For instance, warmer water temperatures have been linked to increased incidences of cholera and other diarrheal diseases, particularly in developing countries where water treatment infrastructure may be inadequate (Colwell, 2019;

Reiner *et al*., 2018). Additionally, higher temperatures can lead to increased mosquito breeding and the spread of vector-borne diseases like malaria and dengue fever (Semenza and Suk, 2018). Extreme pH levels can cause skin irritation, gastrointestinal problems, and respiratory issues when humans are exposed to water with abnormal pH levels (Evans *et al*., 2016). Acidic water can leach metals such as lead, mercury, and aluminum from natural deposits or plumbing systems, leading to increased concentrations in drinking water and subsequent health problems (Reimann and de Caritat, 2019; Spangler *et al*., 2021). Chronic exposure to heavy metals can cause neurological disorders, kidney damage, and developmental problems in children (ATSDR, 2019). Low DO levels can create conditions conducive to the growth of anaerobic bacteria, such as Clostridium species, which can produce toxins harmful to humans (Bricker *et al*., 2020). Hypoxic conditions can also promote the proliferation of harmful algal blooms that produce toxins affecting the liver, nervous system, and skin (O'Neil *et al*., 2012; Backer *et al*., 2020). These conditions can lead to acute poisoning or long-term health issues, including cancer (Codd *et al*., 2005; Stewart *et al*., 2006).

## 2.12 Need for Universally Uniform Regulatory Guidelines

Monitoring fecal pollution of waters used for recreational drinking, and industrial purposes is imperative for public health and economic reasons. Pathogens introduced from fecal contamination leads to diseases in humans (typhoid, Salmonellosis, cholera), livestock and economic losses to industries (Ballester and Sunyer, 2000; Bernhard and Field, 2000). Coliform densities are influenced by seasonal changes. While in the tropical climate coliform levels are higher during monsoon, owing to sewage seepage and runoffs, in the comparatively cooler regions they are higher during summer due to ambient growth temperature (Kar *et al*., 2010; Galadima *et al*., 2011; Panigrahi and Patra, 2013).

This geographically inconsistent season based variation in coliform numbers had led to ambiguous standards which don't hold context in every reference. The stringency in the guidelines was finalized owing to global health concern reports. As per the WHO, there should not be any E. coli or thermotolerant coliform detectable in 100 ml of water sample (WHO, 1998, 2011). While coliforms are established as sanitary standards, the startling issue of multi-drug resistance in them is also was on the rise. Coliforms are extremely receptive and altruistic to horizontal gene transfer in nature (Mishra *et al*., 2013; von Wintersdorff *et al*., 2016). Hence, many aberrations to the stipulated traits of coliforms have been reported. Unusual Sucrose fermentation was reported by Palchaudhuri *et al*. (1977) the multi-drug resistant (MDR) strain of *E. coli* also carried genes for resistance against Streptomycin, Ampicillin, Tetracycline, Chloramphenicol, and sulphonamides. Atypical raffinose fermentation linked with Verocytotoxigenic *E. coli* (de Lopez *et al*., 1982; Saif *et al*., 2008) is plasmid mediated with genes for antibiotic resistance (Orskov and Orskov, 1973; Cornelis *et al*., 1978).

Non-fermentation of rhamnose is a decisive factor of VT+*E. coli 0157* (Chapman *et al*., 1991). The biotype (Rha- and Suc-)" is a quintessential marker for the preliminary detection of SLT-I+ *E. coli* in SLTEC associated diarrhea (Wieler *et al*., 1995). The occurrence of certain atypical carbohydrate fermentation genes is largely coupled with multi-drug resistance. These genes are transferred both intra and inter-specifically through horizontal gene transfer mechanism bestowing multidrug resistance to the isolates which changes their biochemical characteristics. Numerous investigations employ biochemical fingerprinting for identifying coliform (Gililland and Vaughn, 1943; Kühn *et al*., 1991; Guentzel, 1996; Betsy and Keogh, 2005). While results obtained by most of the biochemical tests are qualitative and used mostly for the identification of the bacterial isolate into genus and species but carbohydrate fermentation, antimicrobial

susceptibility, genotypic profiling characterize the bacterial isolate down to the strain level. When coupled together these analyses well substantiate the identification of an individual strain with detailed characterization of its standalone traits. Carson *et al*. (2001) have used 16S rDNA analysis to discriminate fecal E. coli of human source from collective fecal E. coli isolates of non-human origins and have obtained accuracy up to three host sources. 16S rDNA analysis has been used to differentiate isolates within the same species based on taxonomic classification (Laurent *et al*., 1996), epidemiological tracking (Bernhard and Field, 2000; Zhao *et al*., 2005), geographical distribution (Akhtar *et al*., 2000), population biology and phylogeny (Paradis *et al*., 2005; Xia *et al*., 2013).

