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The influence of El Niño induced drought on the limnology and dynamics of cyanotoxins in a shallow tropical reservoir (Koka): Implications for possible public health threat and restoration efforts.

by

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Addis Ababa, Ethiopia
February, 2020

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Samson Tilahun

A Dissertation Submitted to the School of Graduate Studies at Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

Ethiopian Institute of Water Resource
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Examining Committee

Advisor ______________________ Signature_________________Date__________

Examiner _____________________ Signature_________________Date __________

Examiner______________________Signature_________________Date___________

Chairman______________________Signature_________________Date__________

LIST OF APPENDED PAPERS

Samson Tilahun and Demeke Kifle, (2020) Atmospheric dry fall out of nutrients: An overlooked source of eutrophication in tropical lakes with large Catchment to Lake Surface Area. ***Earth System and Environment doi 10.1007/s41748-020-00162-w***

Samson Tilahun and Demeke Kifle-Hydrologic and hydro-chemical responses of Koka Reservoir to extreme hydro-climatic conditions: Implications for biogeochemical phosphorus recycling and management of the reservoir. ***(12th annual conference of the Ethiopian Fisheries and Aquatic Science Association (EFASA) (Proceeding in press)***

Samson Tilahun and Demke Kifle**,** 2019. The influence of El Niño-induced drought on cyanobacterial community structure in a shallow tropical reservoir (Koka Reservoir, Ethiopia). ***Aquatic Ecology, 53****, 1-17.*

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Samson Tilahun[1, 2] Exclusive portioning of intra-and extracellular cyanotoxins: limitation of the conventional procedure published in the ***Journal of Environmental Science and Pollution Research doi10.1007/s11356-020-08256-8***

The influence of El Niño induced drought on the limnology and dynamics of cyanotoxins in a shallow tropical reservoir, Koka: Implications for possible public health threat and restoration efforts

ABSTRACT

Studies predict that future climate-change scenarios such as global warming promote the proliferation and dominance of potentially toxic cyanobacteria due to their influence on the limnology of aquatic environments. Lentic systems in the tropics such as Koka Reservoir are largely of riverine origin. They are, therefore, more susceptible to changes in hydrological cycles or rainfall pattern induced by climate change anomalies such as El Niño induced drought and La Niña induced flooding. This study was conducted on Koka Reservoir, which is one of the most severely impacted water bodies by the recurrence of potentially toxic cyanobacteria. Our study period (May 2015-April 2016) coincided with the recent incidence of El Niño induced drought that was reported to be the worst in decades. Physico-chemical and biological parameters were determined using the standard methods. The drought caused a reduction in the flow of Awash River and amount of sediment transported and drying of Modjo River. This expectedly caused a concomitant reduction in the annual nutrient budget of the reservoir received from riverine sources. The consequent low levels of nutrients in the reservoir, which was most pronounced for nitrogen sources, led to the occurrence of *Microcystis* spp. in unusually low abundance and the emergence and dominance of diazotrophic cyanobacteria, *Cylindrospermopsis* spp.. The low nitrogen levels (µg L^{-1}) observed in the open water (16.5-135) and near-shore (9.69 -250) sites also suggest that riverine input is probably the major source of nutrients to the reservoir. Total phosphorus (µg L^{-1}), however, exhibited better availability, with its levels varying from 532 (Jul) to 668 (Mar) and 500 (Jul) to 698 (Mar) in the open water and near-shore sites, respectively. The large discrepancy between the model predicted phosphorus concentration ($\mathbf{P}_{\lambda}$, 156.6 mg m^{-3}**)** calculated from the total phosphorus input from allochtonous sources (riverine and atmospheric) and the observed in-lake phosphorus concentration (609 mg m^{-3}), with the former constituting only 25.7 % of the latter, also

suggest the supply of phosphorus from autochthonous sources. Frequent horizontal and vertical mixing of the reservoir may have enhanced phosphorus recycling. Horizontal mixing of the reservoir was reflected by the absence of significant differences in limnological parameters. Frequent vertical mixing manifested by turbidity (NTU, 48.39 - 2820 and 48.29l-2970), TSS (g L^{-1}), 0.015l-1.672 and 0.029-1.5), Secchi disk transparency (cm,3-17 and 3.05-17.01) between the open water and near-shore sites, respectively, may have promoted the presumed internal phaphorus loading. Despite the low abundance of *Microystis* spp, the detected intra-cellular concentrations (µg L^{-1}) of the microcystins variants MC-LR (815), MC-YR (466.6) and MC-RR (265.68) by far exceeded the public health safety limit of 1 µg L^{-1}. However, *Dolichospermum,* the co-occurring diazotrophic cyanobacterium, may have also been responsible for the observed levels of MCs although its confirmation requires further study. The extra-cellular toxins were relatively less concentrated and less frequently detected. However, concentrations (µg L^{-1}) of up to 20 of MC-LR, 6.13 of MC-YR and 1.27 of MC-RR, which also exceeded the permissible limit (µg L^{-1}) set by WHO, were measured. The most potent MC-variant, MC-LR, constituted the greatest proportion (52.6-77.6 %) of total microcystins concentrations in the majority of the samples suggesting the extremely high potential public health risk. The strong positive correlation among the extracellular MCs variants (Spearman Rank Order Correlation, $P< 0.05$) suggests the possible involvement of a non-specific release mechanism, namely cell lysis, which may have occurred following the collapse of the bloom in May. This study also revealed that all limnological conditions including water residence time (239.5-439.9 days), temperature (22.4 -31.3 ^{o}C), and salinity except the limiting level of nitrogen, were within the ranges reported to be optimal for the proliferation of *Microcystis* and production of microcystins. The strong, positive and significant correlation between *Microcystis* spp. abundance and nitrogen concentration (Spearman Rank Order Correlation ($r= 0.7$, $P<0.05$), and the lack of association with other limnological features suggest that nitrogen was the key environmental factor in the dynamics of *Microcystis* spp. and microcystins. Absence of correlation between the abundance of diazotrophic cyanobacteria and concentrations of nitrogen ($r=-0.173$, $P>0.05$) but the strong and positive correlation with the concentrations of total phosphorus ($r=0.718$, $P<0.05$) seem to suggest that phosphorus

was the key nutrient influencing this functional group in the reservoir as nitrogen can never be limiting. Considering the long history of sediment deposition and the most likely concurrent nutrient loading since 1960s and the high retention efficiency associated with the prevailing long water residence time, the reservoir may have already accumulated a huge phosphorus reserve in the sediment. Emergence of nitrogen-fixing genera that are capable of satisfying their nitrogen requirement from the inexhaustible atmospheric sources and the presumably huge phosphorus reserve already accumulated in the reservoir implies that reduction in the external input of both nutrients can hardly help in reversing the current situation in Koka Reservoir at least in the near future.

Key words: Atmospheric deposition**,** Climate change, Cyanobacteria, cyanotoxins, diazotrophic, Koka Reservoir, *Microcystis*, Phosphorus recycling, nutrient-limitation, trophic state.

DEDICATION

Dedicated to my mother

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ABBRIVATIONS

APHA	American Public Health Association
ATD	Atmospheric Deposition
BOD	Biological Oxygen Demand
CA:LA	Catchment Area to Lake surface Area ratio
Chl-a	Chlorophyll a
COD	Chemical Oxygen Demand
CSTRS	Continuously Stirred Tank Reactor System
CYN	Cylindrospermopsin
DCA	Detrended Correspondence Analysis
DO	Dissolved Oxygen
EMWIE	Ethiopian Ministry of Water Irrigation and Electricity
ERVL	Ethiopian Rift Valley Lakes
EPA	Envirnnmetal Protection Agency
FR	Flow Rate
GF/F	Glass Fiber Filter
HPLC	High Performance Liquid Chromatography
HRT	Hydrologic Retention Time
LC-ESI-HRMS	Liquid Chromatography Electro Spray Ionization High Resonance Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantitation
max-LT-Av	Maximum Long term Average
MCs	Microcystins
Min-LT-Av	Minimum Long Term Average
NMDS	Non- Metric Multi Dimensional Scaling
NME	National Meteorological Agency of Ethiopia.
NTU	Nephlometric Turbidity Unit
OCHA	Office for the Coordination of Humanitarian Affairs
OECD	Organization for Economic Co-operation and Development
RDA	Redundancy Analysis
RL	Riverine Loading
Sal	Salinity
SPE	Solid Phase Extraction
SRP	Soluble Reactive Phosphate
TDP	Total Dissolved Phosphorus
TDS	Total Dissolved Solids
Temp	Temperature
TP	Total Phosphorus
TSS	Total Suspended Solids
Turb	Turbidity
WHO	World Health Organization

CHAPTER ONE

1. GENERAL INTRODUCTION

1.1. BACKGROUND

The supply of clean water that meets public demand has become a challenge due to water quality deterioration associated with pollutants originating from agricultural and industrial operations (Ahn and James, 2001; Baker and Miller, 2013). Enrichment of water bodies with macronutrients, nitrogen and phosphorus, and the consequent eutrophication-related water quality problem, namely emergence of harmful algal blooms is one of the major environmental problems of global concern (Backer 2002; Carmichael et al. 1997; Paerl and Huisman, 2009). In fresh water systems, harmful algal blooms are largely caused by cyanobacteria of the genera *Anabaena, Aphanizomenon, Cylindrospermopsis, Microcystis, and Planktothrix (Oscillatoria).* They are known to produce a variety of toxins such as hepatotoxins, neurotoxins, and deramtotoxins that cause acute and chronic public health problems and in some cases fatal poisoning in human, aquatic and domestic animals (Backer, 2002; Carmichael et al., 1997). Animal and human poisoning may occur due to ingestion of water contaminated with toxic cells, or toxins released from decaying cells (Oberholster et al., 2006). Consumption of food animals such as fish, which have ingested cyanobacteria and accumulated their toxins, is also another reported route of exposure (Ibelings and Chorus, 2007). Complications associated with cyanotoxins include gastroenteritis, nausea, vomiting, fever, sore throats, blistered mouth, ear and eye irritations, severe systematic effects such as hepatic failure and death (Stone and Bress, 2007), itching, and allergic dermatitis (Soong et al., 1992; Vranješ and Jovanović, 2011). The ecologically damaging situation and the high potential public health risk caused by harmful algal blooms are now being recognized globally due to their ever-increasing trend of occurrence in both frequency and geographical distribution (Paer1 and Huisman, 2009). The globally worsening situation could be due to the combined effect of anthropogenic activities and climate change (Carmichael et al., 2001; Huisman and Hulot, 2005). The recent increase in the temporal and spatial recurrence of harmful algal bloom is often associated with climate change such as global warming (Zhang et al., 2012 and the references therein). Changes in hydrological cycles or rain fall pattern induced by climate change anomalies such as El Niño induced drought and La Nina induced flooding have cascading effects on the physico-chemical environment (Paerl and Paul, 2012). Impacts of severe drought on the limnology of lakes and reservoirs in arid and semi arid regions consequent dominance of cyanobacteria has been studied by several authors including (Brasil et al., 2016; Bouvy et al., 2003). Cyanobacteria may take advantage of their adaptive features to survive and dominate in such changing environment. For instance, the increase in temperature due to global warming, for example, decreases water viscosity. As this condition decreases resistance to vertical migration in the water column, cyanobacteria can move up and down with relative ease. This helps them to harness light from the surface and acquire nutrients from the deeper part of the water bodies (Pearl and Paul, 2012). Other meteorologically-induced physico- chemical changes such as nutrient availability (Conley et al., 2009; Scott and McCarthy, 2010; Wang and Wang, 2009, temprature (Paerl and Paul, 2012), pH, mixing regime etc. also promote the dominance of cyanobacteria. The role of meteorological events in structuring phytoplankton communities was largely studied for marine ecosystems (e.g. Barber et al., 1996; Blanchot and Rodier, 1996; Liu et al., 1998).

There are relatively fewer similar reports on fresh water ecosystems, particularly the Great Rift Valley lakes. Furthermore, studies reported on the influence of climate anomalies on the cyanobacterial community structure of Ethiopian rift valley lakes are non -existent. Protection of public health, wild life, and aquatic ecosystems from the adverse effects of harmful algal blooms calls for an effective intervention strategy, which efficiently mitigates occurrence of blooms. This requires knowledge of environmental factors that dictate cyanobacterial dynamics, which may emanate from long-term data and identification of major sources of pollution. Reports show that inappropriate measures taken to control harmful blooms have exacerbated the situation. For instance, application of copper sulfate in an attempt to destroy the cyanobacterial blooms that developed in a water supply reservoir worsened the problem by causing disruption of cells and release of the toxins, with consequent higher concentrations of the toxins in drinking water (Falconer, 1999). One possible approach for the protection of public health from the potentially harmful algal blooms could be developing an early warning system at the time and place of high public health risk. This calls for long-term sampling to study the dynamics of cyanobacterial blooms and the key factors dictating their dynamics. Conflicting reports on several studies made on bloom formation indicate that there is no single cause for the occurrence of harmful algal blooms. It is rather the result of the complex interaction and the combined effect of various biotic and abiotic factors (Figueredo and Giani, 2009). Therefore, occurrence of blooms and production of toxins reported for one lake may not, therefore, necessarily represent the facts for other lakes. These variations may be enormous even within a lake depending on the dominant cyanobacterial strains, which in turn are influenced by temporal variations in physico-chemical and biological factors. This implies that generalization that can be applied for all lakes in space and time may not be possible. Therefore, every lake should be considered unique when it comes to cyanobacterial bloom formation and toxicity potential (Kotak et al., 1995). This suggests the need for detailed study on the key environmental drivers of bloom formation and toxin production in relation to conditions specific to local scenario. Thus, the present study focused on the limnology of Koka Reservoir with emphasis on understanding the dynamics of cyano bacterial blooms and intra and extra-cellular microcystin in relation to the key environmental factors in Koka Reservoir. The study also attempts to estimate nutrients inputs to Koka Reservoir from riverine and atmospheric sources and asses their potential implication for eutrophication related water quality problem of the reservoir.

1.2. Statement of the Problem

The water bodies in the Ethiopian rift valley have immense ecological, economic and public health importance as they are used for drinking water supply, sanitation, irrigation, livestock watering and fisheries (Willén et al., 2011). However, due to rapid population growth, urbanization and increased industrial operations, eutrophication- related water quality deterioration is emerging in most parts of Ethiopia. This is particularly evident in water bodies of the Ethiopian rift valley due to pollution by chemicals, which originate from domestic sewage, industrial and agricultural operations (Seyom Leta et al., 2003; Zinabu G/Mariam and Pearce, 2003). For instance, deaths of zebras in Lake Chamo, one of the lakes in the Ethiopian rift, was reported to have been caused by *Microcystis aeruginosa*, the cyanobacterium often dominant in the Lake (Amha Belay and Wood, 1982a; Wood and Talling, 1988). Koka Reservoir, the subject of the present study, is among the water bodies in the Ethiopian rift valley that have been severely impacted by the recurrence of potentially toxic cyanobacterial blooms (Willén et al. 2011; Yeshiembet Major, 2016). The Reservoir was found to support a phytoplankon community dominated by *Microcystis*, which produced microcystins whose concentration greatly surpassed WHO permissible level for drinking water supply. In fact, according to Willén et al., (2011), human and cattle illness around Koka Reservoir has already been reported by the local inhabitants since early1980s.

Protection of public health and livestock either through early warning system and/or mitigation of bloom formation could be one possible approach. This calls for the generation of long-term data on cyanobacteria and type and level of the associated toxins and understanding the key factors dictating their dynamics. Thus, as indictaed above, the present study aimed to study the limnology of the reservoir with particular emphasis with identifying the major environmental drivers dictating the dyanmics of cyanobacterial blooms and Microcystins (both cell bound and extracellular MCs) concentrations in Koka Reservoir. Furthermore, the study also aimed to assess the possible impact of the major sources of eutrophication (atmospheric and riverine sources) on Koka reservoir.

