EUTROPHICATION STIMULATION EFFECTS ON RIVER ERUVBI PHYTOPLANKTON

BY

Ijeboi Omole Sefi

SR\1985\RPR\22\72

University Of Benin

Benin City

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IJEBOI OMOMLE SEFI

A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FUILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (BSC.) IN PLKANT BIOLOGY AND BIOTECHNOLOGY

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CERTIFICATION

We certify that this research work was carried out by Sefi O. Ijeboi of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

Signed: **……...** **.....**

PROF M.O. Kadiri Date

Project Supervisor

……

Signed: **.....** Date

Prof D. E. Vwioko

Head of Department

DEDICATION

This work is dedicated to god almighty who has been my help through this phase of my life. Also to my mother Mrs Ayi Ijeboi for her endless help in making sure I achieve all my goals.

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All glory and adoration been giving to God almighty for his benevolent grace and mercy upon my life, He has been a great support for me throughout my university life

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A special thanks to my HOD and all my lectures for impacting me with knowledge, discipline and training throughout my years in the university.

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ABSTRACT

The study eutrophication stimulation effect on river Eruvbi phytoplankton was done to observe the growth response of the phytoplankton to different nutrient enrichment. Water samples were collected from the river site as well as phytoplankton samples. The phytoplankton was subjected to microscopic examination. The study involved natural phytoplankton population and introduced algae of (*Scenedesums acutus* and *Chlorella vulgaris*). The nutrient concentration used were 2.4mg/L nitrate, 1.2 mg/L phosphate and 2.4 mg/L nitrate + 1.2 mg/L phosphate, growth measurement was done optically using spectrometry at 750nm. The physiochemical composition of the river was also carried out, with some done *in situ* and others done in the laboratory. The result of the stimulated experiment showed that indigenous phytoplankton growth was best stimulated by nitrate enrichment, while introduced phytoplankton responded better to phosphate enrichment. The phytoplankton composition of river Eruvbi has a low biodiversity and the algae present show the river to be oligotrophic in nature. Some physiochemical parameters such as dissolved solid, total alkalinity, total hardness and turbidity fell within the WHO guidelines for drinking water, while others like dissolved oxygen and pH were above the WHO guidelines for drinking water. It was concluded from the results that river Eruvbi which is oligotrophic as at present will under eutrophication if subjected to nitrate enrichment which will also cause a rapid bloom in the phytoplankton species of the river. Therefore extra care must be taken to prevent the eutrophication of the river.

CHAPTER 1

1.0. INTRODUCTION

1.1. EUTROPHICATION

The term eutrophication originates from two Greek words ‘eu’ meaning ‘well’ and ‘trope’ meaning ‘nourishment’. Eutrophication is the process by which nutrients enrichment [nitrogen and phosphorus] causes a decline in water quality which leads to an increase in the growth of algae and other aquatic plants which can have a number of negative effects on the environment.

Over several centuries human population growth and its activities have altered landscape, hydrologic cycle and the flux of riverine constituents at increasing rates (Galloway and Cowling., 2002, Galloway *et al.,* 2003, Green *et al.,* 2004, Meybeck., 2003, Seitzinger *et al.,* 2002, 2005, Syvitski *et al.,* 2005). Due to rapid urbanization, industrialization and intensifying agricultural production, water eutrophication has been greatly accelerated by human activities thus increasing the rate of nutrients input in water bodies (Yang *et al.* 2008).

Eutrophication is a global problem aggravated by contamination and other sources of pollution around the world (Mendiondo 2008). A significantly higher amount of nutrients (Nitrogen and Phosphorus) have found their way into the coastal oceans, especially in the last half of the 20th century. By mid 1960s, eutrophication became an evident problem in lakes in countries around the world. The investigation from UNEP (United Nation Environmental Protection) indicates that about 30% - 40% of the lakes and reservoirs have been affected more or less by water eutrophication all over the world. (Yang *et al*., 2008). The magnitude of nutrients mentioned above no longer causes positive response from the ecosystem such as increase in fish production but rather leading to

eutrophication of water, which have symptoms of poor water quality, noxious algal bloom, oxygen depletion and sometimes loss in fish production.

One of the main effects of eutrophication on phytoplankton is an increase in their growth. This is because nutrients, such as phosphorus and nitrogen, are essential for phytoplankton growth. When the nutrients are present in excess, phytoplankton can grow rapidly, leading to blooms. Phytoplankton blooms can have a number of negative effects on the environment. They can block light penetration, which can lead to death of underwater plants and animals. They can also produce toxins that can harm humans and animals. In addition, phytoplankton blooms can deplete oxygen levels in the water, which can lead to fish kills. The effects of eutrophication on phytoplankton can be seen in many lakes and rivers around the world. In some cases, these effects have been so severe that they have lead to the closure of beaches and swimming areas.

1.1.1. FACTORS INFLUENCING EUTROPHICATION

Eutrophication is a common stress that involves the addition of nutrients to water bodies. Eutrophication which is basically a chemical stress is categorized according to the sources: point source, non-point source, long range atmospheric transport.

Point source includes sewage, industrial discharges and municipal waste water.

Long range atmospheric transport of contaminants, such as sooth, dust, ash etc. which is the most difficult to measure and control (Mendiondo, 2008).

Non – point sources such as runoff from agriculture, construction site and urban areas. The most important of non – point's sources include the wet and dry deposition from the atmosphere, land erosion, weathering of minerals and anthropogenic sources. An important driver of non – point

nutrient input is the excessive application of fertilizers and manure which causes phosphorus to accumulate in the soils (Bennett *et al,.* 2001). Today, non point has become a major factor to water quality degradation. Agricultural activities are the major agents for nutrient and sediment exports, which increases the rate of eutrophication in surface water.

There are a number of things that can be done to reduce the effects of eutrophication on phytoplankton. One important step is to reduce the amounts of nutrients that enter water bodies. These can be done by reducing the use of fertilizers and manure on the land, and by improving water waste treatment. In addition, it is important to protect wetlands and other areas that filter nutrients from the water. By taking these steps, we can help to reduce the effects of eutrophication on phytoplankton and protect our water resources.

1.1.2. EFFECT OF EUTROPHICATION ON WATER BODIES

Eutrophication which results in an excessive growth of phytoplankton's and their subsequent death form a greenish slime layer over the surface of the water body. The slime layer reduces light penetration and reduces reoxygenation through air currents. The death and decay of aquatic plants produces a foul smell and makes the water more turbid. (Beeby, 1995; Roa, 1998).

Eutrophication of drainage ditches by over fertilization of nitrogen and phosphorus causes a shift in mainly submerged aquatic vegetation to a dominance of floating duckweed. This could result in anoxic conditions, loss of biodiversity and hampering of the agricultural functions of such ditches (Janse & Puijenbroek., 1998). Change in eutrophic conditions can be observed in the occurrence pattern of distribution and diversity of biotic community (Tiwar., 1998).

Many natural water bodies are described as oligotrophic. Oligotrophic water bodies contain less than 5-10µg L^{-1} of phosphorus and less than 250 -600 µg L^{-1} of nitrogen. The primary productivity

in oligotrophic waters is reported between 50 -300 mg Carbon m^{-2} day^{-1}. In moderately eutrophic bodies the phosphorus content is 10 – 30 µg L^{-1} and nitrogen content is 500 – 1100 µg L^{-1}, primary productivity is reported to be above 1g Carbon $m^{-1}day^{-1}$ (Likens *et al.,* 1977).

1.1.3. BIOINDICATORS OF EUTROPHICATION

Bioindicators act as a means to measure environmental conditions. They are also called 'ecological indicators'. They are used to observe the functioning and cause – and – effect relationships within an ecosystem. Bioindicator of eutrophication may be single species or a variety of several species. Indicators for eutrophication differ in rivers and lakes. For a river ecosystem bioindicators describe the diversity and occurrences of life cycles, phytoplankton, aquatic vegetation and fishes play an important role as indicators of eutrophication. Some biotic components used as bioindicators of eutrophication include; algae, macrophyte, diatoms, plant pigments, ecosystem functioning

1.1.4. CONTROL MEASURES OF EUTROPHICATION

Phosphorus is one major nutrient which controls algae growth. Reduction of the amount of phosphorus in water bodies, greatly reduce the growth rate of aquatic plants and phytoplankton. Also removal of bloomed algae which is phosphorus bounds and sediments before anoxic state from the bottom of rivers may also be of great significance in the remediation of eutrophic waters. Other control measures include;

Biological control: This involves the use of algal, macrophyte to reduce phosphorus and nitrogen quantities in the river. Also phytoplanktivorous fishes can be for used weed management. Periphyton can also be maximized.

Mechanical control: Sewage, fertilizers and detergents effluents should be treated to minimize the

phosphorus and nitrogen content before being discarded into water bodies.

Legislative measures: Laws should be passed out against the use of phosphate detergents or equivalent laws should be placed to restrict the use of such detergents. As these detergents are major sources of phosphate nutrients enrichment in water bodies.

1.2. LITERATURE REVIEW

Anderson *et al.,* (2005) carried out a research on eutrophication of rivers using an ecological perspective in the United Kingdom (England and Wales). This report provided an overview on the ecological imparts of eutrophication in rivers. It established the causes of eutrophication in rivers, which include agricultural runoff, sewage discharge, and atmospheric deposition, while also giving an imparts of eutrophication on the ecology (aquatic ecosystem), which includes reduced biodiversity, changes in water quality, increased in the growth rate of phytoplankton and other aquatic plants, harm to fish and other aquatic animals and an increases in health hazard to humans. They concluded with the challenges of managing eutrophication in rivers and the approaches which can be implemented to help with this problem, which includes; reduction of nutrients inputs in water bodies [rivers], improving of water quality, restoring river natural habitats and educating of the public about the dangers of eutrophication.