## 2.13 ANTIBIOTIC RESISTANCE IN GUT COLIFORM

Though tagged as "miracle drugs," antibiotics are increasingly being misused. Their unsystematic use and dumping has led to multi-drug resistance among the indigenous microbiota, in case of a re-infection these antibiotics become futile for therapy. The increasing use of antibiotics in clinical, agricultural and veterinary sectors is analogous with the escalated resistance of bacteria to these frequently used antibiotics (Dhanorkar and Tambekar, 2004; Ahmed *et al*., 2010). This has exaggerated the expenditure of therapy, the peril of spreading MDR, mortality from diseases through the MDR strains (Martínez and Baquero, 2002; White, 2006; Hawkey and Jones, 2009; Kolar *et al*., 2010). Antibiotic resistance has been established to be broadcasted in the gut of the warm-blooded animals (Chopra and Roberts, 2001; Launay *et al*., 2004; Fukao and Yajima, 2012).

Studies by Larsson and Fick (2009) and Laxminarayan and Heymann (2012) have reported seepage of about 45 kg of Ciprofloxacin quotidian from factories into the nearby water bodies. Spread of environmental pollution has led to the unearthing of antibioticresistant bacteria in

uninhabited lands like Antarctica (Sjölund *et al*., 2008; Hernández *et al*., 2012). Prolongation of disease either due to scarcity of antibiotics or superfluous use of antibiotics in circumstances where their use is not obligatory is common. Antibiotics usage has seen a precipitous boost over the past few years. Sale of cephalosporins amplified by 60% over 2005– 2009, sales of other last line antibiotics escalated six-fold from 2005 to 2010 (Ganguly *et al*., 2011; Westly, 2012). Drug resistant coliform are the most commonly infecting bacteria, infection is contracted by exposure to unhygienic food, water, to the bacteria or its carriers and unwise use of antibiotics. Hannah *et al*. (2005) have investigated the association of drug resistant E. coli infections with epidemiological factors: demographic variables, diet, healthcare and antibiotic usage and have found positive correlation between infection with MDR E. coli and contact with infected persons, contaminated food, ambulatory visit, antimicrobial misuse, prolonged medication and frequent meat consumption with self-prescription of drugs. Over-consumption of antibiotics kills the beneficial bacteria in our system enhancing the growth of pathogens which may be insensitive to the antibiotics used thus eventually becoming resistant. Consuming antibiotics lesser than the prescribed amount does not kill the pathogen rather confers resistance upon it. In both the cases treating the MDR bacteria becomes arduous with often, fatal consequences. Antimicrobial susceptibility with the production of Extended Spectrum Beta-Lactamases (ESBL) among the coliform isolates causes concern as they render therapy with beta-lactum antibiotics inefficient (Pitout and Laupland, 2008; Peirano and Pitout, 2010; Rogers *et al*., 2011; Shibl *et al*., 2012).

Many investigations have stressed on the concern of multidrug resistant coliforms in water (Manji *et al*., 2012; Mishra *et al*., 2013; Al-Agamy *et al*., 2014; El-Zanfaly, 2015; Madec *et al*., 2016; Azzam *et al*., 2017; Jutkina *et al*., 2018). Manji *et al*. (2012) have emphasized concern over the prevalence of multi-drug resistant coliforms and *Staphylococcus aureus*, in rural water

