Field and experimental studies on phytoplankton-zooplankton interaction in Lake Koka, with particular references to control of *Microcystis* species

A thesis submitted to the Joint MSc Program of Addis Ababa University and Bahir Dar University in partial fulfillment of the requirements for the Master of Science in Aquatic Ecosystems and Environmental Management (AEEM)

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This is to certify that the thesis prepared by Aynalem Tilaye,Field and experimental studies on phytoplankton-zooplankton interaction in Lake Koka, with particular references to control of *Microcystis* species , and Submitted to the Department of Zoological Sciences in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Aquatic Ecosystem and Environmental Management).

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TABLE OF CONTENTS

Contents **Pages**

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Blooms of Microcystis aeruginosa frequently occur in many eutrophic lakes in Ethiopia,however, there is very little experimental study on the relationship between Microcystis and Zooplankton from Ethiopian waters. The effects of different concentrations of toxic M. aeruginosa on two common freshwater zooplankton Cladoceran (Moina micrura)and Cyclopoid were investigated in laboratory experiments.

The main purpose of this study was to examine if there is a difference in the development of tolerance to toxic Microcystis among two species of zooplankton copepoda (Cyclopoid) and a cladocera (Moina micrura)exposed to toxic Microcystis. We tested whether exposure to a toxic strain of cyanobacteria (Microcystis) affects survival, and growth, of a common herbivore, Cyclopoid and cladoceran(Moina micrura). In the experiments, cultures from the lakes will be test at five concentrations of toxic Microcystis aeruginosa: 0%, 20%, 50%, 80% and 100%.After 16 days, we compare the ability of these two populations to withstand the toxic Microcystis by assessing survivorship, and growth rate.Field study showed that Surface water temperatures of all sampling sites were within the range of variation reported for most tropical water bodies (23-25ºC). Surface water DO(mg L^{-1}), which was varied from 5.48 of the HEPP site to 6.99 of the MetoAleka shore site. Mean levels of nitratevaried between 0.11 HEPP and 0.224 mg L^{-1} Tannery, while those of ammonia ranged from 0.054 of the MetoAlekashore site to 0.033 Tannery mg L^{-1}. Mean SRP (in mg L^{-1}) 0.083 and 0.09 MetoAleka shore, TP, ranged from 0.254 mg L^{-1} Tannery of the site to 0.298 mg L^{-1} of the MetoAleka shore). The phytoplankton communities of all sampling sites were dominated, in terms abundance, by three alga groups, Cyanobacteria (blue-green algae), Bacillariophyceae (diatoms) and Chlorophyceae (green algae). Chlorophyceae was the most species-rich taxonomic group, followed by Cyanophyceae and Bacillariophyceae. The most abundance species were Microcystis sp, and Planktothrix cf.agardhii in Lake Koka. The zooplankton community in Lake Koka was dominated by the copepod which accounted for about 56% of total zooplankton abundance. From experimental result we get more survival rate for cladoceran treatment and low for copepod treatment. It can be suggested that biomanuplation controlling methods can be used to improve water quality in Lake Koka .

Key words*: biomanuplation,cyanobacterial bloom,*eutrophication*, grazing, Microcystis spp, zooplankton.*

1. INTRODUCTION

1.1. Background and justification

Fresh water is one of the most important resources on the planet and has many ecological and economical uses and values. Lakes, reservoirs, rivers and streams, and wetlands provide water used for drinking, washing, cooking, irrigation, sanitation and recreational purpose.

Ethiopia has a number of lakes, most of which are found within the rift valley, that are central for socio-economic developments in the region. Lake Koka (Koka Reservoir), an artificial lake located in the upper part of the Ethiopian rift valley, was initially created in 1960 by damming the Awash River for hydroelectric power generation although it has also been used for fulfilling different social and ecological needs including drinking water supply, livestock watering, irrigation, etc. (Seyoum Letta *etal*., 2003). Lake Koka, like the other lakes of the same region, also supports large commercial fishery providing food and income for thousands of local inhabitants (Feyissa, 1983)

Despite their great importance to economic development, Ethiopian lakes including Lake Koka have been subjected to natural and anthropogenic threats due to overfishing, water abstraction largely for irrigation, water pollution through direct discharge of domestic and industrial wastewaters and extensive use of pesticides and fertilizers on agricultural lands located within their catchment areas and overgrazing and deforestation that led to siltation and nutrient enrichment. Developmental activities taking place within the catchment areas of lakes including industries and agriculture represent the most serious threats to aquatic resources. For example, the tanneries that are established on both sides of the course of Mojo River are damping their wastes including toxic chemicals into the river. Seyoum Letta *etal*., (2003) reported that pollution parameters along the Modjo River, which eventually flows into Lake Koka, were too high to meet the provisional discharge limits set by the Ethiopian Environmental Protection Agency (EEPA) especially for most toxic substances such as chromium, sulphide and ammonia (Seyoum Leta *etal*., 2003). In addition to tanneries, there are also other industries, horticultures and small scale factories producing soaps and plastics, which are operationally situated along the banks of lakes and its feeder rivers. Water quality deterioration resulting from the afore-mentioned human activities has consequently become a problem of nation-wide concern.

Pollution of water bodies in Ethiopia, particularly Koka Reservoir has led to eutrophication and the consequent excessive proliferation of algae, primarily cyanobacteria, which adversely affect the suitability of its water for drinking and livestock watering and the fish that can be harvested from it. Studies made on Ethiopian rift valley lakes reported the development of cyanobacterial blooms dominated by *Microcystis aeruginosa* (Hadgembs Tesfay, 2007;Willén *etal*., 2011; Yeshiemebet Major *etal*., 2016) and presence of *Microcystins* in four of the lakes of the Ethiopian Rift Valley (lakes Chamo, Langano, Ziway, and Koka). Furthermore, cyanobacterial blooms can have considerable impact on both biodiversity and ecosystem functioning, as well as ecosystem services, such as recreation and drinking water supply, making the water resource less desirable and even harmful(Anderson *etal*., 2002).

Therefore, the development of mitigation strategies to reduce and prevent occurrences of the cyanobacterial blooms in water systems is an urgent and serious need. Much effort is, therefore, invested in preventing or controlling cyanobacterial blooms. Studies made so far have suggested different methods to control cyanobacteral blooms such as physical, chemical, and biological control methods. As environmentally-friendly methods of control of toxic cyanobacterial blooms are the most desirable methods, several recent studies have focused on the potential agents of biological control for the prevention of cyanobacterial blooms(i.e. bacteria, viruses and unicellular grazers) (e.g. Nishibe *etal*.,2004; Choi *etal*., 2005; Tucker and Pollard 2005; Honjo *etal*., 2006; Zhang *etal*., 2008; Van Wichelen *etal*., 2010), exploitation of allelopathic interactions (Wu *etal*., 2011) or manipulation of fish stocks (Jeppesen*etal*.,2007; Kasprzak *etal*., 2007). Trophic cascade effect through biomanipulation and grazer-mediated bloom control is among the different methods that are used for controlling blooms.

The biomanipulation controlling methods is simply the reduction of planktivorous fishes, which results in higher densities of herbivorous zooplankton and consequently in lowered densities of algae due to zooplankton grazing (Shapiro *etal*., 1975; McQueen, 1990).

Numerous experiments have shown that cyanobacteria, and especially *Microcystis*, reduce zooplankton fitness via defensive traits such as toxicity and morphological features(filament length or colony size), as well as low nutritional quality(Fulton and Paerl, 1987; Ghadouani *et al*.,2004; Ger*etal*., 2010). However, increasing reports of high zooplankton biomass co-occurring

with toxic blooms suggest that tolerant zooplankton may be common in bloom dominated waters (Bouvy *etal*.,2001; Souza *etal*., 2008;Lacerot*etal*.,2013).

Several studies indicate that *Daphnia* may control the development of *Microcystis* blooms (e.g. Sarnelle, 2007; Dejenie *etal*., 2009; Peretyatko *etal*., 2010). Sarnelle & Wilson (2005) suggested that *D. pulicaria* populations, exposed to high cyanobacterial levels over long periods of time, can adapt in terms of being more tolerant to dietary toxic cyanobacteria. Gustafsson *etal*.,(2005) conducted a study in which *Daphnia magna* individuals were exposed to toxic *Microcystis* and found that *Daphnia* were able to develop and pass the defense mechanisms to their offspring. Other studies, however, reported that zooplankton feed on small seston particles and grazing on colonial or filamentous cyanobacteria does not seem to be the general pattern in tropical freshwater ecosystems (Yeshimebet Major *etal*.,2017). This could be due to the dominance of small zooplankton in tropical waters including Lake Koka. Moreover, maximum zooplankton herbivory is not generally achieved in natural environments due to fish predation (Jeppesen *etal*., 2000), which makes it difficult to quantify the potential of large, efficient zooplankters to regulate the growth of cyanobacteria. For example, body-size is a critical trait shaping consumer-prey interactions and size-dependent predation by fish preferentially eliminates large-bodied grazers, such as the crustacean Daphnia (Brooke *etal*., 1965). Large-bodied generalist grazers like *Daphnia* have higher grazing rates than smaller-bodied zooplankters (e.g., copepods and small cladocerans) and may dominate plankton communities at low levels of fish predation (Jeppesen *etal*., 2000).

Analysis of the zooplankton community in Lake Koka by Yeshiemebet Major (2016) revealed the dominance of cladocerans by small species, *Ceriodaphnia cornuta*, *Diaphanosoma excisum* and *Moina micrura*,with rare occurrence of the large cladoceran *D. barbata* and the dominance of copepods by *cyclopoid s*sp. The present study aimed to investigate the existing condition of Lake Koka, with particular focus on physico-chemical variables, and composition and density of phytoplankton and zooplankton and examine the potential of selected zooplankton taxa to control the frequently bloom-forming cyanobacterial genus *Microcystis.*

1.2. Statement of the problem

The ongoing and predicted global increase in cyanobacterial dominance is one of the most visible threats to the ecology of lakes, rivers, and some estuaries (Paerl and Huisman, 2008).

Massive proliferations of cyanobacteria caused by continuing Eutrophication have strong impacts on food web interactions and ecosystem function, as well as on ecosystem services, through increased hypoxia and 'dead zones' .

Cyanobacteria can produce toxic compounds known as cyanotoxins such as neurotoxins, cytotoxins, dermatotoxins, and hepatotoxins. Globally, the most frequently found cyanobacterial toxins in blooms from fresh and brackish waters are the *microcystins*, which are generally produced by *Microcystis* spp. (Chorus and Bartram, 1999).

Current occurrences of toxic algal blooms of *Microcystis aeruginosa* in Lake Koka have been reported by several authors(Hadgembs Tesfay, 2007;Willén*etal*., 2011; Yeshiemebet Major,2016). Willén *etal*.(2011) reported that the *Microcystis aeruginosa* concentration up to 100,000µgL^{-1}, which were above WHO guidance and also responsible for the death of livestock. The cause for this algal bloom seem to be the result of pollution of Lake Koka with nutrients originating from nearby agricultural lands on which fertilizers were applied(Hadgembs Tesfay, 2007). Most people use this lake for drinking; washing purpose as result this could cause health problem for human and the aquatic animals. Therefore there is an urgent need to find suitable methods to control the frequency of harmful cyanobactera (microcystis).

The present study attempts to investigate the existing condition of Lake Koka focusing on physico-chemical variables, phytoplankton and zooplankton composition and density, and conduct grazing laboratory experiment to examine the potential of tropical freshwater zooplankton to control cyanobacterial bloom development. For the grazing experiment, dominant species of zooplankton (copepods, cladocerans) were selected as target species to assess its grazing effect on cyanobacteria (*Microcystis*), which frequently bloom in Lake Koka (Yeshiemebet Major,2016).

Resistance to toxins has previously been tested by exposing *Daphnia* to toxins during a period (in the order of weeks or months) of culturing (Guo 2006; Gustafsson and Hansson 2004; Sarnelle 2005). While the concept is similar, this study will include *cladocera and copepod* which have exposure to microcystis as a result of bloom formation in lakes. Studying the effects of zooplankton on Microcystis was significant in determining its effectiveness in controlling algal blooms and to improve water quality.

1.3. Research questions and objectives

1.3.1. Research questions

- How are the physico-chemical variables such as major algal nutrients in Lake Koka?
- How is the phytoplankton and zooplankton community structure in Lake Koka?
- How is the biomass of phytoplankton and zooplankton communities in Lake Koka?
- Do zooplanktons influence the growth rate of *Microcystis*?
- How are the survival rate and grazing efficiency of a copepod (*cyclopoid spp.*) and a cladoceran (*Moina micrura*) at different concentrations of *Microcystis?*

1.3.2. Objectives

General objective

To assess the grazing effect of tropical zooplankton on *Microcystis* and examine their potential use as biomanipulation technique to control algal blooming in rift valley lakes, which are affected by eutrophication and algal blooming.

Specific objective

- To investigate physico-chemical variables including major algal nutrients in Lake Koka
- To determine the species composition of algal and zooplankton communities of Lake Koka
- To estimate the biomass of phytoplankton as chlorophyll-a concentration.
- To determine the influence of zooplankton on *Microcystis* growth.

- To investigate the survival rate and grazing efficiency of a copepod (*cyclopoid*) and a cladoceran (*Moina micrura*) at different concentrations of *Microcystis.*

1.3.3. Hypothesis of the problem

- **For field work**

It is hypothesized that there are changes in physico-chemical water quality and phytoplankton community structure in the Lake Koka and significant variations among sampling sites due to the impacts of agricultural practices, industrial and domestic influents.

- **For laboratory experiment**

It is hypothesized that *Cladoceran* can develop tolerance to microcystin exposurediet then *copepod.*

2. LITERATURE REVIEW

2.1. Distribution of Harmful algal blooms throughout the World

Have toxic algal blooms increased in frequency during the last 30 years?