Haer *et al.,* (2018) reported on the responses of aquatic plants to eutrophication in rivers using a conceptual model. The model working with previous knowledge on this topic inputted new findings on the role of physical habitat in curbing the effects of eutrophication on aquatic plants. While observing three main pathways in which eutrophication can have an effect on aquatic plants, which include:

Nutrient enrichment: an increase in the nutrient level of rivers, leads to a drastic increase in the growth rate of algae which outcompetes the aquatic plants for light and nutrients.

Physical habitat: changes in the physical habitat like turbidity has an effect on the growth of aquatic plants, as increases in turbidity can reduces light penetration which in turn reduces photosynthesis of the plants.

Multiple-stressor interactions: eutrophication being one sources of stress on the aquatic ecosystem, as a result interaction with other stressor like pollution or climatic change can occur which will have further impact on the ecosystem [plants]. Using this model to identifies some responses of aquatic plants to eutrophication, which include tolerance and sensitivity.

The report carried by Wayne *et al.,* (2019) on the nutrients, eutrophication and harmful algal blooms along freshwater to marine continuum, by using current knowledge about the relationship between eutrophication and harmful algal bloom (HABs) in freshwater and marine ecosystem they provided an overview on role of eutrophication on HABs, by giving a link between nutrient enrichment and an increase in algal growth and toxin production. They reported on the challenges faced in managing eutrophication and HABs, while also giving an approach in cubing these challenges. Some of these approaches includes reduce nutrient levels i.e. agricultural practices, storm water management and wastewater management, as well as the challenge of monitoring HABs. It was concluded with a charge for the need for more research on eutrophication and HABs, while highlighting on the need to understand the link between nutrient enrichment, algal growth and toxin production and also highlighted on the need to develop more effective means of managing eutrophication and HABs.

Astuti *et al.,* (2022) researched on the water quality and eutrophication in Jatiluhur reservoir, West

Java, Indonesia. Water samples were collected from nine station in the reservoir and analyzed using different parameter (transparency, total phosphorus, temperature, pH, conductivity, chlorophyll-a, and dissolved oxygen). Carlson Trophic State Index (TSI) was used to analyze the eutrophication status of the reservoir. The result showed the reservoir to be eutrophic with its TSI vale ranging from 4.4 to 5.3, which indicates a high level of nutrient enrichment. The results from the parameters showed the reservoir had high temperature, pH was shown to be alkaline, dissolved oxygen was very low and the total phosphorus and chlorophyll-a concentration were high which are always expressed in a eutrophic condition. The poor water quality was attributed to a number of factors, including

- The inflow of nutrients from the Citarum River, this is the most polluted river in Indonesia.
- Discharge of agricultural and industrial wastewater in the catchment area of the reservoir.
- Growth of aquatic plants which consumes oxygen and release nutrients into the reservoir.

A number of negative consequences due to the eutrophication of the reservoir were giving by the authors, including

- Death of aquatic life and fishes
- Growth and spread of harmful algal blooms.
- Contamination of drinking water.
- Degradation of the ecosystem.

The authors concluded with a number of recommended measures to help improve the water quality of the reservoir which includes: reduction of the inflow of nutrients from the Citarum River, treatment of wastewaters before discharge, controlled growth rate of aquatic plants, increase awareness of the dangers of eutrophication to the public and policymakers.

González *et al*., (2019) investigated the relationship between eutrophication and phytoplankton: some generalities from lakes and reservoirs in Americas. It was noted that eutrophication has a direct effect on the increase in the biomass and community composition of phytoplankton. It also gave some ecological consequence of eutrophication, ranging from change in the food web, to a decrease in water quality, to development of harmful algal bloom. It augured on a combination approaches needed for the management of eutrophication, some of which include reduces inputs of nutrients n water bodies, restoring of damaged ecosystem, control of algal blooms. The report concluded by stating how eutrophication is problem to lakes and reservoirs in America while calling for necessary action to be taking in curbing these challenges.

Following a research carried out by Rabalais *et al.,* (2007) on the 'sediments tell the history of eutrophication and hypoxia in the northern gulf of Mexico' it discussed the role of sediments in eutrophication processes, it also reported on the use of sediments cores in the study of eutrophication in the northern Gulf of Mexico. The study also found that nutrients levels in the northern Gulf of Mexico has been on the increase since 1950s while linking these rise to the increase flow of nutrients from the Mississippi river. The report concluded on the severity of eutrophication in the northern Gulf of Mexico, with these problems increasing in the nearest future. It also sounded an alarm on the need to reduce flow of nutrients from the Mississippi river which in turn reduces the nutrients input in northern Gulf of Mexico.

Dodds *et al.,* (2015) analyzed the role of nitrogen, phosphorus, and eutrophication in streams. They reviewed the evidence of nitrogen (N) and phosphorus (P) as important factors in the growth rate of algae in streams. this was done by citing studies on the correlation of algal biomass to the concentration of N and P in water column, also cited studies on the synergistic effects of N and P on the algal growth, which means that the combination effects of both nutrient is greater than their

individual effects. They also reviewed on the effects of both nutrients on the eutrophication of streams, also stating that the relationship of N and P on the growth rate of algae is not linear, rather it depends on other factors which could include intensity of light and water temperature among others. The authors also stated that both nutrients should be considered in the managements of eutrophication as it is difficult to determine which nutrients is the limiting factor of that stream, but considered P to be the more limiting nutrient in eutrophication as it is less soluble in water than N which makes it easily lost in water column. It was noted that in eutrophication management phosphorus control will likely be more effective.

An overview by Conley *et al,.* (2016) on the controlling of eutrophication: nitrogen and phosphorus discussed on the role of these nutrients in the eutrophication process and augured on the importance of these nutrients on algal growth. It was sated that phosphorus is considered to be the more limiting nutrients in many aquatic ecosystems. The authors stated the challenges of eutrophication control, by pointing out that these nutrients comes from a variety of sources (agricultural runoff, sewage discharge and atmospheric deposition), stating that a combination of approaches is needed for the control of eutrophication and the effectiveness of these control measures is dependent on specific circumstances. These control measures includes; reduced nutrient inputs, improvement of water quality by treating, creation of artificial wetlands for the absorbing of nutrients, promotion on the use of less polluting agricultural practices.

Domingues *et al.,* (2011) investigated on ammonium, nitrate and phytoplankton interactions in freshwater tidal estuarine zone: potential effects of cultural eutrophication. It was found that phytoplankton in this ecosystem preferred ammonium over nitrate, and this prefers is group-specific. Green algae and cyanobacteria were found to prefer ammonium, while diatoms and dinoflagellates have a preference for nitrate. They also found that ammonium can inhibit the uptake of nitrate, with

this inhibition more pronounced at a level of low light. They discussed on the potential implication of these findings on cultural eutrophication, it was suggested that cultural eutrophication can lead to an increase in the abundances of ammonium loving phytoplankton which in turn can have a negative effect on the ecosystem as the prevalent of these phytoplankton which is the cyanobacteria produces toxins which are harmful to humans and other organism. It was concluded with an argument on the importance of understanding the roles and effects of cultural eutrophication on the ecosystem and a need for more research to be done on this topic.

A report by Frost *et al*., (2010) on eutrophication research impact assessment gave an assessment on the impact Water Research Commission (WRC) research on eutrophication in South Africa. The report showed that WRC had a significant role in the understanding and management of eutrophication in South Africa. The report highlight some achievement of the WRC research: improvement in the understanding of the causes and consequences of eutrophication, development of new methods for monitoring and managing of eutrophication, helped in the development of policies and regulation addressing eutrophication, raising awareness about eutrophication problem among stakeholders. The report concluded that WRC research has made a significant contribution which has helped in the understanding and managing eutrophication in South Africa, it also gave a number of recommendations on how the WRC could improve its impact eutrophication research.

Erhumwunse *et al.,* (2013) discussed on managing eutrophication in Nigeria inland waters, by giving the causes of eutrophication in Nigeria [agricultural runoff, swage discharge and industrial wastewater], while also giving the consequences of eutrophication in Nigeria waters, these includes decreased of water clarity, decreased of biodiversity, increases algal blooms, and fish kills. While also stating the potential health risks associated with eutrophication because of the toxins produces by some algae. The authors gave a number of strategies in the management of eutrophication in

Nigeria, they includes reducing the inputs of nutrients into water bodies, using of treatments to improvement of water quality, use of less agricultural practices creating artificial wetlands for nutrient absorption, and raising public awareness of the problem of eutrophication. The authors called for concerted efforts in addressing the problems of eutrophication in Nigeria.

Ugochukwu *et al.,* (2019) investigated the eutrophication potential of nutrients in Oij River in Enugu, Nigeria using various methods to assess the nutrient level in the river, which are electrical conductivity (EC), pH, total dissolved solids (TDS), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), dissolved nitrate (NO3-N), nitrite (NO2-N), ammonium NH4-N, phosphate (PO4-P) and silica (SiO2-Si). It was found that Oji River has a high nutrient level and is at a risk of eutrophication. The main sources of nutrient enrichment in the river are agricultural runoff, sewage discharge and industrial wastewater. It was found that the algal biomass is high which indicates a risk of further eutrophication. Using a water quality model the author's stimulated the effects of different nutrients loading scenarios on the eutrophication potential of the river, the results showed that even a small increase in the nutrients loading could lead to a significant increase in the algal biomass and eutrophication. It was conclude that Oji River is at a high risk of eutrophication and it's a serious threat to the rivers ecosystem, while also recommending measures to reduce the nutrient loading of the river.

