# THE EFFECT OF AQUACULTURE ON PHYTOPLANKTON DIVERSITY AND ABUNDANCE IN LAKE KARIBA, ZAMBIA

BY

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A Dissertation Submitted to the University of Zambia in partial fulfillment of the Requirements for the Degree of Master of Science in Tropical Ecology and Biodiversity

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## DECLARATION

I, Chikungu Joel, do hereby declare that this dissertation represents my own work and it has not been previously submitted for a Master's Degree or other qualification at this University or any other University.

Signature .....

Date .....

## APPROVAL

The University of Zambia approves this dissertation of CHIKUNGU JOEL as fulfilling part of the requirements for the award of the Degree of Master of Science in Tropical Ecology and Biodiversity.

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## ABSTRACT

This study investigates the effect of aquaculture on Phytoplankton diversity and abundance in Lake Kariba. Water parameters that were monitored included: phosphate, nitrate and silica concentrations. Water samples were analysed from an aquaculture station at Yalelo fish cages located at Kamimbi area and a non-aquaculture station at Sampa Kaluma Island on Lake Kariba in Siavonga. A total of 44 species were identified belonging to 24 taxonomic groups. *Oscillatoria sp.* dominated the phytoplankton community in terms of relative abundance and contributed 29.6 per cent of the total observed phytoplankton at Kamimbi area and 24.3 per cent at Sampa Kaluma Island. The dominance was followed by *Microcystis aeruginosa* and *Chlorella sp.* Many of the algae are grazed by protozoa. Cyanobacteria such as *Oscillatoria* sp. and *Microcystis aeruginosa* are not grazed to the same extent as other algae, hence out competing other organisms (phytoplankton). They require little energy for maintenance of cell function and hence having high growth rate than other phytoplankton. They were also other species whose relative abundance was quite low.

Results showed that concentration of nitrate was the same in the two stations, at Kamimbi area where there are fish cages the assimilation capacity for nitrate was not exceeded by the input of the nutrient from aquaculture hence having a constant concentration in the two stations. The constant concentration of nitrate resulted in a condition of having no correlation  $(r^2 = 0)$  between nitrate and diversity and abundance of phytoplankton in both stations. Results also showed that there was a significant difference (P < 0.05) in concentrations of phosphate and silica between the two stations. There was more phosphate at Sampa Kaluma Island than at Kamimbi area. The concentration at Kamimbi remained at 0.01 mg/cm<sup>3</sup> at all depths because of assimilation capacity which was not exceeded by the input from aquaculture while at Sampa Kaluma Island the high concentration resulted from hydrology causing diffusion from the sediments. Kamimbi area had more concentration of silica because of its close proximity to the shore which was the source and also as a result of diffusion from the sediments that are not as deep as that of Sampa Kaluma Island sediments.

There was no correlation  $(r^2 = 0)$  between phosphate and diversity and abundance of phytoplankton at Kamimbi area due to constant concentration of phosphate, however there was a very low correlation  $(r^2 = 0.086)$  between phosphate and abundance of phytoplankton at Sampa Kaluma Island, because of variation in terms of concentration. There was also a correlation  $(r^2 = 0.172)$  between phosphate and diversity of phytoplankton at Sampa Kaluma Island due to higher concentration of phosphate. There was a correlation between silica and abundance of phytoplankton in both stations (Kamimbi area;  $r^2 = -0.120$  and Sampa Kaluma Island;  $r^2 = 0.57$ ). From the results obtained, it shows that aquaculture has had no effect on the concentration of nitrate and phosphate. There has been an input of phosphate and nitrate from aquaculture, but without a net change in nutrient balance. Aquaculture on Lake Kariba still remains at a small scale; no wonder the lake is still oligotrophic. However, the abundance of phytoplankton is higher at Kamimbi area compared to Sampa Kaluma Island.

Keywords: Aquaculture; Phytoplankton; Nutrients; Abundance; Diversity

## **DEDICATION**

To my father Kabamba Kenneth Chikungu, my mother Clementina Mwewa Chama, my wife Julian Malama, my children Geoffrey, Mirriam, Esther, Chama and Stella, I thank you so much for your encouragement and moral support.

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# ACRONYMS

| FAO     | Food Agriculture Organisation                              |
|---------|--|
| FNDP    | Fifth National Development Plan                            |
| MDG     | Millennium Development Goals                               |
| SADC    | Southern African Development Coordination Conference       |
| SASSCAL | Southern African Science Service Centre for Climate Change |
|         | and Adaptive Land Management                               |
| SP      | Species  |
| WD      | Water Division   |
| ZACPLAN | Zambezi River Action Plan                                  |
| ZACPRO  | Zambezi River Action Plan Project                          |

### **CHAPTER ONE**

#### **INTRODUCTION**

Aquaculture is currently one of the fastest growing sectors of agriculture in Zambia due to increased demand for fish. Currently aquaculture is taking place actively on Lake Kariba in Siavonga district, two major companies were introduced in 2011 and these are Lake Harvest Ltd and Yalelo Ltd (Genschick *et al*, 2017). Aquaculture introduces metabolic wastes from fish and food remains into previously oligotrophic lake water, which increases phosphorus and nitrogen levels and cause oxygen depletion due to increase of organic matter (Axlex *et al*, 1996). An increase of nutrients in a water body poses a serious threat to freshwater ecosystem due to high levels of nitrogen, phosphorus and silicon. These inorganic nutrients accelerate algal growth and lead to changes in aquatic ecosystem (Kalff, 2002). Increase of phytoplankton concentration in a water body can reduce water quality (pollution of water) and also make water purification processes expensive. Aquaculture has benefits such as creation of employment, increase of food (animal protein) and increase of revenue for the local authority which allow Municipality to improve on service delivery to local residents (African Development Bank Group, 2011).

The study was mainly based on the effect of aquaculture on the phytoplankton of the Zambian part of Lake Kariba. The effect of aquaculture in the lake was investigated in order to determine the impact on phytoplankton diversity and abundance, as well as concentration of nutrients in Lake Kariba. The study was undertaken in order to investigate the effect of aquaculture on phytoplankton diversity and abundance in Lake Kariba.

Lake Kariba is one of the largest man-made lakes in the world. The Kariba dam was constructed for generating electricity for Zambian copper mines and industries in Zimbabwe. It is monomictic with a mean surface temperature of about  $25^{\circ}$ C (Balon and Coche, 1974). The maximum depth is 120m and the mean depth is 29m. Temperature stratification is usually at a depth of 10 - 15m, with a surface temperature of  $28 - 32^{\circ}$ C during the hot – wet season, December – March. During the cool – dry season, April – July, surface temperature declines to a minimum of about  $22^{\circ}$ C and whole mass mixes sometime during July. The temperature increases in the beginning of the hot – dry season and in August a new stratification is established (Ramberg *et al*, 1987).

Lake Kariba is economically important to Zambia in terms of food by providing fish. In 1967 *Limnothrissa miodon*, a freshwater sardine locally known as Kapenta was introduced into Lake Kariba from Lake Tanganyika where it is endemic (Bell-Cross and Bell-Cross, 1971). The introduction of *Limnothrissa miodon* was successful. The Kapenta in Lake Kariba currently supports a commercial fishery based on its exploitation producing over 30,000 tonnes annually for both Zambia and Zimbabwe (Machena and Konondo, 1991).

The catchment area of the Zambezi River and its tributaries in Zambia has three seasons: A cool dry period from May to July, a hot dry season from August to October and a rainy season from November to April (Archer, 1971). The Lake Kariba area and the whole of the Middle Zambezi are regarded to be a low rainfall area in Zambia and receives less than 700 mm rainfall annually.

Changes in the phytoplankton community of large freshwater lakes have been long recognised as a good indicator of trophic status and environmental quality (Reynolds, 1996). Aquatic environments are subject to high temporal variation with frequent reorganisation of relative abundance and species composition of phytoplankton, as a result of interaction between physical, chemical and biological variables (Reynolds *et al*, 2000). Phytoplankton abundance and taxonomic diversity depend upon the supply of nutrient in natural waters, where abundance increases with increasing nutrient concentration (Abu *et al*, 2005).

Cage aquaculture is currently going on in Kamimbi area in Namachembele Village in Siavonga District, Zambia (Yalelo, 2012). Cage aquaculture has the potential to offset the large deficit of affordable fish within Zambia and can contribute significantly to the development of a rural economy of Siavonga by providing 260 new jobs (Yalelo, 2012). Yalelo limited is the producer of tilapia fish in Zambia. It grows organic tilapia fish in Lake Kariba, Zambia. Over fishing is a problem in Zambia. Yalelo is directly reversing the trend by breeding and growing sustainable tilapia in order to strengthen food security (Yalelo, 2012). It is estimated that Zambia currently produces 85,000 tonnes of fish per year against an estimated demand of 145,000 tonnes. Approximately 6,000 tonnes of fish is currently being imported annually from China, India and Zimbabwe (Yalelo, 2012). Major environmental effects are generally associated with high input and high output intensive system. Their effects include a build-up of anoxic sediments and a change in benthic communities and eutrophication of lakes (FAO, 2014). The composition of phytoplankton

community of Lake Kariba has been investigated (Thomasson, 1965). However, there is need to investigate the effect of increased levels of nutrients on phytoplankton community.

