Diurnal Variation In Physico-Chemical Parameters and Soil Sediment Bacterial Population Of Magada Lake, Ibafo

BY

MUSE IFEOLUWA SHALOM

Matric No – 15010101007

A PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR

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DEPARTMENT OF BIOLOGICAL SCIENCE AT THE COLLEGE OF BASIC AND APPLIED SCIENCE,

MOUNTAIN TOP UNIVERSITY, MOWE IBAFO, OGUN STATE.

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ABSTRACT

Due to environmental and health challenges associated with water bodies which include lakes, the ecological and microbial factors need to be given prior attention. The ecological factors have the potential to influence bacterial population associated with water bodies. However, the effect of ecological factors on soil sediment bacterial population have not been investigated in Magada lake which passed through Mountain Top University. Soil sediment samples were collected at different time points of 12am, 6am, 12pm and 6pm to determine the physico-chemical parameters and bacterial population using standard methods. In addition, in-situ physico-chemical parameters such as air temperature, water temperature, salinity, conductivity, depth, dissolved oxygen and turbidity were determined at the point of collection. Remarkably, only pH was significantly different ($p<0.05$) in comparison to the others. It was observed at 6am partly 12pm that the bacterial population, phosphate/sulphate/nitrate/magnesium and calcium were on the high side. Similarly, the heavy metal content indicates that cadmium/iron/lead/chromium were on the high side at – and -. in addition, water hardness, alkalinity, biochemical oxygen demand, total dissolved solids were outstanding in their values at 6am and 12am in comparism to the control at different time points. Thus, in time-dependent ecological factors is more pronounced on bacterial population at 6am and 12pm. In conclusion, further studies are expected to ascertain other factors (e.g. anthropogenic activities) that may be contributing to ecol bacterial population of Magada lake at.

CERTIFICATION

This is to certify that this research project report titled **"DIURNAL VARIATION IN THE PHYSICO-CHEMICAL PARAMETERS AND SOIL SEDIMENT BACTERIAL POPULATION OF MAGADA LAKE"** was carried out by Muse Ifeoluwa Abdulsalam with matric number 15010101007. This project report meets the requirements concerning the award of Bachelor of Science degree in Department of Biological Sciences, Program of Microbiology, Mountain Top University, Ogun state Nigeria and is approved for its contribution to knowledge and literary presentation.

……. **…….**

DR. F. H IBADIN **DATE**

PROJECT SUPERVISOR

……. **…….**

DR. M. A ABIALA **DATE**

CO-PROJECT SUPERVISOR

…… **……..**

DR. ADEIGA **DATE**

(HEAD OF DEPARTMENT)

DEDICATION

I dedicate this project first and foremost to God Almighty for His protection and support to me throughout the course of my four years of study in Mountain Top University.

ACKNOWLEDGEMENT

I wish to express my sincere gratitude first to the Almighty God for his infinite mercies and grace, and for wisdom to carry out this work.

I am grateful to my parents, Mr. and Mrs. Muse for their love and encouragement and most especially, financial support during this period.

I am also very grateful to my H.O.D, Dr. ADEIGA and also my able supervisors Dr. F.H IBADIN and Dr. M.A ABIALA for their interest, guidance and encouragement in ensuring a successful completion of this work.

I also want to appreciate my sponsor, Mr Olujimi Adebowale for his unending support towards me and for financing my four years of study and also Dr. D.K Olukoya for his input both direct and in direct on my life.

Finally, to my co-supervisee Ogaleye Blessing, my friends (Bayonle Anuoluwapo, Adekunle Rotimi, Akorede Breakthrough, Ajayi Oluwatoye, Adebayo Glory and Arugbemi Bunmi) and my siblings for always pushing me to do more, for always being a source of encouragement.

CHAPTER ONE

STATEMENT OF PROBLEM

Water is an important component in any community of which Magada Lake that passed through Mountain Top University is not an exception. In addition to the time dependent variation, that is expected in Magada Lake of Mountain Top University, heterotrophic bacteria are also expected to be an important component of the soil sediments from this lake. Globally, ecological (physico-chemical) factors have the potential to influence the bacterial population of this lake. Consequently, the bacterial population associated with these soil sediments can be a pressing challenge to the environment and aquatic life and processes of this lake.

RESEARCH QUESTIONS

1. Does the ecological parameter encourage the bacterial population?
2. What are the most prominent or prevalent population or bacteria at each time
3. Which of the time favour bacterial population and which one disfavours bacterial population.

JUSTIFICATION

The influence of Magada lake ecological factors on bacteria population associated with soil sediments have not been evaluated since the inception of Mountain top University. The ore, this study aimed to evaluate the effect of diurnal ecological factors on bacterial population associated with the soil sediments of Magada Lake that passed through Mountain Top University.

OBJECTIVES OF STUDY

1. To determine the diurnal variation of the physico-chemical factors associated with the water in MTU Lake.
2. To determine the diurnal variation of the bacteria population of MTU lake water.
3. To assess the influence of physico-chemical parameters on the bacteria population.

INTRODUCTION

The Earth's surface is said to be covered by 70.9% of water, and is essential for all known forms of life ("United Nations". Un.Org. 2005-03-22). On the surface of the Earth, it is mostly found in oceans and large bodies of water, also, 1.6% of water can be found below ground in aquifers and 0.001% is found in the air as water vapor, clouds (formed by solid and liquid water particles suspended in air), and precipitation (Water Vapor in the Climate System, Special Report, [AGU], December 1995). Oceans are known; to hold 97% of surface water, glaciers and polar ice caps to hold 2.4%, and other land surface water such as rivers, lakes and ponds to hold 0.6%. A very minor amount of the Earth's water is contained within biological bodies and manufactured products. Liquid water is found in bodies of water, such as an oceans, seas, lakes, rivers, streams, canals, ponds, or puddles. The majority of water on Earth is said to be sea water. Water is also present in the atmosphere in solid, liquid, and vapor states. It also occurs as groundwater in aquifers. Water plays a significant role in the world economy, as it functions as a solvent for a wide variety of chemical substances and facilitates, industrial, cooling, and transportation. Approximately 70% of freshwater is consumed by agriculture. (Baroni et al., 2007).

A lake (from the Latin lacus) is a huge body of water within a body of land. Like rivers, some lakes do not flow, but many lakes have rivers that flow into and out of them. Most of the Earth's surface lakes are fresh water, most of them in the Northern Hemisphere. Over 60% of the world's lakes are in Canada. Finland is known as the Thousand Lakes Land (Finland has 187,888 lakes, 60,000 of which are large. From **worldatlas.com**).

Many lakes are man - made reservoirs built for electricity generation, recreation, or use of water for irrigation or industry, or in homes.

The easiest classification is based on the size of a lake. There are numerous types, kinds and categories of lakes in the world. They can be classified on the basis of the following which are:

a) Origin
b) Trophic levels
c) Mixing of water.
d) Nature of Inflow-outflow.

They can also be further classified based on each of the above listed categories, the ore;

i. Based On Origin

i. Lakes formed due to landslides
ii. Salt lakes
iii. Oxbow lakes
iv. Crater lakes
v. Sinkhole lakes
vi. Lakes formed due to erosion
vii. Kettle lake
viii. Artificial lake
ix. Fjord lake
x. Tectonic lakes

Soil covers the Earth's surface and is a precious ecosystem. Soil consists of minerals, nutrients, water, air, organic matter and micro-organisms.

It is said to be a natural body made up of layers (soil horizons) of variable thickness mineral constituents that differ in their morphological, physical, chemical and mineralogical characteristics from the parent materials (Birkeland and Peter, 1999).

It is composed of broken rock particles that have been altered by weathering and erosion in chemical and environmental processes. Because of interactions between lithosphere, hydrosphere, atmosphere, and biosphere, soil differs from its parent rock (Chesworth, 2008). It is a mixture of solid, gaseous and aqueous mineral and organic constituents (Voroney et al., 2006).

Soil is also known as Earth: it is the substance that takes its name from our planet. Little of planet Earth's soil composition is older than the Tertiary and no older than the Pleistocene (Buol et al., 1973). Soil is called regolith or loose rock material in engineering.

There are various soil types which include the following:

1. Sandy soil type,
2. Silty soil type,
3. Clay soil type ,
4. Peaty soil type,
5. Saline soil type,
6. Loam soil type.

CHAPTER TWO

Literature review

Water And Associated Soil

The availability of good quality water is a necessary feature for preventing diseases and improving quality of life (Oluduro and Aderiye, 2007). Metals for example, can be introduced into aquatic system through several ways which include, weathering of rocks and leaching of soils, dissolution of aerosol particles from the atmosphere and also from several human activities, including mining, processing and the use of metal-based materials (Ipinmoroti and Oshodi, 1993; Adeyeye, 1994; Asaolu et al., 1997).

Water is a very important resource for industries, agriculture, manufacturing and many other human activities. Many research works have been carried out on lakes by scientists all around the world. But much erence will be made on works done in Nigeria.

The water bodies (including rivers, lakes, dams and estuaries) are endlessly exposed to dynamic state of transformation with respect to the geological age and geochemical characteristics. This is established by constant rotation, conversion and buildup of energy and matter through the intermediate of living things and their various activities. The self-motivated balance in the aquatic ecosystem is upset by anthropogenic activities, which are consequential in pollution. Aquatic ecosystems are affected by several health stressors that significantly deplete biodiversity. In the future, the loss of biodiversity and its effects are predicted to be greater for aquatic ecosystems than for terrestrial ecosystems (Sala et al., 2000). The quality of water is usually determined by its physico-chemical characteristics. It is a well-established fact that domestic-sewage and industrial effluent discharged into natural water result in deterioration of water quality and cultural eutrophication (Shaw et al., 1991). The other important sources of water pollution include mass bathing, disposal of dead bodies, rural and urban waste matters, agricultural run-off and solid waste disposal (Tiwana, 1992)

Rivers are exposed to numerous natural processes taking place in the environment, such as the hydrological cycle. As a consequence of unparalleled development, humans are responsible for choking several lakes to death. Storm water runoff and release of sewage into rivers are two common ways that various nutrients enter the aquatic ecosystems thereby resulting in the pollution of those systems (Sudhira and Kumar, 2000; Adeyemo, 2003).

Water quality is best defined in relations with the physical, chemical and biological factors which are associated with the water, these factors include physico-chemical factors like temperature, pH, conductivity, salinity, hardness, etc. there are discrepancies on these factors when there are seasonal variations, they also vary based on geographic areas and on anthropogenic activities. Hence, the contamination of water is amplified due to increasing human populations, industrialization, the use of fertilizers in agricultural practices, improper disposal of waste and other man-made activity.

The adversative effects of human influences on the aquatic systems comprise; water – borne diseases, alteration of aquatic biota composition, eutrophication, reduction or destruction of ecosystem integrity (Sridhar et al., 1981; Egborge, 1991; Oduwole, 1997; Ekpo et al., 2012).

In water testing it is not possible to clearly divide amid true suspension substances and temporary suspension substances stirred-up from the sediments. Sediment testing is not, or only minimally, affected by other influences. The suspended and precipitated (non-floating) substances and organic substances in waters are capable of adhering pollutant particles (adsorption). The sediments, both suspended and precipitated stored on the water bottom, form a reservoir for many pollutants and trace substances of low solubility and low degree of degradability (Biney et al., 1994; Barbour et al., 1998, 1999). Pollutants are conserved in sediments over long periods of time according to their chemical

persistence and the physical-chemical and biochemical characteristics of the substrata (O.K Adeyemo et al, 2008). This can also allow for conclusions to be drawn regarding sources of contamination.

Though water is a renewable resource, reckless usage and improper management of water systems may cause serious problems in availability and quality of water. Water may be contaminated by various means, chemically or biologically and may become unfit for drinking and other uses. In our environment, 70% of the water is seriously polluted and 75% of illness and 80% of the child mortality is attributed to water pollution.

Sediments makeup a natural buffer and filter system in the material cycles of waters. Sediment in our rivers is a significant habitat as well as a chief nutrient source for aquatic organisms. Furthermore, sediments have an impact on ecological quality because of their quality, or their quantity, or both (Stronkhorst et al., 2004). Waters are subject to strong differences of flow rate, substance input, transport, and sedimentation. Sediment analysis is increasingly important in evaluating qualities of the total ecosystem of a body of water, in addition to the water sample analysis practice which has been practiced for years. In contrast to water testing, sediment testing mirrors the long-term quality condition autonomous of current inputs (Hodson, 1986; Haslam, 1990).

Types of soil

Soil is basically of 5 types, these are: -

1. Sandy Soil:

   Sandy soil is light and dry in nature. It does not have high humidity and warms up rapidly in the spring. The ore, it is good for the production of early crops. Sandy soil is appropriate for cultivating any time of the year and is relatively easier to manage. Since it absorbs water rapidly, the plants rooted in it need to be watered regularly.

2. Clay Soil:

   Clay soil is also called late soil, since its wet nature makes it appropriate for planting seeds in late autumn. The soil supports as an outstanding retort for the dry season, as it has a high-water retaining quality. It is essential to drain clay soil regularly, for improving its consistency. The soil becomes uncontrollable during rainy season, as it becomes sticky. On the other hand, throughout draught, it becomes rock solid.

3. Loam Soil:

   Being the perfect soil for agricultural practices, Loamy soil is a mixture of all the three, which are - sandy soil, clay soil and silt soil, in the proportion of 40:40:20. It is apt for any and every kind of crops. A union of three soils, loam soil has best of the properties of all the groups of soils. It has high nutrients content, warms up swiftly in summers and infrequently dries out in the dry weather. It has become the perfect soil for cultivation.

4. Peaty Soil:

   Peaty soils are acidic in content, which makes them sour. This is the most outstanding feature of Peaty soils. It is usually found in low-lying areas, these soils require proper drainage, as the place is familiarized to a lot of water clogging. Though peaty soils have low nutrient content, they warm up rapidly in the spring, making them appropriate if right amount of fertilizers are added.

5. Chalky Soil:

   Chalky soil is basic in nature and typically poor in nutrients. It requires nourishment, in the form of additional nutrients and soil improvers, for better quality. The soil becomes dry in summers, making it very hard, and would require too much of watering for the plants to grow. The only

benefit which such a soil has is its lime content. When deep-rooted, Chalky soil becomes excellent for plant growth and favors good growing conditions as well.

Economic importance of soil sediments

Sediment are particles (such as sand and other soils) which settle, or are deposited, on the sides and bottom of water bodies. It is important in the formation of beaches, spits, sand bars and estuaries and provides substrates for aquatic plants and animals. Sediment also provides nutrients and minerals vital to the health of downstream ecosystems.

Sediment reaches aquatic areas in three main ways:

1. watershed erosion.
2. mass wasting events, such as landslides.
3. shoreline erosion.

The main places that sediment comes from are:

1. steep slopes with unstable or unprotected soils (such as feeder bluffs).
2. landslide hazard areas.
3. and unarmored channels.

Sediment processes are an extremely vital part of many ecosystems, as well as of primary importance to particular species.

For example, various organisms in both marine and freshwater environments rely on replacement of soil sediments for their reproductive habitat. Fluctuations to sediment content (either too much or too little) can change substrates or cause sediment not to be deposited in the appropriate locations. Sediment

moves through the ecosystem and is sometimes stored in wetlands, streams, lakes, and the banks of the shorelines.

The amount of sediment reaching these areas is primarily altered by draining or filling wetlands, loss of shoreline roughness (for example, the removal or loss of large woody debris), channelization of streams, shoreline armoring, dams, and the development of structures like boat ramps and groins which are oriented perpendicular to the marine shoreline. Dredging and bulkheads can also affect how much sediment is present in aquatic shoreline areas.