Ihejirika *et al.,* (2011) invested on the seasonal influences on levels and eutrophication potential of nutrients in Imo River, Nigeria using various method including chemical analysis, algal biomass measurement and water quality modeling to asses nutrients level of the river. The found that the nutrient level of the Imo river varies with season, with the highest level during the rainy season. The main source of nutrients are agricultural runoff, industrial wastewater and sewage discharge, it was also found that the algal biomass in the river varies with the seasons with the highest during the

rainy season. The use of a water quality model to stimulate the effect of nutrients loading on the eutrophication potential of the river showed that a small increase in the nutrient loading on the river will lead to a significant increase in the algal biomass and eutrophication of the river.

An investigation was carried out by Ekere., (2012) on the level of nitrates in the water sources in rural communities in Uzouwani, Nigeria, by using various assessment methods like chemical analysis and spectrophotometer he was able to determine the nitrate levels in the water bodies. It was found that nitrate levels in the water sources are high and exceed the world health organization's (WHO) guideline value for drinking water. Main source of nutrients inputs include agricultural runoff, industrial wastewater, sewage discharge, nitrate level was found to vary with season with the highest season during the rainy season. He concluded that the level of nitrate in the water sources are a serious health risk to the inhabitants of Uzouwani and measures should be taken to reduce the nitrate level.

Investigation was carried out by Ude et al., on the trends in nitrate-nitrogen, nitrite-nitrogen and phosphorus concentration of the Ebonyi River in Nigeria. By using various method including chemical analysis and spectrophotometric analysis they were able to assess the nutrient level of the river. The nutrient level of the river was found to have increased over time which is likely due to the increased in agricultural and industrial activates in the area. The nutrient level was found to vary with season, with the highest point during the rainy seasons due to and increased in agricultural runoff. They concluded that the increases nutrients level of the Ebonyi River is of great concern which could lead to eutrophication of the river. Recommend measures were giving to control the nutrient level of the river.

Olu., (2021) investigated on the concentration of nitrate in groundwater used by residents around a

dumpsite in Lagos, Nigeria and the health risk associated with exposure to nitrate. It was found that the concentration of nitrate in the dumpsite was high and exceeded the World Health Organization's (WHO) guideline value for drinking water. The main source of nitrate in the groundwater is likely the dumpsite which is a major source of organic material that can be converted to nitrate by bacteria. The concentration of nitrate in the groundwater was proportional to the distance from the dumpsite. This means the closer a house is to the dumpsite the higher they are exposed to high level of nitrate in the drinking water. Using a health risk assessment model to estimate the health risk associated with high nitrate intake, showed that exposure to nitrate from the groundwater around the dumpsite could increases the risk of methemoglobinemia, this causes a condition called cyanosis (blue skin) and death in infants, he also found that the health risk associated with the consumption of the groundwater contaminated with nitrate is higher in infants and pregnant women. He concluded that the high level of nitrate pollution in the groundwater around the dumpsite is a serious health risk to residents who drinks the water and recommend that measures should be taken to reduce the nitrate level.

An investigation carried out by Adesuyi *et al.,* (2015) on nitrate and phosphate pollution in surface water of Nwaja Creek, Port Harcourt, Niger Delta, Nigeria using a variety of methods including chemical analysis and spectrophotometric analysis, it was found that the nitrate level of the creek was high and exceeded the World Health Organization's (WHO) guideline value for drinking water. They also discovered that the nitrate and phosphate level varies in season with the highest point during the rainy season. They concluded that the high level of nitrate and phosphate poses a serious risk to the ecosystem of the creek and recommended some measures to put in place to reduce the risk.

Investigation by Omoruyi *et al.,* (2018) 0n the concentration level of phosphate and nitrate in

Ikpoba river of Edo state, Nigeria, by using different methods including chemical analysis and spectrophotometric analysis to assess the nutrient level of the river. They found concentration level of phosphate and nitrate in the Ikpoba River are high and exceed the Health Organization's (WHO) guideline value for drinking water. It was also found that the concentration of the nutrients varies with season with the highest level during the rainy season. It was concluded that the high concentration level of phosphate and nitrate in the river is a serious threat to the ecosystem and measures should be taken to reduce it.

Akhere *et al.,* (2020) investigated the use of phytoplankton for the determination of the trophic status and portability of Aiakhuakhuari River, Benin cit, Edo state, Nigeria. By using a variety of methods including phytoplankton composition, chlorophyll-a concentration, and nutrient levels, the river was found to be eutrophic and the dominant phytoplankton composition of the river to be cyanobacteria, which is an algae that produces toxins. They concluded with some recommended measures to take in the management of the nutrient loading in the river, while also emphasize on the risk the eutrophic condition has on the river ecosystem.

1.3. AIM AND OBJECTIVES

The aim of these study was to determine the susceptibility rate of river Eruvbi to eutrophication and its effects on the phytoplankton composition

OBJECTIVES

The objectives of the study were to:

1. Determine the responses of introduced phytoplankton to nutrient enrichment;
2. Examine the response of the natural phytoplankton to nutrient enrichment.; and
3. Assess water quality of the river.

CHAPTER 2

2. MATERIALS AND METHODS

2.1. STUDY AREA

River Eruvbi is located in Iguosa community, between latitude N 6^{0}27’08” of the Equator and longitude E 5^{0}36’37” of the Greenwich meridian, in Ovia East local government area of Benin City, Edo state, Nigeria at about 15 kilometer from the Benin City center. The river is fast flowing and turbulent, with some clear and transparent portions and the other portions turbid with lots of suspended particles and dissolved materials which is said to be from 7-up bottling company situated close to the vicinity. It has a sandy river bed with some patches of clay stones. The river is said to have shrunk from its original size of about 100 meters to its current size of about 2 meters in size due to serious gully erosion upstream, this is seen by the large area of low-laying flat land which is devoid of vegetation surrounding the river on one side. The most conspicuous plant species found within the area, is the palm tree (*Elaeis guinesis*), others include aquatic macrophyte (*Nymphaeae lotus*), some grasses like (*Panicum maximum*) and also several trees and climbing plants. The geology is identified by the top reddish earth and weathered sedimentary rock which is composed of ferruginized clay sand with an undulating topography. Human activities consist of normadism

within the catchment area down-hill, with faming activities, fetching of fire wood among others up-hill. The settlement within the area is rapidly developing with modern architecture and is relatively organized, the river servers as a source of drinking, irrigation and religious purposes for the inhabitants which live around the area.

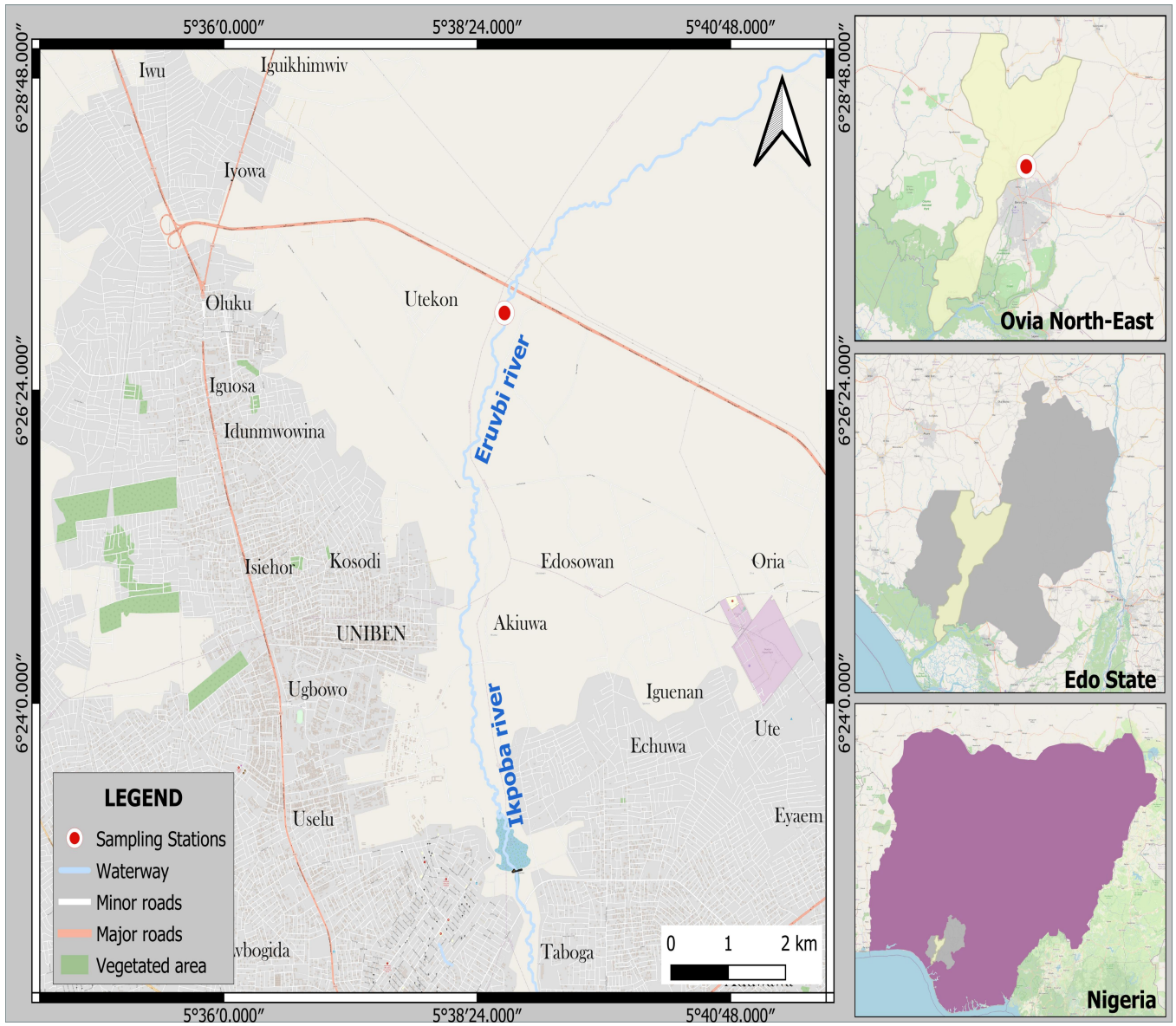

FIGURE 1: MAP OF STUDY AREA SHOWING SAMPLING LOCATION

PLATE 1: RIVER ERUVBI.

