PHYTOPLANKTON COMPOSITION, GROWTH RATES AND OIL PRODUCTION POTENTIAL OF FAST GROWING SPECIES FROM LAKE NAIVASHA AND WATER RESERVOIRS AT EMBU UNIVERSITY COLLEGE

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree or any other award in any other university.

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DEDICATION

I dedicate this work to my father Josphat Yumya, my mother Elizabeth Mbithe, my sisters Betty and Fridah and my brothers Peter and Massive who have been of great inspiration to me.

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ABBREVIATIONS AND ACRONYMNS

ANOVA	Analysis of Variance
APHA	American Public Health Association
Ca	Calcium
$CaCO_3$	Calcium carbonate
Cu	Copper
EUC	Embu University College
Fe	Iron
GJ	Gigajoules
IDH	Intermediate Disturbance Hypotheses
K	Potassium
Mg	Magnesium
Mg L^{-1}	Milligrams per liter
Ml	Milliliters
Mn	Manganese
N	Nitrogen
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NPK	Nitrogen, Phosphorus and Potassium
P	Phosphorus
PAST	Paleontological Statistics Software Package
pH	Potential of hydrogen
PO_4-P	Phosphate phosphorus
S	Sulphur
SAS	Statistical Analysis System
SE	Standard Error
SW index	Shannon Wiener index
TA	Total Alkalinity
USA	United States of America
UV	Ultraviolet Visible
Zn	Zinc

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ABSTRACT

The demand for non-renewable fossil fuel has greatly increased in the last few years from 84 million barrels of fossil fuel per day in 1980's to over 100 million barrels per day in 1990. To supplement this demand, research on alternative sources has been going on since 1960's in different parts of the world. Among the alternative sources, the phytoplanktons have shown great promise due to their high oil yield in comparison to energy crops. In Kenya, despite the occurrence of highly productive eutrophic lakes, no research has been carried out to assess the potential of phytoplankton species in oil production. This study therefore aimed at identifying and assessing the oil production potential of fast growing phytoplankton species in a species rich natural freshwater lake (Lake Naivasha) and man-made reservoirs at Embu University College. Data was collected from December 2014 to May 2015. During each sampling trip, selected physico-chemical parameters were measured and water samples collected for analysis of nutrients as well as species identification, composition, isolation and culturing in the laboratory. The samples were inoculated in phytoplankton growth media and cultured under 14:10 light: dark photoperiod. The fast growing species were identified through screening the biomass accumulated. These species were then cultured for lipid extraction. Results on physico-chemical characteristics revealed that, inflows during the wet months (December 2014 and April 2015) resulted in low Secchi depth, conductivity and total alkalinity. Highest values were recorded during the dry month of February 2015. High values of pH (8.6 both in lake Naivasha and EUC dams were recorded). High total nitrogen (TN range =0.18-0.271 mg L^{-1} in Lake Naivasha and 0.091-0.097 in EUC dams) and total phosphorous (TP range =0.069-0.093 mg L^{-1} in Lake Naivasha and 0.004-0.073 in EUC dams) ranges were recorded during the wet months. Results showed that phytoplankton species composition consisted of a total of 134 species in Lake Naivasha and 122 species in EUC reservoirs. Species diversities ranged from 1.29 to 1.68 with Lake Naivasha registering a mean of 1.5 which was not significantly different ($p < 0.158$) from the mean of 1.44 in EUC Dams. The fastest growing phytoplankton species in both Lake Naivasha and EUC reservoirs included; *Closteriopsis acicularis* (0.64 mg L^{-1} d^{-1}), *Chlorella saccharophilla* (0.53 mg L^{-1} d^{-1}), *Chlorella vulgaris* (0.49 mg L^{-1} d^{-1}), *Cosmarium contractum* (0.42 mg L^{-1} d^{-1}) and *Scenedesmus ellipticus* (0.37 mg L^{-1} d^{-1}). Oil production potential recorded by *Nannochloropsis* sp, *Chlorella saccharophilla, Chlorella vulgaris* and *Scenedesmus ellipticus* was 30%, 10.8%, 8.7% and 5.4% respectively. The results also showed that mean oil produced by phytoplankton isolates from Lake Naivasha and EUC reservoirs did not differ significantly($p< 0.05$). The findings of phytoplankton species from Lake Naivasha and EUC reservoirs having oil production potential opens an opportunity for discussion and further research on how to incoporate recent technology in phytoplankton biofuel production to offer a solution to the experienced energy crisis.

CHAPTER ONE: INTRODUCTION

1.1 Background to the study

The demand for non-renewable fossil fuel has increased greatly in the last few decades – from 84 million barrels per day, equivalent to 13.3 billion litres to over 100 million barrels per day (Butterwick *et al*., 2005; Goto *et al*., 2010). According to the Central Intelligence Agency (CIA) 2009 fact book, out of these recorded values, USA consumes 18.7 million barrels per day, Europe 13.6 million barrels per day and China 8.2 million barrels per day. African continent consumes 3.2 million barrels per day with Kenya consuming only 78,000 barrels per day. It is estimated that by 2030, the global oil consumption will have increased by more than 20%. As at January 2008, total worldwide oil reserves were estimated at 1,332 billion barrels (International Energy Agency, 2008). Based on the present and projected oil demand, it is estimated that these reserves can only last for between 40 to 60 years. Therefore unless new sources of fuel are discovered, the world faces an imminent danger of running out of this principal source of energy to cater for the world's needs (Abdullah, 2005). To supplement this demand, research on biofuel production from such products as corn, sugarcane, wheat, *Jatropha* sp. and phytoplankton has been ongoing especially in Europe and USA (Demirbas, 2007; Kajikawa and Takeda, 2008; Fischer *et al*., 2009).

Bio-fuel production from food crops such as corn, sugarcane and wheat obtained through abstracting their starch, sugar and oil then converting it to biodiesel began in the early 1950's (Gressel, 2008). Use of food crops as biofuel source has, however been reported to be non-promising due to competition between supplying food to man, and also the limited availability of farmland for growing crops for bio-fuel production. This is because most arable land is utilized for food crop production as well as the construction of infrastructure and industries (Mohr and Raman, 2013). Use of food crops for fuel production has the potential of raising the price of animal feed and the cost of food in general (Fischer *et al*., 2009). In addition, when the total carbon dioxide emission of growing, harvesting and processing is factored into the cost of bio-fuel, it becomes clear that bio-fuel from food crops is not environmentally sustainable.

The limitations of using food crops as a source of biofuel lead to a search for non-food crop sources of biofuel such as *Jatropha* sp. However, the major drawback with non-food

crops grown on land as sources of biofuel is the competition they offer to food crops in the agricultural land and time taken to accumulate biomass for oil extraction (Ariyadej *et al*., 2004; Domingues, 2005; Huber and Dale, 2009). Due to these challenges, its production has not been sustainable.

Research on some phytoplankton species as a potential source of biofuel began in early 1960's (Sialve *et al*., 2009; Carriquiry *et al*., 2011). A renewed interest in the subject was witnessed in the 1970s when several research projects were implemented in the USA to investigate the potential of the phytoplankton as a source of biofuel (Benemann *et al.,* 1982; Regan and Gartside, 1983; Sheehan *et al*., 1998). These studies revealed that phytoplankton have the potential for producing a high volume of bio-fuel at a low production cost (Metting, 1996; Spolaore *et al*., 2006 and Gao *et al*., 2012).

The potential of phytoplankton as a source of bio-fuel is based on several advantages that include; high growth rates, formation of large amounts of biomass, ability to be grown on non-arable land, use of non-potable water and the fact that they do not compete food crops for space (Spolaore *et al.,* 2006). Further, their production is not seasonal and they can accumulate large quantities of oil in a short time hence they can be harvested regularly (Metzger and Largeau, 2005; Krienitz and Wirth 2006; Greenwell *et al*., 2010; Abubakar *et al*., 2012).

1.2 Problem statement and justification

Globally, oil demand has been increasing greatly in the last few decades. However the available non-renewable fossil fuel resources cannot last for many years hence the need for renewable energy sources is important. Phytoplankton species are known to have a high oil yield and therefore can be a potential source of the renewable energy source (Spolaore *et al*., 2006). Countries in the tropical region are endowed with different water bodies with high diversity of phytoplankton species (Dawson and Spannagle, 2008). Despite these, few studies have explored their oil production potential. In Kenya particularly, oil production potential of the phytoplankton species remains largely unknown. Lake Naivasha, a freshwater lake in Kenya is known to have high phytoplankton diversity (Hubble and Harper, 2002). Yet no research on their cellular oil content has been investigated. Similarly, Embu University College reservoirs, which can represent readily available inland water bodies in the country have not been investigated

on their phytoplankton species composition and the oil production potential. Characterization of the species present for their oil production potential and fast growing habits in the selected water bodies was therefore desirable. This contributed additional information on the potential of phytoplankton as a source of biofuel in general and specifically identified the species with the highest oil yield. In addition, the information on the seasonal changes shed some light on the ideal period to collect the species of interest.

1.3 Research questions

1. Do phytoplankton species composition present in Lake Naivasha and Embu University College dams exhibit pronounced temporal changes from wet to dry months?
2. Are there variations in growth rates of phytoplankton species in Lake Naivasha from those in Embu University College dams under laboratory conditions?
3. Is there any relationship in the cellular oil contents of fast growing phytoplankton species in Lake Naivasha and EUC reservoirs?

1.4 Hypotheses

1. The phytoplankton species numbers of Lake Naivasha and Embu University College dams does not increase over a transition period from wet to dry months.
2. There are no significant differences in the growth rates of fast growing phytoplankton species common to Lake Naivasha and Embu University College dams.
3. There are no significant differences in cellular oil content of fast growing phytoplankton species obtained from Lake Naivasha and EUC reservoirs.

1.5 Objectives

1.5.1 General objective

To determine oil production potential of fast growing phytoplankton species isolated from Lake Naivasha and Embu University College reservoirs under laboratory conditions.

1.5.2 Specific objectives

1. To determine the changes in phytoplankton composition in Lake Naivasha and Embu University College reservoirs over wet to dry months.
2. To identify fast growing phytoplankton species in Lake Naivasha and Embu University College dams under controlled laboratory conditions.
3. To determine the cellular oil contents of fast growing phytoplankton species from Lake Naivasha and Embu University College reservoirs.

CHAPTER TWO: LITERATURE REVIEW

2.1 Phytoplankton species composition in tropical natural lakes and man-made reservoirs

Tropical water bodies generally have a high phytoplankton species composition as compared to temperate ones (Talling, 2001; Borges *et al.*, 2003; Garibaldi *et al.*, 2003). In general, the phytoplankton species composition of tropical regions is similar to that of temperate lakes during summer (Calijuri *et al.*, 2002). However, the actual composition is greatly influenced by the nutrient availability and the physico-chemical characteristics of the aquatic system (Becker *et al.*, 2010; Rigosi and Rueda, 2012). Many studies have attempted to relate temporal variations in phytoplankton composition to changes in environmental conditions. For example temperature and density stratification of water bodies has been reported to influence vertical distribution of phytoplankton species across the water column. Under conditions of stable thermal stratification, the distribution of most phytoplankton is generally limited to the well mixed euphotic layer. An exemption to this is the distribution of heterocystous cyanobacteria which have the ability to regulate their vertical location in the water column using aerotopes (Salmaso, 2002). Similarly flagellated species (euglenophytes, dinoflagellates and chrysophytes) can actively migrate along the water column with the aid of their flagellas (Salmaso, 2000). Tiwari and Chauhan (2006) have reported a positive correlation between wind speed and the abundances of diatoms. Overall, phytoplankton community shifts linked to environmental variations are still not well understood in the tropical water bodies (Schagerl and Oduor, 2008).

Several studies in African tropical lakes have shown that cyanobacteria and chlorophytes are generally more abundant (Downing *et al.*, 2001; Brookes *et al.*, 2003; De Figueiredo *et al.*, 2004). The species compostion show marked temporal changes. It has been reported that in deep tropical lakes, phytoplankton composition dominance starts with a community of diatoms and cryptophyta during turn over event corresponding with nutrients enrichment from rain. This is followed by chlorophyta, as stratification develops and then cyanophyta, ending with the dominance of dinoflagellates when stratification is fully established. In shallow tropical lakes, where stratification is shortlived and unpredictable, it has been observed that diurnal cycles rather than the seasonal weather changes are

dominant and as such, limited seasonal changes in phytoplankton composition is registered in the course of the year (Jeppesen *et al.*, 2005).

In Lake Tanganyika for example the larger phytoplankton is mainly composed of chlorophytes and diatoms (*Nitzschia* sp.) with large blooms of filamentous cyanobacteria periodically observed during September to November at the onset of rainy season (Ndembele *et al.*, 2010). This has however changed recently as a result of changes in water column stability (Ramberg, 1987). These seasonal succession of major phytoplankton groups corresponding to weather changes has similarly been shown in many African lakes such as Lakes Edward, Kivu, Malawi, Kariba and Victoria (Beadle, 1981).

In lake Naivasha, studies on phytoplankton species composition have been carried out extensively since the 1960's (Gaudet and Melack, 1981). Results from these studies have shown that due to changing water quality, phytoplankton species composition both vertically and horizontally have been highly dynamic. Harper *et al.* (1990) have for example been following the phytoplankton species composition and have reported extensive changes over time, attributed to the progressive increase in nutrient concentration over time (Ballot *et al.*, 2004). According to Ballot *et al.* (2004), changes in phytoplankton communities of lake Naivasha can be contectualized to studies on equilibrium phases which reflect degradation scenarios (Padisak *et al.*, 2003). For instance, the phytoplankton community in 1980's showed a seasonal shift between diatom and cyanobacterial dominance (Hubble and Harper, 2002). Moreover, the introduction of water hyacinnth (*Eichhornia crassipes*) into the lake in 1988 caused the lakes phytoplankton productivity to reduce as a result of mat shadding of the phytoplankton cells (Mironga *et al.*, 2011). Ballot *et al.* (2004) found out that, cyanobacterial *Cyanocatena planktonica*, Chlorophyte *Pediastrum simplex* and Bacillariophyta *Aulacoseira granulata* together reached 75% of the total biomass. Moreover, *Botryococcus terribilis* reached a biomass of more than 90% in September 2002. From the comparison of the changes in species abundance, it is evident that there is a clear change in species numbers and dominance (Harper, 1992). Hubble and Harper (2002) reported that, 170 phytoplankton species have been identified in lake Naivasha. Currently, the water quality of Lake Naivasha has been seriously affected by the rising water levels that occurred in 2012 due to geological factors (Onywere, 2013). These changes are bound to

have profound effects on phytoplankton composition similar to those which occurred on earlier years.

Generally, limnological studies on man made reservoirs is limited particularly in Africa due to the historical bias of limnologists towards studying riverine and lake ecosysytems (Mwaura *et al.*, 2004). Studies which have been undertaken in Kenya have mainly focused on the chemical composition of reservoirs without dwelling much on the issue of phytoplankton species composition and seasonal changes. In the few studies conducted, phytoplankton species composition and diversity in the reservoirs has been reported to differ from that of natural lakes. Comparing natural lakes and man-made reservoirs, phytoplankton species composition and diversity is lower in man made reservoirs than in natural lakes (Mwaura *et al.*, 2002). This is as a result of change in status from lotic to lentic states upon impoundment of the running stream by the constructed dam. However, a study by Mavuti and Uku (1994) showed similar species composition in Masinga and Ruiru dams to those of lake Naivasha. In a different study, Silva (2007) observed that adjoining reservoirs also have phytoplankton species composition and diversity that differs. Generally, in reservoirs cyanobacterial dominance has been reported with chlorophytes being more prominent in young reservoirs (Pacini, 1994; Mwaura *et al.*, 2002). A study by Mwaura *et al.* (2004) in 3 small man made high altitude reservoirs in the Kenyan rift valley reported a similar phytoplankton community to that of lake Naivasha. The phytoplankton community was dominated by chlorophytes, cyanophytes and chrysophytes with a few diatoms occuring. Further, Cyanophyta dominance was observed during the dry season with both chlorophytes and cyanophytes occuring at the onset of rains. In addition, the low dominance of diatoms observed is similar to findings by Pacini, (1994) who also reported poor diatom abundance in reservoirs in a study in Masinga Dam, Kenya.

2.2 Phytoplankton growth strategies and growth rates

Phytoplankton species show wide variations in growth rates (Hinder *et al.*, 1999; Vaulot, 2001; Scheer and Sterling, 2005; Smil, 2006). Calijuri *et al.* (2002) classified phytoplankton species based on their growth rates into three groups as follows: C-strategists, S-strategists and R-strategists. C- Strategists are small phytoplankton species, with a high surface area/volume ratio, they grow quickly and are selected by conditions of both high light availability and nutrients concentration. On the other hand, S-strategists are

slow growing, large unicells which can be colonial with a low surface area/volume ratio, they are able to dominate under conditions of high light availability and low nutrients concentration. Lastly, R-strategists are generally large, elongated unicells and colonies or filaments with high surface area/volume ratio, which are adapted to low light availability and high nutrients concentration.

Cell size in phytoplankton species affects physiological rates, and ecological functions like metabolic rates which include: growth, photosynthesis and respiration (Hein *et al.*, 1995; Finkel, 2001). Studies on phytoplankton growth rates depending on their cell size and metabolic rates has yielded a wide range of differences. For example Tang (1996) recorded that dinoflagellates generally have lower growth rates than diatoms of comparable size. Study by Kagami and Urabe (2001) showed that phytoplankton growth rates decrease with increasing cell size. Similarly studies comparing single celled and colonial/ filamentous species revealed that single celled species were fast growers as compared to the colonial ones (Needoba *et al.*, 2003).

Study of phytoplankton growth rates in the wild environment, where conditions show wide variations is difficult (Crookes, 2006; Matthew, 2008). For easy monitoring and determination of phytoplankton growth rates, laboratory experiments are appropriate. In the laboratory, different species can be isolated to form uni-algal cultures which can be studied seperately. In addition, growth conditions can be manipulated and easily controlled to investigate a particular behaviour. For instance, a study by Regan (2013) to investigate diatom growth rates *in situ* modified the conditions by using incubation chambers similar to those in the laboratory conditions. The findings showed that growth rates were significantly high in the laboratory conditions as compared to the ones in the *in situ* trial.

Phytoplankton growth rates differ among species (Calijuri *et al.*, 2002). Chlorophytes exhibit higher growth rates compared to cyanophytes at varying light regimes, with most cyanophyta species recording low growth rates at high light intensities (Deblois *et al.*, 2013). Moreover, small sized clorophytes with flagella show high growthrates as compared to large sized ones (Raven and Kubler, 2002). A study in the USA using phytoplankton species to reduce carbon dioxide emmissions showed *Chlorella vulgaris* to have the fast growth rates of 1.775×10^3 cells mL^{-1} within five days of culture followed by *Chlorella pyrenoidosa* and *Chlorella sorokiniana* (Dowing *et al.*, 2001; Cong *et al.*, 2015).

2.3 Potential for bio-fuel production by phytoplankton species

Research extending from 1960's has shown that phytoplankton have high potential in bio-fuel production (Brown and Zeiler, 1993; Aresta *et al.*, 2005; Grover and Chrzanowsk, 2006). Some phytoplankton species have been shown to have oil content of up to 60% of their dry weight (Metting, 1996; Van Gerpen, 2005; Spolaore *et al.*, 2006; Griffiths and Harrison 2009). Studies conducted in the US have shown that the annual lipid productivity and oil yield of phytoplankton cells is far greater than that of seed crops (Borowitzka, 1997). A study by Haag (2007) and Chang (2007) showed that phytoplankton species can produce a yield of 90,000 litres per hectare which is by far higher than the 6,000 litres produced by canola and palm crops. Dermibas (2009) observed that typical oil content of phytoplankton cells is usually 20-50% of their dry weight.

In Japan and USA, the governments have invested over US$117 million and US$25 million respectively for technologies to culture phytoplankton cells in photobioreactors coupled with open ponds in a two stage process. In this investment, *Haematococcus pluvialis* has shown to be a promising species with >420 GJ $ha^{-1}yr^{-1}$ (Huntley and Redalje, 2007). Other studies by Christi, (2007) showed some phytoplankton species to accumulate large quantities of lipids e.g. *Botryococcus braunii* accumulates (25–75%), *Chlorella* sp. (28–32%), *Nitzschia* sp. (45–47%), *Phaeodactylum tricornutum* (20–30%), *Schizochytrium* sp. (50–77%). However, under the right conditions, some strains can accumulate oils of up to 80% of their dry weight (Metting, 1996; Spolaore *et al.*, 2006; Campbell, 2008; Bajhaiya *et al.*, 2010).