The answer to this question is obviously yes as the frequency of toxic algal blooms has increased. The increase in the number of toxic algal taxa, number of affected regions and reports of fish kills, and mussel intoxication of larger mammals also corroborates the above argument.

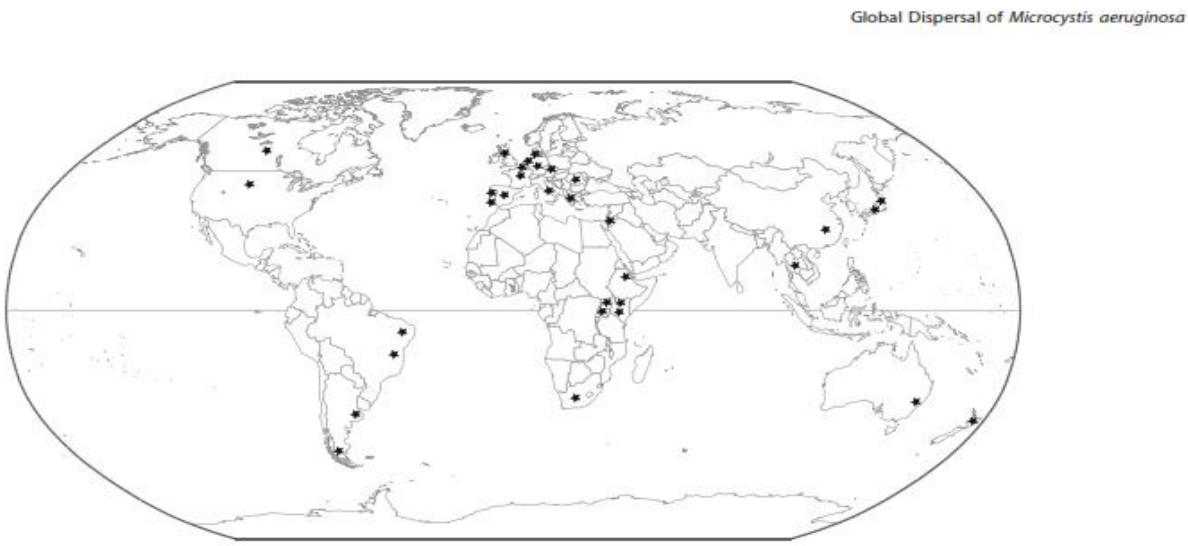

Figure 1.Map indicating the origin of the Microcystis ITS sequences used in this study.doi:10.1371/journal.pone.0019561.g001

Several decades ago relatively few countries appeared to be affected by HABs, but now most coastal countries are threatened, in many cases over large geographic areas and by more than one harmful or toxic species. Many countries are faced with a bewildering array of toxic or harmful species and impacts, as well as disturbing trends of increasing bloom incidence, with larger areas affected, more fisheries resources impacted, and higher economic losses. In recent years, surveys have been carried out in a number of countries in South America, Africa, Australasia, Asia and Europe.

2.3. Cyanobacterial blooms in Africa

In most African countries wars, poverty, water pollution and disease outbreaks have become common with rapid population increases, as most countries are developing. This, of course, ties in with nutrient loading into waters from wastes and emerging contaminants of concern, as well as added pressures on the efficiency of current wastewater treatment facilities.

In Ethiopia, Lake Tana is the largest water body and had reports of *Microcystis aeruginosa* occurrences, with microcystin concentrations of up to 2.65µgL^{-1}(Mankiewicz-Boczek *etal*., 2014). Earlier studies conducted to determine whether prior animal deaths were due to cyanotoxins also indicated the presence of microcystin-producing strains in seven rift valley lakes, with Lake Koka being a high risk lake with cyanobacterial cell numbers exceeding100,000cells mL^{-1} and total microcystins concentration of 45µgL^{-1}(Willén *etal*.,2011).

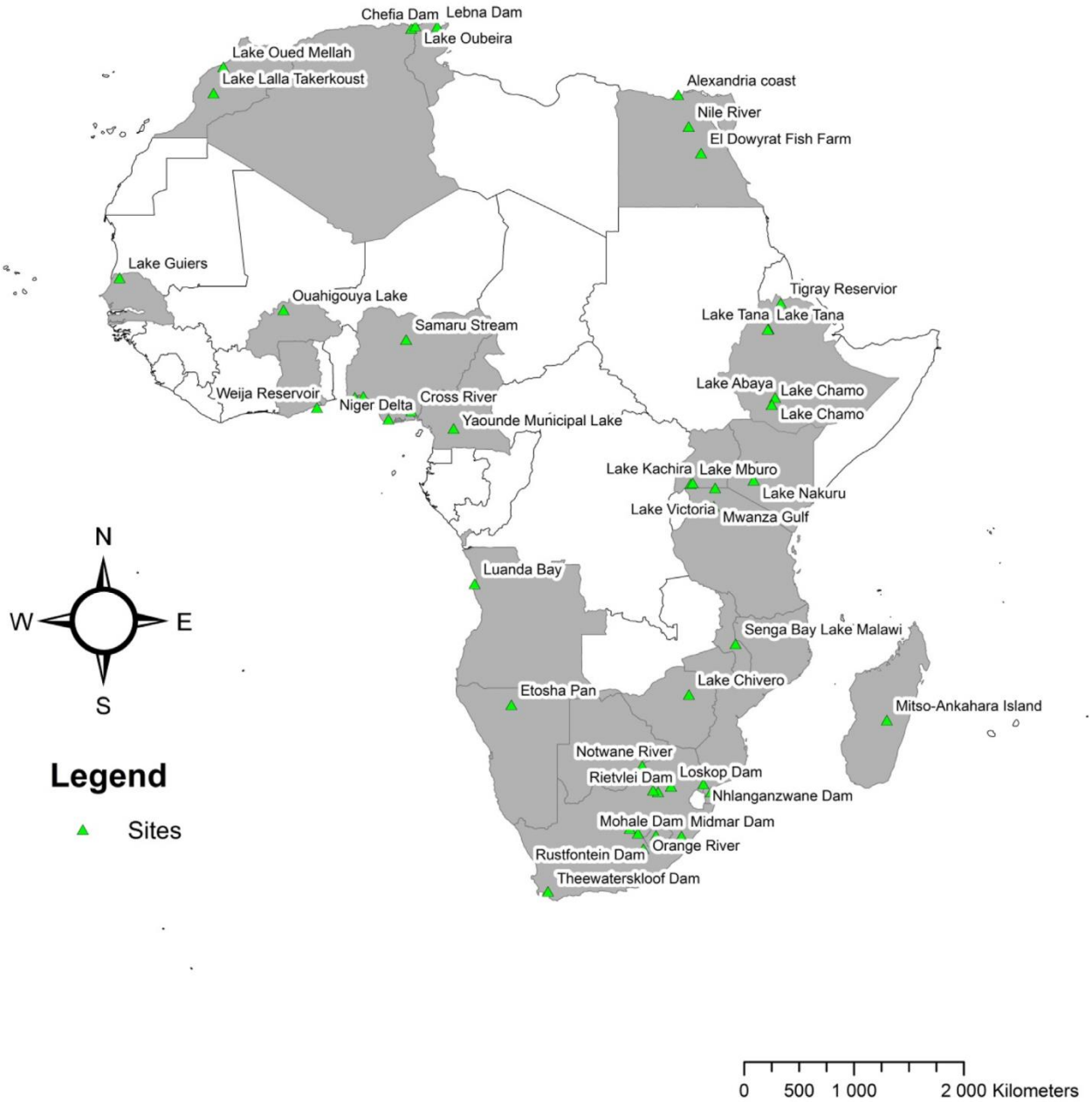

Figure 2.Summary of areas affected by cyanobacteria blooms in Africa(Ndlela *etal*.,2016)

2.4.Cyanotoxins and Effects on Aquatic Communities

2.4.1.Cyanobacteria and their toxins (Cyanotoxins)

The toxins produced by cyanobacteria are commonly known as cyanotoxins. Cyanotoxins are generally divided into categories based on their modes of action in mammalian test systems (Codd, 1995; Sivonen and Jones, 1999):hepatotoxins, dermatotoxins and neurotoxins. Although

the most studied cyanobacterial toxins are the hepatotoxins and neurotoxins, other cyanobacterial endotoxins called lipopolysaccharides (LPS) have also been implicated in human illness.

Table 1. General features of the cyanotoxins(WHO,1999)

Toxin group	**Primary target organ in animals**	**Cyanobacterial genera**
Microcystins	Liver	*Microcystis,Anabaena,Planktothrix(Oscillatoria),Nostoc,Hapalosiphon,Anabaenopsis*
Nodularin	Liver	*Nodularia*
Anatoxin-a	Nerve synapse	*Anabaena,Planktothrix(Oscillatoria),Aphanizomenon*
Anatoxin-a(S)	Nerve synapse	*Anabaena*
Aplysiatoxins	skin	*Lyngbya,Schizothirx,Plankthrix(Oscillatoria)*
Cylindrospermopsins	Liver	*Cylindrospermopsis,Aphanizomenon,Umezakia*
Lyngbyatoxin-a	Skin,gastro-intestinal tract	*Lyngbya*
Saxitoxins	Nerve axons	*Anabaena,Aphanizomenon,Lyngbya,Cylindrospermopsis*
lipopolysaccharides (LPS)	Potential irritant ; affect any exposure tissue	*All*

Table 2.WHO recreational water guidelines for human health risk (WHO,2003)

Probability of adverse health effects	**Cell concentration (per ml)**	**Chlorophyll-a concentration(µg/L)**
Relatively low	<20,000cells	<10
Moderate	20,000-100,000cells	10-50
High	>100,100cells	Visible scum

2.4.2. Effects of cyanobacteria on aquatic communities

The excessive growths of cyanobacteria and eukaryotic algae have important consequences on the physical and chemical characteristics of water bodies. Common effects include the decrease in water transparency, dissolved carbon dioxide concentration and an increase pH, and the alteration of biogeochemical cycles.

Effects on Zooplankton

The study of the interactions between cyanobacteria bloom and zooplankton has become important because of the potentials for top-down control of cyanobacterial blooms by zooplankton (Burns, 1987). A large body of research has been directed at understanding the mechanisms by which zooplankton affect cyanobacteria and vice versa.

Cyanobacteria are characterized by several adaptations that may make them less sensitive to zooplankton grazing than other phytoplankton groups. Many cyanobacterial species form colonies that are often too large to be ingested by zooplankton (Gliwicz, 1990, Gliwicz and Lampert, 1990). In addition, several species produce toxins (Ferreira*etal*., 2001, Pereira *etal*., 2000), the effect of which ranges from a reduction in feeding activity (Haney *etal*., 1994) to immediate death (Reinikainen *etal*., 1994). For example, the filamentous morphology of certain cyanobacteria has been shown to negatively affect large cladocerans more than small cladocerans through reduced fecundity (Webster and Peters, 1978, Gilbert, 1990). Two major mechanisms have been proposed for the greater susceptibility of large cladocerans to colonial or filamentous blue-green algae (Webster and Peters 1978, Richman and Dodson, 1983, Porter and McDonough, 1984): 1) colonial or filamentous algae clog the filtering appendages of larger cladocerans, reducing their feeding rates on co-occurring nutritious food sources or increasing their respiration rates or both, 2) larger cladocerans ingest more readily colonial or filamentous blue-greens and are, therefore, more strongly affected by any toxic chemicals that these algae may possess. These characteristics suggest that cyanobacteria may be quite resistant to zooplankton grazing, and this has indeed been reported by many studies (Sarnelle, 1993; DeMott, 1999). Yet, several studies report that the development of cyanobacteria can be suppressed by zooplankton grazing (Haney, 1987, Matveev *etal*., 1994).

A number of studies suggest that the dominance of *Daphnia* in the zooplankton community results in a reduced likelihood of cyanobacterial blooms (MacKay and Elser, 1998, Smith, 1983). This may at first sight seem counter-intuitive, because it has been shown that especially large-bodied cladocerans such as *Daphnia* suffer from clogging of their filtration apparatus by cyanobacterial filaments (Gliwicz, 1990). Yet, *Daphnia* are also the most efficient grazers of phytoplankton, and one likely scenario may be that grazing by *Daphnia* may prevent cyanobacteria to develop dense populations and produce filaments (Gliwicz, 1990). If so, timing of the presence of *Daphnia* can be important, because efficient grazing could then prevent bloom formation but not suppress an existing bloom. As such, *Daphnia* would promote dominance of green algae over cyanobacteria indirectly, through a bottom-up effect.

Cyanobacteria species such as *Microcystis* have been shown to be polymorphic with respect to the production of toxins, with non-toxic and toxic strains often coexisting and showing seasonal changes in relative abundance (Kirk and Gilbert, 1992, Barreiro *etal*., 2007). There is a need for studies that systematically investigate the relationship between zooplankton, and especially *Daphnia*, and the occurrence of cyanobacteria in the phytoplankton community, their tendency to bloom, and the degree to which these blooms are toxic. It is currently insufficiently known whether grazing of zooplankton actually reduces or enhances cyanobacteria blooms and to what extent this is dependent on species composition of the zooplankton community.

Effects on Fish

Fish, especially phytoplanktivorous species, can be exposed to *Microcystins* while feeding or by passive means when the toxins pass through the gills during breathing. Fish in the early stages of life are generally more sensitive to toxic compounds than adults and juveniles, probably due to their thin epithelial layer combined with a relatively large body surface area and high metabolic rate (Malbrouck and Kestemont, 2006).