#### **1.1** Back ground to the study

This study was intended to contribute to the knowledge on the level of nutrients (phosphorus, nitrate and silica) and its effect on the phytoplankton community of Lake Kariba on the Zambian side as a result of aquaculture that is taking place there. Lake Kariba is one of the largest man – made lake in the world. It was constructed in 1958 for the generation of hydroelectric power for the mining and industrial development in Zambia and Zimbabwe (Hakan *et al*, 1996). In 1968 a Sardine *Limnothrissa miodon*, locally known as Kapenta was introduced from Lake Tanganyika and it proved to be a success (Marshall *et al*, 1982). Currently aquaculture is taking place on Lake Kariba as one way of supplementing the much needed protein to the Zambian people (Yalelo, 2012).

#### **1.2** Statement of the problem

Aquaculture is the answer to increasing demand for more fish . The huge volume of water in Lake Kariba could easily contribute to meeting the rising demand for fish. However it is not known whether the concentration of nutrients has increased in the area as a result of aquaculture. An increase in phosphorus and nitrogen levels can increase phytoplankton concentration and it also encourages development of algal blooms. Algal blooms affect water quality and aquatic life and can have an adverse effect on human health and livestock. Decay of algal blooms is known to lead to low oxygen levels and fish being killed. With excessive phytoplankton growth, there is a risk that water may contain toxic substances and might also affect other aquatic organisms.

It is not known whether phytoplankton density has changed due to aquaculture on Lake Kariba. Furthermore it is not clear whether phytoplankton diversity has changed since the earlier studies that were conducted by Thomasson in 1965. During the filling phases of Lake Kariba, samples of water analysed were found to contain a high population of desmids (Thomasson, 1965). The dominating algal species in 1984 – 85 consisted of blue – green algae, mainly heterocyst forming *Gloeotrichia* sp., followed by a number of *Oscillatoria* sp. and *Lyngbya* species (Ramberg et al, 1987). The effect of depth on phytoplankton density and concentration of nutrients in Lake Kariba is not known. The levels of nutrient concentrations

and phytoplankton density will also tell whether the lake has been polluted due to aquaculture.

## **1.3** General Objective

To assess the effect of aquaculture, on phytoplankton diversity and abundance of Lake Kariba.

## **1.4 Specific Objectives**

- i. To determine the effect of aquaculture on the phytoplankton diversity and abundance of Lake Kariba.
- To determine the effect of aquaculture on nitrate, phosphate and silica concentrations in Lake Kariba

## 1.5 Hypotheses

The study tested the following hypotheses:

- i. There exists no correlation between concentration of nutrients and phytoplankton diversity and abundance in Lake Kariba.
- Aquaculture has no effect on nitrate, phosphate and silica concentrations in Lake Kariba.

## **1.6** Research questions

- i. Does Aquaculture have an effect on phytoplankton abundance on Lake Kariba?
- **ii.** Does Aquaculture have an effect on phytoplankton diversity on Lake Kariba?
- iii. What is the effect of Aquaculture on the concentration of nitrate, phosphorus and silica on Lake Kariba?

## **1.7** Limitation to the study

Collecting of all water samples in one day for the entire study could have affected the density of phytoplankton, since temperature changes may have affected the type of species found at a particular depth and area.

#### **1.8** Significance of the study

Lake Kariba supports aquaculture industry which is important for improvement of food security. Aquaculture industry offers creation of rural employment. However, it may be under threat from the introduction of nutrients such as nitrogen, phosphorus and silicon due to human activities. Lake Kariba is important for hydroelectric power generation, artisanal and subsistence fishing, Kapenta industrial fishing, tourism, water supply, and lake transport (Musando, 1996).

Since aquaculture is currently taking place on Lake Kariba at Kamimbi area, it is necessary to find out the present nutrient levels and the concentration of phytoplankton in Lake Kariba. The findings will explain whether the lake is being polluted or not.

The protection of Lake Kariba and the maintenance of its resources are of paramount importance. The study assesses the impact of aquaculture on the abundance of nutrients in the lake and its effect on phytoplankton population. Phytoplankton have a critical role in primary production, nutrient cycling, and in food webs. It makes a significant proportion of the primary production in aquatic systems (Dawes, 1998). The pelagic fishery of Lake Kariba is based on a simple food chain. The phytoplankton are grazed by the zooplankton which are taken up by the introduced *Limnothrissa miodon* (Ramberg *et al*, 1987).

The study also compared the phytoplankton diversity in aquaculture and non-aquaculture areas. Biological monitoring is an increasingly important tool for assessing the impact of man's environment (Lange and Lambert, 1995). Phytoplanktons have been used as an effective bio indicator that is sensitive to environmental changes (Mai, 2003). Monitoring the phytoplankton is necessary in order to evaluate environmental impact of cage aquaculture and other agricultural activities in and around the lake. Organisms in our environment can serve as unique indicators of the habitability of the environment due to their strict dependence on it (William *et al*, 1973).

## **CHAPTER TWO**

#### LITERATURE REVIEW

#### 2.1 Lake Kariba

Lake Kariba is situated between latitudes, 16°15' to 18°04'S and longitudes 26°42' to 29°03'E. It has a surface area of 5,364 square kilometres with a maximum width of 40 km and a maximum depth of 120 metres. (Ramberg *et al*, 1987). At the time of its creation, it was the largest man-made lake in the world. It is currently the world's largest artificial lake by volume. Lake kariba was filled between 1958 and 1963 after the completion of the Kariba Dam.

Lake Kariba was created for hydroelectric power generations. Fishing has been encouraged on the lake as a way of compensating lakeshore communities most of whom were displaced from their traditional homes when the lake was formed. Before the lake filled, large areas of land were cleared of the vegetation and were burnt to facilitate fishing operations. This may have contributed significantly to the high nutrient content of the water in the initial phases of the lake formation.

## 2.2 Mixing of stratified water of Lake Kariba

Lake Kariba is a warm monomictic lake and stratifies during the warm seasons from September to April. Complete mixing takes place during the cold season from May to August (Harding, 1961, 1964; Coche1968, 1969, 1974; Begg, 1970). The depth of the mixed layer not only affects production, but also causes phytoplankton loss (Diehl *et al*, 2002). Three factors deserve consideration as important mechanisms controlling the growth of phytoplankton: Nutrient availability, light availability and metabolic rate as affected by temperature. Nutrient availability is markedly influenced by change in mixing depth which is defined as the surface layer over which nutrients and gases are freely redistributed within 24 hour span (Lewis, 1978).

#### 2.3 Phytoplankton of Lake Kariba during the filling phases of the lake

During the filling phases of the Lake Kariba, phytoplankton quality and quantity were evaluated (Thomasson, 1965). These samples consisted largely of Desmids. In 1980 Thomasson again analysed additional samples from Lake Kariba that showed a decrease in the phytoplankton species. This was attributed to the declining levels of nutrient in the lake.

The first quantitative investigations of the phytoplankton of Lake Kariba were done in the northern basins of the lake between 1982 and 1983 (Ramberg, 1984, 1987). Mean biomass of the phytoplankton was estimated low at  $0.29 \text{mg l}^{-1}$  with about 60% of the biomass consisting of the blue green algae. The highest biomass coincided with the rainy season (December – January). The diatoms were present throughout the year but were maximum during the phase of isothermy in June.

Investigations were conducted in all basins of Lake Kariba between 1986 and 1990 and the results showed that pelagic biomass of phytoplankton in Lake Kariba were as low as  $0.2 - 2mg l^{-1}$ (Cronberg, 1997). The highest bio-mass was recorded in October to November and lowest in March. The algal flora was dominated by diatoms during the phase of circulation. *Melosira* sp. was dominant and *Cyclotella fragilaria* and *Synedra* sp. were subdominant (Hancock, 1979).

#### 2.4 Economic benefits of Lake Kariba

Some large-scale irrigation schemes have been implemented close to Lake Kariba to take advantage of the waters of the lake. Such projects also have the capacity to contribute significantly to the eutrophication of the lake which uses large amounts of fertilizers. In 1987, SADC adopted ZACPLAN as part of economic integration cooperation and development of Southern African Countries. The objective of ZACPLAN is to achieve environmentally sound planning and management of water and related resources in the Zambezi river basin. Through ZACPLAN a number of projects have been undertaken (Euroconsult Mott MacDonald, 2007). According SADC – WD/ZACPRO Number 6.2 (Euroconsult Mott MacDonald, 2007), Zambia and Zimbabwe, among other Basin countries, have prioritized agriculture as the main vehicle to attain MDG Number 1. In the case of Zambia the Zambian Vision 2030 and FNDP place irrigated agriculture as a priority in order to achieve food security.

The major socioeconomic benefit of aquaculture conducted on Lake Kariba is creation of employment. The local authority also benefit from increased revenue from Lake Harvest (for example Municipal rates, water and sewerage charges). Additional employment has been created through industries that provide support to the fisheries industry. These include suppliers, feed transporters, metal fabrication companies, fish distributors and maize/soya beans farmers (African Development Bank Group, 2011). One of the main industries in Siavonga tied closely to the lake is Kapenta Fishing. There are several commercial Kapenta fishing companies in the town producing one of Zambia's high protein foods. Kapenta is usually sun dried and packed in bulk ready for market. Some companies also supply frozen Kapenta pre – packed for the supermarkets. The Lake Kariba is the third largest lake in terms of fish output.