1.3. Significance of the Study

Despite the potentially high public health risk associated with the use of the reservoir water, relevant studies particularly on the Koka town side of the reservoir is scanty. The few sporadic studies on this aspect of the reservoir are of cross-sectional type and/or lack comprehensiveness with respect to the dynamics of the bloom and associated toxins both in space and in time. Studies on the dynamics of intra- and extra-cellular toxins in the reservoir, which need serious attention, are also non-existent. The mere absence of visible bloom in the reservoir may not guarantee public health safety as the extra-cellular cyanotoxins dissolved in water may remain high enough to pose potential public health risk. This is particularly true immediately following the collapse of the bloom as the cell bound toxins are released due to cell lyses. This condition may put the public even at higher risk as there is no visual cue of visible bloom, which could be misleading and give a sense of safety.

The results of the present study, therefore, provide scientific information to the health workers, policy makers, environmentalists and the public at large on the potential public health risk associated with cyanotoxins during both the bloom and non-blooming season. The study also provides scientific information on the key environmental variables dictating dynamics of cyanobacterial abundance and the intra and extracellular micrcystins concentrations in Koka Reservoir. Our study period (May 2015- April-2016) coincidently overlapped with El Niño induced drought. Thus, the results of the present study are also expected to give a good opportunity to have an insight into how shallow lakes and reservoirs in tropical region would likely respond to such climate change anomaly. The response of the reservoir with respect to nutrient availability, the dynamics of potentially toxic cyanobacteria and their toxins have great implication for public health and restoration effort of Koka reservoir.

1.4. Research questions and Objectives

1.4.1. Research Questions

- Do the physico-chemical characteristics of the study reservoir show temporal and spatial variations?

- What is the water residence time (WRT) of Koka Reservoir?
- What are the major cyanobacterial genera in the reservoir? Do they show any spatial and temporal variations?
- Do microcystins concentrations show any temporal variations in Koka Reservoir?
- Do the dynamics of the major cyanobacterial taxa and the concentrations of microcystins in Koka Reservoir have any association with physico-chemical characteristics of the reservoir water?
- How does Koka reservoir respond to nutrient input exclusively from riverine sources?
- How important is atmospheric nutrient input through dry fall to Koka Reservoir?

1.5. Objectives

1.5.1. Overall Objective

To study the limnology of Koka Reservoir, a shallow tropical reservoir, with emphasis on the spatio-temporal dynamics of nutrients availability, cyanobacteria and cyanotoxins as well as the possible impact of the major sources of eutrophication (atmospheric and riverine sources) and assess their implications for public health threat and restoration effort.

1.5.1.1.Specific Objectives

- To study the temporal and spatial variations in physico-chemical characteristics of the reservoir.
- To investigate the temporal and spatial dynamics of major cyanobacterial genera
- To Identify and quantify the major variants of intracellular and extra-cellular microcystins in the reservoir

- To investigate the associations between the physico-chemical factors and the dynamics of cyanobacteria and concentrations of intracellular and extracellular microcystins in the reservoir.

- To estimate riverine and atmospheric nutrient input to the reservoir and model trophic state response of the reservoir.

1.6. Organization of the Dissertation

This PhD dissertation resulted from studies carried out on "the influence of the El Niño induced drought on the limnology of Koka, reservoir, a shallow tropical reservoir, with emphasis on the spatio-temporal dynamics of nutrients, cyanobacteria and cyanotoxins and their implications for public health risk and restoration effort".

The dissertation comprises seven chapters.

Chapter One.

General Introduction

This chapter deals with general introductory information and justification (research gaps), statement of the problem, significance of the study, research questions and research objectives and this section, organization of the PhD dissertation.

Chapter Two.

Literature Review

In this chapter, a review of recent articles on the current state of knowledge on limnology lentic systems with emphasis on eutrophication and eutrophication related water quality problems.

Chapter Three.

Reverine and atmospheric input of nutrients to Koka reservoir.

This chapter deals with the extent of pollution by nutrients originating from riverine and atmospheric (through dry fall) sources and their potential affects Koka Reservoir. The following manuscript, which emanated from this chapter has been published in the journal ***Earth System and Environment***

Samson Tilahun and Demeke Kifle. Atmospheric Dry Fallout of Macronutrients in a Semi-Arid Region: An Overlooked Source of Eutrophication for Shallow Lakes with Large Catchment to Lake Surface Area Ratio
Earth Systems and Environment Doi 10.1007/s41748-020-00162-w

Chapter Four.

Physical and chemical limnology of Koka Reservoir during extreme hydro-climatic conditions: implications for biogeochemical phosphorus recycling and management of the reservoir.

This chapter addresses the hydrologic and hydro-chemical response of Koka Reservoir to the extreme hydro-climatic event, namely El Niño induced drought, which was reported to be the worst in decades. The chapter also deals with the possible implication of the observed response of the reservoir for nutrient availability, nutrient recycling and management of the reservoir. The following manuscript, which originated from data of this chapter, has been presented in the 12th Annual conference of of the Ethiopian Fisheries and Aquatic Science Association (EFASA) under theme

“**Changing Aquatic Resources and Development and Management in contemporary Ethio-Global scenario.”**

Samson Tilahun and Demeke Kifle-Hydrologic and hydro-chemical responses of Koka Reservoir to extreme hydro-climatic conditions: Implications for biogeochemical phosphorus recycling and management of the reservoir. ***(12th annual conference of the Ethiopian Fisheries and Aquatic Science Association (EFASA) (Proceeding in press)***

Chapter Five.

The influence of El Niño-induced drought on cyanobacterial community structure in a shallow tropical reservoir (Koka Reservoir, Ethiopia)

In this chapter, I reported the influence of the El Niño induced drought on cyanobacteria community structure of the reservoir and discussed possible factors associated with the shift in dominance from non-diazotrophic cyanobacterial genus, *Microcystis* Spp. that had been dominant over several years in the reservoir to the unusual diazotrophic cyanobacterium *Cylindrospermopsis* and co-occurrence of other heterocystous cyanobacterial genera. The following manuscript, which originated from this chapter has been published in the ***Journal of Aquatic Ecology.***

Samson Tilahun and Demke Kifle**,** 2019. The influence of El Niño-induced drought on cyanobacterial community structure in a shallow tropical reservoir (Koka Reservoir, Ethiopia). ***Aquatic Ecology, 53****, 1-17.*

Chapter Six.

Temporal dynamics of intra-and extra-cellular microcystins concentrations in Koka Reservoir (Ethiopia): Implications for public health risk

This chapter presents data that demonstrated the temporal dynamics of microcystin concentrations in both algal seston and water samples in relation to the physico-chemical condition of the reservoir and the associated public health risk. The following two articles have been published from this chapter.

Samson Tilahun, Demeke Kifle, Tigist W. Zewde, Jostein A. Johansen, Taye B. Demissie, and Jørn H. Hansen **(2019).** Temporal dynamics of intra-and extra-cellular microcystins concentrations in Koka Reservoir (Ethiopia): Implications for public health risk. ***Toxicon, 168:****83-93*

Samson Tilahun[1, 2] Exclusive portioning of intra-and extracellular cyanotoxins: limitation of the conventional procedure published in the ***Journal of Environmental Science and Pollution Research doi10.1007/s11356-020-08256-8***

Chapter 7

Synthesis and Summary

This chapter summarizes the major findings of the PhD dissertation and recommends research aspects that need to be addressed in future investigations.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Introduction

Due to rapid population growth, urbanization and increased industrial operations (Paerl and Huisman, 2009) and global warming (Paerl et al., 2011), eutrophication related water quality deterioration, namely the occurrence of potentially toxic cyanobacterial blooms, has become a global concern. The recurrence of toxic cyanobacterial blooms is becoming increasingly common in tropical lakes and reservoirs in general (Bouvy et al., 2003; Figueredo and Giani, 2009a) and in Ethiopian rift valley lakes in particular (Willén et al., 2011) due to pollutants particularly macronutrients (nitrogen and phosphorus) originating from both diffuse and point sources. The dominance of harmful algal blooms in this region seems to have been favored by the high water temperature, high level of pollution, and the adaptive strategies evolved by cyanobacteria to cope up with prevailing conditions. In this review, major sources of nutrients for tropical lakes and environmental factors dictating dynamics of cyanobacteria and toxin production are briefly discussed.

2.2. Eutrophication of Tropical lakes and Reservoirs

Enrichments of tropical water bodies with macronutrients such as nitrogen and phosphorus is increasingly becoming a common phenomenon particularly in areas where extreme land degradation associated with intense agricultural operations has occurred (Ahn and James, 2001; Baker and Miller, 2013). Studies show that riverine input and atmospheric deposition are the major routes of nutrient inputs to inland waters. The relative importance of the two sources may depend on such factors as catchment area to lake surface area ratio, CA:LA (Kalff, 2002; Redfield, 2002), and flow regime (Das et al., 2011). It is also greatly influenced by meteorological factors (Amodio et al., 2014) such as wind speed, and rain fall pattern (Anderson and Downing, 2006; Ravi et al., 2004), land use (Baker and Miller, 2013) and vegetation cover (Yan et al., 2013). Lakes and reservoirs in the tropical region are of riverine origin (Lewis Jr, 1996), which may put them at a higher risk of pollution from both diffuse and point sources than lakes of other regions. Conversion of vegetated land to agricultural land causes changes in the watershed hydrology through its effect on the rate of canopy interception, infiltration, evapo-transpiration, and ground water recharge, all converging to increasing nutrient transportation (Baker and Miller, 2013). Several African rift valley lakes have thus been impacted by nutrient loads originating from diffuse sources due to the ever increasing land use changes (Baker and Miller, 2013; Muvundja et al., 2009). Koka Reservoir, which is located downstream of the upper Awash catchment, is among the water bodies in the Ethiopian rift valley that have been impacted by nutrient loads from both diffuse and point sources. The upper Awash River catchment, which has an area of around 11, 000 km^2, is largely dominated by agricultural land use and is consequently progressively degraded due to forest clearing for additional agricultural land and fuel wood (Halcrow and Pattern, 1989). This has caused transportation of huge amount of sediment and siltation in the reservoir particularly during the rainy season (Halcrow and Pattern, 1989). Most of the previous reports on the impact of such land use and land cover changes in the upper Awash catchment (Abebe, 2001; Dilnesaw Chekol et al., 2007; Kebede Wolka , 2012) aimed at investigating the sediment yield and the consequent impact on the water-holding capacity of Koka Reservoir in connection with hydroelectric power generation. However, such equally important water quality issues as impact of land use changes in the upper Awash catchment on nutrient fluxes and the consequent water quality responses of the reservoir have not been given much attention. As a result, the contribution of Awash River to the annual nutrient budget of the reservoir is not known. Nevertheless, circumstantial evidences such as recurrent cyanobacterial blooms over the last several decades in the reservoir seem to suggest that there has been a concomitant transport of huge amount of nutrient to the reservoir. Furthermore, several industries discharge their untreated wastewaters into the nearby rivers which eventually end up in lakes and reservoirs. For instance, there are over 20 tanning industries concentrated in the Ethiopian rift valley lakes area, over 90% of which lack wastewater treatment system of any kind (EP**A**, 2003). Mojo River, which is one of the rivers that feed Koka Reservoir, currently revieves untreated wastewater from many of the tanneries established on both sides of its course. As a result, the river, which eventually ends up in Koka Reservoir, is one of the most polluted rivers in Ethiopia (Seyoum Leta et al., 2003; Amanial Haile, 2015; Zinabu G/ Mariam and Pearce, 2003). It is characterized by high concentration of nitrogen and phosphorus and other pollutants including the heavy metals Cr and Cd and high BOD and COD (Seyuom Leta et al., 2003; Amanial Haile, 2015). Thus, Koka reservoir presumably receives much of the nutrients and other pollutants originating from point sources through this river. Fig. 1 shows Awash and Modjo River entry points to Koka Reservoir. One may also anticipate that those factors reported to have increased sediment yield from the agricalural dominated upper Awash catchment may have similarly influenced nutrient transport. Furthermore, nutrients accumulated over the years in lakes and reservoirs may serve as a source of nutrient input through internal recycling (see chapter 4). Several authors (Jeppesen et al., 1999; Phillips et al., 1999; Søndergaard et al., 2003; Søndergaard et al., 1993; Welch and Cooke, 1999) reported the role of nutrient recycling between the sediment and the water column as a possible source of nutrients. In fact, nutrient recycling, particularly supply of phosphorus through internal loading in shallow lakes has been implicated for the failure or delayed water quality improvement upon reduction in external input (Jeppesen et al., 1999; Phillips et al., 1999; Søndergaard et al., 2003; Søndergaard et al., 1993; welch and Cooke,1999).

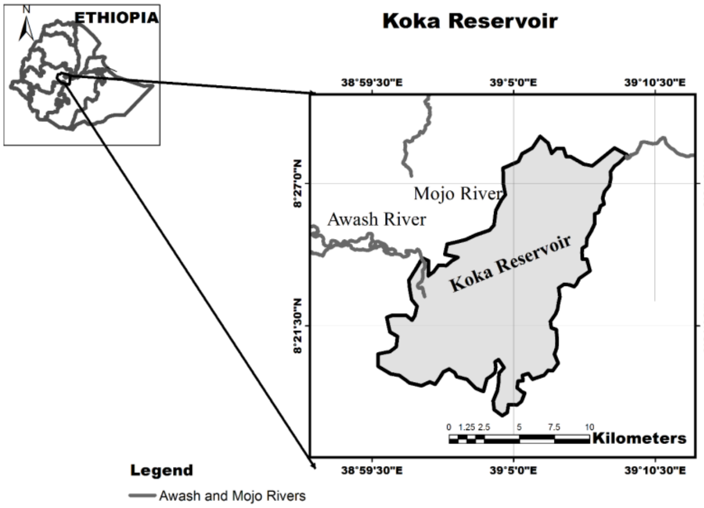


Figure 2.1. Koka Reservoir, its feeder rivers and a partial view of its catchments

Apart from riverine sources, nutrient inputs through atmospheric deposition has also been implicated as possible source of pollution for inland waters in several studies (Jassby et al., 1994; Muvundja et al., 2009; Tamatamah et al., 2005). Atmospheric deposition of nutrients onto lakes and reservoirs may take place through snow fall or rainfall, which is collectively termed wet deposition, while that, which takes place through gaseous phases and/or as particle-bound, is known as dry deposition (Zhai et al., 2009). In lakes located in regions with tropical climate, deposition through rainfall and deposition through gaseous phases and as particle-bound are the relevant mechanisms of nutrient inputs of wet and dry depositions, respectively. Atmospheric deposition may have significant contribution to total nutrient inputs to lakes and reservoirs (Anderson and Downing, 2006), with its contribution sometimes exceeding that of riverine loading (Jassby et al., 1994; Muvundja et al., 2009; Tamatamah et al., 2005). This is possibly because unlike riverine sources, wind blown nutrients may also come from areas far away from the catchments (Reynolds et al. 2006), which could be regional or continental. The conceptual model depicting the potential source areas for atmospheric and riverine inputs is shown below (Fig. 2.1).

