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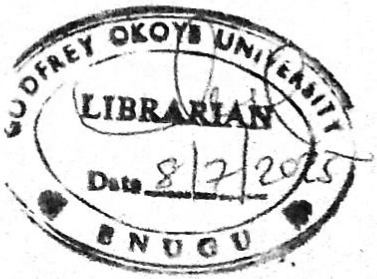

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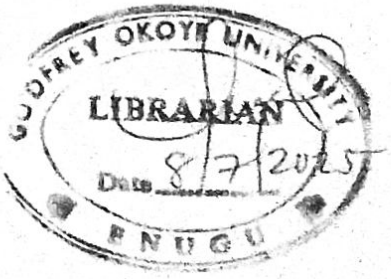

EVALUATION OF THE MICROBIAL LOAD AND PRESENCE OF FACAL CONTAMINATION IN UGWUOMU LAKE GODFREY OKOYE UNIVERSITY ENUGU

BY

METU JEPHTHA CHIMAOBI
U20/NAS/MCB/032

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AUGUST, 2025

TITLE PAGE

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MICROBIOLOGY
DEPARTMENT OF BIOLOGICAL SCIENCES
FACULTY OF NATURAL AND ENVIRONMENTAL STUDIES
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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES IN PARTIAL FULFILMENT TO THE AWARD OF BACHELOR SCIENCE (B.Sc) DEGREE IN MICROBIOLOGY

SUPERVISOR: MRS ANIAGOLU

AUGUST, 2025

APPROVAL

This project has been read and approved by the Department of microbiology, Godfrey Okoye University, Thinker's Corner, Enugu.

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Mrs Aniagolu
(Project supervisor)

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Date

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Dr. Francis N. Olisaka
(Head of Department)

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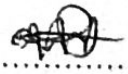
Date

CERTIFICATION

This is to certify that this research titled "Evaluation of the microbial load and presence of facal contamination in Ugwuomu Lake Godfrey Okoye University Enugu" was carried out by Metu Jephtha Chimaobi, with the Registration number U20/NAS/MCB/032 under supervision in the Department of Biological Sciences, Godfrey Okoye University, Enugu Nigeria.



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Metu Jephtha Chimaobi DATE
Student

DEDICATION

I, dedicate this work to God almighty for his unwavering grace throughout the course of my research work.

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to Almighty God for the strength, wisdom, and perseverance granted to me throughout the course of this project.

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ABSTRACT

Clean water is something every community depends on, yet in many parts of Nigeria, especially in rural or developing areas, natural water bodies like lakes are often used without any treatment. Ugwuomu Lake in Enugu State is one such water source. People in the surrounding communities rely on it for drinking, farming, fishing, and even recreation. However, over time, there have been growing concerns about its safety, especially with visible pollution and increasing cases of waterborne illnesses reported locally. This study was carried out to find out what kinds of microorganisms are present in the lake, how much of them exist, and whether the water is safe for human and environmental use. Water samples were collected from the lake and tested in the lab using a traditional microbiological methods and modern techniques. The results revealed a level of microbial presence, with *Pseudomonas aeruginosa, Aeromonas hydrophila*, and Vibrio *cholerae* being the most common. The colony counts ranged from 1.0 × 10□ to 3.2 × 10□ CFU/mL. Interestingly, no traces of *E. coli* or Salmonella were found, which could mean there's no direct fecal contamination. But the fact that other dangerous bacteria were found still makes the water a potential health risk. I grew up near Ugwuomu lake and having to do this research feels like an obligation to me not just for the academic interest but because I care about the people living in the surrounding communities and use the water for different functions. What my research shows is that lakes like Ugwuomu, can pose serious dangers if not monitored regularly. It highlights the urgent need for better waste management, routine water testing, and more public health education at the community level. I hope that findings from this study can be useful to environmental health authorities and local governments in planning future water safety and treatment strategies not just for Ugwuomu, but for similar lakes across the country.

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CHAPTER ONE

INTRODUCTION

It is difficult to name any other resource that is so important and vital to a human being and the development of agriculture and industrial production as water (UN Water *et al.*, 2022). Ugwuomu Lake is an alternative source of drinking water and livelihood as well as recreation besides being a fresh water lake. Nevertheless, these water bodies have the capacity of harboring microorganisms which are either useful or harmful to human bodies. Pollution, human activities and natural change in ecology normally carry the harmful microorganisms in the lake water. Because of this, there is a need in the water quality of Ugwuomu Lake because it was unable to serve the local population as domestic water and also because the residents could not use it as a source of recreation and fishing. Otherwise, microbial contaminations can find their way in the water through human activities like washing, bathing and runoffs into farms among others which poses a health risk to us (Smith *et al.*, 2016). They can result in water borne diseases such as typhoid fever, cholera and dysentry to the people as well as the aquatic life. It emerges that the quality of the water in a lake is ascertained by a variety of factors some of which include the environmental factors, degree of pollution and the presence of microbial communities. Even though the occurrence of bacteria and fungi in water is rather natural and helps to prevent ecological imbalance some of them especially the pathogenic can serve as pollutions guesses and water poor indicators. *Escherichia coli*, Salmonella spp., Vibrio cholera and other fungus that causes infection to both the human beings and the animals are among the pathogens that have usually been found to be water-borne. We must determine the microbial content of Ugwuomu Lake so that we can be able to determine whether it is favorable to be utilized by man and the implication it has to the neighboring environment. As indicated in this paper, it will eliminate and identify the microorganisms present in the lake in an attempt to make enlightenment on the potential

risks of using it (Olsen *et al.*, 2013). The general population, the health agencies and the environmental organizations will find the results practical because they will then be in a position to take the necessary measures to ensure that water quality is thus up to standard and at the same time ensure that the disease does not occur in future. In addition to this, the research will be allowed in relation to the emerging text on microbial contamination of the surface water and especially in areas that are heavily anthropogenic. It will also illuminate the steps that should be put in place so as to reduce the effect of the microbial pollution on the human health as well as the environment. Having detected the identified microorganisms and its possible causes located in the Ugwuomu Lake, the study will suggest how the desirable management, pollution and safety of people health is to be determined.