2.4 Phytoplankton cultures

Phytoplankton growth culture is prepared by use of different types of growth medium since phytoplankton species have varying nutritional requirements (Gouveia and Oliveira 2009; Singh *et al.*, 2013). In order to achieve the best growth results in phytoplankton culture, an understanding of their ecophysiology is important and key in designing the best growth conditions to optimize the production of algal biomass in large scales. Therefore, maximum production with minimum costs incurred is key issue of consideration in phytoplankton culture. Several media formulations for cultivation of phytoplankton have been proposed, many of which are derived from analysis of chemical environment in natural habitats where the species thrive (Vonshak, 1986).

Elements required for growth of most green algae are N, P, K, Mg, Ca, S, Fe, Cu, Mn and Zn (Eyster *et al.*, 1958). Both macro and micronutrients play significant roles in phytoplankton growth. Extensive studies on phytoplankton growth at various concentrations of macro and micronutrients and how the nitrogen and phosphorous concentrations affect the growth pattern have been conducted (Eyster *et al.*, 1958; Jeanfils *et al.*, 1993; Tam and Wong, 1996). It has been established that microalgae respond differently alterations in the environmental conditions where they grow (Scragg *et al.*, 2002). Therefore the culture conditions can be manipulated in order to control phytoplankton biochemical composition and growth, focusing on the specific elements with high productivity. According to Harrison and Berges (2005), culture maintenance, algal biomass yield and growth experiments are a good way to search for ideal growth conditions. These can be achieved by use of alternative media such as inorganic fertilizers like NPK and a mixture of other micronutrients extracts (Sipaúba Tavares *et al.*, 2015).

2.5 Isolation of phytoplankton

The most widely used methods of isolation are capillary pipette cell isolation and serial multiple dilution into new sterile containers after growth of the isolates (Andersen, 2005). The other common method is streaking of cells in sterile Petri-dishes half-filled with solidified media containing 1.0 -1.5% Agar. In capillary pipette cell isolation technique, a sterile pipette attached to silicone rubber tubing is used to pick a single cell of a target species under the microscope by mouth pipetted up the cell into the micropipette and transferred to a drop of sterile medium on a glass slide. The cell isolation process is repeated to wash the cell in order to free it from bacterial contamination. After every cell transfer, the micropipette is sterilized using hot distilled water pipetted in to the micropipette and blown out.

2.6 Oil extraction methods

There are a wide variety of methods for extracting oil from phytoplankton biomass (Aresta *et al.*, 2005; Govindarajan *et al.*, 2009; Gupta *et al.*, 2012). Physical extraction involves mechanical crushing of dried algae after which the oil can be pressed out with an oil press. An expeller press is a screw type machine, which presses the concentrated dried microalgae through a caged barrel-like cavity. This machine uses friction and continuous pressure from the screw drives to move and compress the dried cells. The oil seeps through small openings that do not allow cell fiber solids to pass through. Afterward, the

pressed dry cells are formed into a hardened cake, which is removed from the machine (Popoola and Yangomodou, 2006; Milledge, 2011). In addition to the physical extraction, chemical extraction methods have also been used which involve use of chemical solvents. Solvent extraction is achieved through grinding of the dried cells. The ground cells or cake is then purged or washed with a petroleum distillate (the most common chemical used is hexane) which releases the oil in the cells. The solvent is then "flashed off" by heating the oil in a sealed chamber. The oil/solvent blend is next heated to (100 ºC) to distill off the solvent. Hexane has been reported to be the most efficient solvent in extraction as it has one of the highest extraction capabilities and is of low cost with the advantage of being recycled (Serrato, 1981; Demirbas, 2009; Luisa and Ana, 2009).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study areas

Lake Naivasha is a shallow freshwater lake situated in a warm and semi-arid region in the Eastern Rift valley of Kenya, at an altitude of approximately 1890 m a.s.l, and latitude 0° 45' S, longitude 36° 20' E (Fig. 3.1). It is located about 80 km Northwest of Nairobi at the rain shadow of the Aberdares range. The total surface area of the lake is 139 km² and is surrounded by a swamp that covers an area of 64 km^2. However, the swamp area varies depending largely on the amount of rainfall received. The rainfall pattern in the area surrounding the lake is bimodal, peaking in April and October with considerable inter-annual irregularity. Evapotranspiration rate in this area is known to exceed local rainfall amounts (Harper *et al.*, 1995). The mean temperature around the lake is approximately 25 °C with the maximum temperature of 30 °C with December to March being the hottest period. July is the coldest month with a mean temperature of 23 °C.

Lake Naivasha is a closed basin lake (endorheic) that is fed by two perennial rivers, Malewa and Gilgil. The two rivers discharge 80% and 20% of the total inflow volume respectively (Becht, and Harper, 2002; Becht *et al.*, 2005). A key feature of this endorheic lake is the presence of a subterranean outlet (Melack, 1979; Gaudet and Melack, 1981) which enables the lake to remain a freshwater ecosystem.

The main habitat of Lake Naivasha is a freshwater lacustrine wetland with a fringing shoreline vegetation dominated by papyrus and many other emergent plants, floating leaved wetland plants and submerged species of *Potamogeton* and *Najas pectinata*. The wetland is in most areas surrounded by fringing woodland of *Acacia xanthophloea* including the flood plains of the rivers which run in to the lake dominated on the edges by papyrus species. The surrounding areas are mainly dry shrub with horticulture and planted shade and ornamental trees in some places (Harper *et al.*, 1995). The wetland soils are mainly sediments of a former larger lake, influenced highly by the quaternary deposits of lacustrine volcanic origin of the basin rocks and soils (Boar and Harper, 2002).

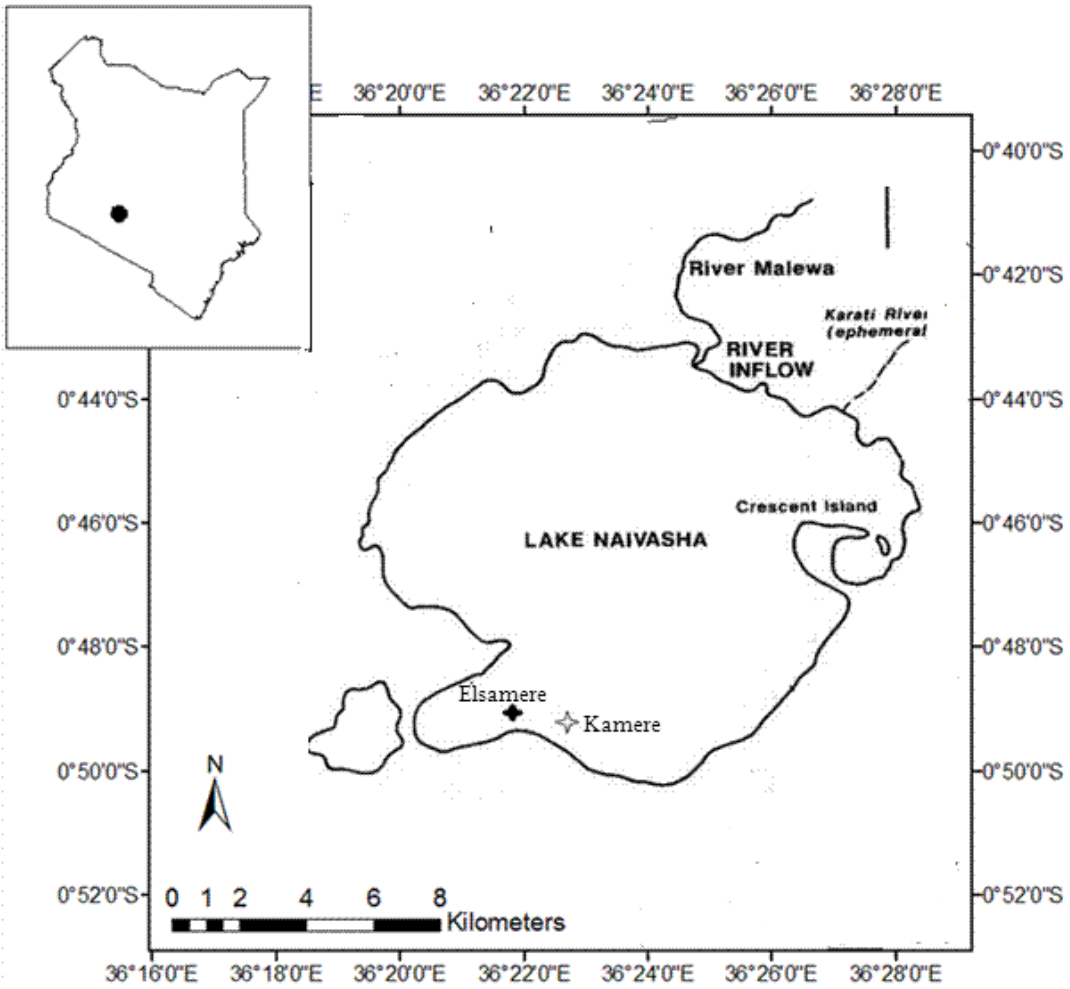

Fig. 3.1: Map of Lake Naivasha showing the two sampling sites (Elsamere and Kamere sites).
Map re-drawn from (Mavuti *et al.*, 1996).

The other study site was the man-made reservoirs at Embu University College, located in Embu County, at the south eastern side of Mount Kenya, along the Nairobi Meru highway at a distance of about 128 km from Nairobi. The College is located at an altitude of about 1324 m a.s.l., latitude 0° 30' S and longitude 37° 45' E (Fig. 3.2). There are four water reservoirs in the College, referred to as Dams 1, 2, 3 and 5, all of which are connected to each other in series. The reservoirs are made of varying surface areas with Dam 5 being the biggest covering an area of over 700 m^2 and Dam 4 the smallest covering an area of about 100 m^2.

The region receives a bi-modal rainfall pattern with the long rain season in between March to June while the short rains fall between October and December. Temperature in this

region ranges from a minimum of 12 °C in July to a maximum of 30 °C in March with an annual mean of about 21 °C. The source of water for the reservoirs is mainly through underground seepage into the first reservoir and surface runoff during the rainy season which then feeds the other reservoirs downstream through a seasonal stream.

The aquatic macrophytes community composition in EUC reservoirs is not well established as there is no distinct zonation pattern from the shoreline to the land. Next to the reservoirs is a surrounding of short bushes mainly composed of *Lantana camara*. The land next to the dams is farm land which is utilized mainly for growing of crops e.g. beans, maize, potatoes vegetables like cabbages and Sukuma wiki. Further, coffee and dairy cattle keeping is practiced in the university farm. The soils of the region are mostly andosols, developed from parent materials of Mt. Kenya.

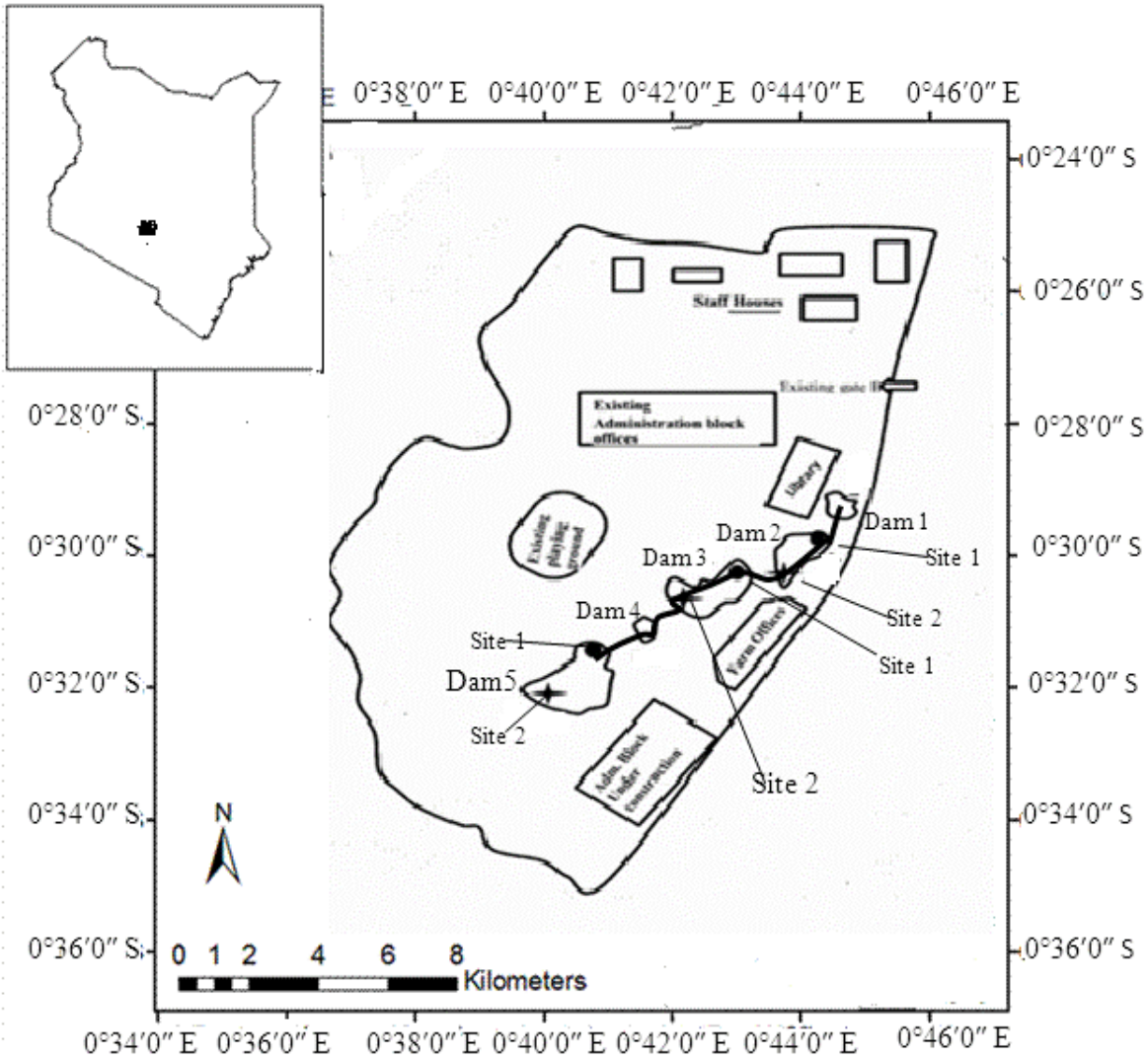

Fig. 3.2: Map of Embu University College showing the sampling sites in Dams 2, 3 and 5.

3.2 Description of the sampling stations

Sampling in Lake Naivasha was carried out at Kamere beach and Elsamere conservation centre (Table 3.1). The two sampling sites were selected to represent differences in human activities. Kamere beach is characterized by a number of human disturbances ranging from laundry, fish landing, watering and grazing of both domestic and wild animals hence was expected to support phytoplankton species that are characteristic of disturbed sites. Fish landing, which is the main activity at this site has greatly affected the nature of the site. The landed fish is gutted and sold mainly at the site after initial biomass measurement by the Fisheries Department officers at the ground. As a result of the many activities involved in the preparation and selling of the fish at the site, a lot of fish wastes find their way back

to the lake. Due to the large amounts of organic wastes, the site is heavily infested by marabou storks (*Leptoptilos crumenifer)* which occur in hundreds. The high amounts of nutrients, resulting from the organic wastes has caused the excessive phytoplankton growth observed at the sites. The littoral area of the site is heavily covered by a thick papyrus swamp. Beyond the papyrus swamp is famous for hippo watching and this has opened up the site for tourism activities. The other site was at Elsamere Conservation centre. This site is well protected with limited human activities hence was expected to support phytoplankton species that prefer undisturbed sites.

At Embu University Reservoirs, two sampling sites were chosen in each of the dams 2, 3 and 5. In each reservoir, site one was at a point near the water inlet while site two was at a point where the water exits the reservoir. The choice of the sampling sites was designed to increase the chances of sampling most of the phytoplankton species present in the reservoirs.

Table 3.1: Sampling sites GPS coordinates.

Station	Coordinates	Site description
Kamere site	0° 48' 83"S; 36° 19' 33"E	Main access point to the lake, characterized by several activities ranging from laundry, fish landing, watering and grazing of both domestic and wild animals. The site supports phytoplankton species that are characteristic of disturbed sites.
Elsamere site	0° 49' 04"S; 36° 20' 23"E	Found in well protected area with limited human activities. Therefore supports phytoplankton species that prefer undisturbed sites.
EUC dam 2 site 1	0° 30' 63"S; 37° 27' 18"E	Point of water inflow into dam 2, within 5 metres from the edge of the dam
EUC dam 2 site 2	0° 30' 22"S; 37° 27'47"E	It is at the exit from the dam to the stream connecting the subsequent dams located within 5 metres from the edge of the dam.
EUC dam 3 site 1	0° 30' 53"S; 37° 27' 00"E	Point of water inflow into dam 3, within 5 metres from the dams' edge.
EUC dam 3 site 2	0° 30' 21"S; 37° 27' 33"E	This is a site at the point of water exit from the dam to the stream connecting the subsequent dams located within 5 metres from the edge of the dam.
EUC dam 5 site 1	0° 30' 59"S; 37° 27' 94"E	This site is at the point of water inflow into dam 5, within 5 metres from the point of entrance of the inflowing stream.
EUC dam 5 site 2	0° 30' 88" S; 37° 27' 64" E	Site located at the point of water exit from the dam to the stream connecting the subsequent dams located within 5 metres from the edge of the dam.

3.3 Collection of the samples from the sites

Sampling was carried out once a month for a period of six months from December 2014 to May 2015 in both Lake Naivasha and Embu University College reservoirs. At each sampling site, some selected physico-chemical variables (temperature, pH, conductivity, water transparency and salinity) were measured using a portable pH conductivity meter (WTW Multiline P4 meter/ Wissenschaftlich Technische Werkstätten, Weilheim, Germany). Appropriate probe was lowered into the water and allowed to stabilize for between one to two minutes before recording the values of the various parameters. Water transparency was determined with the aid of a Secchi disc. At each site, Secchi disc was lowered and water transparency computed as the average of the depth at which the disc ceased to be visible on being lowered and the depth of re-appearance when being raised (Parsons *et al.*, 1984). Three water samples were collected randomly at each site during each sampling for nutrient analysis. For qualitative analysis of phytoplankton species, samples were taken using a phytoplankton net of mesh aperture 10 µm at the uppermost 10 cm of the water column in a uniform way. The net was pulled over a distance of 2 m for 5 minutes. The phytoplankton samples were preserved in the samples bottles using Lugols' Iodine solution. The collected samples were transported to the laboratory for further analysis within 24 hours.

3.4 Laboratory analysis

3.4.1 Total alkalinity

Total alkalinity was determined by titrating a known volume of the water sample against 0.02 N hydrochloric acid using few drops of mixed bromocresol blue/methyl red indicator to determine the titration end-point with the color changing from blue to yellow. Total alkalinity was calculated as the amount of acid used to reach the titration end point (mL) × Normality of acid (eq L^{-1}) × 50,000 (mg $CaCO_3$ eq^{-1}) / sample volume (mL) (APHA, 2005).

3.4.2 Nutrient analysis

3.4.2.1 Total phosphorus

All forms of phosphorus in unfiltered samples were first oxidized to phosphate P (PO_4^+-P) by autoclaving 50 mL of water sample at 140 ºC, 15 pounds pressure for 40 minutes in the presence of ammonium persulfate oxidizing agent. The concentration of the resultant soluble reactive P was determined as an intensely blue complex by the colorimetric ascorbic acid reduction procedure (APHA, 2005). The absorbance of each treated sample was measured at 690nm using UV/VIS spectrophotometer (MRC Spectro V-11D, China). Standards for the determination of actual concentration as well as reagent blank of distilled water were subjected to the same treatment as the samples. A calibration curve of absorbance against phosphate concentration was plotted and used to establish the concentrations of total phosphorus in water samples (APHA, 2005).