CHAPTER THREE

MATERIALS AND METHODS

The Study Area

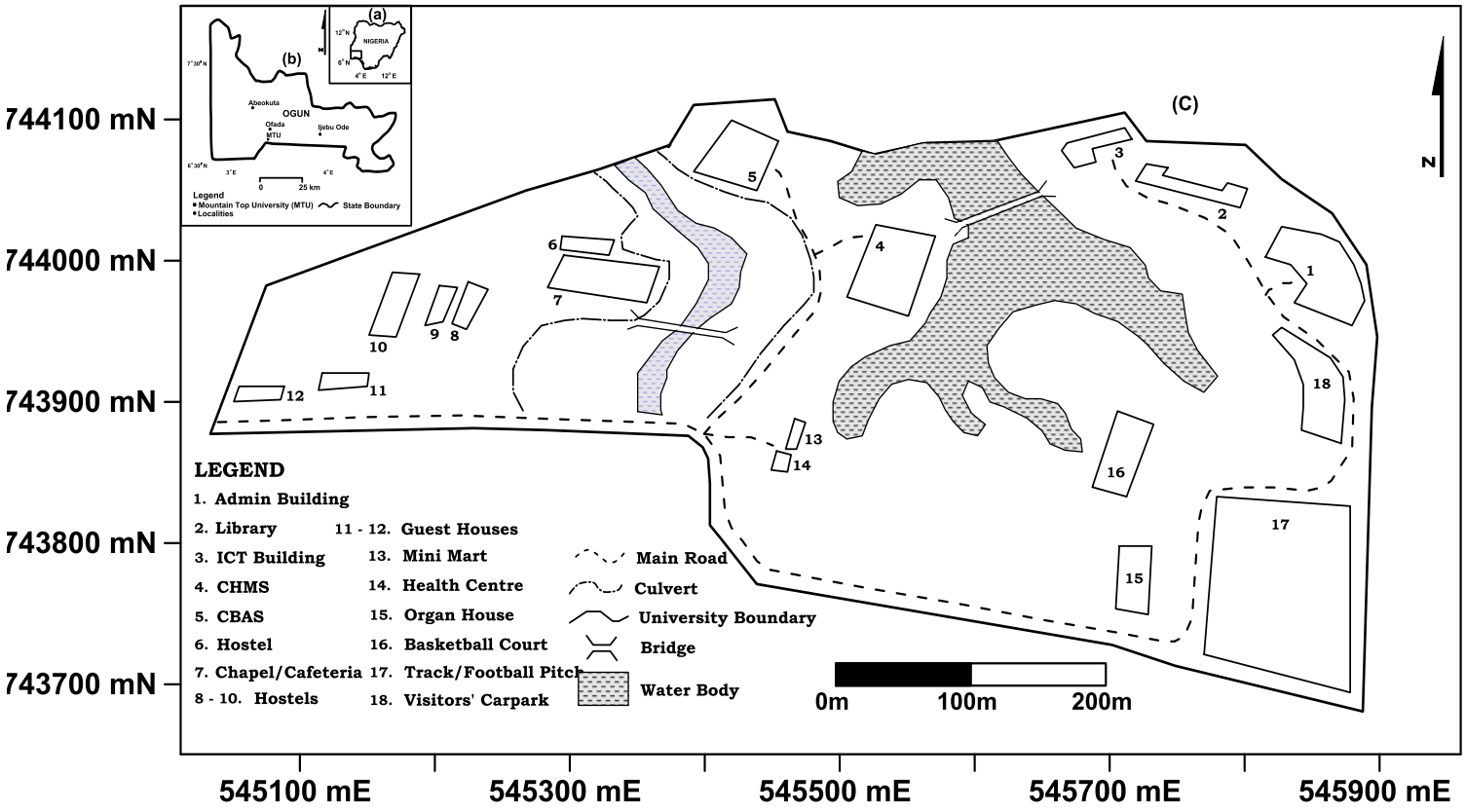

Fig. 1: Map of (a) Nigeria showing the Location of Ogun State; (b) Ogun State identifying MTU location; (c) MTU campus.

The study was carried out in the month of may 2019 in Mountain Top University (MTU), in which samples come from four (4) stations at different points in the lake. The water body is located within Mountain Top University Campus which is bounded with Latitudes 743700 and 744100mN and Longitude 545100 and 545900mE. The Mountain Top University Campus is located at Prayer City, Kilometre 12 Lagos- Ibadan Express Way, Ogun State. The water body is typically lentic and thus does not have a defined unidirectional flow pattern, except limited movement as a result of wind action.

Map of the four Lakes and Location of Mountain Top University, Ibafo, Ogun state, Nigeria

Figure 1 composite sampling site for station1 in Magada Lake flowing into MTU Lake.

Figure 2 composite sampling site for station 2 in Magada Lake flowing into MTU Lake.

Figure 3 composite sampling site of station 3 in Magada Lake flowing into Mtu.

Figure 4 composite sampling site of station 4 in Magada Lake flowing into Mtu.

Summary Of Physico-Chemical Methods

S/N	Analysis	Reagents & Materials
1	Total suspended & dissolved solids	Beakers, measuring cylinder, oven, analytical balance
2	pH	pH meter, pH electrode, beaker, buffer 4,7 & 10 solution.
3	Turbidity	
4	Conductivity	
5	Dissolved oxygen	Test kit 3688-SC
6	BOD	Manganese II chloride, Sodium hydroxide solution containing potassium iodide, Sodium thiosulphate.
7	Total alkalinity	0.02M HCl, CO_2-free distilled water, phenolphthalein indicator, bromophenol blue indicator.
8	Salinity	
9	Nitrate	Brucine-Sulfanilic acid solution, Sulphuric acid solution, Sodium chloride solution 30%, standard solution.
10	Sulphate	Barium chloride crystals, conditioning reagent
11	Phosphate	Vanadate molybdate, standard phosphate solution
12	Magnesium	
13	Sodium	
14	Potassium	
15	Calcium	Test kit

16	Copper	Citric acid solution, 1:3 H_2So_4, ammonium chloride solution, chloroform, sodium diethyl-thiocarbamate solution.
17	Zinc	Conc. HCl, sodium acetate solution, sodium thiosulphate solution, dithizone solution, standard zinc solution.
18	Cadmium	Test kit 4017-01
19	Iron	Ortho- phenanthroline, Hydroquinone solution, buffer solution containing Acetic acid and sodium acetate and 2M sodium acetate, nitric acid, 1:1 hydrochloric acid.
20	Lead	Conc. HCl, ammonium solution
21	Chromium	NaOH solution, potassium permanganate solution, methanol, HCl, di-sodium hydrogen phosphate solution, phosphoric acid, diphenyl-carbamide solution, standard chromium solution
22	Indole test	Indole kovac's reagent
23	Urease test	Urea broth
24	Starch hydrolysis test	
25	Sugar fermentation test	
26	MRVP test	
27	Catalase test	
28	Oxidase test	
29	Citrate utilization test	

Methodologies Applied

Soil sample collection:

1. Bamboo stick was tied with a nylon at the open end. The prepared bamboo stick was then thrust into the base of the water body so as to pick soil from the base of the water. When this was done, the bamboo stick was gently brought up to empty the soil into the designated containers. The container was capped and stored in an ice-packed cooler

Physico-chemical parameters:

These factors are categorized into two groups which consist of the following:

1. In-situ parameters (parameters which can be taken at the site).
2. Ex-situ parameters (parameters which have to be taken to lab for analysis).

The In-situ parameters include:

1. pH
2. temperature
3. Depth
4. Salinity
5. Conductivity
6. Turbidity
7. Dissolved oxygen

pH

The pH of the soil sediments samples collected at each station was done in-situ using a probe, The probe was dipped into a beaker containing the soil sediments samples and left for 2-3 minutes before readings were taken. This process was repeated three times.

Temperature

The air and water temperatures were taken at each station where by the probe, the probe was dipped into a beaker containing the soil sediments samples and left for 2-3 minutes before readings were taking. For air temperature, the probe was left in the air and left for 2-3 minutes before readings were taken. This was done in triplicate.

Depth

The depth was measured using a straight standardized wooden pole, which was then marked on the pole and the reading was taken for each station.

Turbidity

Turbidity was carried out using a turbidimeter, where the soil sediments samples were dispensed in a sample compartment and the result was observed and read, this sampling was done in triplicate for every station and time variance.

Conductivity

The samples were measured using a HACH Conductivity metre which was calibrated by dipping the conductivity probe into a beaker that containing the soil sediments samples and the readings were taken.

Salinity

The salinity probe was dipped into a beaker containing the soil sediments samples, which was done in triplicate for the stations at different time frame. This was left for 2-3 minutes before readings were taken.

Dissolved Oxygen

A reagent bottle was immersed beneath the water surface, to exclude air bubbles, the cover was opened beneath the water and it was stoppered tightly. The samples were then placed in a cell and put in a sample compartment and then recorded after 1-2 minutes, which was done for the stations at different time.

The Ex-situ parameters include:

1. B.O.D
2. Heavy metals
3. Nutrients

Ex- situ determination of physico-chemical parameters

In the duration of this research, some ex situ analysis were carried out, they include: Heavy metals (Zn, Fe, Pb, Arsenic, Cr, Cu), nutrients (nitrate, phosphate, sulphate, calcium, magnesium) and BOD5.

Biochemical Oxygen Demand (BOD_5)

1. Dark reagent bottles were used to amass soil sediments samples from the various stations,
2. They were draped in three black polythene bags.
3. They were then taken back to the laboratory and kept in a dark room where there is no light penetration.

4. After 5 days the oxygen was fixed and determined by the same process as that of dissolved oxygen, then the results were compared with dissolved oxygen.

This was calculated as thus;

$$BOD_5\,(mgl) = DO_0 - DO_5$$

Where DO_0 = The dissolved oxygen at the time the soil sediments samples were collected

DO_5 = The dissolved oxygen after 5 days

Phosphate

25ml of the water sample was with 1ml of ethanol added to the samples. A ready mixture of A (ammonium molybdate and antimony potassium tartate) and ascorbic acid was made and 1ml was added to the 25ml of the sample ethanol. Absorbance interpretation using a Milton Roy 21D Spectrophotometer at 952nm was noted and this was in turn multiplied by a multiplying factor of 30.6 to know the value of phosphate.

Nitrate

A beaker containing 10ml of water sample was heated to dry in an oven. After been brought out, to extract nitrate, 1ml of phenyldisulphuric acid was added to the beaker. A paste was formed by using a glass rod and the paste was diluted with 5ml of distilled water, then 3ml of concentrated ammonia solution was added to the sample and a yellow colour was developed. Using a spectrophotometer, absorbance reading was taken at 410nm and the obtained value was multiplied by a multiplying factor of 4.43 to get the value of the nitrate.

Sulphate

25ml of the samples were measured in a conical flask, then 50ml glycerol, 30ml conc. Hydrochloric acid, 300ml distilled water, 175g NaCl and95% alcohol of condition reagent in 1.25ml were added. Using spatula, few grams of barium chloride was added and absorbance reading of the sample was taken at 530nm wave length and the value was multiplied by a multiplying factor of 100 to get the value of sulphate.

Calcium

50mls of water sample was put in a 250ml Erlenmeyer flask in addition with 2ml of 1M sodium hydroxide solution with some grams of about 0.2g of the murexide indicator mixture and 1ml of hydroxylamine chloride which will serve as the incubator. Thereafter, the 0.01M EDTA was added until the pink colour of the sample turned to purple. By multiplying the litre value of 0.4008, the concentration of calcium in mgl^{-1} was obtained.

Magnesium

Using the EDTA method, this was also discovered. In this method, to obtain magnesium, the calcium and magnesium concentrations were determined together before exploitation. Thereafter, 50ml of sample was put in an Erlenmeyer flask and 3ml of ammonical buffer was added followed by 2 drops of Eriochrome Black T, the indicator/inhibitor solution. A sky blue end point was gotten from a wine red

color when the titre 0.01M EDTA solution was added. The following equation was how the value of magnesium was obtained:

$$Mg = (a-b)\ 0.2432$$

Where k= value of magnesium in mgl^{-1}

a= titre volume for calcium and magnesium titration

b= titre volume for calcium titration only.

Heavy Metal

Using the Aluminium Block Digester BD 110, ten millilitre of water sample was digested having added 4ml perchloric acid, 20ml concentrated nitric acid and 2ml concentrated sulphuric acid. The white fume evolved and a clear solution was obtained when the mixture was heated. Using Milton Roy Spectrophotometer, Iron (Fe) and Copper (Cu); Ffe by Orthophenamthroline method and Cu by 2, 2^{-1} biquinomyl method. Also, using the Milton Roy 21D Spectrophotometer, the Pb, Cr, Zn, and Cd were determined.

COLLECTION OF SOIL SEDIMENTS SAMPLES FOR MICROBIAL ANALYSIS

Soil sediment samples were collected from different points of Mountain Top University Lake. The soil sediments samples were collected in a clean sterilized MacCartney bottles from sampling stations 1,2,3 and control at time variations of 6:00am in the morning, 12pm in the afternoon, 6:00pm in the evening and 12am in the morning from MTU Magada Lake, Lagos-Ibadan express way, Ogun state. Analytical study of the soil sediments samples were carried out in the Microbiology laboratory of Mountain Top University for identification of possible bacteria.

PREPARATION OF MEDIA

The media used for isolation was Nutrient Agar. The Petri dishes and Durham bottles to be used for isolation were sterilized using the dry heat sterilization method (Oven) at 160°C for 1hr. It was prepared by weighing 28g of Nutrient Agar in 1000ml in a conical flask using a weighing balance and they were sterilized respectively and kept in the water bath for homogenization after which they were transferred to the autoclave to sterilize at 120mmHg for 1hr and then brought out and poured into the petri dishes aseptically and when not needed they were transferred to the water bath to maintain the temperature and prevent them from solidifying.

SERIAL DILUTION AND ISOLATION OF BACTERIA

Serial dilution of the soil sediments samples were made from stock, by putting 1ml of the water sample into 9ml of distilled water in a test tube. Using a five-fold dilution, 10^{-1}dilution factor was made by taking 1ml of water from the stock and then 10^{-2} dilution factor was made by taking 1ml of water from 10^{-1}dilution factor until 10^{-5} dilution factor was made. Isolation was carried out using the pour plate method whereby 0.1ml of the soil sediments samples were siphoned from the dilution factors $10^{-1}$and dispensed in a petri dish using a micropipette, and the agar was allowed to cool but not solidify and 20ml was aseptically poured into the sterilized glass Petri dishes containing dilution factors 10^{-1} and allowed to set and the process was done for 10^{-3} and $10^{-5}$and also for the control which was taken from the untreated borehole water. This process was done aseptically to avoid contamination. The Petri dishes were left for 20mins before inverting them and transferring into the incubator at 37oC for 24-48hrs. Colonies grown on media were enumerated and calculated as colony forming units per cubic meter (CFU m^{-3}). The results were interpreted and documented

SUBCULTURING OF ISOLATES

Distinct colonies from each culture plates were sub-cultured by streaking them on fresh agar plates in order to obtain pure culture to carry out biochemical tests to identify bacteria. The purpose of sub-

culturing colonies is to isolate a colony from various colonies inside a medium and plate inside a fresh nutrient medium. The pure isolates were transferred onto agar slant in McCartney, inoculated for 14-18hrs before transferred into a rigerator at 4°C to serve as stock culture for subsequent test during identification. The sub-culturing process was carried out aseptically to prevent contamination. The Petri dishes were inverted and transferred into the incubator at 37^{o}C for 24hrs

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SUBCULTURING OF ISOLATES

Distinct colonies from each culture plates were sub-cultured by streaking them on fresh agar plates in order to obtain pure culture to carry out biochemical tests to identify bacteria. The purpose of sub-culturing colonies is to isolate a colony from various colonies inside a medium and plate inside a fresh nutrient medium. The pure isolates were transferred onto agar slant in McCartney, inoculated for 14-18hrs before transferred into a rigerator at 4°C to serve as stock culture for subsequent test during identification. The sub-culturing process was carried out aseptically to prevent contamination. The Petri dishes were inverted and transferred into the incubator at 37^{o}C for 24hrs.

BIOCHEMICAL TESTS FOR IDENTIFYING THE ISOLATES

The tests carried out in identifying the isolates are: Gram staining, catalase test, oxidase test, sugar fermentation test, and indole test

GRAMS STAINING:

Reagents used were: Crystal violet, Gram's iodine, safranin and 70% alcohol. The inoculating loop was sterilized on a flame of a bunsen burner, then a pure culture was smeared on a sterile slide and heat fixed by passing it across the flame quickly with the smear facing up. The slides were placed on the staining rack for staining. The smear was covered with crystal violet stain and allowed to stand for 1 minute, then washed off ca ully under running tap water. Gram's iodine was then used to flood the smear and allowed to stand for 1 minute, and then drained off under gentle running tap. Afterwards, the slide was flooded

with decolorizing agent (70% alcohol) and the allowed to stand for 10 seconds. The slide was then washed under running tap water, drained completely and counterstained with safranin for 30 minutes. Then, slide was washed under gently running tap water until no colour appears in the effluent and then blot dried with filter paper and then viewed under the microscope

CATALASE TEST**:**

Reagent used: Hydrogen peroxide

The pure culture was smeared on a sterile slide using a sterilized inoculating loop. Then, a drop of hydrogen peroxide was dropped on the smear. The result was then observed. The presence of oxygen bubbles showed the presence of catalase and the absence of the oxygen bubbles showed the absence of catalase.