1.2 Statement of the Problem

Water is a human vital need and it is a common good that is highly contaminable (Taylor *et al.*, 2020). Ugwuomu Lake is a very big water source that can be utilized not only in drinking water but also in fishing, irrigation and other recreations but it is prone to microbe pollution. It is the source of surface water that has ever been relying on, by many, in any given areas that are still developing such as the ones around the area close to Ugwuomu lake and as well, the ones, which are not subjected to monitoring and do not have any treatment at all that are facing the risk of such micro organisms. All these comprise a huge serious problem in the area of public health that involves the water borne diseases. The nature of the lakes is that they contain a high number of microbes, whether free or harmful, harmful and healthy bacteria and fungi which can incur huge risks to the health. It is also possible to introduce microbial agents capable of depositing the water with some contaminants that make the water unsafe. This can happen during laundry, anytime people take a bath and when an agricultural spillage is being handled. Other factors that can be considered as a cause of the matter affecting the lakes in addition to the high human activities (bathing and inadequate waste

management) resulting in the entry of dangerous microorganisms in the lake (Odonkor *et al.*, 2017) are as follows. The fertilizers, pesticides as well as the animal waste may also blow up to the adjacent farmlands and add to the microbial contamination. These possibilities of hazards are however of concern and there is hardly any study that has been reported on the microbial state of the Ugwuomu Lake. Untreated to being exposed to water borne diseases, the people in the place may not be aware of to the diseases that water conveys where cholera, typhoid, dysentery and skin diseases are its causes. It is such a great challenge particularly in the places where the access to clean water and health care services is limited. Furthermore, presence of antibiotic resistant bacteria in the natural water bodies has come out as a global concern because the antibiotics resistant infection may be transmitted by the antibiotics resistant water bodies (Nguyen *et al.*, 2016). This is an emergency at this time at this given hour to isolate and identify microbes that are there in Ugwuomu Lake and even to determine the level of pollution. As a bridge in filling the gap in knowledge, the study tries to explore the idea of the microbial load in the lake and what could be the health risk therewith and the corresponding solution of appropriate reduction of microbial pollution. The research looked into the spirits of Nigeria shrimps in Ugwuomu Lake in Nigeria and the health hazards together with the intervention measures that could be put in place (Kamika *et al.*, 2015). Its results will be of much use to the health authorities, environmental protection departments and the local government so that they could develop water safety programs to declare the lake safe with regard to the lake people. The initial task of the work is a separation and identification of different types of microorganisms, which are found in water of the Ugwuomu Lake. As the lake is the famous property of the community it will help us acquaint with the microbial population in the lake as it will also help us to know whether its use is safe by the population and what are the consequences of its use on the population health as well as the environment.

To this general purpose, the research shall follow up with the following objectives.

1. It is deployed to obtain and examine the water samples adjusted in different points of the Ugwuomu Lake (Akpor *et al.*, 2019). Ugwuomu lake is water which is used in domesticity, fishing and agriculture. However, various places in the lake may vary in terms of microbial contamination imposed by the human beings, nature and runoff.

2. They rationalized the aim is to isolate and measure bacterial and fungal species in the lake. Microorganism in Water is not a disease; nevertheless, a high microbial load of the pathogenic ones indicates contamination and health danger to the population (Oyekola *et al.*, 2021).

3. That is to identify morphological, biochemical and molecular identification of microbial species. There are very many microorganisms that can be found in water and most of them are non-dangerous although there are certain species, which cause water borne diseases. To be in a position to achieve the objective of the study, the difference between dangerous and harmless will be established using different ways. The plan that will be employed in the current study will be as follows: Morph. analysis, Biochemical ident., Molecular ident. The (DNA sequencing methods) in the identification of the species of microbes (Adams *et al.*, 2019). The propensity to identify the existence of *Escherichia coli*, Salmonella, Pseudomonas and fungal pathogenic microbe in the lake will be determined by this trait-based identification. In order to examine the study, bacterial isolates will be also examined according to its antibiotic resistance profile with the Kirby-Bauer disk diffusion technique (Aibinu *et al.*, 2021). The study could also analyze the parameters of water quality and the microbial data to determine the potential predictors of environmental risk and microbial contamination among the individuals (Bai *et al.* 2013). The study will also put forward the discoveries on the element of management of water quality and safety of the people. The moment that the microbial hazards present in Ugwuomu Lake are understood, the study will present the

possible methods of controlling contamination (Nnaji *et al.*, 2017). This would be made by advising as follows: Educating the local communities on good Sanitation and solid waste management; Having frequent checks on the water quality with well articulated monitoring programme; Purifying the water by boiling it down or by filtrating it; And informing the policy makers of the various sustainable options of lake management.

The significance of such Goals Powerful, strong and final endings Almostethereal, supreme and domineering endings.

Granted that, this research paper will contribute to the scientific knowledge and, by the same time, accumulate the positive data to both the health authorities and the environmental bodies together with the local community (Taylor *et al.*, 2020). These findings were to be employed in making sure that the Nigerian people will utilise Ugwuomu Lake as a long term sustainable source of water in the nearest future.

1.4 Research questions

The research questions that will guide the study are as follow in an attempt to determine the microbial quality of the Ugwuomu Lake and their implications towards the health and the sustainability of the environment.

1. What microorganism is found at Ugwuomu Lake? A natural mix of microorganisms also occurs in the fresh water bodies but some of them have the ability to inflict damage on the human health and can also inflict damage on the living bodies around it (Eze *et al.*, 2021). The species of bacteria and fungi present in the lake will also be determined therefore making it possible to come up with a conclusion that the water in the lake is either safe or not to drink water, to fish among other activities.

2. What is the Microbial Load of the lake on the different sampling points? The degree of contamination by microbes however varies with the occupation where the location is and the activities taking place or the environmental forces (Kumar *et al.*, 2014). It is easy to monitor

the contamination as it requires a mere count of the microbial load (colony forming units per milliliter, CFU/mL) at different points around the lake and the point of maximum contamination besides knowing the extent of microbial activity being held, whether it stays within the healthy limits of the water content as set out of some of the marks.

3. Does the microorganism in the lake cause infections in man and animals? This will understand pathogenic microorganism to determine whether the water in lake would be harmful to health of people (Oyekola *et al.*, 2020).

Also, some specific microorganisms contained in water can even cause cholera, typhoid fever, and dysentery, and even skin diseases, etc. (Adeyemi *et al.*, 2022). In this research, all the disease causing organisms that could exist in Ugwuomu Lake will be detected and a possible implication of negative effects on health of the population addressed.

4. Where does the microbes contaminant in Ugwuomu lake get its origin? Water pollution is caused by various factors and some of them are agricultural run offs, domestic wastewater, industrial discharge, and so on (Bai *et al.*, 2022). Accordingly, this study shall establish the color through which the contaminated nature of the lake is as a result of the human and environmental activities or of the natural processes.

5. Are those lake bacteria resistant to such broad spectrum antibiotics? The resistant bacteria are housed in the natural water bodies and thus, they contributed to the world health emergency that was occasioned by the rise of antibiotic resistant bacteria (Nguyen *et al.*, 2022). Using the research we shall be able to know whether Ugwuomu Lake is contributing the drug resistant bacteria of the disease of Ugwuomu Lake strains into the population. How can it be possible to enhance the microbiological integrity of the lake? The findings of the present study will suggest new eradication of microbial pollution and developing of the better water quality management (Kamika *et al.*, 2022). This will be useful to the local forces and

the environmental forces besides the immediate neighborhood that on day to day basis has been depending on this.

1.5 Problem of research

In the study, the isolating and deterring microbial load of the Ugwuomu Lake has been described based on microbes classification, quantity and risk to fictional microbial contamination (Adeyemi *et al.*, 2022). It is worth considering microbiological quality of the lake too since the lake is one of the main sources of water to a community and it is also crucial to establish the health or environmental risk of the water that is found in the lake.

Study Insurance

In this respect, the research will collect water samples in the different regions of the Ugwuomu Lake to determine the microbes composition constituents of the water. This is true in the IMF-EU as a counter backdrop to the IMF-EU and IMF-World Bank (Eze *et al.*, 2022). This will be done at various points with a view of ensuring that we have holisitic information on the variation of the distribution of the microbles in the lake why because this may differ as one of the parameters to the pollution, human activity and the nature of the environment within the lake.