Impacts on Human Health

Microcystins have caused human poisoning worldwide. The main routes for cyanobacterial toxins in humans are ingestion (toxins in water, contaminated food), inhalation (water sports, bathing in contaminated waters), skin contact (recreation, bathing) and intravenous introduction (dialysis). The liver is the most affected organ in humans, but exposure to the toxin is likely to

affect such other organs as kidneys and colon, as evidenced by *in vivo* and *in vitro* studies. As a result, the illnesses attributed to *microcystin* poisoning are gastroenteritis and related diseases, allergic and irritation reactions, and liver diseases. Some lesions can evolve into tumors and primary liver cancer and colorectal cancers in human populations have been related to microcystin exposure and toxicity (Campos, and Vasconcelos,2010).

Exposure to cyanobacterial toxins through consumption of contaminated drinking water has caused serious poisoning in humans, even leading to fatalities(Falconer *etal*.,2005). Therefore, in 1997, the World Health Organization established a provisional reference value of 1 µg/L for microcystin-LR in drinking water (WHO,2012). In addition to the oral route, there is the possibility of the parenteral route of exposure, when surface waters infested by cyanobacteria are used for hemodialysis, considerably increasing the internal dose of toxins, which enter the blood and therefore, endanger the life of patients (Funari, and Testai, 2008). Various other cases of human poisoning due to the presence of cyanotoxins dissolved in water have been described.

However, beyond the possibility of human contamination through the water supply, there is also the risk of contamination through the food chain. Bioaccumulation of cyanotoxins and transfer through the food chain has been demonstrated in several studies. There is a possibility that these toxins reach humans by the consumption of fish. Some, like tilapia and carp, can consume the cells of cyanobacterial toxins in water, accumulating them in the liver, kidneys, muscles, and viscera (Ferrão-Filho,2009) .

2.5. Control methods

Methods to control these Harmful algal blooms, to minimize the potential adverse effects on the environment and to mitigate economic loss would be advantageous. Physical, chemical and biologicalmethods have been used in freshwater and marine systems for small and large-scale control of HABs due to their significant public health, economic and ecosystem impacts (Chorus and Bartram ,1999).

2.5.1. Physical(Mechanical)Control

Various mechanical bloom control strategies have been identified, but it is not known the extent to which these can be applied to particular harmful algal bloom species in specific environments or habitats. Physical control involves removing the harmful algal cells via filtration, skimming, ultrasound, electrolysis or similar methods (Rey, 2007). Pumping of surface algal scums from in shore areas hasproven be an effective mechanism to temporarily protect recreational users of freshwater lakes from exposure to toxic cyanobacteria and filtration has been used effectively in purification of drinking water supplies (CENR, 2000).

2.5.2. Chemical Control

Chemical control is the use of chemicals to kill or reduce the density of HAB cells. A variety of chemicals such as copper sulfate, sterol surfactants, sodiumhypochlorite, magnesiumhydroxide and others have been tried for control of HAB organisms. In general, most chemicals tried have been too expensive and too non-specific (causing damage to non-target components of the ecosystem) for effective use against HABs (Rey, 2007).Chemicalshas been used to treat blooms in drinking water supplies and other enclosed freshwater systems. These include copper compounds, barley straw and chemical oxidants such as chlorine, peroxide, ozone and chloramines (Chorus *etal.*,1999)

2.5.3. Biological control

It has been argued that a reduction in both external and internal phosphorus sources is the best way for a long term reduction of cyanobacterial blooms in alake (Cooke *etal*.,2005). But, the dominance of blue-greens in alake is determined not only by the availability of nutrients, but also by the proper structure of food webs in the ecosystem, *e.g.*by the appropriate zooplankton:phytoplankton ratio. There are a lot of cases where, after a reduction in nutrient loading, cyanobacterial blooms are present, mainly because of a dense stock of planktivorous fish in the lake. The fish suppress the zooplankton population, so the factor, which can effectively control the phytoplankton population is lacking (Jeppesen *etal*.,2000).

Biomanipulation is the most often used method in the biological control of algal blooms. The method consists of reducing the pressure of planktivorous fish on crustacean plankton, which feed on phytoplankton (Shapiro ,1990). The desired result can be achieved by stocking the predatory fish and/or by removing the planktivorous fish. Many authors have suggested that

Daphnia spp. is one of the foremost contributors to the success of biomanipulation (Matveev *etal*. 1994 ; Sarnelle 2005).

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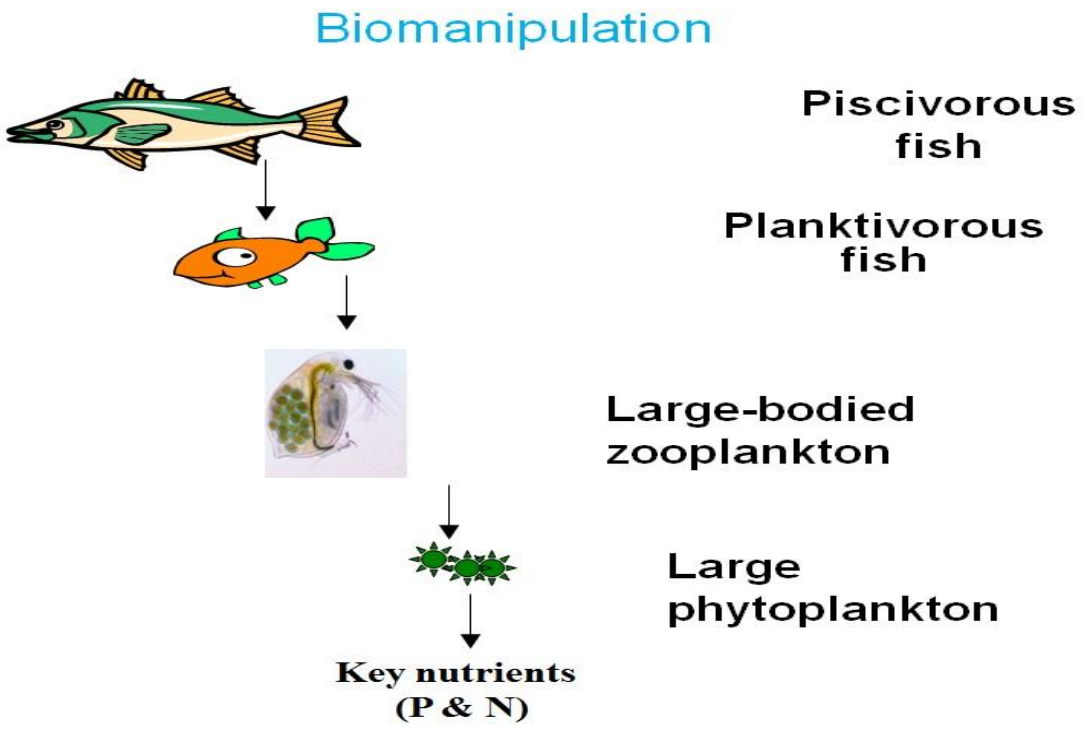

Figure 3.Schematic representation of Biomanipulation

3. MATERIALS AND METHODS

3.1. Study site

The tropical Lake Koka, also called Lake Galilea in the old literature, is located some 90 km Southeast of Addis Ababa in the Ethiopian Rift Valley at a geographical position of 08°23'22" N - 39°05'15" E and at an altitude of 1590 m a.s.l.. There are two inflowing rivers: Awash (major) and Modjo (minor), which flow into the lake in its western part. The Reservoir has an outflow called Awash River (Melaku Mesfin *etal*., 1988).

The reservoir area is characterized by a bimodal rainfall pattern with a short minor rainy season (March-May), and a long major rainy season (June to September), with the dry period extending from October to February.

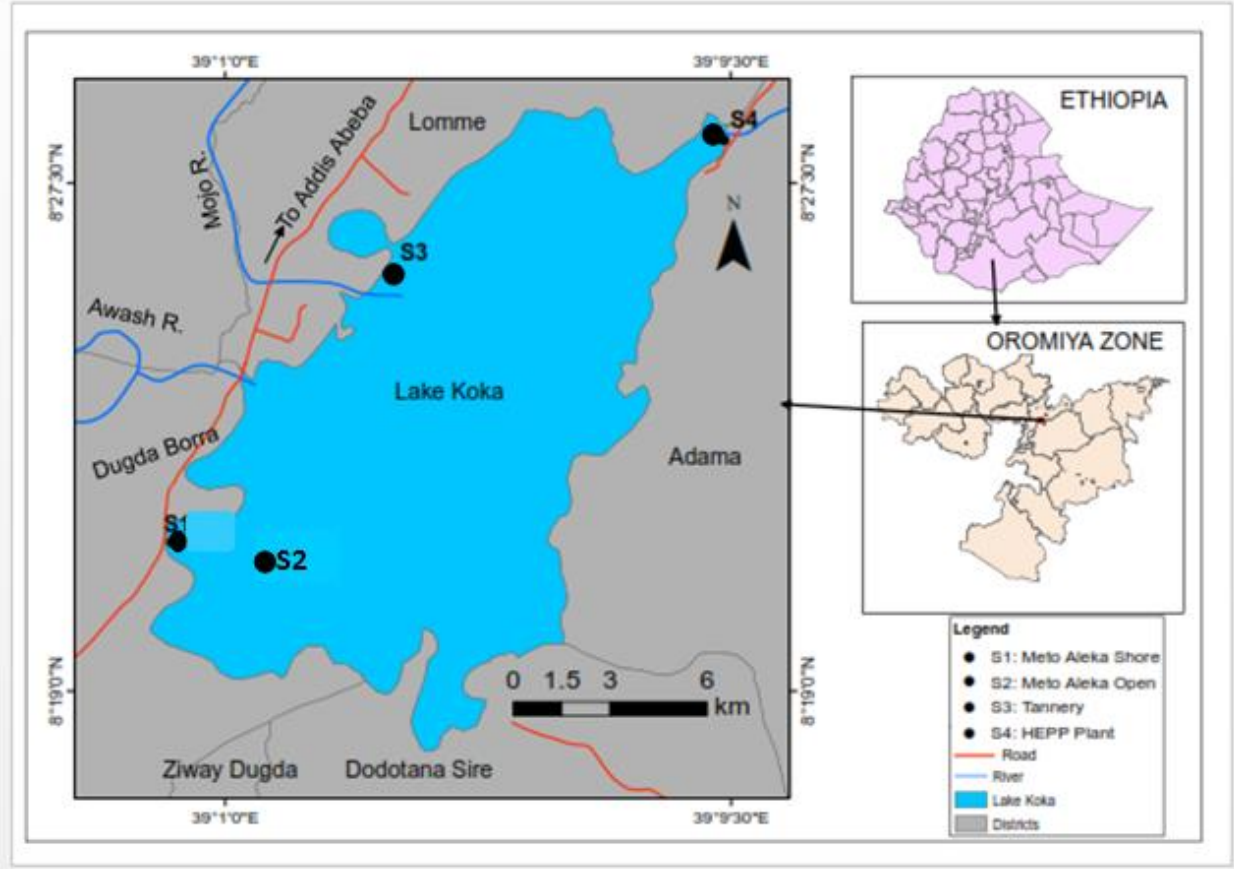

Figure 4.Location map of Lake Koka with sampling sites indicated as dots.

3.2. Sampling protocol

In the present study, four sampling sites were selected (Table 6) based on the type and degree of human impacts. All of the Four sampling sites (Tannery, HEPP and Meto Aleka shore,) are found littoral parts of the lake affected by agricultural practices and presence of industrial and domestic effluents. Meto Aleka Open, the fourth sampling site, is a relatively open water site and represents less impacted site. At all four sites, monthly sampling was carried out from May, 2017 to August, 2017.

Table 3. Description of sampling sites

Sampling site	Name	Altitudes (m)	Coordinates
S1	Meto Aleka shore	1590	08^{o}21'.442"N,039^{o}00'.098"E
S2	Meto Aleka open	1588	08^{0}21'.437"N,039^{0}00'.336"E
S3	Tannery	1583	08^{0}27'.138"N,039^{0}04'.818"E
S4	HEPP(hydro electric power plant)	1585	08^{0}28'.188"N,039^{0}09'.382"E

Samples were collected using a bucket of 10 liter capacity at the sampling sites and placed inside plastic bottles for chemical analysis. Then the samples were poured into plankton net with a mesh size of 15µm. A 100ml concentrate was produced from the sample collected at the bottom of the net. This concentrated sample was used for the determination of abundance of phytoplankton taxa. For zooplankton sample taken vertically by using mesh size of 30µm. The filtered sample placed inside 100ml bottle immediately preserved phytoplankton with Lugol's iodine solution and zooplankton with formalin (5%), respectively. All water and plankton samples were transported in cool icebox to the Limnology laboratory of Addis Ababa University.

3.3. Measurement of Physico-chemical parameters

Measurements of important physicochemical parameters were concurrently made. Lake water transparency was estimated with a standard Secchi disk of 20 cm diameter. DO (mg L^{-1}), conductivity (µS cm^{-1}), and pH were measured with a digital multi-parameter meter (HQ40d).

Unfiltered water samples were used to analyze total phosphorus(TP), while filtered water samples were used to determine soluble reactive phosphate-phosphorus (PO_4-P, SRP) with the Ascorbic Acid method (APHA, 1999). The Soluble reactive silica (SiO_2) was measured using molybdosilicate method (APHA 1999), nitrate-nitrogen(NO_3-N) was analyzed using the sodium-salicylate method (APHA 1999). nitrite-nitrogen(NO_2-N,) analyzed using the reaction between sulfanilamide and N-naphthyl-(1)-ethylendiamin-dihydrochloride and ammonia-nitrogen (NH_4^+-N,) was analyzed with the indo-phenol blue method (APHA, 1999).