OXIDASE TEST**:**

Reagent used: Oxidase reagent

The pure culture was smeared on the filter paper and few drops of the oxidase reagent was added and the results were observed. A purple colouration was produced within 10 seconds by oxidase positive cultures. Absence of purple colouration was produced by oxidase negative cultures

INDOLE TEST:

Reagent used: Kovac's Reagent

Peptone water was poured in to 19 test tubes and inoculated with a loopful of the 19 isolates respectively. Then, it was incubated for 5-7 days at 37^0C. Afterwards, 0.5ml of Kovac's reagent was added to the test tubes and gently shook and allowed to stand. The colour was observed

CHAPTER FOUR

RESULTS

Summary of Insitu physico-chemical characteristics of Magada Lake AS indicator For Soil Sediment

Physico-Chemical Parameters	Time			p-Value
Air Temperature(°c)	12AM	25.6222	±0.78975	P<0.05
	6AM	27.8667	±0.88882	
	12PM	26.3889	±1.03297	
	6PM	23.6111	±3.77629	
	CONTROL	27.3000	±0.000	
Water Temperature(°c)	12AM	27.0333	±0.43333	
	6AM	27.9333	±0.53645	
	12PM	26.8222	±0.60123	
	6PM	27.0556	±1.02974	
	CONTROL	28.2667	±0.000	

pH	12AM	7.6333	±0.31798
	6AM	6.9889	±0.15396
	12PM	7.1667	±0.65574
	6PM	6.3444	±0.45010
	CONTROL	5.8833	
Salinity(mgl^{-1})	12AM	7.3333	±0.25166
	6AM	130.4444	±48.55962
	12PM	113.7778	±54.75839
	6PM	80.6144	±78.44701
	CONTROL	86.0000	±0.000
Turbidity(NTU)	12AM	17.9478	±8.07649
	6AM	7.9178	±3.74110
	12PM	6.5844	±4.68186
	6PM	18.6700	±15.26461
	CONTROL	.0700	±0.000
Dissolved oxygen(mgl^{-1})	12AM	9.6800	±1.13210
	6AM	11.5367	±3.77971
	12PM	15.3222	±3.01300
	6PM	13.5778	±4.99793
	CONTROL	0.0667	±0.000
Conductivity(μscm^{1})	12AM	0.6544	±0.68406
	6AM	0.2656	±0.10178
	12PM	0.1467	±0.08083
	6PM	0.1456	±0.07042
	CONTROL	0.8467	±0.000

Depth(cm)	12AM	3.6000	±1.47309
	6AM	3.6000	±1.47309
	12PM	3.4667	±1.30512
	6PM	3.4667	±1.30512
	CONTROL	4.5000	±0.000

Table 4.2: Summary of Nutrients characteristics in Magada Lake

PHYSICO-CHEMICAL PARAMETERS	TIME	MEAN	STD. DEVIATION	P -VALUE/ SIG
Phosphate(mgl^{-1})	12AM	0.0090	±0.00624	p>0.05
	6AM	0.0087	±0.01016	
	12PM	0.0092	±o.00366	
	6PM	0.0085	±0.00585	
	CONTROL	0.0140	±0.00000	
Sulphate(mgl^{-1})	12AM	3.4810	±3.00319	
	6AM	4.9139	±2.14602	
	12PM	4.8880	±4.12575	
	6PM	6.2476	±0.57927	
	CONTROL	2.5447	±0.00000	
Nitrate(mgl^{-1})	12AM	4.0456	±3.60322	
	6AM	7.1667	±2.43333	
	12PM	8.6278	±01.1219	

	6PM	7.5356	±0.12760
	CONTROL	817.88	±0.00000
Magnesium(mgl^{-1})	12AM	1.5033	±1.29950
	6AM	2.6300	±0.59851
	12PM	4.5956	±03.4622
	6PM	2.2989	±0.29410
	CONTROL	2.3800	±0.00000
Calcium(mgl^{-1})	12AM	17.344	±1.52036
	6AM	14.094	±2.12723
	12PM	16.377	±2.93359
	6PM	15.744	±3.73651
	CONTROL	10.000	±0.00000

Table 4.3 : Summary of Ex situ physico-chemical characteristics of Magada Lake

PHYSICO- CHEMICAL PARAMETERS	TIME	MEAN	STD. DEVIATION	P- VALUE
Cadmium(mgl^{-1})	12AM	0.0032	±0.00291	p>0.005
	6AM	0.0077	±0.01002	
	12PM	0.3747	±0.64894	
	6PM	0.3760	±0.63945	
	CONTROL	0.0000	±0.00000	
Iron(mgl^{-1})	12AM	0.7310	±0.63950	
	6AM	1.0457	±0.97769	
	12PM	0.8386	±0.62883	
	6PM	0.3401	±0.42383	
	CONTROL	0.0000	±0.00000	

Lead(mgl^{-1})	12AM	0.0003 ±0.00058
	6AM	0.0010 ±0.00173
	12PM	0.0043 ±0.00751
	6PM	0.0363 ±0.05950
	CONTROL	0.0000 ±0.00000
Chromium(mgl^{-1})	12AM	0.0367 ±0.06351
	6AM	0.0750 ±0.07744
	12PM	0.0241 ±0.03835
	6PM	0.7102 ±0.61796
	CONTROL	0.000.000
Hardness(mgl^{-1})	12AM	12.6778 ±4.47367
	6AM	14.8200 ±2.25868
	12PM	12.2611 ±5.88837
	6PM	14.9844 ±5.70780
	CONTROL	10.3167 ±0.00000
Alkalinity(mgl^{-1})	12AM	1.3656 ±0.37144
	6AM	1.0200 ±0.19757
	12PM	1.5289 ±0.01388
	6PM	0.5622 ±0.45928
	CONTROL	1.1300 ±0.0000

Table 4.4: Summary of other Ex situ physico-chemical characteristics of Magada Lake

PHYSICO-CHEMICAL PARAMETERS	TIME	MEAN	STD. DEVIATION	P VALUE
BOD_5	12AM	0.0556	±0.09623	P< 0.05
	6AM	0.0722	0.048110	
	12PM	0.1667	±0.10000	
	6PM	0.1556	±0.03849	
	CONTROL	0.0000	±0.00000	
TDS (mg^{-1})	12AM	33.2611	±14.20043	
	6AM	16.0400	±8.20978	
	12PM	13.6989	±8.67599	
	6PM	34.3611	±27.08495	
	CONTROL	0.866± .		
TS (mg^{-1})	12AM	20.3289	±9.82747	
	6AM	8.6356	±4.15949	
	12PM	7.3856	±5.84371	
	6PM	20.7033	±18.00330	
	CONTROL	0.0500	±0.000000	
Total solid (mg^{-1})	12AM	53.5900	±24.01837	
	6AM	24.6756	±12.36826	
	12PM	21.0511	±14.53360	
	6PM	55.0611	±45.07567	