## 2.5 Aspects of Chemical Composition of Lake Kariba Waters

A detailed account of the chemical composition of Lake Kariba waters was first provided by Coche (1968, 1969 and 1974). During the early stages of the lake formation, the nutrient content of the lake was high and progressed towards oligotrophy with time. The hydrography and fish production during the early stages of Lake Kariba were studied by Coche (1974) which led Coche to concentrate on these ions because they increase the production of phytoplankton (Ramberg, 1984). The nitrate contents were found to be low ranged from 0.002 to 0.06 mg/cm<sup>3</sup> in the surface waters. Mean phosphate contents were consistently below 0.025 mg/cm<sup>3</sup> in the pelagic zone. The silica contents were also very low with values ranging between 10 - 15 mg/cm<sup>3</sup>. Phosphorous concentration of 0.005 - 0.015 mg/cm<sup>3</sup> is categorised as oligotrophic, 0.01 - 0.03 mg/cm<sup>3</sup> mesotrophic, 0.030 - 0.1 mg/cm<sup>3</sup> as eutrophic. Above 0.1 mg/cm<sup>3</sup>, phosphorus content is considered as pollution of a lake. Nitrogen concentrations in lakes vary from about 0.1 mg/cm<sup>3</sup> and over 6 mg/cm<sup>3</sup>. Above 15 mg/cm<sup>3</sup> nitrogen in lake suggests the lake to be polluted (Bronmark and Hansson, 2005).

Lindmark (1977) recorded low phosphate concentrations ranging between 0.001 - 0.007 mg/cm<sup>3</sup> in the epilimnion and 0.004 - 0.051 mg/cm<sup>3</sup> for layers below 30 meters. Nitrate concentrations ranged between 0.001 - 0.03 mg/cm<sup>3</sup> for the epilimnetic zone and between 0.001 - 0.215 mg/cm<sup>3</sup> in the hypolimnion.

#### 2.6 Effect of nutrients on phytoplankton population due to aquaculture

Confield (1983) demonstrated that concentration of nitrogen and phosphorus are important factors influencing phytoplankton abundance in Florida lakes. All aquaculture systems involving feeding or nutrient addition can impact water quality (Axlex *et al*, 1996). Organic enrichment in intensive commercial aquaculture operations comes primarily from uneaten food and fish faeces (Rosenthal *et al*, 1988).

Phytoplankton abundance and taxonomic diversity depend upon the supply of nutrient in natural waters, where abundance increases with increasing nutrient concentration (Abu *et al*, 2005). Nutrient enrichment due to input of nutrients and organic material from uneaten fish waste can stimulate excessive growth of algae (African Development Bank Group, 2011). Phosphorous and nitrogen are generally two nutrients that can lead to limit of algal growth (Wetzel, 2001). Two of the most consistent eutrophication effects are a shift in algal species composition and an increase in the frequency and intensity of nuisance algal blooms (Huisman *et al*, 2005). These nutrients make a water body eutrophic and enable it to support a large variety of phytoplankton assemblage (Abu *et al*, 2005). One of the most serious threats to freshwater ecosystem is the over abundance of nitrogen, phosphorus and silicon. Phosphorus is the main contributing factor to the eutrophication of freshwater environments (Kalff, 2002). Eutrophication is defined as a process by which a body of water becomes enriched with dissolved nutrients that stimulate the growth of aquatic plant life.

It is generally accepted that the communities of aquatic organisms can serve as indices of pollution (Wu, 1984). The pattern of phytoplankton and water quality is interdependent (Venneaux, 1976). The dominance of certain species and the frequency of the presence of such species are considered as the characteristics of polluted water (Wu, 1984). Planktonic microalgae qualify as suitable indicators in that they are simple, capable of quantifying changes in water quality, applicable over large geographic areas (Onyema, 2007). Changes in water quality can be reflected by the species type presence, abundance, absence or their distribution pattern (Onyema, 2013). In South Western Nigeria (Nwankwo, 2004) recorded a number of blue – green algae associated with different degrees of water pollution.

## **CHAPTER THREE**

#### MATERIALS AND METHODS

## 3.1 Study area

The study area is located in Siavonga District in Southern Province on the northern shore of Lake Kariba, Zambia. Lake Kariba lies 480 metres above sea level and is over 380 kilometres long and 40 kilometres wide. It covers an area of 5,364 square kilometres and its storage capacity is 185 cubic kilometres. The mean depth of the lake is 29 metres with a maximum depth of 120 metres (Ramberg *et al*, 1987). Figure 1 shows the study area in Siavonga.



Figure 1: Map of study area showing location of the Lake Kariba and sampling sites. Source: Cartographic office – Geography Department UNZA The six red doted spots on the map are the areas where water samples were collected from. The exact locations for collection of water samples on Lake Kariba was at Kamimbi area in Namachambele village where there is aquaculture activity, were site C with coordinates  $16^0$  30.109'S and  $28^0$  38.655'E, site D with coordinates  $16^0$  30.274' S and  $28^0$  38.537'E and site E with coordinates  $16^0$  30.336'S and  $28^0$  38.597'E.

The exact locations for collection of water samples on Lake Kariba at Sampa Kaluma Island where there was no aquaculture activity, were site A with coordinates  $16^0$  33.218'S and  $28^0$  39.817'E, site B with coordinates  $16^0$  33.013'S and  $28^0$  40.119'E and site F with coordinates  $16^0$  32.944'S and  $28^0$  40.683'E south of Siavonga. Water samples were collected from Siavonga and Sampa Kaluma Island which is about 30 kilometers south of Siavonga. From each of the two stations, three sampling points were picked as shown by the GPS above. Each sampling site in a station was about 100m away from each other. Water samples from each site were collected with a water sampler from depths of 0m, 5m, 10m, 15m, 20m and 25m. Water samples were analysed from each depth.

A boat was hired from the Marine Department of Police Service in Siavongo which is a property of the Ministry of Home Affairs for the collection of water samples on the lake. Water samples were collected in the hot season on 12<sup>th</sup> October, 2016 for analysis of phytoplankton concentration and identification. All the water samples were collected in one day. Water samples were put in opaque plastic bottles of 500cm<sup>3</sup> each. Water samples for phytoplankton analysis were preserved immediately in Lugol's solution in the field. These were labelled according to location and depth collected. The volume of water sample collected for nutrient analysis was also 500cm<sup>3</sup> and no Lugol's solution was added. The water samples were obtained. Samples for nutrient were labelled according to location and here water samples for phytoplankton analysis were obtained. Samples for nutrient were labelled according to location and transported to University of Zambia within 24 hours.

Nutrient (nitrates, phosphates and silica) analysis was done at Environmental Engineering Laboratory, School of Engineering, University of Zambia. The analysis was done by using standard procedure for laboratory tests. The named nutrients were the only ones that were analysed because they have shown significant effect on algal growth. Wasted nitrogen and phosphorus from feed, faeces, and excretion may also contribute to eutrophication problems in nutrient limited lakes (Phillips *et al*, 1985).

Chemical analysis for phosphate, silica and nitrate of water quality was done as well as qualitative and quantitative analysis of Phytoplankton.

## **3.2 Identification of phytoplankton**

The water samples were examined at Chilanga Fisheries Limnology Laboratory using a stereo microscope (M 150 C - 1, 40X - 1000X Biological Compound Microscope) to identify phytoplankton. The algal species were identified at family level, and where possible also to genus or species level, by using pictorial and taxonomic keys (Prescott, 1978).

#### 3.3 Counting phytoplankton

Three sub samples were used to find the average density for each depth, and it was used to find density (unit/cm<sup>3</sup>) of phytoplankton. In one station three areas of the same depth were used. The water samples were transported to Chilanga Fisheries Limnology Laboratory for analysis. The phytoplankton were counted using a microscope (PX - 38 Series, Biological Microscope) according to Utermohl's (1958) method. Counting of the phytoplankton was done by using ultermohl chambers that hold a known amount of water (unit/cm<sup>3</sup>). The method first described by (Utermohl, 1958) is utilised to identify and enumerate phytoplankton community from many different types of aquatic habitats. In order to have a good concentration of sample for counting in a chamber, each bottle was shaken and 100cm<sup>3</sup> sub sample was collected and allowed to settle for 24hrs. The volume of water for each sample was 2cm<sup>3</sup>. The chamber had grids and algae were counted by determining number of algae (colonies, filaments or single cells) per grid. In each field 10 grids were skipped before reaching a grid where counting was done. Where a unique species was observed, it was counted without considering the skipping of 10 grids. Each observed taxon was recorded. The algae were identified to family or genus level and where possible to species level by using pictorial and taxonomic keys (Prescott, 1978). The formula below was used to calculate the density of phytoplankton.

Individual unit Density =U/(A \* G \* C/B)

Where :

- U = Total number of cells of a taxon counted in all grids
- A = Area of the grid used
- G = Number of grids counted
- C = Volume of Utermohl chamber used
- B = Total basal area of Utermohl chamber

Results: Cell density for a taxon i.e. unit/cm<sup>3</sup>

Total unit Density =  $\sum -(i = 1)^n M U/(A * G * C/B)$ 

Where:

- U = Total number of cells of all taxa counted in all grids
- A = Area of the grid used
- G = Number of grids counted
- C = Volume of Ultermohl chamber used
- B = Total basal area of the Ultermohl chamber
- n = The total number of taxa
- i = Beginning with the 1<sup>st</sup> taxon

Results: The total cell density for a sample (unit/cm<sup>3</sup>).

The concentration (density) obtained by using the formula was used to make a comparison of the phytoplankton abundance for the two stations, using the student's test (d – statistics).

#### 3.4 Correlation between nutrients and phytoplankton diversity and abundance

In order to test whether a correlation existed between nutrient concentration and phytoplankton diversity and abundance, the same method of collection of water samples was carried out as in 3.2.