3.4.2.2 Total nitrogen

All forms of nitrogen in unfiltered samples were first oxidized to nitrate by autoclaving 50 mL of water sample at 140 ºC, 15 pounds pressure for 40 minutes in the presence of sodium hydroxide and potassium persulfate. The concentration of the resultant soluble reactive nitrate was maintained at pH 2-3 by addition of concentrated hydrochloric acid. Concentration of nitrates was determined by measuring the absorbance of each treated sample at 220 nm using UV/VIS spectrophotometer (MRC Spectro V-11D, China) according to (Crumpton *et al.*, 1992). Standards for the determination of actual concentration as well as reagent blank of distilled water were subjected to the same treatment as the samples. A calibration curve of absorbance against phosphate concentration was plotted and used to establish the concentrations of total nitrogen in water samples (APHA, 2005).

3.4.3 Phytoplankton analyses

3.4.3.1 Phytoplankton composition

Determination of phytoplankton species composition was carried out under an inverted light microscope (Nikon Eclipse Ti2) for identification. The preserved phytoplankton sample was mixed gently by inverting the sample bottle for 45 seconds. Five milliliters

was then measured using a measuring cylinder and loaded to the sedimentation chamber. These was then mounted on the microscope and scanned through at 100× magnification using parallel strips of 10 mm per strip. The observed cells were identified with the aid of identification keys by Green (1984); Tomas (1997); Desai *et al*. (2001); Verlecar and Desai (2004) and John *et al*. (2011).

3.4.3.2 Phytoplankton density and abundance

Quantitative determination of cell density was carried out on preserved sampled. A 10 mL well-mixed phytoplankton sample was introduced into an Utermöhl chamber and left standing for 12 hours for complete cell sedimentation to occur before counting. The cells were counted under an inverted microscope at 400 × magnification and the cell numbers per ml computed and recorded (Utermöhl, 1958; Lund *et al*., 1958; Parson *et al*., 1984).

3.5 Phytoplankton culture experiments

3.5.1 Phytoplankton uni-algal establishment

To determine the phytoplankton species with the ability to survive under culture conditions, fresh phytoplankton samples were cultured directly in sterile 250 mL conical flasks. For each of the collected samples, 40 mL was introduced into four conical flasks with the Modified Bourelly growth media. The conical flasks were maintained under 14:10 hour light: dark photoperiod at room temperature for one week. During this period, the culture flasks were agitated every morning and afternoon to ensure that the cells do not settle at the bottom of the flask. After one week, a sterile glass capillary (sterilized by plugging its tip with cotton wool and autoclaving at 126°C for 15 minutes) was fitted with soft silicon rubber tubing. This was used to isolate single cells of the dominant surviving species. Gentle suction was applied to pick single cells via the glass capillary. The picked cells were transferred through drops of sterile bold basal medium arranged in a series on a glass slide to clean the cells a method known as wash transference technique (Andersen, 2005). The procedure was repeated several times so as to free the cells from contamination. The isolated species were allowed to grow in modified Bourrelly medium in vial culture vessels in the laboratory under 14:10 hour light: dark photoperiod.

3.5.2 Identification of fast growing phytoplankton species

After a period of two weeks, eleven pure isolates consisting of *Closteriopsis acicularis, Chlorella saccharophilla, Chlorella vulgaris, Cosmarium contractum, Nannochloropsis* sp*, Scenedesmus ellipticus, Coenococcus polycoccus, Actinastrum hantzschii, Navicula capitata, Diatoma tenuis and Scenedesmus communis* that survived under the laboratory conditions were innoculated into sterile 250 mL conical flasks with Modified Bourrelly medium. They were maintained in the laboratory controlled environment for three weeks, under 14:10 hour (light: dark) photoperiod at room temperature. The culture flasks were agitated twice a day in the morning and in the evening to maintain the cells in suspension. Phytoplankton biomass was determined as the actual dry weight of cells in 200 mL of the culture sample on a weekly basis. The dry weight of filter paper was determined and then 200 mL of culture sample filtered through the filter paper to trap the cells. The filter paper with micro-algae was dried to a constant weight and the dry weight recorded. Phytoplankton growth rate was determined as the biomass accumulated over the growth period. In addition, the cultured isolates, direct cell count was performed also to determine the growth rate. Phytoplankton cells were counted using an inverted microscope at 400X magnification (Utermöhl, 1958; Lund *et al*., 1958; Parson *et al*., 1984). The above process was repeated every week during the growth period. An amount of 10 mL cultured cells was introduced into an Utermöhl chamber and left standing for 12 hours for complete cell sedimentation. Cell counting was done under the inverted microscope and cells numbers recorded per mL of culture sample.

3.5.3 Mass growth of phytoplankton in different media types

From the eleven unialgal isolates, six isolates (*Chlorella saccharophila, Chlorella vulgaris, Scenedesmus ellipticus, Closteriopis acicularis, Cosmarium contractum and Nannochloropsis* sp.) with the highest biomass accumulation in the laboratory were subjected to media A- comprising of micronutrients media only (Table 3.2), B- consisting of a concentration of 1.09 mg L^{-1} ortho-phosphate obtained by dissolving 5 g of the NPK (23-23-23) fertilizer in one litre of distilled water, C- consisting of a concentration of 1.09 mg L^{-1} ortho-phosphate obtained by dissolving 5 g of the NPK (23-23-23) fertilizer in one litre of distilled water with addition of 0.5 mL of micronutrients, D- consisting of a concentration of 4.8 mg L^{-1} obtained by dissolving 25 g of NPK 1:2:1(40) fertilizer in one litre of distilled water and additional micro nutrients and E- having an ortho-phospate

concentration of 2.4 mg L^{-1} ortho-phosphate obtained through dissoving 25 g of NPK 2:1:1(40) in one litre of distilled water and additional micro nutients. An amount of 0.5 mL of micronutrient solution was applied to each media.

Table 3.2: Concentration of micronutrients for the culture of fresh water algae

Nutrient	Conc. in mg L^{-1}.
Iron	0.654
Zinc	0.005
Manganese	0.05
Molybdenum	0.003
Cobalt	0.003
Copper	0.003

(Source: Andersen *et al.*, 2005a)

The six identified fast growing phytoplankton species (*Chlorella saccharophila, Chlorella vulgaris, Scenedesmus ellipticus, Closteriopis acicularis, Cosmarium contractum and Nannochloropsis* sp.) common in both Lake Naivasha and EUC reservoirs were innoculated into the five media types in three replicates and cultured under the same light regime for seven weeks. On weekly basis phytoplankton biomass was determined as the actual dry weight of cells in 200 mL of the culture sample. The dry weight of filter paper was determined and then 200 mL of water sample filtered through it filter paper. The wet weight of the cells was determined after which the filter paper with micro-algae was dried to a constant weight and the dry weight recorded. Cell maturity was reached during the sixth week when the cell numbers and biomass declined. At this point the cells were harvested. The growth rate for each species was calculated from the dry cell weight as an average of the biomass divided by the number of days the biomass was established.

3.5.4 Phytoplankton harvesting

After six weeks of culture, the phytoplankton cells were harvested for oil extraction. Laboratory cultures were harvested via flocculation method. Ten grams of aluminum sulphate (alum) were added to 10 L of culture solution and stirred to dissolve the flocculants and allowed to stand for 30 minutes then decanted. The decanted cells were spread on aluminum foil and air dried for 48 hours (Eldridge *et al.*, 2012).

3.6 Oil extraction from phytoplankton

Oil was extracted from the harvested biomass of each phytoplankton species by solvent extraction method. The dried algal cells were weighed using a weighing balance and soaked in hexane for 24 hours in a ratio of 1:2 w/v, then filtered. The filtrate was introduced into a vacuum rotary evaporator at 40 °C to allow for solvent recovery, leaving the oil in the rotary bottle. The oil was measured in measuring cylinder and stored in a refrigerator in vial bottles.

3.7 Data analysis

Data on phytoplankton species composition was grouped based on the presence or absence of species. For the present phytoplankton species, cells were enumerated to determine the abundance of each individual species. Shannon Wiener diversity index, dominance and evenness were calculated using PAST software (version 3) (Hammer *et al.*, 2001). For each of the measured physico-chemical parameters, the recorded data was arranged per site and the mean values during each sampling period computed. Similarly, means and standard deviations of the experimental data on the growth rates of different phytoplankton species in the different growth media were computed. Significant differences on phytoplankton growth rates in different media concentrations, as well as significant differences in physico-chemical properties and species diversity indices in the different study sites and significant differences in oil production potential of the different species were determined using one way ANOVA test using Statistical Analysis System software (SAS) version 12.1. Highly significant differences in the tests were determined using Tukey's Honest Significance Difference (HSD) at 5% probability level to separate the means. The oil yield differences of Lake Naivasha and Embu University College reservoirs isolates was determined using a two sample t test.

CHAPTER FOUR: RESULTS

4.1 Temporal variation in physico-chemical characteristics

4.1.1 Electrical conductivity

Mean conductivity in Lake Naivasha was 265 µS ± 0.07 cm^{-1} with the lowest and highest values of 202 and 289 µS cm^{-1} recorded in April 2015 and February 2015 respectively (Fig. 4.1A). At Embu University College reservoirs, conductivity ranged between 118 and 172 µS cm^{-1}. The conductivity values in Dams 2, 3 and 5 were 120.5 to 133 µS cm^{-1}, 127.5 to 152.5 and 162 to 175 µS cm^{-1} respectively. Low conductivity values were recorded in April 2015 while the highest values were recorded in February 2015. A one way ANOVA test revealed temporal variations in mean conductivity values of Lake Naivasha and EUC dams 2, 3 and 5 was significant (p<0.05, df=44). Post Hoc test using Tukey's mean separation method revealed that the conductivity value of Lake Naivasha was significantly higher than those of the EUC reservoirs, while Dam 5 at EUC also had a significantly higher mean conductivity than Dams 2 and 3 (Table 4.1). In general, high conductivity values were observed towards the end of dry season in February 2015, during the period characterized by low river inflow and no surface runoff. Low values were recorded at the end of the wet season in April 2015.

4.1.2 Secchi depth

Mean Secchi depth in Lake Naivasha was 58.9 ± 0.03 cm with the highest and lowest mean values of 62.0 and 57.3 cm recorded in February and April 2015 respectively (Fig. 4.1B). At Embu University College reservoirs, mean monthly Secchi depth ranges in Dams 2, 3 and 5 were 28.5 to 36 cm, 27.8 to 36.1 and 30.0 to 35.3 respectively. The high Secchi depth values were recorded in February 2015 while the low values were recorded in April 2015. A one way ANOVA test revealed that the variations in Secchi depth of Lake Naivasha and EUC dams 2, 3 and 5 was significant (p<0.05, df=44). Post Hoc test using Tukey's mean separation method revealed that values for Lake Naivasha was significantly higher than those of EUC reservoirs while Dam 5 also had a significantly higher value than Dams 2 and 3 (Table 4.1). High Secchi depth values were observed towards the end of dry season in February 2015 during the period characterized by low river inflow and no surface runoff. Low values were recorded at the end of the wet season in April 2015.

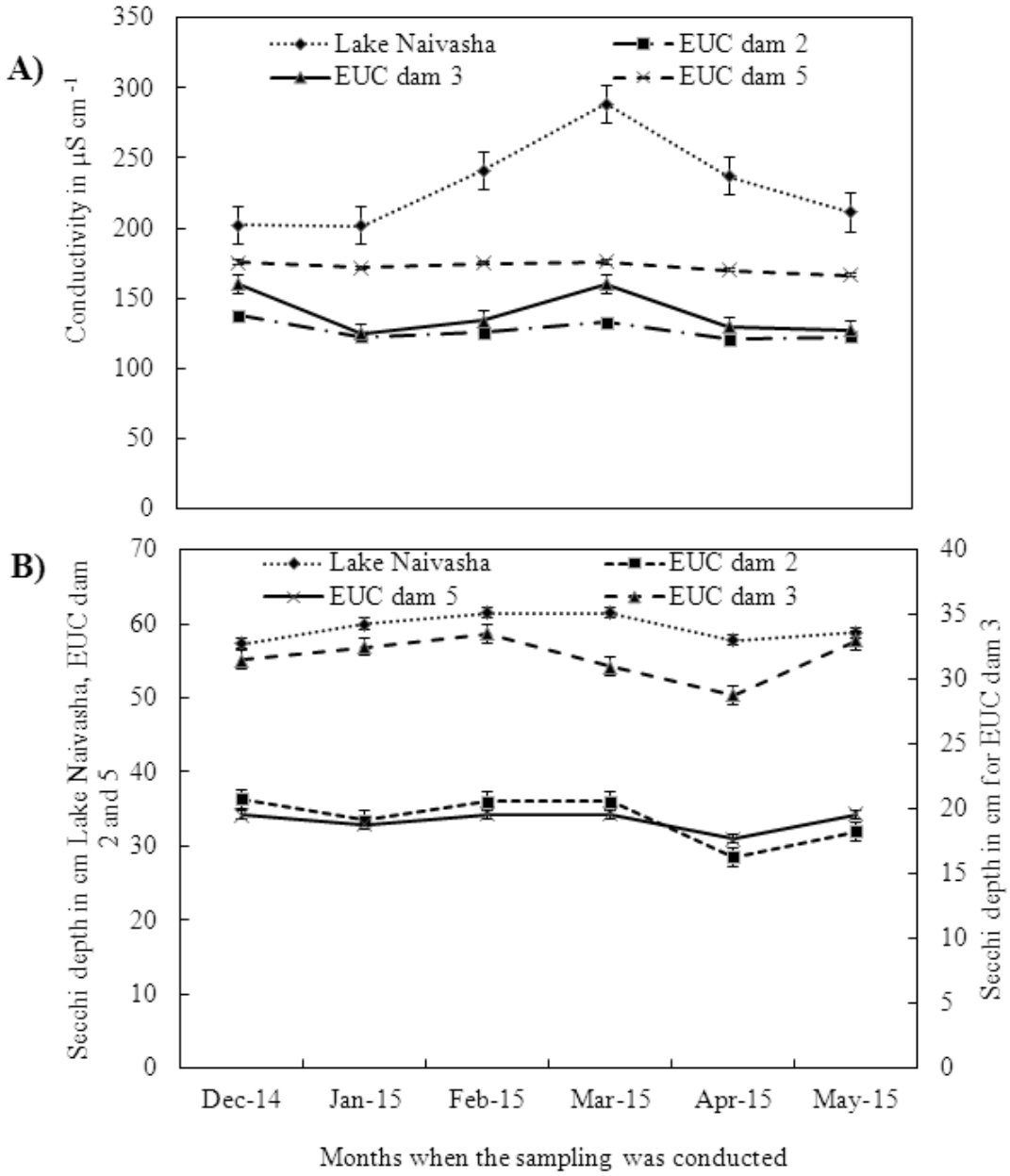

Fig. 4.1: Temporal changes in conductivity and Secchi depth measurements for Lake Naivasha and EUC Dams 2, 3 and 5 from December 2014 to May 2015.
(A) Mean conductivity values (B) Mean Secchi depth with the primary vertical axis representing the Secchi depth for Lake Naivasha and EUC dams 2 and 5 while the secondary vertical axis plots the Secchi depth for EUC dam 3. The error bars show the standard error of the mean.

4.1.3 Total alkalinity

Generally total alkalinity (TA) values registered a progressive increase at all sampling stations over the period from December 2014 to March 2015, with a decline registered at the onset of rains in April 2015. Total alkalinity in Lake Naivasha was highest (73.5 mg L^{-1} of $CaCO_3$) in March, 2015 and lowest (65.8 mg L^{-1} of $CaCO_3$) in May, 2015. In Embu University College reservoirs, Dam 5 recorded the highest value (63.5 mg L^{-1} of $CaCO_3$) in March 2015 and lowest value 60.7 mg L^{-1} of $CaCO_3$ in April, 2015. The highest TA value at Dam 2 (60.2 mg L^{-1} of $CaCO_3$) was recorded in February 2015 while the lowest value of 55.3 mg L^{-1} $CaCO_3$ was recorded in May 2015. In Dam 3, only a small change in the alkalinity values were recorded with the highest and lowest values of 60.4 mg L^{-1} $CaCO_3$ and 59.2 mg L^{-1} of $CaCO_3$ being recorded in December, 2014 and May, 2015 (Fig. 4.2A). A comparison of the mean TA values of the four sampling sites (Lake Naivasha and EUC dams 2, 3 and 5) using a one way ANOVA test revealed a significant difference in mean TA values ($p<0.05$, df=44). Post Hoc test using Tukey's mean separation method revealed that the monthly mean TA value of Lake Naivasha was significantly higher than that of the EUC reservoirs (Table 4.1).

4.1.4 pH values

In Lake Naivasha, pH values ranged from 7.7 recorded in March and May 2015 to 8.6 recorded in April, with a median value in Lake Naivasha of 8.2. At the Embu University College reservoirs, a median pH value of 7.3 was recorded. In Dam 2, pH values ranged from 6.5 recorded in January and February 2015 to 8.5 recorded in December 2014. In Dam 3, the highest pH value of 8.6 was recorded in April 2015 while the lowest value of 7.4 was recorded in March 2015. In Dam 5 the highest and lowest value pH values of 7.9 and 8.1 were recorded in February and April of 2015 respectively. Temporal changes in pH were not different (Fig. 4.2B).

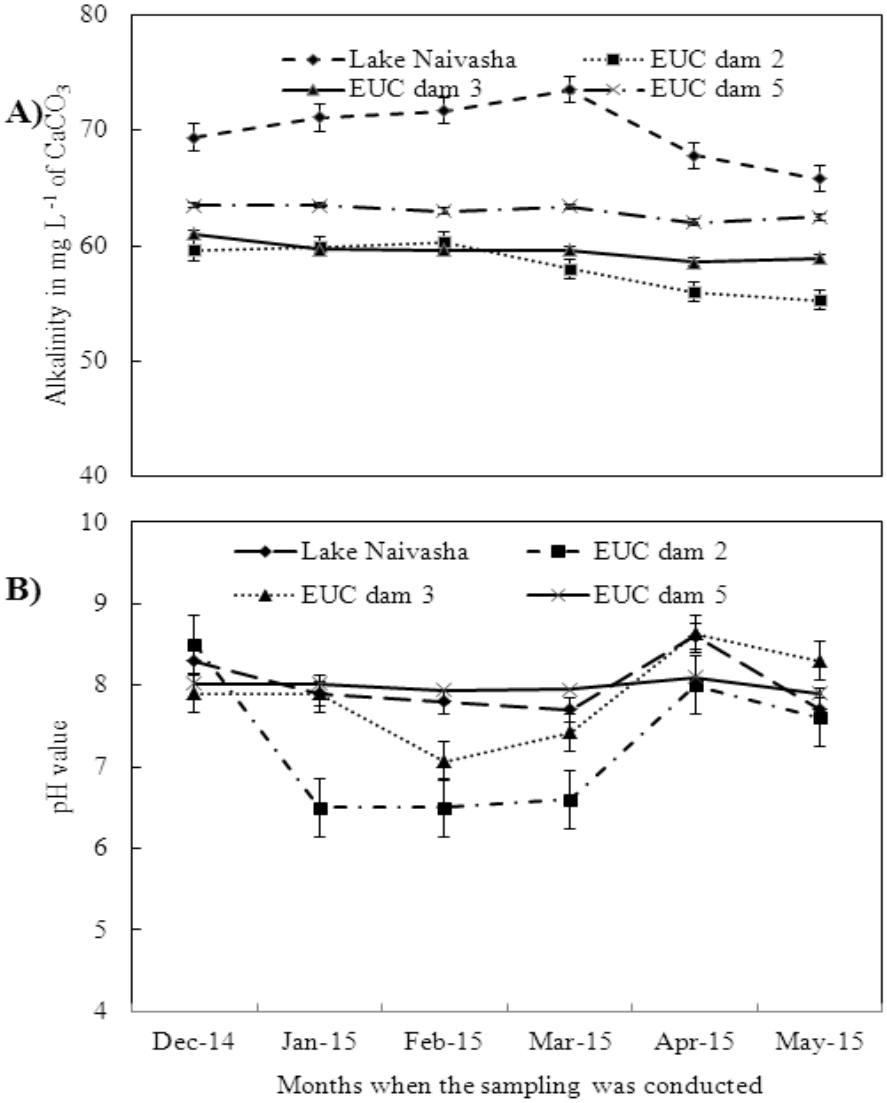

Fig. 1.2: Alkalinity and pH for Lake Naivasha and EUC Dams 2, 3 and 5 from December 2014 to May 2015.
(A) Mean monthly Alkalinity (B) Median pH value. The error bars show the standard error of the means.