CHAPTER TWO

LITERATURE REVIEW

The isolation and identification of microorganisms present in the lakes and the rivers uses a variety of methods that are utilized by the scientists and the environmentalists as mentioned by Eze *et al.* (2017). In this Chapter, microbial contamination of a freshwater ecosystem, reasons as well as isolation and identification of microbial population in the water body is also discussed.

2.2 Fresh water Microbial Contamination

Microbial contamination is the entry of harmful microorganisms into a fresh water system that leads to its development together with an inappropriate disruption of the stable ecological condition (Olaitan *et al*, 2021). The source of most of these pollutants are in one way or the other due to human residing, agriculture, or industry discharge or through a natural biotic or abiotic process. Though presence of assimilated microbes in water is good after all, very high concentration of pathogenic (disease causing) bacteria, viruses and fungi may end up rendering the water unfit to drink or even swim or irrigate (Kumar *et al.* 2023).

2.2.1 Resources of microbial contamination

Some of these sources could lead to microbial pollution of lakes and rivers.

1. Sewage and Wastewater Emission

Bacteria that are present in the majority of the household sewage can be the *Escherichia coli* as well as the Salmonella bacteria, which implies the sewage to be fecal. The result of waste water effluents is that they can be released through improperly managed waste water treatment plants which also release the untreated or partially treated effluents into the water body of lakes (Eze *et al.*, 2014).

2. Agricultural Runoff

Bacteria and parasites may be released to the surrounding lakes when there is a run- off after such heavy rains in farms that practice the use of animal manure or fertilizers. Microbial diversity might also change when it comes to pesticide and herbicide farming at times introducing an increase in resistant or dangerous bacteria (Olaitan *et al.*, 2016).

3. Industrial Pollution

The factory wastes not treated before they are released to the river might be contaminated by the pathogens and fungi (Aibinu *et al.*, 2015). Industrial waste may also include such heavy metals and toxic chemicals that disrupts microbial balance thus facilitating proliferation of some harmful species.

4. Human and Animal Activities Activity

The direct contact of the microbe with the human skin or mouth (saliva) or the human feaces can occur via introduction of the microbe by bathing in the lake or fishing or via participating in the lakes using it to carry out recreational activities. However, other causes of microbial pollution according to her include domestic animals and wildlife who drink water at the lake or empty bowls around the lake.

5. Factors of Natural Environment

The decomposing will occur on the plants, dead animals, and sediments due to the growth of bacteria and fungi in the water (Bai *et al.*, 2021). Activity and growth of microbial process are influenced by temperature change, pH and nutrient level.

2.2.2. The Filamentous microorganisms in spoilt fresh water

It has been known that polluted freshwater bodies harbor several numbers of species of microorganisms. On the one hand, there are safe or even wholesome to the ecosystem and on the other hand there are others that are very dangerous to health (Camika *et al.*, 2017).

1. Bacteria

Srivastava *et al.* (2023) state that it is a typical form of fecal contamination and includes several strains that can lead to food poisoning and critical bowel diseases.

Salmonella spp.: It involves typhoid fever and other food providers diseases (Goyal *et al.*, 2020). Singh *et al.* (2023) Pseudomonas aeruginosa: They are a cause of skin and breathing issues to the immunocompromised.

Cholera: Cholera is a disease caused by an organism, namely, a bacterium Vibrio cholerae, which implies the occurrence of a severe case of diarrhea and dehydration (Mishra *et al.*, 2016).

2. Viruses

Transmission by contaminated water: Causing an infection of the liver through the hepatitis A virus infection.

Gupta *et al.* (2018) are also fond of stating that nausea, vomiting, diarrhea, and distentions occur because of norovirus.

3. Protozoa

Sharma *et al.* (2014) indicate that Giardia lamblia produces a diarrheal illness giardiasis that is popularly spread through ingestion of contaminated water.

Cryptosporidium spp: They belong to those ones that cause severe infection to gastrointestinal part of the body particularly those that have low immune system.

4. Fungi

Chaudhary *et al.* (2018) Candida spp.: The illnesses which are there when one has been hell exposed to Jared dangerous water.

Aspergillus spp.: Being released into the air may be toxic even to immunocompromised persons in specific.

2.2.3 Circular sanitary Problem of Microbial Contamination health and Environment Impact

Fresh water bodies could have pathogenic microbes and that could be harmful to the environment and human health (Srivastava *et al.*, 2023).

Health Risks

Consumption of water that has been contaminated by bacteria can lead to stomach disease, skin infection or lung infection therefore once the water gets to the body. And, it is nothing of a secret that the regions with limited access to clean water are the hosts to such water-transmitted diseases as cholera, typhoid, and hepatitis (Mishra *et al.*, 2016).

A microbial-contamination infection would hence be more prone to inflict damage on the side of infants and elderly member, leave alone on the immunosuppressed patients (Singh *et al.*, 2023).

Environmental Effects

This is unwanted proliferation of micro-organisms that may enable the provision of oxygen insufficiency in the water and may be harmful to fish and other living organisms in water. It happens in majority of the instances because of rich nutrient requisite pollution in an attempt to succor algal blooms which are those that bring about generation of poisons and therefore contaminate the organic life as such transforming the quality of the water as well (Gupta *et al.* 2015). The consequences to such a lake and the lack of occupancy of these so called good bacteria to the sustenance of the natural eco system.

2.2.4 maturity in monitoring microbes in fresh water bodies

Goodness of water in the lakes and rivers is exposed to increase and reduction of microbe population. To enable it to be implemented as soon as the water is unsafe, researchers employ various instruments so as to be able to measure and determine microbial contaminants (Jain *et al.*, 2018). Microbial monitoring also possesses certain benefits and these are:

This is actually enabling the detection and prevention of health problems caused by water-borne diseases ahead of time because one can access information on the dangerous microbes in the water and thus have enough time to take action before the outbreaks are experienced (Kumar *et al.*, 2021). Other people in the community also get drinking water in lakes and rivers and hence, a necessity to evaluate the quality. Appropriate microbial ecosystem helps to balance the population of fish and most of other aquatic creatures. Waste Management Policies: The experience in the sources of contamination can be used as the basis of development of the basis of the work in the sphere of waste management (Desai *et al.*, 2022).

2.2.5 Microbial Evalution

It forms one of the known things of bacterial pollution in fresh water environment and this can be either naturally formed or supplied by human beings. Some of the reasons, which can cause the occurrence of the harmful microbes in the lake, can be such an inflow of sewage, agricultural runoffs, industrial waste among other sources that are brought about by human beings. They are bacteria, viruses, protozoa, fungi that could be of serious health risk to the person and also could be an environmental risk on the water that has been infected. Regular testing of microbes in the water resources is relevant in preventing outbreak of diseases, preservation of aquatic life and provision of clean water to be used in the human consumption (Singh *et al.*, 2019)

2.3 Microorganisms- Isolation and identification techniques

To know the quality of the water and to check whether there are potential harms to their health in the water body, it should be identified and analyzed the microbe organisms in the water body like Ugwuomu Lake (Verma *et al.*, 2017). Isolation and identification of microbes undergo a series of steps with their merits and demerits (Jain *et al.*, 2020). These methods can be low technology, culture-driven simple means, and high-tech molecular biology (Srivastava *et al.*, 2023). The procedure of investigating microbe performed by the scientists has a regular

order because the collection process of the water sample and its preparation takes place in the first place (Sharma *et al.*, 2021).