supplies of Nigeria. Over the period of several months their analysis involved bacteria counting, antibiogram and MIC analysis. The presence of pathogens as *S. aureus, Bacillus* sp., *Pseudomonas aeruginosa*, with umpteen antibiotics resistant *E. coli* strains warrant immediate treatment as they pose momentous public health repercussions. Mishra *et al*. (2013) have worked on the prevalence of antibiotic resistant *E. coli* in the River Mahanadi. The water there unintentionally experiences influx of miscellaneous composition. They have employed MPN, Modified Eijkman test, biochemical fingerprinting, antimicrobial susceptibility testing and 16S rDNA ribotyping methods to isolate, enumerate and identify the coliform isolates to be E. coli with established resistance to betalactum antibiotics, carboxypenicillin coupled with β-lactamase inhibitor, glycopeptides, carbapenems, macrolides, till fourth generation of fluoroquinolones cephalosporins. They have also reported appalling resistance rates of the isolates against 42 indigenously used antibiotics with MAR indices ranging from 0.51 to 0.90 which indicate severe pollution and risk to the public health. Transfer of antibiotic resistance cannot be ascribed to horizontal gene transfer mechanisms alone rather integrons too play an important role in certain cases. Xia *et al*. (2013) used 16S rDNA analysis to accurately isolate coliforms, harboring class 2 integrons and investigated their molecular architecture.

Al-Agamy *et al*. (2014) have characterized the prevalence of ESBL *Escherichia coli* (ESBL-EC) in Riyadh. They used antimicrobial susceptibility and pulsed field gel electrophoresis (PFGE) to type and investigate the epidemiology of ESBL-EC and the prevalence of ST131 in them. They have also expressed concern over the dearth of local reports on the prevalence of ESBL-EC despite its universal predominance. Such state of affairs demands the routine and vigilant inspection of the environment. El-Zanfaly (2015), apprehends that resistance to antibiotics may actually escalate during the sewage treatment processes his study has employed enumeration,

antimicrobial susceptibility, granular activated carbon test and transferability of antibiotic resistant character tests to characterize ampicillin, sulfaguanidine penicillin, 2-sulfanilamide pyrimidine, tetracycline, chloramphenicol, neomycin and streptomycin resistant coliforms. Activated carbon application in pilot water treatment plant experiment showed their prevalence with easy transferability of resistance. The work insists on including antibiotic resistance in coliforms as a deciding criterion for judging water quality standards. Madec *et al*. (2016) have stressed on the implication of antimicrobial resistance in drinking water as a potent risk indicator for humans in low-income nations. A particular *E. coli* isolate ST48, procured from drinking water in France, resistant to ceftiofur, tetracyclines and sulfonamides was found to harbor the blaCTX-M-1 IncI1/ST3 plasmid. The plasmid is analogous to the other blaCTX-M-1 IncI1/ST3 plasmids that have been reported in other animals and humans. Based upon their findings they suggest the possibility of human transfer of ESBL genes through drinking water in developing and under-developed nations. Jutkina *et al*. (2018) conducted a series of conjugation and MIC assays of both donor and recipient strains to investigate the latent of cefotaxime, ciprofloxacin, gentamicin, erythromycin, sulfamethoxazole, trimethoprim, and chlorhexidine digluconate, hexadecyl trimethyl ammonium chloride and triclosan to stimulate horizontal gene transfer of antibiotic resistance genes.

## 2.14 Mitigation Strategies for Coliform Contamination

Mitigating coliform contamination in rivers requires a multifaceted approach that includes improving wastewater treatment, managing agricultural practices, and implementing effective stormwater management strategies. Upgrading sewage treatment plants to include tertiary treatment processes, such as disinfection, can significantly reduce the levels of coliform bacteria discharged into rivers. In agricultural settings, best management practices such as buffer strips,

controlled grazing, and proper manure management can help reduce runoff and limit the entry of fecal coliforms into water bodies (Mallin *et al*., 2000; Yates *et al*., 2023).

Additionally, public education and awareness campaigns are essential to promote safe hygiene practices, proper waste disposal, and the protection of water resources. Governments and local authorities must also enforce regulations and provide adequate infrastructure to prevent contamination and protect public health (Dufour *et al*., 2012; Parnell *et al*., 2023).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 STUDY AREA AND SAMPLE COLLECTION

Samples was collected from three (3) locations (down, middle and upstream) of Ikpoba River in Benin City metropolis and was transported aseptically to the microbiology laboratory of University of Benin for microbial evaluation and assessment. This study was conducted in Benin City, Edo State, Nigeria.

## 3.2 Sterilization of materials

All culture media was prepared according to the manufacturer's instructions. Sterilization will be at 121ºC at 15psi for 15 min unless otherwise stated by manufacturer.