4.1.5 Total phosphorus

Total phosphorus (TP) concentration ranged from 0.069 to 0.093 mg L^{-1} in Lake Naivasha. The highest concentration of 0.093 mg L^{-1} was recorded in December 2014 and April 2015 while the lowest concentration of 0.069 mg L^{-1} was recorded in February 2015. In the Embu University College reservoirs, the concentration of total phosphorus ranged between 0.044 and 0.073 mg L^{-1}. In Dam 2, TP values ranged from 0.056 mg L^{-1} recorded in April 2015 to 0.044 mg L^{-1} recorded in February 2015. In Dam 3, the highest TP value of 0.06 mg L^{-1} was recorded in April 2015 while the lowest value of 0.042 mg L^{-1} was recorded in

February 2015. A similar trend was observed in Dam 5 with the highest value of 0.073 mg L^{-1} being recorded in April 2015 and the lowest value of 0.061 mg L^{-1} being recorded in February and March 2015 (Fig. 4.3A). A one way ANOVA test revealed that the temporal variations TP values of the four sampling sites (Lake Naivasha and EUC dams 2, 3 and 5) was significant (p >0.05, df=44). Post Hoc test using Tukey's mean separation method revealed that the concentration of TP in Lake Naivasha was significantly higher than that of the EUC reservoirs (Table 4.1).

4.1.5 Total nitrogen

Total nitrogen (TN) concentration ranged between 0.18 and 0.271 mg L^{-1} in Lake Naivasha. The highest value of 0.271 was recorded in April 2015 and lowest value of 0.18 in February 2015. At the Embu University College reservoirs, the concentration ranged from 0.091 to 0.097 mg L^{-1}. Dam 2 recorded a high value of 0.096 mg L^{-1} in April 2015 and low value of 0.092 mg L^{-1} in February and March 2015. In Dam 3, the highest value of 0.95 mg L^{-1} was recorded in April 2015 while the lowest value of 0.092 mg L^{-1} recorded in February 2015. Similarly, Dam 5 recorded a high value in April (0.097 mg L^{-1}) and lowest value of 0.091 mg L^{-1} in March 2015. A comparison of the mean total nitrogen concentration values of the four sampling sites (Lake Naivasha and EUC dams 2, 3 and 5) using a one way ANOVA test revealed a significant difference in total nitrogen concentration values (p<0.05, df=44). A Post Hoc using Tukey test revealed that total nitrogen value of Lake Naivasha was significantly higher than that of the EUC reservoirs (Table 4.1).

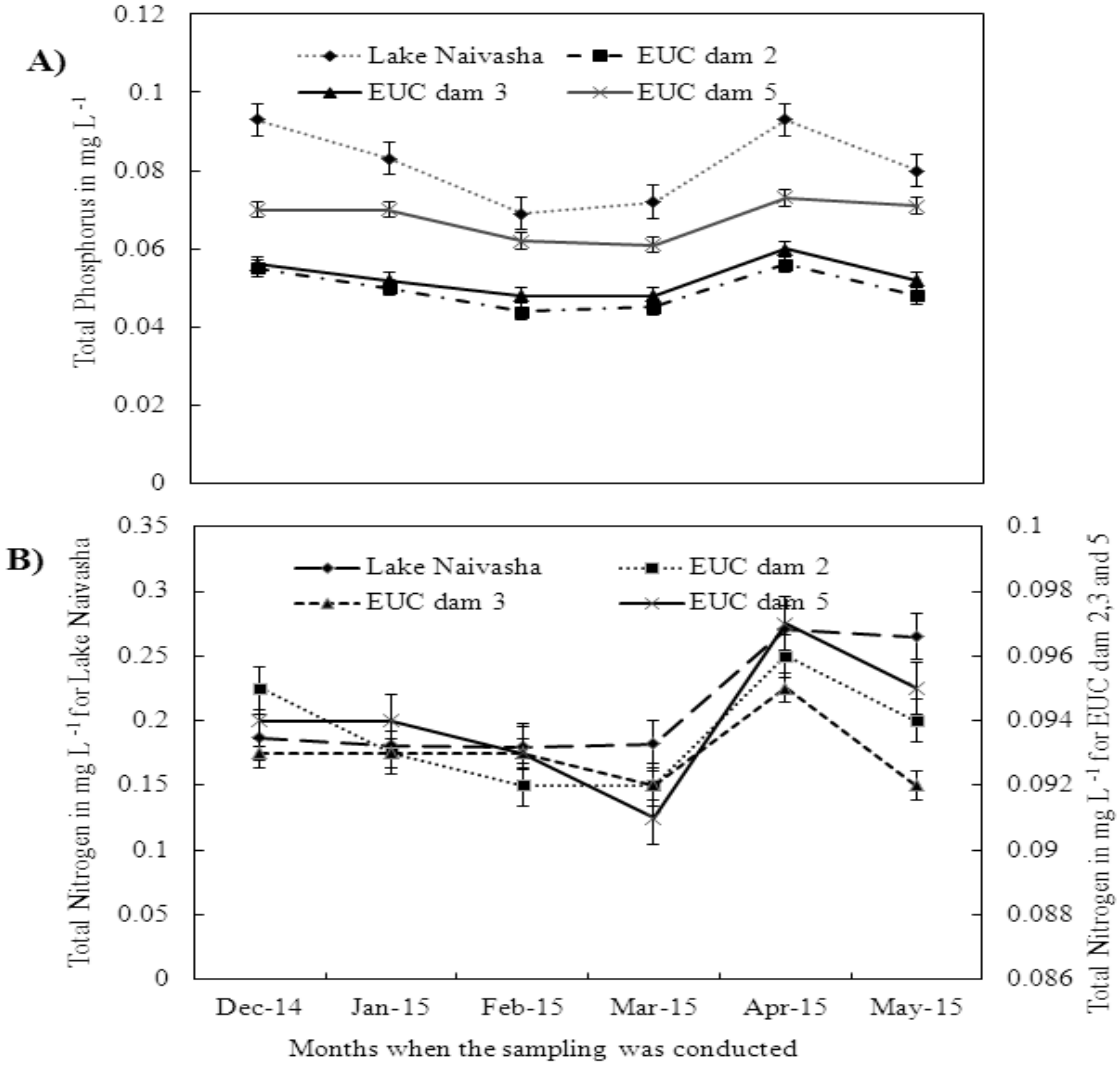

Fig. 4.3: Total phosphorus and total nitrogen for Lake Naivasha and EUC Dams 2, 3 and 5 over the period December 2014 to May 2015.

(A)Mean total phosphorus. The primary vertical axis shows the seasonal changes in TP (B) Mean total nitrogen. The primary vertical axis shows the seasonal changes in TN for Lake Naivasha while the secondary vertical axis shows the seasonal changes in EUC dams 2, 3 and 5. Error bars show the standard error

Table 4.1: Mean levels of the physico-chemical properties and diversity indices of Lake Naivasha and EUC reservoirs.

Values (Means ±SE) followed by dissimilar letters along the rows are significantly different at $p < 0.05$.

Parameter	EUC Dam 2	EUC Dam 3	EUC Dam 5	Lake Naivasha	*p* value
Conductivity	127 ± 6.8c	139.1 ±16.1c	172.4 ±3.9b	238.7 ± 3.1a	<0.0001
Secchi depth	33.9 ± 3.2b	31.7 ± 1.8b	33.4 ± 0.9b	58.9 ± 2.1a	<0.0001
Total phosphorus	0.0185 ±0.03b	0.019 ±0.02b	0.0193 ±0.03b	0.153 ±0.06a	<0.0001
Total nitrogen	0.092 ±0.03b	0.1 ±0.01b	0.095 ±0.03b	0.26 ±0.1a	<0.0001
Total alkalinity	55.17 ±2.05b	53.7 ±1.02b	57.9 ±0.6b	69.5 ±02.7a	<0.0001
Shannon Wiener Index	1.44 ±0.08a	1.48 ±0.09a	1.5 ±0.09a	1.44 ±0.1a	<0.158

4.2 Seasonal changes in phytoplankton species composition

4.2.1 Lake Naivasha

The phytoplankton composition of Lake Naivasha over the period between December 2014 and May 2015 consisted of a total of 126 species identified fully to species level and 8 identified to genus level. They belonged to 7 phyla (Chlorophyta, Cyanophyta, Bacillariophyta, Xanthophyta, Euglenophyta, Phyrrophyta and Chrysophyta) (Table 4.2). The highest number of phytoplankton species was recorded in February 2015 when a total of 89 species were recorded. The number of species declined to 63 taxa in April 2015 after which it rose again to 78 species in May 2015.

Among the 7 phyla recorded, the Chlorophyta registered the highest number of species throughout the sampling period. However, in April 2015, Cyanophyta recorded its highest number of species as compared to other months. Bacillariophyta had the highest number of species in May 2015 as compared to the recorded species in the other months of study (Table 4.2). In general, chlorophyta had the highest number of species in the dry months of January, February and March 2015 while the cyanobacteria had their highest number of species in the rainy months of December 2014 and April 2015. At the later stage of the rainy season during the months of May 2015, most species belonged to the Bacillariophyta group. Among, the Cyanophyta the most common species, which were present throughout the study period was *Microcystis aeruginosa* while *Scenedemsus* sp. and *Chlorella* sp. were the most common members of the Chlorophyta. Species of the Chrysophyta were recorded only in December 2014 at Elsamere site.

Table 4.2: Temporal changes in the phytoplankton composition of Lake Naivasha over the period December 2014 to May 2015.
(+ denotes presence while - is absence)

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Cyanophyta						
Anabaena circinalis	+	+	+	-	+	-
Anabaena sphaerica	+	+	-	-	+	-
Anabaena spiroides	+	-	+	-	+	+
Anabaena spiroides	+	-	+	-	+	+
Anabaenopsis elenkinii	+	+	-	-	+	-
Aphanothece clathrata	+	-	+	-	-	-
Aphanothece ellipsoidea	+	+	+	-	+	-
Aphanothece nidulans	-	-	-	-	+	-

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Chlorogloea sp.	-	+	+	+	+	+
Chroococcus cronbergae	-	-	-	-	+	+
Chroococcus dispersus	+	+	+	+	-	+
Chroococcus limneticus	+	-	-	-	+	+
Chroococcus miutus	+	-	+	-	+	-
Chroococcus turgidus	+	-	-	-	-	+
Homoethrix varians	-	-	-	+	+	+
Lyngbya epiphytica	-	-	+	+	-	-
Merismopedia sp.	+	+	+	-	-	+
Microcystis aeruginosa	+	+	+	+	+	+
Microcystis biformis	+	-	+	+	+	+
Microcystis flos-aquae	+	+	+	-	+	+
Nostoc coeruleum	-	-	+	+	+	-
Nostoc parmelioides	-	-	-	+	+	-
Oscillatoria jenneri	-	-	+	+	+	-
Oscillatoria redekei	-	+	-	+	+	-
Spirulina sp.	-	-	-	+	-	+
Synechococcuss sp.	+	+	-	+	+	-
Total	16	11	15	12	20	13
Chlorophyta						
Actinastrum hantzschii	+	+	+	+	+	+
Ankistrodesmus falcatus	+	+	+	+	+	+
Ankistrodesmus fusiformis	-	+	+	+	+	+
Ankrya ancora	+	+	+	+	-	-
Ankrya judayi	+	+	+	+	-	-
Chlamydomonas debaryana	+	-	-	-	+	-
Chlorella minutissima	+	+	+	+	+	+
Chlorella saccharophilla	-	-	+	+	+	+
Chlorella vulgaris	+	+	+	+	+	+
Closteriopsis acicularis	+	+	+	+	+	+
Closterium acerosum	+	-	+	-	+	-
Closterium setacum	+	-	+	-	+	-
Coelastrum sp.	-	-	+	-	+	+
Coenococcus polycoccus	-	+	+	+	+	+
Cosmarium contractum	+	+	+	+	+	+
Crucigenia sp.	-	-	-	-	-	+
Franceia amphitrichia	-	-	+	+	-	+
Kirchneriella aperta	-	+	+	+	-	-
Kirchneriella contorta	-	-	+	-	-	-
Kirchneriella irregularis	-	+	+	+	-	-
Lagerheimia subsalsa	+	-	+	+	+	-
Monoraphidium contortum	+	+	+	+	-	+
Monoraphidium griffithii	+	+	+	-	-	+
Oocystis marsonii	+	-	+	-	-	-
Pediastrum duplex	+	+	+	+	-	-

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Pediastrum boryanum	-	-	+	-	-	-
Pediastrum simplex	-	+	+	+	-	-
Scenedesmus acuminatus	+	+	+	+	+	+
Scenedesmus bernadii	-	+	+	+	+	+
Scenedesmus bicaudatus	+	+	+	+	+	+
Scenedesmus communis	+	+	+	+	+	+
Scenedesmus dimorphus	-	-	+	-	+	-
Scenedesmus ellipticus	+	+	+	+	+	+
Scenedesmus falcatus	+	+	+	+	-	+
Selenastrum bibraianum	-	-	+	-	-	+
Selenastrum capricornatum	-	+	+	-	-	+
Selenastrum gracile	-	-	+	-	-	-
Staurastrum lunatum	-	+	+	-	+	-
Staurastrum selbaldi	-	+	+	-	+	-
Staurodesmus sp.	-	+	+	-	+	+
Tetraedron caudatum	-	+	+	-	-	+
Tetraedron minimum	-	+	+	+	-	-
Total	21	28	40	25	23	24
Xanthophyta						
Botrydiopsis arrhizal	+	+	+	+	+	-
Goniochloris fallax	+	+	+	+	+	-
Goniochloris mutica	+	+	+	+	+	-
Pseudostaurastrum haustatum	+	+	+	+	-	-
Pseudostaurastrum lobulatum	+	+	+	-	-	+
Tetraedriella sp.	-	+	+	-	+	+
Tetraplektron torsum	+	+	+	-	+	+
Total	6	7	7	4	5	3
Bacillariophyta						
Achnanthes brevipes	+	+	+	+	+	+
Achnanthes exigua	+	+	+	+	-	+
Achnanthes inflate	+	+	+	+	+	+
Achnanthes minutissima	+	+	+	+	+	+
Aulacoseira granulata	+	+	+	+	+	+
Aulacoseira italica	+	+	+	+	+	+
Cocconeis placentula	+	-	+	-	-	+
Cyclotella meneghiniana	+	-	+	-	-	+
Cyclotella stelligera	+	-	+	-	-	+
Cymbella lacustris	+	-	+	-	-	+
Denticula pelagica	+	-	-	-	-	-
Denticula thermalis	-	-	+	-	-	+
Diatoma tenuis	+	+	+	+	+	+
Fragilaria capucina	-	-	+	-	-	+
Melosira ambigua	+	+	+	+	+	+
Navicula capitate	+	+	+	+	-	+
Navicula fonticola	-	+	+	+	-	+

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Navicula lanceolata	-	+	+	+	-	+
Navicula protractoides	-	+	+	+	-	+
Navicula pygmae	-	+	+	+	-	+
Navicula radiosa trivallis	-	+	+	+	-	+
Nitzschia sp.	+	+	+	+	-	+
Pinnularia sp.	-	-	-	-	+	+
Stephanodiscus neostraea	+	-	-	-	-	+
Surirella angusta	-	-	+	+	+	+
Surirella linearis	-	+	+	+	+	+
Synedra acus	-	+	+	+	-	+
Synedra capitate	+	+	+	+	+	+
Synedra pulchella	-	+	+	+	-	+
Synedra ulna	-	+	+	+	-	+
Total	17	20	27	21	11	29
Euglenophyta						
Euglena oxyuris	+	-	-	-	-	+
Euglena polymorpha	-	-	-	-	-	+
Euglena texta	-	-	-	-	-	-
Euglena variabilis	-	-	-	-	-	+
Euglena virdis	+	-	-	-	-	+
Phacus acuminatus	+	-	-	-	-	-
Phacus meson	-	-	-	-	-	-
Phacus tortus	-	-	-	-	-	-
Scystomonas gibberosa	-	-	-	-	-	+
Scystomonas pusillus	-	-	-	-	-	+
Scystomonas quadrangularis	-	-	-	-	-	+
Strombomonas acuminata	-	-	-	-	-	+
Trachelomonas cylindrica	+	-	-	-	-	-
Trachelomonas globularis	+	-	-	-	-	-
Trachelomonas granulata	+	-	-	-	-	-
Trachelomonas hispida	+	-	-	-	-	-
Total	7	0	0	0	0	8
Pyrrophyta						
Ceratium cornutum	+	+	-	-	+	+
Ceratium hirudinella	+	+	-	-	+	+
Chroomonas sp.	+	-	-	-	-	-
Gymnodinium chiastosporum	-	-	-	+	-	+
Gymnodinium inversum	-	-	-	+	-	+
Peridiniopsis borgei	+	-	-	+	-	+
Peridiniopsis cunningtonnis	+	-	-	+	+	+
Peridinium volzii	+	-	-	+	+	+
Tetragodinium verrucatum	+	-	-	-	-	-
Total	7	2	0	5	4	7
Chrysophyta						
Chromulina pyriformis	+	-	-	-	-	-

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Chrysococcus rufescens	+	-	-	-	-	-
Chrysomonas ellipsoidea	+	-	-	-	-	-
Chrysospora fenestrata	+	-	-	-	-	-
Epicystis sp.	+	-	-	-	-	-
Mallomonas leboimei	+	-	-	-	-	-
Ochromonas viridis	+	-	-	-	-	-
Total	7	0	0	0	0	0

4.2.2 EUC reservoirs

The phytoplankton community in Embu University College reservoirs over the period December 2014 to May 2015 comprised a total of 115 species identified up to the species level and 7 species identified to genus level. They belonged to 6 phyla (Chlorophyta, Cyanophyta, Bacillariophyta, Xanthophyta, Euglenophyta and Pyrrophyta). Each reservoir recorded different numbers of phytoplankton species in different months of the study. In Dam 2, the Pyrrophyta was absent throughout the study. Arranged in order of abundance from the most abundant to the least abundant, the following order was recorded; Chlorophyta (most abundant), Cyanophyta, Bacillariophyta, Euglenophyta and Xanthophyta (least abundant). The highest number of phytoplankton taxa was recorded in February 2015 with a total of 68 species. The number declined to 33 species in April 2015 after which it rose to 63 in May 2015 (Table 4.3). Chlorophyta registered the highest number of species in February 2015, with Cyanophyta registering its highest number of species in April 2015 as compared to the other months of the study. Similarly, Bacillariophyta registered its highest species numbers in May 2015 as compared to the other months during the study, while Euglenophyta had their highest number of species in December 2014 and absent in February and March 2015. Species of Xanthophyta were present in December 2014, March and April 2015 although absent in January, February and May 2015.