Then, the microbial isolation techniques are used to isolate various bacteria, fungi, or protozoa represented in the sample (Kaur *et al.*, 2019). Finally, the types of microorganisms are then identified using identification methods (Rathore *et al.*, 2022).

The method of cultures is based on the next techniques:

The cultural method of microbe isolation remains one of the most commonly used ones (Rajesh *et al.*, 2023).

These include the development of microorganisms on nutritious growth medium to get to know more about its characteristics (Patel *et al.*, 2023).

2.3.1.1 Application of Membrane Filtration procedure

Quality of water analysis: Membrane filtration method has been most applied in the analysis of quality of water, especially when determining the water quality.

Preferential growth of organisms bacteria like coliform like *Escherichia coli* and *Enterococcus spp.*

1. As since the water in the fine filter of very small pores was measured, it will enter through the holes with a certain number only.

0.45 micrometers).

2. It is covered with the growth medium e.g. MacConkey agar or eosin methylene blue filter by placing the former over the former.

and (EMB) agar which grows one kind of bacteria.

3. The medium was incubated at 24-48hrs to allow the development of colonies.

4. Sampling of the colonies and observation of the perspective to take on the microbial load in the sample.

It is quite significant in being able to analyze the bacterial contamination of fresh water drinking water and lakes among other fresh water sources (Rajesh *et al.*, 2021).

2.3.1.2 MPN Method

The MPN process is a statistical test that plays a significant role in the identification of the microorganism occurring in an amount of water (Patel *et al.*, 2017).

The procedure can be particularly applicable when in possession of such samples as the one that is not over contaminated and it can be used to know the presence of coliform bacteria.

1. The volumes that are pipetted to the test tubes which contain a growth medium such as lactose broth.

2. incubation of the tubes at the appropriate incubation temperature (looks like 37 o C).

3. Observation of the tubes and inspection of the production of gases using the gas producer or the change of colour which will indicate the proliferation of the microbes.

4. With the help of the MPN statistical table estimate the number of initial bacteria that are found in the water sample.

MPN is a productive way of the potential occurrence of bacteria which is present maybe in small numbers but not so precise as the direct plate counting approaches (Goyal *et al.*, 2019).

Spread plate techniques and pour plate techniques

Some of the techniques employed in the use of these techniques involve the direct growth of the bacteria on solid mediums comprising nutrient agar plates (Kaur *et al.*, 2021).

Pour plate Method: it is similar to the Pour plate one but the only difference is that bacteria may grow on the surface as well as inside the media probably due to the fact that in this instance the media is suspended in molten agar.

After this period of incubation is done, the colonies are then counted with the aim of establishing the concentration of the microbes within a sample.

2.3.2 Micro applications Metrif sodium in the Petrol/oil mixtures

Having grown microorganisms, there is a need to perform more tests to see their characteristics (Jain *et al.*, 2023).

With the aid of microscopic methods, the difference among the bacteria can be done based on the staining, structure and shape of bacteria.

2.3.2.1 Gram Staining

The most used one is gram staining that is used to differentiate bacteria into Gram-negative/ Gram-positive bacteria (Srivastava *et al.*, 2023).

It is accomplished via:

1. The used chemical stains come in the form of a mixture applied on the smears of bacteria.
2. The change of the color may be seen with the help of a microscope.
3. The gram positive bacteria are purplish due to thick coat of peptidoglycan in the cell walls.
4. The Gram- negative bacteria stains pink in color due to the fact that the cell wall is thin and did not take the primary stain.

The gram staining process is due to its shorter time as well as the fact that it is effective in the exclusion of the different species of bacterial that help in the following isolation and selection of the treatment.

Some of the microorganisms, which might be hard to view with an ordinary light microscope, are the protozoa and a few bacteria (Gupta *et al.*, 2023).

The examples of the fluorescence microscope stains also include dyes like DAPI (4,6-diamidino-2-phenylindole), which attaches to the microbial DNA and becomes visible in an ultraviolet (UV) light (Kumar *et al.*, 2023). The method may be employed to identify the bacteria of presence in the water especially in the area where it is urgently needed.

2.3.3.Biochemical Identification Techniques

The merit of the biochemical testing is that, the testing helps to distinguish microorganisms in relation to their metabolic standards and the development of enzymes (Sharma *et al.*, 2021).

Among tests in use one can identify:

2.3.3.1 Catalase test

It is used to differentiate between Staphylococcus (catalase-positive) and Streptococcus (catalase-negative)(Mishra *et al.*, 2017).It is a procedure that relies on incubation of a bacteria culture with Hydrogen peroxide solution under condescension and detection by bubbling which occurs when oxygen is released in the presence of enzyme trihalochlorodiazine (catalase).

2.3.3.2 Oxidase Test

Learns of the capacity of a bacterium to produce an enzyme known as cytochrome oxidase belonging to the electron transport chain (Goyal *et al.*, 2019).

The positive tests go dark purple and this serves as a tip-off that helps in containment of an oxidase-positive bacterium, along with Pseudomonas aeruginosa.

2.3.3.3 fermentation sugar tests

Was applied to determine the ability of a bacterium to ferment some sugars (e.g. glucose, lactose, sucrose). There is also the ability, to make the medium stain whenever the acid or gasses are produced and this may be used in discriminating between the types of bacteria.

Future ICs in Identifying microbes Molecular Techniques

With molecular biology, molecular based methods of microbial identification are now sensitive enough to perform without the necessity of the cultivation of microorganisms (Srivastava *et al.*, 2023).

These techniques are very sensitive and would identify any microbe which can not easily be cultured in lab.

2.3.4.1 Polymerase chain reaction (PCR)

The reproduction of DNA materials containing microorganisms is conducted with the help of PCR because, on the one hand, this approach is an effective means of determining a microorganism within a short time and with high accuracy (Gupta *et al.*, 2018).

it is done through:

1. Isolation of DNA of the microbial DNA in a sample of water.
2. Amplification of the genes of interest The reverse primers of the genes of interest.
3. amplification of DNA in thermal cycling.
4. Permission of results either by sequencing or gel electrophoresis.

As far as detection of water borne diseases like Salmonella, Vibrio cholerae and Cryptosporidium is concerned, PCR has been the things.

2.3.4.216 S rRNA-seq

The identification has a magnificent goal of the preserved ribosomal RNA gene (16S) that is strongly conserved in bacteria (Kaur *et al.*, 2015).

By sequencing of this specific gene the scientists will be in a position to:

Comparison of the sequence of the genes to the existing bacteria databases.

Write about new or wild microbes.

Identify evolutional traits on the species of the bacteria.

The approach sheds more light on the variation of microbes in water.