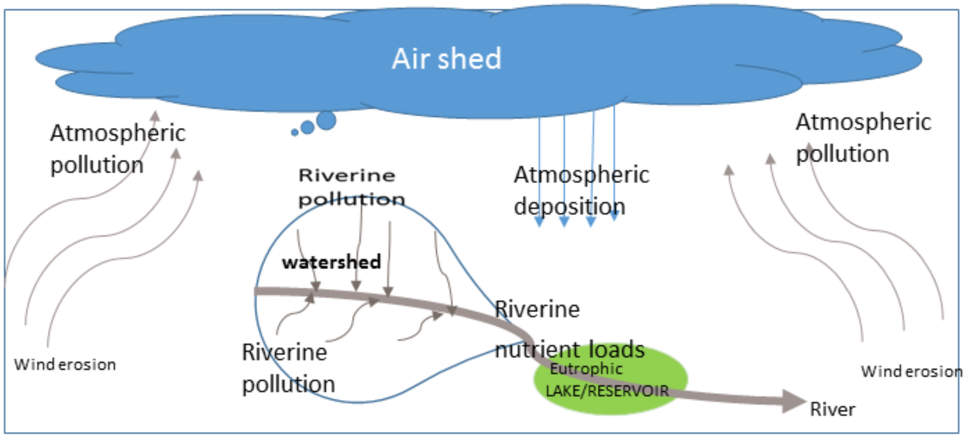


Fig. 2.2. Conceptual model depicting relative source areas for atmospheric and riverine input of nutrients

Furthermore, atmospheric input of nutrients may also originate from sources other than agricultural practices such as automobile emission exhaust and biomass burning (Tamatamah et al., 2005), and unpaved roads (Herut et al., 1999). For instance, large scale deforestation and biomass burning have been suggested as sources of atmospheric deposition in African great lakes such as lake Victoria (Tamatamah et al., 2005). Similar studies on the role of atmospheric input of nutrients to lakes and reservoirs in Ethiopia are nonexistent. However, considering the meteorological conditions such as rainfall pattern, wind speed and other local conditions, wind-induced erosion in the region is expected to be high suggesting that atmospheric deposition of nutrients could possibly be considerable (see chapter 3).

2.3. Potentially Toxic Cyanobacteria (blue green algae)

One of the water quality problems associated with eutrophications of aquatic environment, which has become a serious issue of global concern, is the occurrence of harmful algal blooms formed priamrily by potentially toxic cyanobacteria (Carpenter et al., 1998; Heisler et al., 2008). Cyanobacteria are prokaryotic primarily photosynthetic algae, which produce chlorophyll-*a* and the blue-colored phycobilin known as phycocyanin, which is responsible for their blue-green coloration. The taxonomic position of cyanobacteria seems to be controversial in light of the fact that they exhibit features of both photosynthetic bacteria and eukaryotic algae (Krogmann, 1981; Palinska and Surosz, 2014; Walter et al., 2017). That is, they are unique among all organisms in being prokaryotic and at the same time capable of synthesizing chlorophyll-*a* and producing oxygen during photosynthesis (Palinska and Surosz, 2014; Walter et al., 2017). Their cellular organization is more like bacterial cells than any other group of algae indicating the close evolutionary relationship between blue-green algae and bacteria (Bold and Wynne, 1978; Palinska and Surosz, 2014). Some biologists including (Sterner, 1989), in fact, state that “These organisms are not algae. Their taxonomic association with eukaryotic groups is an anachronism formally equivalent to classifying the bacteria as a constituent group of the fungi.” Therefore, this group of organisms can be recognized as a major group of bacteria in view of their cellular organization and peptidoglycan cell wall. Phycologists, however, are reluctant to classify these organisms as bacteria owing to their possession of chlorophyll-*a* and evolution of oxygen during photosynthesis, characteristic features of green plants and eukaryotic algae and are used as a justification for continuing to refer to them as algae (Bold and Wynne, 1985).

2.3.1. Cyanobacteria, their Distribution and Survival Strategy

Cyanobacteria are ubiquitous in their distribution reported both from aquatic and terrestrial environments. Members of this group have also been reported from both temperate and tropical regions (Paerl, 1997; Wiedner et al., 2007; Willén et al., 2011) which may suggest their wide geographical distributions. Cyanobacteria are one of the major groups of algae inhabiting Ethiopian Rift Valley lakes (Elizabeth Kebede, 2002) and Bishoftu Crater lakes. As a group, cyanobacteria are known to be the most competent group compared to other phytoplankton in the aquatic environment (Pearl and Husein, 2009). Their wider distribution and dominance over other phytoplankton groups are associated with their adaptive features and strategies to survive under varied environmental conditions.This in turn is possibly due to their long history of evolution, which according to fossil record (Fig. 2.1) can be traced back to 3.5 billion years in time.

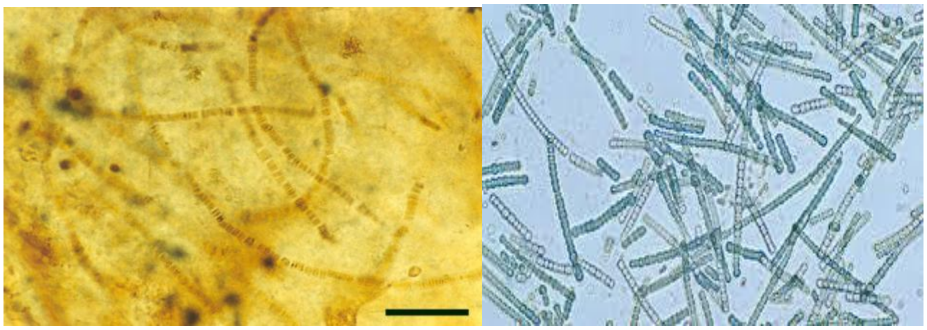


Plate. 2.1 Fossilized cyanobacteria, Source: sciencenews.org

During this long history of evolution, they are believed to have developed a wide range of both structural and physiological adaptations to survive under varied and extreme environmental conditions (Carey et al., 2012; Paerl and Paul, 2012). Some members of this group, for instance, were found to survive in such extreme environments as frozen lakes where the temperature goes below zero degree centigrade and in hot springs where the temperature exceeds 85 ^{o}C (Cronberg and Annadotter, 2006). Other members of this group also survive extremely acidic, basic and salty conditions (Tonk et al., 2007). Cyanobacteria genera such as *Microcystis and Anabaena* are salt tolerant (Tonk et al., 2007). *Microcystis aeruginosa*, for instance, was reported to tolerate salt concentration as high as 9.8g/l (Orr et al., 2004). Several members of cyanobacteria tolerate salty and dry conditions by forming dormant seeds in desiccated sediments of soils (Potts, 1994). They can also survive poor light and nutrient deplete conditions by virtue of regulating their buoyancy. Possession of gas vesicles enables them to migrate vertically in the water column to harness light energy required for photosynthesis and to exploit nutrients in the deeper part of the water bodies (Reynolds, 2006). They also constitute the only group of photosynthetic organisms capable of fixing atmospheric nitrogen, which give them a survival advantage at times of nitrogen depletion (Fogg, 1969; Gallon, 1992). *Anabaena, Aphanizoonmon, Cylindrospermopsis and Nudularia* are among members of cyanobacteria that are capable of fixing nitrogen from the atmosphere (Pearl et al., 2011). Furthermore cyanobacteria outcompete other phytoplankton community through high consumption of CO_2 during their blooming time, which is accompanied by high pH that causes the concentration of carbon dioxide to be less than 1% (Paerl and Ustach, 1982). This makes the concentration of CO_2 in the aquatic environment to be too low for other phytoplankton community to carryout photosynthesis. Their bloom also acts as a physical barrier preventing the diffusion of CO_2 from the atmosphere.

2.3.2. Determinants of Cyanobacterial bloom formation and toxin production

Reports on some field and laboratory studies show that such environmental variables as nutrients, light, culture age, temperature, and pH, and water residence time greatly influence cyanobacterial bloom formation and production cyanotoxins (Park et al., 1993). In the following sections, the influence of some of these factors on cyanobacterial bloom dynamics and toxin production are briefly discussed.

2.3.2.1. Nutrients

Algae and other aquatic plants need more than 20 different kinds of nutrients as their growth requirements (Lewis, 2000). Given optimal environmental conditions, the potentially maximum growth of aquatic plants may be limited by one or more of these nutrients, which largely depends on the ratio of the demand for a given nutrient to how much available in the environment. This is known as the Liebig`s law of the minimum (Danger et al., 2008). A nutrient is, therefore, said to be growth limiting if it is exhausted first in relation to other nutrients considering its normal cellular stoichiometric concentration (Salas and Martino, 1991) and if the supply of which induces growth response (Lewis, 2000). In this regard, nitrogen and phosphorus are most often reported as growth limiting nutrients compared to other nutrients, for example, carbon which is abundantly available and silicon which only affects a certain taxonomic group, the diatoms (Lewis, 2000). This is possibly because nitrogen and phosphorus are required to make or synthesize some vital macro molecules such as proteins (Conley et al., 2009), nucleic acids (RNA, DNA), photosynthetic pigments (Chlorophyll) and ATP, the energy currency of a cell, (Conley et al., 2009). These makes nitrogen and phosphorus the vital nutrients required by all phytoplankton taxonomic groups. Furthermore, these nutrients are relatively in short supply in relation to the high demand by various taxonomic groups of phytoplankton including cyanobacteria. Therefore, enrichment of water bodies with macronutrients (nitrogen and phosphorus) has been implicated as one of the most important factors inducing cyanobacterial bloom formation, and dictating their spatio-temporal dynamics, (Conley et al., 2009; Scott and McCarthy, 2010; Wang and Wang, 2009) and toxin production (O'neil et al., 2012); Wang et al., 2010). Cyanobacteria are diverse, with varying response to nutrient availability. For instance, studies have revealed that the availability nutrients influence cyanobacteria community structure (Schindler et al., 2008; Wang and Wang, 2009) favoring one group over another. According to these studies, alteration of external input of nutrients, as for example, reduction in the external input of nitrogen has resulted in a shif from a cyanobacterial community dominated by non-diazotrophic to that dominated by diazotrophic cyanobacterial genera (Schindler et al., 2008; Wang and Wang, 2009). This is because diazotrophic cyanobacteria can take advantage of their capability to replenish their nitrogen supply through nitrogen fixation from the abundantly available atmospheric nitrogen. For such a group, nitrogen is never limiting and hence phosphorus must be the key limiting nutrient. However, members of the non-diazotrophic cyanobacteria including one of the most ecologically damaging species, *Microcystis aeruginosa,* may be severely impacted by nitrogen limitation. Reports also show that response to nutrients depends on strains within a given species (Xu et al., 2010). For instance, de Figueiredo etal., (2004), in their short communication, suggested that toxin production by *Microcystis* could be influenced by variation in nitrogen and phosphorus concentration, with responses differing with strains. Yoshida et al., (2007) also reported that increased growth of the toxic genotypes within the *Microcystis* community was attributed to high

NO_3-N. Furthermore, there are also some cyanobacterial genera such as *Cylindrospermopsis,* which are able to use nitrogen when it is available and resort to nitrogen fixation when it is scarce in the aquatic environment (Moisander et al., 2008). The key nutrient that dictates cyanobacterial community structure, bloom dynamics and toxin production may, therefore, depend on nutrient availability, cyanobacterial genera, and strain present in the given system (Xu et al., 2010).

2.3.2.2. Temperature

Temperature is another environmental factor that has a dictating role in cyanobacterial dynamics. As it is the case in other prokaryotes, high temperature favors the growth and rapid proliferation of cyanobacteria as opposed to eukaryotic algae (Paerl and Paul, 2012). A review by Paerl and Paul, (2012) indicates that temperature exceeding 25 °C resulted in a decline in the growth rate of the eukaryotic algae, *Golenkiniia radiata,* the diatom *Skelelonema constatum* and the dinoflagellate *Prorocentrum minimum,* while increasing proliferation of *Microcystis*. Yamamoto and Shiah, (2012) also studied factors related to the dominance of *Cylindrospermopsis* in a shallow pond in northern Taiwan and reported that the dominance of the genus could be due to its competitive advantage over other phytoplankton groups under condition of high water temperature. They also suggested trichome size adjustment as a possible survival mechanism at high water temperature. In another instance, a survey conducted on 143 lakes of varying trophic states indicated that within total phytoplankton biomass, the biomasses of members of cyanobacteria were found to increase with increasing temperature (Kosten et al., 2012). This positive correlation between cyanobacterial biomass and temperature was also observed by (Robarts and Zohary, 1987). Cyanobacteria are also able to cause localized increase in temperature by virtue of their photosynthetic and photoprotective pigments (Kahru et al., 1993). These and other studies may suggest the key role played by temperature in bloom formation (Stüken et al., 2006). The persistent dominance of members of cyanobacteria in several of the Ethiopian rift valley lakes (Elizabet Kebede and Willén, 1998; Yeshemebet Major et al., 2017; Willén et al., 2011) may, therefore, be partly attributed to the prevailing high water temperature throughout the year.

2.3.2.3. Salinity

Salinity is one of the limnological variables that influence phytoplankton growth. Although cyanobacteria as a group are known to tolerate high salt concentration (Moisander et al., 2008), some members may be adversely affected by high salinity conditions. Experimental study on the degree of salt tolerance of some selected cyanobacteria carried out by Moisander et al., (2008) revealed that *Anabaena* tolerated salinity conditions of 15-20 g/l, whereas *Cylindrospermopsis* tolerated salinity of only 2-6g/l. Some members of nitrogen-fixing cyanobacteria were, reported to be rare or less abundant in water bodies of high salinity such as estuarine (Conley et al., 2009). This could possibly due to survival mechanisms such as active sodium transport, synthesis of stress protein, and production of osmolytes demanding high metabolic cost, which may compromise the growth rate and partly explain the rare occurrence of cyanobacteria in such water bodies (Moisander et al., 2008).

2.3.2.4. Light

Underwater light is one of the most important environmental variables that influence phytoplankton growth and their dynamics (O’Farrell et al., 2007). Studies show that members of cyanobacteria are well adapted to a wide range of irradiance levels (Padisák, 1997). Some members such as *Cylindrospermopsis* can tolerate low irradiance by taking advantage of their adaptive features to survive and proliferate in water bodies with low transparency (Dokulil and Teubner, 2000; Fabbro and Duivenvoorden, 2000; Padisák, 1997). According to Yamamoto and Shiah, (2012), poor light condition that results from an increase in cell density of *C. raciborskii* may limit the growth of other phytolankton taxa, which further enhances the dominance of the genus in water bodies with low water transparency. Other members like *Microcystis* are able to regulate their buoyancy by virtue of their gas vesicles, which enable them to migrate vertically in the water column to harness light energy required for photosynthesis (Reynolds, 2006). The reported dominance of *Microcystis* spp. in many of the Ethiopian rift valley lakes including Koka Reservoir (Elizabeth Kebede and Willén, 1998; Melaku Mesfin et al., 1988; Willén et al., 2011) and Lake Chamo (Amha Belay and Wood, 1982), despite the poor underwater light condition, might be attributed to their capability to regulate their buoyancy.

2.3.2.5. Water Residence Time (WRT)

Water residence time is the average time required to refill a basin if it were emptied (Kalff, 2002). The average water residence time can be calculated from the basin volume (V, m^3) and the average annual outflow (Q, $m^3\ yr^{-1}$) (Kalff, 2002).

$$\tau = \frac{V}{Q}$$

Where: - τ = water residence time (year)
V= Volume of the basin (V, m^3)
Q= Out flow rate (Q, $m^3\ yr^{-1}$)

Water residence time (HRT) greatly influences the limnology of the aquatic systems due to its direct effect on the fate of chemicals entering the lake (Rangel et al., 2012), their concentration and sedimentation, accumulative capacity of inorganic nutrients and thermal stratification (Ambrosetti et al., 2003), primary productivity, and phytoplankton community structure (Kalff, 2002; Rangel et al., 2012). Lakes and reservoirs with short residence time have less sediment and nutrient retention capacity, which may also influence phytoplankton community structure (Kalf, 2002). Short water residence time, for instance, may affect the diversity of aquatic biota, if their doubling time is longer than the water residence time, directly through hydrologic washout before they acclimatize and establish themselves (Kalf, 2002). In this regard, members of cyanobacterial genera such as *Microcystis* need long water residence time to acclimatize and establish in the aquatic system (Romo et al., 2013). Long water residence time also affects the aquatic system with its cascading effects on other limnological variables in such a way that other members of the phytoplankton community cannot cope-up with. However, members of cyanobacteria can take advantage of their adaptive strategy to deal with such limnological changes. For instance, water bodies with longer water residence tend to get warmer and stratify. In such cases, some members of cyanobacteria such as *Microcystis* may benefit from their capability to remain in the epiliminion (Visser et al., 2016) where sufficient light is available by virtue of their positive buoyancy. They can also benefit from the high water temperature as they have higher optimal growth temperature (Paerl and Huisman, 2009). The reported dominance of such members of cyanobacteria as *Microcystis* in several of the Ethiopian rift valley lakes (Elizabeth Kebede and Willén, 1998) may suggest that several of the lakes have long water residence time at least to allow the establishment of members of this group.