3.3. Measurement of Biological Parameters

3.3.1 Species composition and abundance of phytoplankton

Samples used for the identification and estimation of the abundance of the different taxa of the phytoplankton community in the reservoir were collected with a plankton net of 15 µm mesh size and stored in brown bottles. The samples were immediately preserved using Lugol's solution and placed in a refrigerator. The identification of phytoplankton species using these preserved samples involved the use of various taxonomic references(e.g. Gasse,1986;Komarek *etal.*, 2002 and Cronberg and Komarek,2004).The major phytoplankton species were enumerated on a Sedgewick-Rafter cell with an inverted microscope (A.Kruss optronic) at a magnification of x40/0.60 in randomly selected grids(squares). Additionally for further magnification we use x100 by using oil immersion. As above mentioned the water sample was taken by 10L bucket (i.e 10,000ml). From the concentrated sample, 1 ml subsample was taken with a pipette to enumerate phytoplankton on the Sedgewick- Rafter cell. The phytoplankton species were counted as individual (cells, colonies or filaments) in about 50 random grids then the estimation of phytoplankton abundance was made using the following formula (Hotzel and Croome, 1999).

$$C\ [\text{cells mL}^{-1}] = \frac{N*1000mm^3}{A*D*F*\text{Concentration factor}}$$

Where,

N = number of cells counted

A = area of grid (mm)

D = depth of a grid (Sedgwick-Rafter chamber depth) (mm)

F = number of grids counted.

$$Concentration factor = \frac{volume of lake water filtered\ (ml)}{volume of concentrate\ (ml)}$$

=10000/100

=100ml

3.3.2 Biomass of phytoplankton

Phytoplankton biomass was estimated as chlorophyll-a (Chl-a) concentration spectrophotometrically using100 ml water samples filtered through glass fiber filters (GF/F) and

frozen immediately. Filters containing seston were manually ground in a small volume of 90% acetone with a glass rod to enhance the extraction of chlorophyll-a. The extracts were transferred into a centrifuge tube and centrifuged at 3000 rpm for 10 minutes. Phytoplankton biomass, in terms of chl-a concentration was estimated from absorbance readings at 665 and 750 nm and the concentration of chlorophyll a were calculated according to Talling and Driver, (1963), without phaeopigments correction.

3.3.3 Estimation of zooplankton abundance

Zooplankton Samples were collected using a 30 μm mesh size plankton net towed vertically from Littoral site (4.3m in HEPP,2.7m in MetoAlekashore, 1.3m from Tannery) and open water site (5.3m in MetoAleka open) depth to the surface**.** The samples were immediately preserved in 5 % formalin. For estimation of abundance, sub-samples of 20 ml were taken for counting using a pipette with wider mouth and poured into a gridded Petri dish. Three grids were counted for each sample using well-mixed samples. Counting was done with 50 X magnification under a WILD stereoscope microscope in the Limnology Laboratory of the Department of Zoological Sciences, AAU. Zooplankton species were identified using keys (Fernando, 2002). The volume of water filtered was determined using the following formula:

$$V=\prod r^2 d$$

Where, V is the volume of the water filtered (m^{-3})

r is the radius of the net (m).

d the distance through which the plankton net is towed (m).

Estimation of zooplankton abundance (individual's m^{-3} of lake water) was computed for each month using the formula of (Edmomdson and Winberg 1971).

$$\text{Ind./m3}=\frac{(N*k)}{V}$$

N=the total number of counts; k=the proportion of total volume to subsample volume; v is the amount of water filtered by the sampling net in m^3. Then, the number of organisms per m^{-3} of the lake was calculated.

3.4. Laboratory Experiments

3.4.1. Isolation and culturing of phytoplankton

Parallel to the field work, *Scenedesmus* and *Microcystis* spp., isolated from samples collected from the Lake Koka, were cultured in the Limnology Laboratory of the Zoological Sciences Program Unit, AAU. *Scenedesmus* and *Microcystis* spp., were isolated using micropipette with serial dilution technique (Andersen and Kawachi 2005). BG-11 medium (Rippka *etal*., 1979) was used for culturing *Scenedesmus* and *Microcystis* spp(fig.5). A drop of sample was transferred onto a glass slide and placed under the inverted microscope; the micropipette was used to pick up different cells and then drop onto the side of the glass slide. The individual cells were transferred to multi-well plate, which contained BG-11 medium for isolation. A simple technique to purify a contaminated strain is to proceed with repeated sub-cultures obtained by progressive dilutions of the original sample. Each cycle dilutes the original sample and increases the probability of single-cell isolation; the cycle stops when it is probable that no cell will be transferred. Finally, after a series of similar dilutions and observations, some isolates were picked up with the micropipette and introduced into 250 ml flasks containing 100ml of the BG-11 medium. The cultures were kept at a temperature of 25°C and at an illumination of 70 μmol quanta m^{-2} sec^{-1} (produced by six fluorescent lamps, 58 W each) in 12:12 hours light: dark cycle and shaken three times a day by hand. The cultures were more than 90% of pure *Scenedesmus* and *Microcystis* taxa.

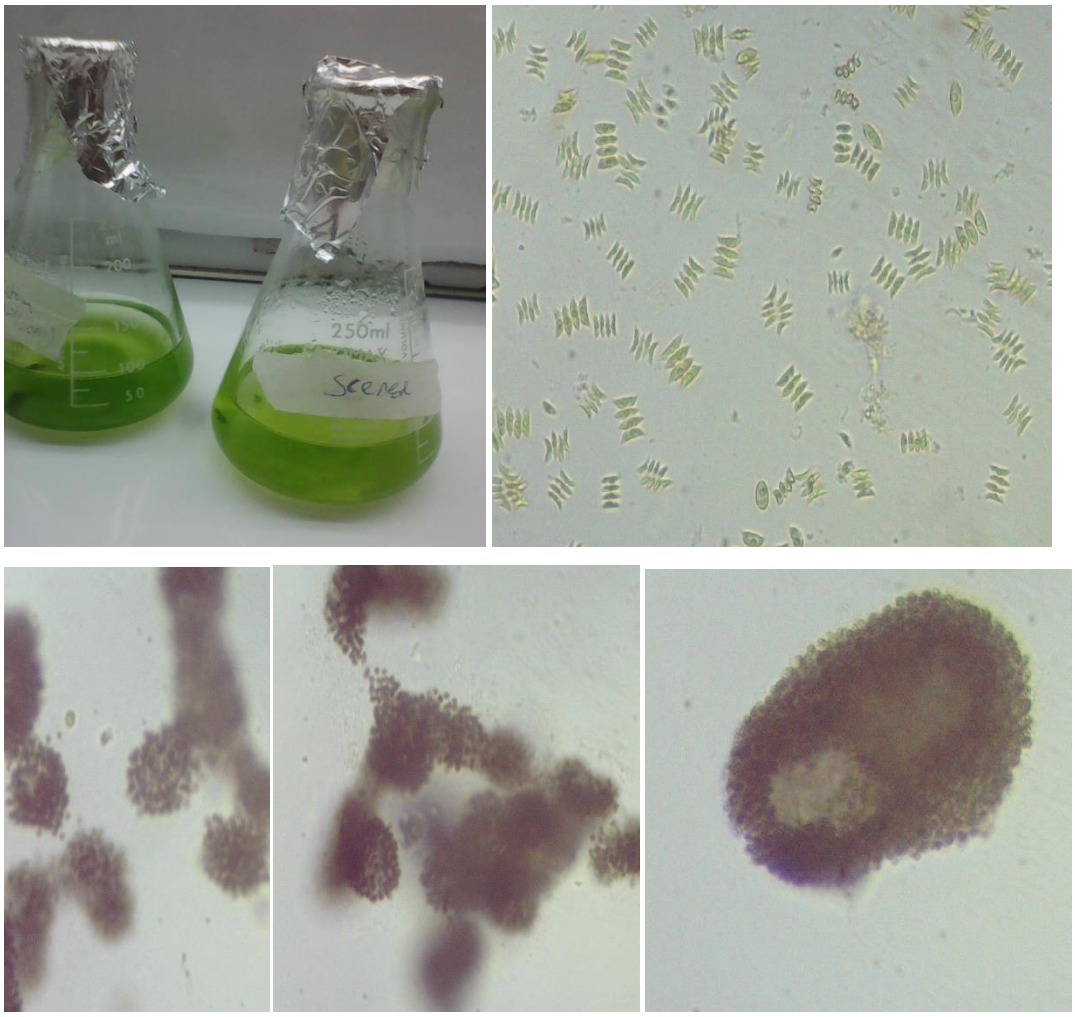

Figure 5.Isolates of *Microcystis* spp. and *Scenedesmus* spp. from Lake Koka used for experimental culture.

3.4.2. Culturing zooplankton

The zooplankton species, a copepod (*cyclopoid spp*) and a cladoceran (*Moina micrura*), were collected from Lake Koka with 30µm mesh size plankton net and used for the feeding experiment(fig 6). The zooplanktons were identified using keys and transferred into Petri dishes using wider mouth pipette. Finally, after isolation, the two zooplankton spp. were separately cultured in beakers under a culture condition, which was the same as that for the phytoplankton taxa:25^{o}C and light: dark cycle of 12: 12 and fed with *Scenedesmus* once every two days (Guo and Xie 2006).

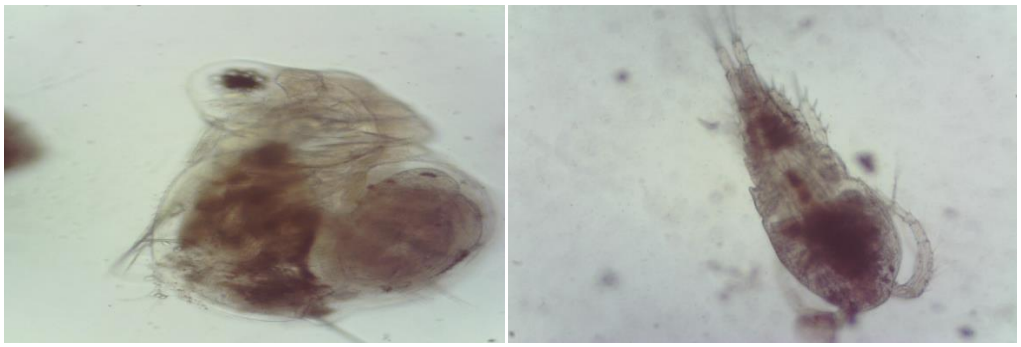

Figure 6.Isolates of cladocera (Moina micrura) and a copepod (Cyclopoid.)from Lake Koka used for experimental culture.

3.4.3 Experimental setup

The present experiment was designed to evaluate the grazing effect of a cladoceran (*Moina micrura*) and a copepod (*Cyclopoid spp.*) on *M. aeruginosa* of different concentrations. This study was composed of two independent treatment (Fig. 7). Each treatment was carried out in triplicate, with each replica having10 adult *Moina micrura* and 10 adult *C*individuals. Before the *Cyclopoid*commencement of the experiment, initial chlorophyll-a concentration was determined. Abundance of *Microcystis* and *Scenedesmus* was monitored using samples taken before the start of the actual experiment (in the inoculums) and during the experiment on days 1, 3, 6, 10 and 13.Phytoplankton spp. cell density was determined prior to dilution aimed at precluding variation in cell concentration that may arise during the addition of fresh food. The experiments for all treatments were conducted in 250 mL flask filled with 100ml algal suspensions constituted by different proportions of the two phytoplankton taxa (*Microcystis* and *Scenedesmus*). *Microcystis* and *Scenedesmus* were mixed with the aim of producing cultures in which *Microcystis* accounted for 20, 50, 80 and 100% of the cultures. The flasks were placed, at a temperature of 25 ^{o}C and photon flux density of 70 µE m^{-2} s^{-1}at a 12 L: 12 D cycle. The experiments lasted for 16 days, as suggested by (Guo and Xie 2006)

As control, the *Microcystis* and *Scenedesmus* without zooplankton was also included to compared the phytoplankton biomass by using Chl-a. At experiment, the conditions were changed by adding various proportions of *Microcystis* (20, 50, 80 and 100%) (Fig. 7). During the experiments, the cladoceran and copepod populations were fed with

0% *Microcystis* and 100% *Scenedesmus* on the 1st day,

20% *Microcystis* and80%*Scenedesmus* on the 3rd day,

50% *Microcystis* and50%*Scenedesmus* on the 6th day,

80% *Microcystis a*nd20%*Scenedesmus* on the10th day, and 100%*Microcystis* on the 13th day.