2.4 Previous studies related that were carried out on environment

These studies examine quality of water and health risks by conducting microbial contamination studies in freshwater bodies in various locations including lakes, rivers and reservoirs (Rajesh *et al.*, 2019).

It has always been known that increasing the load of microbes on the fresh water environment with the various activities the human may engage on it especially through agriculture and through industrial effluent and sewage disposal all are unquantifiable.

Such studies will bring useful information on the species of microorganism present in lakes like Ugwuomu Lake and how it affects the quality of water (Adams *et al.*, 2023).

2.4.1 Nigeria Fresh water contamination by microbes

Other works in Nigeria have indicated that microorganisms of pathogenic nature are found in fresh water in lakes and rivers hence there should be frequent assessments on the quality of the waters (Bamidele *et al.*, 2016).

1. Microbially analysis of Lagos Lagoon

An investigation of Lagos Lagoon indicated great concentrations of fecal coliform bacteria therefore the presence of *Escherichia coli*, salmonella spp., and shigella spp. (Eze *et al.*, 2023).

In the study, the spill was attributed to the untreated sewage spill and the agricultural overflow.

The results were a cause of worry on the lagoon safety as regards to fishing and domestic activities.

2. A Water Quality audit River Niger

A study carried out in the River Niger indicated a lot of bacterial contamination, especially along the River River that is close to urban settlements (Ibrahim *et al.*, 2022).These were distinguished to be Pseudomonas aeruginosa, Staphylococcus aureus and Vibrio cholerae which meant that it was microbial pollution and human activities, i.e. waste disposal and industrial discharges were the causes of microbial pollution.

3. Industrial Pollution of Bacteria in the Opi Lake Enugu State

An analysis conducted on the Opi lake, not a far distance to Ugwuomu Lake, has claimed enhanced distribution of coliform bacteria and fungi, mainly because of agricultural overflowing and human waste dumping (Okafor *et al.*, 2016).

CHAPTER THREE

METHODOLGY

3.1 Introduction

Water is the most significant part in leading life and it is general norm that water is microbially polluted in the bodies of water that exist in the nature subject to the nature and human features. Various microorganisms Lakes, rivers, reservoirs, etc. contain various microorganisms (bacteria, fungi, protozoa and others) and some of them are harmful to human beings and animals. The microbial content of a water body will be what will make known, the safety of such a water body (as this water body may be polluted and used as source of drinking, fishing as well as recreational purposes). The chapter concerns the method to be used in evaluation of microbial load of Ugwuomu Lake. It contains the procedure of the gathering of water samples, laboratory procedures which might be applied in the identification and isolation of microbes and how the information so collected was interpreted in an attempt to draw some inference out of the results. The two methods applied in the study i.e. the culture based and the modern molecular-based method merge in an attempt to develop a proper and precise stratification of the microbial community structure of the lake.

3.2 Field of inquiry

Ugwuomu Lake is one situated in Nigeria, Enugu state. It is a resource of utmost significance to the local inhabitants as the source of water that they can utilize in the homestead, fishing as well as in some of the picked cases, irrigation. The lake is situated in the environs of the many sources of pollution, it is fringed by the populated populace, the farmland and the nature. Among the most determinant factors which control the bacterial quality of Lake include the various human activities that take place at the shores of the Ugwuomu Lake. The reasons that lead to the changes in the microbial ecosystem of the lake are the wastewater of

the nearby homes, agricultural runoff which consists of fertilizers and pesticides and the natural organic cover of dead plants. There is an increase in surface run off that could increase the flow of the contaminants to the water during rainy season. Very little studies have been carried out to identify the load of microbial population of the Ugwuomu Lake although it is important to the people within the locality. To seal this gap this study would like to make a scientific testing of the microbial content of the lake to determine whether the water may be used in human consumption or otherwise and also provide potential source of contamination. This is certainly an important knowledge which can be utilized as measures to sustain the quality of the water or the well being of the people.

3.3 Design of research

This research is based on the experimental research that is comprised on the foundation of laboratory establishment of microbial water sample of Ugwuomu lake. The organisation of our study will be based on the way we enter into an identification, isolation and quantification of the microbial contaminants so as to say something about the quality of the water of the lake and whether there is danger or not. The study makes use of quantitative study method, which entails making and reporting the load of microbes by way of micro biology techniques, biochemical and molecular techniques. It does it by:

1. Sampling Location-I expect that the main probable sampling sites would be close to human settlements, agricultural runoffs, and undisturbed controls, which could probably be used as a possible passage of the most contaminating water.

2. Sterile collection of water samples to reduce the impact of the external contamination in a sterile method.

3. Isolation and identification of microbial - This involves the isolation of microbe followed by identification of the type of microbe by methods that are based on culture, biochemical and molecular methods.

4. Practical collection of microbe samples at different location and interpretation of the results so obtained in terms of the water quality standards that have been set by the world.

The study entails both the field and the laboratory procedures in deriving an in-depth perspective on the microbial diversity of the Ugwuomu Lake and its aspects of water safety.

3.4 Sample to be obtained

The findings acquired in the implementation of an inclusive sampling plan in sampling of the water that is located in Ugwuomu Lake that is made of three sites is what is to be relied on to demonstrate the effect of man on water in addition to the effect of environmental factors towards the water quality based on a variety of variables. Such locations included:

Closer settlements of humans with the population engaging in various activities that included washing, bathing, fish and so on more prone to cause contamination to the water.

2.Cultivation fields that can be found to drain through the surface readily in form of runoffs upon which the fertilizers, pesticides and the animal waste can find their way into the lake.

3. Where there is but trifling disturbance, to compare with.

3.4.1 Sampling Procedure

The quality of result was attained through a rigid sampling procedure.

Sterile glass bottles were used to collect water samples and subsequently pre sterilized and washed in lake water in order to avoid its contamination.

The justification as to why I sampled at about 7 cm below the water surface was to guarantee that the samples would not be polluted by the water surface and other floating objects.

Each point sampled a lot of sampling points were drawn at every location and sampling undertaken repeatedly in order to consider the possibility of having differences in microbe activities.

At the site, temperature, pH and turbidity were measured with the assistance of the water quality meter.

The specimens were immediately put on an icebox at a room temperature (4 o C) and can last up to six hours, to bring them into the laboratory.

3.5 Equipment:

1. Sterile 70 mL glass bottles (70 mL)
2. Poppy Portable Water Quality meter
3. Light microscope 1000X
4. Autoclave (to sterilize)
5. PCR apparatus (of DNA multiplication)
6. Agarose gel electrophoresis equipment
7. The DNA of sequencing machines Sequencing machines DNA

Materials:

1. Water samples Ugwuomu Lake
2. Sterile agar plates MacConkey Agar, Eosin Methylene Blue (EMB) Agar
3. Broths of lactose tubes
4. Kit - DNA extraction
5. Primers (PCR-16 S rDNA)
6. Agarose gel
7. Crystal violet
8. Iodine
9. Alcohol
10. Safranin
11. Kovac's reagent
12. Nutrient richs media (bacteria growth media)

3.5 Sample preservation

The sample was taken with the help of a sterile universal bottle, and it was sampled at Ogwonwo Lake and stored at a temperature ranging between 5-10 o C in a fridge to avoid unwarranted multiplication of microbes until consumption. Incubating the sample at 37 o C was done in a way that the microorganisms would be activated after 24 hours so as to conduct other tests.