## 3.3 Preparation of culture media

Media for microbiological analysis was prepared according to the manufacturer's specifications. The media used were MacConkay agar and Nutrient agar.

### 3.3.1 Nutrient agar

Twenty-eight grams (28 g) of nutrient agar was dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium was placed in an autoclave to sterilize it for 15 min at 121 °C. After sterilization, the flask was allowed to cool.

### 3.2.2 MacConkay agar

52g of MacConkay agar was dissolved in 1000ml of distilled water and stir to completely dissolve all component and autoclave for 15 min at 15 psi (121$^{o}$C). It was allowed to cooled at 60$^{o}$C and poured into sterile Petri dishes.

## 3.3 PHYSICOCHEMICAL ANALYSIS

### 3.3.1 **Determination of pH**

The pH of the water sample was determined using a digital Denver pH meter model 20 with glass electrode. This was done according to standard method (APHA, 2008). The pH is a measure of the hydrogen ion concentration or activity of an aqueous solution and every aqueous solution can be measured to determine its pH value. This value ranges from pH 0-14 with values below pH 7 exhibiting acidic properties and values above pH 7 exhibiting basic or alkaline properties. pH 7 is the centre of the measurement scale; it is neither acidic nor basic. The pH scale is traceable to a set of standard solutions whose pH is established by international agreements. pH standard values were determined using a concentration cell with transference by measuring the potential difference between a measuring electrode and a reference electrode such as the silver chloride electrode, hence measurement of pH for aqueous solutions were done with a glass electrode and a pH metre using indicators [water testing kit]. (Roselló-Soto *et al*., 2019)

### 3.3.2 **Determination of Temperature**

The temperature was determined with a centigrade thermometer capable of reading from 0$^{oC}$ to 110$^{o}$C. The thermometer was dipped into the diluted samples and the reading taken after equilibrium (Asante, 2013).

### 3.3.3 **Determination of Suspended Solid**

This was determined by the use of a 100-mL pipette and a single 47-mm circle of Wathman 934-AH filter paper (1.5-lmcutoff) for sample collection and separation of suspended solids, respectively. The following three types of pipettes were used to examine the effect of pipette type on the TSS measurement: (i) An original or unmodified pipette, (ii) A pipette that had been cut off in the middle of the tip contraction and fire polished, and (iii) An open pipette that had been cut off just above the tip contraction (Roselló-Soto *et al*., 2019).

### 3.3.4 **Determination of Alkalinity Content of the Samples**

This was determined using argentiometric titration according to BP, 1993. To make 500 mL of a 0.01 M solution, 1.0 M AgNO3 was measured. Deionized water was placed in an amber 500 mL bottle. It was mixed and the solution was read for the result. (Roselló-Soto *et al*., 2019)

### 3.3.5 **Determination of Total Hardness**

This was determined using complexometric titration. The burette was filled with the usual EDTA solution to the zero level. To check for excessive calcium content, 50 ml of sample water was placed in a flask. Subsequently, smaller quantities were taken and diluted to 50 ml. 5-6 drops of the Ericrome black-T indicator and 1 ml of ammonia buffer were also added. The solution now has a wine-red hue. It was recorded the initial reading. The substance was tested in comparison to an EDTA solution. The final point is where the color changes. Blue replaces wine red as the color value. To reach the final volume, distilled water was added after boiling 50 ml of the sample in another flask (Roselló-Soto *et al*., 2019)

### 3.3.6 **Electrical conductivity**

The electrical conductivity of each sample was determined using a portable conductivity meter. Fifty milliliter of the sample was collected with a beaker, the plastic electrode probe was inserted

into the sample, and the result in microsiemens (µs/cm-1) read from the screen. The meter was calibrated using distilled water after each measurement according to the American Public Health Association (APHA, 2018).

### 3.3.7 **Turbidity**

The turbidity of the respective water samples were be determined using spectrophotometric method. Five milliliter of the sample was dispensed into a curvette and placed in the light chamber and the absorbance was measured at a specific wavelength (450 nm) using distilled water as blank. The turbidity values were recorded in nepholometric turbidity unit (NTU) (American Public Health Association, 2003).