CONTROL	0.93670 ±.0.00000

Figure 5 showing water temperature

Figure 6 Showing Air Temperature

Figure 7 showing pH

Figure 8 showing Salinity

Figure 9 Showing Turbidity

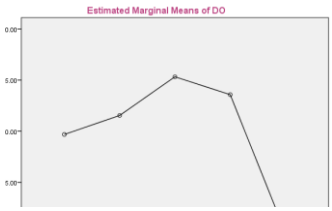

Figure 10 showing Dissolved Oxygen

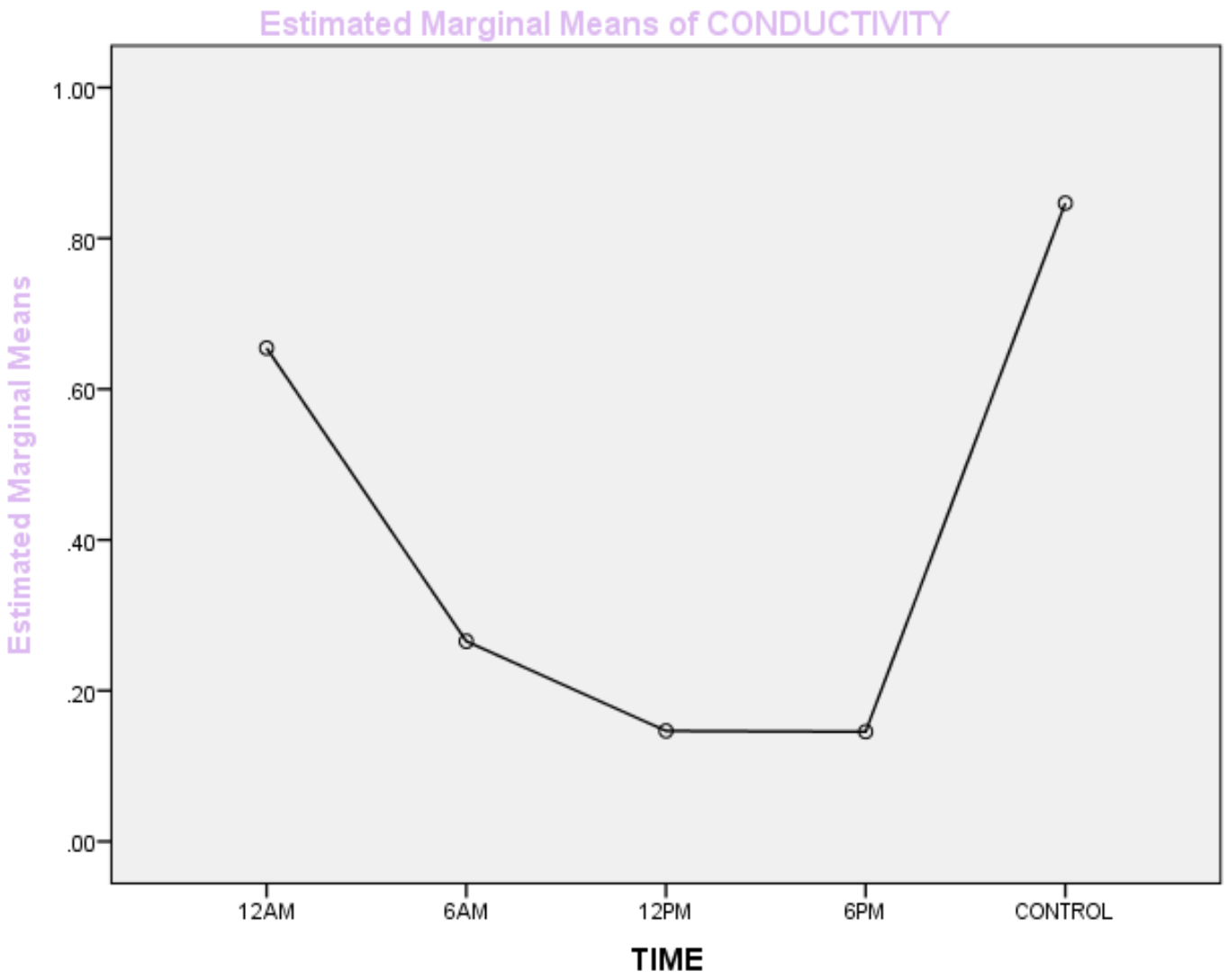

Figure 11 showing Conductivity

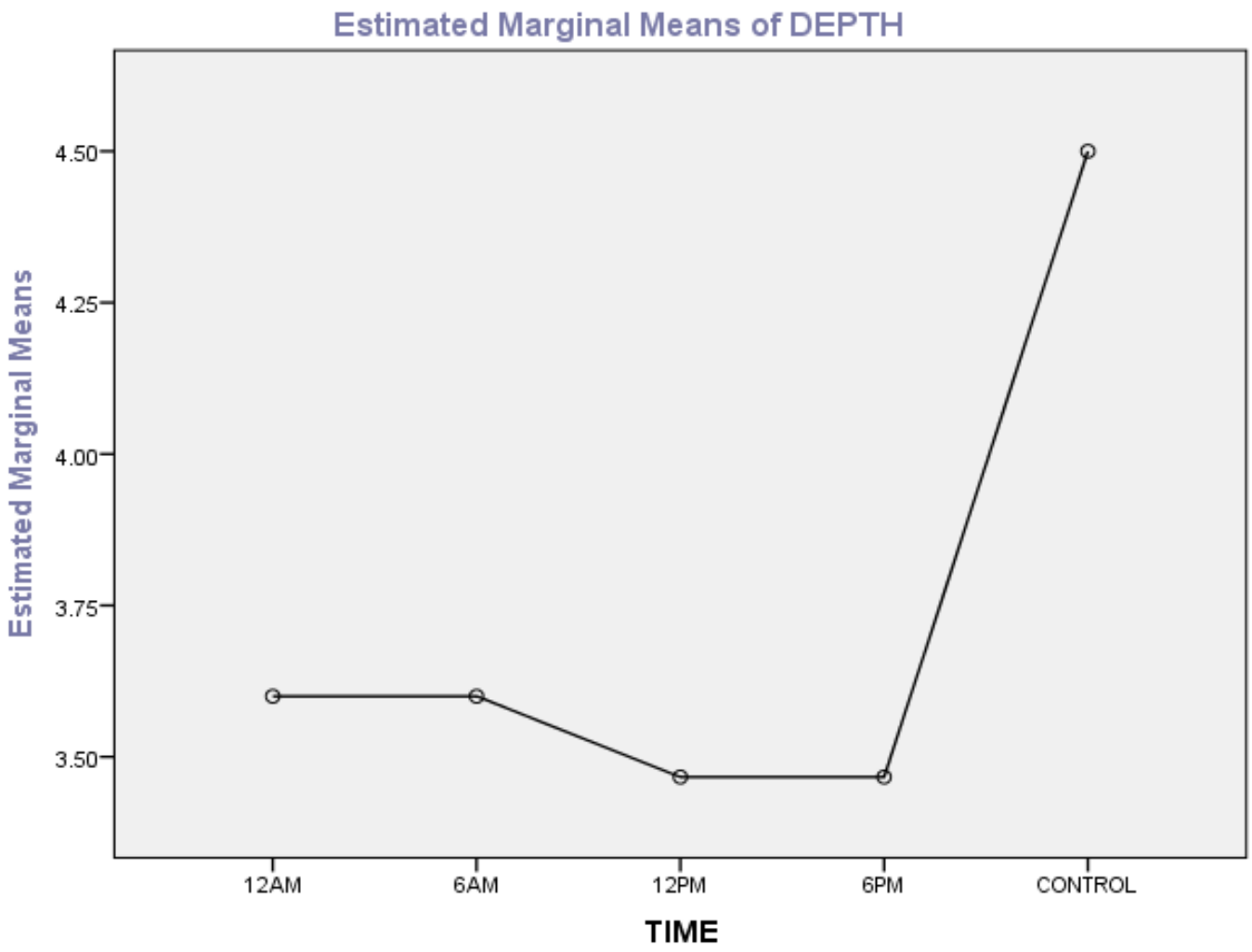

Figure 12 showing Depth

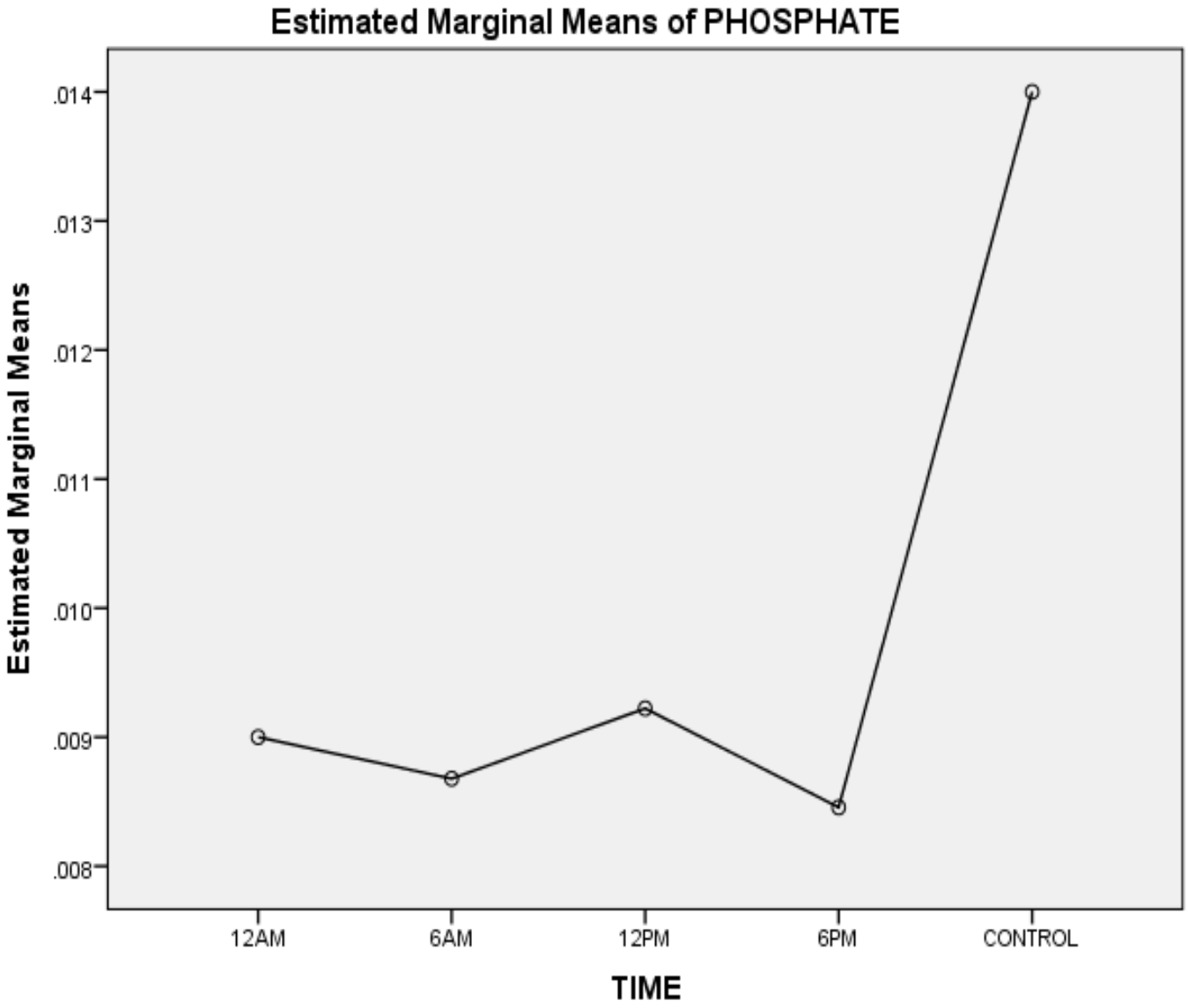

Figure 13 showing Phosphate

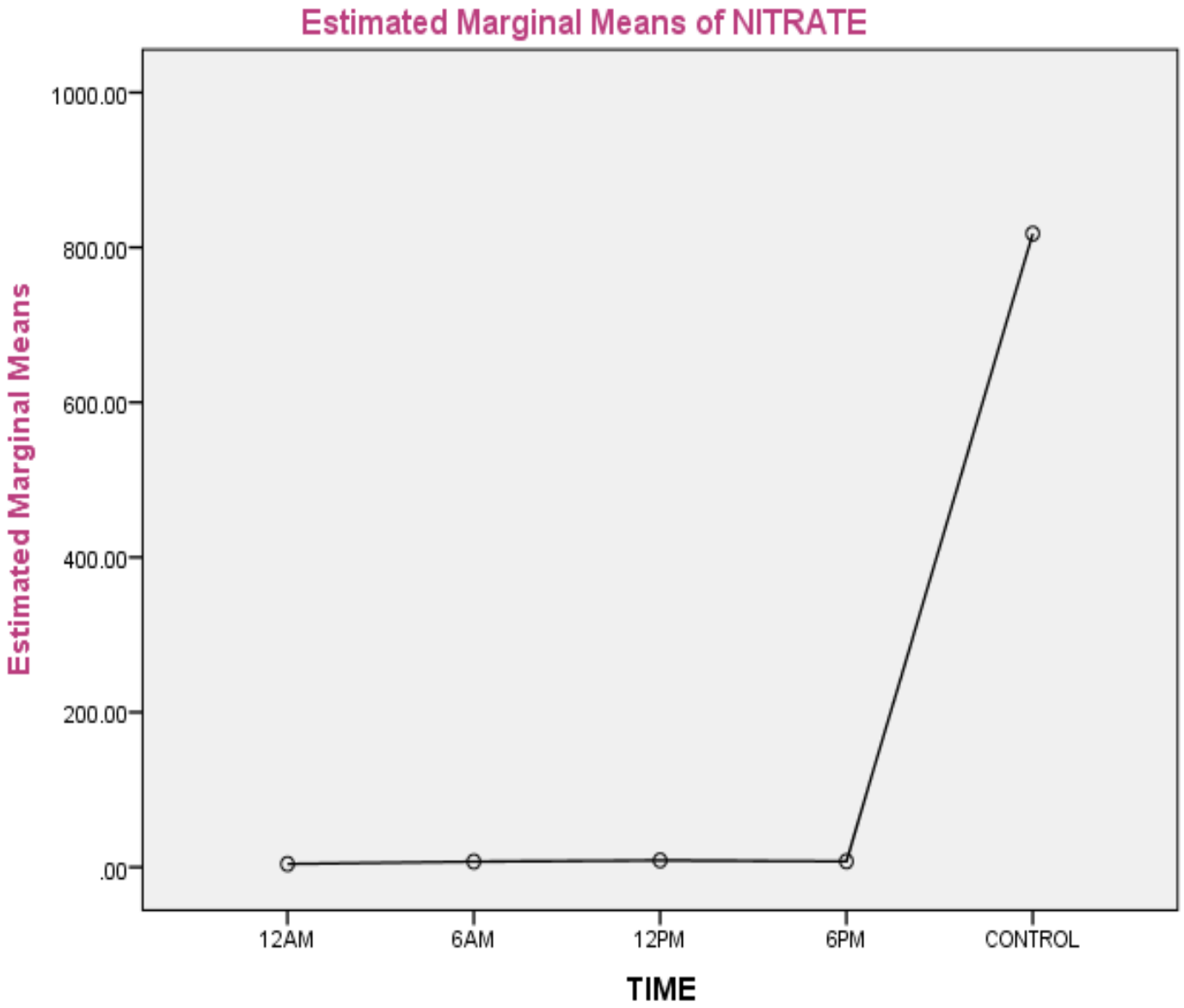

Figure 14 showing Nitrate

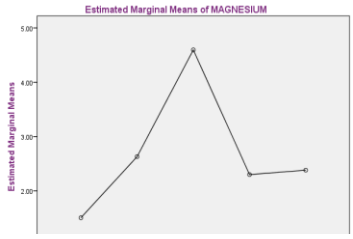

Figure 15 showing Magnesium

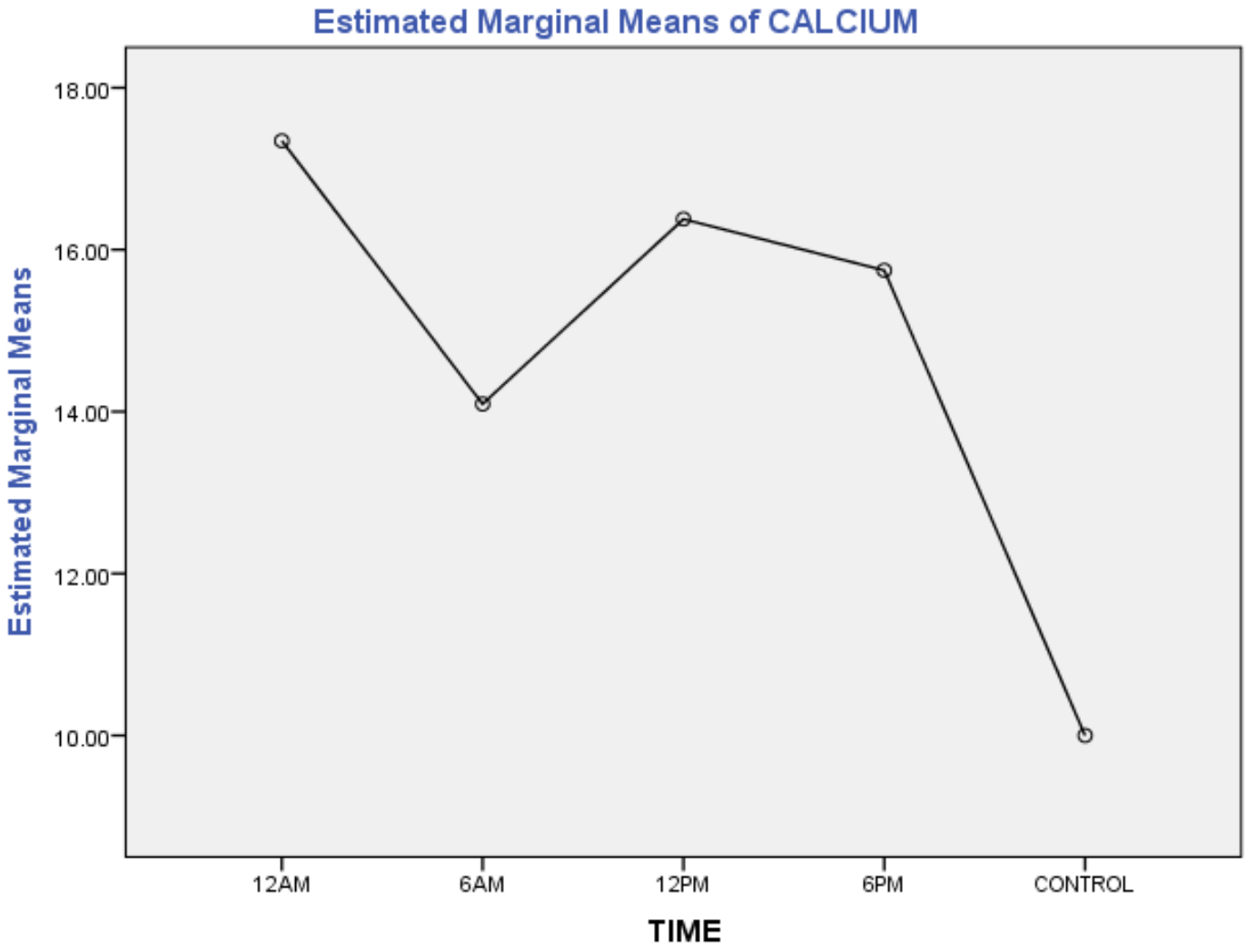

Figure 16 showing Calcium

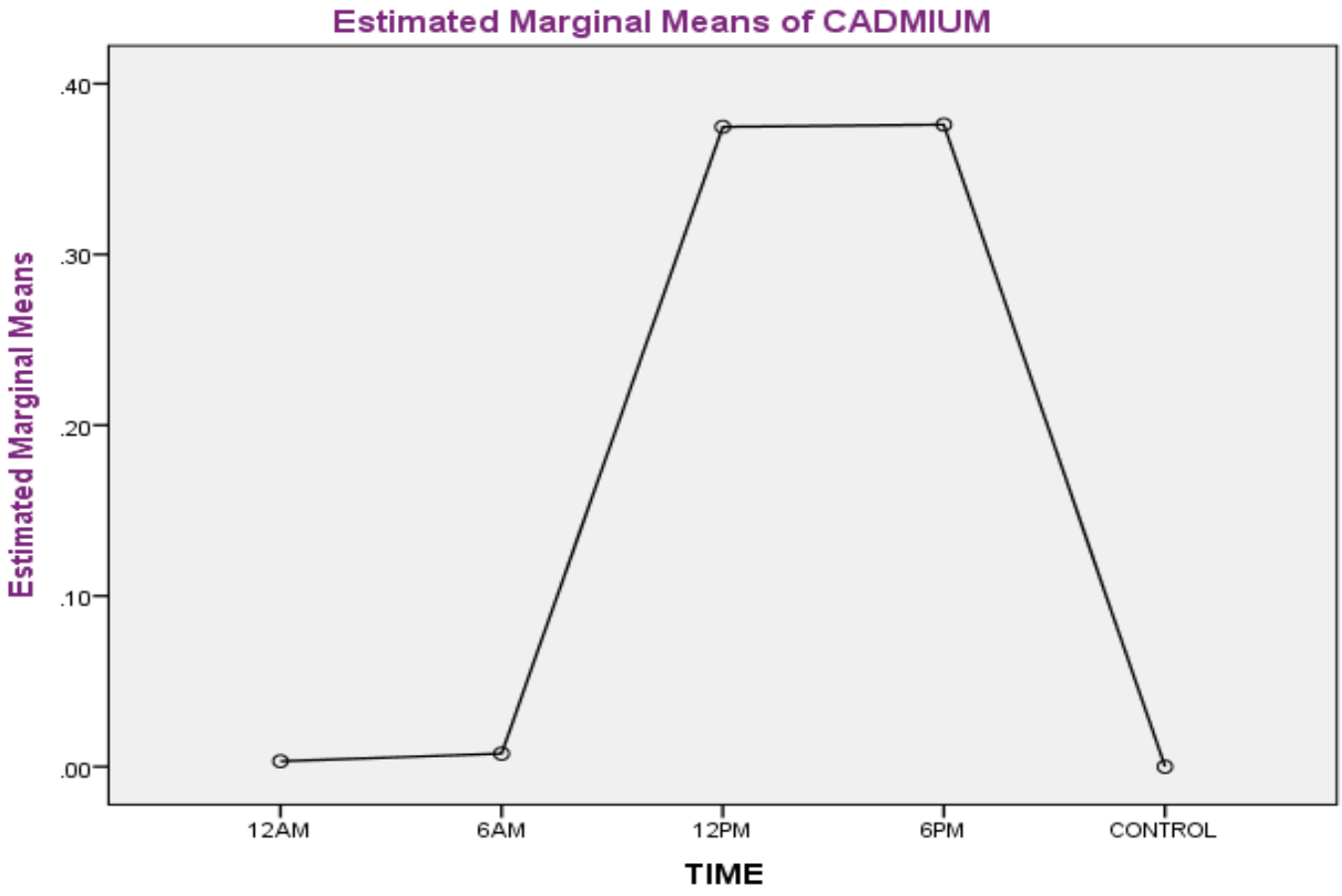

Figure 17 showing Cadmium

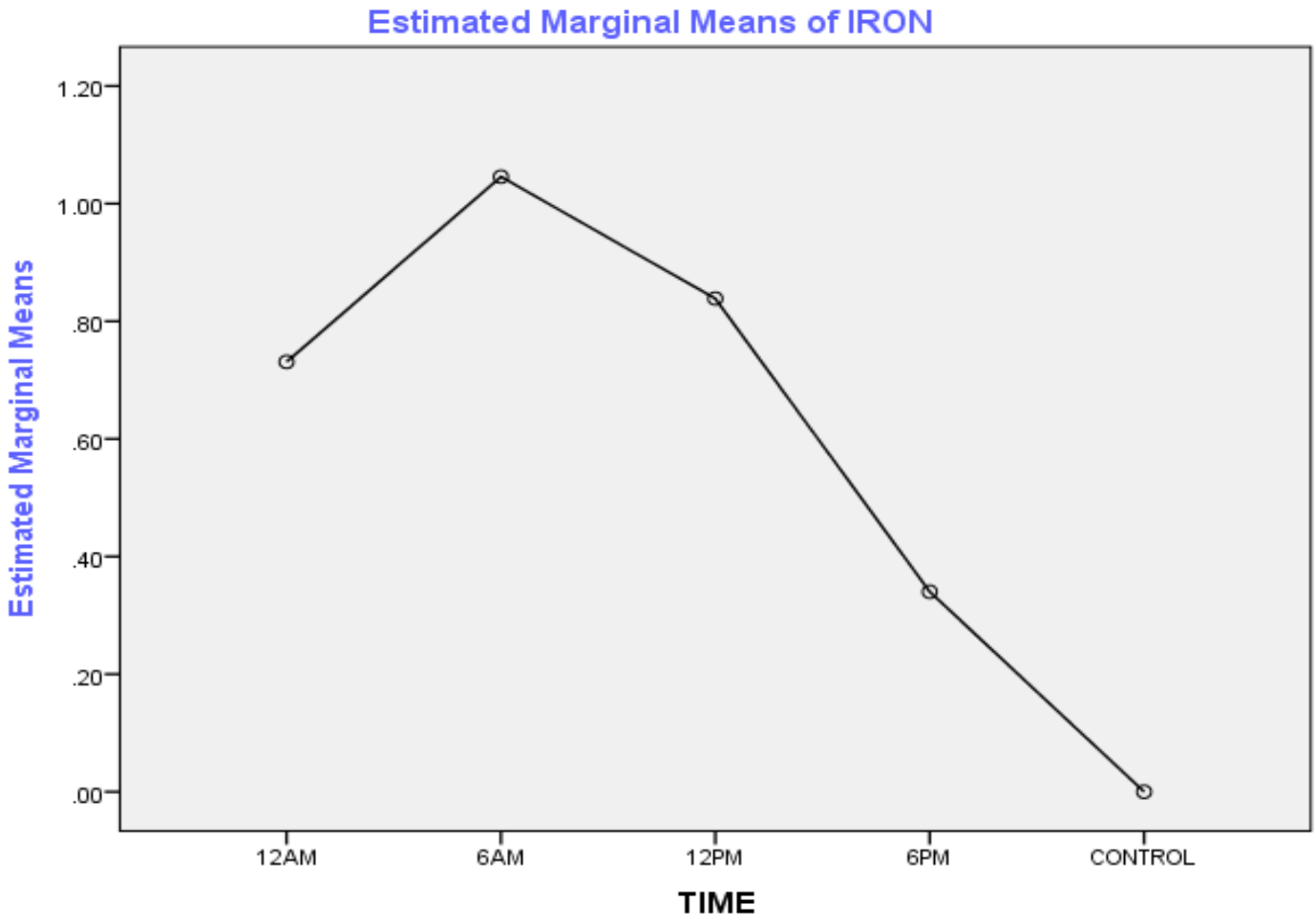

Figure 18 showing Iron

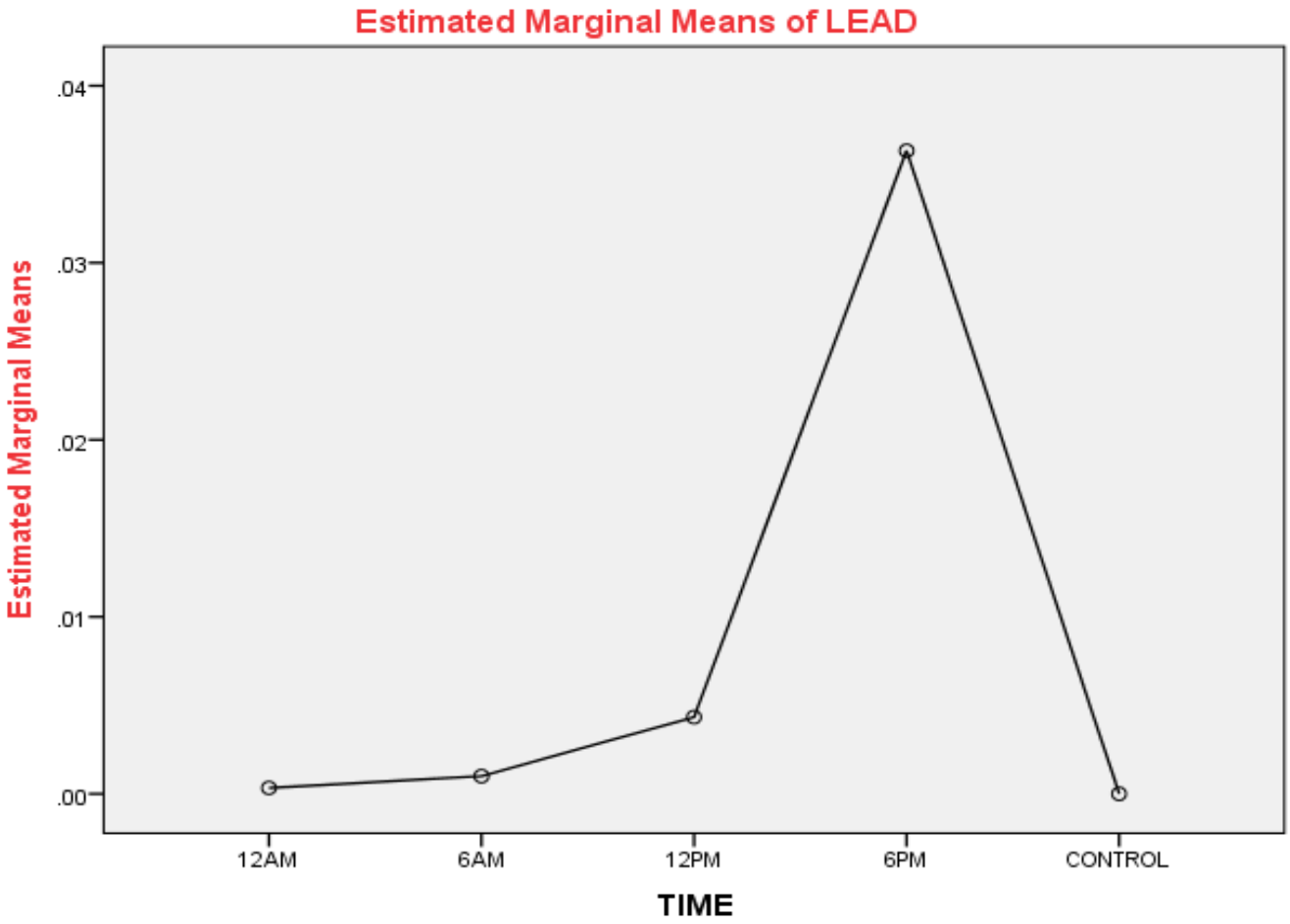

Figure 19 showing Lead

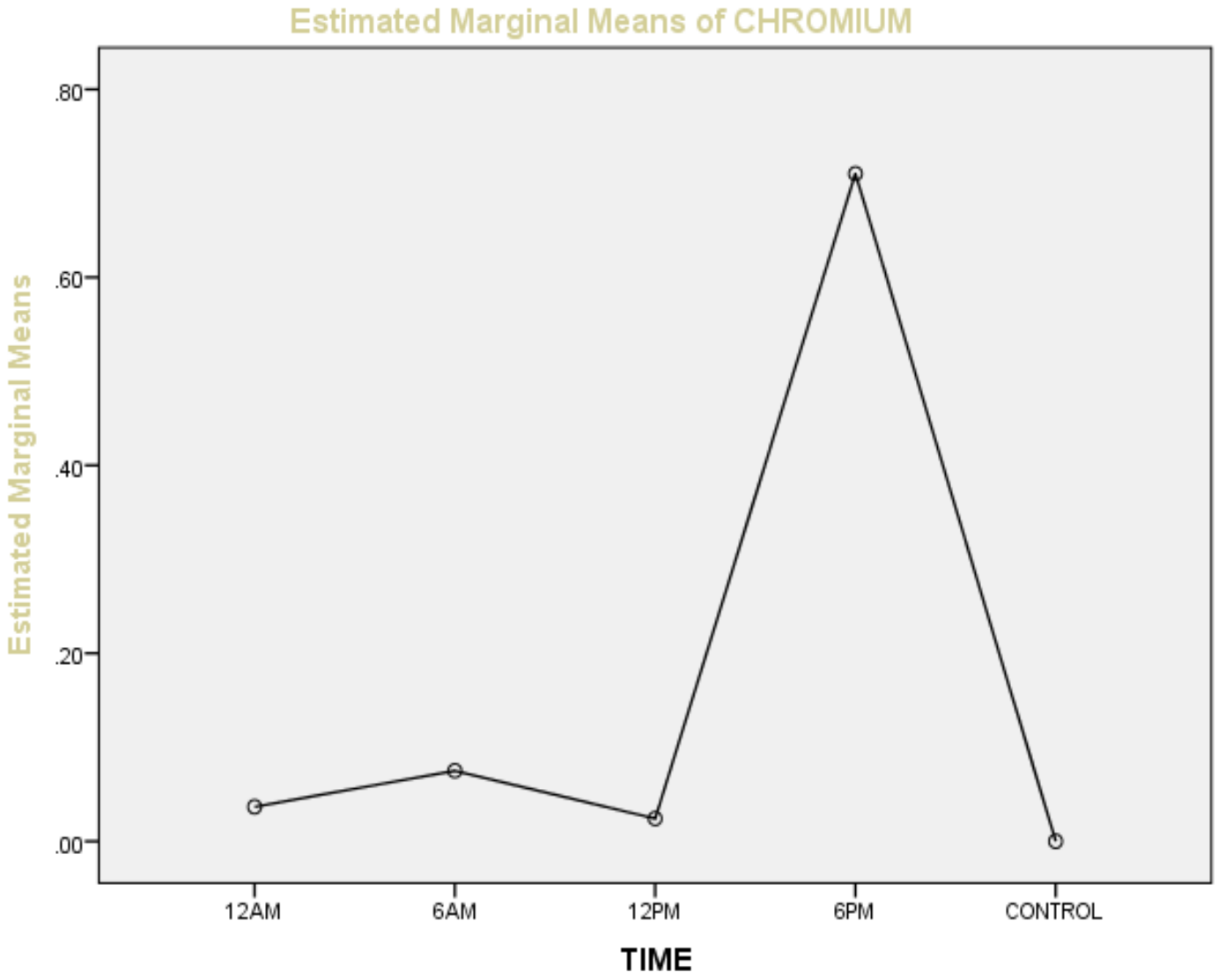

Figure 20 showing Chromium

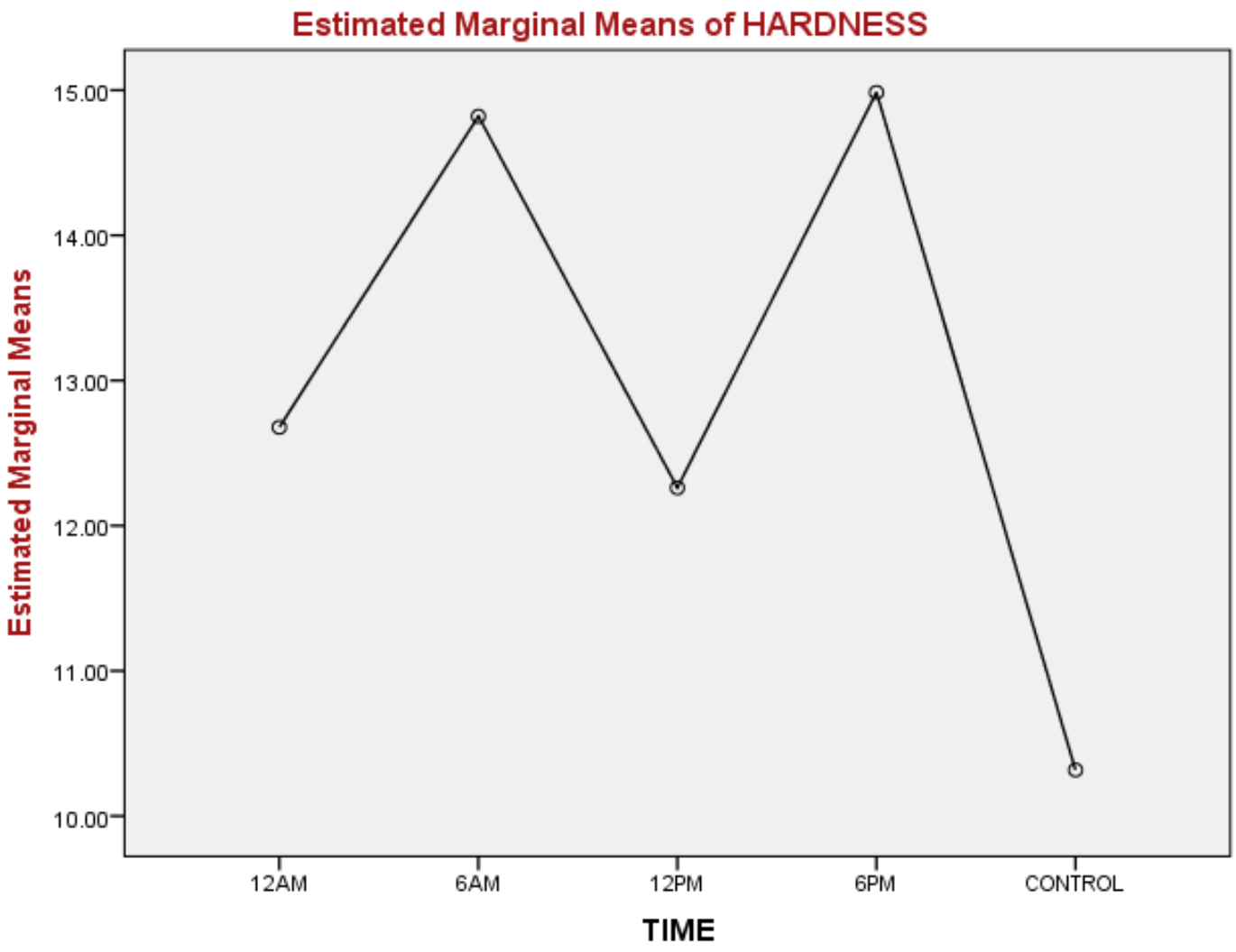

Figure 21 showing Hardness

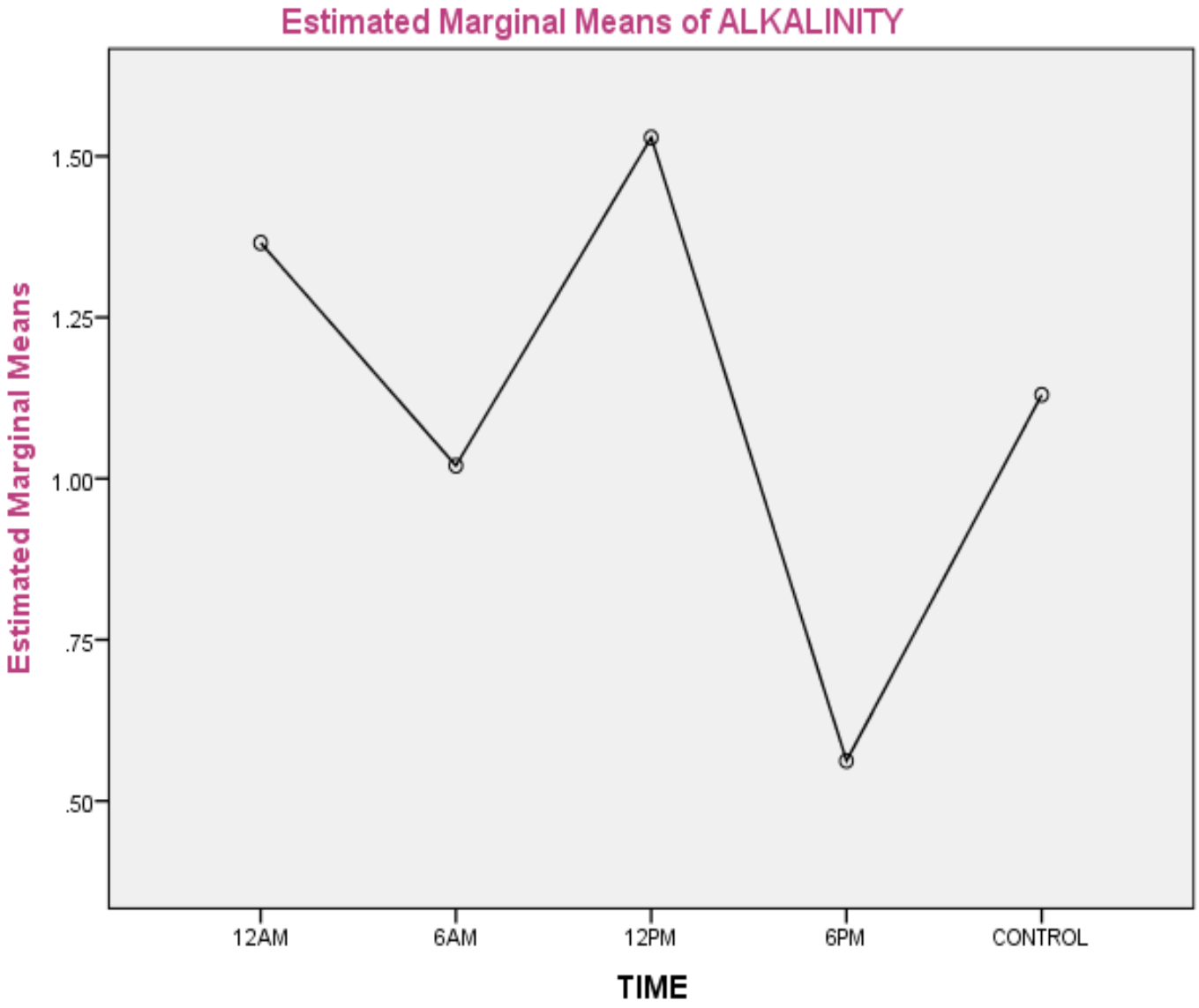

Figure 22 showing Alkalinity

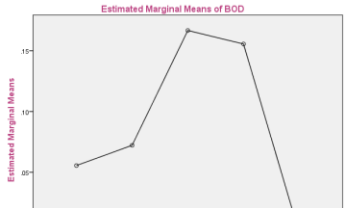

Figure 23 showing B.O.D

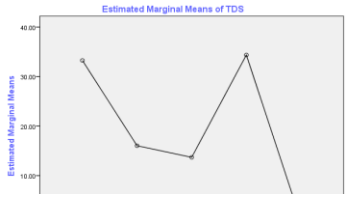

Figure 24 showing Total Dissolved Solids

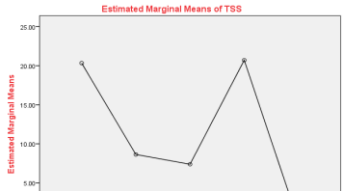

Figure 25 showing Total Suspended Solids

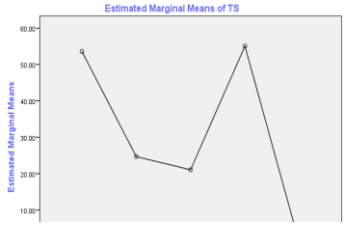

Figure 26 showing Total Solids

In-situ physic-chemical parameters were determined with respect to time. The air temperature was high at 6am which is also similar to that of the control. However, the air temperature at 12am and 12pm are similar, while that of 6pm was the least in diurnal variations. The water temperature of the control was unique in comparism to 12am, 6am, 12pm and 6pm. Though 12pm was the lowest. At 12am and 12pm, the pH value is neutral. The pH at 6pm was relatively acidic in comparison to the control that was completely acidic. At 6am, the salinity was high with a value of 130.4 mgl^{-1} which is closely followed by 12pm. The salinity at 6pm is nearly comparable with the control while that of 12am is the smallest with value of 12.63 mgl^{-1}. The turbidity at 12am and 6pm are relatively close. Though 6pm was having the highest value of 18.60. Similarly. control has the lowest value of 0.07. Dissolved oxygen is outstanding at 12pm and is closely followed by 6pm, However D.O at 6am is also significantly higher than that of 12am,in all these, control is having the least value .Conductivity at 12pm is similar to that of 6pm having the least values. The conductivity at 6am increases above that of 12pm and 6pm. The conductivity of the control is the highest value which is then followed by the conductivity at 12am (Table 1).