2.3.2.6. Climate Change and Harmful Algal blooms

The recent increase in the temporal and spatial recurrence of harmful algal bloom is often associated with climate change such as global warming and the subsequent changes in hydrological cycles or rain fall pattern (Zhang et al., 2012 and the references therein). This is attributed to the influence of climate change on the physico-chemical environment and the biological processes such as growth rate and bloom formation (Paerl and Paul, 2012). The influence of meteorological events such as the El Niño-Southern Oscillation (ENSO) cycles, which play a role in global climate, on the structure of phytoplankton communities has recently been studied in marine ecosystems (e.g. (Barber et al., 1996; Blanchot and Rodier, 1996; Liu et al., 1998). Climate change predictions indicate that Climate change anomalies such as El Niño induced drought and La Niña induced flooding are expected to increase in the future (Salinger, 2005). They may greatly modify the limnology of lentic systems such as Lakes and reservoirs in a way that it not only promotes the dominance of cyanobacteria but will also have great influence by fine-tuning cyanobacterial community structure. Furthermore, climate change also enhances the competitive advantages of cyanobacterial dominance in the aquatic environment. The increase in temperature due to global warming, for example, decreases water viscosity. As this condition decreases resistance to vertical migration in the water column, cyanobacteria can move up and down with relative ease. This helps them to harness light from the surface and acquire nutrients from the deeper part of the water bodies (Pearl and Paul, 2012). The other climatic condition associated with harmful algal bloom is the change in the hydrological cycle (Shaw et al., 2001). High rainfall rates may mobilize land nutrients and increase nutrient loading into the receiving water bodies, which in turn induces cyanobacterial bloom formation (Reichwaldt and Ghadouani, 2012). Changes in the physico-chemical characteristics of water bodies due to rainfall depend on land use in the catchment area. Land use in the proximity of water bodies and nutrient export, for example, have strong relationship, which in turn has a strong influence on cyanobacterial bloom dynamics (Bowes et al., 2005; Howarth, 1998; Kato et al., 2009). Rainfall events in the catchment areas with livestock production, for example, export more ammonium (NH_4) than nitrate (NO_3) into water bodies compared to land used for agricultural purposes (Shaw et al., 2001). This condition favors the proliferations of cyanobacteria, which are able to take up NH_4 more efficiently than other phytoplankton taxa (Présing et al., 1996).

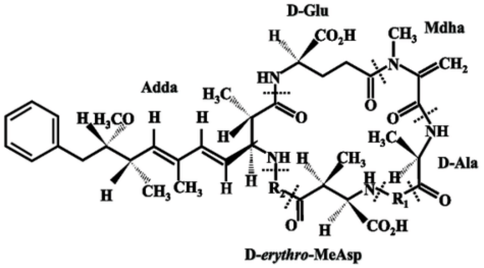
In summary inconsistencies in the reports on the key factors dictating cyanobacterial bloom dynamics and toxin production seem to suggest that there is no single cause for the occurrence of harmful algal blooms and toxin production. It is rather the result of the complex interaction and the combined effect of various biotic and abiotic factors. The occurrence of blooms and production of toxins reported for one lake may not, therefore, necessarily represent the facts for other lakes. These variations are so enormous even within a lake at different times of the year depending on the dominant cyanobacterial strains, which in turn are influenced by temporal variations in physico-chemical and other environmental factors. According to Xu et al., (2010), for instance, the concentrations of cyanotoxins seem to be affected by the strains of the toxin-producing blooms, which vary from lake to lake, both spatially and temporally, even within a given lake. Therefore, every lake should be considered unique when it comes to cyanobacterial bloom formation and toxicity potential (Kotak et al., 1995)**.**

2.3.3. Impacts of harmful algal blooms and their toxins

The occurrence of harmful algal bloom episodes is showing an increasing trend both in frequency and geographical distribution thereby threatening the healthy functioning of the aquatic ecosystem (Qin et al., 2010; Willén et al., 2011) and bringing high risk to public health (Backer, 2002; Qin et al., 2010; Willén et al., 2011). In the following sections, the socio-economic, environmental as well as public health problems induced by harmful algal blooms and their toxins are briefly discussed.

2.3.3.1. Cyanobacterial toxins (cyanotoxins)

Several cyanobacterial genera are known to produce a variety of toxins. *Anabaena, Aphanizomenon, Cylindrospermopsis, Microcystis, Nodularia, Planktothrix (Oscillatoria)* are among the toxin-producing taxa (Backer, 2002). The major groups of toxins produced by cyanobacteria include hepatotoxins and neurotoxins. The cyanobacterial hepatotoxins are cyclic or ringed heptapeptides. Hepatotoxins produced by different cyanobacterial genera comprise different number of amino acids. For instance, hepatotoxins produced by *Anabaena, Microcystis and Planktothrix* contain seven amino acids and are known as *microcystins* whereas those hepatotoxins produced by *Nodularia spumigena* contain five amino acids and are known as nodularins. Cyanotoxins also vary in their chemical structure and are not present as a single compound. They exist as numerous variants. For example, microcystin has more than 47 variants (Rinehart et al., 1994). The molecular structures of the three major and most common microcystin variants, MC-LR, MC-RR, and MC-YR are shown below (Table 2.1).



	R1	R2
Microcystin-RR	arginine	arginine
Microcystin-LR	Leucine	arginine
Micrcystin-YR	tyrosine	arginine

Table 2.1. Molecular structure of the three major microcystin variants (MC-RR, MC-LR, MC-YR).

2.3.3.2. Eco-physiological Functions of Cyanobacterial toxins

Some attempts have been made to understand the ecological and/or physiological purpose of toxin production by cyanobacteria. However, the role of the toxins is still remains largely unknown (de Figueiredo et al., 2004). Many of these studies conducted to understand the eco-physiological functions of cyanotoxins were carried out on *Microcystis* (El-Shehawy et al., 2012) known to be one of the most ecologically damaging species due to its highly potent toxins, microcystin. According to these studies, microcystin are synthesized non-ribosomally by microcytin synthetase that is encoded by MC gene cluster (de Figueiredo et al., 2004; Rinta-Kanto et al., 2005; Rinta-Kanto et al., 2009). The gene encoding for microcystin was identified and sequenced in many cyanobacterial genera including *Microcystis aeruginosa*, *Planktothrix agardhi* and

Anabaena species (Christiansen et al., 2003; Rouhiainen et al., 2004; Tillett et al., 2000). Other similar toxins identified and sequenced are nodularins, which are synthesized by *Nodularia spumigena* (Lyra et al., 2005; Moffitt and Neilan, 2001) and Cylindrospermopsin produced by *Cylindrospermopsis*. Based on their findings, different researchers have forwarded different speculative theories to explain why these groups of organisms produce the toxins. Some suggested that the toxins are meant to deter bacteria/ algae that compete for the same resources such as nutrients (N, P) and space (Engström-Öst et al., 2011); Cole 1982). Others claim that the toxins are synthesized in iron-depleted environments and during fierce competition for it with other bacteria. In this regard, some bacteria are able to secrete soluble iron chelators known as siderophores (Braun et al., 1998). The synthesis of microcystin is, therefore, meant to compete for iron as the toxins have iron-chelating (scavenging) properties (Humble et al., 1997; Utkilen and Gjølme, 1995). Still others associate the synthesis of the toxins with accumulation of surface proteins, which serve as means of colony formation (Kehr et al., 2006; Zilliges et al., 2008). Another possible explanation suggested by (Schatz et al., 2007) is that microcystin may serve as an extracellular signaling molecule. Although the physiological and molecular details of the mechanisms of signaling is not well understood, it is suggested that when some cyanobacterial cells undergo apoptotic cell death as a result of environmental stress, microcystin and other peptides are released into the aquatic environment. The rest of the population senses the released toxin and peptides as a signal of environmentally stressful conditions. The perceived signal induces the regulation of biosynthesis of microcystin as a defense mechanism (Schatz et al., 2007), for example, for avoiding grazing by zooplankton (Sterner, 1989).

2.3.3.3. Socio-economic Impacts of Cyanobacterial bloom

The occurrence of harmful algal blooms causes a lot of damage to recreational, as well as to drinking water bodies. They decrease the water quality and increase the risk of toxicity to aquatic and terrestrial organisms including humans (Carmichael et al., 1997)**.** Among cyanobacterial taxa, *Microcystis aeruginosa* (Plate 1), is the most ecologically damaging species due to its wide distribution and degree of toxicity (Briand et al., 2003).

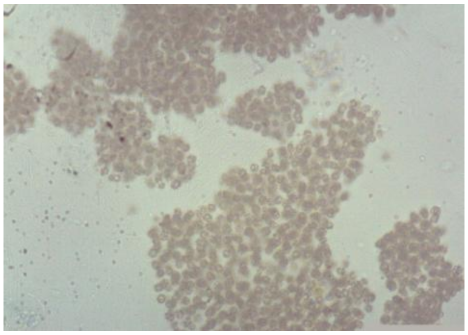


Plate 2.2. *Microcystis* sp. from Koka Reservoir at 40X magnification (Photo by Samson Tilahun)

Poisoning of animals results from the ingestion of water contaminated with toxic cells, or toxin released from decaying cells (Oberholster et al., 2006). Unexplained deaths of pond-grown catfish, for example, have occurred for many years, some of which may be attributed to the cyanobacterial toxin, microcystin (Zimba et al., 2001)**.** A report by Peuthert et al., (2007) indicated that MC-LR, one of the most studied toxins, is absorbed by roots of 11 agricultural plants studied and subsequently translocated to the shoots of the seedlings. The phyto-toxicity of the toxin is due to the inhibition of phosphotase enzyme and oxidative damage caused to the tissues. This may reduce agricultural productivity and end up in humans through the food chains. Apart from the economic loss due to reduced productivity of plants, consumption of the plants might cause serious public health problem (Chen et al., 2012). Similarly, this productivity loss and public health risk associated with cyanobacterial toxins apply to fish species grown in water bodies with harmful algal blooms (Watkinson et al., 2005). The socio-economic impacts of such damages in the US have been reported to be worth more than 2 bln US dollars (Dodds et al., 2008). In a similar report, Lake Taihu, China, which serves as a source of drinking water supply for more than 10 million people, and used for fishing, and tourism, is experiencing a widely spreading bloom of *Microcystis*. The blooms have caused a lot of environmental, societal as well as economic losses. Animal poisoning by cyanobacteria leading to deaths of domestic animals like dogs and cattle upon exposure to microcystins produced by *Microcystis aeruginosa* in England (Edwards et al., 1992) and nodularins by *Nodulariaspumigena* in Norway (Nehring, 1993) have also been reported. Although there are some reports on the occurrence of harmful algal blooms in many of the rift valley lakes of Ethiopia (Elizabeth Kebede and Willén, 1998; Melaku Mesfin et al., 1988; Willén et al., 2011), the actual environmental and economic damage, as well as the public health problems caused by the blooms have not been reported and/or documented. The only report by (Amha Belay and Wood, 1982; Wood and Talling, 1988) documented the death of zebras around Lake Chamo caused by the bloom of *Microcystis aeruginosa*, the cyanobacterium often dominant in the Lake.

2.3.3.4. Human Exposure Routes to Cyanotoxins

Although there are several ways, the most common route of human exposure to cyanotoxins is through drinking and recreational water (Funari and Testai, 2008). These involve direct ingestion of water containing cyanobacterial cells and toxins. Children are particularly at risk from ingesting large quantities of cyanobacterial cells, while playing in shallow waters along lake shores where toxic cyanobacterial scum accumulates (Oberholster et al., 2006). A reservoir which was drinking water supply source for a rural town of Australia which became eutrophicated and supported a bloom of *Microcystis aeruginosa* resulted in liver damage in the exposed population, (Falconer, 1983). Furthermore, nowadays there is a growing interest in the use of cyanobacteria as dietary supplements. However, there exists possible contamination of these supplements with cyanotoxins. Microcystin, for instance, was detected in such algal supplements (Gilroy et al., 2000). Consumption of these dietary supplements is, therefore, one possible direct route of exposure (Backer, 2002) to cyanotoxins. Consumption of food animals such as fish, which have ingested cyanobacteria and accumulated their toxins, is also another reported route of exposure (Ibelings and Chorus, 2007). Use of untreated water sources for irrigation and lawn watering may also put people at risk of exposure to cyanobacteria and their toxins through aerosols (Backer, 2002). Another route of exposure reported by (Jochimsen et al., 1998) is the use of water contaminated with cyanotoxins in dialysis treatments of patients, which caused serious illness and even deaths.

2.3.3.5. Public Health Impacts of Cyanobacterial blooms

With the advent of climate change, pollution associated with industrialization and agricultural practices, the occurrence of harmful algal blooms is showing an increasing trend both in frequency and in geographical distribution (Pearl and Paul, 2012). As a result, the impacts of these blooms are now being felt and reported all over the world. Complications associated with cynaobactrial toxicity are varied including gastroenteritis, nausea, vomiting, fever, sore throats, blistered mouth, ear and eye irritations, severe systematic effects such as hepatic failure and death (Stone and Bress, 2007). However, there are only few epidemiological data on the public health impacts of cyanobactrerial toxicity. This is possibly because many of the clinical symptoms of cyanotoxins overlap with those of other common causes such as enteric infections or illnesses associated with agricultural and industrial pollutants (Texeira et al., 1993). For instance, people swimming in water bodies with cyanobacterial bloom may experience itching, allergic dermatitis (Soong et al., 1992), which may be confused with cercarial penetration during *Schistosoma* infections (Stone and Bress, 2007). Similarly, headache, abdominal cramps, nausea, vomiting, diarrhea are also reported by people exposed to cyanobacterial toxins.

In Table 2.3. cyanobacteria responsible for fresh water algal blooms and human illnesses caused by their toxins are presented. The symptoms experienced are confused with illnesses associated with enteric infection. As a result, when an outbreak of human illness occurred in the past, cyanobacterial toxicity as a possible cause was less suspected and hence only few documentations and reports on this aspect are available. Nevertheless, there are a few reports.

Table 2.3. Public health problems associated with cyanotoxins reported based on studies on animal models (Source: Becker et al, 2002)

Toxin	Organisms	Acute effect	Signs and symptoms of intoxications
Anatoxin-a	*Anabaena* *flos-aquae*	Neuro toxicity	Progression of muscle fasciculation decreased movement abdominal,
Anatoxin-a	*Anabaena*	Neuro toxicity	Hyper salivation, mucoid nasal discharge,
Cylindrospermopsin	*Cylindrospermopsis raciborskii*	Hepatotoxicity Chromosome breakge	Huddling, anorexia, slight diarrhea, gasping respiration, enlarged liver,
Microcystins	*MicrocystisSpp* anabena spp	hepatotoxicity	
Nodularin	*Nodularia spumigena*	Hepatotoxicity	Skin and eye irritations
Cylindrospermopsin	*Cylindrospermopsis raciborskii*	Hepatotoxicity Chromosome breakge	Huddling, anorexia, slight diarrhea, gasping respiration, enlarged liver, malaise

For example, several people in Australia were reported to have suffered series complications associated with cyanobacterial toxicity (Falconer et al., 1983). In a similar report, outbreak of gastroenteritis was reported in people who were using water from eutrophicated Ohio River (Falconer, 1999). In another report from Brazil, dialysis patients were affected due to the use of contaminated water with cyanobacterial toxins. The patients suffered severe hepatic failures >50 of the cases were reported to have died. Latter investigations revealed cyanotoxins, cylinderospermopsin and microcystin were responsible for the reported illnesses and deaths (Carmichael et al. 2001; Jochimsen et al. 1998).