### 3.3.8 **Phosphate**

Ten (10) ml of the sample was dispensed onto a clean curvette. Four milliliter of phosphate reagent containing ammonium molybdate, antimony potassium tartate and ascorbic acid was also added to the curvette containing the water sample. One milliliters of 95 % ethanol and 1 ml of concentrated $H_2SO_4$ was then added. It was shaken, and then left for 5 min to allow for color development. The absorbance was determined at a specific wavelength (560 nm) using a spectrophotometer (Asante, 2013).

### 3.3.9 **Nitrate**

Ten (10) ml of the sample was placed in a test tube, followed by the addition of 2 ml NaCl solution, this mixture was swirled and 10 ml of $H_2SO_4$ solution was also added. The resultant solution was swirled and allowed to stand. A sample blank was also prepared. To the first test tube containing the mixture of the sample, NaCl and $H_2SO_4$, 0.5 ml of brucine –sulphanilic acid reagent was added and the test tube was swirled and left to stand for about 20 minutes. The test

tubes were allowed to develop color and the absorbance reading of the solution was taken using a spectrophotometer at a specified wavelength (450 nm) (Asante, 2013).

3.3.10 **Sulphate**

Ten (10) ml of water was decanted onto a clean curvette. One milliliter ml of 95 % isopropyl alcohol, 0.5 ml of glycerol and 5 ml of conditioning reagent, which consist of NaCl, BaCl and Citric acid, were added to the curvette containing the sample. The solution was left to stand for 5 min to allow colour development, after which the absorbance was read at a specific wavelength (540 nm) using a spectrophotometer (American Pharmacists Association, 2003).

3.3.11. Determination of Chromium

10ml of water was added to buffer. Phenanthroline was added and mixed. The mixture was transferred to cuvette where the wavelength was measured using spectrophotometer (Roselló-Soto *et al*., 2019).

3.3.12 **Calcium**

EDTA was dissolved in deionized water, a drop of buffer was added and the pH adjusted to 10, and the solution was titrated as the as the calcium ion started reacting with EDTA forming colour change. The resulting positive result was a change of colour from wine red to blue or purple (Asante, 2013).

3.3.13 **Determination of magnesium**

50ml of water was added to Erlenmeyer flask, few drops of magnesium indicator were added and a small amount of buffer was added to the flask. The mixture was thoroughly mixed and titrated. The process was repeated to obtain duplicates (Asante, 2013).

3.3.14 **Determination of chlorine**

Two drops of potassium iodide was added to 50ml of water, the mixture was diluted with sulphuric acid. The flask was thoroughly swirl to mix. 2 drops of starch indicator was added and titrated by adding sodium thiosuphate in drops. This was done till the colour changed from blue black.

3.3.15 **Determination of Fe**

10ml of water was added to buffer. Phenanthroline was added and mixed. The mixture was transferred to cuvette where the wavelength was measured using spectrophotometer (Roselló-Soto *et al*., 2019).

3.7 **Enumeration and isolation of total heterotrophic bacterial count**

Appropriate media was used for fungal and bacterial enumeration. Tryptone soy agar (supplemented with fluconazole) for bacteria Plates were cultured at 28±2$^0$C for 24 hours. The number of colony forming unit per milliliter (cfu/ml) was calculated using the formula below:

$$\frac{cfu}{ml} = \frac{number\ of\ colonies\ x\ dilution\ fold/series}{volume\ of\ inoculum}$$

(Willey *et al.,* 2008)

**3.8 Phenotypic Identification of Bacteria from Samples**

Following successful pour plate technique, isolation and culture was made from a single colony and characterized using cultural, morphological and biochemical methods using the Bergey's manual. Several tests such as Gram reaction, catalase, urease, indole, oxidase, sugar fermentation, citrate utilization, respective reaction on triple sugar iron agar tests were carried out to presumptively identify bacterial isolates (Holt *et al*., 1994).

### 3.9 Morphology identification

The morphological identity of each bacteria isolate was obtained by Gram staining so as to know the gram reaction, cell morphology and arrangement by viewing under the microscope. The Gram stain procedure is as follows:

A smear of the bacteria isolate was made on grease free slide and heat fix by passing over flame. The smear was flooded with crystal violet which is the primary stain for 1min then washed with distilled water.

Subsequently the slides was flooded with Lugol's iodine solution for 30sec and then washed off with distilled water.

95% alcohol was used for decolorization for 10sec and immediately washed off with distilled water.