Ex-situ physic-chemical analysis revealed the nutritional status with respect to diurnal variations. The phosphate level at 12am and 12pm are similar, in comparism with the phosphate level at 6am and 6pm that also exhibits similar status with the control. The sulphate level at 6pm is greater than the rest of the diurnal periods, with the control being the list. 12am is slightly above the control with 6am and 12pm is similar in comparison with each other. The control of nitrate has a remarkably high value.12pm is incomparable to the control but is the highest among the other time.12am is the lowest ;with 6am and 6pm and control are similar in values which are slightly above 12pm.At 12am ,the level of calcium is remarkable and is incomparable with the control.12pm is next in value after 12am. At 6pm the value is incomparable with 6am. Hence, 6pm has a higher value than 6am (table 2)

Ex-situ physic-chemical parameter precisely heavy metals with respect to time. 12am and 6pm have similar cadmium content which are the highest content on the table. 12am and 6am also have low content of cadmium; hence they are significantly lower in value than 6pm and 12pm, which the control detected .6am followed by 12pm and 12am descend in iron content respectively with 6pm having a significantly lower value than the rest of the time variations, with the control still not detected. The content of lead as at 6pAt time m happens to significantly vary from the rest of the time variations with 12pm, 6am, and 12am decreasing respectively as the control still not detected. 6am is outstanding and is significantly higher than the rest of the time variations. Next in line is 6am, and 12am and 12pm decrease respectively with the control not detected. 6pm and 6am are similar 12am and 12pm are also similar. 6pm and 6am respectively possess a high degree of hardness while 12pm and 12am are respectively lower than the former, with the control having a value of 10.13. 12pm possess the highest value of alkalinity while 6pm is lowest on the time variation .12am and 6pm are incomparable with 12am slightly higher than 6am-6pm has the lowest degree of alkalinity. The value for the control 1-13. At 12pm, the biochemical oxygen demand is outstanding closely followed by 6pm. 6am and 12am decrease respectively with the control not detected. Total dissolved solid at 6pm is outstanding and is closely followed by 12am, 6am, and 12pm respectively with control having the lowest value. 6pm and 12am happened to be significantly higher in total suspended solid than other values with 6pm higher than 12am, and 6am higher than 12pm, and control having the lowest value.6pm has a remarkably high level of total solid which is closely followed by 12am. The level of solid in 6pm and 12pm are incomparable with 12am and 6pm. 6am is higher than 12pm in total solid. The control is the least value.