The correlation between the concentration of nutrients and phytoplankton diversity and abundance was assessed by determining Correlation Coefficient and regression by using two variables i.e. concentration of nutrients and diversity and abundance of phytoplankton (independent variable; concentration of nutrients in water, dependent variable; diversity and abundance of phytoplankton, data from 3.2), the statistical tools of Microsoft Excel and SPSS Statistics version 20.

## 3.5 Effect of aquaculture on nutrient concentration in Lake Kariba

The quantity of nutrients was compared by using the student's test (d - statistics). Concentrations of nutrients from the two stations were used to determine the effect of aquaculture on nutrient quantity in the Lake.

# CHAPTER FOUR RESULTS

# 4.1 Effect of aquaculture on nitrate, phosphate and silica concentrations in Lake Kariba

There was significantly more phosphate at Sampa Kaluma Island compared to Kamimbi area (calculated t – value 7.43 > tabulated t – value; p< 0.05). At site A of Sampa Kaluma Island where there is no aquaculture activity there was a variation of phosphate concentration as depth increased, at this site surface water had the highest concentration. At site B and F the concentration for phosphate was the same at all depths measured (Table 1 and 2).

At Kamimbi area where aquaculture activities are taking place (sites C, D and E), the concentration of phosphate  $(0.01 \text{ mg/cm}^3)$  was the same at all depths (Table 1, and 3).

There was significantly more silica at Kamimbi area compared to Sampa Kaluma Island (calculated t – value 10.94 > tabulated t – value 2.23; p < 0.05). Silica's concentration increased as depth increased in both stations (Tables 1, 2 and 3). The concentration for nitrate was the same (0.01 mg/cm<sup>3</sup>) at Kamimbi area and Sampa Kaluma Island. The trend was the same at all depths (Tables 1, 2 and 3).

| Table | 1: | Average | concentration | of | nutrients | at | Sampa | Kaluma | Island | and | Kamimbi |
|-------|----|---------|---------------|----|-----------|----|-------|--------|--------|-----|---------|
| area  |    |         |               |    |           |    |       |        |        |     |         |

|       | $PO_4^{-3}(mg/cm^3)$ |         | Silica (mg/cm <sup>3</sup> ) |         | $NO_3$ N (mg/cm <sup>3</sup> ) |         |
|-------|----------------------|---------|------------------------------|---------|--------------------------------|---------|
| Depth | Sampa Kaluma         | Kamimbi | Sampa Kaluma                 | Kamimbi | Sampa Kaluma                   | Kamimbi |
| (m)   | Island               | area    | Island                       | area    | Island                         | Area    |
| 0     | 0.3                  | < 0.01  | 2.24                         | 3.48    | <0.01                          | < 0.01  |
| 5     | 0.03                 | < 0.01  | 2.38                         | 3.48    | < 0.01                         | < 0.01  |
| 10    | <0.01                | < 0.01  | 2.49                         | 3.51    | < 0.01                         | < 0.01  |
| 15    | 0.05                 | < 0.01  | 2.50                         | 3.55    | < 0.01                         | < 0.01  |
| 20    | < 0.01               | <0.01   | 2.55                         | 3.69    | < 0.01                         | < 0.01  |
| 25    | < 0.01               | < 0.01  | 2.57                         | 3.88    | <0.01                          | < 0.01  |

The concentration for phosphate showed a variation as depth increased at Sampa Kaluma Island. Surface water had the highest concentration. Kamimbi area showed a constant concentration  $(0.01 \text{ mg/cm}^3)$  of phosphate at all depths. Concentration of silica was relatively high at both stations. However as depth increased the concentration of silica also increased. The concentration for nitrogen was the same  $(0.01 \text{ mg/cm}^3)$  in both stations at all depths.

|       |             | ~ .    |            | 1           | ~ -    |            | 1           | ~ -    |            |
|-------|-------------|--------|------------|-------------|--------|------------|-------------|--------|------------|
|       |             | Site A |            |             | Site B |            |             | Site F |            |
| Depth | $PO_4^{-3}$ | Silica | $NO_3^- N$ | $PO_4^{-3}$ | Silica | $NO_3^- N$ | $PO_4^{-3}$ | Silica | $NO_3^- N$ |
| (m)   | (mg/l)      | (mg/l) | (mg/l)     | (mg/l)      | (mg/l) | (mg/l)     | (mg/l)      | (mg/l) | (mg/l)     |
| 0     | 0.98        | 2.28   | < 0.01     | < 0.01      | 2.10   | < 0.01     | < 0.01      | 2.33   | < 0.01     |
| 5     | 0.07        | 2.52   | < 0.01     | < 0.01      | 2.22   | < 0.01     | < 0.01      | 2.39   | < 0.01     |
| 10    | < 0.01      | 2.64   | < 0.01     | < 0.01      | 2.34   | < 0.01     | < 0.01      | 2.49   | < 0.01     |
| 15    | 0.14        | 2.66   | < 0.01     | < 0.01      | 2.35   | < 0.01     | < 0.01      | 2.50   | < 0.01     |
| 20    | < 0.01      | 2.72   | < 0.01     | < 0.01      | 2.38   | < 0.01     | < 0.01      | 2.55   | < 0.01     |
| 25    | < 0.01      | 2.72   | < 0.01     | < 0.01      | 2.40   | < 0.01     | < 0.01      | 2.59   | < 0.01     |

 Table 2: Concentration of nutrients at Sampa Kaluma Island (non - aquaculture area)

| <b>Table 3: Concentration</b> | of nutrients at Kamimb | i area (aquaculture area) |
|-------------------------------|------------------------|---------------------------|
|-------------------------------|------------------------|---------------------------|

|       |             | Site C |          |             | Site D |          |             | Site E |          |
|-------|-------------|--------|----------|-------------|--------|----------|-------------|--------|----------|
| Depth | $PO_4^{-3}$ | Silica | $NO_3 N$ | $PO_4^{-3}$ | Silica | $NO_3 N$ | $PO_4^{-3}$ | Silica | $NO_3 N$ |
| (m)   | (mg/l)      | (mg/l) | (mg/l)   | (mg/l)      | (mg/l) | (mg/l)   | (mg/l)      | (mg/l) | (mg/l)   |
| 0     | < 0.01      | 3.28   | < 0.01   | < 0.01      | 3.88   | < 0.01   | < 0.01      | 3.27   | < 0.01   |
| 5     | < 0.01      | 3.33   | < 0.01   | < 0.01      | 3.84   | < 0.01   | < 0.01      | 3.28   | < 0.01   |
| 10    | < 0.01      | 3.34   | < 0.01   | < 0.01      | 3.90   | < 0.01   | < 0.01      | 3.28   | < 0.01   |
| 15    | < 0.01      | 3.40   | < 0.01   | < 0.01      | 3.90   | < 0.01   | < 0.01      | 3.34   | < 0.01   |
| 20    | < 0.01      | 3.44   | < 0.01   | < 0.01      | 4.21   | < 0.01   | < 0.01      | 3.42   | < 0.01   |
| 25    | < 0.01      | 2.46   | < 0.01   | < 0.01      | 4.28   | < 0.01   | < 0.01      | 4.90   | < 0.01   |



Figure 2: Comparison of phosphate, silica and nitrogen at Sampa Kaluma Island

Figure 2 shows the concentration of nutrients at Sampa Kaluma Island. Surface water had the highest concentration of phosphate compared to all depths. The concentration for silica increased as depth increased. The concentration for nitrogen was very low and constant at all depths. Silica concentration was high compared to phosphate and nitrogen.



Figure 3: Comparison of phosphate, silica and nitrogen at Kamimbi area

Figure 3 shows the concentration of nutrients at Kamimbi area. Phosphate and nitrogen concentrations were the same  $(0.01 \text{mg/cm}^3)$  and very low at Kamimbi, however the concentration for silica was high and it increased as the depth increased.

## 4.2 Correlation of nutrients and phytoplankton diversity and abundance

A total of 44 species belonging to 24 taxonomic groups were observed in the water samples analysed. The taxonomic groups observed were as follows: Bacillariophyceae, Stephanodiscaceae, Naviculaceae, Sphaeropleaceae, Oscillatoriaceae, Zygnemataceae, *Microcystaceae*, Fragilariaceae, *Cryptomonadaceae*, *Oocystaceae*, *Closteriaceae*, Scenedesmaceae, Chlamydomonadaceae, *Hydrodictyaceae*, Volvocaceae, Euglenaceae, Ulotrichaceae, *Pleaurochloridaceae*, *Chrococcaceae*, Desmidiaceae, Microsporaceae, Chlococcaceae, Nostacaceae and Peridiniaceae. The genus/species identified from the water samples collected were of various shapes. Correlation coefficient and regression were calculated by using the statistical tools of Microsoft Excel and SPSS Statistics version 20.

There was no correlation between the concentration of phosphate and phytoplankton diversity at Kamimbi area ( $r^2 = 0$ ). There was also no correlation between the concentration of nitrate and phytoplankton diversity ( $r^2 = 0$ ). However, there was a negative correlation between concentration of silica and phytoplankton diversity at Kamimbi area ( $r^2 = -0.250$ ) and there was no significant regression of silica on phytoplankton diversity (p = 0.984; p > 0.05).

At Sampa Kaluma Island there was a correlation between the concentration of phosphate and phytoplankton diversity ( $r^2 = 0.172$ ) and there was no significant regression of phosphate on phytoplankton diversity (p = 0.226; p > 0.05). There was also a correlation between silica and phytoplankton diversity ( $r^2 = 0.221$ ) and there was no significant regression of silica on phytoplankton diversity (P = 0.201; P > 0.05). However, there was no correlation between the concentration of nitrate and phytoplankton diversity ( $r^2 = 0.201$ ; P > 0.05).