2.3.3.6. Harmful algal blooms and their impacts in Ethiopian lakes

The abundance/dominance of potentially toxic cyanobacteria is also becoming increasingly common in many of the Ethiopian rift valley lakes (Elizabeth Kebede and

Willén, 1998; Melaku Mesfin et al., 1988; Willén et al., 2011). Out of the 206 species of phytoplankton identified in samples collected from Ethiopian Rift valley lakes, 61 species belonged to cyanobacteria (Elizabeth Kebede and Willén, 1996). *Microcystis* spp. are among the most important component species of the phytoplankton communities supported by the Ethiopian Rift Valley lakes (Elizabeth Kebede and Willén, 1996). As most of these water bodies are currently serving as sources of freshwater used for drinking water supply, watering livestock, irrigation, fisheries, sanitation and recreation (Fig. 2.3), the potential public health hazard and economic loss is immense. For instance, incidence of massive fish kills in Lake Chamo, one of the rift valley lakes in Ethiopia, and deaths of terrestrial wild animals around this lake in the year 1978 were linked to cyanotoxins (Amha Belay and Wood, 1982). A cross sectional survey carried out on seven of the Ethiopian rift valley lakes has demonstrated the dominance of toxic cyanobacteria and presence of associated toxins at concentrations much higher than the permissible level in most of the water bodies (Willén et al., 2011). This suggests the potential public health hazard associated with the use of these water bodies. According to Willén et al. (2011), Koka Reservoir, was in the worst situation, with *Microcystis aeruginosa,* overwhelmingly dominating the cyanobacterial assemblage and with the levels of total microcystins (MCs, 45–54 µg L^{-1}) greatly surpassing the permissible level set by WHO. This implies that domestic use of the reservoir, as a source of fresh water for the afore-mentioned purposes may be potentially hazardous. In fact, according to Willén et al., (2011), human and cattle illness around Koka Reservoir has already been reported by the local inhabitants since early1980s. There is, therefore, an urgent need to protect public health, aquatic and terrestrial life.

CHAPTER THREE

3.0 RIVERINE AND ATMOSPHERIC INPUT OF NUTRIENTS TO KOKA RESERVOIR AND THEIR POTENTIAL IMPACT

3.1. Introduction

Atmospheric deposition and riverine loading are the major routes of nutrient input to inland waters, particularly in areas where extreme land degradation associated with intense agricultural operations has occurred (Ahn and James, 2001; Baker and Miller, 2013). The relative importance of atmospheric and riverine sources may depend on several factors. Catchment area to lake surface area ratio, CA:LA (Kalff, 2002; Redfield, 2002), flow regime (Das et al., 2011), meteorological factors (Amodio et al., 2014) such as rain fall pattern (Anderson and Downing, 2006; Ravi et al., 2004), land use (Baker and Miller, 2013) and vegetation cover (Yan et al., 2013) are among such factors. Atmospheric deposition of nutrients onto lakes and reservoirs may take place through snow fall or rain fall, which are collectively termed as wet deposition, while that, which takes place through gaseous phases and/or as particle-bound, is known as dry deposition (Zhai et al., 2009). In lakes located in tropical climate such as Koka Reservoir, the subject of the present study, deposition through rainfall and gaseous phases and as particle-bound nutrients are relevant mechanisms of nutrient inputs of wet and dry depositions respectively. Atmospheric deposition may have significant contribution to total nutrient inputs to lakes and reservoirs (Anderson and Downing, 2006), with its contribution sometimes exceeding that of riverine loading (Jassby et al., 1994; Muvundja et al., 2009; Tamatamah et al., 2005). One possible reason for this is that atmospheric nutrients may also originate from sources other than agricultural practices such as automobile emission exhaust and biomass burning (Tamatamah et al., 2005), and unpaved roads, (Herut et al., 1999). For instance, large scale deforestation and biomass burning have been suggested as sources of atmospheric deposition in African great lakes such as Lake Victoria (Tamatamah et al., 2005), which could also be true as such practices are becoming increasingly common in the Awash basin. Furthermore, unlike riverine sources, wind blown nutrients may also come from areas far away from the catchments (Reynolds et al. 2006), which could be regional or continental. The airshed that could be possible source of nutrients for atmospheric deposition is 10-20 times larger than the watershed (Paerl et al. 2002). This seems to be in agreement with the situation in Koka Reservoir, the subject of the present study. The watershed, which is relevant to nutrient input into Koka Reservoir through Awash River and Modjo river is the upper Awash catchment located upstream of the reservoir covering an area of 10, 800 km^2 excluding the reservoir. (Fig 3.2). The airshed, which could be the potential source of nutrients through atmospheric deposition may, however, originate from the entire Awash basin covering an area of around 110, 000 Km^2, which is more than 10.2 times the watershed area (Fig 3.2). As tropical lakes are generally of riverine origin (Lewis Jr, 1996), they are also at a higher risk of pollution from riverine sources. Conversion of land with natural vegetation to agricultural land causes changes in the watershed hydrology through its effect on the rate of canopy interception, infiltration, evapo-transpiration, and ground water recharge, all enhancing nutrient transportation (Baker and Miller, 2013). Several African rift valley lakes have been impacted by nutrient loading from diffuse sources associated with land use changes (Baker and Miller, 2013; Muvundja et al., 2009). Koka Reservoir is among the Ethiopian Rift Valley Lakes (ERVL) that have been impacted by nutrient loading from both diffuse and point sources.

The upper Awash River catchment is largely dominated by agricultural land use and is progressively degraded due to forest clearing for additional agricultural land and fuel wood (Halcrow and Pattern, 1989). This has caused transportation of huge amount of sediment and siltation in the reservoir, particularly during the rainy season (Halcrow and Pattern, 1989). Furthermore, several industries dischatge their wastewaters into the rivers that feed the lakes (EPA, 2003). Mojo River, one of the rivers that feed Koka Reservoir, is currently receiving wastewater from several tanneries. As a result, Mojo River is one of the most polluted rivers in Ethiopia, with high concentrations of nitrogen and phosphorus (Seyoum Leta et al., 2003; Amanial Haile, 2015; Zinabu G/Mariam and Pearce, 2003). Presumably, Koka Reservoir receives much of the nutrients from point sources through this river. Thus, this study aimed at estimating nutrients inputs from riverine and atmospheric sources and their potentials to cause eutrophication related water quality problems of the reservoir.

3.2. MATERIAL AND METHODS

3.2.1. Description of Study area

3.2.1.1. Koka Reservoir and Awash River Basin

Koka Reservoir

Koka Reservoir (Fig. 3.1**)** is located some 100 km southeast of Addis Ababa at 8°23'38.4''N, 39°04'51.6''E and at an altitude of 1590 m a.s.l. It was constructed in early 1960s across Awash River for hydroelectric power generation, and flood control (Halcrow and Pattern, 1989).

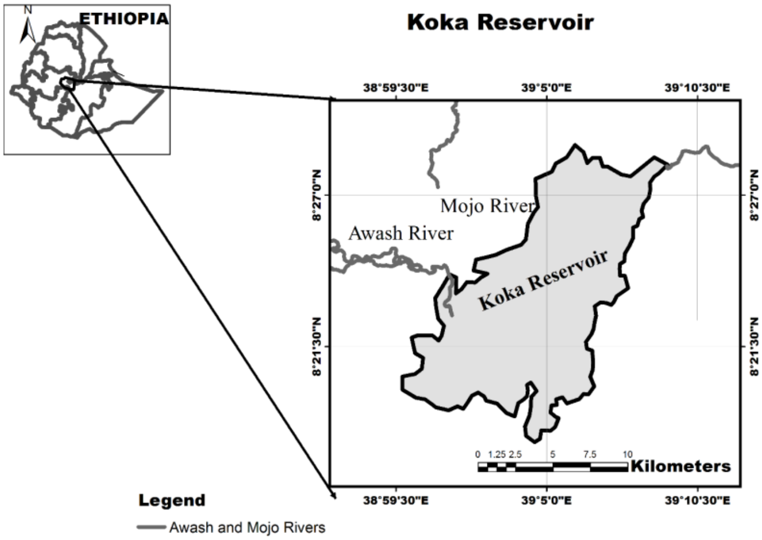


Figure 3.1. Koka Reservoir, its feeder rivers and a partial view of its catchments

However, thousands of the local inhabitants use the reservoir as a source of fresh water supply, and for livestock watering and make their living through subsistence commercial fisheries (plate 3.1).

Plate 3.1 Livestock drinking water at the near-shore site of the reservoir (top right), Fetching water for household purposes (top left); bloom of Microcystis; patches and remnants of Microcystis scum at the near-shore site after the collapse of the bloom (bottom left). **Photos by Samson Tilahun**

The reservoir had an initial volume of 1650 Mm^3 (Abebe, 2001), and surface area of 200 km^2 (Elizabeth Kebede and Willén, 1996; Willén et al., 2011). The morphometric characteristics of the reservoir have been changing due to progressive silt deposition through Awash River and a minor feeding river, Modjo River (Fig. 3.1). According to a review by Michael Abebe, (2001), the reservoir had lost 28.9 % of its water holding capacity by the year 1998 primarily due to siltation. Expectedly, the reservoir has also been receiving huge amounts of nutrients from tanneries and flower farms and agricultural runoff causing eutrophication-related water quality problems such as recurrent cyanobacterial blooms over the last several years.

The Awash basin

The Awash basin covers an area of 110, 000 km^2 **(Table-3.1)** and is located between latitudes 7°53′N and 12°N and longitudes of 37°57′E and 43°25′E in Ethiopia (Fekadeselassie Berhe et al., 2013). It is divided into three distinct zones known as upper Awash, middle Awash, and lower Awash **(Fig.3.2)** based on several factors including location, altitude and climate (Desalegn Chemeda et al., 2010).

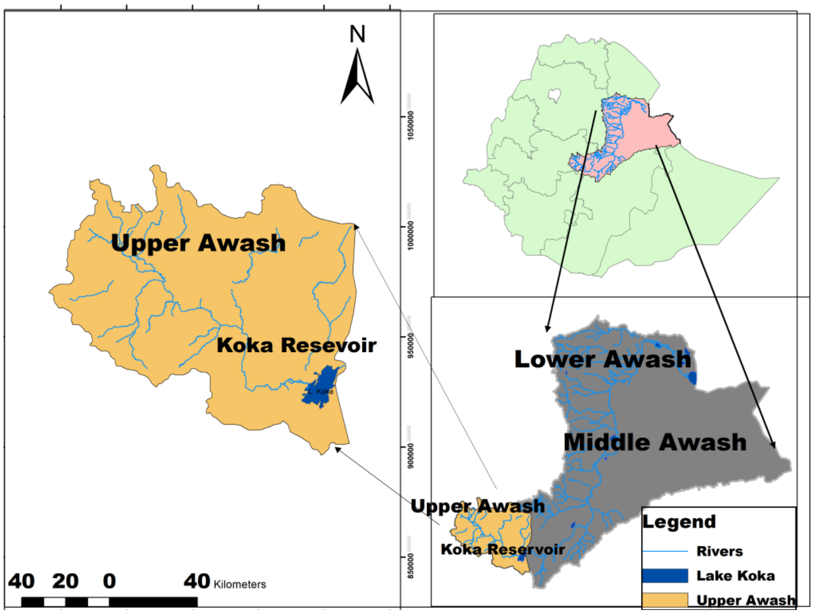


Fig. 3.2. Upper Awash sub-basin and Koka Reservoir: potential riverine source area (left), the entire Awash basin (Bottom right), which is the potential source area for atmospheric input of macronutrients and total suspended solids.

The climate of the Awash basin varies from humid subtropical over central Ethiopia to arid over the Afar lowlands (Gonfa Lemma, 1996). Long-term wind speed record at Bole airport indicates that the greatest frequency (**Table 3.2**) of wind speed exceeding the erosive threshold value of 5.3 m/s (an approximate wind speed for wind erosion) occurs during the dry months (Halcrow and Pattern, 1989). According to this record, the mean maximum dust speed during these months is 16 m/s, which is also much higher than the erosive threshold speed. It is also noted that the transport rate of wind-carried dust increases as a cube of surpassing the threshold levels (Halcrow and Pattern, 1989). Koka Reservoir is located wthin the upper Awash catchment (**Fig. 3.2)**. Long-term hydrological data record at Hombole, the most downstream of upper Awash gauging station, obtained from Ministry of Water, Irrigation, and Electricity (MWIE) indicate that the flow rate of the river exhibited considerable seasonal fluctuations, which is attributed to the rainfall pattern of the basin **(Fig. 3.3)**.

Table: 3.1–Morphometric and hydrological parameters of Koka Reservoir and Awash basin

Parameter	Value
Surface area[1]	200 Km^2
Mean depth of the reservoir [2]	5.93m
Watershed area (upper Awash catchment excluding the reservoir)reservoirreservoir)[2]	10,800 Km^2
entire Awash basin[3] (airshed)	110,0000 km^2
The mean annual runoff to the reservoir[4]	1650Mm^3
Mean annual riverine sediment load to the reservoir [5]	17 Mm^3/yr.

1.Willén et al.(2011), 2. Ministry of Water Irrigation and Electricity (1998), 3.Halcrow and Pattern (1989), 4.Girma Taddese (2001), 5. Wolancho (1993).

According to this data record, the average maximum and minimum discharges are 235 m^3/s and 3.76 m^3/s, during the rainy and dry seasons, respectively. The flow of the river is more erratic during the rainy season (Jun to Sept) than the long dry season as it is reflected in the greater standard error (see Fig. 3.3). During the long dry period, the flow of the river is limited to base flow rather than surface flow. The total inflow to the reservoir during the long dry season constitutes only 17 % of the total capacity of the reservoir suggesting the riverine input of nutrient to the reservoir is greatly reduced during the dry months. **Fig 3.3** shows the typical flow regime of Awash River and the long-term monthly mean rainfall at Hombole gauging station. The reservoir also receives the greatest proportion of its sediment load during the rainy season, June-September and possibly a major portion of its nutrient budget from riverine resources corresponds to these peak flow months. With increasing population and anthropogenic impact, the entire basin is being progressively degraded (Halcrow and Pattern, 1989). Forest clearing for additional agricultural land and fuel wood (Halcrow and Pattern, 1989) is a common practice. Extreme vegetation loss exposed the soil to severe water and wind induced erosion in the basin (Hailemariam, 1999; Halcrow and Pattern, 1989).

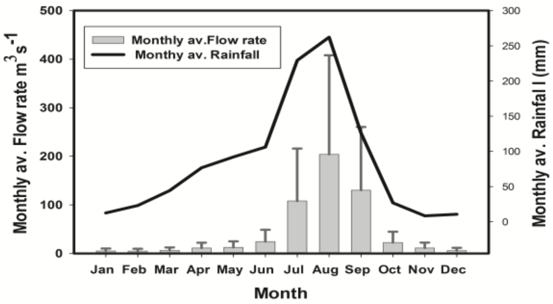


Fig. 3.3. Long-term monthly average flow rate of Awash River and monthly mean rainfall at Hombole gauging station

Table-3.2:- Seasonal trends in the frequency of wind speed in the Awash basin (Source: Halcrow, 1989).

Month	**%frequency Mean wind speed**	**% of months with easterlies wind**	**% of wind from E>5.3m/s**
Jan	47	82	75
Feb	82	76	79
Mar	88	82	80
Apr	65	82	73
May	88	71	73
Jun	18	12	67
Jul	6	0	-
Aug	6	0	-
Sept	29	24	60
Oct	94	76	76
Nov	88	88	93
Dec	53	94	89

The huge soil loss through runoff (Halcrow and Pattern, 1989) and possibly wind erosion in the basin has greatly impacted Koka Reservoir through siltation **(chapter 4)** and nutrient input with consequent water quality deterioration associated with eutrophication, namely the occurrence of harmful algal blooms **(Chapter 5).** In this chapter, we report the results of the study on the possible impact of nutrient inputs from riverine and atmospheric sources on Koka Reservoir.