Table 4.3: Temporal changes in the phytoplankton composition of the Embu University College Dam 2 over the period December 2014 to May 2015.
(+ denotes presence while - is absence)

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Cyanophyta						
Anabaena circinalis	+	-	-	-	+	+
Anabaena elliptica	+	-	-	-	+	+
Anabaena sphaerica	+	+	-	-	+	+

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Anabaena spiroides	+	+	-	-	+	+
Anabaenopsis elenkinii	+	+	-	-	+	-
Aphanocapsa elachista	+	-	-	-	+	-
Aphanothece clathrata	+	+	-	-	+	-
Aphanothece ellipsoidea	+	+	-	-	+	-
Aphanothece nidulans	+	+	-	-	+	-
Chlorogloea sp.	-	+	+	+	+	+
Chroococcus dispersus	-	+	+	+	+	+
Chroococcus limneticus	+	+	-	-	+	+
Chroococcus turgidus	+	+	+	+	+	+
Homoethrix varians	-	+	+	-	+	+
Lyngbya epiphytica	+	+	-	-	-	-
Microcystis aeruginosa	+	+	+	+	+	+
Microcystis flos-aquae	+	+	-	-	+	+
Nostoc coeruleum	-	-	+	-	+	+
Oscillatoria redekei	-	-	+	+	+	+
	14	14	7	5	18	13
Chlorophyta						
Actinastrum hantzschii	+	+	+	+	+	+
Ankistrodesmus fusiformis	+	+	-	-	-	+
Ankrya ancora	+	+	+	-	+	+
Chlorella minutisssima	-	+	+	+	+	+
Chlorella saccharophilla	+	+	-	-	+	+
Chlorella vulgaris	+	+	+	+	+	+
Chlorococcum minutum	-	-	-	-	-	+
Closteriopsis acicularis	+	+	+	+	+	+
Coelastrum sp.	-	+	+	+	-	+
Coenococcus polycoccus	-	+	+	+	-	+
Cosmarium ccontractum	+	+	+	+	+	+
Crucigenia tetrapedia	-	+	+	+	-	+
Franceia amphitricha	+	+	+	-	+	+
Kirchneriella aperta	-	+	+	-	-	+
Kirchneriella contorta	-	+	+	-	-	+
Kirchneriella irregularis	-	+	+	-	-	+
Lagerhemia ciliata	+	-	+	-	+	+
Lagerhemimia subsalsa	+	-	+	-	+	+
Monoraphidium arcuatum	+	+	+	-	-	-
Monoraphidium contortum	+	+	+	+	+	+
Monoraphidium griffithii	+	+	+	-	+	+
Pediastrum duplex	-	+	+	-	-	-
Pediastrum simplex	+	+	+	-	-	-
Scenedesmus acuminatus	+	+	+	+	+	+
Scenedesmus bernadii	+	+	+	+	+	+

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Scenedesmus communis	+	+	+	+	+	+
Scenedesmus dimorphus	+	+	+	-	-	+
Scenedesmus ellipticus	+	+	+	+	+	+
Scenedesmus protuberans	-	+	+	-	-	+
Selenastrum bibraianum	-	+	+	-	-	+
Selenastrum capricornatum	-	+	+	-	-	+
Selenastrum gracile	-	+	+	-	-	+
Staurastrum lunatum	+	+	+	-	+	-
Tetraedron caudatum	-	+	+	+	+	+
Tetraedron minimum	+	+	+	-	+	+
	21	32	32	14	19	31
Bacillariophyta						
Denticula pelagica	-	+	-	-	+	-
Diatoma tenuis	+	+	+	+	+	+
Navicula capitata	+	+	+	+	+	+
Navicula lanceolata	+	+	+	+	+	+
Navicula pygmaea	+	+	-	-	+	+
Nitzschia acicularis	+	+	+	+	+	+
Nitzschia amphibia	+	+	-	-	+	+
Nitzschia fonticola	+	+	+	+	+	+
Nitzschia linearis	+	+	-	+	+	+
Surirella angusta	-	+	+	+	-	+
Surirella linearis	-	+	+	+	-	+
Synedra acus	+	+	+	+	+	+
Synedra capitata	+	+	+	+	+	+
Synedra pulchella	+	-	-	-	-	-
Synedra ulna	+	-	+	+	+	+
	12	13	10	11	12	13
Euglenophyta						
Euglena oxyuris	-	+	-	-	-	+
Euglena polymorpha	-	+	-	-	-	+
Euglena texta	-	+	-	-	-	+
Euglena variabilis	+	+	-	-	+	+
Euglena viridis	+	+	-	-	+	+
Phacus acuminatus	+	-	-	-	+	-
Phacus tortus	+	-	-	-	+	-
Scytomonas pusillus	-	-	-	-	+	+
Trachelomonas cylindrica	+	+	-	-	+	-
Trachelomonas globularis	+	+	-	-	+	-
Trachelomonas granulata	+	+	-	-	+	-
Trachelomonas hispida	+	+	-	-	+	-
	8	9	0	0	9	6

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Xanthophyta						
Botrydiopsis arrhiza	+	-	-	+	-	-
Goniochloris fallax	-	-	-	+	+	-
Goniochloris mutica	+	-	-	+	+	-
Pseudostraurastrum haustatum	-	-	-	-	+	-
Pseudostraurastrum lobulatum	-	-	-	-	+	-
Tetraedriella sp.	+	-	-	-	-	-
	3	0	0	3	4	0

In Dam 3, the highest number of phytoplankton taxa was 62 species recorded in February 2015, which declined in April 2015 to 51 species and then rose again to 61 species in May 2015 (Table 4.4). All the six registered phytoplankton phyla in EUC reservoirs were present in Dam 3. Phylum Chlorophyta was the most abundant in terms of species numbers throughout the sampling period. However, Cyanophyta recorded its highest species numbers in April 2015 with Bacillariophyta recording its high species numbers in May 2015. The chlorophyte dominance in terms of species numbers recorded an increase during the dry season from December 2014 to February 2015. This was later followed by a decline in species numbers in March and April 2015. Xanthophyta species were present in December 2014, March, April and May 2015 but absent in January and February 2015. Pyrrophyta species were present in January, February, March and May 2015 but absent in December 2014 and April 2015.

Table 4.4: Temporal changes in the phytoplankton composition of the Embu University College Dam 3 over the period December 2014 to May 2015.
(+ denotes presence while -is absence)

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Cyanophyta						
Anabaena circinalis	+	-	-	-	+	-
Anabaena sphaerica	+	-	+	-	+	+
Anabaena spiroides	+	-	+	-	+	+
Anabaenopsis elenkinii	+	-	+	-	+	+
Chroococcus dispersus	+	+	+	+	+	-
Chroococcus limneticus	+	-	-	+	+	+
Chroococcus turgidus	+	-	-	+	+	+
Homeothrix varians	+	+	+	-	+	+
Merismopedia sp.	+	-	+	-	+	-
Microcystis aeruginosa	+	+	+	+	+	+
Microcystis flos aquae	-	+	+	+	-	-
Nostoc coeruleum	+	+	+	+	+	-
Nostoc parmeliodes	-	+	+	+	+	+

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Oscillatoria jennerii	+	-	+	-	+	+
Oscillatoria redekei	+	+	+	+	+	+
Synechoccus sp.	-	-	+	-	+	+
	13	7	13	8	15	11
Chlorophyta						
Actinastrum hantzschii	-	+	+	+	-	+
Ankistrodesmus falcatus	-	+	+	+	-	+
Ankistrodesmus fusiformis	+	+	+	+	+	+
Ankrya ancora	+	+	-	-	+	+
Chlorella minutisssima	-	+	+	+	+	-
Chlorella saccharophilla	+	+	+	+	+	+
Chlorella vulgaris	+	+	+	+	+	+
Chlorococcum minutum	+	+	-	+	+	+
Closteriopsis acicularis	+	+	+	+	+	+
Coelastrum microporum	-	+	+	+	-	-
Coelastrum reticulatum	-	+	+	+	-	-
Coenococcus polycoccus	-	+	+	+	-	-
Cosmarium contractum	-	+	+	+	-	-
Crucigenia sp.	-	+	+	+	-	-
Kirchneriella aperta	+	+	+	+	+	+
Kirchneriella contorta	+	-	+	-	+	+
Kirchneriella irregularis	+	+	+	+	+	+
Lagerhemia ciliata	-	-	+	-	-	+
Lagerhemia subsala	-	-	+	-	-	+
Monoraphidium contortum	+	+	+	+	+	+
Monoraphidium griffithii	+	+	+	+	+	+
Oocystis marsonii	-	+	-	-	-	-
Pediastrum duplex	+	+	+	+	+	+
Pediastrum simplex	-	+	+	+	+	+
Pediastrum tetras	+	-	-	-	+	+
Scenedesmus acuminatus	+	+	+	+	+	+
Scenedesmus bernadii	+	+	+	+	+	+
Scenedesmus bicaudatus	+	+	+	+	+	+
Scenedesmus communis	+	+	+	+	+	+
Scenedesmus dimorphus	+	+	+	+	+	+
Scenedesmus ellipticus	+	+	+	+	+	+
Selenastrum gracile	+	+	+	+	+	+
	20	28	28	26	22	25
Bacillariophyta						
Aulacoseira granulata	+	-	+	-	+	+
Aulacoseira italica	+	-	+	-	+	+
Cyclotella meneghiniana	+	-	-	-	+	+
Cyclotella stelligera	+	-	-	-	+	+
Diatoma tenuis	+	+	+	+	+	+
Navicula capitata	+	+	+	+	+	+

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Navicula lanceolata	+	+	+	+	+	+
Navicula radiosa trivalis	+	-	-	-	+	+
Nitzschia acicularis	+	+	+	+	+	+
Nitzschia amphibia	+	+	+	+	+	+
Nitzschia fonticola	-	+	+	+	-	+
Nitzschia linearis	-	+	+	+	-	+
Surirella angusta	-	+	+	+	-	+
Surirella linearis	-	+	+	+	-	+
Synedra acus	-	+	+	+	-	+
Synedra capitata	+	+	+	+	+	+
	11	11	13	11	11	16
Xanthophyta						
Botrydiopsis arrhiza	-	-	-	+	-	-
Goniochloris fallax	+	-	-	+	+	+
Goniochloris mutica	+	-	-	+	+	+
Pseudogoniochloris sp.	-	-	-	-	+	+
Pseudostaurastrum haustatum	+	-	-	-	-	-
	3	0	0	3	3	3
Euglenophyta						
Euglena oxyuris	-	+	+	-	-	-
Euglena polymorpha	-	-	+	-	-	-
Euglena variabilis	-	+	-	-	-	-
Euglena viridis	+	+	+	-	-	-
Phacus acuminatus	+	+	+	-	-	-
Trachelomonas globularis	+	-	-	-	-	-
Trachelomonas granulata	+	-	-	-	-	-
Trachelomonas hispida	+	-	-	-	-	-
	5	4	4	0	0	0
Phyrrophyta						
Ceratium cornutum	-	-	-	-	-	+
Ceratium hirudinella	-	-	-	-	-	+
Chroomonas minuta	-	+	+	+	-	+
Chroomonas rubra	-	+	+	+	-	+
Peridiniopsis borgei	-	+	+	+	-	+
Peridinium volzii	-	+	+	+	-	+
	0	4	4	4	0	6

In Dam 5, the highest number of phytoplankton species was recorded in February 2015 represented by 72 species, the number declined in April 2015 to 59 species with a subsequent rise to 60 species in May 2015 (Table 4.5). Among the six registered phyla, Chlorophyta was present in highest numbers throughout the sampling period. However, Cyanophyta species were highest in April 2015 as compared to other months.

Bacillariophyta species recorded their high numbers in May 2015. Xanthophyta species were present in January to April 2015 but absent in December 2014 and May 2015. Euglenophyta species were present in December 2014, February and March 2015 although absent in January, April and May 2015. Moreover, Pyrrophyta species were present in months of January, February, March and May 2015 but absent in months of December 2014 and April 2015.

Table 4.5: Temporal changes in the phytoplankton composition of the Embu University College Dam 5 over the period December 2014 to May 2015.
(+ denotes presence while - is absence)

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Cyanophyta						
Anabaena circinalis	+	+	+	+	+	+
Anabaena elliptica	+	+	+	-	+	+
Anabaena sphaerica	+	+	+	+	+	+
Anabaena spiroides	+	+	+	+	+	+
Anabaenopsis elenkinii	+	-	+	-	+	-
Aphanothece ellipsoidea	+	+	+	+	+	+
Aphanothece nidulans	-	-	-	-	+	-
Chlorogloea sp.	+	-	+	-	+	+
Chroococcus dispersus	+	+	-	+	+	+
Chroococcus limneticus	+	+	+	+	+	+
Chroococcus turgidus	+	+	+	+	+	-
Homeothrix varians	+	+	+	-	+	+
Lyngbya epiphytica	+	-	-	-	+	-
Merismopedia sp.	-	+	+	-	+	-
Microcystis aeruginosa	+	+	+	+	+	+
Microcystis flos aquae	+	+	+	-	+	-
Nostoc coeruleum	+	+	+	+	+	+
Oscillatoria jennerii	+	+	+	-	+	+
Oscillatoria redekei	+	+	+	+	+	+
Synechoccus capitatus	+	-	-	-	+	+
	18	15	16	10	20	14
Chlorophyta						
Ankistrodesmus fusiformis	+	+	+	+	+	+
Ankyra ancora	+	+	+	-	+	+
Ankyra judayi	+	-	+	-	+	+
Chlorella saccharophilla	+	+	+	+	+	+
Chlorella vulgaris	+	+	+	+	+	+
Closteriopsis acicularis	+	+	+	+	+	+
Closterium setaceum	-	-	+	+	-	+
Coelastrum microporum	-	-	+	-	-	+
Coelastrum reticulatum	-	-	+	+	-	+
Coenococcus polycoccus	+	-	+	+	+	-

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Cosmarium contractum	+	+	+	+	+	+
Crucigenia sp.	-	-	+	+	-	+
Franceia amphitricha	-	-	+	+	-	+
Kirchneriella aperta	+	+	+	+	+	+
Kirchneriella irregularis	+	+	+	+	+	+
Monoraphidium contortum	+	+	+	+	+	+
Monoraphidium griffithii	+	+	+	+	+	+
Pediastrum duplex	+	+	+	+	+	+
Pediastrum tetras	+	+	+	+	+	+
Scenedesmus acuminatus	+	+	+	+	+	+
Scenedesmus bernadii	+	+	+	+	+	+
Scenedesmus bicaudatus	+	+	+	+	+	+
Scenedesmus communis	+	+	+	+	+	+
Scenedesmus dimorphus	+	+	+	+	+	+
Scenedesmus ellipticus	+	+	+	+	+	+
Selenastrum bibraianum	-	+	+	+	-	+
Selenastrum gracile	-	+	+	+	-	+
	20	20	27	24	20	26
Bacillariophyta						
Achnanthes exigua	+	+	+	-	+	+
Aulacoseira granulata	+	+	+	+	+	+
Aulacoseira italic	+	+	+	+	+	+
Cyclotella stelligera	+	-	+	-	+	-
Cymbella lacustris	+	-	+	-	+	-
Diatoma tenuis	+	+	+	+	+	+
Melosira ambigua	+	-	-	-	+	-
Navicula capitata	+	+	+	+	+	+
Navicula lanceolate	+	+	+	+	+	+
Navicula protractoides	-	-	+	-	-	+
Navicula pygmaea	-	+	-	-	-	+
Navicula radiosa trivalis	+	+	+	+	+	+
Nitzschia acicularis	+	+	+	+	+	+
Nitzschia pusilla	-	+	+	-	-	+
Synedra capitata	+	+	+	+	+	+
Synedra pulchella	+	+	+	-	+	+
Synedra ulna	+	+	+	+	+	+
	14	13	15	9	14	14
Euglenophyta						
Euglena oxyuris	-	-	+	+	-	-
Euglena polymorpha	-	-	+	+	-	-
Euglena variabilis	-	-	+	+	-	-
Euglena viridis	-	-	+	+	-	-
Phacus acuminatus	+	-	+	+	-	-
Trachelomonas hispida	+	-	-	+	-	-
	2	0	5	6	0	0

Species	14-Dec	15-Jan	15-Feb	15-Mar	15-Apr	15-May
Xanthophyta						
Botrydiopsis arrhiza	-	+	+	+	+	-
Goniochloris fallax	-	+	+	+	+	-
Goniochloris mutica	-	+	+	+	+	-
Pseudostraurastrum haustatum	-	+	-	+	+	-
Pseudostraurastrum lobulatum	-	+	+	-	-	-
Tetraplektron torsum	-	-	+	+	+	-
	0	5	5	5	5	0
Pyrrophyta						
Ceratium cornutum	-	-	-	-	-	+
Ceratium hirudinella	-	-	-	-	-	+
Chroomonas minuta	-	+	+	+	-	+
Chroomonas rubra	-	+	+	+	-	+
Peridiniopsis borgei	-	+	+	+	-	+
Peridium volzii	-	+	+	+	-	+
	0	4	4	4	0	6

4.3 Phytoplankton species abundances

During the dry months of January, February and March 2015, Chlorophyta species were more abundant in both Lake Naivasha and Embu University College reservoirs followed by species of Bacillariophyta (Figs 4.4 A and B). In Lake Naivasha, Chlorophyta cell count ranged from 1486 to 2782 cells L^{-1} with *Scenedesmus* being the most abundant genus with cell density of between 369 and 690 cells L^{-1}. Bacillariophyta abundance varied from 665 to 798 cells L^{-1} with dominant genus being *Aulacoseira* varying from 118 to 440 cells L^{-1} (Figs 4.4 A and B).

In Embu University College Dam 2, Chlorophyta density ranged from 1088 to 1527 cells L^{-1} with *Chlorella* being the most abundant genus with a cell density of between 346 to 722 cells L^{-1}. Bacillariophyta cell density ranged from 690 to 743 cells L^{-1} with *Navicula*, the dominant genus, ranging between 78 to117 cells L^{-1} (Fig. 4.4 C and D). In Dam 3, Chlorophyta abundance varied from 1405 to 1839 cells L^{-1} with *Scenedesmus* being the dominant genus with a cell density of between 387 and 528 cells L^{-1}. Bacillariophyta abundance varied from 657 to 693 cells L^{-1} with *Navicula*, the dominant genus, ranging from 73 to 171 cells L^{-1} (Fig. 4.4 E and F). In dam 5, Chlorophyta abundance varied from 1279 to 1527 cells L^{-1} with *Chlorella*, the dominant genus, varying from 204 to 450 cells

L^{-1}. Bacillariophyta abundance varied from 650 to 685 cells L^{-1} with *Aulacoseira* being the dominant genus with a cell density ranging from 97 to 281 cells L^{-1} (Fig. 4.4 G and H). During the wet season months of December 2014 and April 2015, Cyanophyta had the highest cell numbers in both Lake Naivasha and Embu University College reservoirs. Mean phytoplankton cell density in Lake Naivasha and Dams 2, 3 and 5 was 882, 802, 757 and 862 cells L^{-1} respectively. The dominant genus at each site was *Microcystis*.

4.4 Phytoplankton species diversity indices

In general, diversity indices indicated that Lake Naivasha had higher mean evenness in January and March 2015, higher dominance in April 2015 and higher Shannon Wiener diversity index in February 2015. In EUC reservoirs, high mean evenness were in December 2014, high dominance in April 2015 and high Shannon Wiener diversity index in February 2015 (Fig. 4.4; Table 4.6). A one way ANOVA test revealed that the difference in mean Shannon Wiener diversity indices of the four sampling sites (Lake Naivasha and EUC dams 2, 3 and 5) was not significant (p<0.05, df 44) (Table 4.1).