The taxonomic groups identified were *Bacillariophyceae* with three genera (*Gomphonema* sp. *Nitzschia* sp. and *Rhopalodia* sp.). All these three are unicellular and pennate diatoms. *Stephanodiscaceae* had one genus (*Cyclotella* sp.), this is a unicellular and centric diatom. *Naviculaceae* had three genera (*Calones* sp. *Gyrosigma* sp. and *Navicula* sp.). These three are

also unicellular and pennate diatoms, *Gyrosigma* sp. is unique because it is narrow and sigmoid

Sphaeropleaceae had one species (Sphaeroplea annulina) which is filamentous. Oscillatoriaceae had one genus (Oscillatoria sp.) which is a blue – green algae and also filamentous. Zygnemataceae had one genus (Mougeotia sp.) that is filamentous green algae. Oocystaceae had two genera (Chlorella sp. and Treubaria sp.). Chlorella sp. is unicellular green algae and is spherical in shape. Treubaria sp. is unicellular and triangular with spines at each angle. Microcystaceae had one species (Microcystis aeruginosa), these are cells that are organised as colonies. Fragilariaceae had one genus (Synedra sp.), this species is unicellular and needle – like. Cryptomonadaceae had one genus (Cryptomonas sp.), this species is unicellular oval shaped.

*Closteriaceae* had four genera (*Closterium aciculare*, *Closterium leibleinii*, *Closterium maniferum* and *Closterium* sp.). These are unicellular green algae that are crescent shaped or elongate. *Scenedesmaceae* had five genera (*Scenedesmus acuminatus*, *Scenedesmus quadricauda*, *Scenedesmus obliquus Scenedesmus* sp. and *Tetrastrum elegans*), *Scenedesmus* is a genus of green algae and it is colonial with cells arranged in such a way that they alternate with the long axis parallel to one another. *Tetrastrum elegans* is green algae that form a colony of four cells forming a quadrangular plate. The cells are oval with spines.

*Chlamydomonadaceae* had one genus (*Carteria* sp.), it is a species of green algae that is unicellular and the cell body is spherical. *Hydrodictyaceae* had two genera (*Pediastrum simplex* and *Pediastrum* sp), this genus is a green algae with a fixed number of cells making it colonial. *Volvocaceae* had two species (*Pandorina morus* and *Pandorina* sp.), the green algae in this family forms a colony. *Pleaurochloridaceae* had one species (*Tetraedriella laevis*), it unicellular and tetragonal.

*Euglenaceae* had one species (*Trachelomonas volvocina*), which is unicellular and spherical. *Ulotrichaceae* had two species (*Geminella interrupta* and *Ulothrix zonata*) and both of these species are filamentous. *Chrococcaceae* had one genus (*Chroococcus* sp.), which is found in colonies of four cells that are spherical.

Desmidiaceae had six genera (Cosmarium magaritatum, Cosmarium sp. Mesotaenium sp. Staurastrum pseudosebaldi, Staurastrum sp. and Roya sp.). The genus Cosmarium is unicellular having semi cells joined by a narrow isthmus. Mesotaenium sp. and Roya sp. is

unicellular and cylindrical. *Staurastrum* sp. has semi cells with an urn shape. *Microsporaceae* had one genus (*Microspora* sp.) a green alga which is filamentous. *Chlococcaceae* had one genus (*Tetraedron* sp) which is a green unicellular alga with a tetrahedron shape. *Nostacaceae* had one species (*Anabaena smithii*) which is filamentous and *Peridiniaceae* had one species (*Peridinium aciculiferum*) which unicellular and ovoid. The taxonomic group with the highest number of species was *Desmidiaceae*. Appendix 1 shows species belonging to the respective taxonomic groups. And appendix 2 shows the presence of algae according to depth at the two stations.

The type of algae that was most abundant at Yalelo fish cages was *Oscillatoria* sp. with 29.6 per cent and at Sampa Kaluma Island it was also *Oscillatoria* sp. with 24.3 per cent. At Kamimbi area the second most prominent group was *Microcystis aeruginosa* with an abundance of 19 per cent; this was followed by *Chlorella sp*. which contributed 16 per cent. At Sampa Kaluma Island the second most prominent group was *Chlorella sp*. which had an abundance of 12 per cent; this was followed by *M. aeruginosa* with an abundance of 11 per cent and *Carteria* sp. with an abundance of 7 per cent. Appendix 1 shows the genera/species that were identified at Kamimbi area and at Sampa Kaluma Island. Appendix 1 also shows the relative abundance percentage of the species observed in both Stations.

Diatoms found in the two stations were both centric and pennate. Diatoms identified were *Cyclotella sp., Synedra* sp., *Navicula* sp., *Gyrosigma* sp., *Gomphonema* sp., *Rhopalodia* sp., *Caloneis* sp., *and Nitzschia* sp. Appendix 1 also shows the Stations where they were absent and present.

Filamentous algae identified were *Microspora sp. Anabaena smithii, Mougeotia* sp. *Sphaeroplea annulina, Ulothrix zonata, Geminella interrupta* and *Oscillatoria* sp. *Oscillatoria* sp. *Mougeotia* sp. *Sphaeroplea annulina and Geminella interrupta* were recorded from the two Stations sampled. *Microspora* sp. was only present at Kamimbi area, while *Anabaena smithii* and *Ulothrix zonata* were only recorded at Sampa Kaluma Island.

Desmids (*Closterium aciculare, Closterium* sp., *Staurastrum pseudosebaldi, Cosmarium* sp., *Mesotaenium* sp. *Closterium leibleinii, Cosmarium magaritatum, Roya* sp. and *Closterium maniferum*) were also identified. Sampa Kaluma Island had the highest species diversity of desmids while Kamimbi area recorded *Closterium* sp with the highest abundance of 3.49 per cent.

Two species of *Pediastrum* sp. (Hydrodictyaceae) were recorded at Kamimbi area while one was recorded at Sampa Kaluma Island. This species forms a colony that has a fixed number of cells of 8, 16, 32 and sometimes 64. The Scenedesmaceae family had higher species diversity and abundance on Sampa Kaluma Island as compared to Kamimbi area. *Scenedesmus quadricauda* had the highest abundance of 1.10 per cent amongst Scenedesmus family. *Scenedesmus* sp. was only present at Kamimbi area while *Tetrastrum elegans, Scenedesmus quadricauda*, *Scenedesmus acuminatus* and *Scenedesmus obliquus* were only recorded at Sampa Kaluma Island.

*Cryptomonas* sp., with an abundance of 0.57 per cent, was present only at Kamimbi area. *Pandorina morus* and *Pandorina* sp. were present only at Sampa Kaluma Island. *Peridinium aciculiferum* was present in both Stations but had the highest abundance of 6.4 per cent Sampa Kaluma Island. *Tetraedron* sp. occurred in both Stations, but Sampa Kaluma Island showed higher density. *Treubaria* sp. and *Chroococcus* sp. were present only at Kamimbi area.

Figure 4 is the picture showing the Fish cages at Kamimbi area. Some of the phytoplanktons that were identified are shown in figures 5, 6, 7, 9, 10, 11 and 12.



Figure 4: Fish cages at Kamimbi area (Yalelo fish cages).



## Figure 5: Naviculaceae



FIGURE 6: Hydrodictyaceae







FIGURE 8: Desmidiaceae



FIGURE 9: Closteriaceae



FIGURE 10: Volvocaceae



FIGURE 11: Scenedesmaceae



FIGURE 12: Peridiniaceae



Figure 13: Rank abundance showing relative species abundance visualising species richness and species evenness at Kamimbi area.

The rank abundance at Kamimbi area is shown in Figure 13. The species richness at Kamimbi area is higher (more genera/species) than at Sampa Kaluma Island and species evenness is high (abundances of different genera/species are similar) between the 1<sup>st</sup> rank and 9<sup>th</sup> rank. *Oscillatoria sp.* had the highest density, followed by *Microcystis aeruginosa* and *Trachelomonas volvocina* had the least density.



Figure 14: Rank abundance showing relative species abundance visualising species richness and species evenness at Sampa Kaluma Island.

The rank abundance at Sampa Kaluma Island is shown in Figure 14. Species richness at Sampa Kaluma Island is lower (less genera/species) than at Kamimbi area and the species evenness is low (high – ranking species have higher abundances than the low – ranking species). *Oscillatoria sp.* had the highest density, followed by *Chlorella sp.* and *Scenedesmus acuminatus* had the least density. Kamimbi area has more species than Sampa Kaluma Island.



Figure 15: Rank abundance curves (Whittaker plot) for Kamimbi area and Sampa Kaluma Island.

The comparison of the rank abundance in the two stations is shown in Figure 15. The rank abundance curve displays relative species abundance, it also shows species richness and species evenness. The horizontal axis shows relative abundance and the vertical axis show number of genera/species identified. The two curves show some difference in specie evenness between 1<sup>st</sup> rank and 9<sup>th</sup> rank. The steep gradient at Sampa Kaluma Island display low evenness as the higher – ranking species have much higher abundance than the low – ranking species. The shallow gradient at Kamimbi area shows high evenness as the abundances of different species are similar.