2.2. SAMPLE COLLECTION

Water samples was collected from river Eruvbi on the 11th of July, 2023 using two 20L containers which were placed horizontally on the surface of the water until they were filled up, they were labeled and taken to the laboratory for analysis. The Dissolved Oxygen (DO) water samples were collected in small dark sample bottles, this was achieved by completely immersing the sample bottles in the water until it was filled and stopped bubbling. The DO bottles were fixed instantly with 1ml each of Winker A and Winker B reagents, then covered and shaked about 5-6 times.

Phytoplankton were also collected from river Eruvbi into 50cl bottle from the water surface and preserved with few drops of formalin

2.3. EFFECTS OF NUTRIENTS ON NATURAL PHYTOPLANKTON

Water samples were taking to limnology laboratory university of Benin. 1800ml of the water samples were measured using a measuring flask which was divided into nine 35cl bottles each containing 200ml of water samples; this was labeled as ‘control’. 1800ml of sample water was measured and placed in a conical flask and 2.4 ml of nitrate was added which was divided into nine 35cl bottles each containing 200ml of water and was labeled ‘N’. 1800ml was measured and 1.2ml of phosphate was added and divided into nine 35cl bottles containing 200ml of water labeled as ‘P’. 1800ml was measured with 2.4ml of nitrate and 1.2ml of phosphate added and divided into nine 35cl bottles labeled ‘NP’ containing 200ml of water. These samples were placed under the sun to allow photosynthesis occur and were checked every two days using a spectrophotometer with a wavelength of 750nm. The readings were recorded and were used to determine the growth rate of the natural phytoplankton in each water samples.

2.4. EFFECTS OF NUTRIENTS ON INTRODUCED PHYTOPLANKTON

The water samples were filtered using a filtering machine and 10ml of phytoplankton [Scenedesums A.] was added [introduced phytoplankton - IP], 1800ml of the filtered water sample with IP was measured and divided into nine 35cl bottles labeled as 'Scenedesums control' which contained 200ml of the samples. 1800ml of samples water with IP was measured which was spiked with 2.4ml of nitrate and divided into nine 35cl bottles of 200ml each labeled as 'Scenedesums N'. 1.2ml of phosphate was added to 1800ml of water samples with IP and was divided into nine 35cl of 200ml each labeled 'Scenedesums P'. 2.4ml of nitrate and 1.2ml of phosphate was added to 1800ml of water samples with IP and divided into nine 35cl bottles of 200ml each labeled 'Scenedesums NP'.

This was also done for Chlorella V. with each labeled as [Chlorella control, Chlorella N, Chlorella P, Chlorella NP]. Both samples were placed under the sun for photosynthesis to occur and were checked every two day using a spectrophotometer with the wavelength of 750nm. This were recovered and used in determining the growth rate of the introduced phytoplankton [Chlorella V. and Scenedesums A.].

PLATE 2: SAMPLE BOTTELS.

PLATE 3: HACH 2000 SPECTOPHPOTMETER

PLATE 4: FILLTERING MACHINE.

2.5. PHYSICAL PARAMETERS

2.5.1. Air and Water Temperature

This was done in situ using a mercury-in-glass thermometer. The air temperature was measured by holding the thermometer above the river and recorded when the mercury ball was stable. The water temperature was measured by placing the thermometer in the river with the mercury ball completely immersed, the temperature was recorded thereafter. Both temperatures were recorded in Celsius (^{0}C).

2.5.2. Total Dissolved Solids (TDS)

This was measured using a HACH Conductivity/TDS/Salinity meter. A small quantity of the water sample was placed in a conical flask and the HACH Conductivity/TDS/Salinity meter probe was dipped into it. The meter was set to record TDS and the TDS was recorded in mg/L.

2.5.3. Turbidity

Turbidity of the samples was measured using HACH DR 2000 spectrophotometer. Samples were well shaken before measurements were taken; the spectrophotometer was used on a wavelength of 750nm. Two cuvette was used with one having distilled water for blanking and the other with 20ml of the sample water and placed in the machine for measurement. The turbidity result was recorded in NTU.

2.5.4. Colour

The water sample true colour was measured using a HACH DR 2000 spectrophotometer. The samples were not shaken before measurements were taken, with the spectrophotometer on a

wavelength of 750nm. Distilled water was used for blanking and 20ml of the sample water was placed in the machine. Colour values were recorded in Platinum Cobalt units (PtCoU).

2.6. CHEMICAL PARAMETERS

2.6.1. pH

pH was measured using an HANNA pH meter. A little quantity of the water samples was placed in a conical flask and the probe of the pH meter was inserted, the result was displayed on the pH meter and recorded when stable.

2.6.2. Conductivity

This was measured using a HACH Conductivity/TDS/Salinity meter. The probe was placed into a conical flask containing the water sample after setting the meter on conductivity. The record was displayed on the meter and recorded. Conductivity was measured in µS/cm.

2.6.3. Dissolved Oxygen (DO)

Fixing of DO was done *in situ* by adding 1ml of Winkler A (Manganous sulphate) and 1ml of Winkler B (Alkaline iodide). In the laboratory, 2ml of concentrated sulphuric acid was added to the DO sample and thoroughly mixed to get a pale yellow colouration, 20ml of the digested sample was measured, few drop of the indicator (starch) was added which turned the sample blue-black, and the solution was titrated with 0.02M of Sodium thiosulphate until a colourless liquid was gotten. Dissolved Oxygen was then calculated with the formula;

$$\text{Dissolved Oxygen (mg/L)} = \frac{A \times N \times 8 \times 1000}{\text{Volume of water sample (20ml)}}$$

Where A= Average titre value

N= Normality of titrant

2.6.4. Total Alkalinity

Total alkalinity of the water sample was measured using titration, 20ml of the water sample was measured and few drop of methyl orange indicator was added. The sample was titrated with 0.1M of sulphuric acid until there was colour change from orange to pink. Total alkalinity was measured with the formula;

$$\text{Total Alkalinity (mg/L)} = \frac{A \times N \times 50{,}000}{\text{Volume of water sample (20ml)}}$$

Where A = Average titre value

N = Normality of titrant

2.6.5. Total Hardness

Total hardness of water was determined using EDTA titration method. 20ml was water sample was measured into a conical flask and 1ml of Ammonium buffer solution was added, few drops of the indicator (Eriochrome Black T) was added and titrated with 0.01M of EDTA until colour changed from purple to blue end point. Total Hardness was calculated with the formula;

$$\text{Total Hardness (mg/L)} = \frac{A \times 1000}{\text{Volume of water samples (20ml)}}$$

Where A = Average titre value

2.6.6. Sulphate

Sulphate value was determined using the turbidimetric method. A pinch of barium chloride was added to 20ml of water sample. A timer of 5 minutes was set on the HACH DR 2000 spectrophotometer to allow the $BaCl_2$ to dissolve in the water sample; a wavelength of 680nm was set. The original water sample was used for blanking; the cuvette with prepared sample ($BaCl_2$ × water) was placed in the machine. The sulphate valve was displayed on the machine and recorded in mg/L.

2.6.7. Nitrate

Nitrate content of the water sample was measured with the cadmium reduction method by a HACH 2000 spectrophotometer. For this 20ml of the water sample was measured into two cuvette, with one for blanking and to the other NitraVerS Nitrate reagent Powder Pillow was added. The colorimeter was set to measure NO_3^- and a 5 minutes timer was set on the machine. The prepared sample cell was placed in the cell holder after being wiped clean. The result was displayed on the machine and recorded in mg/L.

2.6.8. Phosphate

The phosphate content of the water sample was measured using the ascorbic acid method with the HACH colorimeter. This entailed taking 15ml of ascorbic acid solution was mixed with 10ml of sulphuric acid, 2.5ml potassium antimonyl tartrate solution and ammonium molybdate solution. 4ml of the test reagent was added to 20ml of the water sample and mixed thoroughly and left to stand for 5min then poured into the cuvette. The spectrophotometer was set on PO_4^{3-} and the result was recorded as in mg/L.