Finally, the smear was counter stained with saffranin for 1min and washed off.

The slides were allowed to air dry before observing under the microscope using an oil immersion objective lens of ×100 magnifications to view the slides.

### 3.10 Biochemical identification

Biochemical test was carried out so as to help in the identification of the bacteria isolates as phenotypic (cultural) characteristics is not sufficient. The various biochemical test carried out are shown below;

### 3.10.1 Oxidase test

This is mainly used to differentiate between *pseudomonas* from other gram-negative rod bacteria. Oxidase test was carried out to identify bacteria species that will produce cytochtome oxidase enzyme. *Staphylococcus aureus* and *Escherichia coli* which are gram positive and gram negative respectively were employed as control. A piece of filter paper using sterilizes wire loop 2-3 drops of freshly prepared oxidase reagent (1% aqueous tetramethyl-3-phenyl nediamine dichloride) was added. A positive oxidase test is indicated by purple colouration within 10 seconds.

### 3.10.2 Urease test.

This is used to test organisms that have the abilities to produce the enzyme urease which catalyzes the breakdown of urea to produce ammonia. The test is usually used to differentiate organisms like *Proteus mirabilis* from other non-urease positive organism. A sterilized medium was dispensed into test tubes aseptically and the test bacteria isolated were inoculated into the medium and incubated at 37 degree centigrade for 24 hours. A change in colour from yellow to red-pink confirmed the presence of urease.

### 3.10.3 Indole production test

This test was used to determine which of the isolates has the ability to split indole from tryptophan present in peptone water. The best is usually used in differentiating gram-negative bacilli especially those of enterobacteriaceae. Five grams of commercially available peptone broth was dissolved in 1litre of distilled water. The medium was then sterilized by autoclaving at 121 degree centigrade for 15 minutes. The 4 ml of the medium was dispensed into sterile test tube and each of the bacteria isolates was inoculated into the peptone broth. The inoculated

media was incubated 37 degree centigrade for 24 hours after which few drops of KOVAC reagent will be added. KOVAC reagents consist of 150ml of amylalcohol, 10g dimethylamino benzaldehyde and 150ml of concentrated hydrochloric acid. Positive test was indicated by the red colouration that occurs immediately at the upper part of the test tube.

### 3.10.4 Citrate utilization test

This test is used to identify which of the isolate can utilize citrate as the sole source of carbon for metabolism. The medium used for this test is simon`s citrate agar. In the preparation, 22 grams of commercially available simon`s citrate agar was dissolved in litre of distilled water and sterilized by autoclaving at 121 degree centigrade for 15 minutes. The medium is dispensed into test tubes and the test organism was inoculated by stabling the medium on the tubes using sterile straight inoculation wire containing culture. The tubes were incubated at 37 degree centigrade for about 24 hours. Positive result is indicated by a change in colour from green to bright blue colouration.

### 3.10.5 Catalase test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdowns of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

### 3.10.6 Sugar fermentation and production of gases using Triple sugar iron agar (TSI)

TSI was prepared following manufacturer’s instruction and the prepared media was placed in a test tube and kept in a slant position for it to solidify. The slant and butt of the medium was

inoculated with the test bacterium using a sterile loop and it was incubated for 18- 24 hr. The results were read on the basis of acid or alkaline production in the slant or butt region of the tube and gas production was confirmed by the presentence of crack or air bubbles in the slant or but region. More so, production of hydrogen sulphide was confirmed by the blackening of the medium. A prepared laboratory chat was used for result interpretation in line with microbiological standard protocol as well as other biochemical tests carried out on the isolates to confirm or ascertain their identity.

### 3.11 Statistical analysis

The data were analysed using the SPSS package version 21.0. All data are mean of three replicates. The mean, range and standard deviation of each parameter was determined.

# CHAPTER FOUR

# RESULTS

Table 1 represents the physicochemical anlysis result of the Ikpoba river water samples

Table 2 represents the total coliform bacteria count obtained from the Ikpoba river water samples in Benin city, Edo state.

Table 3 represents the cultural, morphological and biochemical characteristics of the bacteria isolates obtained in this study.