#### **4.3** Density of Phytoplankton according to depth

| Depth | Site C                  | Site D                  | Site E                  | Average density at              |
|-------|-------------------------|-------------------------|-------------------------|---------------------------------|
| (m)   | (Unit/cm <sup>3</sup> ) | (Unit/cm <sup>3</sup> ) | (Unit/cm <sup>3</sup> ) | Kamimbi (Unit/cm <sup>3</sup> ) |
| 0     | 7787                    | 3166                    | 8764                    | 6572                            |
| 5     | 2061                    | 2984                    | 3274                    | 2773                            |
| 10    | 3583                    | 2160                    | 4607                    | 3450                            |
| 15    | 3080                    | 1461                    | 1983                    | 2175                            |
| 20    | 2808                    | 2210                    | 2096                    | 2371                            |
| 25    | 6747                    | 1444                    | 1228                    | 3140                            |

Table 4: Total unit density of algae at Kamimbi area at diferent depths of Lake Kariba

Surface water had the highest density of phytoplankton, but as the depth increased there was some fluctuation of density of phytoplankton as shown in site C, D and E (Table 4).

| Depth | Site A                  | Site B                  | Site F                  | Average density at Sampa              |
|-------|-------------------------|-------------------------|-------------------------|---------------------------------------|
| (m)   | (Unit/cm <sup>3</sup> ) | (Unit/cm <sup>3</sup> ) | (Unit/cm <sup>3</sup> ) | Kaluma Island (Unit/cm <sup>3</sup> ) |
| 0     | 3599                    | 4507                    | 3708                    | 3938                                  |
| 5     | 2843                    | 4619                    | 3923                    | 3795                                  |
| 10    | 3165                    | 3483                    | 4669                    | 3772                                  |
| 15    | 2671                    | 3330                    | 3571                    | 3191                                  |
| 20    | 3437                    | 1816                    | 3051                    | 2768                                  |
| 25    | 2669                    | 2000                    | 2536                    | 2402                                  |

Table 5: Total unit density of algae at Sampa Kaluma Island

The density of algae at different sites and depths are shown in Table 5. At site B the highest density was at a depth of 5m and site C had the highest density of phytoplankton at a depth of 10m. The average showed highest density of phytoplankton from surface water, which was followed by a fluctuation of density of phytoplankton.

 Table 6: Average Density totals at Kamimbi area and Sampa Kaluma Island

| Depth | Average at Kamimbi area                  | Average at Sampa Kaluma |
|-------|--|-------------------------|
| (m)   | from the 3 Sites (Unit/cm <sup>3</sup> ) | Island from the 3 Sites |
|       |  | (Unit/cm <sup>3</sup> ) |
| 0     | 6572                                     | 3938                    |
| 5     | 2773                                     | 3795                    |
| 10    | 3450                                     | 3772                    |
| 15    | 2175                                     | 3191                    |
| 20    | 2371                                     | 2768                    |
| 25    | 3140                                     | 2402                    |

The average density of algae for the two stations is shown in Figure 6. The average density of phytoplankton at Kamimbi area and Sampa Kaluma Island showed that the surface water had the highest density. There was a reduction of phytoplankton density at 5m in both stations. Kamimbi station had a drastic reduction while Sampa Kaluma Island had a minimal reduction. According to the trend of Sampa Kaluma Island, there was gradual reduction of density as depth increased. Kamimbi Station's density fluctuated as depth increased.

At Kamimbi area there was no correlation between phosphorus and phytoplankton abundance,  $(r^2 = 0)$ . There was also no correlation between nitrate and phytoplankton abundance  $(r^2 = 0)$ . There was a negative correlation between silica and phytoplankton  $(r^2 = -0.12)$  and there was no significant regression of silica on density of algae (P = 0.53; P > 0.05).

There was a correlation between phosphate and phytoplankton abundance at Sampa Kaluma Island ( $r^2 = 0.086$ ), however there was no significant regression of phosphate on phytoplankton abundance (P = 0.29; P > 0.05). There was no correlation between nitrate and phytoplankton abundance ( $r^2 = 0$ ). There was also a correlation between silica and phytoplankton abundance ( $r^2 = 0.57$ ), but was no significant regression of silica on phytoplankton abundance (P = 0.051; P > 0.05).

## **CHAPTER FIVE**

#### DISCUSSION

#### 5.1 Concentration of Nutrients

At Kamimbi area phosphate concentration had no effect on phytoplankton diversity, there was no correlation because the concentration of phosphate was too small and constant. However, there was a weak positive correlation between phosphate and phytoplankton diversity at Sampa Kaluma Island, because higher concentration of phosphate had a small influence on phytoplankton diversity.

The level of phosphate at Kamimbi area was found to be small at all depths (Tables 1 and 3) probably because of the assimilation capacity of the Lake. Phosphate assimilation capacity of the local environment remains unchanged despite the increased nutrients inputs from fish farms (Hakan *et al*, 1996). Phosphate assimilation capacity is based on phosphorus intake through photosynthesis by aquatic plants. Phosphate assimilation capacity is also based on efficient retaining of phosphorus from feed by fish, bone is comprised mainly of calcium phosphate. Phosphorus is also quickly and strongly adsorbed by sediments, phosphate from sediment has low solubility which makes it unavailable to re-enter the water column (Claude, 2001).

There was more phosphate at Sampa Kaluma Island compared to Kamimbi area, because of the difference in hydrology and hydrodynamics of the two stations particularly site A of Sampa Kaluma Island. Hydrology refers to physical movement of a body of water, including changes in water level and properties of water. Hydrodynamics refers to the motion of fluids and the forces acting on solid bodies immersed in fluids. Site A was located where Zambezi River passes. The movement of water of Zambezi River causes disturbances in sediments and the vertical distribution of phosphate is possibly attributed to hydrology of water. Hydrology and Hydrodynamics cause disturbances in the sediments and intensify phosphorus exchange and diffusion at the sediment water interface and when environment changes the dynamic balance is disturbed causing phosphorus to be released into water through a number of complex chemical processes that can cause pollution (Wang and Liang, 2015). The high concentration of phosphorous at Sampa Kaluma Island compared to Kamimbi area is

attributed to hydrology and hydrodynamics, which enhances disturbances in the sediments diffusion of phosphorus up wards. Table 1 shows the average concentration of phosphorus in this Station. Coche (1968, 1969 and 1974) carried out a research on Lake Kariba and found that the phosphorus content ranged from 0.002mg/cm<sup>3</sup> to 0.06mg/cm<sup>3</sup> in the epilimnion (surface water). Lindmark (1977) recorded phosphorus levels between 0.001mg/cm<sup>3</sup> to 0.07mg/cm<sup>3</sup> in the epilimnion and 0.004mg/cm<sup>3</sup> to 0.051mg/cm<sup>3</sup> in the deep layers below 30 meters. From these results shown in Tables 2 and 3, the levels were within range except for site A (Table 2) which had higher phosphate levels depending on depth.

The general reduction of species with decreasing pH and the tolerance limits for low pH are known for major aquatic taxa such as fish, zooplankton and algae (Schindler, 1988). There were more diatoms at Kamimbi area than Sampa Kaluma Island because of the higher concentration of silica at Kamimbi area. Having stated the source of silica in both stations above, it means that aquaculture had no effect on the Phytoplankton diversity of diatoms of Lake Kariba i.e. were silica concentration is concerned at Kamimbi area. There is a direct relationship between concentration of silicates and density of diatoms (Munawar, 1974). However, aquaculture is not a source of silica in both stations.

Kamimbi area had more silica than Sampa Kaluma Island, probably due to proximity to the lake shore. The fish cages at Kamimbi area are about 50 meters from the lakeshore and in such a case chances of silica coming from the Lakeshore are very high. Near the Lakeshore, high volume of silica tends to come from detrital components washed into the Lake such as Siliciclastic sand and mud (http://www.saltworkconsultants.com/blog/silica-mobility). The pH levels were also low at Kamimbi area compared to Sampa Kaluma Island (Appendices 3, 4 and 5). This situation is also a contributing factor to having high level of silica. Robert, (1980) determined that in all river sediments sampled decrease in pH and increase in dissolved silica coincided with a change in sediment colour from brown to black an apparent increase in organic decay.

A gradual increase of silica was observed in the two Stations (Kamimbi area and Sampa Kaluma Island), as the depth increased the concentration of silica also increased. Robert, (1980) conducted a test in the laboratory and in Lake Seneca and determined that silica concentration in interstitial waters of rivers and lake sediments increased continuously with depth until an apparent maximum was attained. He further went on to say that the gradient observed in silica suggested that this substance is transported from the sediment into the

overlying water by diffusion, with rates of diffusion differing in the top 8cm from that of below 8cm. The fish cages at Kamimbi area are very close to the lakeshore, the first cage is about 50 meters away from the lakeshore as the rest of the cages follow in wards the lake and Sampa Kaluma Island is about 30 kilometres away from the lakeshore and that makes it deeper than Kamimbi area.

Despite aquaculture at Kamimbi area, the concentration remains the same as that of Sampa Kaluma Island probably because of the following factors. Nitrogen enters into aquaculture system through rainfall, in-situ nitrogen fixation, river run- off, diffusion from sediments, uneaten feeds and fish wastes; it is largely controlled by redox reactions mediated by phytoplankton and bacteria (http://www.ep.gov/bioindicators). Nitrogen is removed from the water in a water body by a number of ways, ammonia which contains nitrogen is taken up by algae and other plants as a nutrient for growth. It can also be transformed into nitrite and then nitrate (Hargreavs and Tucker, 2004). Under anaerobic conditions, such as sludge at the bottom of lake nitrate is converted to nitrite and to nitrogen gas thus removing total nitrogen from the aquatic system (http://www.alken-murray.com/TESTSO1.). Coche (1968, 1969 and 1974) did a study on Lake Kariba and recorded nitrate ranging from 0.002 to 0.06mg/cm<sup>3</sup> in the surface water. Despite aquaculture taking place concentration of nitrate has not changed much. Lake Kariba is considered to be oligotrophic (Machena and Kautsky 1988; Marshall, 1994) with phosphorus probably being the limiting nutrient (Magadza et al, 1989). Relatively low nutrients levels are probably due to high uptake of nutrients by phytoplankton, especially Microcystis species (Imai et al, 2009). Low level of nutrients could also be attributed to global warming. Ndebele (2010) carried out a study on the Zimbabwean side of Lake Kariba and showed that high evaporation coupled with reduction of rainfall may cause reduced water flow and water level in the lake and thus reduce the nutrient concentration in water and consequently phytoplankton biomass.