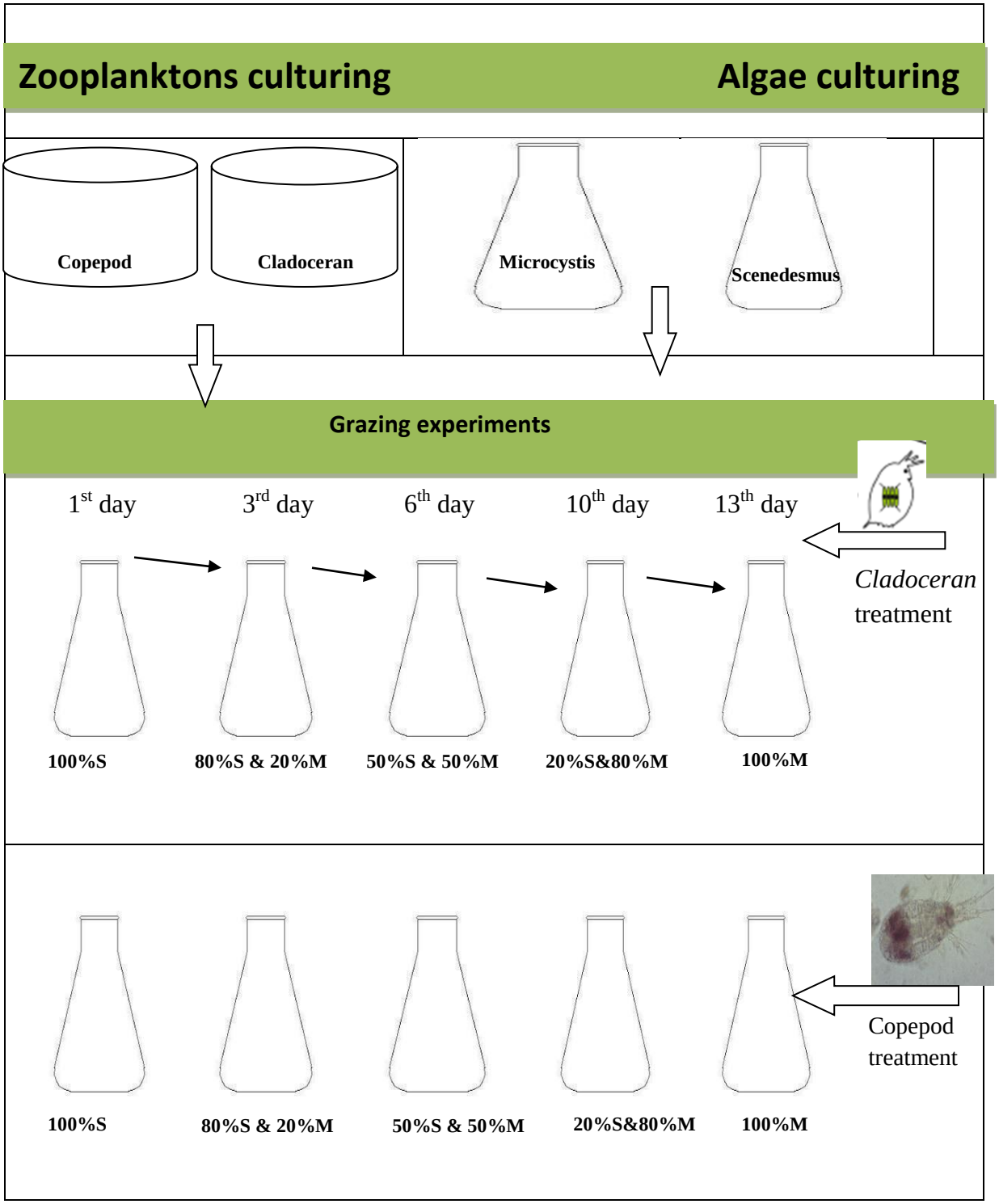

Figure 7.Experimental setup

After the end of the experiment, the cultured samples (50 mL) were filtered through a GF/F glass-fiber filter for chlorophyll-a analysis. The surviving animals were counted, and on the 16th day the surviving animals were killed using 5% Formalin solution.

3.5 Data Analysis

Descriptive statistics was used to analyze the physicochemical data. The variation in physicochemical parameters among the sampling sites was analyzed by using One-Way ANOVA using SPSS version 20. The means physicochemical were separated using Tukey HSD. A Kaplan-Meyer test was used to determine if there were statistically significant differences in the survivorship of the copepod (*Thermocyclops*) and cladoceran (*Moina micrura*) between the two treatments. The Kaplan-Meyer test takes into account the censored survivorship data (Hosmer, 1999).

4. RESULTS

4.1 Physicochemical parameters

The physicochemical parameters measured in the present study exhibited spatial variations (Table 7).

Table 4. Variations in physicochemical parameters measured *in situ* among the sampling sites

Sampling Sites	Physicochemical Parameters				
	pH	**Temperature(^{o}C)**	**Conductivity(µScm^{-1})**	**DO(mg/l)**	**Secchi Depth(cm)**
MetoAleka Shore	8.68a±0.147	25.16a±3.206	126.74a±40.76	6.90a±0.302	7.22a±0.622
MetoAleka Open	8.62ab±0.257	23.32a±4.313	123.24a±38.99	6.99a±0.608	9.45b±0.868
HEPP Shore	8.39b±0.500	23.24a±1.203	86.54b±22.72	5.48a±3.0.65	9.58b±1.886
Tannery	8.45ab±0.169	24.72a±1.755	85.43b±17.18	6.57a±1.209	9.56b±1.672

NB: Means within a column followed by the same letter are not significantly different ($p > 0.05$)

Water temperature and DO values recorded during the study did not show any significant variations among the sampling sites (p=0.152 and p=0.051, respectively). The mean values of pH ranged from 8.39±0.5 at HEPP shore to 8.68±0.15 at MetoAleka Shore, with the value at MetoAleka Shore being significantly higher than those at HEPP Shore and Tannery (at p=0.028) Conductivity measured at the sampling sites, which ranged from 85.43±17.18 at Tannery site to 126.74±40.77 at MetoAleka Shore, varied spatially with MetoAleka Shore and MetoAlekaOpen having significantly higher values than HEPP Shore and Tannery(p=0.00). The minimum Secchidepth(cm) was recorded at MetoAleka Shore (7.22±0.62), while the maximum was observed at HEPP Shore (9.58±1.89). Secchi depth showed variations among the sampling sites (p=0.00), with MetoAleka Shore having significantly lower transparency than the other sites although the variation in the Secchi depth values among the latter was not statistically significant (Table 7).

4.2. Inorganic nutrients

Table 5.Spatial variations in the levels of inorganic nutrients

Sampling Sites	Inorganic nutrients					
	NO_2-N(mgL^{-1})	**NO_3-N(mgL^{-1})**	**NH_4^+-N(mgL^{-1})**	**SiO_2(mgL^{-1})**	**TP(mgL^{-1})**	**SRP(mgL^{-1})**
MetoAleka Shore	0.326cd±0.538	0.186a±0.036	0.054cb±0.020	0.298a±0.032	0.597a±0.188	0.179a±0.002
MetoAleka Open	0.206c±0.258	0.211a±0.088	0.046c±0.014	0.284ac±0.037	0.671a±0.194	0.090a±0.002
HEPP Shore	0.079ac±0.066	0.110a±0.079	0.039bc±0.066	0.289ac±0.041	0.233c±0.057	0.089a±0.001
Tannery	0.107bc±0.120	0.224a±0.116	0.033ac±0.022	0.254bc±0.021	0.253bc±0.154	0.083a±0.001

NB: Means within a column followed by the same letter are not significantly different (p > 0.05)

The levels of nitrate and SRP did not show significant variation among the sampling sites (p=0.74, and p=0.96, respectively). The concentration of nitrite (mgL^{-1}) at the sampling sites ranged from 0.079±0.066 at HEPP Shore to 0.326±0.054 at MetoAleka Shore, with significant variations among the sampling sites (p=0.001). MetoAleka Shore had significantly higher nitrite concentration than the other sites, while MetoAleka Open was characterized by significantly

higher nitrite levels than HEPP Shore and Tannery sites although the variation in nitrite concentrations between the latter two sites was not statistically significant (Table 8).

Silica and TP concentrations (mg L^{-1}) of the sampling sites ranged from 0.254±0.021 at the Tannery site to 0.297±0.032 at MetoAleka Shore site and from 0.232±0.574at HEPP site to 0.671±0.195 at MetoAleka Open site, respectively. The values of silica showed significant variations among the sampling sites (p=0.02), with significantly higher concentration at MetoAleka Shore site than at the Tannery Site although its differences from those of MetoAleka Open and HEPP Shore sites were not statistically significant. The silica level at MetoAleka Open site did not also differ significantly from those of HEPP Shore and Tannery sites. Similarly, TP values of the sampling sites showed significant variation (p=0.002), with the MetoAleka Open site having significantly higher value than HEPP Shore and Tannery sites (Table 8).

4.2 Biological parameters

4.2.1. Species composition and abundances of Phytoplankton

Five phytoplankton taxa, namely Bacillariophyceae (diatoms), Cyanophyceae or cyanobacteria (blue green algae), Chlorophyceae(green algae), Cryptomonaceae (Cryptomonads), and Euglenophyceae (Euglenoids),represented by a total of 37 major species constituted the phytoplankton community of the lake(Table 9).The most species-rich group was Chlorophyceae (with 13 species), followed by Cyanophyceae and Bacillariophyceae, with 12 and 6 species, respectively. The other phytoplankton taxa, which constituted the phytoplankton community of Lake Koka, euglenoids (Euglenophyceae), and cryptomonads (Cryptophyceae), were relatively poorly represented (4 and 1 species, respectively).

Table 6. List of phytoplankton species/taxa identified in samples collected from Lake Koka (May 2017-April 2017).

Phytoplankton group	Identified Species
Blue-green algae (Cyanophyceae)	*Anabaena cf. spiroides* Kleb. *A. circinalis* Aphanocapsa sp. *Cylindrospermopsis africana (*Komarek and Kling). *C.curvispora* (Watenabe). *C.raciborskii* (Seenayya and Subba) *Microcystis aeruginosa Kutzing.* *M. flos-aquae*(Kirchner and Forti)

	M. wesenbergii (Cronberg and Komarek). *Planktothrix cf.agardhii* *Pseudoanabaena* sp. *Raphidiopsis curvata* (Fritsch and Rich)
Diatoms (Bacillariophyceae)	*Aulacoseira granulata (Ehr.) Simons.* *Cyclotella meneghiana Kutz* *Fragilaria sp.* *Navicula sp.* *Nitzchia sp.* *Syndera ulna (Nitzsh.)Lange-Bert*
Green algae (Chlorophyceae)	*Actinastrum hantzschii Lagerh.* *Closterium cf.acutumBreb.* *Coelastrum sp.* *Monoraphidiumsp.Oocystis sp* *Pediastrum duplex v. clathratum Meyen* *P. simplex Meyen* *Scenedesmus ecornis (Ralfs) Chod.* *S. acuminatus (Lagerh.) Chod.)* *S.bijugatus* *S.obliquust* *S.quaricauda(Turp.)Breb.* *Staurastrum sp.*
Euglenoids(Euglenophyceae)	*Euglena spirogyra* *Phacusaccuminatus* *Phacus longicauda* (Ehr.) Duj. *Trachelomonas sp*
Cryptomonads(Cryptophyceae)	*Cryptomonas ovate Ehrenb*

The highest total phytoplankton abundance at the MetoAlekaOpen and MetoAleka Shore sites was observed in June, concurrently with the highest peak of cyanobacterial abundance (Fig. 8). The highest total phytoplankton abundance at the HEEP and Tannery sites, however, occurred in

May. Slightly lower peaks of total phytoplankton abundance, which concurred with similarly lower abundance of blue green algae (Cyanobacteria), were also observed at all sites in July or August. Although not as abundant as the cyanobacteria, diatoms, were far more numerous than the other algal groups represented in the phytoplankton community of the study lake. Euglenoids and cryptomonads were the least abundant in the alga flora of Lake Koka.

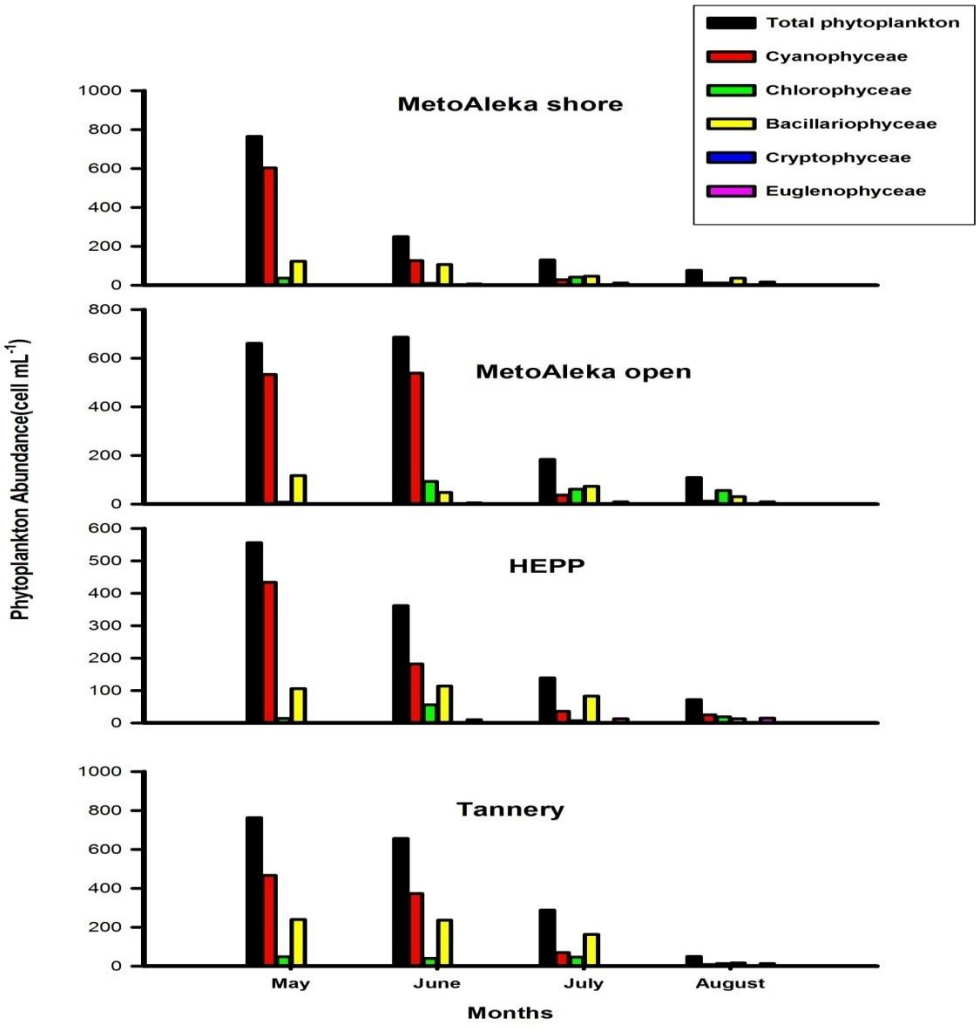

Figure 8.The abundance of phytoplankton at the different sampling sites of Lake Koka

The most abundant algal group, Cyanobacteria (blue - green algae) (with 60.63% contribution, Fig. 9), was dominated by two species of *Planktothrix* and *Microcystis.* The second most abundant algal group was the Bacillariophyceae(Diatoms) , with mean percentage contribution to total phytoplankton abundance of 27.03 % (Fig. 9). Diatoms were most abundant during May and June(Fig.6)and were constituted primarily by *Aulacoseria granulata*. The third most

important algal group was Chlorophyceae(green algae, Fig. 7), with species richness comparable to that of Bacillariophyceae (Table 9).