Table 1: physicochemical parameters of water sample

| Sample | River water | WHO |
|---|---|---|
| pH | 5.0 | 6.5-8.5 |
| Electronic Conductivity | 62 | 1500 |
| Organic Carbon | 0.28 | - |
| Organic Matter | 0.48 | - |
| Total Phosphorus | 6.37 | - |
| Total Nitrogen | 4.74 | 50mg/l |
| Calcium | 0.08 | - |
| Magnessium | 0.72 | 10-50mg/l |
| potassium | 38 | >10mg/l |
| Sodium | 0.04 | >200mg/l |
| Exchangeable Acidity | 0.16 | - |
| Iron | 64.74 | >0.3mg/l |
| Zinc | 26.45 | >3mg/l |
| Copper | 8.73 | >2mg/l |
| Lead | 0.39 | >0.01mg/l |
| Cadmium | 0.042 | >0.003mg/l |
| Cromium | 3.95 | >0.05mg/l |
| Manganese | 2.68 | >0.4mg/l |

Table 2: Mean coliform bacteria count (cfu/ml)

| SAMPLE CODE | SAMPLE |
|---|---|
| UP | $4.7 \times 10^4 \pm 2.00$ |
| MIDDLE | $6.0 \times 10^4 \pm 0.00$ |
| DOWN | $7.7 \times 10^4 \pm 1.00$ |

Key: up= upstream

Middle= middle stream

Down= down stream

Table 3: cultural and morphological characteristics of bacterial isolates

| Cultural characteristics | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Colour | Cream | Cream | Golden yellow | Cream | Yellow |
| Shape | Circular | Circular | Circular | Circular | Circular |
| Elevation | Convex | Convex | Convex | Convex | Convex |
| Margin | Entire | Entire | Entire | Entire | Entire |
| Size | Small | Small | Small | Small | Small |
| KOH | + | + | + | + | + |
| Gram stain | - | - | - | - | - |
| Cell morphology | Rod | Rod | Rod | Rod | Rod |
| Cell arrangement | Single | Single | Clusters | Single | Clusters |
| Biochemical characteristics | | | | | |
| Catalase | + | + | + | + | + |
| Coagulase | - | - | + | - | - |
| Indole | + | - | - | - | - |
| Oxidase | - | - | - | - | - |
| Citrate | - | + | + | + | + |
| Urease | - | - | - | - | - |
| $H_2S$ production | - | + | - | - | - |
| Glucose | + | + | + | + | + |
| Lactose | + | + | - | - | + |

| | | | | | |
|---|---|---|---|---|---|
| Sucrose | - | + | + | + | + |
| Mannitol | + | + | + | + | + |
| Identity | *E. coli* | *Citrobacter* sp | *Enterobacter* sp | *Serratia* sp. | *Klebsiella* sp |

Key:

+=positive

-=negative

## CHAPTER FIVE

## DISCUSSION

Coliform bacteria contamination in river water is a significant public health concern, as it often indicates the presence of fecal pollution, which may harbor pathogenic microorganisms. Coliform bacteria, especially *Escherichia coli*, are commonly used as indicator organisms for water quality assessment due to their presence in the intestines of warm-blooded animals and, therefore, in their waste. High coliform counts in river water suggest that the water might be contaminated by untreated sewage, agricultural runoff, or stormwater, each of which can introduce harmful pathogens that pose risks to human health (Edberg *et al*., 2000). This study was aimed at evaluating the physicochemical and coliform contamination of river water. The physicochemical result obtained in this study showed that pH was at 5.0, as against the WHO limit of 6.5-8.5 thus making the water dangerous for consumption by humans and aquatic lives. The electronic conductivity of the water was recorded at 62, against the WHO limit of 1500, this was below the permissible limit. turbidity was at 6.37. This was in agreement with Rabalais *et al*. (2002) in his study of physicochemical evaluation of river water. Copper, zinc, chromium and cadmium result in this study were 8.73, 26.45, 3.95, 0.042 respectively. This was against the WHO recommendation of >2mg/l, >3mg/l, >0.05mg/l and >0.003 respectively for copper, zinc, chromium and cadmium. They were all out of specification. Concentrations of metals and

organic pollutants may still pose risks to aquatic organisms and human health, particularly in sensitive ecosystems or vulnerable populations. Bioaccumulation and biomagnification of contaminants through food webs can lead to chronic toxicity, reproductive impairments, and ;Ldevelopmental abnormalities in aquatic biota. The coliform bacteria count obtained in this study showed that the bacteria count ranged at 4.7 X $10^4$± 2.00 cfu/ml -7.7 X $10^4$± 1.00 cfu/ml for all sections of the river. These figures were in agreement with study conducted by Dwivedi *et al*. (2019) while evaluating microbial population of up and downstream. In this study, downstream had the highest microbial population. Downstream river segments often receive inputs of untreated or partially treated sewage effluent from urban and industrial sources (Pandey *et al*., 2018).