#### 5.2 Abundance of Phytoplankton

At Kamimbi area phosphate had no influence on abundance of phytoplankton because phosphate assimilation capacity was not exceeded by the input from aquaculture. This implies that aquaculture had no effect on the abundance of phytoplankton at Kamimbi area of Lake Kariba. At Sampa Kaluma Island there was an influence of phosphate on the abundance of phytoplankton, because there was a higher concentration that was available. The high concentration of phosphorous was because of hydrology i.e. physical movement of a body of water and properties of water which results into diffusion of phosphate from the sediment of the station (Wang and Liang, 2015). Aquaculture had no effect on the abundance of phytoplankton at Sampa Kaluma Island, the effect was as a result of phosphate diffusing from the sediment and it exceeded the phosphate assimilation capacity.

The negative relationship (correlation) between silica and phytoplankton abundance at Kamimbi area was because of the fluctuation of the density of phytoplankton as depth increased (Table 5). At Sampa Kaluma Island there was positive correlation between silica and phytoplankton abundance, there was a gradual decrease of the phytoplankton density as depth increased (Table 6). As stated above aquaculture is not the source of silica. Physico – chemical parameters such as turbidity, nutrients and depth play a pivotal role in determining abundance of certain phytoplankton groups and taxa in a given area with prevailing resources (Alvin *et al*, 2016).

Nitrate did not have a correlation with phytoplankton abundance in both stations probably because of same concentration (constant values) found in the water, which was low. However the phytoplankton abundance was higher at Kamimbi area compared to Sampa Kaluma Island. The input of nitrate from aquaculture has not gone beyond 0.01mg/cm<sup>3</sup> because of assimilation capacity and other reasons given above.

Figure 13 and 14 shows that the composition of phytoplankton has changed. However aquaculture has not caused that situation. The dominating species in the two stations was *Oscillatoria* sp. A survey carried out 1984 – 85 showed that the dominating algal species consisted of *Gloeotrichia* sp., followed by a number of *Oscillatoria* and *Lyngbya* species (Ramberg *et al*, 1987).

Figure 15 shows some difference between  $1^{st}$  rank and  $9^{th}$  rank, the composition was different. Appendix 1 shows the composition of phytoplankton, a number environment factors such as turbidity, nutrients and depth can determine the abundance of certain phytoplankton groups (Alvin *et al*, 2016). The two curves also show that most species are rare (those with very low abundance percentage).

## **CHAPTER SIX**

## 6.1 CONCLUSION

Aquaculture activities have had no effect on the phytoplankton diversity and abundance at Kamimbi area of Lake Kariba. Aquaculture has not changed the net nutrient balance in Lake Kariba. The lake still remains oligotrophic, despite aquaculture activities. The density of phytoplankton was dominated by *Oscillatoria* sp., followed by *Microcystis aeruginosa* and *Chlorella* sp. During the filling phases of the Lake, water samples consisted largely of Desmids (Thomasson, 1965). At Kamimbi area 31 species were identified and 28 species at Sampa Kaluma Island.

A study done in Lake Buhi in Philippines with aquaculture activities found a diverse and high phytoplankton density, there was a high occurrence of nutrients and suspended pollutants (Alvin *et al*, 2016).

## 6.2 Recommendations

- i. It is important for the Fisheries Management authorities to monitor nutrients from fish feed. The use of good quality feed and avoidance of over feeding should be practiced by the companies involved in aquaculture. Concentration of nutrients and density of algae especially blue green algae must be regularly monitored.
- ii. There is also need to do a similar research, which should allow water samples to be collected in different seasons. And time of the day when water samples are collected must be the same and more funds must be availed for such a research.

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## APPENDICES

Appendix 1: Phytoplankton identified at Kamimbi area and Sampa Kaluma Island and their average relative abundances (%) in the month that water samples were collected.

| Family             | species                   | Kamimbi area | Sampa         |
|--------------------|---------------------------|--------------|---------------|
| (Taxonomic group)  |                           | (%)          | Kaluma Island |
|                    |                           |              | (%)           |
|                    | Gomphonema sp.            | 0.82         | 0.0           |
| Bacillariaceae     | Nitzschia sp.             | 0.0          | 0.37          |
|                    | Rhopalodia sp.            | 0.28         | 0.0           |
| Chlamydomonadaceae | <i>Carteria</i> sp.       | 6.75         | 7.30          |
| Chlococcaceae      | Tetraedron sp.            | 0.33         | 0.76          |
| Chrococcaceae      | Chroococcus sp            | 0.33         | 0.0           |
|                    | Closterium aciculare      | 1.13         | 0.0           |
| Closteriaceae      | Closterium leibleinii     | 0.0          | 0.71          |
|                    | Closterium maniferum      | 0.0          | 1.73          |
|                    | Closterium sp.            | 1.20         | 3.49          |
| Cryptomonadaceae   | Cryptomonas sp.           | 0.57         | 0.0           |
|                    | Cosmarium magaritatum     | 0.33         | 0.0           |
|                    | Cosmarium sp.             | 0.0          | 2.95          |
| Desmidiaceae       | Mesotaenium sp.           | 0.0          | 2.42          |
|                    | Staurastrum pseudosebaldi | 0.54         | 0.0           |
|                    | Staurastrum sp.           | 0.0          | 1.05          |
|                    | Roya sp.                  | 0.33         | 0.0           |
| Euglenaceae        | Trachelomonas volvocina   | 0.28         | 0.0           |
| Fragilariaceae     | Synedra sp.               | 7.44         | 6.07          |
| Hydrodictyaceae    | Pediastrum simplex        | 0.82         | 0.37          |
|                    | Pediastrum sp.            | 0.33         | 0.0           |
| Microcystaceae     | Microcystis aeruginosa    | 18.82        | 10.57         |
| Microsporaceae     | Microspora sp.            | 0.55         | 0.0           |
|                    | Caloneis sp.              | 0.28         | 0.0           |
| Naviculaceae       | Gyrosigma sp.             | 0.89         | 0.0           |
|                    | Navicula sp.              | 1.10         | 0.68          |
| Nostocaceae        | Anabaena smithii          | 0.0          | 0.39          |

| Oocystaceae         | <i>Chlorella</i> sp.    | 15.87 | 11.84 |
|---------------------|-------------------------|-------|-------|
|                     | <i>Treubaria</i> sp.    | 0.66  | 0.0   |
| Oscillatoriaceae    | Oscillatoria sp.        | 29.64 | 24.29 |
| Peridiniaceae       | Peridinium aciculiferum | 0.66  | 6.40  |
| Pleaurochloridaceae | Tetraedriella laevis    | 0.82  | 2.54  |
|                     | Scenedesmus acuminatus  | 0.0   | 0.37  |
|                     | Scenedesmus obliquus    | 0.0   | 1.08  |
| Scenedesmaceae      | Scenedesmus quadricauda | 0.0   | 1.10  |
|                     | Scenedesmus sp.         | 0.56  | 0.0   |
|                     | Tetrastrum elegans      | 0.61  | 0.39  |
| Sphaeropleaceae     | Sphaeroplea annulina    | 1.20  | 4.96  |
| Stephanodiscaceae   | Cyclotella sp.          | 0.33  | 0.0   |
| Ulotrichaceae       | Geminalla interrupta    | 2.44  | 4.61  |
|                     | Ulothrix zonata         | 0.0   | 1.08  |
| Volvocaceae         | Pandorina morus         | 0.0   | 0.37  |
|                     | Pandorina sp.           | 0.0   | 0.68  |
| Zygnemataceae       | Mougeotia sp.           | 4.47  | 1.11  |