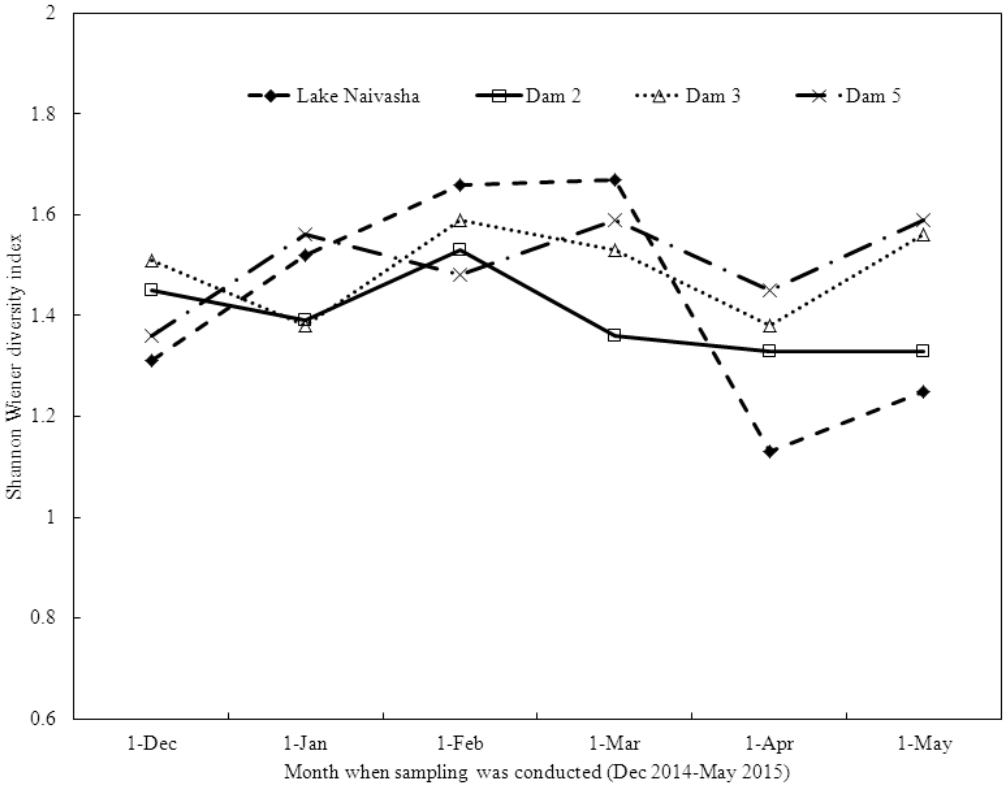

Fig. 4.4: Shannon wiener diversity index in Lake Naivasha and EUC Dams 2, 3 and 5 from December 2014 to May 2015

Table 4.6: Mean diversity indices for Lake Naivasha, EUC dam 2, EUC dam 3 and EUC dam 5 during the period December 2014 to May 2015
(The values are means of two sampling stations for each sampling trip)

Month	Diversity indices	Lake Naivasha	Dam 2	Dam 3	Dam 5
14-Dec	Dominance	0.32	0.27	0.28	0.27
	SW index	1.31	1.45	1.51	1.36
	Evenness	0.74	0.86	0.79	0.87
15-Jan					
	Dominance	0.26	0.29	0.3	0.24
	SW index	1.52	1.39	1.38	1.56
	Evenness	0.76	0.8	0.79	0.79
15-Feb					
	Dominance	0.24	0.25	0.23	0.26
	SW index	1.66	1.53	1.59	1.58
	Evenness	0.71	0.77	0.78	0.81
15-Mar					
	Dominance	0.22	0.3	0.28	0.23
	SW index	1.63	1.36	1.53	1.57
	Evenness	0.76	0.78	0.77	0.82
15-Apr					
	Dominance	0.41	0.32	0.31	0.28
	SW index	1.13	1.33	1.38	1.45
	Evenness	0.62	0.75	0.8	0.85
15-May					
	Dominance	0.35	0.34	0.25	0.23
	SW index	1.25	1.33	1.56	1.56
	Evenness	0.64	0.69	0.78	0.82

4.5 Identification and biomass accumulation of fast growing algal isolates

A total of eleven pure isolates comprising *Closteriopsis acicularis*, *Chlorella saccharophilla*, *Chlorella vulgaris*, *Cosmarium contractum*, *Nannochloropsis* sp, *Scenedesmus ellipticus*, *Coenococcus polycoccus*, *Actinastrum hantzschii*, *Navicula capitate*, *Diatoma tenuis and Scenedesmus communis* survived growth under laboratory conditions. In general, biomass accumulation by each of the eleven unialgal isolates was characterized by a progressive increase for the first 5 weeks of culture with the optimum biomass reached in week five of culture. *Closteriopsis acicularis* recorded the highest biomass in the first five weeks while *Scenedesmus communis* recorded the lowest. In weeks 6 and 7 *Cosmarium contractum* and *Chlorella vulgaris* recorded the least decline in biomass respectively (Table 4.7). A comparison of the mean biomass values of the 11

isolates using a one way ANOVA test revealed significant differences in the mean biomass values of each of the seven weeks (p<0.05, df 110). A Post Hoc test using Turkey's mean separation method revealed that the mean biomass value of *Closteriopsis acicularis* and *Chlorella saccharophila* was significantly higher than that of the other isolates in each week of the seven weeks (Table 4.7). Over the seven week culture period, the phytoplankton species with the highest biomass accumulation were *Closteriopsis acicularis*, *Chlorella saccharophila*, *Chlorella vulgaris*, *Cosmarium contractum*, *Nannochloropsis* and *Scenedesmus ellipticus* (Table 4.7; Plate 4.1).

Table 4.7: Mean biomass in g L^{-1} of 11 phytoplankton species cultured for a seven week period in 500 ml glass conical flasks in Modified Bourelly medium under laboratory conditions

Values (Means ±SE) followed by dissimilar letters in each column are significantly different according to Tukey's Honestly Significant Difference (HSD) at p <0.05.

Species	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Actinastrum hantzschii	0.04±0.001c	0.74±0.3bc	1.51±0.06d	2.51±0.07e	3.13±0.03g	2.8±0.02d	1.8±0.042d
Chlorella saccharophilla	0.06±0.002c	1.23±0.02b	3.77±0.13a	6.04±0.06a	6.43±0.11b	5.51±0.06a	4.13±0.05ab
Chlorella vulgaris	0.07±0.001c	0.87±0.01d	2.72±0.22b	6.04±0.04a	6.32±0.13b	5.73±0.04a	4.58±0.093a
Closteriopsis acicularis	1.31±0.019a	2.49±0.14a	4.07±0.06a	6.17±0.03a	6.97±0.03a	5.65±0.06a	3.68±0.18b
Coenococcus polycoccus	0.05±0.003c	0.11±0.02d	1.34±0.05d	3.31±0.01d	3.99±0.07f	3.64±0.02b	2.87±0.021c
Cosmarium contractum	1.08±0.032b	2.2±0.06a	3.9±0.01a	5.44±0.04b	6.08±0.08c	5.77±0.03a	3.62±0.044b
Diatoma tenuis	0.04±0.001c	0.08±0.03d	1.14±0.02d	2.07±0.06g	3.06±0.04g	2.5±0.08d	1.42±0.03d
Nannochloropsis	0.05±0.01c	1.11±0.05b	3.03±0.04b	5.95±0.06a	6.05±0.04c	5.72±0.11a	3.93±0.26b
Navicula capitate	0.05±0.001c	0.1±0.02d	2.04±0.04c	3.57±0.04c	4.65±0.06e	3.11±0.01c	1.47±0.021d
Scenedesmus ellipticus	0.09±0.002c	0.91±0.06cd	1.51±0.1d	3.17±0.04d	5.02±0.07d	3.67±0.12c	2.8±0.04c
Scenedesmus communis	0.03±0.001c	0.07±0.02d	0.19±0.01e	2.41±0.05e	3.08±0.01f	2.56±0.32d	1.33±0.04d
p.	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

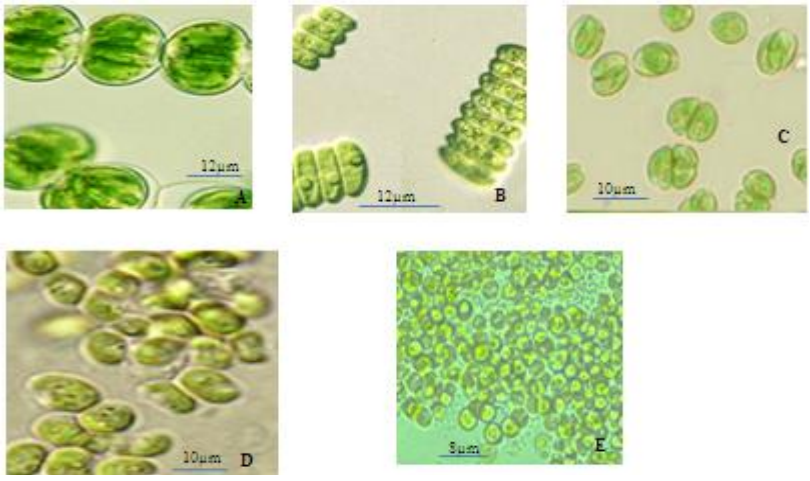

Plate 4.1: Isolated phytoplankton species from Lake Naivasha and EUC water reservoirs as viewed under light microscope:
A) *Cosmarium contractum*, B) *Scenedesmus ellipticus*, C) *Nannochloropsis* sp. D) *Chlorella saccharophilla* and E) *Chlorella vulgaris*.

4.6 Growth rates of fast growing phytoplankton species

From the experiments on the rate of biomass accumulation, five species with the highest biomass accumulation were selected for further trials on growth rates. The species identified (*Chlorella saccharophila*, *Chlorella vulgaris*, *Scenedesmus ellipticus*, *Closteriopis acicularis* and *Cosmarium contractum*) both from Lake Naivasha and EUC reservoirs were grown. For each of the phytoplankton strain from the different sites, which were cultured in replicates of three were harvested after six weeks. An average of the accumulated biomass of each phytoplankton species was calculated. Samples from Lake Naivasha recorded mean biomass values of 6.51, 6.32, 5.07, 6.91 and 6.08 mg L^{-1} for *Chlorella saccharophila*, *Chlorella vulgaris*, *Scenedesmus ellipticus*, *Closteriopis acicularis* and *Cosmarium contractum* respectively while the isolates from EUC reservoirs recorded 6.43, 6.27, 5.12, 6.88 and 5.99 mg L^{-1} respectively.

A two sample t test revealed a significant difference in the growth rates of the two strains of *Chlorella saccharophila* from Lake Naivasha and EUC reservoirs ($t = 3.26$ $p = 0.047$). Similarly, the growth rate of *Cosmarium contractum* from Lake Naivasha was significantly different ($t = 0.85$ $p = 0.041$) from that of the strain from EUC reservoir. However, the growth rates of strains of *Chlorella vulgaris*, *Closteriopis acicularis* and *Scenedesmus ellipticus* from Lake Naivasha and EUC reservoirs were not significantly different ($t = 0.29$ $p = 0.796$; $t = 1.05$ $p = 0.370$ and $t = 1.14$ $p = 0.337$ respectively).

Growth rates calculated as the biomass obtained from one litre of culture cells after a specific growth period showed that *Closteriopis acicularis* had highest growth rate of 1.34 mg L^{-1} d^{-1}, followed by *Chlorella vulgaris* (1.23 mg L^{-1} d^{-1}) then *Cosmarium contractum* and *Chlorella saccharophilla* with (1.11 mg L^{-1} d^{-1}) second last was *Scenedesmus ellipticus* with a growth rate of 0.96 mg L^{-1} d^{-1} and *Nannochloropsis* sp which recorded the lowest growth of 0.79 mg L^{-1} d^{-1} (Fig. 4.5).

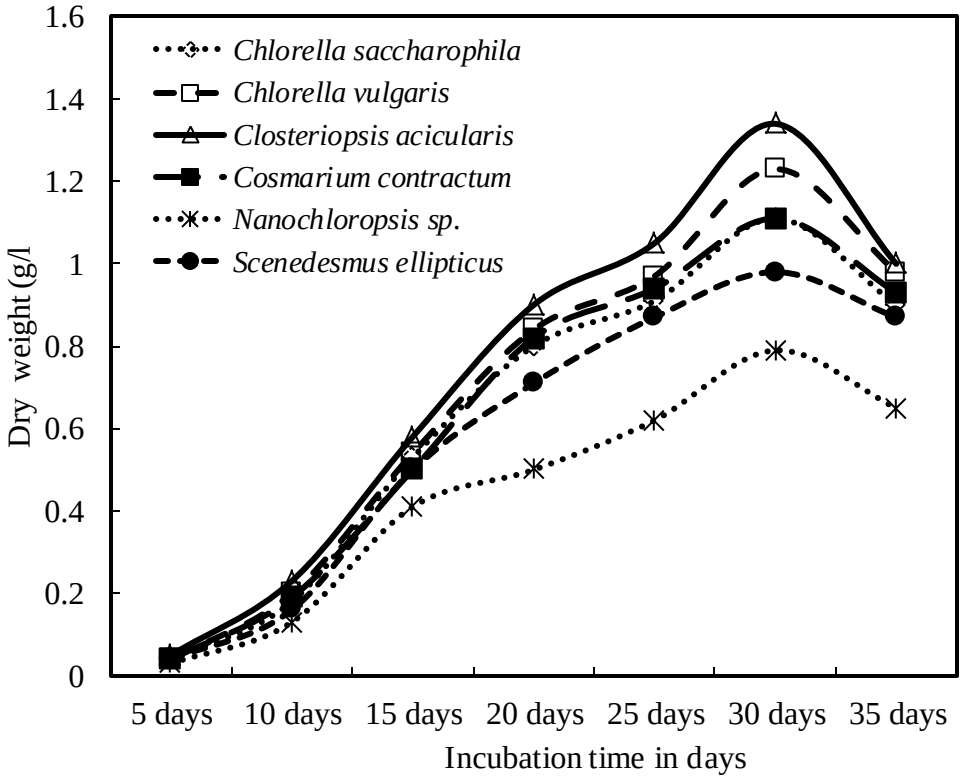

Fig. 4.5: Phytoplankton growth rates (as biomass accumulation) against incubation time in days in Modified Bourelly medium.

4.7 Growth behavior of fast growing phytoplankton species in different media concentrations

The growth behavior of *Closteriopsis acicularis* under five different types of media formulations (A, B, C, D and E) was generally characterized by a progressive increase in biomass for the first 5 weeks. Optimum growth was recorded in week five in culture Media D and E. A progressive increase in biomass was also recorded in Media A from week one to week three, while in media B and C biomass increase went on up to week 4. Means were calculated from the biomass recorded by each replicate for different media types. The lowest mean biomass values (g L^{-1}) recorded for weeks 1, 2, 3, 4, 5, 6 and 7 were 0.08, 1.17, 2.82, 2.25, 2.13, 1.62 and 1.57 respectively all in Media A while the

highest mean biomass values were 1.31, 2.50, 4.07, 5.17, 6.97, 5.65 and 3.68 respectively in Media D. Mean biomass was computed from the biomass recorded by each of the replicates of the experiments in each treatment. A one way ANOVA test revealed that the difference in mean biomass of *Closteriopsis acicularis* in the five media types in each week was significant (p<0.05, df = 50). Post Hoc test using Tukey's mean separation method revealed that the mean biomass in Media D and E were significantly higher than that of the media A, B and C (Table 4.8; Fig. 4.6A).

Table 4.8: Biomass in g L^{-1} of *Closteriopsis acicularis* in different media types measured weekly for a seven week period for cultures in 1000 mL glass conical flasks under laboratory conditions

Values (mean ±SE) followed by dissimilar letters in each week are significantly different according to Tukey's Honestly Significant Difference (HSD) at p <0.05.

Media	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
A	0.08±0.07b	1.17±0.05b	2.82±0.04c	2.25±0.07c	2.13±0.02c	1.62±0.11c	1.57±0.09c
B	0.46±0.03b	2.12±0.09a	2.97±0.02c	4.36±0.03b	4.24±0.04b	4.14±0.01b	3.09±0.05b
C	1.19±0.03a	2.44±0.05a	3.26±0.07b	4.53±0.25b	4.32±0.15b	4.16±0.09b	3.36±0.14ab
D	1.31±0.02a	2.5±0.14a	4.07±0.06a	5.17±0.03a	6.97±0.03a	5.65±0.06a	3.68±0.18a
E	1.26±0.03a	2.5±0.06a	3.98±0.08a	5.12±0.02a	6.83±0.04a	5.53±0.1a	3.537±0.09a
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Estimates of the growth rate of *Chlorella saccharophila* (as dry cell weight) in 5 different culture media formulations (A, B, C, D and E) revealed a difference in biomass accumulation in these media. In general, the growth pattern was characterized by an increase in biomass in all the five media. Media A recorded an increase up to week 3 with media B and C recording an increase up to week 4. During week one, Media E recorded the highest growth of 0.07±0.03 g L^{-1} while media A had the lowest growth of 0.05±0.03 g L^{-1}. In weeks 2, 3, 4, 5, 6 and 7, Media A had the least growth of 0.19±0.08, 2.97±0.04, 2.39±0.01, 2.07±0.05, 1.97±0.19 and 1.35±0.09 g L^{-1} respectively, while highest growth rates were recorded in Media D in weeks 2, 4 and 5 while the highest in weeks 3 and 7 was recorded in media E. A comparison of the mean biomass values of the five media types using a one way ANOVA test revealed a significant difference in mean biomass values (p<0.05, df = 50). Post Hoc test using Tukey's mean separation method revealed

that the mean biomass value of Media D and E was significantly higher than that of media A, B and C (Table 4.9; Fig. 4.6B; Plate 4.2).

Table 4.9: Biomass in g L^{-1} of *Chlorella Saccharophilla* in different media types measured weekly for a seven week period for cultures in 1000 mL glass conical flasks under laboratory conditions

Values (mean ±SE) followed by dissimilar letters in each week are significantly different according to Tukey's Honestly Significant Difference (HSD) at $p < 0.05$.

Media	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
A	0.05±0.03b	0.19±0.08a	2.97±0.04c	2.39±0.1b	2.07±0.05d	1.97±0.19a	1.35±0.09a
B	0.05±0.01b	1.19±0.05b	3.29±0.08bc	4.53±0.03b	4.37±0.15bc	4.24±0.02b	3.42±0.03b
C	0.06±0.02a	1.2±0.01b	3.30±0.04bc	4.52±0.05b	4.45±0.11b	4.30±0.05b	3.67±0.12b
D	0.064±0.02a	1.23±0.02b	3.77±0.13a	6.04±0.06a	7.14±0.11a	6.51±0.06c	4.13±0.05c
E	0.07±0.03a	1.21±0.02b	3.42±0.13ab	6.02±0.04a	6.83±0.03a	6.57±0.05c	4.19±0.02c
p.	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Biomass accumulation by *Nannochloropsis* sp. increased progressively in the 5 different media formulations (A, B, C, D and E). In each media there were variations in the recorded biomass over the weeks. Media A recorded an increase in biomass from week one to week three with a similar trend of increasing biomass registered in media B and C up to the fourth week although the mean biomass accumulated in C was slightly higher than that in B. Media D and E recorded a progressive increase in biomass up to week five with the optimum biomass recorded in week 5. A one way ANOVA test revealed that the difference in mean biomass accumulation recorded over the weeks in the five media types was significant ($p<0.05$, df = 50). A Post Hoc test using Tukey's mean separation method revealed that the mean biomass value of Media D and E was significantly higher than that of media A, B and C (Table 4.10; Fig. 4.6F).

Table 4.10: Biomass in g L^{-1} of *Nannochloropsis* sp. in different media types measured weekly for a seven week period for cultures in 1000 mL glass conical flasks under laboratory conditions

Values (mean ±SE) followed by dissimilar letters in each week are significantly different according to Tukey's Honestly Significant Difference (HSD) at $p < 0.05$

Media	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
A	0.038±0.02c	0.098±0.02b	2.12±0.04c	2.08±0.12c	1.96±0.13c	1.72±0.1c	1.25±0.04c
B	0.038±0.03c	0.19±0.04b	2.46±0.04b	4.3±0.22b	4.12±0.08b	4.06±0.14b	2.42±0.08b
C	0.043±0.02bc	0.21±0.06b	2.96±0.05a	4.5±0.07b	4.24±0.03bc	4.03±0.01b	2.37±0.03b
D	0.054±0.01a	1.31±0.05a	3.13±0.04a	5.9±0.06a	7.05±0.04a	6.52±0.12a	3.93±0.26a
E	0.052±0.01a	1.30±0.04a	3.13±0.08a	5.9±0.08a	6.99±0.05a	6.073±0.06a	3.91±0.26a
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Increase in biomass for *Cosmarium contractum* in the 5 different formulations (A, B, C, D and E) was recorded for the first 5 weeks of culture in Media D and E. In Media A the biomass increased from week one to week three while in media B and C a progressive increase was realized up to the fourth week. The mean dry weight values (g L^{-1}) for weeks 1, 2, 3, 4, 5, 6 and 7 were 0.1±0.03, 0.18±0.01, 2.24±0.01, 2.09±0.11, 1.74±0.04, 1.28±0.06 and 1.09±0.03 respectively all recorded in Media A. while 1.08±0.03, 2.2±0.06, 3.92±0.01, 5.44±0.04, 7.08±0.08, 5.77±0.03 and 3.62±0.044 respectively were recorded in Media D. A one way ANOVA test revealed that the difference in mean biomass of *Cosmarium contractum* in the five media types during each week was significant ($p<0.05$, df = 50). Post Hoc test using Tukey's mean separation method revealed that the mean biomass in Media D and E was significantly higher than that of the media A, B and C (Table 4.11; Fig. 4.6D).

Table 4.11: Biomass g L^{-1} of *Cosmarium contractum* in different media types measured weekly for a seven week period for cultures in 1000 mL glass conical flasks under laboratory conditions

Values (mean ±SE) followed by dissimilar letters in each week are significantly different according to Tukey's Honestly Significant Difference (HSD) at $p < 0.05$

Media	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
A	0.1±0.03b	0.18±0.01d	2.24±0.01d	2.09±0.11d	1.74±0.04c	1.28±0.06c	1.09±0.03d
B	0.1±0.02b	1.82±0.05c	2.91±0.03c	3.497±0.11c	3.28±0.02b	3.13±0.1b	3.04±0.04c
C	0.1±0.03b	1.95±0.05bc	3.19±0.01b	4.28±0.04b	4.19±0.02b	4.04±0.13b	3.23±0.06b
D	1.08±0.03a	2.2±0.06a	3.92±0.01a	5.44±0.04a	7.08±0.08a	5.77±0.03a	3.62±0.04a
E	1.05±0.02a	2.14±0.03ab	3.84±0.07a	5.41±0.05a	6.067±0.06a	5.69±0.04a	3.603±0.03a
p.	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

The growth behavior of *Chlorella vulgaris* under the 5 different media formulations (A, B, C, D and E) was generally characterized by a progressive increase in biomass over the first 5 weeks of culture in Media D and E. The progressive increase in biomass was also recorded in Media A from week one to week three only while in media B and C biomass increase went on up to week 4. The recorded mean biomass values (g L^{-1}) in media A for weeks 1, 2, 3, 4, 5, 6 and 7 were 0.04, 0.13, 2.15, 1.6, 1.21, 1.08 and 0.79 respectively, with media D recording 0.07, 0.17, 2.7, 5.04, 6.32, 5.73 and 4.58 for respectively. A one way ANOVA test revealed that the difference in mean biomass of *Chlorella vulgaris* in the five media types in each week was significant ($p<0.05$, df = 50). A Post Hoc test using Tukey's mean separation method showed that the mean biomass in Media D and E was significantly higher than that of the media A, B and C (Table 4.12; Plate 4.3; Fig. 4.6C).