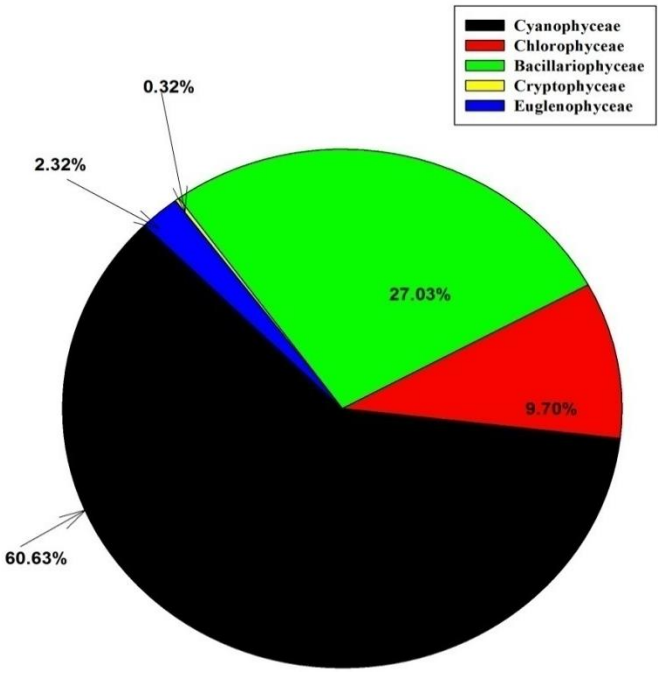

Figure 9.Percentage contribution of Phytoplankton groups to total phytoplankton abundance

The largest peak of abundance of Cyanobacteria occurred at the four sampling sites on different sampling dates (Fig.9). The most dominant cyanobacteria were *Planktothrix* and *Microcystis*spp.The highest peak of abundance of *Planktothrix spp.* occurred in May and June at the MetoAleka open and Tannery. The second most abundant taxa were *Microcystis* spp. with highest peak of abundances at MetoAleka Shore and Tannery sites. The third most abundant group was *Cylindrospermopsis* spp., which were more important at MetoAleka shore and MetoAleka open sites. The other taxa of cyanophyceae (*Anabaena, Pseudoanabaena and Aphanocapsa*) were at relatively low levels of abundance at the four sampling sites (Fig.10).

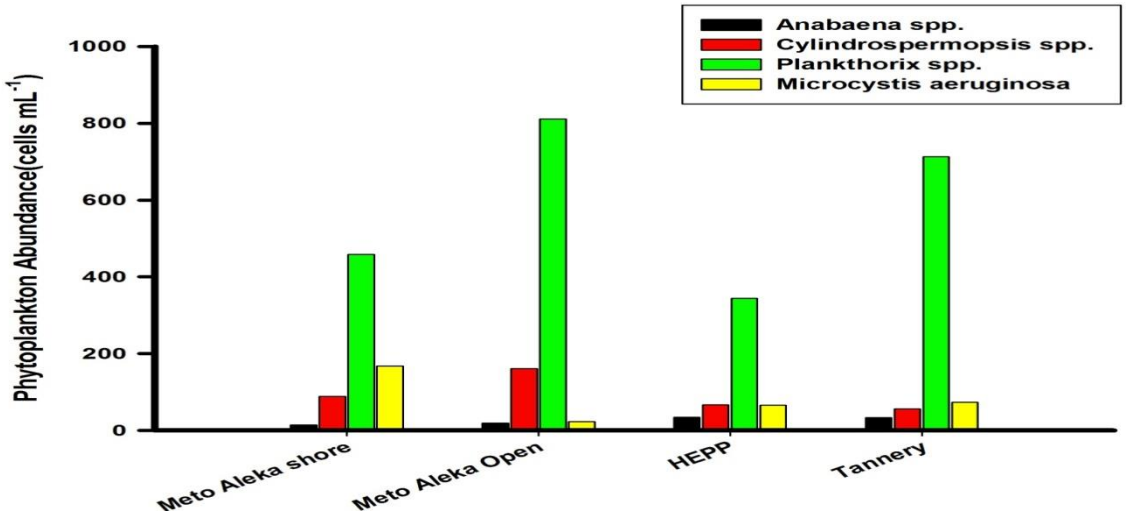

Figure 10.The abundance of major species of the dominant taxonomic groups in Lake Koka

4.2.2. Biomass of phytoplankton

In the present study, phytoplankton biomass of46.56 µg/L, 25.02 µg/L, 7.64 µg/L and 4.17 µg/L were recorded for MetoAleka shore, MetoAleka open, HEPP and Tannery sites, respectively. The Chl-a values recorded ranged from 4.17µg/L in August to 46.56µg/L in May. The chl-a values were lower during the months of the major rainy period(July and August)(Fig. 11).

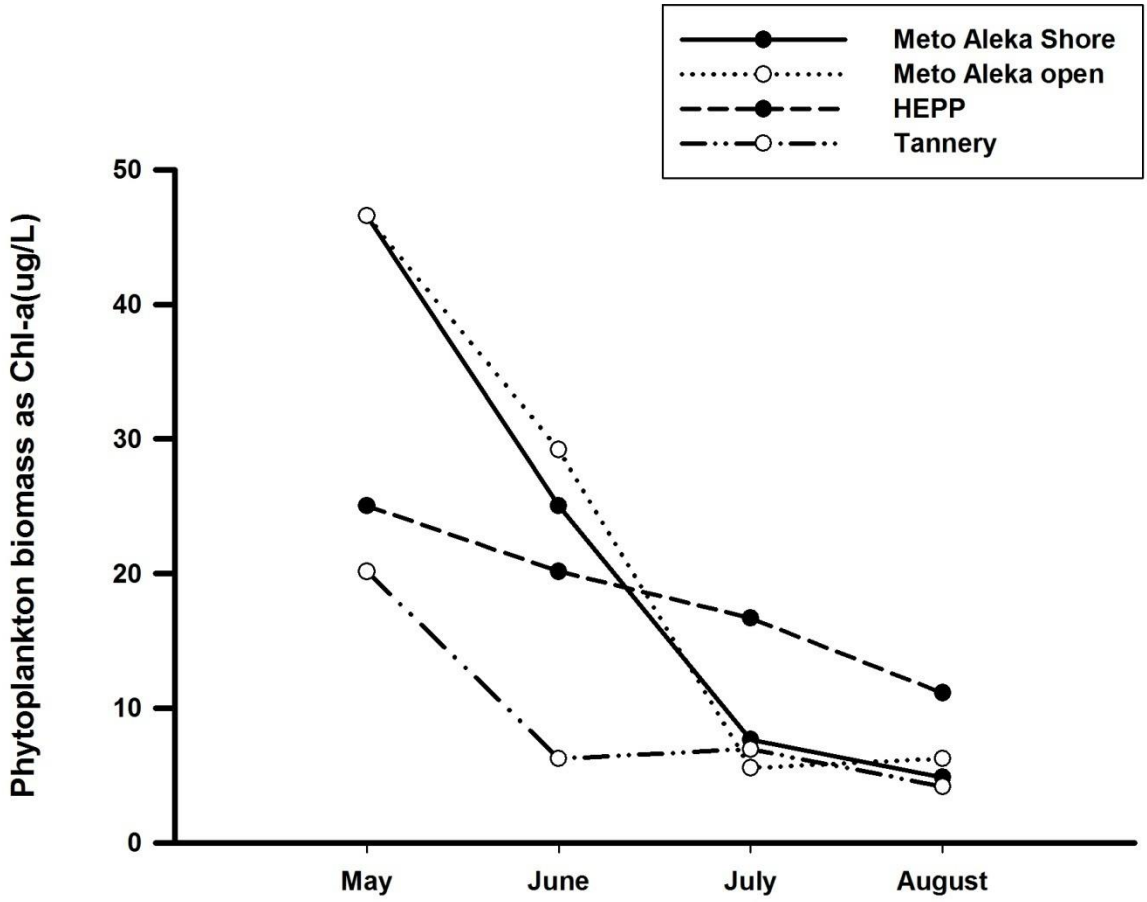

Figure 11.Temporal variations in Chl-a concentration at the four sampling sites (Lake Koka)

4.3 Zooplankton

4.3.1 Taxonomic Composition and abundance of Zooplankton

During the study period, a total of 23 species, with 14 species of rotifers, 4 species of copepods and 5 species of cladocerans, were identified (Table 7).

Table 7. List of zooplankton taxa identified in samples collected from Lake Koka over the study period.

Rotifera	**Copepoda**	**Cladocerana**
Anuraeopsisflissi	Cyclopoida	*Bosmina sp.*
*Asplanchna*sp.	*Thermocyclops*sp.	*Ceriodaphnia sp.*
Brachionus	Calanoida	*Daphnia barbata*
calyciflorus	*Nauplii*	*Diaphanosomaexcisum*
B.caudatus		*Moinamicrura*
B.falcatus		
B.pilicatilis		
Filina spp		
Hexarthra spp		
Keratella tropica		
Keratella avalga		
Polyarthra vulgaris		
Trichocerca Spp.		

The high peaks of abundance of copepods were observed at the tannery, MetoAleka Shore and MetoAleka open sites in July and/or August. Similarly, Rotifers abundance peaked at the MetoAlekaShore and MetoAlekaOpen sites in July and August, respectively. The highest peak of abundance of Cladocerans was observed at MetoAlekaOpen site in June, while those of copepods and Rotifers were recorded in July at the Tannery and MetoAleka Shore, respectively (Fig.12).

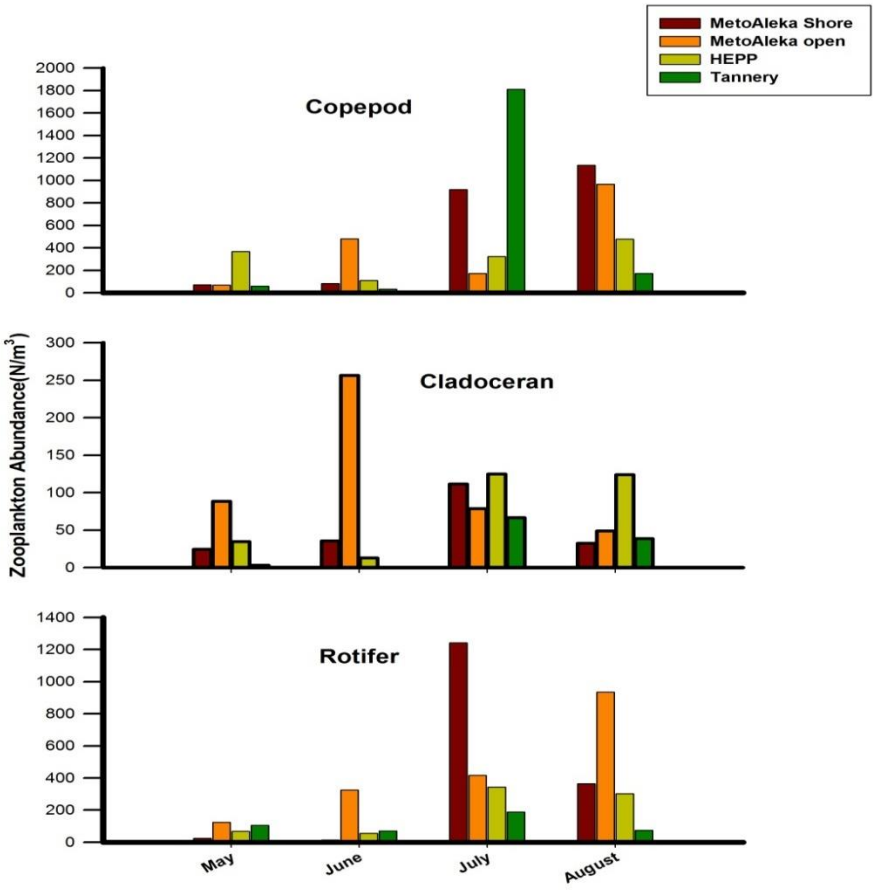

Figure 12.Temporal variations in the abundance of zooplankton taxa at the four sampling sites of the study on Lake Koka

4.4. Experimental results

Comparison of the colony counts of *Microcystis* and counts of surviving individuals of the cladoceran and copepod at the start of the experiment, day zero, and at the end of the experiment, day 16across the concentration gradients of *Microcystis* revealed variations(Fig. 13).