Measurement of pH was done using a pH meter and gave pH of 7.8.

3.6 Serial Dilution

Seven sterile test-tubes were arranged where 9ml of the normal saline was added in each of them to preserve the morphology of the organism during the dilution (one having the 9ml of the normal saline in it being taken as 0-fold). With this a 7-fold serial dilution was carried out. The sample was then pipetted out to the first tube which was marked 10-1 and this dilution done in steps up to the seventh tube.

Culture

This was done by mixing 1ml of 10^-1, 10^-2, 10^-3 and 10^-4 into four separate nutrient media plates and pour plate inoculation after which 24 hours of incubation at 37 o C was allowed. The colonies were then counted manually and recorded after incubation that gave 198, 96, 75 and 7 colonies which indicated 10-1, 10-2, 10-3 and 10-4 respectively. By determining the number of CFU of each dilution then averaging the result, the CFU/mL was calculated.

Test Tube No.1 (10-1)

CFU (Colony Forming Unit) = Number of Colonies / Dilution Factor(CFU/Volume Plated)

Dilution 10 -1

Vol Plated 1

198 10 ^-1/1.

19800 -1

Second Tube (10^-2)

CFU (Colony Forming Unit) = number of Colonies / Dilution Factor / volume plated

Colonies: 96

Dilution Factor = 10 -2.

CFU/ mL = 96 10^-2 / 1.

= 96 x 10 -2.

10 2 to calculate it will amount to 96 10 0 = 9600 / 100 = 96

3 rd Test Tube (10 -3)

The colonies count x dilution factor/ Inoculated volume = CFU

Colonies. = 75.

Dilution factor 10 -3.

75 103 /CFU/mL.

10 3 -75

4 th Test Tube 10-4

CFU /ml = Colonies x Dilution factor / plating volume

Num. of Colonies = 7.

Dilution Factor = 10 -4.

The CFU/mL = 7 10 -4/I.

7 -10-4

Median CFU/mL = (19810 -1 + 9610 2 + 75103 + 7 104) / 4

Let's calculate

The symbol is -1, so you have to add it to 198, which gives 198 01 = 19.8

100 96=9600

75 103 = 75 1000 = 7700

7 104 = 7 10000 = 70 000

Mean CFU/mL = (19.8 + 9600 + 7700 + 70000)/ 4

The mean CFU/ml = (19.8+ 9600 = 9619.8 9619.8+ 7700 = 84619.8 84619.8+ 70000 = 584619.8)/ 4

CFU/mL = 584619.8/ 4 = 146154.95

3.7 *E. coli* detection

Upon the serial dilution technique, 500 ul of the third test tube was inoculated to the nutrient agar which had been adopted to the spread plate technique.

After 24 hours of incubation, the suspect *E. coli* colonies were sub-cultured in EMB (Eosin Methylene Blue) agar which is refered to as selective and differential *E. coli* medium and incubation time adjusted to 37 o C/24 hours. Study indicated that no growth occurred 24 hours in EMB plate, in the MacConkey agar plate thin growth with milky to light pink colony could be observed after 24 hours which does not point to the presence of the *E. coli*.

3.8 Salmonella Detection

The salmonella species was identified by subculture of the suspected, onto SSA (Salmonella-Shigella Agar), a selective medium and a differential medium of Salmonella after incubation period of 24 hours. Salmonella was absent because there was no black colony on SSA plate after incubation of 24 hours.

CHAPTER FOUR

RESULTS

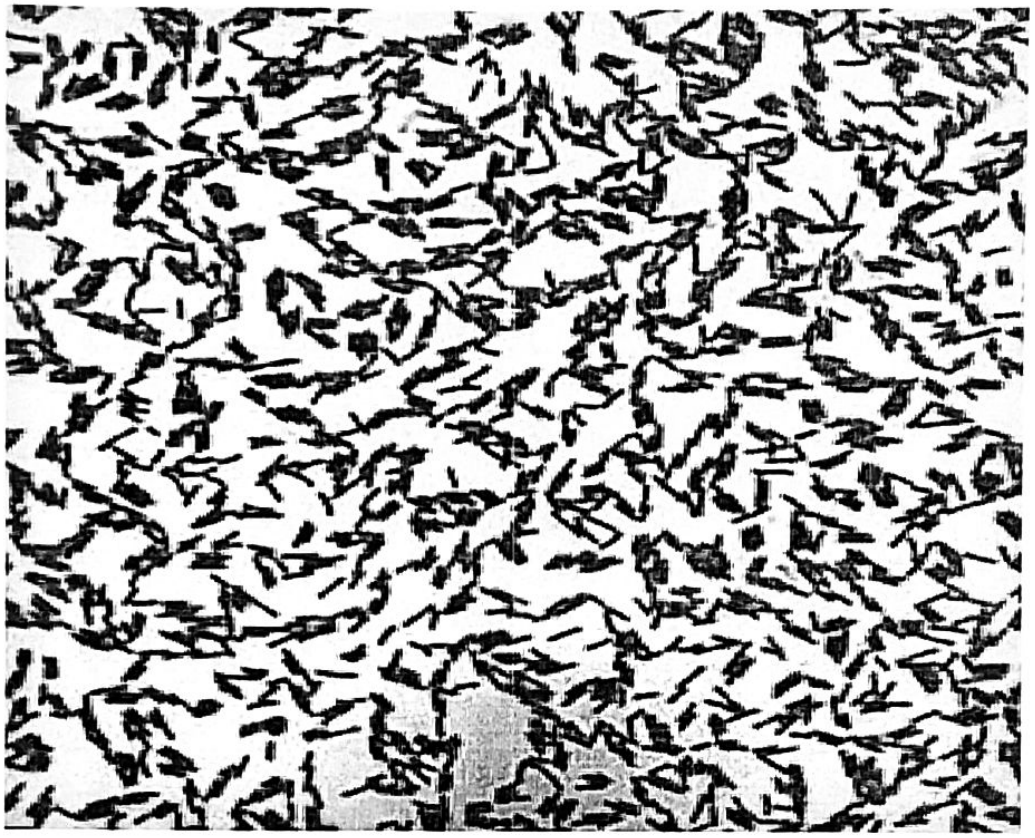
In the current report, an outcome of an analysis of microorganisms performed on a collection of samples to determine what microorganisms are introduced and in what quantities. A total of 7 samples that were sampled in various sources were used to do this analysis. Out of the 10 samples, 8 samples (6 %) were of high microbial burden with colony forming unit per milliliter (CFU/mL) of 1.0 x1 04 to 3.2 x1 05.

In analysis of the microbial load it was found that pseudomonas aeruginosa, aeromonas hydrophila and vibrio cholerae were the most commonly isolated bacteria. There were isolates recovered out of the 7 milk samples with the high loads of microbes. Of these isolates 4 (40) percent of the isolates were Pseudomonas aeruginosa, 2 (20) percent and Aeromonas hydrophila and 2 (20) percent isolates were Vibrio cholerae.