CHAPTER 3

RESULTS

3.1. RESPONSE OF INDIGENOUS PHYTOPLANKTON TO NUTRIENT ENRICHMENT

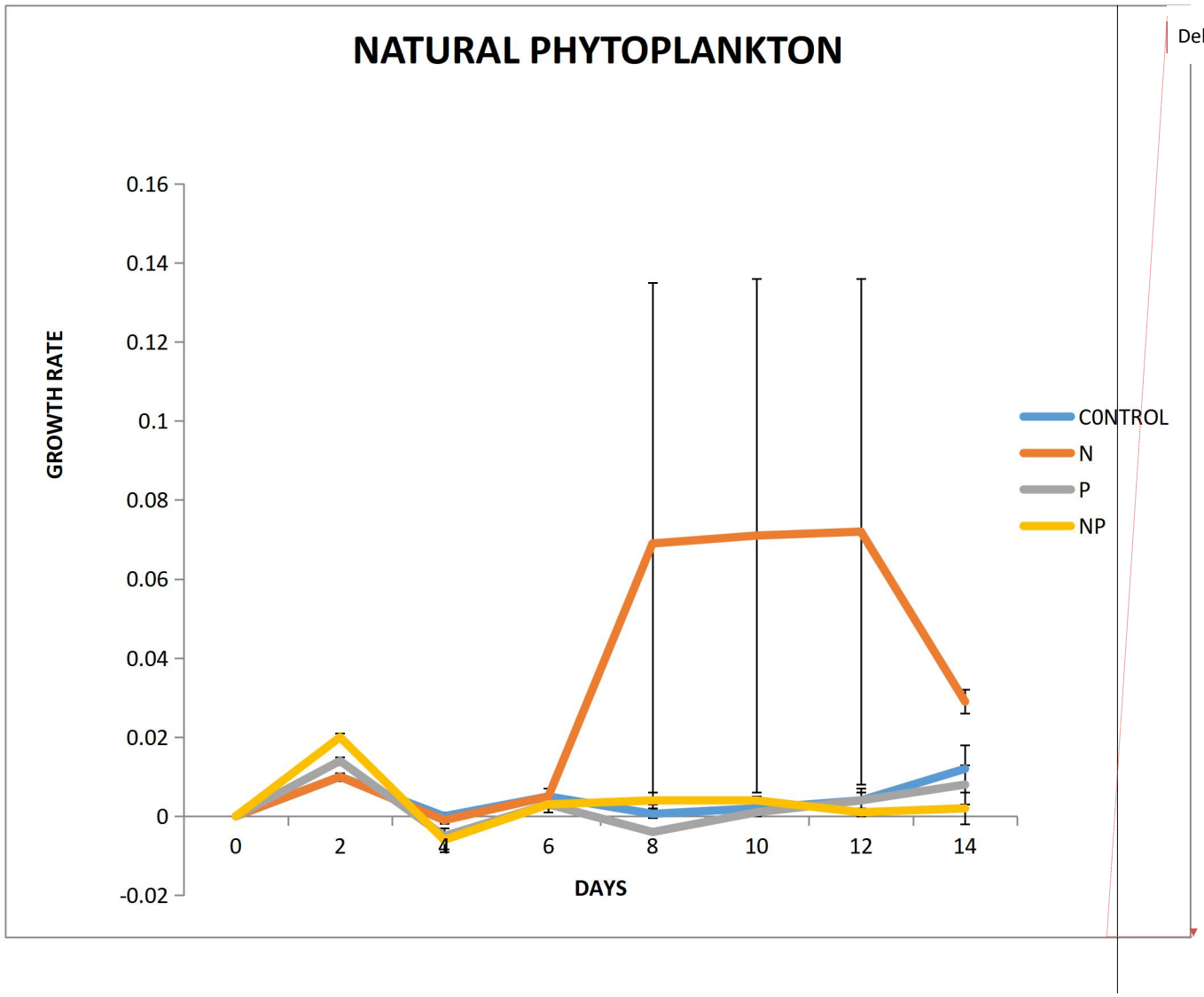

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Fig 2. Growth response of natural phytoplankton to nutrient enrichment

The above line graph shows the growth rate of natural phytoplankton against number of days. Four water samples were used for the experiment which were labeled as control, nitrate [N], phosphate [P] and nitrate-phosphate [NP]. All experiment started growth from day 0 with NP having the highest growth by day 2, followed by P, while control and N had the lowest growth. By day 4 there was a decline in growth by all parameters with NP as the lowest. A slight increase was seen by all parameters between day 4-day 6 with NP as the lowest, on day 6 N had a very drastic increases in growth to a point of 0.069, and remained on a slow steady growth of about 0.003 growth from day 8 to day 12, and at day 12 there was a drastic decline in growth which ended on day 14.

3.2. RESPONSE OF INTRODUCED PHYTOPLANKTON TO NUTRIENT ENRICHMENT

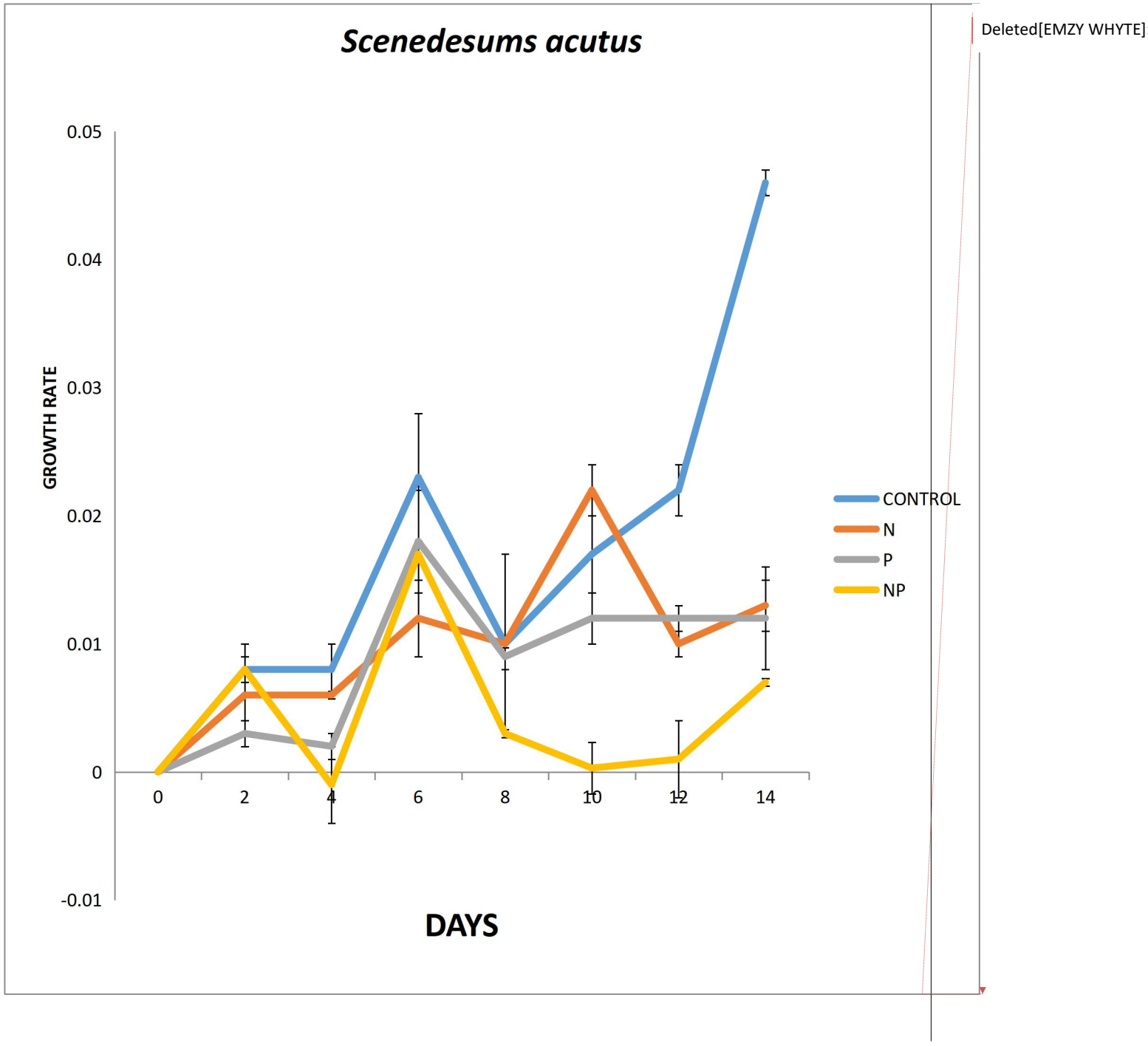

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Fig 3. Growth response of *Scenedsums acutus* to nutrient enrichment

The above line graph showed the growth rate of *Scenedesums acutus* against the number of days cultured. The different treatment were experiment and were labeled control, nitrate [N], phosphate [P], and nitrate-phosphate [NP]. Growth rate was observed from day 0 with a steady increase in day 2, P had a low growth rate at day 2 followed by NP as the lowest. From day 2-day4 there was a steady growth in control and N, while P had a very slight decrease and NP had a decline in growth. From day 4 there was an increase in growth for all samples till day 6. From day 6 to day 8 a decline in growth were observed with NP as the lowest. From day 8 each sample had a different growth curve, control had a steady increase from day 8 to day 14, N had an from day 8 to day 10 and reduced on day 12 then had a slight increase on day 14, in sample P a slight growth to a point of 0.012 which remained steady till day 14, sample NP had a decline in growth in day 10 which continued till day 12 and at day 12 there was an increase till day 14.