3.2.2. Sampling of Awash River water

River water sampling for nutrient analysis was carried out monthly at the outlet of the watershed of Awash River in the upper Awash catchment located at the most downstream gauging station at Hombole **(Fig. 3.2).** Sampling was carried out at about the middle of the course of the river without touching its bed. Cable car was used for easy access to the middle of the river (see appendix). Sampling was made using a clean plastic container after rinsing it with the river water itself. All samples were transported to the laboratory in cool icebox and analyzed immediately upon arrival to avoid any possible change.

3.2.3. Sampling for Atmospheric deposition

Samples for atmospheric deposition (dry fall) were collected on the Koka town side of the reservoir on monthly basis. Samples were collected with bowls properly cleaned with phosphate-free detergent and rinsed several times with distilled and de-ionized water. There are several techniques of collection of samples for the determination of atmospheric input of nutrients through dry deposition. In this study, atmospheric deposition on wet surfaces was assumed to be the most appropriate and relevant to input of nutrients through reservoirs' surface (Cole et al., 1990). Therefore, the containers were filled with a pre-defined volume of distilled water (1L) to simulate the collecting property of lake surface (the reservoir surface) (Cole et al., 1990; Jassby et al., 1994). It was then exposed to open air 2.5 m above the ground far away from any physical objects such as trees to minimize local contamination such as birds dropping (Anderson and Downing, 2006) for 24 hours (Anderson and Downing, 2006; Muvundja et al., 2009). At the end of the 24-hour exposure, the final volume was noted and immediately transferred to a clean plastic container. All samples were transported to the laboratory in cool icebox for analysis on same day of sample collection. Rainwater-contaminated samples were discarded.

3.2.4. Acquisition of Hydrological and Meteorological data

Long-term daily weather data record (including data of our sampling period) at the Koka dam station was obtained from the National Meteorological Agency (NMA). Long-term flow rate data of Awash River (including data of our sampling period) recorded at Hombole gauging station was obtained from the Ethiopian Ministry of Water, Irrigation and Electricity (EMWIE).

3.2.5. Analysis of Inorganic Nutrients

The detailed procedure followed for the analysis of inorganic nutrients is described in chapter 4.

3.2.6. Estimation of riverine nutrient load

The monthly nutrient loading from upper Awash catchment was calculated by multiplying the monthly concentration, C (mg/m^3) by monthly discharge.

The monthly nutrient load (ton) was calculated as

$$M_{Rl} = K * \sum_{i=0}^{n} Q_i * C$$

Where: MR_l= Monthly riverine loading, Q_i= daily flow rate (m^3/d), C=concentration (mg/m^3), n= number of days of the month (30) and K is a factor for conversion to ton.

3.2.7. Estimation of Atmospheric Nutrient Load

Nutrient load through atmospheric deposition per day per unit area was calculated as described in (Anderson and Downing, 2006).

$$At_{dd} = {C * V_f}/{A * \Delta t}$$

Where At_{dd}= Atmospheric dry deposition (mg $m^{-2}d^{-1}$), V_f= Final volume after 24 hrs (m^3) of exposure to open air, A= Area of the bowls (m^2), and C= Concentration of nutrients (mg m^{-3}), Δt = 1 day

Extrapolation to monthly load of the dry days of the month and to the entire reservoir surface was given by

$$M_{Atl} = A_r * K * n * {C * V_f}/{A_c * \Delta t}$$

Where M_{Atl} = monthly atmospheric load (ton),V_f= final volume after 24 hrs (1 day). A_c= area of the container (m^2), C= concentration of nutrients (mg m^{-3}), K, = factor for conversion to ton. A_r= Area of the reservoir (m^2), Δt = 1 day and n = number of dry days during the month

3.2.8. Modeling the Impact of Atmospheric and Riverine Sources of Pollution

The possible trophic state response of Koka Reservloir to total phosphorus input from allochthonous sources (atmospheric and riverine sources) was analyzed using a trophic state model proposed by (Salas and Martino, 1991). A detailed account of the model (Salas and Martino, 1991) is given in **Chapter 4.**

3.2.9. Statistical Analysis

Spearman rank order correlation analysis was used to determine the possible association of nutrient loads from atmospheric and riverine sources with monthly precipitation at Koka Reservoir. Statistical analyses were carried out using SPSS ver. 20. Sigmaplot Ver. 10 was used for plotting graphs.

3.3. RESULTS

3.3.1. Flow Rate

The present study period, May, 2015 to April, 2016, coincided with the El Niño induced drought, which occurred in 2015 (Gleixner et al., 2017). The level of precipitation recorded at Koka dam station was much lower than the long-term (2004-2014) average determined for the same area, while the air temperature documented during the present study period was higher than the long-term (2004-2014) average **(Fig. 3.4).**

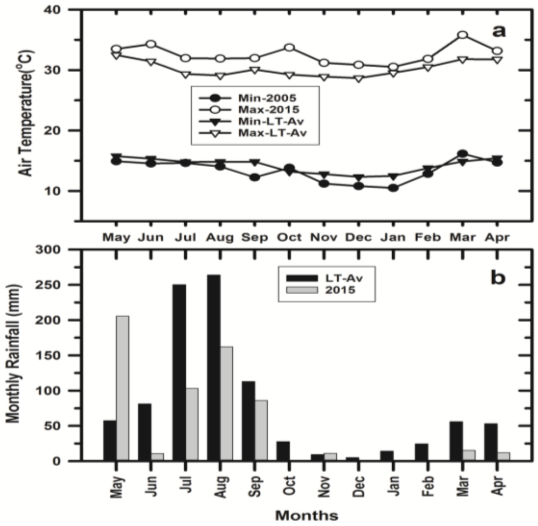


Fig. 3.4. Monthly mean maximum (Max-2015) and minimum (Min-2015) air temperature and monthly rainfall of the El Niño induced drought year (2015) in relation to long-term (2004-2014) average values of monthly mean maximum long-term average (Max-LT-Av) and minimum long-term average (Min-LT-Av) air temperature and long-term monthly rainfall (LT-Av) of the study area.

The mean monthly flow rate of Awash River recorded at Hombole gauging station followed similar pattern (relatively lower flow rate than the long-term (2004-2014) with the rainfall recorded during the present study **(Fig. 3.5).** The mean flow rate of Awash River during the present sampling months (26.46 m^3/s) was much lower than the average (45.5m^3/s) of the observations made for similar months over long-term period (2004-2014). The observed low flow rate concurred with reduced sediment transport relative to the long-term scenario. Modjo River, a minor feeding river to the reservoir, dried up completely during the same sampling months (see plate 3.1).

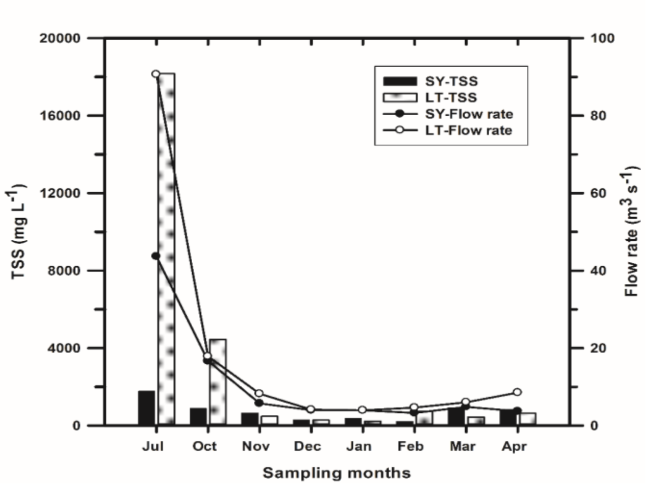


Fig. 3.5 Variations over the sampling year (SY) (2015) in monthly average total suspended solids (TSS-2015) and flow rate (FR-2015) in relation to the long-term (LT) (2004-2014) monthly average of TSS (TSS-2004-2014) and flow rate (FR-(2004-2015).

Plate 3.2 Impact of El Niño induced drought: Complete dry-up of one of the feeder rivers, Modjo River, close to Koka Reservoir (March, 2016 of the minor rainy period).

3.3.2. Riverine Loads of Macronutrients

Monthly riverine input of TP and SRP (in tons) at Hombole gauging station ranged from 1.12 (Feb) to 70.3 (Jul) and from 1.07 (Feb) to 42.8 (Jul), respectively, while nitrate (NO_3-N) and ammonia (NH_3+NH_4^+-N) varied between 0.00431 (Feb) and 1.7 (Jul) and 0.037 (Feb) and 7.78 (Jul), respectively (Fig. 3.6).

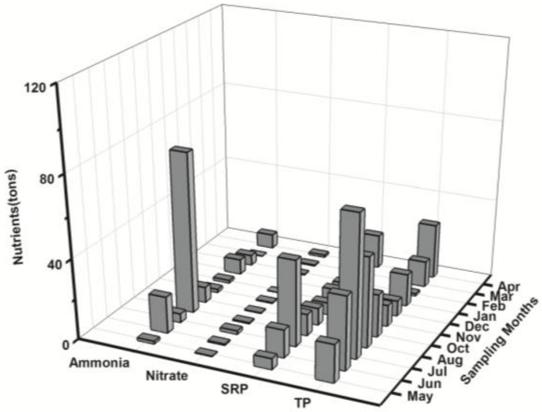


Fig. 3.6 Monthly variations in riverine loadings of macronutrients to Koka Reservoir.

Nutrient inputs from riverine sources showed no association with the monthly rainfall record at Koka Dam station (Spearman Rank Order correlation, $p > 0.05$, Table 3.3). However, strong positive association (Spearman Rank Order correlation, $p < 0.01$) among the nutrients (Nitrate and SRP, TP) and TSS (Table 3.3) was observed. The areal total phosphorus load exclusively from atmospheric source, L (P), during the present study was 2410 mg/m^2. Using the trophic state model developed for tropical warm lakes (Salas and Martino, 1991), in Lake TP concentration, $P_{\lambda,}$ was predicted to be 146.6 mg/m^3, which surpassed the cutoff point of 100 mg/m^3 for eutrophic state. The reservoir is, therefore, categorized as hyper-eutrophic lake based on the probabilistic distribution of trophic state (see Fig. 4.2, Chapter 4) as modified by Salas and Martino (1991) for warm water tropical lakes.

Table 3.3. Spearman Rank Order Correlation of monthly rainfall and riverine nutrient concentrations

	Monthly Rain fall	**TP(ton)**	**SRP**	**Nitrate**	**Ammonia**	**TSS**
Monthly	1	0.453	0.216	0.123	0.435	-.0773
TP(ton)		1	**0.895****	**0.790****	0.402	0.523
SRP			1	**0.909****	0.0206	**0.766****
Nitrate				1	-0.00879	**0.796****
Ammonia					1	-0.304
TSS(ton						1

3.3.3. Atmospheric Loads

This study is probably the first report on the possible impacts of nutrient input exclusively from atmospheric sources on a tropical reservoir in Ethiopia. Atmospheric inputs (tons) of macronutrients and TSS during the dry days of the sampling months are presented (Fig.3.7 and Fig.3.8). TP and SRP varied between 0.317 (Jul) and 8.77(Feb) and between 0.105 (Jul)–2.74 (Feb.), respectively.

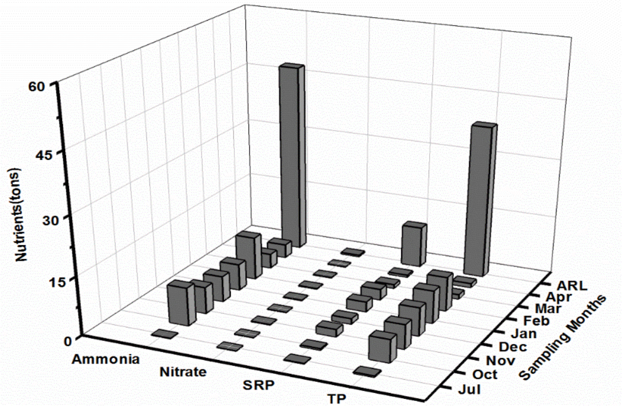


Fig. 3.7. Monthly variations in the atmospheric loading of macronutrients and annual atmospheric loading of the same in Koka Reservoir.

Nitrate loading (tons) ranged from 0.003 (Jul) to 0.093 (Feb), while that of ammonia varied between 0.048 and 11 tons with the lowest and highest loads occurring in July and February, respectively. As atmospheric samples contaminated with rainwater were not included in the estimation of atmospheric input of nutrients, the annual nutrient and TSS loads are expected to be much higher than reported here. However, the areal total phosphorus load exclusively from atmospheric source, L (P), during the present study was 195.05 mg/m^2. Using the trophic state model developed for tropical warm lakes (Salas and Martino, 1991), in Lake TP concentration, $P_{\lambda,}$ was predicted to be 11.84 mg/m^3, which surpassed the cutoff point of 10 mg/m^3 for oligotrophic state. The reservoir is therefore, categorized as mesotrophic lake based on the probabilistic distribution of trophic state as modified by Salas and Martino (1991) for warm water tropical lakes.

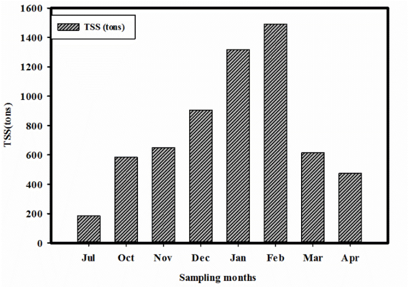


Fig. 3.8. Total TSS loads through dry fall during sampling months

Similar to those from riverine source, NO_3, SRP and TSS from atmospheric source exhibited strong association (Spearman Rank Order correlation, (*P*<0.001, Table 3.4).

Table 3.4. Spearman Rank Order Correlation between rainfall and atmospheric nutrient input

	Monthly rain fall	TP	SRP	NO_3	NH_3	TSS
Monthly Rain fall	1.000					
TP	-0.164	1.000				
SRP	-0.082	**0.905****	1.000			
NO_3	-0.3.00	**0.830***	**0.952***	1.000		
NH_3	0.470	**0.786***	0.619	0.586	1.000	
TSS	-0.164	**0.976****	**0.952****	**0.903****	0.690	1.000

**Correlation significant at 0.01; *Correlation significant at 0.05

3.4. DISCUSSION

3.4.1. Riverine loads of macronutrients

The observed low precipitation and high mean minimum and maximum air temperature of the present study, compared to long-term average, is associated with the El Niño induced drought in Ethiopia. The relatively low flow rate and sediment transport of Awash River observed compared to long-term records **(Fig.3.5),** and the complete dry up of Modjo River, the minor feeder river to Koka Reservoir, could possibly be the reflection of low precipitation at the upper Awash catchment associated with the then severe drought in Ethiopia. Furthermore, the observed average discharge rate (26.46 m^3/s) was also very low compared to that reported for Lake Kivu ($28.8m^3/s$) considering the large catchment area of the upper Awash (10,800 km^2), which is more than twice the catchment area of Lake Kivu (5090 km^2) (Muvundja et al., 2009). This further corroborates the association of reduced flow rate of Awash River with the extended drought. The relatively higher flow rate and sediment flux observed in July, October, November, March and April may be attributed to the relatively higher precipitation levels within the catchment area during these months or the aftereffect of rainfall events of the preceding months. Similar to hydrological features and sediment transport, nutrient transport is also expected to be greatly influenced by weather conditions. However, historical data on nutrient loading into the reservoir through Awash River are nonexistent. Therefore, the influence of the drought on nutrient transport into the reservoir could not be unraveled through comparison with the nutrient load estimated in the present study. However, circumstantial evidence such as reduced river flow rate and sediment yield estimated during the present study (**Fig. 3.5)** relative to those of earlier historical records seem to suggest that there was a concomitant reduction in nutrient transport as well. Furthermore, several studies made on the flow of Awash River and sediment yield at Hombole gauging station, with the aim of assessing the impact of siltation on the reservoir, reported that the highest flow and sediment yield occurred during the peak rainy season (Jul to Oct, Melesse and Abtew, 2016), with 90% of the annual runoff occurring during these months. In the present study riverine input during these months, constituted more than 72 and 74% of the total TP and SRP loads, respectively and accounted for more than 72.4% of the total riverine TSS load. This seems to suggest that rainfall greatly influences surface runoff, sediment yield and presumably external nutrient loading in the upper Awash catchment. Lack of positive association between the rainfall data recorded at Koka dam station with monthly nutrient loads could possibly due to the fact that rainfall events at Koka dam station may not necessarily represent the precipitation level of the entire source area, namely the upper Awash catchment. The strong positive association (Spearman rank order Correlation, $p<0.01$) among the nutrients (nitrate and SRP, TP) and TSS may suggest that they have a common source and mechanism of transport **(Table 3.3).** Total nutrient loading observed in the present study could also be considered low compared to that reported for Lake Kivu (Muvundja et al., 2009). The loading (tons) into Koka Reservoir during the sampling months of the present study totaled 93.6 (SRP), 167.9 (TP), 4.2 (nitrate), 35.2 (ammonia) and 63112.9 (TSS**)**. The annual riverine loadings (tons) into Lake Kivu were estimated at 40 (SRP), 599 (TP) and 134 (ammonia) and 115,000 (TSS). The total nutrient and sediment loading can, therefore, be considered very low given the large catchment area of the reservoir (10800 km^2), which is more than twice that of Lake Kivu. Therefore, the expectedly low nutrient input during the present study could partly be attributed to the severe drought, which greatly reduced surface runoff. The flow of Awash River was probably restricted to base flow for an extended period. This might have considerably reduced the annual riverine nutrient input into the reservoir. Das et al., (2011) compared base flow and surface flow with respect to nutrient and sediment transport. They concluded that nutrients (nitrogen and phosphorus) and sediment loads were significantly greater in surface flow than in base flow. This was attributed primarily to the mobilization of nutrients and sediments from diffuse sources, re-suspension of nutrients from the stream bed and streambed erosion associated with rainfall events and increased stream flow (Das et al., 2011). The presumed low nutrient input during our sampling period was also evident in the low levels of nutrients (see chapter 4), particularly nitrogen, within the reservoir. The relatively low nutrient level compared to ealier record for the reservoir triggered the unusual proliferation of diazotrophic cyanobacteria (Samson Tilahun and Demeke Kifle, 2019; Chapter 5).