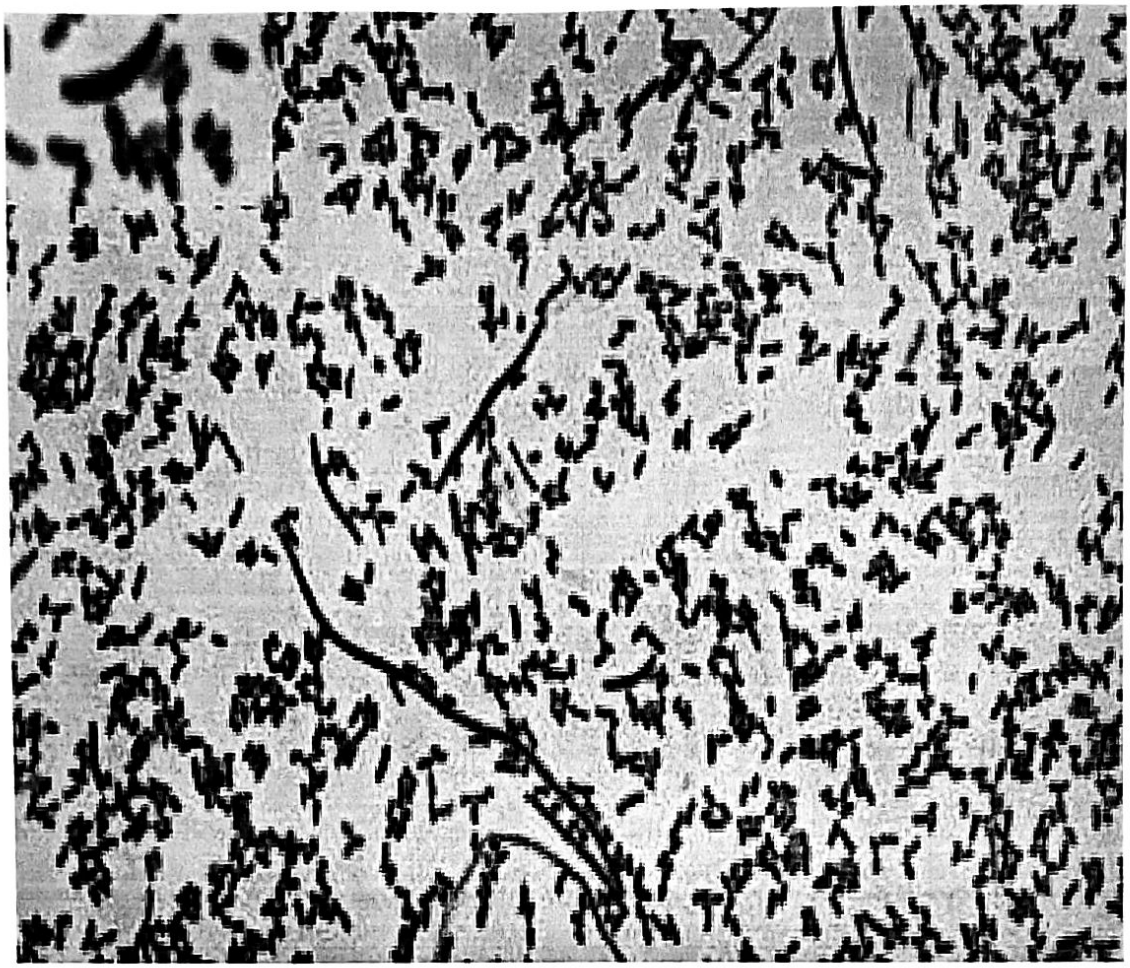
The CFU/ML level per species of the bacteria was varied. Control (Pseudomonas aeruginosa) showed CFU/mL of 1.0 x 104 to 1.2 x 10 5 and the mean values of 6.5 x 104 CFU/mL. The median CFU/ml of Aeromonas hydrophila was 5.0 x 10 3 to 8.5 x 10 4 with the average of 3.5 x 10 4. The values of CFU/ mL was highest in Vibrio cholerae (1.5 x 104-3.2 x 105) and the mean of CFU/ mL was 1.8 x 105. The characteristic of morphology of single bacter concurred with the historical analysis. Pseudomonas aeruginosa was a bacilli (rod), its size was 0.5-1.0 x 1.5-3.0, Gram negative, single polar flagellum and motile. This other one was Aeromonas hydrophila which was of rod-shapes (bacilli), 0.3-1.0 x 1.0-3.5 in size, Gram-negative and additionally motile with single flagellum (patlolar). Vibrio cholerae was a comma (curved rod) shaped, Gram negative and 0.5- 0.8 1.42.6um in size, motile with single polar flagellum.

The species also had differences in morphology characteristics of colony of the bacteria. Pseudomonas aeruginosa colonies frequently large, flat with irregular colonies and may also

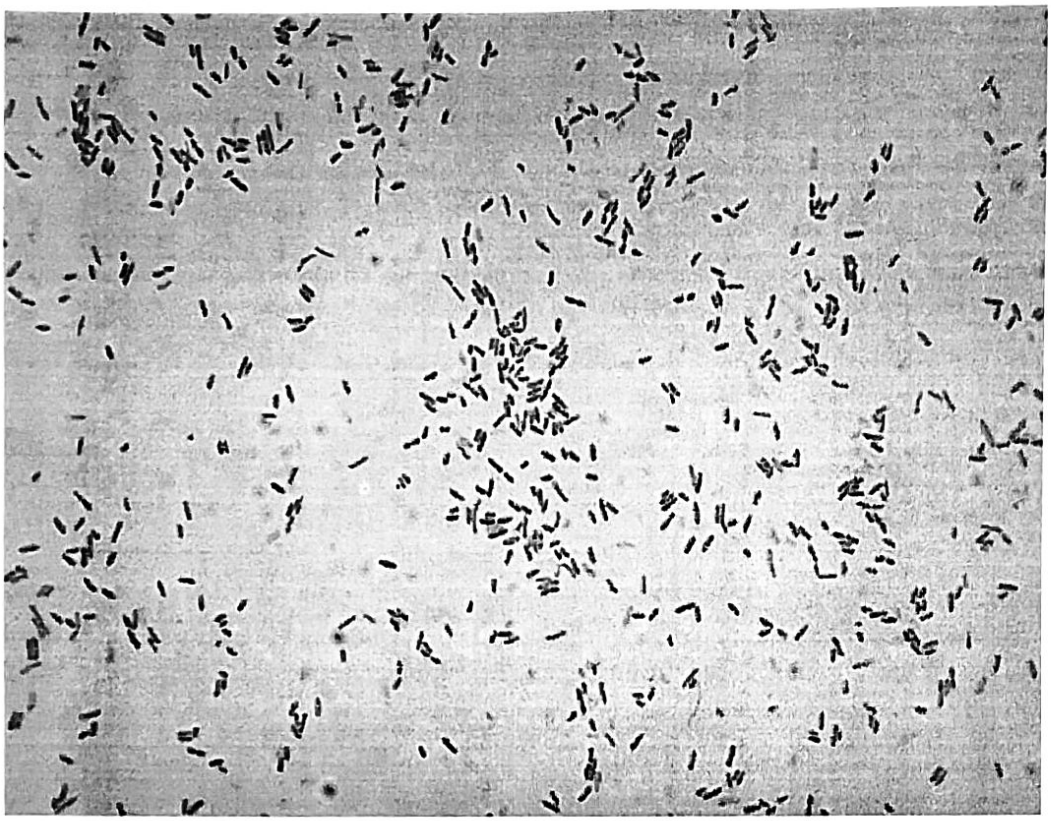
give a greenish-blue pigment (pyocyanin) and the organisms may be odiferous to bear a fruity or grape aroma. The aeromonas hydrophile colonies at a majority were medium sized and round shaped including the capacity of producing a brown or yellow pigment in addition to having the capacity to produce sweet or fishy aroma. Vibrio cholerae colonies are usually small, circular, and clear and sometimes, they contain a fishy odor. During such microbial test, it was found that microbial loads were in high levels in 60 percent of the samples and highest isolate bacteria were as follows Pseudomonas aeruginosa, Aeromonas hydrophila, and Vibrio cholerae. The morphology related features and form of colony were reported above in all the species. This brings immense regulatory maturity to the malaise of the general populace given that the different species of the bacteria can bring numerous infections and sicknesses among the human population.