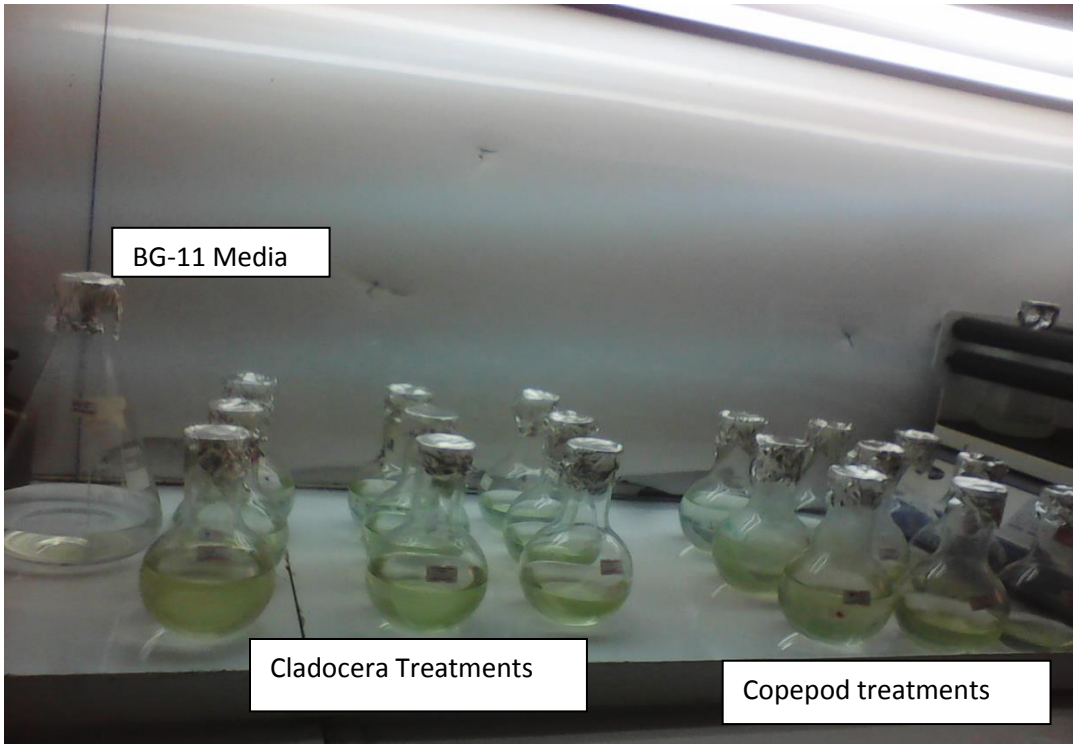

Figure 13**.** Grazing experiment

The survival rate of the copepod was affected by the concentration of *Microcystis*. As the concentration of *Microcystis* increased, the survival of the copepod decreased.At the beginning of the experiment, all animals were censored, but with an increase in *Microcystis* concentrations,t he survival of the copepod went down. In the treatment with 100% *Microcystis*, the copepod did not survive up to the end of the experiment. At the lower concentrations of 80% and 50% *Microcystis*, however, the copepod managed to survive. However, the animals of the cladoceran treatment (Group 2) showed higher survival compared with those of the copepod treatment, Group 1 (Fig. 14). In the treatments with 20%, 50%, 80% and 100% M. *aeruginosa*, the cladoceran showed high survival probability.

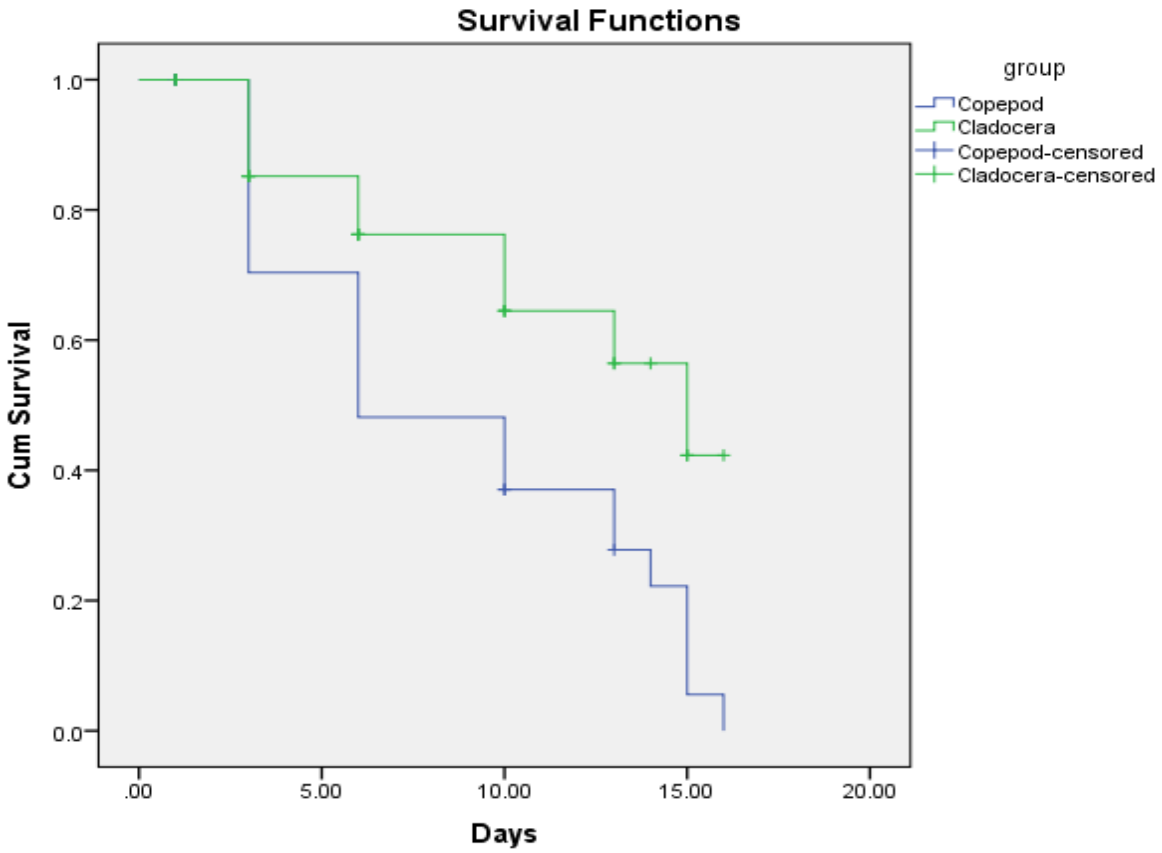

Figure 14.Survival curve of zooplankton in the two experimental treatments (Copepod and Cladoceran)

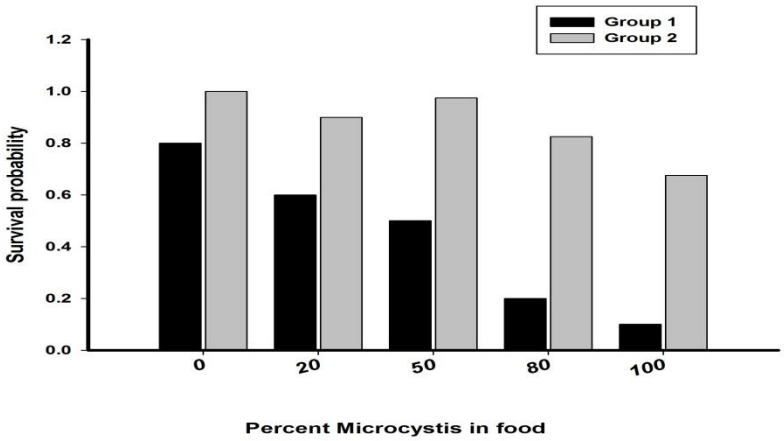

Figure 15.Changes in 13-day survival probability of the *Cyclopoids* and the cladoceran (Moina)in relation to the proportion of *Microcystis* in their food.

The chl-a concentration showed variations among the treatments. In the control experiment, (phytoplankton without zooplankton), the chl-a value was higher than those of the various treatments with zooplankton. The cladoceran treatments had lower values of Chl-a than the copepod treatments(Fig.16).

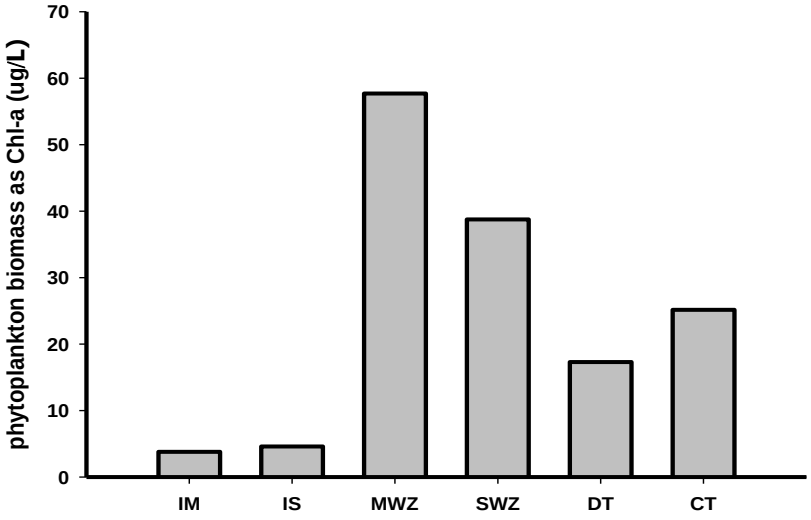

Figure 16.Chl-a biomass of phytoplankton (IM= initial*Microcystis*, IS = initial *Scenedesmus*,MWZ=(*Microcystsis* without zooplankton, SWZ=(*Scenedesmus* without zooplankton),DT=Cladoceran treatment, and CT=Copepod treatment

5. Discussion

5.1. Physical parameters

Temperature is an important physical property of water because it regulates the amount of dissolved oxygen and photosynthesis by phytoplankton. Natural variations in water temperatures mainly occur in response to seasonal and regional climate. The water temperature in of Lake Koka varied from 23.24^{o}C to 25.16^{o}C during the rainy period from May to August, 2017(Table. 7). Yeshiemebet Major (2016) also recorded the highest water temperature(24ºC) during the month of the major rainy period (August) and the lowest (20.2ºC) in April. Hadgembes Tesfaye (2007), however, reported minimum water temperature of 26.2 ºC observed in December, while the highest surface water temperature(28.7^{o}C) was recorded in April. These contrasting observations seem to have resulted from changes in climatic conditions of the study area. The Secchi disk visibility provides an estimate of water transparency, and it is a standard indicator of water clarity, which is strongly correlated with biomass and annual productivity of suspended

algae (Peck ham *etal*., 2006). The Secchi depth(cm)of this study, which varied from 7.22,of the Metoaleka shore site to 9.56 of the Tannery site, is broadly similar to those recorded by Yeshiemebet Major (2016) for months of the same period for the same water body (7 cm in June and July, 2013).The present Secchi depth values are considerably lower than that recorded previously (28 cm) by Elizabeth Kebede *etal*., (1996) for the same reservoir, which may be siltation has been increased due to catchment degradation and agriculture activities .

The range of dissolved oxygen (DO) concentration of the present study (6.57-.6.99 mg/l) is similar to that reported previously for Lake Koka during June-August (6.35-7.52);Yeshiemebet Major,2016). In the study by Handgembes Tesfaye (2007),maximum surface oxygen concentration of 11.37mg L^{-1} in December, 2005 and a minimum of 5 mg L^{-1}in February 2006. According to WHO (2006), a healthy water body has DO concentrations within the range of 5-14.6 mg/l, which implies that Lake Koka exhibits the feature of a healthy water body at least during the months of the major rainy period although the ever-increasing human activities taking place around the lake are likely to lead to drastic changes in its ecological health.

The range of pH of the surface water of Lake Koka recorded in the present study(8.39 to 8.68)is similar to that reported in anearlier investigation on the same lake(8.11 to 8.6; Hadgembes Tesfaye, 2007). However, Yeshiemebet Major *etal*., (2017) reported a range of higher pH values (8.82 to 9.04) for Lake Koka, which is comparable to those documented for other Ethiopian rift valley and crater lakes: Babogaya (8.84-9.09; Yeshiemebet Major, 2006) and Chamo (8.53-9.44; Eyasu Shumbulo, 2004). Such high pH levels were not observed in the present study as its sampling program did not include the months of the dry period, which are often, associated with heavy cyanobacterial blooms.

5.2. Inorganic nutrients

Mean values (mg L^{-1}) of nitrate for Lake Koka ranged from 0.110 to 0.224, with the maximum values generally occurring at Tannery site, which is probably related to the additional input of nutrients from the tanning industry. Lower concentrations of nitrate ranging from 0.004 to 0.044 mg L^{-1}were reported for Lake Koka by Fasil Degefu *etal*., (2011), while Hadgembes Tesfaye (2007) reported a range of mean nitrate levels (0.073 to 0.267mgL^{-1}) for the major rainy period,

the upper boundary value of which is considerably higher.A more recent study by Yeshiembebet major, (2016) has also recorded levels of nitrate, which are much higher than those observed in the present study and averaging 0.512mgL^{-1}. The nitrate levels observed in the present study are not, however, comparable to those documented for a similar water body, Legedadi Reservoir (240 to 1850 µg L^{-1}, Adane Sirage, 2006).The nitrate concentrations of the present study are below the maximum level tolerable by fish and other aquatic organisms (<5 mg L^{-1}, Chattopadhyay and Banerjee, 2007) and the guideline value set to protect public health (< 50 mgL^{-1}, WHO, 1996).The observed high nitrate levels in Lake Koka seem to be the result of inputs from the surrounding agricultural lands on which fertilizers are commonly applied to boost crop yield. During periods of high rainfall, nutrients may also be washed into the reservoir through runoff and may also be introduced through Awash River from the upper catchments areas. The differences in the levels of nitrate recorded for Lake Koka by different investigators may be attributable to differences in the location of sampling sites, timing of sample collection, and variations in hydrological (water input-output) conditions in the reservoir.