# Appendix 2: Presence of algae according to depth at the two stations

| Depth | Kamimbi area                    | Sampa Kaluma Island           |
|-------|---------------------------------|-------------------------------|
| (m)   | (Relative abundance of algae)   | (Relative abundance of algae) |
| 0     | Carteria sp. 4.21%              | Anabaena smithii 1.85%        |
|       | Chlorella sp. 36.70%            | Carteria sp. 7.53%            |
|       | Chroococcus sp. 1.10%           | Chlorella sp. 5.04%           |
|       | Closterium sp. 1.10%            | Closterium maniferum 1.85%    |
|       | Geminella interrupta 2.22%      | Closterium sp. 3.78%          |
|       | Microcystis aeruginosa 5.63%    | Geminella interrupta 3.78%    |
|       | Mougeotia sp. 4.18%             | Microcystis aeruginosa 10.10% |
|       | Oscillatoria sp. 37.28%         | Oscillatoria sp. 39.04%       |
|       | Pediastrum sp. 1.10%            | Peridinium aciculiferum 6.26% |
|       | Staurastrum pseudosebaidi 1.10% | Scenedesmus obliquus 1.85%    |
|       | Synedra sp. 3.18%               | Scenedesmus quadricauda 5.54% |
|       | <i>Tetraedron</i> sp. 1.10%     | Sphaeroplea annulina 5.53%    |

|    | Treubaria sp. 1.10%           | Synedra sp. 6.04%             |
|----|-------------------------------|-------------------------------|
|    |                               | Tetraedriella laevis 1.85%    |
| 5  | Carteria sp. 8.08%            | Carteria sp. 3.87%            |
|    | Chlorella sp. 10.52%          | Chlorella sp. 21.13%          |
|    | Cosmarium margaritatum 2.62%  | Closterium maniferum 1.95%    |
|    | Cryptomonas sp. 2.67%         | Closterium sp. 4.88%          |
|    | Cyclotella sp. 2.62%          | Cosmarium sp. 1.95%           |
|    | Geminella interrupta 2.62%    | Geminella interrupta 5.78%    |
|    | Microcystis aeruginosa 12.02% | Mesotaenium sp. 1.95%         |
|    | Microspora sp. 2.67%          | Microcystis aeruginosa 10.44% |
|    | Oscillatoria sp. 50.82%       | Oscillatoria sp. 25.87%       |
|    | Synedra sp. 5.36%             | Peridinium aciculiferum 5.78% |
|    |                               | Scenedesmus obliquus 1.92%    |
|    |                               | Sphaeroplea annulina 6.76%    |
|    |                               | Staurastrum sp. 1.92%         |
|    |                               | Synedra sp. 3.90%             |
|    |                               | Tetrastrum elgans 1.92%       |
|    |                               |                               |
| 10 | Carteria sp. 6.03%            | Carteria sp. 7.22%            |
|    | Chlorella sp. 25.24%          | Chlorella sp. 6.60%           |
|    | Closterium aciculare 2.11%    | Closterium leibleinii 1.93%   |
|    | Cyrosigma sp. 4.21%           | Closterium moniferum 1.97%    |
|    | Geminella interrupta 2.16%    | Closterium sp. 1.98%          |
|    | Gomphonema sp. 2.11%          | Cosmarium sp. 3.91%           |
|    | Microcystis aeruginosa 12.89% | Geminella interrupta 3.92%    |
|    | Navicula sp. 1.93%            | Mesotaenium sp. 2.64%         |
|    | Oscillatoria sp. 21.47%       | Microcystis aeruginosa 9.38%  |
|    | Peridinium aciculiferum 2.11% | Mougeotia sp. 1.96%           |
|    | Scenedesmus sp. 4.20%         | Navicula sp. 1.93%            |
|    | Sphaeroplea annulina 4.83%    | Oscillatoria sp. 29.38%       |
|    | Synedra sp. 8.61%             | Peridinium aciculiferum 5.87% |
|    | Tetraedriella laevis 2.11%    | Scenedesmus acuminatus 1.93%  |
|    |                               | Scenedesmus obliquus 1.93%    |
|    |                               | Sphaeroplea annulina 5.83%    |

|    |                               | Staurastrum sp. 1.96%         |
|----|-------------------------------|-------------------------------|
|    |                               | Synedra sp. 5.83%             |
|    |                               | Tetraedriella laevis 1.93%    |
|    |                               | Tetrahedron sp. 1.93%         |
| 15 | Carteria sp. 11.39%           | Carteria sp. 9.31%            |
|    | Closterium aciculare 10.22%   | Chlorella sp. 11.49%          |
|    | Closterium sp. 3.34%          | Closterium leibleinni 2.28%   |
|    | Cryptomonas sp. 3.40%         | Closterium moniferum 2.32%    |
|    | Microcystis aeruginosa 21.31% | Cosmarium sp. 4.56%           |
|    | Mougeotia sp. 10.15%          | Geminella interrupta 4.56%    |
|    | Navicula sp. 6.82%            | Mesotaenium sp. 3.49%         |
|    | Oscillatoria sp. 15.33%       | Microcystis aeruginosa 8.53%  |
|    | <i>Roya sp.</i> 3.34%         | Mougeotia sp. 2.28%           |
|    | Synedra sp. 11.36%            | Nitschia sp. 2.28%            |
|    | Tetrastrum elegans 3.34%      | Oscillatoria sp. 16.66%       |
|    |                               | Pandorina morum 2.28%         |
|    |                               | Pandorina sp. 2.32%           |
|    |                               | Peridinium aciculiferum 6.98% |
|    |                               | Sphaeroplea annulina 4.56%    |
|    |                               | Straurastrum sp. 2.32%        |
|    |                               | Synedra sp. 6.94%             |
|    |                               | Tetraedriella laevis 4.60%    |
|    |                               | Ulothrix zonata 2.28%         |
| 20 | Carteria sp. 9.31%            | Carteria sp. 7.62%            |
|    | Closterium sp. 3.07%          | Chlorella sp. 23.15%          |
|    | Cyrosigma sp. 3.07%           | Closterium sp. 2.52%          |
|    | Geminella interrupta 3.07%    | Cosmarium sp. 5.09%           |
|    | Microcyctis aeruginosa 22.65% | Geminella interrupta 6.39%    |
|    | Mougeotia sp. 9.25%           | Mesotaenium sp. 2.52%         |
|    | Navicula sp. 3.07%            | Microcystis aeroginosa 11.18% |
|    | Oscillatoria sp. 13.23%       | Mougeotia sp. 2.59%           |
|    | Pediastrum simplex 6.13%      | Navicula sp. 2.52%            |
|    | Peridinium aciculiferum 3.12% | Oscillatoria sp. 10.95%       |
|    | Sphaeroplea annulina 3.12%    | Pandorina sp. 2.52%           |

|    | Synedra sp. 11.75%            | Peridinium aciculiferum 5.09% |  |
|----|-------------------------------|-------------------------------|--|
|    | Tetraedriella laevis 3.12%    | Sphaeroplea annulina 2.52%    |  |
|    | Trachelomonas volvocina 3.07% | Synedra sp. 7.73%             |  |
|    | Treubaria sp. 3.07%           | Tetraedriella laevis 2.52%    |  |
|    |                               | Tetraedron sp. 2.52%          |  |
|    |                               | Ulothrix zonata 2.52%         |  |
| 25 | Caloneis sp. 2.32%            | Carteria sp. 9.52%            |  |
|    | Carteria sp. 2.36%            | Chlorella sp. 3.18%           |  |
|    | Geminella interrupta 4.62%    | Closterium moniliferum 3.12%  |  |
|    | Gomphonema sp. 2.32%          | Closterium sp. 9.40%          |  |
|    | Microcystis aeruginosa 49.59% | Cosmarium sp. 3.12%           |  |
|    | Mougeotia sp. 6.44%           | Geminella interrupta 3.12%    |  |
|    | Oscillatoria sp. 18.33%       | Mesotaenium sp. 6.30%         |  |
|    | Rhopalodia sp. 2.32%          | Microcystis aeruginosa 14.63% |  |
|    | Sphaeroplea annulina 2.32%    | Oscillatoria sp. 13.02%       |  |
|    | Synedra sp. 7.09%             | Pandorina sp. 3.12%           |  |
|    | Terastrum elegans 2.32%       | Pediastrum simplex 3.12%      |  |
|    |                               | Peridinium aciculiferum 9.50% |  |
|    |                               | Sphaeroplea annulina 3.12%    |  |
|    |                               | Synedra sp. 6.38%             |  |
|    |                               | Teraedriella laevis 6.24%     |  |
|    |                               | Ulothrix zonata 3.12%         |  |

## Appendix 3: Limnology Data at Sampa Kaluma Island

| Parameter                     | Site A | Site B | Site F |
|-------------------------------|--------|--------|--------|
| рН                            | 8.14   | 8.23   | 8.28   |
| Temperature ( <sup>0</sup> C) | 27.6   | 27.7   | 27.7   |
| Conductivity (µS)             | 87.1   | 87     | 87     |
| D.O. (mg/l)                   | 9.9    | 10.1   | 10.5   |

| Appendix 4: Limnolog | y Data at Kamimbi area |
|----------------------|------------------------|
|----------------------|------------------------|

| Parameter                     | Site C | Site D | Site E |
|-------------------------------|--------|--------|--------|
| рН                            | 7.63   | 7.96   | 8.11   |
| Temperature ( <sup>0</sup> C) | 25.9   | 26.4   | 26.8   |
| Conductivity (µS)             | 91.2   | 87.6   | 86     |
| D.O. (mg/l)                   | 8.9    | 8.8    | 10     |

## Appendix 5: Limnology Data at Kamimbi area and Sampa Kaluma Island

| Parameter                     | Kamimbi area | Sampa Kaluma Island |  |
|-------------------------------|--------------|---------------------|--|
|                               | (Average)    | (Average)           |  |
| pH                            | 7.9          | 8.22                |  |
| Temperature ( <sup>0</sup> C) | 26.37        | 27.67               |  |
| Conductivity (µS)             | 88.27        | 87.03               |  |
| D.O. (mg/l)                   | 9.23         | 10.17               |  |

## **Appendix 6: Format of data collection sheet**

| Depth | NO <sub>3</sub> <sup>-</sup> N | PO <sub>4</sub> <sup>3-</sup> | Silica | Density/<br>Population<br>of algae |
|-------|--------------------------------|-------------------------------|--------|------------------------------------|
|       |                                |                               |        |                                    |
|       |                                |                               |        |                                    |
|       |                                |                               |        |                                    |
|       |                                |                               |        |                                    |
|       |                                |                               |        |                                    |