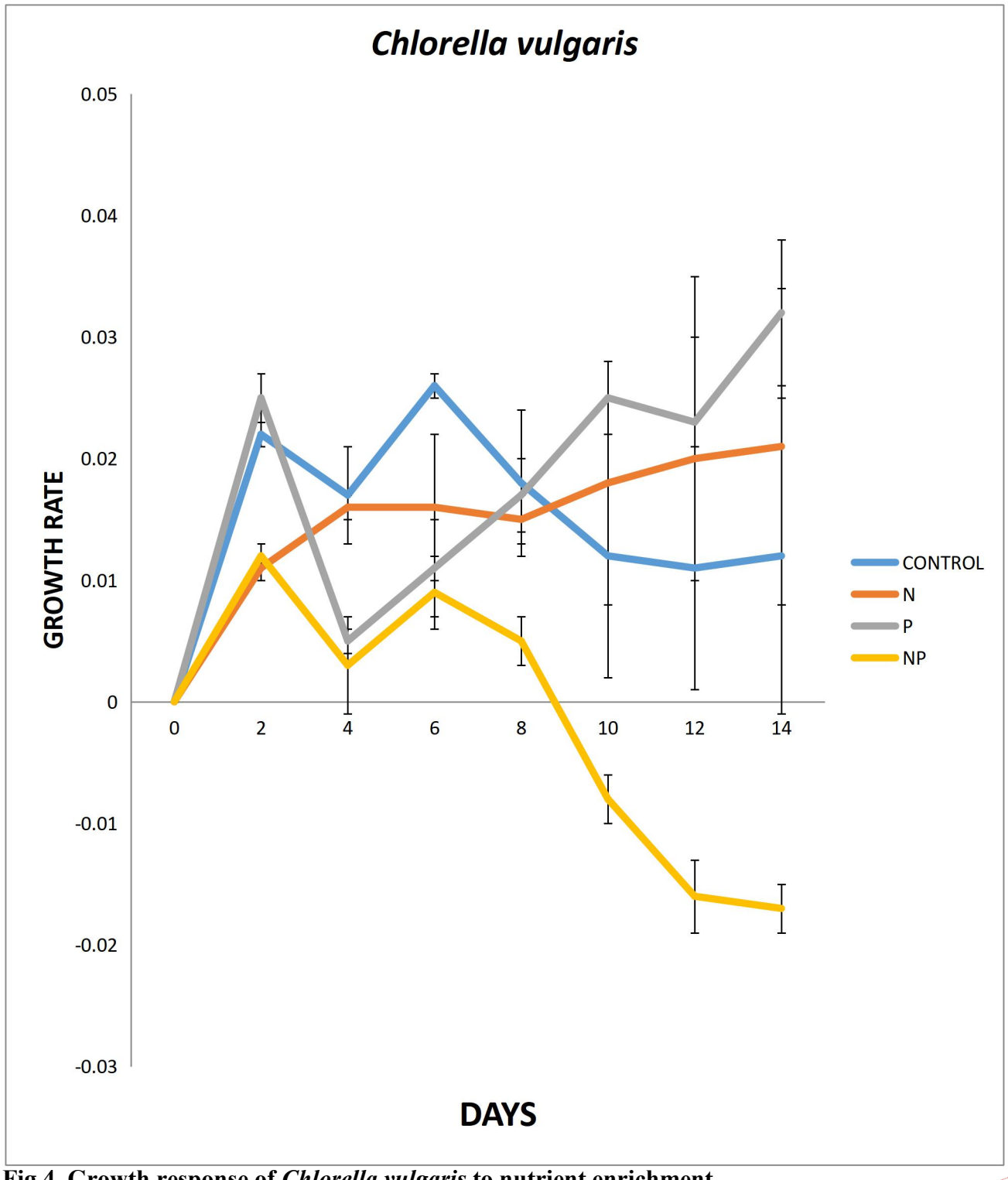

Fig 4. Growth response of *Chlorella vulgaris* to nutrient enrichment

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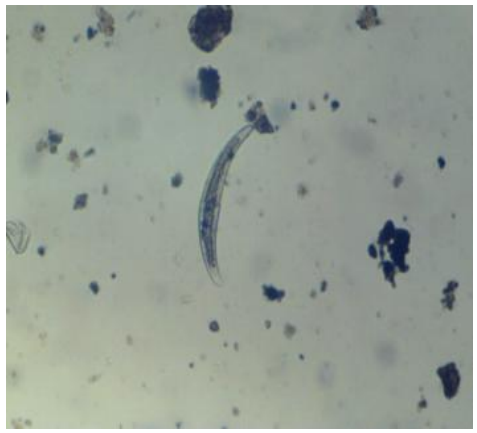
The above line graph depicts the growth rate of *Chlorella vulgaris* against the number of days cultured. The treatment were labeled control, nitrate [N], phosphate [P] and nitrate-phosphate [NP]. All samples started growth from day 0 and had a steady growth till day 2 with P and control as the highest, from day 2 to day 4 control had a decrease in growth to a point of 0.016, which increased to 0.026 on day 6 and had a decline on day 10, which decreased slight on day 12 and increased on day 14. N had an increase from day 2 to day 4, which remained steady till day 6 and had a slight decline on day 8, by day 10 there was an increase in growth with a difference of about 0.003 and it continued on the increases till day 14. P had a decline by day 4 and increased from day 4 and continued its increases till day 10, a slight decrease of about 0.002 was observed by day 12 which increased till day 14. NP had a decline in growth by day 4 and increased again by day 6, by day 8 there was a decline which continued till day 14.

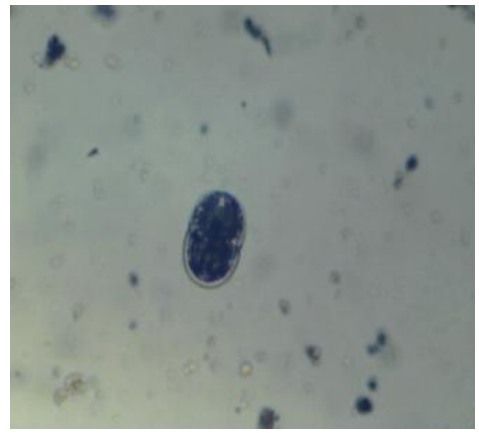
3.3. PHYSICAL AND CHEMICAL PARAMETERS

Table 1: Physiochemical parameters

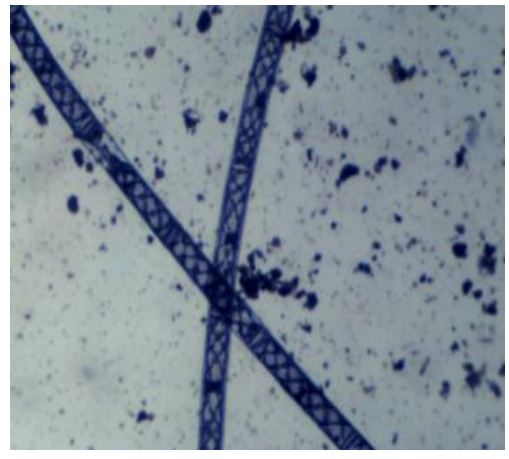
PARAMETERS	VALUES
Air temperature (^{0}C)	35^{0}C
Water temperature (^{0}C)	29^{0}C
Total Dissolved Solids (ppm)	29.5ppm
Turbidity (NTU)	7 NTU
Colour (PtCoU)	4 PtCoU
pH	5.35
Conductivity (µS/cm)	59µS/cm
Dissolved Oxygen (DO)	6.3mg/L
Total Alkalinity (mg/L)	62.5mg/L
Total Hardness	30mg/L
Sulphate (mg/L)	5 mg/L
Nitrate (mg/L)	0.2 mg/L
Phosphate (mg/L)	0.48mg/L

3.4. PHYTOMICROGRAPY OF RIVER ERUVBI PHYTOPLANKTON

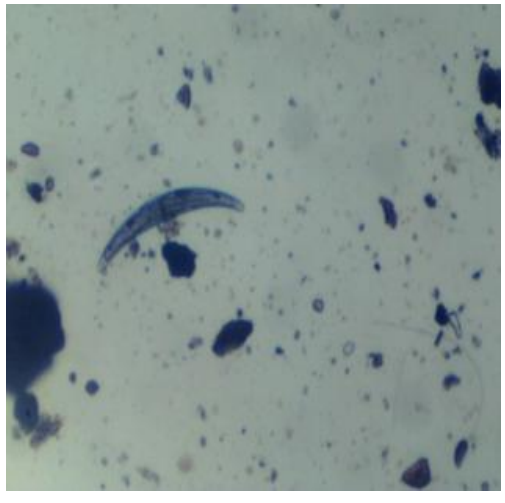




A.



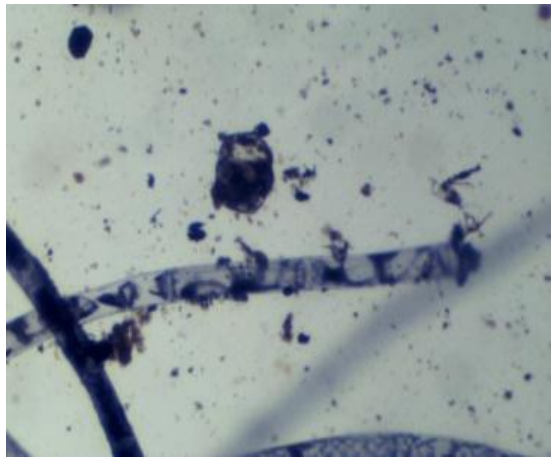
B.