Table 4.12: Biomass in g L^{-1} of *Chlorella vulgaris* in different media types measured weekly for a seven week period for cultures in 1000 mL glass conical flasks under laboratory conditions

Values (mean ±SE) followed by dissimilar letters in each week are significantly different at according to Tukey's Honestly Significant Difference (HSD) at p <0.05

Media	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
A	0.04±0.001a	0.13±0.01a	2.15±0.01a	1.6±0.14c	1.21±0.12c	1.08±0.06d	0.79±0.11c
B	0.05±0.03b	0.16±0.02b	2.16±0.11a	3.6±0.04c	3.43±0.07b	3.23±0.05c	3.12±0.01b
C	0.06±0.04bc	0.167±0.08b	2.5±0.24ab	3.72±0.03c	3.55±0.04b	3.28±0.09c	3.14±0.06b
D	0.07±0.02c	0.17±0.08b	2.7±0.22c	5.04±0.04a	6.32±0.13a	5.73±0.04a	4.58±0.09a
E	0.06±0.02c	0.07±0.09b	2.7±0.01c	4.9±0.02b	5.893±0.05a	4.79±0.17b	4.39±0.09a
p.	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<.0001

Scenedesmus ellipticus recorded increase in biomass in the 5 different media formulations (A, B, C, D and E) over the growth period. In the first 5 weeks of culture in Media D and E there was a progressive increase in the cell dry weight. Similarly, media A recorded an increase within the first three weeks of culture while media B and C recorded a trend of increasing biomass up to week four. The mean cell dry weights (g L^{-1}) recorded for weeks 1, 2, 3, 4, 5, 6 and 7 were 0.06±0.01, 0.11±0.08, 2.26±0.03, 2.13±0.05, 2.07±0.04, 1.7±0.14 and 1.06±0.04 respectively all in Media A. while 0.07±0.02, 0.21±0.06, 2.71±0.01, 4.71±0.04, 5.02±0.07, 4.67±0.12 and 2.8±0.04 respectively were recorded in Media D. A one way ANOVA test revealed that the five media types had significant differences on the mean biomass values recorded over the different weeks (p<0.05, df = 50). Mean biomass value of Media D and E was significantly higher than that of media A, B and C as revealed by A Post Hoc test using Tukey's mean separation method (Table 4.13; Fig. 4.6E).

Table 4.13: Biomass in g L^{-1} of *Scenedesmus ellipticus* in different media types measured weekly for a seven week period for cultures in 1000 mL glass conical flasks under laboratory conditions

Values (mean ±SE) followed by dissimilar letters in each week are significantly different according to Tukey's Honestly Significant Difference (HSD) at p <0.05.

Media	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
A	0.06±0.01b	0.11±0.08a	2.26±0.03a	2.13±0.05a	2.07±0.04c	1.7±0.14c	1.06±0.04c
B	0.06±0.03b	0.12±0.01a	2.28±0.02a	3.41±0.05b	3.73±0.03b	3.41±0.10c	2.39±0.02c
C	0.065±0.01b	0.13±0.01a	2.37±0.18b	4.13±0.13c	3.83±0.04b	3.3±0.04b	2.39±0.15c
D	0.07±0.02a	0.21±0.06b	2.71±0.01c	4.71±0.04d	5.02±0.07a	4.67±0.12a	2.8±0.04a
E	0.069±0.03a	0.21±0.03b	2.46±0.04c	4.63±0.02d	5.01±0.03a	4.48±0.1a	2.72±0.04b
p.	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

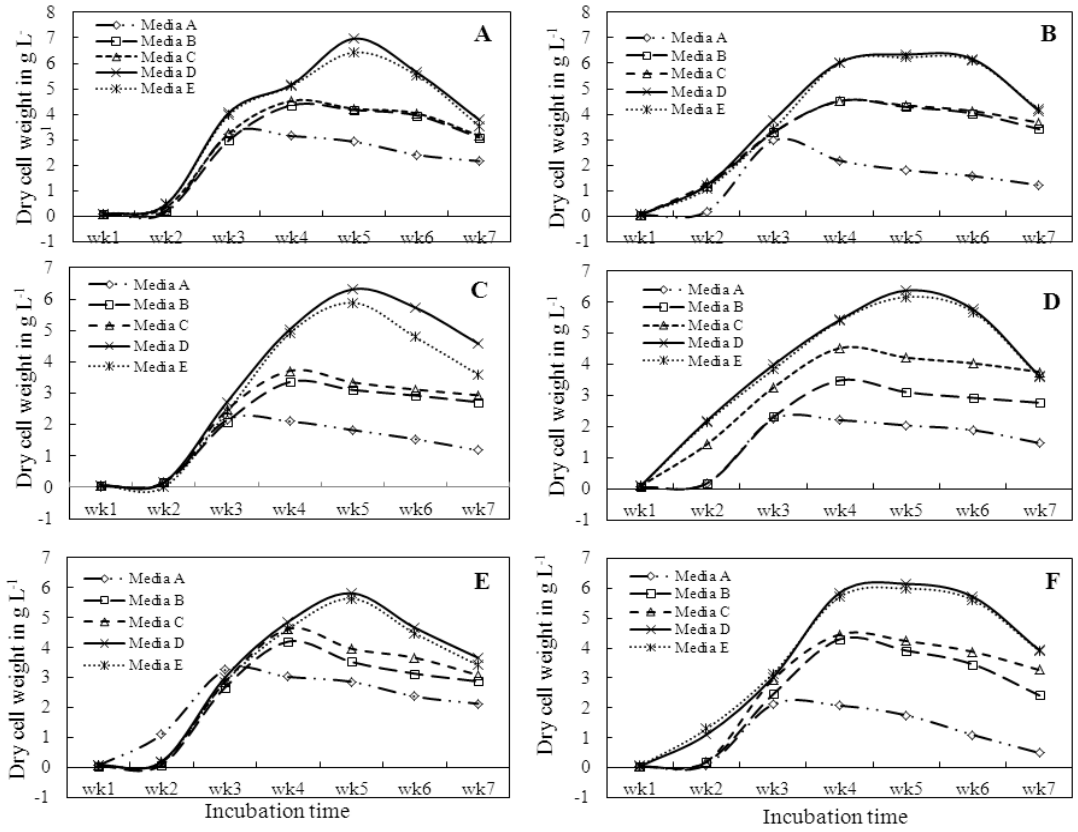

Fig. 4.6: Changes in total biomass (dry cell weight) in g L^{-1} for the selected fast growing phytoplankton species under different media formulation.
(A) *Closteriopsis acicularis*; (B) *Chlorella saccharophilla*; (C) *Chlorella vulgaris*; (D) *Cosmarium contractum*; (E) *Scenedesmus ellipticus* and (F) *Nannochloropsis* sp grown for seven weeks in media A, B, C, D and E.

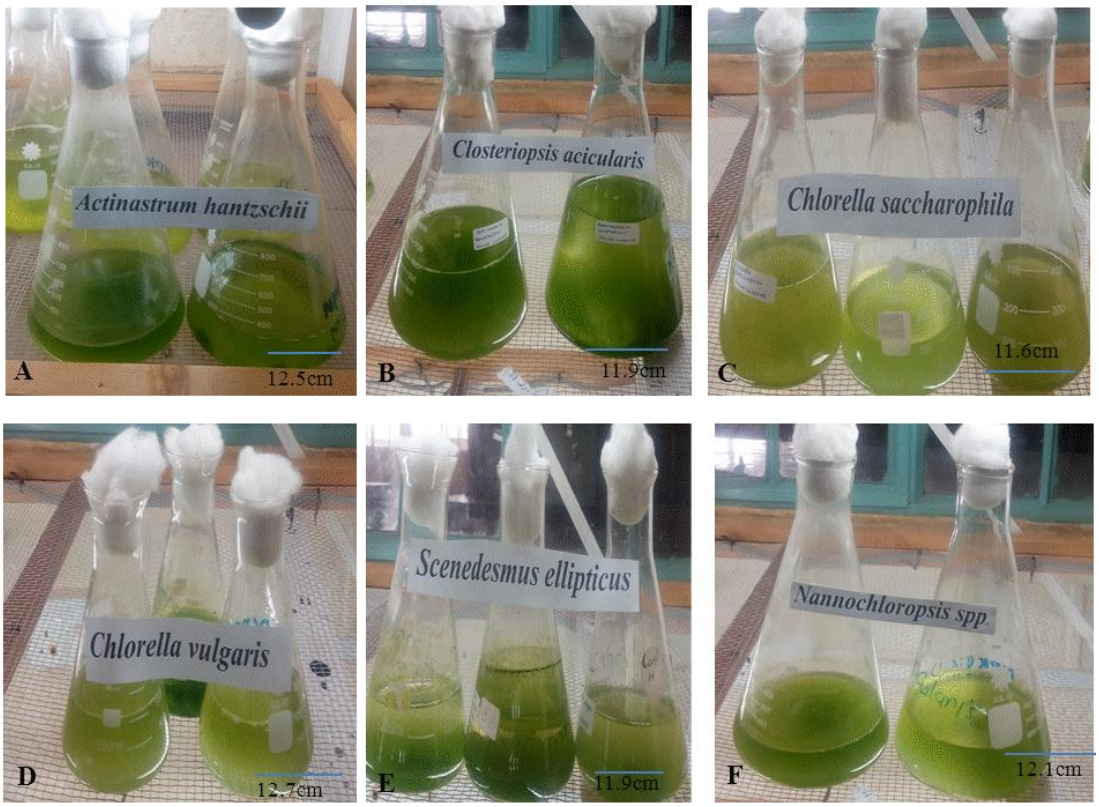

Plate 4.2: Isolated unialgal cultures of A) *Actinastrum hantzschii*; B) *Closteriopsis acicularis*; C) *Chlorella saccharophilla*; D) *Chlorella vulgaris*; E) *Scenedesmus ellipticus* and F) *Nannochloropsis* sp.

4.8 Cellular oil content of fast growing phytoplankton species

Oil extracted from the biomass of the fast growing species using hexane solvent yielded different quantities per unit biomass. Based on actual oil yield values, the lipid content of *Nannochloropsis* sp., *Chlorella saccharophila*, *Chlorella vulgaris* and *Scenedesmus ellipticus* were computed to account for 30%, 10.8%, 8.7% and 5.4% of their dry weight respectively. *Closteriopis acicularis* and *Cosmarium contractum* species did not produce measurable quantities of oil. The mean extracted oil for *Nannochloropsis* sp. in mL g^{-1} was 0.3 ml ± 0.07 with *Chlorella saccharophila* recording 0.109±0.02. These were followed by *Chlorella vulgaris* and *Scenedesmus ellipticus* with 0.088±0.01 and 0.054±0.02 respectively. A one way ANOVA test revealed that the difference in mean oil extracted from the four isolates which produced oil (*Nannochloropsis* sp, *Chlorella saccharophila*, *Chlorella vulgaris*, and *Scenedesmus ellipticus*) was significant (p<0.05, df 44). Post Hoc test using Tukey's mean separation method revealed that the mean oil

extracted of *Nannochloropsis* sp was significantly higher than that of the other species (Table 4.14; Plate 4.3).

Table 4.141: Quantity of oil (in mL g^{-1}) extracted using hexane extraction technique from six phytoplankton strains

Values (mean ±SE) followed by dissimilar letters along the columns are significantly different at according to Tukey's Honestly Significant Difference (HSD) at p <0.05.

Species	Extracted oil in mL g^{-1} ±SE	Lipid content %
Chlorella saccharophilla	0.109±0.02b	10.8
Chlorella vulgaris	0.088±0.01b	8.7
Nannochloropsis sp.	0.3±0.07a	30
Scenedesmus ellipticus	0.054±0.02c	5.4
P	<0.001	

4.9 Relationship of the produced biomass to the extracted phytoplankton oil

A linear regression plot of extracted oil against the dry weight showed that there was no significant (R^2 = 0.092, p = 0.087) relationship between the dry weight and the extracted oil, with the extracted oil decreasing with increase in dry weight (Fig. 4.7)

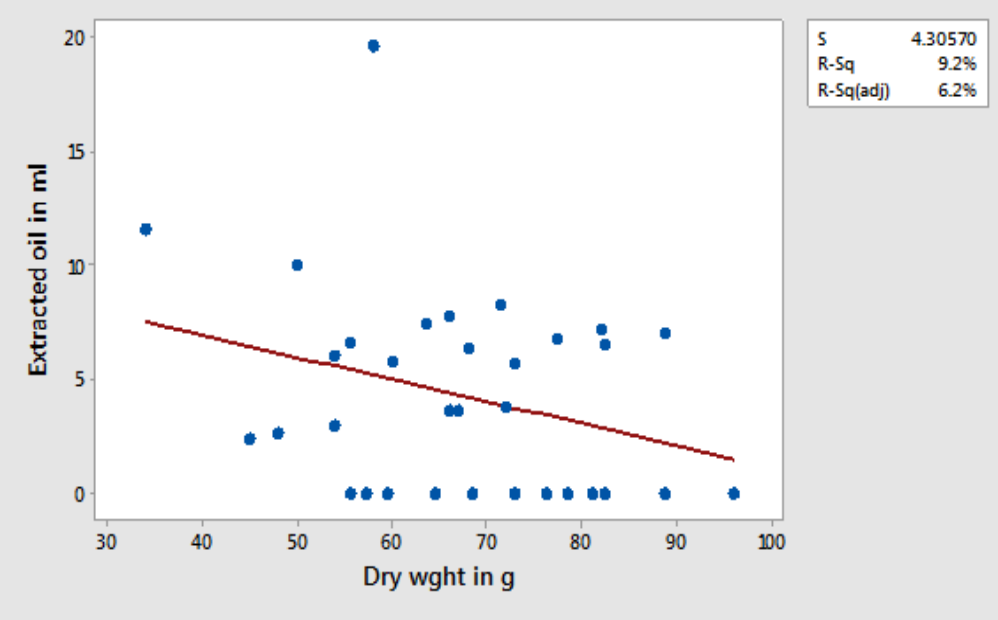

Fig. 4.7: Linear regression analysis of the extracted oil from harvested phytoplankton biomass

4.10 Oil production potential in relation to the site of collection

Mean oil production (per unit of dry weight) by strains of *Chlorella saccharophila, Chlorella vulgaris* and *Scenedesmus ellipticus* isolated from Lake Naivasha were 0.101, 0.097 and 0.054 mL g^{-1} respectively while the strains isolated from EUC reservoirs produced 0.112, 0.078 and 0.053 mL g^{-1} respectively. A two sample t test revealed that mean oil volume extracted from *Chlorella saccharophila, Chlorella vulgaris* and *Scenedesmus ellipticus* from Lake Naivasha was not significantly different from that of EUC reservoirs with the following values respectively (T= 1.72, df = 4, P = 0.160; T = 2.74, df = 4, P= 0.052 and T =2.96, df = 4, P = 0.042.

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Seasonal variation in physico-chemical properties

5.1.1.1 Conductivity

The recorded mean conductivity range of 210 to 265 µS cm^{-1} in Lake Naivasha is comparatively lower to a range of 284 - 374 µS cm^{-1} reported by Ballot *et al.* (2004) and similar to the range of 200 - 245 µS cm^{-1} recorded by (Becht and Harper, 2002). A much higher mean value of 552 µS cm^{-1} was recorded by Ndun'gu *et al.* (2013) in a study carried out between July and November 2011 in Lake Naivasha. The lower conductivity values recorded during the present study as compared to the measurements recorded in 2011 can be attributed to an increase in the water level which started in 2012 (Onywere, 2013) and as at the time of the present study was still high. This has caused a dilution of the ions determining the conductivity hence the low recorded conductivity.

Lower conductivity values in EUC reservoirs as compared to Lake Naivasha can be linked to the shorter residence time of water in the water reservoirs, a feature attributable to the small size as well as the presence of a distinct inlet and outlet. The seasonal changes in conductivity values characterized by low levels during the wet season (with a low of 121 µS cm^{-1} in April to a high value of 177 µS cm^{-1} the dry month of February 2015) can be explained by the dilution influence of rainfall and runoff that depresses conductivity on one hand and the evaporative concentration during the dry season (Mavuti and Uku, 1994).

5.1.1.2 Water transparency

Variations in water transparency (measured as a Secchi depth) during the present study resulted from changes in the amount of suspended solids that is linked to seasonal changes in surface run-off as well as phytoplankton production. At the beginning of the study period in December 2014, the study areas had increased inflows from surface runoff and discharge from rivers as a result of the short rains experienced. These inflows were characterized by increased suspended solids and soil particles from the surrounding agricultural lands, which caused the water in the study sites to be turbid resulting in low

Secchi depth values. As the study progressed in January to March 2015, a dry period with little or no inflow was experienced. This resulted in the sedimentation of the suspended soil particles leading to more clear waters and thus the recorded increase in Secchi depth in January through March 2015. In April 2015, more rain was experienced that brought in more suspended materials to the water bodies which significantly lowered the water clarity hence the recorded low Secchi depth. Fluctuations in Secchi depth values have been reported by Bendorf *et al.* (2002) and confirmed to persist in water bodies in regions where loose soil particles from agricultural and other practices are present. The significant difference in the mean Secchi depth of Lake Naivasha and EUC reservoirs is attributable to size of the water bodies. According to Bruce and Allan (1984), reservoirs have a comparatively lower Secchi depth than natural lakes, which is as a result of a difference in the drainage area to surface area ratios. The drainage area to surface area ratio is higher in reservoirs than lakes leading to a higher sediment input per unit in reservoirs.

5.1.1.3 Total alkalinity

Differences in TA of Lake Naivasha and EUC reservoirs as recorded in the study can be attributed to the differences in the nature of the catchment bedrock and soil types. This observation was reported by Kotut *et al.* (1999) in a study in Turkwell Gorge reservoir. In addition, the recorded low TA values in April 2015 compared to the high values in February 2015 in all the study areas are attributable to the change in inflow volumes. Low TA values attributable to the dilution effects of high volume of inflows have been recorded in Turkwel Gorge reservoir (Kotut *et al.*, 1999).

5.1.1.4 pH

Among the factors that possibly led to the observed increase in pH levels during the rainy season in both Lake Naivasha (from 7.7 to 8.6) and Embu University College (from 6.5 to 8.6) are the reduced disturbance of the water bodies during the wet season, increased water depth and increased phytoplankton activity. A reduced disturbance of the water bodies by wildlife watering led to a reduction of bottom up mixing which usually introduces reduced substances from the sediment surface that depress water pH. This has been reported by Santana-Casiano *et al.* (2009) where mixing as a result of upwellings release carbon dioxide from the sediments to the overlying surface water causing significant decrease in the observed pH. Increased water depth similarly led to an increase in phytoplankton

photosythesis, which elevates water pH. A review by Hinga (2002) in twenty one studies on the effects of pH on marine phytoplankton as well as the change in the pH as a result of carbon dioxide utilization by phytoplankton reported a rise in pH from 8.1 to 8.5.