3.4.2. Atmospheric Loads of macronutrients

Several factors might be responsible for the severe wind-induced soil erosion and the expected concomitant transport of nutrients. Dry soils are more vulnerable to wind-induced erosion (Ishizuka et al., 2005**.** According to Halcrow and Pattern, (1989), the greatest wind-induced erosion in Awash basin occurs during the dry months as the highest frequency of wind speed exceeding erosive threshold level occurs during these months **(Table 3.2).** The extended dry condition of these months may also cause loss of moisture from the soil and the consequent detachment of soil particles making them even more susceptible to blowing thereby soothing the situation for nutrient transport (Ishizuka et al., 2005). The observed levels of total suspended solids (TSS) and nutrient input through dry fall of the present study may, therefore, be attributed partly to the dry soil conditions and the prevailing strong wind with its speed exceeding erosive threshold (Halcrow and Pattern, 1989; Girma Taddese, 2001). Furthermore, crop harvesting and land preparation for the next cropping season take place during the dry season within the Awash basin. These activities make the soil even more susceptible to wind erosion thereby increasing atmospheric nutrient transport across the basin. Furthermore, unpaved roads are quite common within the agricultural fields. Dust re-suspension triggered by the passingby trucks and cattle further promotes wind-mediated transport of nutrients to distant places, with possible landing onto the reservoir's surface. However, similar to riverine source, no evidence of association between monthly rainfall and nutrient input through dry fall was observed in the present study. The non-significant negative correlation between monthly rainfall and nutrient input through dry fall in the present study (Spearman Rank Order Correlation, $p>0.05$, **Table -3.4**) may be due to the timing of rainfall events at Koka dam station and sample collection. Dry fall samples collected during months with rainfall events that took place a few days before sampling had the lowest nutrient and sediment loads. The relatively lower dry fall of nutrients and TSS in samples collected following rainfall events can be attributed to atmospheric flush out (Anderson and Downing, 2006). According to Anderson and Downing, (2006), atmospheric nutrient inputs even through wet deposition during the first rainfall events are much higher than those occurring during subsequent events of rainfall. Rainfall at source area may also be another factor for the non-significant association of monthly dry fall nutrient inputs with monthly rainfall recorded at Koka dam station. This is possibly due to the influence of soil moisture on wind- induced erodibility of soils (Ravi et al., 2004). Finer particles of soils with high moisture content may tend to stick together and become less prone to blowing thereby increasing the erosive threshold speed required for wind-mediated transport of soil particles (Ishizuka et al., 2005) and hence of associated nutrients. This may imply that the dry fall nutrient input to lakes is also influenced by weather conditions at the source area irrespective of the weather conditions at Koka dam station.

The strong association of nutrients with TSS ($p<0.001$, **Table3.4**) have common source and mechanism of transport. SRP, for instance, constituted only 21% of the total phosphorus. This suggests that the greatest proportion of total phosphorus was in particulate form (>0.7 µm) constituting over 79% of total phosphorus deposited through dry fall. This also signifies the severe wind-induced soil erosion in the Awash basin (Halcrow and Pattern, 1989) as it is reflected in the high level of TSS (over 6222 tons) recorded during our sampling period (**Fig. 3.8).**

The total load of ammonia (48.04 ton) was the highest (**Fig. 3.7**) compared to those of other nutrients and unlike other nutrients, monthly ammonia-nitrogen load did not show any association with the rest of the nutrients and TSS ($P>0.05$, **Table-3.4**). This is possibly because ammonia had additional sources other than those common to other nutrients. Emission of gaseous ammonia from agricultural fields, biomass burning, animals' excreta, volatilization from ammonia-based synthetic fertilizers, have been suggested as possible sources of atmospheric ammonia (Asman et al., 1998; Behera et al., 2013; Paerl et al., 2002). This could be a plausible explanation for the observed relatively greater load of ammonia onto the surface of Koka Reservoir. Farming and animal husbandry are the major economic operations in Ethiopia (Amare Getahun, 1978). In the

Awash basin, irrigation-based agriculture is widely practiced (Fekadeselassie Berhe et al., 2013), while livestock production by pastoralists in the low lands of the Awash basin, particularly in the middle and lower Awash (**Fig.3.2**), is one of the major economic sectors currently under operation (Ebro Abule et al., 2005; Behnke and Kerven, 2013; Halcrow and Pattern, 1989). The observed greater proportion of ammonia associated with atmospheric sources compared to those of other nutrients may have originated from direct emission possibly from fertilized agricultural lands and animal wastes due to the huge number of livestock (cattle, goat, camel) held within the basin. The relatively high ammonia loads to the reservoir during the dry season may have important implications for eutrophication related water quality problems. Availability of ammonia may initiate the occurrence of potentially toxic cyanobacterial blooms. Several studies have shown that ammonia is the most preferred form of nitrogen for cyanobacteria (Rückert and Giani, 2004) which is due to the relatively less energy requirement for its assimilation (Donald et al., 2013). Although the results of the present study may not be fully comparable owing to differences in the number of months of sampling, the observed total loads of nutrients seem to be much lower than those reported for other east African lakes. For instance, Muvundja, et al (2009) reported total atmospheric input (tons) of 2520 (TP), 33(SRP) 634 (nitrate) and 674 (ammonia) for Lake Kivu, while Tamatamah et al. (2005) reported lake-wide TP and SRP inputs through dry fall into Lake Victoria that ranged from 9.3 to 15.4 and from 2.3 to 4.8 kilotons, respectively. This is not surprising given the incomparably large collecting surface areas of lakes Kivu and Victoria, which are more than 11 and 344 times larger than Koka Reservoir, respectively is. Therefore, absolute loads of nutrients into Koka

Reservoir observed in the present study are very low compared to those of such vast lakes as Kivu and Victoria. However, we are of the opinion that nutrient loads relative to lake size are more relevant with regard to eutrophication related water quality deterioration rather than the absolute loads. For instance, the areal total phosphorus load exclusively from atmospheric source, L (P), in the present study was 195.05 mg/m^2. Using the trophic state model developed for tropical warm lakes (Salas and Martino, 1991), in Lake TP concentration, $\mathbf{P}_{\lambda,}$ was predicted to be 11.84 mg/m^3, which surpassed the cutoff point of 10 mg/m^3 for oligotrophic state. The reservoir is, therefore, categorized as mesotrophic lake based on the probabilistic distribution of trophic state as modified by Salas and Martino (1991) for warm water tropical lakes. Therefore, although the loading of phosphorus into Koka Reservoir observed in this study is incomparably very low relative to those reported for the vast lakes in east Africa (Muvundja et al., 2009), the resulting in-lake phosphorus concentration (P_{λ}) predicted by the model still shows that it is sufficient to support potentially toxic cyanobacteria. This signifies that nutrient input related to lake size is more relevant than the absolute load. Furthermore, as only eight months of sampling were considered in this study, it is expected that the total annual load of nutrients and the consequent in-lake concentration would be much higher.

3.5. Uncertainties in the estimation of nutrient input through atmospheric deposition and riverine loadings.

Several authors have identified possible sources of errors and uncertainties in the procedures used for the estimation of nutrient input from riverine and atmospheric sources (Anderson and Downing, 2006; Muvundja et al., 2009). The possible source of uncertainty in the estimation of riverine input of nutrients in the present study is the relatively low temporal resolution. As only monthly samples were used to estimate the monthly load, which was multiplied with the monthly flow, this may somehow bias the estimate. In rivers, which exhibit extreme fluctuations in their flow, a more frequent sampling is required to resolve the consequent variability in their nutrient transport (Richards and Holloway, 1987, cited in Anderson and Downing, 2006). A relatively more frequent sampling program is, therefore, recommended in similar future investigations if resources afford the opportunity.

Uncertainties in the estimation of nutrient inputs through atmospheric deposition may stem from spatial and temporal variability in atmospheric inputs (Anderson and Downing, 2006). The spatial variations in atmospheric deposition were reported to be due to differences in land use activities, which could be considerable particularly in very large water bodies. In lakes and reservoirs, which are quite small like Koka Reservoir, such spatial variations may not be that great. While we have attempted to minimize some of such sources of errors inherent in the procedures as local recycling of nutrients (Anderson and Downing, 2006), we cannot still claim that the estimated nutrient loads from atmospheric sources in the present study are totally free of uncertainties**.** Considering the number of samples used in this study, temporal resolution seems to be low and this may add to the possible sources of uncertainties. Therefore, sampling at more frequent intervals is required in order to come up with data that are more reliable.

3.6. CONCLUSIONS

The El Niño induced drought caused reduction in the flow of Awash River and TSS load to the reservoir and complete drying of Modjo River. Expectedly, nutrient inputs from riverine sources were reduced considerably. However, nutrient input from riverine sources was still greater than the atmospheric input despite the expected reduction.

High frequency of wind speed in the basin during the dry months, crop harvest and land preparation and other agricultural activities that take place during these months, direct emission particularly of ammonia from the huge number of livestock held within the basin (cattle, goats, sheep, and camel), may have enhanced wind- carried transport of nutrient across the basin.

Atmospheric input of nutrients to Koka Reservoir in the present study is very low compared to those reported for vast lakes such as lakes Victoria and Kivu. However, the present results still suggest that the contribution of atmospheric input of nutrients to lakes and reservoirs in arid and semi-arid region is considerable. This may also imply that nutrient input relative to lake size is more relevant than the absolute load with regards to eutrophication-related water quality problem. It may also trigger interest of researchers to study the relative importance of atmospheric input in tropical regions where extended dry weather conditions prevail.

CHAPTER FOUR

4.0 PHYSICAL AND CHEMICAL LIMNOLOGY OF KOKA RESERVOIR DURING EL NIÑO INDUCED DROUGHT: IMPLICATIONS FOR PHOSPHORUS RECYCLING AND MANAGEMENT OF THE RESERVOIR.

4.1. INTRODUCTION

Enrichment of water bodies with nutrients originating from diffuse (e.g. riverine and atmospheric sources) and point (e.g. industries) sources **(Chapter 3)** and the consequent water quality deterioration have been reported by several studies (Ahn and James, 2001; Baker and Miller, 2013). As it indicated in chapters, two and three, water bodies in tropical regions are particularly at higher risk of pollution originating particularly from riverine sources as nearly all of them are of riverine origin (Lewis, 1996). As it is the case in other water bodies located in tropical region, Koka Reservoir, the subject of the present study, is among the Ethiopian rift valley lakes (ERVL) that have been severely impacted by nutrient loading from both diffuse and point sources. The reservoir receives nutrient input from diffuse sources, largely from the degraded upper Awash River catchment (Halcrow and Pattern, 1989) **(Chapter 3).** It also receives nutrient input originating from point sources mainly the tanning industries through Modjo River (Seyoum Leta et al., 2003; Zinabu G/Mariam and Pearce, 2003) **(Chapter 3).** Changes in temperature, rainfall pattern and hydrological cycles due to extreme hydro-climatic conditions may have a profound effect on nutrient transport and consequently on water quality of the reservoir. The El Niño induced drought, which occurred in 2015/16, was reported to be the worst in decades (Philip et al., 2018). The present study looked into the hydrologic and hydro-chemical responses of the reservoir to this extreme hydro-climatic condition, with emphasis on phosphorus availability based on sampling, which was carried out for one year (May 28, 2015-April 28, 2016) during the drought. The results of this study may provide insight into the possible response of tropical reservoirs to such climate anomalies and implications for restoration attempts involving reduction in the external input of nutrients.

4.2. MATERIALS AND METHODS

4.2.1. Koka Reservoir

Detailed disciption of Koka reservoir is presented in the previous chapter (chapter 3).

4.2.2. Sampling sites and sampling Protocol

This study was carried out on the Koka town side of the reservoir for one year between May/2015 and April /2016. Sampling sites were selected on the basis of the likelihood of exposure of humans and livestock to the potentially toxic cyanobacteria and extent of human disturbance. Accordingly, points where the local people fetch water for household purposes, and sites used for livestock watering and sanitation were used as sampling points. Three sampling points were selected near the shore (near-shore sites) and composite samples representing the littoral zone (near-shore sites) were produced by mixing the samples in equal proportions. Similarly, three samples collected from three sampling points in the open water (open water sites) were also mixed in equal proportions to produce composite samples representing the open water. Near-shore sampling points were located along a 1.5 km stretch (transect) with each sampling point being about 0.5 km apart, while open water sampling sites were situated 2 km away from the near-shore sites into the open water (Fig. 4.1). Sampling was carried out on monthly basis. As the reservoir is a polymictic and holomictic as reported by earlier studies (Elizabeth Kebede and Willén, 1996; Melaku Mesfin et al., 1988), samples were collected from the surface using properly cleaned plastic containers in accordance with the recommendations of (APHA, 1999). All samples were transported to the laboratory at College of Natutural and Computational Sciences of Addis Ababa University in cool icebox for laboratory analysis.