Concentrations of nitrite (0.079 to 0.32mg/l), which are unusually high for well-oxygenated waters like Lake Koka, were recorded at all sampling sites of the present study on Lake Koka. These may be due to organic wastes largely associated with intensive livestock operations and the tanning industry, agricultural fertilizers, surface runoff and sewage discharge into the lake through the feeder river. Ammonia concentrations of the present study (0.033 to 0.054 mg L^{-1}) are, however, significantly lower than those documented for the same lake in a previous study (0.197 to 0.557 mg L^{-1}).

SRP concentrations of the present study(0.083 to 0.179 mg L^{-1})are noticeably lower than those reported by Elizabeth Kebede *etal*., (1994) and Hadgembes Tesfaye *etal*.,(2007)(0.224 and 0.194.5 mgL^{-1}, respectively)for an Awash River mouth site of Lake Koka, with low levels generally concurring with high phytoplankton biomass. Yeshiemebet Major (2016) has, however, reported a range of SRP (11.46-262.24) that included levels, which are much lower than those observed in the present study. The inter-annual variations in SRP concentrations seem to be largely a function of the level of precipitation and associated nutrient input through Awash River.

The present silica concentrations(0.254 to 0.298 mg L^{-1})are incredibly lower than those reported previously(2.5 and 17mgL^{-1};Elizabeth Kebede,1994, Fasil Degefu *etal*.,2011, respectively) for the same reservoir. Hadgemest tesfaye (2007) and Yeshiemebet Major (2016) also reported higher concentrations of silica that ranged from 6.25 to 15.35 mg/l and 5.11 to 14.15 mg $L^{-1}$for an offshore site, in Lake Koka, respectively. The reduction of silica concentration to below the level regarded to be limiting to diatom growth (0.5 mg L^{-1}, Reynolds, 2006) is attributable to the greater abundance of diatoms, particularly *Aulacoseira granulata*.

5.3. Biological parameter

5.3.1Species composition and Abundances of Phytoplankton

The species richness of the phytoplankton community in Lake Koka observed in the present study (37 spp.) is higher than that reported previously for the same reservoir (24 spp.) by Hadgemest tesfaye (2007). Phytoplankton species richness in Lake Koka is considerably lower than those reported by Elizabeth Kebede and Willen (1998) for the Ethiopian Rift Valley Lakes Ziway (67), Awassa(70) and Chamo (44).The lowest abundances of several genera of green algae and diatoms were observed in the months of the main rainy season (July and August, Fig.8) probably due to the very low water transparency during this period.

The blue green algae*Microcystis*and*Planktothrix*co dominated the phytoplankton community throughout the study period. The present dominance of the phytoplankton community by blue-green algae is consistent with the findings of previous studies(Hadgemest Tesfaye 2007; Yeshiembebet Major *etal*.,2017), which reported Cyanophyceae asthe most dominant group in Lake Koka . This contrasts with the result of a study made in Lake Koka during the period of short rains in 1991, which reported the diatom *Aulacoseira granulata* as the dominant taxon (Elizabeth Kebede and Willen, 1998).The overwhelming dominance of the phytoplankton community in Lake Koka by *Microcystis spp*, particularly *M. aeruginosa*, was reported previously by Melaku Mesfin *etal*.,(1988)Hadgemest Tesfaye ,(2007), a finding, which contrasts with the results of the present study in which *Planktothrix* spp. were found to be the most dominant phytoplankton taxa, followed by *Microcystis*spp.

Hadgemest Tesfaye ,(2007) reported that the high peak of abundance in December was almost exclusively constituted by species of *Microcystis (M. aeruginosa, M. flosaquae and M. wesenbergii*) while the smaller peak of abundance was due to the diatom *Aulacoseira granulata* and species of *Anabaena (Anabaena circinalis* and *A. cf. spiroides*). During major(August-September) and minor (March-April) rainy periods belonged to the green algal genera *Pediastrum, Selenastrum, Scenedesmus* and *Closterium(*Hadgemest Tesfaye ,2007) but in the present study the larger peak of abundances species was *Planktothrix* spp(fig.8) during major rainy period.

The dominance of cyanobacteria may be associated with several environmental factors such as low turbulence (Reynolds and Walsby, 1975; Reynolds, 1987; Pearl, 1995 ; Oliver and Ganf, 2000), low light (Smith, 1986), high temperature (Shapiro, 1990), low carbon dioxide or high pH (Caraco and Muler, 1998) and high total phosphate (Watson *etal.,* 1997).

In the Lake koka the dominance of *Anabaena* and *Microcystis (*cyanobacteria) is favored byseveral environmental factors such as low light intensity, high water temperature, high pH, and increased TP (Fassil Degefu *etal*.,2011).Similarly in the present study the TP, pH score higher values among the sampling sites and showed significant variation (p=0.002, p=0.028), with the MetoAleka Open sites, MetoAleka Shore respectively (Table 8). Hadgemest Tesfaye ,(2007) reported that the low level of DIC in Koka Reservoir contributed to the dominance and persistence of the scum-forming blue-green alga *Microcystis spp*. under bloom conditions (Paerl, 1983). Generally severalexplanations have been forwarded for the formation of cyanobacterialblooms in Lake Koka. These include: elevated temperature, nutrient enrichment, high total phosphorus, low light energy requirements and high pH and/or low carbon dioxide concentration.

Different reports showed that Lake Koka has been affected by cyanobacterial blooms specially *Microcystis spp.* Willén *etal*.,(2011) reported that in a study conducted in four lakes (Chamo, Langano, Ziway, and Koka), Lake Koka showed the highest concentration of *Microcystis*, which was reflected in its high wet weight, 100,000mg L^{-1}. The microcystins concentrations, especially

in Lake Koka, exceeded the permissible level (1 µg L^{-1}) for humans, cattle, and wildlife (Willén *etal*., 2011) .

In a more recent study, Yeshiemebet Major *etal.*,(2016) also reported that the concentrations of microcystins varied between 312 and 641 µg $(g\ dwt)^{-1}$ and 351 and 709 µg $(g\ dwt)^{-1}$, as measured by HPLC-DAD and LC-MS/MS, respectively.

5.4. Phytoplankton biomass

The phytoplankton biomass value of the present study in Lake Koka was ranged from 4.17 µg/L to 46.56 µg/L .The mean concentration of Chl a was the highest (21.89 µg/L) in Meto Aleka open and lowest at Tannery sites (9.4 µg/L). Fasil Degefu *etal*., (2011) reported chl-*a* concentration (214.10µg L^{-1}) in lake Koka was higher than the values in the present study .

5.5. Zooplankton abundances

In the present study, among zooplankton taxa, copepods were the most abundant contributing up to 55.7% of the total zooplankton abundance, which was mainly constituted by *Thermocyclops spp.*Yeshiemebet Major(2016), however, reported that the most dominant zooplankton were rotifers accounting for more than 58% total zooplankton abundance. This is in agreement with the results of the study made by Fasil Degefu *etal*.,(2011) who also reported that in Lake Koka rotifers were the most dominant zooplankton. The dominance of rotifer in tropical ecosystem is common (Adamneh Dagne, Tadesse Fetahi and Seyoum Mengistou 2012).

5.6. Experimental study

The main observations from our experimental study on the effect of zooplankton on phytoplankton biomass and taxon composition in Lake Koka, Ethiopia are that (1) zooplankton exerts a top-down control on phytoplankton; (2) but this top-down effect is taxon-specific; (3) the potentially toxic cyanobacterium M*icrocystis* is significantly affected by zooplankton in our experiment; (4) our results indicate that survival of zooplankton in our experiment was quite low in copepod treatment and higher in cladoceran treatment; and (5) the concentration of the toxin microcystin was significantly higher in the absence of zooplankton. In the following paragraphs, we discuss these observations.

In temperate environments, biomanipulation is often used to control blooms of toxic cyanobacteria such as *Microcystis* spp. and *Anabaena* spp. (Ekvall *etal*., 2014). The efficiency of this technique relies on the dominance of *Daphnia* in temperate water bodies (Jeppesen *etal*., 2005; Peretyatko *etal*., 2012; Ekvall *etal*., 2014). However, the species of *Daphnia* are scarce or absent in tropical aquatic ecosystems, where zooplankton communities are often characterized by the dominance of rotifers, copepods and small sized cladocerans such as *Diaphanosoma, Ceriodaphnia* and *Bosmina* (Sarma *etal*., 2005). In Ethiopia, the zooplankton community was dominated by small calanoid copepods and small-bodied cladocerans, but some large-bodied Daphnia species were present as well (Jacobus *etal*.,2014; Adamneh Dagne, Tadesse Fetahi, 2010). In Lake Koka also small calanoid copepods, small-bodied cladocerans and rotifer were present (Yeshiemebet Major .2016, Fasil Degefu *etal*.,2011). In the present study copepods were the most abundant zooplankton contributing to >55.79% of the total zooplankton abundance. This was followed by rotifer (35.87%) (Fig.10). However according to Yeshiemebet Major . (2016)and Fasil Degefu *etal*. (2011) rotifer were the most abundant zooplankton next to copepod mainly dominated by Thermocyclops decipiens in Lake Koka.Yeshiemebet Major *etal*.,(2016) reported that the cladoceran zooplankton was dominated by small bodysized species, including C. cornuta and Diaphanosoma excisum, whereas large cladocerans were absent except for the occurrence of Daphnia barbata in December 2014. Similarly in the present study small cladocera species, including Bosmina sp,Ceriodaphina sp. Diaphanosoma excisum and *Moina micrura* observed during field study. Large cladocerans such as Daphnia barbata also present in Lake Koka.

If large-bodied zooplankton species, and especially Daphnia species, are abundant, they may be able to keep the growth of algae and cyanobacteria under control, and prevent blooms from occurring. The size structure and abundance of the zooplankton is, however, often determined by the presence of fish (Carpenter and Kitchell 1993).

The small-scale grazing laboratory experiments showed that the presence of zooplankton has an important impact on phytoplankton composition (*Microcystis*). Overall, zooplankton tends to

suppress the abundance of phytoplankton in our experimental units, but the impact differs strongly depending on the taxon involved (*Monia micrura* treatment or *cyclopoid* treatment).

In this experimental study, the survival rate of the cyclopoidwas lower than that of the cladoceran. The number of individuals of *cyclopoid* spp. decreases from the initial, when the concentrations of *Microcysis* increase from 20% to 100%.Matsumura-Tundisi*etal*., 1997 reported thatCyclopoids can feed on large colonial blue-green algae, common in eutrophic systems, sincethey are raptorial.

However in cladocera treatment the ten *Monia micrura* that were immersed at 20% to 100% concentration of *Microcystis* managed to survive, grow, reproduce and create negative impact on the *Microcystis* population growth. Our results suggest that in conditions of eutrophy and frequent cyanobacterial blooms, smaller and medium-sized species of cladocerans such as *Moina minuta*, succeed in being less negatively influenced by the cyanobacteria, and maintain a high and constant biomass.

There are also other supporting evidences that indicate ingestion of *Microcystis* bycladocera.Kâ *etal*., (2012)found that small cladocerans such as Moina micrura andCeriodaphnia cornuta consume small filaments of cyanobacteriaand argued that grazing by zooplankton can eventually be important in controlling filamentous cyanobacteria in tropical waters.

Similarly ,Guo and Xie (2006) found that small-bodied cladocerans develop some tolerance to toxic strains of cyanobacteria, while Davis and Gobler (2011) observed in a lake with cyanobacteria blooms, extremely small numbers of large-bodied cladocerans such as *Daphnia* and larger numbers of small cladocerans (*Bosmina* and *Moina)* that were capable of consuming toxic as well as non-toxic strains of cyanobacteria.

Paes, T.A.S.V. *etal.*, (2014)suggest that in conditions of eutrophy and frequent cyanobacterial blooms, smaller and medium-sized species of cladocerans such as *Moina minuta* and *Diaphanosoma spinulosum*, succeed in being less negatively influenced by the cyanobacteria, and maintain a high and constant biomass.

The chl-a abundances in the experiment show higher in the absence of zooplankton (control) treatment than the two treatment (cladocera and copepods)(fig.16). At the end of the experiment,in copepod treatment the chl-a abundances were higher than cladocera treatment

when we compare the two groups. This could be the survival rate of copepod were low and as result the growth of phytoplankton were increase. However, in cladocera treatment chl-a abundance were lower than copepod treatments. The results obtained in the present study suggested that the presence of microcystins does not have a large influence on the reduction of cladocera biomass in the experiment. Studies considering the possibility of the formation of genotypes that are resistant to microcystin and on the feeding behavior of these metazoans should be undertaken to elucidate this question. These responses can provide important information about the future use of biomanipulation for the control of algal blooms in Lake Koka.

Conclusion and recommendation

In conclusion, both monitoring- and experimental data show that biomanipulation, through top-down control, can lead to improved water quality expressed both as reduced cyanobacterial biomasses and lowered toxin levels during bloom period. Our results have important implications not only for understanding consumer-prey interactions, but also for lake restoration practices to improve the water quality of eutrophic systems. Our experiments reveal that cladocera survive better than copepod treatment as the concentration of Microcystis increasing to 100% . The result of this study indicates the possibility that *cladocera* can suppress *Microcystis*population.In Lake Koka, cyanobacteria (*Microcystis* and *Planktothrix*) were the dominant phytoplankton and the concentrations of microcystins were previously found to surpass WHO's provisional value of 1 µg L^{-1} set for drinking water quality. Over all, we must correlate this experimental work with field data to understand the interaction of the phytoplankton and zooplankton. A field surveyof zooplankton community structure in Lake Koka provided an opportunity to study trophic interactions of zooplankton with *Microcystis* within the context of the wholeecosystem.

Finally, the necessity of applying *Cladocera* at early phase of algal growth before blooming is recommended to control *Microcystis* bloom. Furthermore study focusing on more sites where more than one species of *Cladocera,* with toxic and non-toxic *Microcystis* is recommended.

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