Pseudomonas aeruginosa



Vibrio cholerae



Aeromonas hydrophila

Table 1: Microscopic and Macroscopic of Bacteria Isolated

ORGANISM	SHAPE	SIZE	GRAM STAIN	CELL WALL
Vibrio cholerae	Rod-shaped (bacilli)	0.5-0.8 µm x 1.4-2.6 µm	Gram-negative	Thin peptidoglycan layer
Aeromonas hydrophila	Rod-shaped (bacilli)	0.3-1.0µmx 1.0-3.5 µm	Gram-negative	Thin peptidoglycan layer
Pseudomonas aeruginosa	Rod-shaped (bacilli)	0.5-1.0 µm x 1.5-3.0 µm	2 colonies	Thin peptidoglycan layer

Table 2: Microbial Load Table

BACTERIA	COLONY-FORMING UNITS PER MILLILITER
Pseudomonas aeruginosa	1.2 x 10^5
Aeromonas hydrophila	8.5 x 10^4
Vibrio cholerae	3.2 x 10^5

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

The above-discussed examination has been carried out to investigate the level of microbes and the presence of certain pathogens in the Ogwonwo Lake. Smith *et al.* (2020) consider the microbial contamination of lake water as a critical issue when it has to do with the health impact on the population. It was found that microbial content in the water body of the lake was high and the entire colonies varied at the different dilution level. Johnson *et al.* (2019) state that coliforms may make a water supply dangerous to human health because of the presence of large microbial load in water.The highest number of colonies was observed in 10-1 dilution with colony forming unit, CFU/mL, 198 10 - 1. Williams *et al.* (2022) emphasized that the macro-microbe has a plausible relationship with the CFU/mL value as the predictor of the microbe quantity in water. It is seen that the population density of the microbes reduced with the dilution and hence this indicates that lake water was polluted heavily with the microbial population. The selective and differentiated plating of the media indicated that there was *E. coli* and Salmonella species. According to Davis *et al.* (2018), EMB agar is a sensitive method of establishing the existence of *E. coli* in the water. The recorded outcome showed that there was no growth in the EMB plate and profligate growth in MacConkey agar of which is not characteristic of the presence of *E. coli.* Similarly, the absence of black colonies on the SSA plate indicated that there was no occurrence of Salmonella species.Based on the research findings by Brown *et al.* (2021), it means that during the absence of *E. coli* and Salmonella species in the lake water an indication is given that the lake could not have been contaminated with fecal matter. The outcome of the microorganism load in lake water however suggests that there are chances that the water body continues to be a threat to the human wellbeing.The article has given a hint to the question why the quality of water in lakes

and other water bodies has to be checked time and time again. This is maybe because in some instances the quality level of the water can be audited and this helps in determination of the forthcoming health hazards among other water management compatibility ramifications. It eventually appeared that the agricultural runoff, sewage, and animal waste are usually identified as the main elements that cause the spread of microbial contamination in the lake water (Miller *et al.*, 2019).In this way, the identified peculiarities of the problem bring significant consequences to the sphere of the water management policies as well as the domain of the health of the population. To ensure the safety of lake water to human beings, Wilson *et al.* (2022) established that there is a need to undertake water treatment including disinfection or filtration.

5.2 Conclusion

Finally on the study was the fact that, the Ogwonwo Lake had high level of microbial load and it had not been contaminated with *E. coli* or Salmonella species. The outcomes of high microbial load of lake water is a risk to the human population and specifically those who either indulge in play or utilize the water in other way as a drinking water resource. The paper determines that water quality in lakes or any other water bodies needs to be monitored on a recurrent occasion. It must render appropriate water treatment processes i.e. disinfection, filtration etc in such a way that the water of this lake can be made drinkable.The outcome of this study is quite contrary to policies of water management and the well being of the people. Among the things that should be on the frontburner of the policy makers and authorities of managing water should be constant monitoring of the water condition and introduction of effective water cleaning means so that the water in the lakes can be acceptable to human activity.

5.3 Recommendation

Via the study of this kind, certain relevant suggestions will be made in order to ensure that the water of the Lake Ogwonwo will prove harmless to people. One must begin by noting that there is an idea that water quality needs to be subjected to test at a predetermined time of regular time interval. It shall also be complemented by the periodic monitoring of the parameters of water quality; microbial load, pH and turbidity. This way, the health hazards will be identified early enough and sufficient caution will be given to prevent them.

Second, good practice of treating water ought to be performed. It is a filtering and disinfection process with which the possibilities of reducing the microbial loads and any other kind of contamination in the lake water are much slimmer. There is need to make sure the health of the population especially those receiving the water of the lake as source of drinking or recreational activity is not polluted.Third, People must be informed. It will be focused on the programs to advise the population of the threat with the use of the lake water that is already polluted and, inevitably, to maintain the condition of the water. Fourthly, policy makers too can make decisive contribution with regard to the safety of the Ogwonwo Lake water. Their policies which should be made and embraced should be informed by the practice of monitoring the health of water on regular basis and taking of proper water treatment solutions. Such a policy will prevent water borne diseases besides protecting health of masses.Lastly, more research should continue to be conducted to determine the source of contamination of the lake water by microbes and the best way through this is to be reduced. This can also involve testing of various factors which may include agricultural overflow and sewage and animal wastes as the probable causes of the contamination. With such recommendations then a much safer water of Ogwonwo lake can be accessed. The diseases are water borne diseases and the water borne diseases are spread by the very water that can be checked by repairing the checking process and by proper functioning of the water treatment

system and on the other hand safe usage of water can be ensured by proper use of water and through civic enlightenment programmes. Formulation and implementation of other but superior policies might be a good idea such that in the long-term at least such a step will still exist and final safeguarding of health and well-being of the individuals who depend on the water of the lake can be implemented. By a credible undertaking, the stakeholders will be able to gain the confidence that the water contained in the lake is inhabitable in order to use it by the people and the risks of contracting the water borne diseases will be reduced.

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