In table 1, comparatively; apart from pH that shows a significant difference ($p<0.05$) others such as air temp, water temperature, salinity, turbidity, dissolved oxygen, conductivity and depth were not significantly different.

In Fig 27: At exactly 12am the bacterial population was on the increase and high side, in comparative to 6am, 12pm and 6pm. Similarly, in station 1, the bacterial load was practically low at 6am. In Fig 28: The bacterial population at 12am is similar to 6pm and 6am at station 2, which is high and the bacterial load reduces at 12pm. Fig 29: Shows the bacterial population in station 3 across the time variation. At exactly 12am the bacterial population is high followed by 6pm and then 12pm. Still in station 3, the bacterial population decreases at exactly 6am. Fig 30: shows the bacterial load in the control at exactly 12am which has high microbial population followed by 12pm, in comparison to 6am. The microbial load reduces at 6pm. Fig 31: shows the overall comparison of bacterial population across all the stations in respect to time.

PRESUMPTIVE IDENTITY OF BACTERIA ASSOCIATED WITH WATER/SOIL

s/n	Lab code	Isolate identity
1	6amSm3c	*Bacillus thuringiensis*
2	6amSm3a	*Bacillus fusiforms*
3	6amSm3d	*Bacillus polymyxa*
4	6pmSm3a	*Bacillus amyloliquefaciens*
5	6pmSm3b	*Bacillus cereus*
6	6pmSm3c	*Bacillus subtilis*
7	6amSm2c	*Escherichia coli*
8	6amSm2b	*Enterococci*
9	6amSm2a	*Bacillus popilliae*
10	12pmSm3b	*Bacillus mesentericus*

11	12pmSm3c	*Bacillus alvie*
12	12pmSm1c	*Escherichia coli*
13	12pmSm1c	*Bacillus larvae*
14	12pmSm1b	*Bacillus spp*
15	12pmSm1a	*Bacillus spp*
16	6amSm2d	*Bacillus spp*
17	6amSm2a	*Bacillus spp*
18	6amSm2b	*Enterobacter spp*
19	12amSm2b	*Bacillus*
20	12amSm2a	*Enterobacter spp*

EX-SITU PHYSICOCHEMICAL PARAMETERS FOR THE LAKE

S/N	STATION	TIME	REPLICATE	CADMIUM (Mg/L)	IRON (Mg/L)	LEAD (Mg/L)	CHROMIUM (Mg/L)	HARDNESS	SALKALINITY
1	1	12am	1	0.002	0.000	0.000	0.000	10.50	+1.78
			2	0.006	0.000	0.000	0.000	13.20	+1.70
			3	0.009	0.000	0.000	0.000	15.20	+1.67
		6am	1	0.001	0.003	0.001	0.000	14.50	+0.90
			2	0.004	0.005	0.003	0.000	14.80	+1.00
			3	0.007	0.007	0.005	0.000	13.20	+1.05

		12pm	1	0.000	1.146	0.000	0.000	16.50	+1.60
			2	0.000	1.187	0.000	0.100	19.60	+1.40
			3	0.000	1.197	0.000	0.105	20.00	+1.60
		6pm	1	1.112	0.005	0.001	1.012	12.66	+0.70
			2	1.114	0.009	0.004	1.003	16.50	+0.75
			3	1.117	0.012	0.007	1.002	18.60	+0.78
2	2	12am	1	0.000	1.184	0.000	0.000	15.60	+1.00
			2	0.000	1.187	0.000	0.000	16.50	+0.98
			3	0.000	1.190	0.000	0.000	18.90	+0.95
		6am	1	0.000	1.185	0.000	0.008	14.90	+1.20
			2	0.000	1.187	0.000	0.100	17.50	+1.50
			3	0.000	1.189	0.000	0.103	19.60	+1.00
		12pm	1	0.000	1.222	0.000	0.000	4.95	+1.50
			2	0.000	1.226	0.000	0.000	7.50	+1.80
			3	0.000	1.230	0.000	0.000	9.00	+1.32
		6pm	1	0.000	0.815	0.000	0.000	8.50	+0.06.
			2	0.000	0.818	0.000	0.000	8.60	+0.03

			3	0.000	0.820	0.000	0.000	9.50	+0.03
3	3	12am	1	0.002	1.003	0.000	0.108	7.20	+1.51
			2	0.004	1.006	0.001	0.110	7.50	+1.20
			3	0.006	1.009	0.002	0.112	9.50	+1.50
		6am	1	0.016	1.940	0.000	0.152	10.00	+0.80
			2	0.019	1.945	0.000	0.155	13.30	+0.85
			3	0.022	1.950	0.000	0.157	15.58	+0.88
		12pm	1	1.120	0.111	0.009	0.001	9.50	+1.15
			2	1.125	0.113	0.013	0.004	10.5	+1.62
			3	1.127	0.115	0.017	0.007	12.8	+1.77
		6pm	1	0.011	0.192	0.103	1.123	17.5	+0.89
			2	0.014	0.194	0.105	1.125	20.5	+0.90
			3	0.016	0.196	0.107	1.127	22.5	+0.92
4	Control		1	0.000	0.000	0.000	0.000	8.95	+1.12
			2	0.000	0.000	0.000	0.000	9.50	+1.13
			3	0.000	0.000	0.000	0.000	12.5	+1.14

MICROBIOLOGICAL CHARACTERISTICS

S/N	ISOLATES	GRAM STAINING +/-	GRAM STAINING SHAPE	COLOUR	SHAPE OF COLONY	APPEARANCE	CATALASE +/-	OXIDASE +/-	INDOLE +/-
1.	6amSm3c	+	Bacilli	White	Circular	Dull	+	+	+
2.	6amSm3a	+	Bacilli	White	Irregular	Shiny	+	_	+
3.	6amSm3d	+	Bacilli	White	Circular	Dull	+	+	_
4.	6pmSm3a	+	Bacilli	Cream	Punctiform	Dull	+	_	+
5.	6pmSm3b	+	Bacilli	Cream	Irregular	Translucent	+	_	_
6.	6pmSm3c	_	Bacilli	yellow	Circular	Shiny	+	+	_
7.	6amSm2c	_	Cocci	White	Lobate	Shiny	_	+	_
8.	6amSm2b	_	Cocci	White	Irregular	Shiny	+	+	+
9.	6amSm2a	+	Bacilli	White	Irregular	Shiny	+	_	+
10.	12pmSm3b	+	Bacilli	Cream	Filamentous	Dull	+	_	+
11.	12pmSm3c	+	Bacilli	Cream	Circular	Shiny	+	+	_
12.	12pmSm3a	_	Cocci	Yellow	Undulate	Shiny	+	_	
13.	12pmSm1c	_	Bacilli	Orange	Circular	Shiny	+	_	_
14.	12pmSm1b	+	Bacilli	Cream	Irregular	Dull		_	_
15.	12pmSm1a	+	Bacilli	White	Punctiform	Translucent	+	_	_
16.	6amSm2d	+	Bacilli	Cream	Irregular	Dull	+	+	_
17.	6amSm2a	_	Bacilli	White	Punctiform	Dull	_	+	+
18.	6amSm2b	+	Cocci	White	Punctiform	Shiny	_	+	+
19.	12amSm2b	_	Bacilli	Cream	Irregular	Shiny	+	_	_

20.	12amSm2a	_	Cocci	White	Circular	Shiny	_	+	+

Graphical representation of results

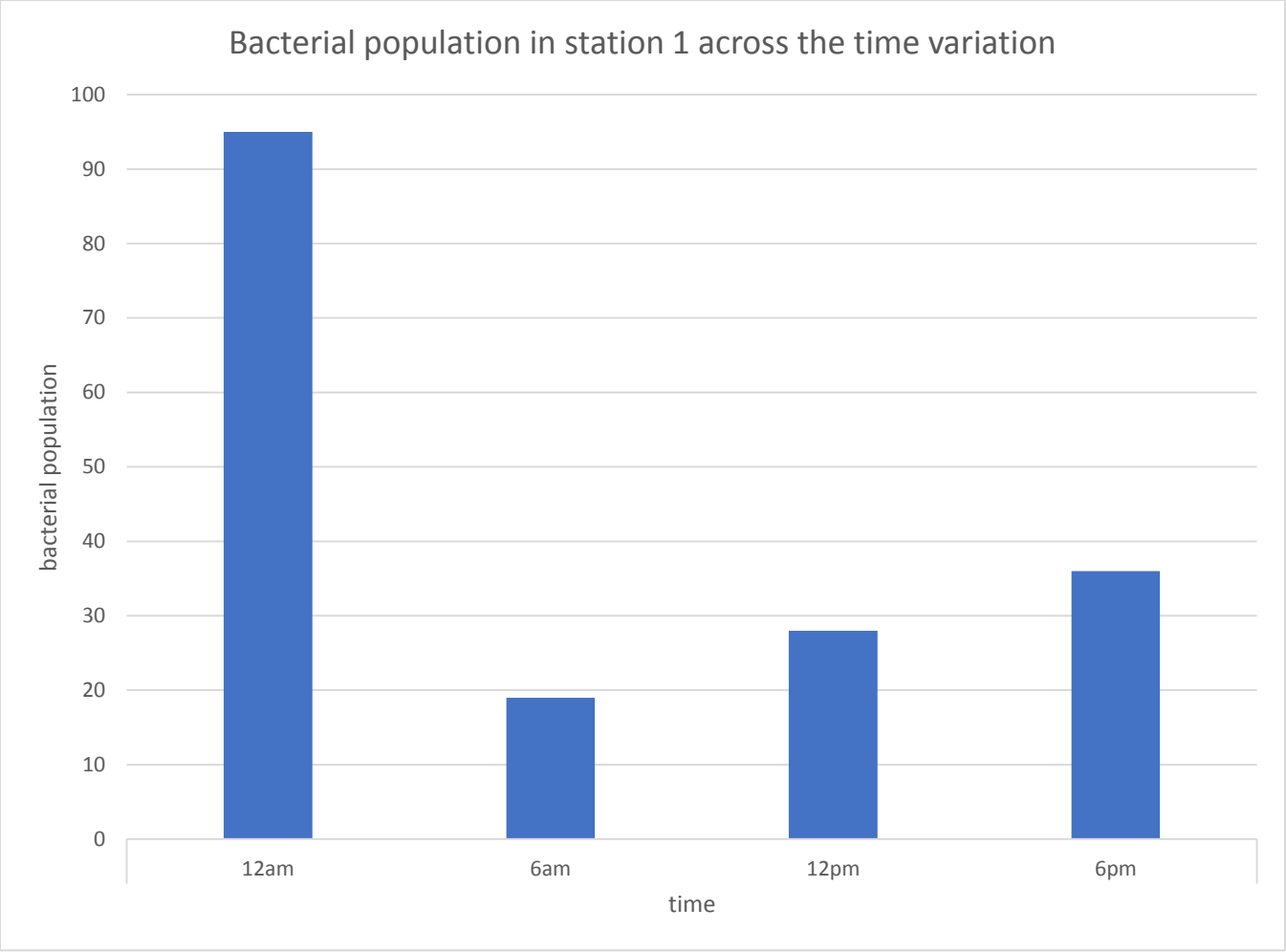

Figure 27 Graph to show bacterial population in station 1 across time variation

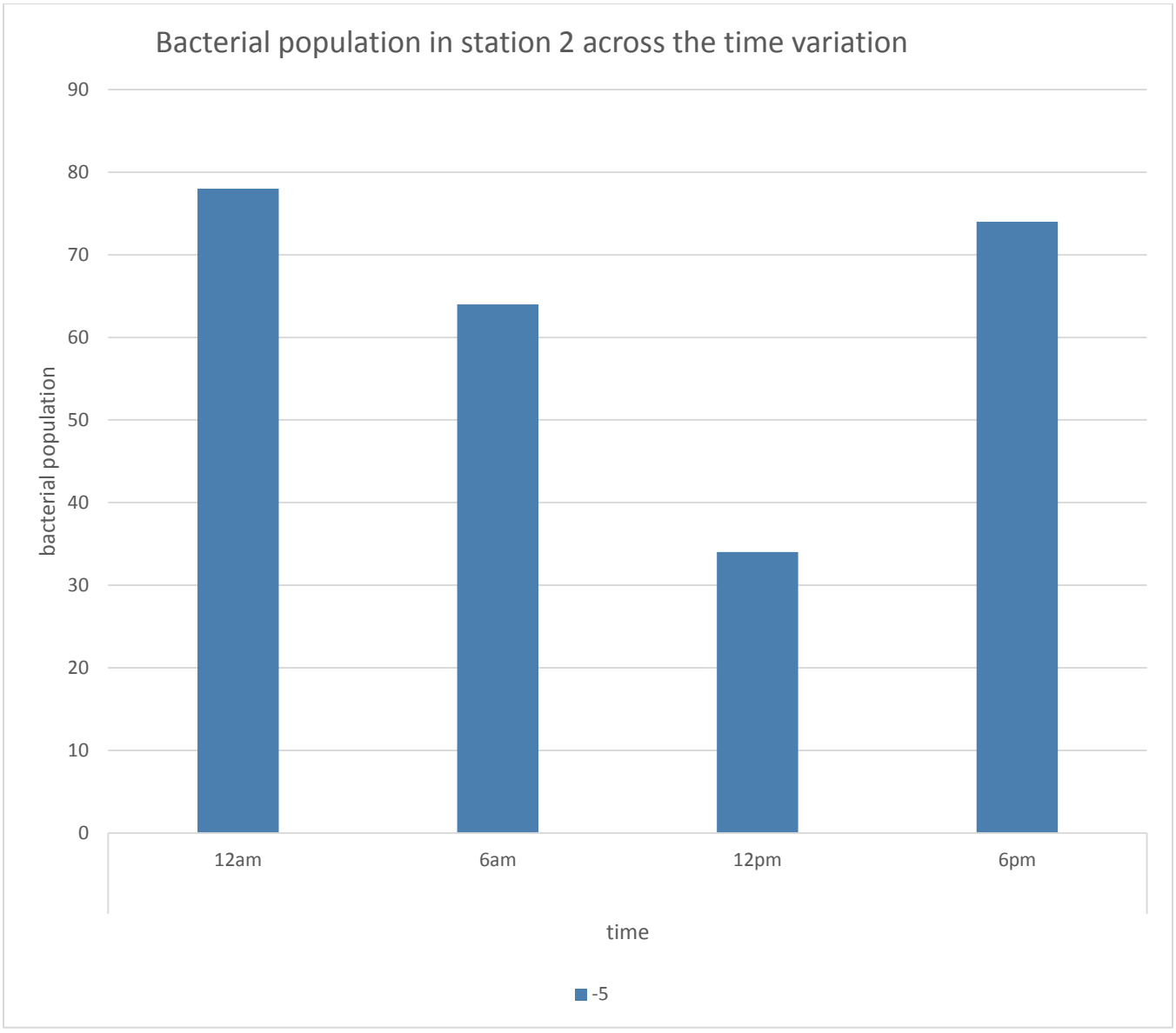

Figure 28 Graph to show bacterial population in station 2 across various time variations