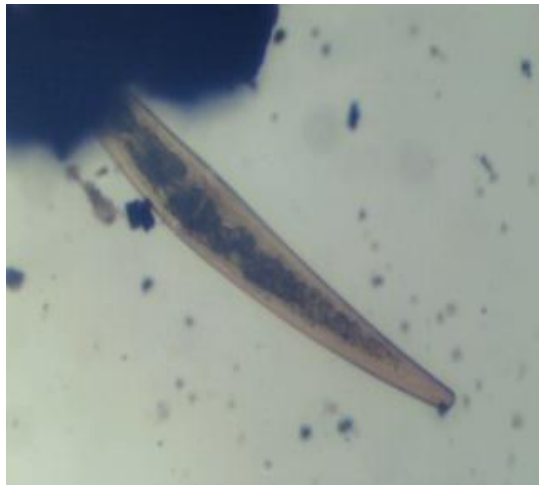


C. D.

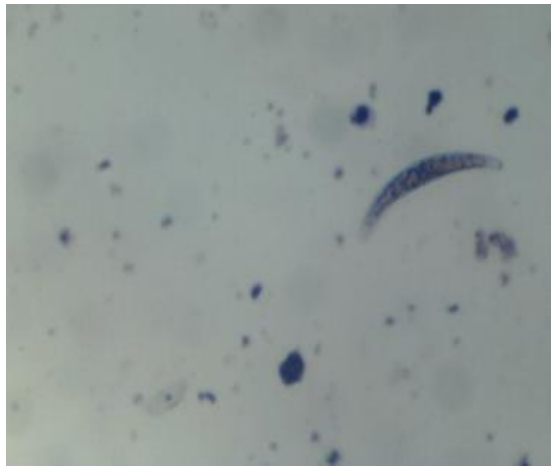
Plate 5: Phytoplankton species of river Eruvbi

Mag ×40

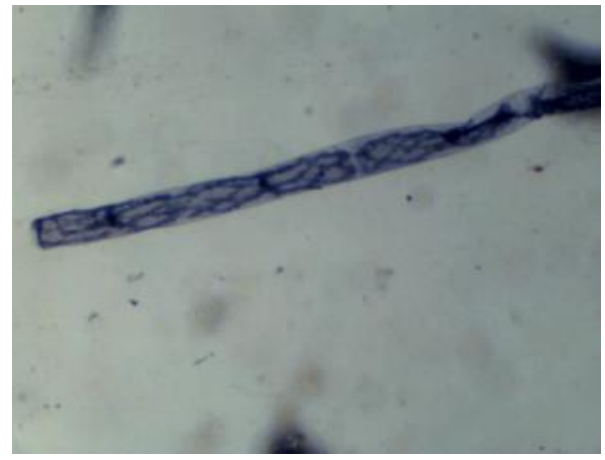




E. F.



G.



H.

Plate 5: Phytoplankton species of river Eruvbi cont.

Mag ×40

A: ***Closterium parvulum***

B: ***Cylindrocystis subryamidata***

C: ***Spirogyra nitida***

D: ***Closterium malmei***

E: ***Spirogyra spreeiana***

F: ***Closterium baillyanum***

G: ***Closterium jennerii***

H: ***Spirogryra rectangularis***

DVISION: CHAROPHYTA

CLASS: Conjugatophyceae

A: ***Closterium parvulum***

ORDER: Desmidiales

FAMILY: Closterium

GENUS: *Closterium*

SPECIES: *Closterium parvulum*

B: ***Cylindrocystis subryamidata***

ORDER: Zgematales

FAMILY: Cylindrocystisaceae

GENUS: *Cylindrocystis*

SPECIES: *Cylindrocystis subryamiata*

D: ***Closterium malmei***

ORDER: Desmidiales

FAMILY: Closteriaceae

GENUS: *Closterium*

SPECIES: *Closterium malmei*

F: ***Closterium baillyanum***

ORDER: Desmidiales

FAMILY: Closteriaceace

GENUS: Closterium

SPECIES: *Closterium baillyanum*

G: ***Closterium jennerii***

ORDER: Desmidiales

FAMILY: Closteriaceae

GENUS: *Closterium*

SPECIES: *Closterium jennerii*

CLASS: Zygnematophyceae

C: *Spirogyra nitida*

ORDER: Zygnematales

FAMILY: Zygnemataceae

GENUS : *Spirogyra*

SPECIES: *Spirogyra nitida*

E: : *Spirogyra spreeiana*

ORDER: Zygnematales

FAMILY: Zygnemataceae

GENUS : *Spirogyra*

SPECIES: *Spirogyra spreeiana*

H: *Spirogryra rectangularis*

ORDER: Zygnematales

FAMILY: Zygnemataceae

GENUS: *Spirogyra*

SPECIES: *Spirogyra rectangularis*

CHAPTER 4

DISSCUSSION

4.1. PHYTOPLANKTON RESPONSE TO EUTROPHICATION STIMULATION

The study was carried with the aim of assessing the River Eruvbi to eutrophication stimulation and also its phytoplankton response, to achieve this, the phytoplankton (indigenous and introduces) where subjected to different nutrient enrichment stimulation. The result of the study showed that the effect of eutrophication stimulantion on phytoplankton of River Eruvbi is significantly high,as seen in the groap above which showed that indigenous phytoplankton of River Eruvbi responsed better to nitrate stimulation compared to the introduced phytoplankton, this may be due to the phytoplankton present in the river (*Closterium* sp and *Spirogrya* sp), studies has shown that these algae grow better in a nitrate condition which is an exception from the general concept of green algae which favors phosphate condition, as according Domingues *et al*. (2005) and Domingues *et al*., (2011), green algae are commonly favored by high Phosphate to low nitrate ratios. Introduced phytoplankton (*Chlorella vulgaris)* had a better growth response to phosphate nutrient condition; this is because phosphate is a limiting nutrient for *Chlorella vulgaris,* meaning *Chlorella vulgaris* grows better in a high phosphate condition than a nitrate condition. In a study done by White *et al*., it was shown that *Chlorella vulgaris* favors phosphate condition to that of nitrate. *Scenedesums acutus* also favored phosphate condition than nitrate condition. The result showed that both introduced algae favors phosphate to nitrate. The result also showed that nitrate-phosphate condition does not stimulate phytoplankton growth, which was unexpected, cause according to Dodds and Smith., (2016) nitrate-phosphate condition is the best eutrophic condition for phytoplankton growth.

4.2. PHYTOPLANKTON COMPOSITION AND DISTRIBUTION

There was low biodiversity of phytoplankton in river Eruvbi. Chlorophyta was the dominant division of river Eruvbi with desmids as *Closterium* spp as the predominant species. These algae are mostly present in oligotrophic waters and are indicators a low nutrient level in the river (Alika and Akoma., 2012). The low biodiversity may also be due to the low conductivity and the acidity of the river.

4.3. PHYSICAL AND CHEMICAL PROPERTIES OF RIVER ERUVBI

Temperature is an important factor of a water body that influences chemical and biochemical activities of the water body (Simpi *et al.,*2011). Air and water temperature of the river was 35^0C and 29^0C respectively. The water temperature of this study was in accordance with the range of inland waters in the tropic as stated by Onyeche and Akankail (2013). Dissolved solid in this study was 29.5mg/L which is in accordance with WHO guideline for drinking water (2008). Dissolved solids of natural water may consist of solutes like calcium, chlorides, sodium etc. Turbidity valeus in this study was 7NTU which exceeds WHO guidelines of 5NTU for drinking water (2011). Colour units of river Eruvbi was at 4PtCoU and may be due to presences of dissolved organic matter (Kadiri., 2000). According to WHO guidelines for drinking water pH value should fall between the range of 6.5 -8.5. In the study however, pH value of river Eruvbi was 5.35 which fell below the guideline value. Conductivity of the water for this study was 59µS/cm; this is within the WHO guidelines for drinking water. Dissolved oxygen is the total oxygen level in the water body. Dissolve oxygen of the study area was 6.3mg/L, and it's below WHO guideline for drinking water. Total alkalinity is the capacity of a water body to neutralize strong acid. The total alkalinity of the

river in this study was 62.5mg/L fell with the approved range of WHO. Hardness is the ability of water to form latter with soap, high hardness content in water increases the boiling rate of the water. The presence of calcium and magnesium determines the total hardness value of water; hardness has been grouped into three [hard, moderate and soft]. The hardness of this study was 30mg/L and is within the WHO guidelines of drinking water. Sulphate is a major anion of natural waters (Ftsum *et al.,* 2015). The sulphate value of river Eruvbi in this study was far below the accorded value of WHO guideline for drinking water. Nitrates in waters are mainly obtained from either natural or manmade sources such as fixation of atmospheric nitrogen by certain microorganisms, plant debris, animal excrements nitrogenous fertilizers and effluents from industries (Ekere., 2012). The value of nitrate in the river of study was 0.2mg/L fell below the approved value for WHO guidelines for drinking water. The presence of phosphate in the water lead to an increase in plant and algae growth in the water.(Adesuyi *et al.,* 2015). Phosphate value was 0.48mg/L, was below the valve of WHO guidelines for drinking water.

4.4. CONCLUSION

In the study of eutrophication stimulation effect on river Eruvbi phytoplankton, water quality parameter and phytoplankton response to eutrophication stimulants was conducted. It was shown

that phytoplankton of river Eruvbi will respond better to nitrate stimulation than any other nutrient. This was found to be due to the low nitrate content of the river. Phytoplankton composition of the river showed that river Eruvbi is oligotrophic in nature and the present of any eutrophic stimulant (nitrate) will lead to eutrophication of the river. These investigations conclude that nitrate is the fastest eutrophic stimulant for the river and adequate care should be taken to prevent the eutrophication of the river, as it is a source of irrigation and drinking for the inhabitants.