Using the standard microbial method which include cultural, morphological and biochemical characterization, the isolates obtained in this study were *Escherichia coli*, *Citrobacter* sp, *Enterobacter* sp, *Serratia* sp and *Klebsiella* sp.

The presence of coliform bacteria such as *Escherichia coli*, *Citrobacter*, *Enterobacter*, *Serratia*, and *Klebsiella* in river water can have serious health and ecological effects. These coliforms are generally indicators of fecal contamination, which can introduce various pathogens into water systems and create significant health risks for humans and animals. Each of these bacteria can play a unique role in waterborne infections and influence water quality by contributing to the spread of infectious diseases, nutrient imbalances, and potential ecosystem disruptions (Edberg *et al*., 2000). *Escherichia coli* (particularly strains such as *E. coli* O157) can cause severe gastrointestinal illness, characterized by symptoms like diarrhea, abdominal cramps, and, in severe cases, hemolytic uremic syndrome (HUS), which can be life-threatening (Feng *et al*.,

2020). *Klebsiella* species, especially *K. pneumoniae*, can lead to respiratory infections, urinary tract infections, and septicemia, posing significant risks, especially for immunocompromised individuals (Podschun & Ullmann, 1998). Similarly, *Enterobacter* species are associated with infections in hospital settings and are known for their resistance to antibiotics, which complicates treatment options (Sanders & Sanders, 1997).

When coliform bacteria proliferate in river water, they can disrupt the natural balance of aquatic ecosystems. The decomposition processes associated with bacterial growth deplete oxygen levels in the water, leading to hypoxic conditions that harm fish and other aquatic life. *Serratia* and *Citrobacter* species, while less commonly associated with severe human illness, are indicators of organic pollution and can contribute to eutrophication by increasing the nutrient loads in water bodies, which can promote the growth of harmful algal blooms (Pandey *et al*., 2014).

The presence of coliform bacteria, including *Escherichia coli*, *Citrobacter*, *Enterobacter*, *Serratia*, and *Klebsiella*, in river water has significant adverse effects on aquatic ecosystems. These bacteria often indicate fecal contamination, which can introduce not only pathogens but also high organic loads, altering the balance of oxygen and nutrients in water systems. When coliform levels are high, increased microbial activity accelerates oxygen consumption in the water, leading to hypoxic or anoxic conditions that can be detrimental to fish and other aquatic organisms (Pandey *et al*., 2014).

Hypoxia, or low oxygen levels, caused by coliform contamination can lead to "dead zones" in rivers and other water bodies. Fish and other aerobic organisms struggle to survive in such conditions, often resulting in mass die-offs or the displacement of species to areas with better oxygen levels. Additionally, coliform bacteria can introduce pathogens that infect fish and other

aquatic life, leading to diseases and impairments in their immune responses, reproductive systems, and growth rates. For example, studies have found that high levels of fecal coliforms disrupt fish populations by reducing water quality and increasing the spread of waterborne pathogens, which can lead to systemic infections in vulnerable aquatic organisms (Vieira *et al*., 2020).

## CONCLUSION

The presence of coliform bacteria, such as *Escherichia coli*, *Citrobacter*, *Enterobacter*, *Serratia*, and *Klebsiella*, along with heavy metals in river water, poses a multifaceted threat to both human health and aquatic ecosystems. Coliforms serve as indicators of fecal contamination and often accompany harmful pathogens, which can lead to gastrointestinal and systemic infections in humans and animals. Their presence also signals poor water quality, which can create hypoxic conditions that stress aquatic life and disrupt biodiversity. Together, the co-occurrence of coliform bacteria and heavy metals indicates pollution, leading to long-term impacts on river water quality, ecosystem balance, and public health. Effective pollution control measures, continuous monitoring, and remediation are essential to mitigate these effects, protect aquatic ecosystems, and ensure safe water quality for human use.

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