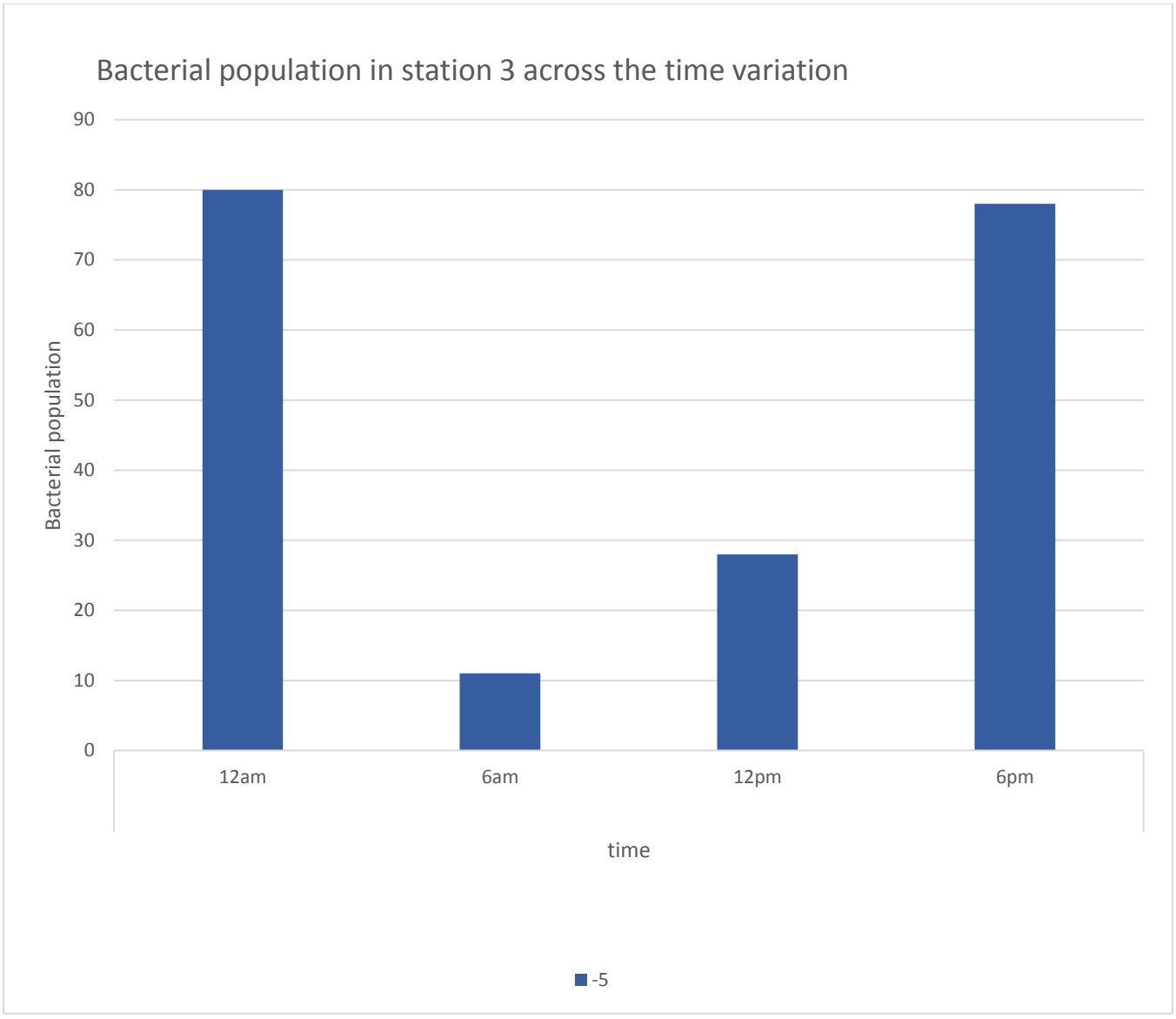

Figure 29 Graph to show bacterial population in station 3 across time variation

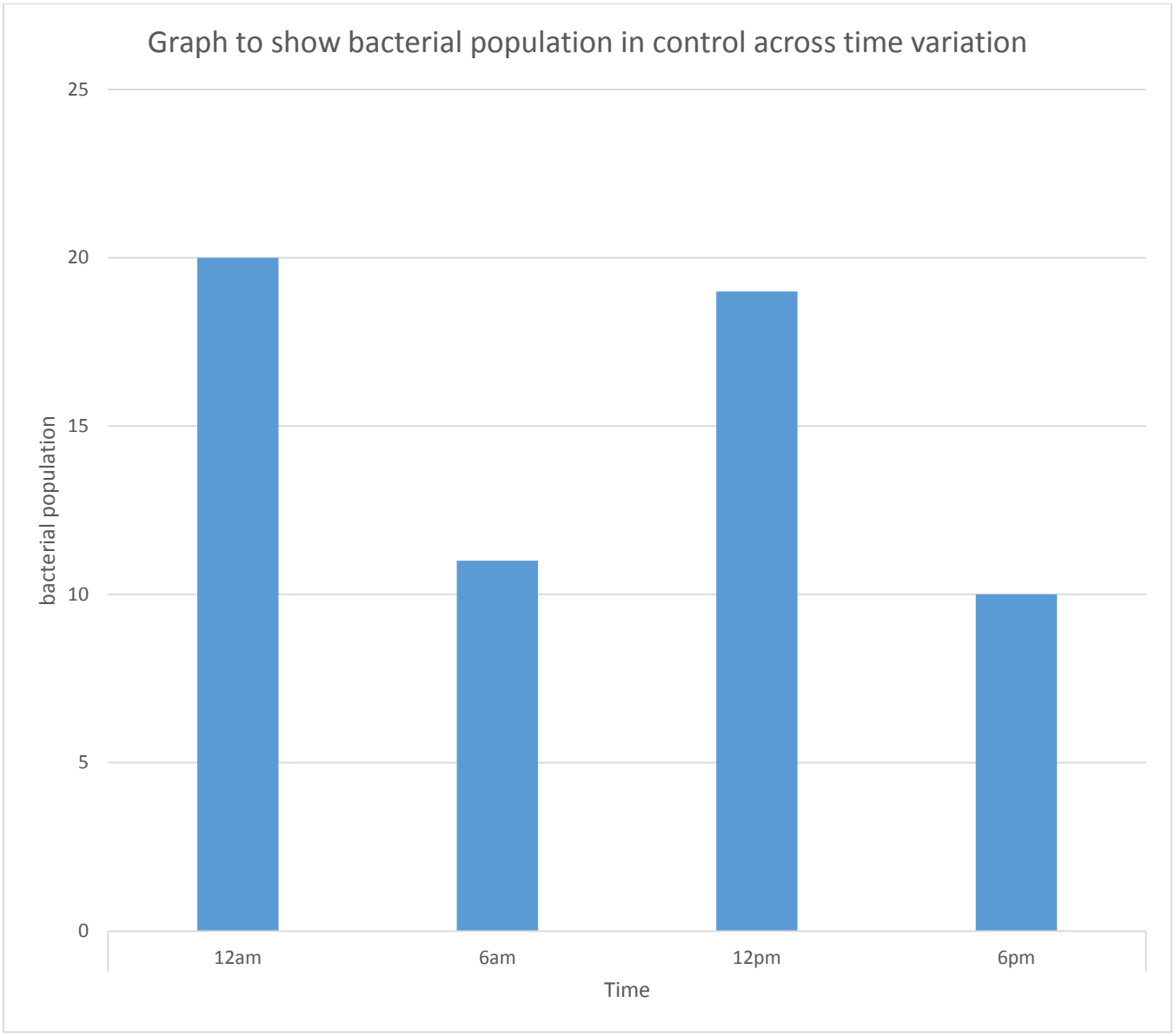

Figure 30 Graph to show bacterial population in control across time variation

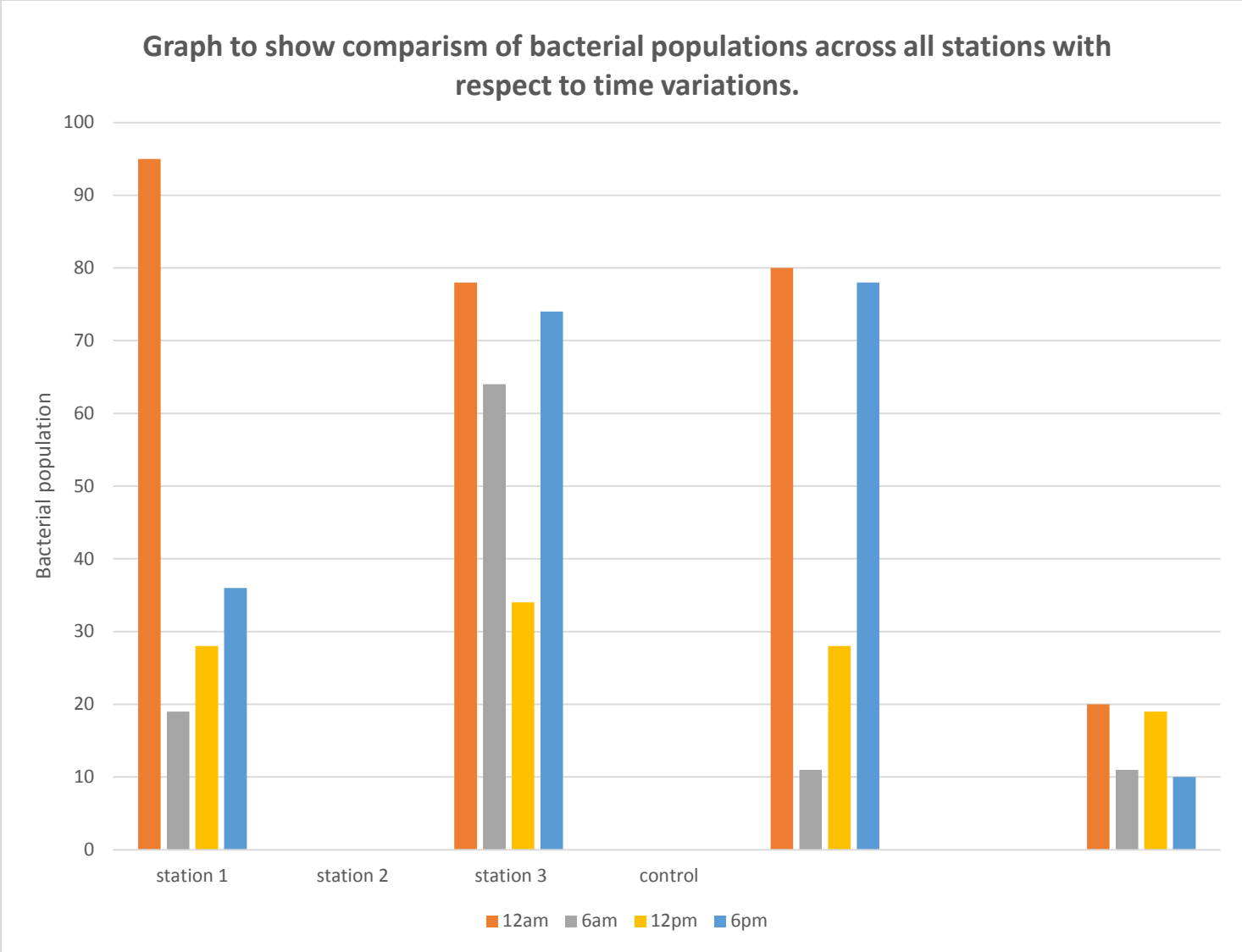

Figure 31 Graph to show comparism of bacterial population across all stations with respect to time variations.

Figure 32 showing pure culture isolates

CHAPTER FIVE

DISCUSSION:

Dissolved Oxygen

According to Mamora (2012) dissolved oxygen is one of the most important parameters in aquatic systems. The mean dissolved oxygen values recorded in Magada lake (9.68 mgl^{-1}) was lowest at 12 am and highest (15.32 mgl^{-1}) at 12 noon. This is at variance with the report of AWQA, 2012 which states that cold water hold more dissolved oxygen than warmers waters. An acceptable reason for this disparity could be as a result of the production of more oxygen in the water due to photosynthesis which is directly proportional to the amount of sunlight, which seemed to be higher at 12 noon. However, all the values recorded exceeded the minimum limit of FEPA and WHO, which is the ore an indication of lesser amount of organic pollution.

BOD

Mahre et al (2007) has stated that BOD_5 of a system is usually increased by the addition of organic and inorganic substances to the system. The BOD5 values ranged from 0.056 mgl^{-1} recorded at 12 am to 0.167 mgl^{-1} recorded at 12 noon. According to them BOD_5 is used as an indication of organic pollution, and the results the ore shows that the BOD_5 levels were far lower than the FEPA and WHO permissible limits for drinking water and aquatic life (Chapman,1996). Thus it can the ore be interpreted that Magada lake was low in organic pollution.

Depth

Depth did not show any form of variation, and ranged from 2.0m (station 1) to 4.9m (station3)

Phosphate

Statement by Turner Designs (2012) indicates that phosphate can be found as free ions in water systems and as a salt in terrestrial environment used in detergents as water softeners Phosphate values ranged

from 0.0085mgl-1 (6 pm) to 0.0092 (12 noon). These values were low compared with the values recorded by Mahre et al, 2007 in River Kaduna (5.5 mgl^{-1} - 44.7 mgl^{-1}). However all the values where lower that the value of the control which was 0.0140mgl-1. The reason for the control value being higher could be as a result of plants and bacteria using up the nutrients for growth, while there were no plants, and negligible bacteria population in the control water (bore hole). The values were also lower than the FEPA and WHO permissible limits for drinking water.

Sulphate

Sulphate levels ranged from 3.48 mgl^{-1} (12 am) to 6.25 mgl^{-1} (6pm). These values are however lover that that on the control (2.54 mgl^{-1}). The slightly high sulphate levels could be due to the usage of fertilizers for horticultural practices around the water body. Another reason for this could also be as a result of burning of fossil fuel for power generation and from transportation around the water body which ends up as runoff water which flow into the water body. The values were however lower that that recorded by Akan *el at* (2007) which ranged from 22.60 mgl^{-1} to 45.45 mgl^{-1}. However the levels were below FEPA and WHO maximum permissible level for drinking water (20 mgl^{-1} and 200 mgl^{-1} respectively). The values were however lower than that of the control (2.54 mgl^{-1})

Nitrate

The nitrate values recorded in this study were between 4.05 mgl^{-1} (12am) to 8.62 mgl^{-1} (12pm). These values were higher than that the control (0.0788 mgl^{-1}). According to Bush and Meyer (1982) nitrate toxicity is capable of causing anemia in infants and pregnant women, and formation of carcinogenic nitrosamines. According to them a nitrate content of more than 100 mgl^{-1} impact bitter taste to water and may cause physiological problem. According to Akan *et al* (2007) can lead to high growth of algae and other organisms which can lead to eutrophication. However, the nitrate levels found in this study were lower than permissible limits of FEPA and WHO (45 mgl^{-1}).

Calcium and Magnesium

Magnesium and Calcium are main indicators of water hardness. The levels of magnesium recorded in the study ranged from 1.50 mgl^{-1} (12 am) to 4.60 mgl^{-1}, while the level of calcium recorded ranged from 14.09 mgl^{-1} (6 am) to 17.34 mgl^{-1} (12 am). The hardness of drinking water can be determined by its calcium and magnesium content, which can be expressed as the equivalent amount of calcium carbonate that is usually formed formed from the calcium and magnesium in solution. One major source of calcium in freshwater is the bedrock and weathering of calcium bearing rocks (Waite, 1984). Calcium is often the most common metallic ion in fresh surface water and among the most common in ground water. However the levels of calcium recorded in this study is relatively high compared to values in other water bodies in Nigeria; Okogwu and Ugwumba (2006) for Ologe Lagoon; Omoigberale and Ogbeibu (2007) for River Osse, who recorded calcium values less than l0 mgl^{-1}. According Omoigberale and Ogbeibu (2007) the level of magnesium recorded in this study is however high when compared with some Nigerian water bodies.

Heavy metals

The lead levels in the study ranged from 0.003 mgl^{-1} (12am) to 0.036 mgl^{-1} (6pm); chromium ranged from 0.024 mgl^{-1} (12pm) to 0.075 mgl^{-1} (6am); Iron ranged from 0.340 mgl^{-1} (6pm) to 1.045 mgl^{-1} (6am) and Cadmium ranged from 0.003 mgl^{-1} (12am) to 0.376 mgl^{-1} (6pm). The heavy metal values recorded in this project did not have any statistical significant difference. Aquatic systems receive large amounts of heavy metals from unregulated sewage, industrial effluents and through runoffs (Tariq *et al*., 1991). Heavy metals are transported dissolved form in water or as an integral part of suspended sediments. Toxic heavy metals dissolved in water have the greatest potential of causing the most deleterious effects. Lead, a cumulative poison is widely distributed in the environment. The source of lead to the aquatic

environment has been traced to automobile exhaust and rusting of lead pipes used in pipe borne water supply (Mombeshora *et al*., 1993).

Iron is a major element in various primary minerals. It reaches natural water mainly from leaching and flaking of rust from iron pipes. The presence of chromium in freshwater bodies could be attributed to the fact that it can be carried as runoff water from industrial sites. Also chromium is transported and deposited in natural debris, which precipitates to the bottom of the water body. Cadmium is a cumulative poison that is emitted into the air from burning of fossil fuels and coal or the use of cadmium containing pesticides. The fact that cadmium gets into aquatic habitat from anthropogenic or aerial emissions have been reported by many authors including Lacerda (1983). Lacerda (1983) also reported cadmium dispersion in the environment and that it enters the aquatic ecosystem from terrestrial ecosystems at a slow rate.

Total Hardness and Alkalinity

The total hardness of a freshwater body can be defined as the total amount soluble magnesium and calcium salts present, and is expressed as $CaCO_3$. In most natural water the predominant ions present are those of bicarbonates which are associated mainly with calcium mainly, and to lesser degree with magnesium and still to a lesser degree with sodium and potassium. The values ranged from 12.261 mgl^{-1} (12am) to 14.984 mgl^{-1} (6pm). The alkalinity values ranged from 0.562 mgl^{-1} (6pm) to 1.528 mgl^{-1} (12pm). According to Wilson (2010) water alkalinity and hardness are primarily a function of the geology of the area where the surface water is located and the dissolution of carbon dioxide (CO_2) from the atmosphere. The stated that the pH, alkalinity and hardness are capable of affecting the toxicity of many substances in water. In line with his observations, the values recorded in this study can be attributed to the influx of runoff water containing large amount of dissolved and suspended materials.

Solids

The values for suspended and dissolved solids ranged from 7.385 mgl^{-1} (12pm) to 20.073 mgl^{-1} (6pm), and 13.698 mgl^{-1} (12pm) to 34.361 mgl^{-1} (6pm) respectively. The presence of high values could be attributed to a high influx of runoff water carrying a lot of dissolved and suspended materials (Patra et al., 2001). These values could also be as a result of the study being done during the rainy season. Suspended solids may settle out on the bottom of the water body thus smothering the benthic organisms and silting up the water body. This may lead to destruction of plant and animal life and the ore the natural food supply of fish. The effects of suspended solids on the delicate respiratory systems of fish has been reported to be lethal at up to 30 mgl^{-1} (Jackson *et al*., 1989). The high level of dissolved solids observed could also be attributed to high influx of rainy season runoff water. The influx of rain is however capable of diluting dissolved solids in water. However, high level of dissolved solids in water may also be harmful, its effect on water density is minimal and can the ore be ignored.