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APPENDIX

Table 2: Growth response of Natural phytoplankton

	DAY	DAY 2	DAY	DAY	DAY	DAY 10	DAY 12	DAY 14
CONTROL								
1	0.011	0.022	0.012	0.015	0.01	0.009	0.011	0.022
2	0.007	0.017	0.008	0.013	0.01	0.012	0.015	0.03
3	0.009	0.017	0.007	0.014	0.009	0.013	0.014	0.03
MEAN	0.009	0.018667	0.009	0.014	0.009 67	0.011 33	0.013 33	0.027 33
NITROGE								
1	0.009	0.018	0.009	0.01	0.01	0.011	0.015	0.044
2	0.008	0.021	0.007	0.017	0.012	0.016	0.02	0.034

3	0.008	0.018	0.005	0.013	0.011	0.012	0.012	0.036
MEAN	0.008 33	0.019	0.007	0.013 33	0.011	0.013	0.015 67	0.038
PHOSPHOUS								
1	0.011	0.025	0.005	0.012	0.006	0.011	0.011	0.011
2	0.011	0.023	0.005	0.015	0.007	0.014	0.02	0.027
3	0.01	0.026	0.006	0.014	0.006	0.01	0.014	0.018
MEAN	0.010 67	0.024667	0.005 33	0.013 67	0.006 33	0.011 67	0.015	0.018 67
NP								
1	0.01	0.025	0.007	0.011	0.015	0.013	0.011	0.026
2	0.011	0.026	0	0.012	0.011	0.016	0.011	0.021
3	0.008	0.027	0.005	0.014	0.014	0.012	0.01	0.032
MEAN	0.009 67	0.026	0.004	0.012 33	0.013 33	0.013 67	0.010 67	0.026 33

Table 3: Absorbance of Natural phytoplankton

	DAY 0	DAY	DAY	DAY	DAY	DAY	DAY	DAY
CONTROL								
1	0	0.011	0.001	0.004	-0.001	-0.002	0	0.011
2	0	0.01	0.001	0.006	0.003	0.005	0.008	0.023
3	0	0.008	-0.002	0.005	0	0.004	0.005	0.002
MEAN	0	0.0096 7	0	0.005	0.0006 7	0.0023 3	0.0043 3	0.0120 3
NITROGE								
1	0.009		0	0.001	0.202	0.202	0.202	0.035
2	0.013		-0.001	0.009	0.003	0.007	0.011	0.025

3	0.01	-0.003	0.005	0.003	0.004	0.004	0.028
		-0.001		0.0693		0.0723	0.0293
MEAN	0.010667	3	0.005	3	0.071	3	3
PHOSPHOR							
1	0.014	-0.006	0.001	-0.005	0	0	0
2	0.012	-0.006	0.004	-0.004	0.003	0.009	0.016
3	0.016	-0.004	0.004	-0.004	0	0.004	0.008
		-0.005		-0.004		0.0043	
MEAN	0.014	3	0.003	3	0.001	3	0.008
NP							
1	0.015	-0.003	0.001	0.005	0.003	0.001	0.016
2	0.015	-0.011	0.001	0	0.005	0	0.01
3	0.019	-0.003	0.006	0.006	0.004	0.002	0.024
		-0.005	0.0026	0.0036			0.0166
MEAN	0.017	7	7	7	0.004	0.001	7

Table 4: Growth response of *Seredesmus acutus*

	DAY	**DAY 2**	**DAY**	**DAY**	**DAY**	**DAY 10**	**DAY 12**	**DAY 14**
CONROL								
1	0.031	0.039	0.036	0.052	0.041	0.042	0.052	0.072
2	0.026	0.036	0.037	0.058	0.037	0.049	0.045	0.074
3	0.026	0.032	0.035	0.041	0.037	0.044	0.051	0.072
MEAN	0.027	0.035667	0.036	0.050	0.038	0.045	0.049	0.072
	67			33	33		33	67
NITROGE								
1	0.03	0.04	0.036	0.047	0.048	0.056	0.038	0.041
2	0.03	0.034	0.036	0.042	0.048	0.051	0.043	0.048

3	0.032	0.036	0.039	0.04	0.028	0.05	0.042	0.044
MEAN	0.030 67	0.036667	0.037	0.043	0.041 33	0.052 33	0.041	0.044 33
PHOSPHO US								
1	0.032	0.033	0.032	0.047	0.042	0.042	0.046	0.044
2	0.032	0.036	0.034	0.045	0.039	0.042	0.043	0.037
3	0.029	0.035	0.034	0.055	0.041	0.045	0.04	0.048
MEAN	0.031	0.034667	0.033 33	0.049	0.040 67	0.043	0.043	0.043
NP								
1	0.034	0.045	0.038	0.052	0.037	0.035	0.04	0.041
2	0.034	0.04	0.028	0.059	0.037	0.03	0.033	0.042
3	0.034	0.041	0.032	0.041	0.036	0.038	0.032	0.041
MEAN	0.034	0.042	0.032 67	0.050 67	0.036 67	0.034 33	0.035	0.041 33

Table 5: Absorbance of *Seredesmus actuus*

	DAY 0	**DAY**	**DAY**	**DAY**	**DAY**	**DAY**	**DAY**	**DAY**
CONTROL								
1	0	0.008	0.005	0.021	0.01	0.011	0.021	0.044
2	0	0.01	0.011	0.032	0.011	0.023	0.019	0.048
3	0	0.006	0.009	0.015	0.011	0.018	0.025	0.046
MEAN	0	0.008	0.0083 3	0.0226 7	0.0106 7	0.0173 3	0.0216 7	0.046
NITROGE								
1	0	0.01	0.006	0.017	0.018	0.026	0.008	0.011
2	0	0.004	0.006	0.012	0.018	0.021	0.013	0.018

3	0	0.004	0.007	0.008	-0.004	0.018	0.01	0.012
MEAN	0	0.006	0.0063	0.0123	0.0106	0.0216	0.0103	0.0136
			3	3	7	7	3	7
PHOSPHOR								
1	0	0.001	0	0.015	0.01	0.01	0.014	0.012
2	0	0.004	0.002	0.013	0.007	0.01	0.011	0.005
3	0	0.006	0.005	0.026	0.012	0.016	0.011	0.019
MEAN	0	0.0036	0.0023	0.018	0.0096	0.012	0.012	0.012
		7	3		7			
NP								
1	0	0.011	0.004	0.018	0.003	0.001	0.006	0.007
2	0	0.006	-0.006	0.025	0.003	-0.004	-0.001	0.008
3	0	0.007	-0.002	0.007	0.002	0.004	-0.002	0.007
MEAN	0	0.008	-0.001	0.0166	0.0026	0.0003	0.001	0.0073
			3	7	7	3		3

Table 6: Growth response of *Chlorella vulgaris*

	DAY	**DAY 2**	**DAY**	**DAY**	**DAY**	**DAY 10**	**DAY 12**	**DAY 14**
CONTROL								
1	0.024	0.049	0.034	0.048	0.053	0.055	0.047	0.061
2	0.024	0.046	0.038	0.048	0.032	0.022	0.016	0.021
3	0.026	0.045	0.04	0.052	0.043	0.034	0.045	0.046
MEAN	0.024	0.046667	0.037	0.049	0.042	0.037	0.036	0.042
	67		33	33	67			67
NITROGE								

1	0.024	0.045	0.042	0.036	0.036	0.057	0.055	0.062
2	0.026	0.046	0.042	0.033	0.039	0.025	0.027	0.021
3	0.027	0.045	0.041	0.055	0.048	0.05	0.055	0.059
MEAN	0.025 67	0.045333	0.041 67	0.041 33	0.041	0.044	0.045 67	0.047 33
PHOSPHO US								
1	0.021	0.044	0.027	0.039	0.044	0.052	0.062	0.065
2	0.021	0.049	0.024	0.028	0.034	0.046	0.05	0.052
3	0.022	0.046	0.028	0.03	0.036	0.041	0.021	0.024
MEAN	0.021 33	0.046333	0.026 33	0.032 33	0.038	0.046 33	0.044 33	0.047
NP								
1	0.029	0.039	0.038	0.042	0.037	0.019	0.015	0.016
2	0.028	0.04	0.033	0.038	0.033	0.024	0.015	0.011
3	0.029	0.042	0.023	0.033	0.03	0.02	0.008	0.008
MEAN	0.028 67	0.040333	0.031 33	0.037 67	0.033 33	0.021	0.012 67	0.011 67

Table 7: Absorbance of *Chlorella vulgaris*

	DAY 0	**DAY**	**DAY**	**DAY**	**DAY**	**DAY**	**DAY**	**DAY**
CONTROL								
1	0	0.024	0.01	0.028	0.029	0.031	0.023	0.037
2	0	0.022	0.014	0.024	0.008	-0.002	-0.008	-0.003
3	0	0.019	0.026	0.026	0.017	0.008	0.019	0.002
MEAN	0	0.0216 7	0.0166 7	0.026	0.018	0.0123 3	0.0113 3	0.012
NITROGE								

1	0	0.021	0.018	0.012	0.012	0.033	0.031	0.038
2	0	0.02	0.016	0.007	0.013	-0.001	0.001	-0.005
3	0	0.018	0.014	0.028	0.021	0.023	0.028	0.032
MEAN	0	0.0196 7	0.016	0.0156 7	0.0153 3	0.0183 3	0.02	0.0216 7
PHOSPHOR								
1	0	0.023	0.006	0.018	0.023	0.031	0.041	0.044
2	0	0.028	0.003	0.007	0.013	0.025	0.029	0.031
3	0	0.024	0.006	0.008	0.014	0.019	-0.001	0.022
MEAN	0	0.025	0.005	0.011	0.0166 7	0.025	0.023	0.0323 3
NP								
1	0	0.01	0.009	0.013	0.008	-0.01	-0.014	-0.013
2	0	0.012	0.005	0.01	0.005	-0.004	-0.013	-0.017
3	0	0.013	-0.006	0.004	0.001	-0.009	-0.021	-0.021
MEAN	0	0.0116 7	0.0026 7	0.009	0.0046 7	-0.007 7	-0.016	-0.017