5.1.1.5 Total phosphorus and nitrogen

Elevated phosphorus and nitrogen levels observed in the month of April in Lake Naivasha and the EUC reservoirs (Fig. 4.2B) can be attributed to the high river inflow and surface run-off associated with the onset of the wet season. In general, nutrient concentration as well as the seasonal variations in a standing water body is usually an indication of fluctuations in loading rate from its catchment. High levels are usually associated with the surface runoff and increased river discharge periods. Past studies have linked the increase in nutrient levels in Lake Naivasha to periods of high river discharge (Becht and Harper, 2002; Kitaka *et al.*, 2002). A study by Kazi *et al.* (2009) concluded that agricultural effluents and domestic wastes from the surrounding horticultural farms and settlement areas around Lake Naivasha add significant amounts of nutrients into the lake ecosystem during periods of high discharge volume. A progressive decline in nutrient levels on moving from the wet to the dry season can be explained by the utilization by the increasing phytoplankton species as water clarity increases as well as the reduced supply through inflows. A general decline in phosphate phosphorus levels linked to phytoplankton uptake was recorded by Romero *et al.* (2013) in a study carried out in rivers in south western Europe.

5.1.2 Seasonal changes in phytoplankton species composition, abundance and diversity

Phytoplankton species composition as well as the total number of species present in the study sites varied with time with the greatest fluctuation recorded during the transition period between the dry and wet months. This is a common trait in tropical water bodies where wet and dry seasons exert the greatest influence on the chemical dynamics of the water column, which ultimately affect the biota (Schagerl and Oduor, 2008). According to Melack (1979), phytoplankton seasonality in tropical lakes can be divided into three broad seasonality patterns; a pronounced seasonality pattern, a muted seasonality pattern and an abrupt switch seasonality pattern. The first two seasonality patterns are characteristic of the phytoplankton species composition of Lake Naivasha. Pronounced seasonal fluctuations occur as a result of variation in inflow or lake mixing, which alters the

physico-chemical conditions resulting in changes in the species and cell numbers. A muted seasonality pattern occurs in buffer fringes, perennial river mouths and areas with sufficient internal recycling of nutrients which results to diel variations which are greater than long term changes which eventually results to the same phytoplankton assemblages persisting for many days as observed in the current study at Kamere beach in Lake Naivasha. The findings of this study are comparable to those by Hubble and Harper (2002) who reported the persistence of most chlorophyte and bacillariophyte species during their study in Lake Niavasha and Oloiden bay. They associated their findings to the high inflow from the perennial rivers and the rains.

The large number of species from the phylum Chlorophyta as compared to other phyla in the study sites, coupled with high number of species of the other phyla in the dry to the wet seasons can be attributed to intermediate disturbance hypothesis (IDH) (Connell, 1978). The hypothesis stipulates that in the absence of disturbance (eternal steady state), competitive exclusion will reduce diversity to minimum levels. This was probably the situation during late period of the dry season in March 2015. Moreover, the hypothesis also points out that under very intense disturbance only few populations of pioneer species could establish themselves after each disturbance event. This would also lead to minimal diversity as observed in December 2014 and April 2015 when increased inflow introduced more nutrients and sediments to the aquatic systems which caused significant disturbance. In addition, the third aspect of IDH points out that if disturbances are of intermediate frequency or intensity, there will be repeated opportunities for the re-establishment of pioneer populations which would otherwise be outcompeted and the populations of the successful competitors could withstand the disturbance without completely taking over the community and thus the peak diversity is realized these time. This was possibly the case during the dry period after the settlement of the sediments introduced by December 2014 rains hence the high species diversity recorded in February 2015. The findings of this study are consistent with observation made in other studies in similar tropical ecosystems (Salmaso, 2000, 2002; Garibaldi *et al.*, 2003; Wondie *et al.*, 2007) in which high species numbers were recorded during the dry period and slightly low species during the wet period. Similarly, Townsend *et al.* (1997) related the occurrence of a high number of species in his work to the effects of the IDH. During the wet seasons, water transperancy was low. These resulted to a comparatively high number of Cyanophyte species (Fig. 4.4). This is perhaps due to the ability of cyanophytes to utilize low light intensities during

photosynthesis, which is made possible by the possession of accessory pigments which helps in trapping light (Scheffer *et al.*, 1997; Soares *et al.*, 2007) as well as the possession of gas filled aerotopes that aid in regulating their depth distribution within the water column (Xiao *et al.*, 2012) hence ensuring that the cells are raised to the euphotic zone.

During the wet season months, cyanophyte species recorded their highest number of species as well as high cell numbers per species, although they did not exceed the numbers recorded by chlorophytes. During these months phosphorus levels increased as a result of runoff from rich agricultural lands surrounding the study areas. A similar observation was made by De Domitrovic (2003) who linked the high Cyanophyta abundance to high phosphorus levels. Similarly, a high cyanophyte species composition and cell numbers during the wet season when the pH values were higher is comparable to findings of Price *et al.* (2008) who reported that high pH enhanced growth of N_2 fixing heterocystous cyanobacteria by over 20-fold biomass. According to Jeanfils *et al.* (1993) and Unrein *et al.* (2010), the ability of cyanobacteria grow faster under pH values greater than 8 can be attributed to their ability to concentrate dissolved inorganic carbon at the site of rubisco enzyme than other taxa.

In the wet season month of April 2015, Chlorophyta species numbers were higher compared to those of Cyanophyta species. The high chlorophyte abundance in this month is probably due to the net loss rate balance between the high growth and loss rate of chlorophytes to low growth and loss rate of cyanophytes. According to Brock (1973) and Havens (2008), a high growth and loss of chlorophytes enables them to out-compete cyanophytes under high phosphorus conditions.

Changes in the dominance by the different phytoplankton phyla as evidenced by the high number of the Chlorophyta during the dry season and the brief dominance by other divisions during the wet season can be linked to changes in the prevailing environmental conditions. Studies by Harney *et al.* (2013), Prassana and Ranjan (2010) and Murugavel and Pandian (2000) concluded that the occurrence of different phytoplankton species is dictated by the prevailing environmental conditions. Jensen *et al.* (1994) and Corell (1998) observed high chlorophyta growth in shallow hypertrophic systems that were associated with the continuous input of nutrient and carbon from sediments when light is adequate. Similarly, surface runoff from the rich agricultural land surrounding Lake Naivasha

releases high nutrient levels into the lake (Kitaka *et al.*, 2002). These greatly affects the water quality and eventually affects species diversity.

During the study period, 126 phytoplankton species were identified fully to species level with other 8 identified up to genus level in Lake Naivasha. The number reported is a slightly lower than a total of 143 species recorded by Kalff and Watson (1986) in the same lake. Ballot *et al.* (2004) identified a lower total of 116 species in lake Naivasha. The lower number of species in the study by Ballot *et al.* (2004) was explained by lower Bacillariophyta species, in which they identified 15 species. In addition, they also observed Cyanophyta blooms which affected presence of other species from other phyla. In the present current study, cyanophyta blooms were not recorded.

Studies on Bacillariophyta dominance have shown that their presence is associated with water turbulence, top down mixing and high silica levels (Garibaldi *et al.*, 2003; Melo *et al.*, 2003; Krienitz *et al.*, 2004). The occurrence of the Bacillariophyta in both the dry and wet seasons as recorded during this study can be attributed to the regular wind induced mixing pattern, which is a common feature of shallow water bodies (Hubble and Harper, 2002). The regular mixing pattern maintains the cells in continuous suspension in the water column. Occurrence of *Aulacoseira* as the most common Bacillariophyta genus in lake Naivasha (Tables 4.1 to 4.4) is consistent with the findings of Gaudet and Melack (1981), Richardson and Dussinger (1986), Gasse *et al.* (1995) and Ballot *et al.* (2004). In the above studies, the genus *Aulacoseira* was represented by three common species; *Aulacoseira granulata*, *Aulacoseira ambigua*, and *Aulacoseira italica*. Presence of Chrysophyta during the dry season only (Tables 4.1 to 4.4) can be attributed to the lower pH during the dry season. Smith (1986) and De M and Nina (1998) reported that chrysophyte species show a negative relationship with pH and trophic state thus high pH values affects their growth.

5.1.3 Growth characteristics of fast growing phytoplankton species

The survival of 11 species under laboratory conditions from an inoculum that comprised over 100 species is attributable to their ability to adapt to the growth medium and the laboratory conditions. In general, the growth of phytoplankton species is greatly affected by the nature of the growth media. According to Bronk *et al.* (2007) varying levels of macro and micro nutrients significantly affect phytoplankton growth by favouring the

growth of certain taxa over others. In addition, phytoplankon cells have previously been reported to grow at different rates in different concentrations of macro and micronutrients (Bronk *et al*. 2007). This was confirmed in the present study when cells in medium A comprising of micronutrients only showed a lower growth rate as compared to cells in media D and E with both micro and macro nutrients.

In the present study, majority of phytoplankton species that survived under laboratory conditions were from the chlorophyta phylum. The ability of members of the Chlorophyta to survive under laboratory has been cited in past studies. For example, Sialve *et al*. (2009) reported that *Scenedesmus* and *Chlorella* species can survive under wide range of laboratory conditions. As observed in the present study, *Chlorella* species have been reported to grow faster in growth media that is rich in phosphates and nitrates (Yang *et al*., 2011).

Increase in phytoplankton cell numbers, as well as the biomass of different species at varying rates as observed in this study can be explained by nature of the cells. Single cells recorded a higher biomass as compared to colonial cells. A study by Mandal and Mallick (2009) focusing on cultures of *Scenedesmus* and *Chlorella* species recorded a higher growth rate for *Chlorella* sp as compared to *Scenedesmus* sp. The authors attributed the high growth rate of *Chlorella* sp. to the nature of cell division where single cells divide faster than colonial or cells forming a coenobia.

A similar growth rate of isolates of the same species from Lake Naivasha and EUC reservoirs as observed in this study confirms the similarity of the species isolates from the two sites and also serves to demonstrate that isolates of the same species exhibit a similar growth pattern when subjected to similar growth conditions. A similar finding was made by Andersen *et al*. (2005b) in a study on the growth rates of *Chlorella* species isolates from eutrophic and oligotrophics lakes under laboratory conditions.

5.1.4 Growth rates of fast growing phytoplankton species in different growth media

The growth rates of the fast growing phytoplankton species in the different growth media resulted to a logistic growth curve (Fig. 4.5) comparable to that reported by Fogg and Thake (1987) with 5 recognized phases; 1- lag or induction phase, 2- exponential phase, 3- phase of declining relative growth, 4- phase of stationary growth and 5- death phase. The lag phase as observed has also been reported by Csavina *et al*. (2011) where cells do not

grow appreciably but become acclimated to their new growing environment. This is probably why in all the different species as well as in the different growth media the biomass accumulation in the first weeks was significantly low. The second phase (exponential) is characterized by a notable increase in cell biomass as recorded in the study. This is followed by declining phase resulting from the limitation of the environmental factors such as light and nutrients. This phase came at different weeks in different media during the experiment probably attributable to limitation of the nutrients which were in different concentrations. The fourth phase (stationary phase) as realized in the study was characterized by an end to biomass accumulation. This was followed by the final phase (death) where cells were no longer dividing but dying. However cell death occurs in all phases, during the death phase (phase 5) there is no growth hence the effect of cells dying is noticeable unlike in other phases (Csavina *et al*., 2011). The characteristic growth curves realized in the study are as a result of the limitation of growth factors as reported by Berges *et al*. (2001) and Harrison and Berges (2005) who noted that whenever a nutrient responsible for growth decreases to levels below saturation in the cell, the maximum uptake velocity of the nutrient in the cell also decreases reaching a limiting minimun hence a decline in cell growth.

The growth rate of phytoplankton species in full media (media D) progressed for five weeks to attain the optimum growth, with that of micronutrients only (media A) progressing for only 3 weeks and attaining a lower biomass as compared to that in media D. This is comparable to the observations by Zeng *et al*. (2009) who recorded a better growth performance of *Chlorella protothecoides* under a full media spectrum of micro and macro nutrients as compared to a simplified media of purely micronutrients. Similarly, Bruland *et al*. (1991), Davies *et al*. (2006) and Bilanovic *et al*. (2009) observed significant increase in biomass of different phytoplankton species cultured in medium made of both macro and micro nutrients. The high growth rate supported by media D compared to other media types is an indication that N and P with addition of micronutrients play significant role in achieving significantly high phytoplankton growth (Tables 4.8 - 4.13). A closely observation was made by Aminu and Ahmed (2001); Kolo *et al*. (2001) and El Nabris (2012) who reported that nitrates, phoshates and micronutrients have significant effect on algal growth.

In the current study, media A made of micronutrients only and media B made of macronutrients only induced growth which was short lived (Fig. 4.6). This can be attributed to the roles played by macronutrients in algal growth. N, P and K support phytoplankton growth with each element affecting growth independently. Both N and P together help in the formation of DNA and also enhance growth, N acts as the source of nutrient with P enhancing synthesis of ATP to provide energy for metabolic activities while K regulates osmotic pressure, enzyme activity and protein synthesis (Larned, 1998; Xu *et al.*, 2010). Micronutrients on the other hand play a significant role in phytoplankton biomass accumulation. This was observed in media B which consisted of only macro nutrients of same concentration as in media C and accumulated slightly lower biomass than media C which had in addition to micro nutrients, some macro nutrients. Similar observations were made by Bruland *et al.* (1991) and Parent *et al.* (1996) while investigating the significance of extremely small quantities (<4ppm) of trace metal ions in the growth of phytoplankton. They observed that trace metal ions induced phytoplankton cell multiplication. In a related study, an investigation by Liu *et al.* (2008) on the effect of micronutrients on algal growth recorded reduced *Chlorella vulgaris* cell growth in the absence of iron as a micro nutrient. Iron limitation significantly depresses photosynthesis electron transfer resulting in reduction in NADPH formation. Iron also acts as a redox catalyst in photosynthesis and nitrogen assimilation that mediates electron transfer reactions in photosynthetic organisms (Terry and Abadía, 1986; Theodrou *et al.*, 1991; Lewis *et al.*, 2011).

5.1.5 Oil production potential of fast growing phytoplankton species

Phytoplankton cells oil content varied with species (Table 4.14) with only four of the six cultured species producing varied quantities of oil per unit of dry cell weight. Variations in cellular oil content can be attributed to the genetic characteristics and physiology of the cells (Lam and Lee, 2012). A study by Krienitz and Wirth (2006) on the oil yield of phytoplankton species in different nutrient concentrations revealed that the oil yield of the same species in different media types was not significantly different. Similarly, Shimizu (2008) cultured phytoplankon cells in non optimized medium (growth medium consisting of the growth elements only) and optimized medium (growth medium consisting of growth elements and additional carbon dioxide supply) with the results of oil yield of similar species not being significantly different.

The lipid content of 30%, 10.8%, 8.7% and 5.4% produced by *Nannochloropsis* sp., *Chlorella saccharophilla, Chlorella vulgaris* and *Scenedesmus ellipticus* respectively are in close agreement with the findings by Pulz and Grass (2004) where green algae grown in nitrogen depleted medium (0.75 mg L^{-1} $NaNO_3$ as nitrogen source) within a similar range to the current study produced 10-23% lipid content of their dry cell weight. The 30% lipid content produced by *Nannochloropsis* sp. in this study is similar to the findings of Wahidin *et al.* (2013) in which *Nannochloropsis* genus grown under 18:6 light:dark cycle at a light intensity of 100 µmol photons m^{-2} s^{-1} produced 31.3% lipid content. However, a higher oil yield of 44.5% lipid was realized by Su *et al.* (2011) when *Nannochloropsis* sp was cultured in 35 g L^{-1} salinity and irradiance of 500 µmol photons m^{-2} s^{-1} and extra supply of carbon dioxide. Similar findings were recorded by Fietz *et al.* (2005) and Bondioli *et al.* (2012). The higher lipid contents registered in these studies can be attributed to the additional supply of carbon dioxide which was not the case in the current study.

Chlorella sp. cultured in different nitrate and phosphate concentrations produces lipids in varying amounts (Javanmardian and Palsson, 1991; Kobayashi *et al.*, 1993). Those grown in nitrogen deprived medium produced 9-18% lipid content (Herrera-Valencia *et al.*, 2011). Although the present study did not involve nitrogen deprivation, the lipid yield recorded was within the same range with *Chlorella saccharophilla* and *Chlorella vulgaris* producing lipid yields of 10.8% and 8.7% respectively. A slightly higher oil yield of 10.64% (compared to an oil yield of 8.7%) was recorded for a culture of *Chlorella vulgaris* grown in a mixotrophic culture (basic growth elements plus 10 g L^{-1} of glycerol) (Heredia-Arroyo *et al.*, 2011 and Kong *et al.*, 2013) on the oil production. The higher production possibly resulted from the additional supply of glycerol.

The lipid content of 10.8% produced by *Chlorella saccharophila* in this study is comparatively lower than the yield of 18.1 % recorded by Chinnasamy *et al.* (2010), under a growth medium containing carpet mill (a source of carbon). The high oil yield recorded by Chinnasamy *et al.* (2010) resulted from the additional carbon contained in the carpet mill, which initiated *de novo* lipid synthesis under conditions of low nitrogen supply. Several studies have reported that some oleaginous microalgae direct their excess carbon and energy to store lipids under nitrogen strained environmental conditions (Illman *et al.*, 2000; Barsanti and Gualtieri, 2005 and Widjaja *et al.*, 2009).

A 5.4% lipid content recorded for *Scenedesmus ellipticus* in this study is similar to a value of 5.39% for *Scenedesmus* sp. grown in autotrophic conditions (Liang *et al.* 2010). However, when the species was cultured in a mixotrophic culture called BG-11 medium (Blue Green medium for cyanobacteria with an addition of 10 g L^{-1} of glycerol), the lipid yield was raised to 15.2%. An interesting observation made during the present study was the low lipid yield by fast growing phytoplankton species (*Closteriopis acicularis* and *Cosmarium contractum*). These findings are consistent with a general observations that under optimal conditions, a large amounts of algal biomass is produced but with relatively low lipid content in their dry cell weight (Lorenz and Cysewski, 2003; Tawfiq *et al.*, 2010; Sharma *et al.*, 2012). A high biomass yield and a low lipid content of 6.5% has been recorded for *Ulothrix* sp. (Rodolfi *et al.*, 2009; Faud *et al.*, 2010; Liu *et al.*, 2011) while a low biomass accummulation and a high oil yield of up to 75% of the dry cell weight has been recorded for *Botryococcus braunii* (Amaro *et al.*, 2011).

5.1.6 Relationship between oil content by phytoplankton species and site of isolation

The findings of this study confirm that the nature of water body does not influence oil produced by algal isolates (Table 4.15). Comparable amounts of lipid produced by algal cells from different aquatic ecosystems can attributted to the genetic make up of the phytoplankton species (Lam and Lee, 2012). However, the nature of environmental conditions found in the water bodies (electrical conductivity, total nitrogen, total phosphorous, pH and dissolved oxygen concentration) could influence cellular oil content. Devi *et al.* (2012) reported that prevailing environmental conditions help phytoplankton cells to undergo metabolic functions that induce the physiological processes which results in synthesis of lipids at same rate. However, the results of this study differed with those of (Devi, 2013) where he compared lipid productivity of algal isolates from lentic with those from lotic ecosystem. The lotic ecosystems produced higher lipid than lentic ones.

5.2 Conclusions

From the results of this study, it can be concluded that:

- The phytoplankton species composition in lake Naivasha and Embu University College reservoirs change with season. In general, the number of species is comparatively higher in the dry season than the wet season months.
- Out of a total of 134 and 122 species identified at Lake Naivasha and EUC reservoirs respectively, the following five species were found to be capable of fast growth under laboratory conditions; *Closteriopsis acicularis, Chlorella saccharophilla, Chlorella vulgaris, Cosmarium contractum, , Scenedesmus ellipticus* and *Coenococcus polycoccus*.
- The difference in the cellular oil content of strains of the five fast growing phytoplankton species cultures from both Lake Naivasha and Embu University College reservoirs was not significant.

5.3 Recommendations

Based on the above conclusions, the study recommends

- Further research should be conducted using different growth media and culture conditions to find out whether there are other phytoplankton species in Lake Naivasha and EUC reservoirs with faster growth rates and oil production potentials besides the five identified in the current study.
- Further research using (4.8 mg L^{-1} of P in NPK fertilizer plus micronutrients) as identified in the study with the highest cell growth rates for mass production of the identified phytoplankton species for oil extraction.
- Further research should be carried out using molecular markers for species identification, isolation and oil production screening to establish other oil producing species.
- Further research should be carried out in other water bodies to establish if there are other phytoplankton species with higher oil production potential.
- From the findings of the research, *Chlorella saccharophilla, Chlorella vulgaris* and *Scenedesmus ellipticus* are recommended as biofuel species when cultured under 14:10 light to dark photoperiod in 4.8 mg L^{-1} of P and 0.5mL $^{-1}$ concentration of micronutrients.

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