In station one,12am recorded the most outstanding bacterial population in comparison with 6am which had the lowest bacterial population while 6pm and 1pm varied slightly. In station two, the bacterial population was remarkable at 12am and was nearly similar with 6pm while 12pm recorded the lowest bacterial population and 6am happened to be an average bacterial population among the diurnal variations. Similar too station one, but with slight variations, station there had as at 12am, the highest bacterial population, while 6am was significantly low in bacterial population, at 12pm, there was an average population while 6pm was nearly similar to 12am. The control had a remarkable bacterial population at 12am, nearly similar with 12pm, 6am and 6pm had similar bacterial population with 6am

slightly higher than 6pm. Across all stations, 12 am recorded outstanding bacterial populations, 6 pm was nearly similar to 12am, while 6am and 12pm varied between the four stations.

CONCLUSIONS:

Physico-chemical and bacterial population of the soil sediments of Magada lake that passed through Mountain Top University were specifically addressed in this study. Diurnal variations were observed as a major determinant of physico-chemical factors. Similarly, the physico-chemical parameters significantly influenced the bacterial population. Specifically, 6am and 12am are the two peak periods that were positively influence and had high levels of bacterial population while 6pm and 12pm were relative to other factors which cannot be ascertained for in this study. Thus, factors contributing to these physico-chemical parameter variations at 6am and 12am indicate chances of bacterial population that can affect the environment. Although, the health status off the water cannot be full ascertained by this study, based on the fact that we did not take into consideration, the seasonal (rainy and dry) variations into consideration. Most of the physico-chemical parameters studied and bacterial population were within the W.H.O acceptable levels for a healthy and safe water.

ERENCES

1. Adeyemo O.K. (2003), Consequences of pollution and degradation of Nigerian aquatic environment on fisheries resources, The Environmentalist, 23(4), 297-306.
2. Adeyeye EI (1994). Determination of heavy metals in Illisha Africana, associated Water, Soil Sediments from some fish ponds. Int. J. Environ. Stud. 45: 231-240.
3. Akan, J. C.; Abdulrahman, F. I.; Ayodele, J. T.; Ogugbuaja, V.O., (2007). Studies on the effect of Municipal waste and Industrial effluents on the Pollutant level of River Challawa, Kano State, Nigeria. Res. J. Appl. Sci., 2 (4), 530-535
4. Alberta Water Quality Awareness (AWQA), 2012, Dissolved Oxygen and Alberta Streams and Rivers, Alberta Surface Water Quality
5. Asaolu SS (1998).Chemical Pollution Studies of Coastal Water of Ondo State. Ph.D Thesis, Fed. Univ. Technol., Akure. (Unpublished).
6. Barbour M.T., Gerritsen J., Snyder B.D. and Stribling J.B. (1999), Rapid bioassessment protocols for use in and wadeable rivers—Periphyton, benthic macroinvertebrates, and fish (2d ed.): U.S. Environmental Protection Agency, Office of Water, EPA 841–B—99–002.
7. Biney C., Amazu A.T., Calamari D., Kaba N., Mbome I.L., Naeve H., Ochumba P.B.O., Osibanjo O., Radegonde V., and Saad M.A.H. (1994), Review of heavy metals in the African aquatic environment, Ecotoxicology and Environmental Safety, 31, 134-159. Barbour M.T., Gerritsen J., Snyder B.D. and Stribling J.B. (1999), Rapid bioassessment protocols for use in and wadeable rivers—Periphyton, benthic macroinvertebrates, and fish (2d ed.): U.S. Environmental

Protection Agency, Office of Water, EPA 841–B—99–002. Biney C., Amazu A.T., Calamari D., Kaba N., Mbome I.L., Naeve H., Ochumba P.B.O., Osibanjo O., Radegonde V., and Saad M.A.H. (1994), Review of heavy metals in the African aquatic environment, Ecotoxicology and Environmental Safety, 31, 134-159.

8. Birkeland, Peter W. ―Soils and Geomorphology‖, 3rd Edition. New York, Oxford University Press, 1999.
9. Buol S.W., Hole .F. D. and McCracken, R. J. (1973), ―Soil Genesis and Classification‖, First Edition. Ames, IA: Iowa State University Press.
10. Bush, D. and M. Meyer, 1982. A case of infertile methemoglobinemia in South Dakota. J. Environ. Health, 44: 310-311.
11. Chapman, D., 1996. Water Quality Assessment: A Guide to the Use of Biota, Sediments and Water in Environmental Monitoring. 2nd Edn., E and FN Spon, London.
12. Chesworth Edited by Ward (2008), ―Encyclopedia of soil science‖, Dordrecht, Netherland.
13. Egborge, ABM (1991). Industrialization and heavy metal pollution in Warri River. Inaugural lecture delivered at the University of Benin, Nigeria. 32p.
14. Ekpo, IE; Chude, LA; Onuoha, GC (2012). The use of multivariate analysis for characterisation and classification of Ikpa River, Nigeria. Elix. Agric.49: 9799-9807.
15. Ipinmoroti K, Oshodi O (1993). Determination of Trace Metals in fish, associated Wanted and Soil Sediments fresh fish ponds. Discovery innovates 5:138.
16. Mahre, M.Y., Akan J.C., Moses, E.A. and Ogugbuaja V.O. (2007): Pollution Indicators in river Kaduna, Kaduna state, Nigeria. Trends in Applied Sciences Research, 2:304-311.
17. Manora-online, 2012, Total Dissolved Solids: Kerala Result 13; (www.manoraonline.com) Retrieved 16th June, 2012

18. Oduwole, GA (1997). Indices of pollution in Ogunpa and Ona Rivers, Ibadan, Nigeria: Physico-chemical, trace metal and plankton studies. Unpublished Ph. D Thesis. University of Ibadan, Nigeria. 236p. Oduwole, GA (1997). Indices of pollution in Ogunpa and Ona Rivers, Ibadan, Nigeria: Physico- chemical, trace metal and plankton studies. Unpublished Ph. D Thesis. University of Ibadan, Nigeria. 236p.
19. Okogwu, OI; Ugwumba, OA (2006). The zooplankton and environmental characteristics of Ologe Lagoon, South West Nigeria. The Zoologist, 1 (4):86 – 92.
20. Oluduro AO, Adewoye BI (2007). Efficiency of moringa Oleifera Sead extract on The microflora of surface and ground water J. plant Sci. 6: 453-438.
21. Omoigberale, MO; Ogbeibu, AE (2007). Assessing the Environmental Impacts of Oil Exploration and Production on the Water Quality of Osse River, Southern Nigeria. Global J of Environ Sciences, 6(1):1 – 13.
22. Omoigberale, MO; Ogbeibu, AE (2007). Assessing the Environmental Impacts of Oil Exploration and Production on the Water Quality of Osse River, Southern Nigeria. Global J of Environ Sciences, 6(1):1 – 13.
23. Patra AK, Sengupta S, Datta T (2001) Physico-chemical properties and ichthyofauna diversity in Karala river, a tributary of Teesta river at Jalpaiguri District of West Bengal, India. Int J Appl Biol Pharmaceutical Technol 2: 47-58.
24. Sala O.E., Chapin III F.S., Armesto J.J., Berlow E., Bloomfield J., Dirzo R., Huber-Sanwald E., Huenneke L.F., Jackson R.B., Kinzig A., Leemans R., Lodge D.M., Mooney H.A., Oesterheld M., Poff N.L., Sykes M.T., Walker B.H., Walker M. and Wall D.H. (2000), Global biodiversity scenarios for the year 2100, Science, 287, 1770–1774.

25. Shaw, B.P., A. Sahu and A.K. Panigrahi (1991). Water quality of the Rushikulya river estuary in relation to waste water discharge from a chlor-aikali plant. Pollut. Res., 10, 139-149.
26. Sridhar, MK; Oluwande, PA; Okubadejo, AO (1981). Health hazards and pollution from open drains in a Nigerian city. Ambio 10(1): 29-33vv
27. Stronkhorst J., Brils J., Batty J., Coquery M., Gardner M., Mannio J., O'Donnell C., Steenwijk J. and Frintrop P. (2004), Discussion document on Sediment Monitoring Guidance for the EU Water Framework Directive. Version 2. EU Water Framework Directive expert group on Analysis and Monitoring of Priority Substances. May 25th, 2004.
28. Sudhira H.S. and Kumar V.S. (2000), Monitoring of lake water quality in Mysore City. In: International Symposium on Restoration of Lakes and Wetlands: Proceedings of Lake 2000, Ramachandra, T.V., Rajasekara, M.C and Ahalya, N. (Eds.), Bangalore, India: Centre for Ecological Sciences, Indian Institute of Science, pp. 1-10.
29. Tariq, J., M. Jaffar and M. Moazzam, 1991. Concentration correlations between major cations and heavy metals in fish from the Arabian Sea. Mar. Pollut. Bull., 22: 562-565,
30. Tiwana, A.J. (1992): Water resource management quality and quality aspects. Proc. Int. Con. Rural works and sanitation in developing countries, IWWA, Nagpur, India. pp. 6-17.
31. Turner Designs, 2012, Phosphate: Trilogy Laboratory Fluorometer, (www.turnerdesigns.com) Retrieved 16th July 201
32. Voroney, R.P., (2006). ‖The Soil Habitat in Soil Microbiology, Ecology and Biochemistry‖.
33. Waite, T D (1984). Principles of water quality. Academic Press. San Diego.
34. Waite, T D (1984). Principles of water quality. Academic Press. San Diego.
35. Wilson CP (2010) Water quality notes: Alkalinity and Hardness; University of Florida.

APPENDIX

B.O.D

PHYSICO-CHEMICAL PARAMETERS	TIME	STATION 1	STATION 2	STATION3	CONTROL
BOD	12am	0±0	0.1±0.1	0±0	0±0
	6am	0.1±0.1	0.1±0.1	0.1±0.1	0±0
	12pm	0.1±0.1	0.1±0.1	0.1±0.1	0±0
	6pm	0.1±0.1	0.1±0.1	0.1±0.1	0±0
TDS (g/l)	12am	40±35.3	32.4±17	26.0±14.9	21.9±14.4
	6am	27.1±10.8	30.2±16.4	24.6±14.9	21.9±14.4
	12pm	25.1±9.7	27.8±16.4	23.5±14.7	21.9±14.4
	6pm	31±18	26.8±15.6	23.1±14.1	21.9±14.4
TSS (g/l)	12am	27.2±22.4	20±11.1	15.6±9.8	13.1±9.3
	6am	16.3±7.1	18.5±10.7	14.8±9.7	13.1±9.3
	12pm	15.1±6.3	16.9±10.8	14.1±9.6	13.1±9.3
	6pm	19±11.6	16.2±10.3	13.8±9.2	13.1±9.3
TS (g/l)	12am	67.2±57.7	52.4±28	41.4±24.8	34.9±23.7
	6am	43.4±17.8	48.7±27	39.2±24.6	34.9±23.7
	12pm	40±15.9	44.7±27.2	37.5±24.2	34.9±23.7
	6pm	49.9±29.5	42.9±25.9	36.8±23.2	34.9±23.7

INSITU

PARAMETERS	TIME	STATION 1	STATION 2	STATION 3	CONTROL
AIR TEMP (°C)	12am	26.53±0.21	25.13±7.75	25.20±7.58	27.13±0.12
	6am	27.57±8.97	27.17±7.76	28.87±7.71	27.13±0.12
	12pm	25.27±8.19	26.60±7.72	26.97±7.71	27.13±0.12
	6pm	26.33±7.95	25.20±7.64	27.63±7.73	27.13±0.12
WATER TEMP					
(°C)	12am	26.53±0.21	27.27±7.92	27.30±7.86	28.27±0.21
	6am	27.77±9.00	27.50±7.91	28.53±7.91	28.27±0.21
	12pm	26.20±8.28	26.87±7.86	27.40±7.90	28.27±0.21
	6pm	25.87±7.98	27.67±7.87	27.63±7.90	28.27±0.21
pH	12am	7.60±0.16	7.33±2.16	7.97±2.13	5.88±0.02
	6am	6.90±2.39	7.17±2.14	6.90±2.11	5.88±0.02
	12pm	7.77±2.31	7.27±2.12	6.47±2.09	5.88±0.02
	6pm	5.90±2.19	6.33±2.09	6.80±2.07	5.88±0.02
SALINITY (ppm)	12am	7.60±0.16	7.30±78.65	7.10±64.56	86.00±2.45
	6am	180.33±91.86	127.67±72.14	83.33±61.20	86.00±2.45
	12pm	177.00±84.16	81.33±66.96	83.00±58.35	86.00±2.45
	6pm	157.67± 74.62	83.33±62.77	0.84±59.87	86.00±2.45
TURB (NTU)	12am	18.543±0.300	25.710±10.588	9.590±9.359	0.070±0.050

	6am	11.230±6.088	8.543±10.245	3.903±9.282	0.070±0.050
	12pm	11.967±5.206	3.456±10.406	4.333±9.126	0.070±0.050
	6pm	36.293±11.252	10.127±9.828	9.590±8.756	0.070±0.050
Do (mg/l)	12am	10.37±0.12	8.37±5.94	10.30±4.93	0.07±0.05
	6am	15.90±5.77	9.27±5.66	9.44±4.79	0.07±0.05
	12pm	18.80±6.00	13.50±5.36	13.67±4.70	0.07±0.05
	6pm	19.33±6.02	11.07±5.12	10.33±4.58	0.07±0.05
CONDUCT					
(ms/cm)	12am	1.423±0.063	0.457±0.441	0.113±0.372	0.847±0.029
	6am	0.260±0.638	0.167±0.417	0.370±0.364	0.847±0.029
	12pm	0.240±0.554	0.100±0.402	0.100±0.353	0.847±0.029
	6pm	0.227±0.496	0.100±0.386	0.110±0.343	0.847±0.029
DEPTH (m)	12am	2.0±0.0	1.90±1.08	2.42±1.31	0.00±0.00
	6am	2.0±0.0	1.90±1.08	2.42±1.31	0.00±0.00
	12pm	2.0±0.0	1.90±1.08	2.42±1.31	0.00±0.00
	6pm	2.0±0.0	1.90±1.08	2.42±1.31	0.00±0.00

MORPHOLOGICAL PAREMETERS

	TIME	DILUTION FACTOR	REPLICATE	NO OF COLONY
Station 1	12am	10^{-1}	Rep 1	TNTC

			Rep 2	TNTC
			Rep 3	TNTC
		10^{-3}	Rep 1	250
			Rep 2	210
			Rep 3	232
		10^{-5}	Rep 1	150
			Rep 2	120
			Rep 3	135
	6am	10^{-1}	Rep 1	270
			Rep 2	265
			Rep 3	250
		10^{-3}	Rep 1	137
			Rep 2	140
			Rep 3	130
		10^{-5}	Rep 1	19
			Rep 2	21
			Rep 3	17
	12pm	10^{-1}	Rep 1	TNTC
			Rep 2	TNTC
			Rep 3	TNTC
		10^{-3}	Rep 1	101
			Rep 2	109

			Rep 3	107
		10^{-5}	Rep 1	35
			Rep 2	29
			Rep 3	21
	6pm	10^{-1}	Rep 1	250
			Rep 2	275
			Rep 3	245
		10^{-3}	Rep 1	180
			Rep 2	172
			Rep 3	150
		10^{-5}	Rep 1	35
			Rep 2	38
			Rep 3	36
2	12am	10^{-1}	Rep 1	172
			Rep 2	181
			Rep 3	200
		10^{-3}	Rep 1	108
			Rep 2	114
			Rep 3	102
		10^{-5}	Rep 1	81
			Rep 2	75

			Rep 3	79
	6am	10^{-1}	Rep 1	210
			Rep 2	205
			Rep 3	200
		10^{-3}	Rep 1	100
			Rep 2	120
			Rep 3	111
		10^{-5}	Rep 1	57
			Rep 2	61
			Rep 3	75
	12pm	10^{-1}	Rep 1	222
			Rep 2	227
			Rep 3	201
		10^{-3}	Rep 1	127
			Rep 2	135
			Rep 3	151
		10^{-5}	Rep 1	47
			Rep 2	34
			Rep 3	21
	6pm	10^{-1}	Rep 1	TNTC

			Rep 2	250
			Rep 3	226
		10^{-3}	Rep 1	145
			Rep 2	132
			Rep 3	139
		10^{-5}	Rep 1	72
			Rep 2	81
			Rep 3	68
3	12am	10^{-1}	Rep 1	TNTC
			Rep 2	342
			Rep 3	TNTC
		10^{-3}	Rep 1	189
			Rep 2	165
			Rep 3	174
		10^{-5}	Rep 1	97
			Rep 2	83
			Rep 3	60
	6am	10^{-1}	Rep 1	100
			Rep 2	119
			Rep 3	99

		10^{-3}	Rep 1	58
			Rep 2	54
			Rep 3	55
		10^{-5}	Rep 1	11
			Rep 2	10
			Rep 3	12
	12pm	10^{-1}	Rep 1	TNTC
			Rep 2	TNTC
			Rep 3	TNTC
		10^{-3}	Rep 1	85
			Rep 2	98
			Rep 3	101
		10^{-5}	Rep 1	41
			Rep 2	22
			Rep 3	21
	6pm	10^{-1}	Rep 1	199
			Rep 2	182
			Rep 3	200
		10^{-3}	Rep 1	111
			Rep 2	109
			Rep 3	120

		10^{-5}	Rep 1	75
			Rep 2	81
			Rep 3	79
control		10^{-1}	Rep 1	
			Rep 2	
			Rep 3	
		10^{-3}	Rep 1	
			Rep 2	
			Rep 3	
		10^{-5}	Rep 1	
			Rep 2	
			Rep 3	