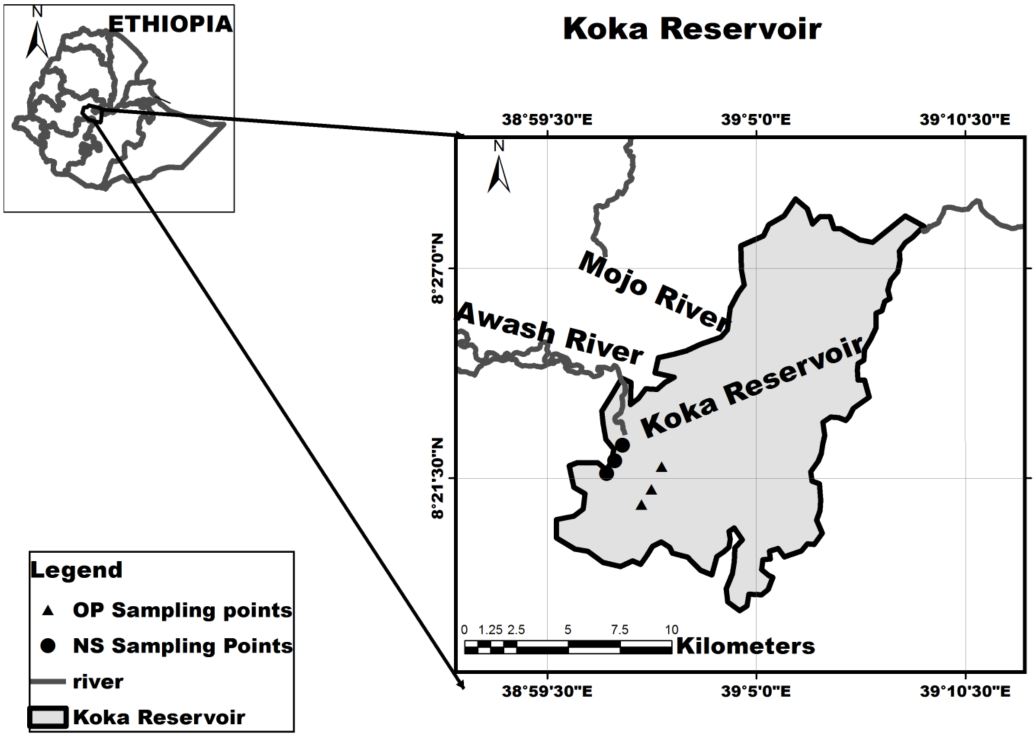


Figure 4.1. Koka Reservoir: its feeder rivers and sampling sites shore (●), open water site (►).

4.2.3. Analytical methods

4.2.3.1. *In situ* Measurements of Physical and Chemical Parameters

Measurements of physical and chemical parameters (Temperature, pH, DO, Salinity, and Turbidity) were carried out on-site with calibrated field meters. Turbidity (NTU) was measured using formazin calibrated portable turbidity meter (model HI 93 703-11). When the turbidity of the samples exceeded the measurement range of the instrument, dilution was carried out with turbidity free water and the actual turbidity of the samples was calculated as per the instruction manual of the instrument. Secchi disk depth (Z_{SD}) was estimated with 30 cm diameter black and white circular disk following the standard procedure (APHA, 1999).

4.2.3.2. Chemical Analysis

Soluble reactive phosphate-phosphorus (SRP) and total phosphorus (TP) in water samples of Awash River and Koka Reservoir were analyzed using the Ascorbic acid method as described in (APHA, 1999). Unfiltered water samples were used for the analysis of total phosphorus (TP) using the Ascorbic acid method, after persulphate digestion (APHA, 1999). Sodium salicylate method was used for the analysis of nitrate (NO_3-N). Silica (SiO_2) was analyzed using the Molybdosilicate method (APHA, 1999), while the Phenate method was used for the analysis of ammonia ($NH_3 + NH_4^+$-N) (Wetzel and Likens, 2000). TSS was analyzed gravimetrically in the laboratory by filtering a known volume of properly mixed sample on pre-weighed glass fiber filter paper (GF/F), which was dried at 105 °C to constant weight (APHA, 1999). Riverine sampling and estimation of nutrient loads were described in **Chapter 3.**

4.2.4. Estimation of morphometric features of the reservoir

There has been a consistent decline in the mean depth and hence in the volume of the reservoir due to the sediment it receives through agricultural runoff (Michael Abebe, 2001). These morphometric changes are expected to influence several limnological parameters of the reservoir including water residence time. Volume of the reservoir at full supply level of 110.3 m during our sampling period was estimated based on the mean annual sediment load to the reservoir suggested by the Ethiopian Ministry of Water, Irrigation and Electricity (EMWIE) (Michael Abebe 2001) in the last bathymetric survey. EMWIE reported mean annual siltation rate of 12.2 Mm^3 in the hydrographic survey report of the year 1998. Assuming a constant siltation rate of 12.2 Mm^3, the total silt deposited since the last bathymetric survey was estimated to be 183 Mm^3. This makes the total silt deposited in the reservoir since 1960 to be 660 Mm^3. The volume of the reservoir at full supply level of 110.3 m was thus estimated at 990 Mm^3 during our sampling period. The daily volume was determined from the daily water level record provided by EMWIE using the following equation.

$$V_d = 990 * {Wl_d}/_{110} \qquad (1)$$

Where:- V_d= Daily volume (Mm^3), Wl_d= Daily water level (m) and 990= the estimated volume of the reservoir (Mm^3) at full supply level of 110m during the present study.

The mean depth was calculated as a ratio of the volume to surface area, assuming a surface area of 200 km^2 indicated in previous reports (Elizabeth Kebede and Willén, 1996; Willén et al., 2011) and the estimated volume of 990 Mm^3 during the present study.

4.2.5. Estimation of Water Residence Time

Water residence time (HRT) refers to the average time required to refill a basin if it were emptied (Kalff, 2002). It is one of the variables that greatly influence the limnology of the aquatic systems due to its direct effect on the fate of chemicals entering lake (Rangel et al., 2012) their concentration, sedimentation, accumulative capacity of inorganic nutrients and thermal stratification (Amborosetti et al 2003), primary productivity, phytoplankton community structure (Kalff, 2002; Rangel et al., 2012). The water residence time (WRT) was calculated using the following equation

$$WRT = {}^{V}/_{Q} \qquad (2)$$

Where: WRT = Water Residence Time (s), V = Volume of the lake (m^3), and Q = the inflow or outflow rate at a steady state (m^3/s).

The equation assumes steady state conditions, and a completely stirred tank reactor system (CSTRS). A CSTRS implies that a substance entering a lake/reservoir is instantaneously mixed throughout the lake volume (Monsen et al., 2002). A CSTRS can be assumed for this reservoir as various studies have shown that Koka Reservoir is a shallow polymictic reservoir exhibiting both horizontal and vertical mixing (Elizabeth Kebede and Willén, 1996; Melaku Mesfin et al., 1988; Willén et al., 2011). The inflow rate, however, exhibits extreme seasonal fluctuations (Fig 3.3, chapter 3) and hence violates the steady state condition. The reservoir is regulated on a daily basis to maintain nearly constant water level and ensure sustainable use of the reservoir for irrigation and power generation. Therefore, reservoir volume and outflow rate were assumed to be constant and this allows a steady state assumption for the daily operation condition. The water residence time was thus estimated on a daily basis (Rangel et al., 2012; Schallenberg and Burns, 1997; Silva et al., 2014) using the following equation (Rangel et al., 2012).

$$WRT_d = {}^{V_d}/_{Q_d} \qquad (3)$$

Where:- WRT_d= the daily water residence time (days), V_d=the daily volume of the lake (m^3), and Q_d=the daily outflow rate at a steady state (m^3/d).

The mean water residence time during the sampling year was calculated as

$$WRT_{\bar{X}} = \frac{\sum_i^n WRT_{di}}{n} \qquad (4)$$

Where; WRTx = the annual mean water residence time
WRT_{di} = Daily water residence time of the day i
n = number of days of the year (365)

4.2.6. Modeling trophic state response

One of the widely used empirical models for the prediction of trophic state response of lakes to external input of phosphorus is that of OECD (1982). However, the model was developed using data collected from the temperate region and may not be applicable for conditions of the tropical region (Salas and Martino, 1991). An analogous empirical model following a similar approach but using data from the tropical region was developed by (Salas and Martino, 1991).

Table 4.1. Working definition and selection criteria used to develop the trophic model for warm Tropical lakes (Salals and Martino, 1991) and the corresponding features of Koka Reservoir

	Min	**Min**	**Altitude**	**Minimum field data**
Salas and Martino (1991)	10	15	<3000	1 year data collected on
Koka Reservoir during the present study	19	22	1590	1 year data collected on monthly basis

The working definition and selection criteria used to develop the model for warm tropical lakes are given in Table 4.1. Koka Reservoir complies with the working definition and fulfills the selection criteria. The trophic state category to which Koka Reservoir belongs was determined according to probabilistic distribution of trophic state proposed by (Salas and Martino, 1991)

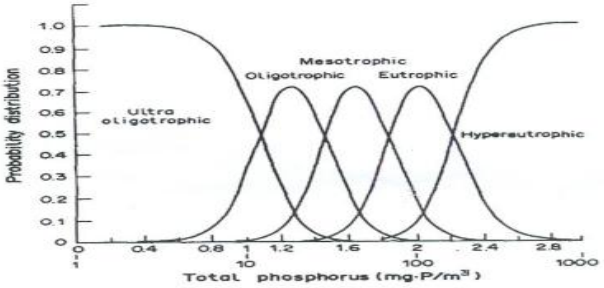


Figure 4.2 Trophic state probability distribution for warm tropical lakes based on total phosphorus (Source: Salas and Martino, 1991)

The modified trophic state model that relates riverine total phosphorus input and in-lake phosphorus concentration proposed by (Salas and Martino, 1991) is

$$P_\lambda = \frac{L(P) * T_w^{3/4}}{3 * Z} \tag{5}$$

Where: - P_λ= In-lake concentration of TP (mg/m^3 or g/m^3), L (P) = phosphorus load (mg/m^2 or g/m^2 per year), Z = Average depth (m), T_w = Water Residence Time (yr.).

4.2.7. Acquisition of meteorological data

Long-term weather data for the study area was obtained from the National Meteorological Agency of Ethiopia, NMAE (**Fig. 3.3, Chapter 3).**

4.2.8. Statistical Analyses

The data sets of the physical, hydro-chemical and hydrologic variables were highly skewed and hence failed to conform to the assumption of normality (Shapiro-Wilk, $p<0.05$). Spearman Rank Order Correlation was, therefore, used for the analysis of the relationships among physical, hydro-chemical and hydrologic variables. The nonparametric test statistics, namely Mann-Whitney Rank Sum test was employed to examine the differences in the levels of environmental variables between sampling sites. Tests were considered significant at $p<0.05$. All statistical analyses were carried out using SPSS ver. 20. Sigma plot Ver. 10 was used for plotting graphs.

4.3. RESULTS

4.3.1. Hydrological and Physico-chemical Features

4.3.1.1. Hydrological features

The mean monthly water level of the reservoir varied between 103.21 and 107.18 m, averaging 105.1±0.401. It was lower than the mean monthly water levels recorded for the two preceding years, (104-109), and 103.9-109 for the year 2013/14 and 2014/15, respectively (Fig. 4.3a). However, the monthly mean water levels of all years varied in a similar temporal pattern with a steady increase during June–September corresponding to the major rainy season, followed by near-consistent decrease during the subsequent months of the dry season (Fig.4.3a). The daily discharge rate of the reservoir during the present study varied within the range 0.176-0.399 Mm^3 and was also much lower than the discharge rate recorded for the earlier years, 2013/14 (0.31-0.645) and 2014/15 (0.251-0.606) Mm^3 (Fig. 4.3b). However, the discharge rate of the dry months of the study period was comparable to those of the earlier years. The calculated water residence time ranged from 239.47 to 540 days, with a mean value of 395±24.5 days. It was significantly longer ($p< 0.05$) than that estimated for the earlier years with mean value 258± 16.1days for the year 2013/14 and 242±15.5 for the year 2014/15 (Fig. 4.3c). The variation in water residence time of the two preceding years seems to follow a seasonal pattern, with longer water residence time generally occurring during the dry period though not in a monotonic manner (Fig. 4.3c). However, the water residence time estimated for our study period exhibited a temporal trend, which contrasts with the water residence time of earlier years (Fig. 4.3c). The water residence time of all years were, however, broadly similar during the months of the dry and minor rainy period (Nov–Apr).

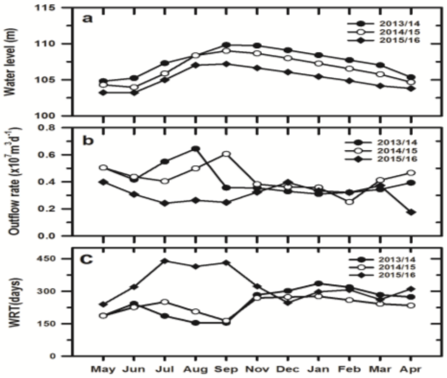


Fig. 4.3. Temporal changes in the water level (a), outflow rate (b) and water residence time (WRT, c) of Koka reservoir

Both mean depth and storage capacity of Koka Reservoir declined almost consistently with time to their lowest value of the present study period corresponding to progressive silting up of the reservoir (Fig. 4.4).

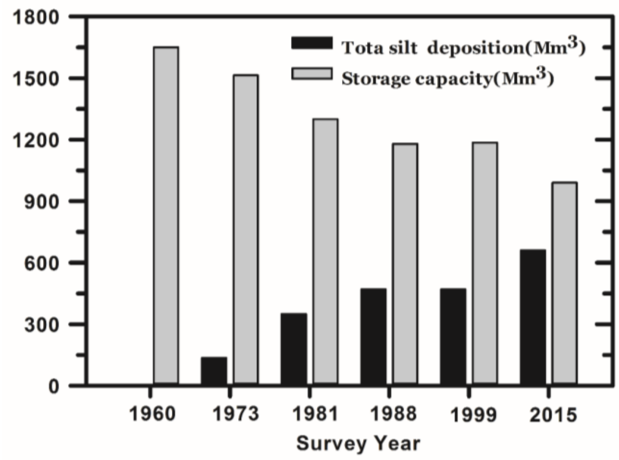


Fig. 4.4.Temporal variations in the amount of silt deposited (Mm3) and storage capacity (Mm3) of Koka Reservoir * Values corresponding to 2015 are estimates of the present study.

4.3.1.2. Physico-chemical features

The water clarity of the reservoir was generally poor as the high TSS and turbidity levels, and low Z_{SD} values seem to suggest (Table 4.2). Higher temperature levels were recorded during the months of the dry period at both sampling sites. The difference in the annual mean water temperature between the open water sites (26.4±2.96) and near-shore sites (25.3±4.8) was not statistically significant (t-test, p=0.89., Table 4.2). The concentrations (µg L^{-1}) of nitrate at the open water site ranged from 16.5 to 135, while at the near-shore site they varied from 9.69 to 250 (Fig. 4.5b). The highest concentration (µg L^{-1}) of nitrate (NO_3-N) was observed in May at both open water (135) and near-shore (250) sites. The annual mean concentrations of nitrate for the open water and near-shore sites were very low (< 55 µg L^{-1}). The difference in the concentrations of nitrate between the two sampling sites was not, however, significant (Mann-Whitney Rank sum test, *p*>0.05, Table 4.2), a trend, which was also observed for other inorganic nutrients. Ammonia concentrations were below the detection limit of the method of analysis used (Phenate method) during most of the sampling months (Fig.4.5a).

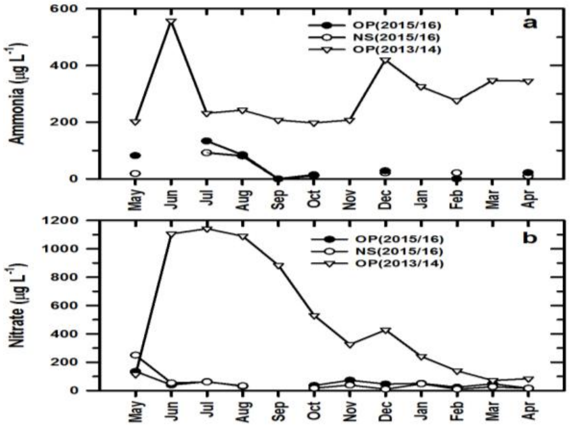


Fig. 4.5. Temporal variations in nitrate and ammonia levels at the open water (OP) and near- shore (NS) sites of the present study on Koka Reservoir in relation to those reported in a previous study (2013/14, Yeshiemebet Major, 2016). Missing data points on the plots of ammonia correspond to undetectable levels.

The observed ranges of SRP concentrations (µg L^{-1}) at the open water and near-shore sites were from 28 to 48 and from 29.2 to 45, respectively (Fig.4.6a).

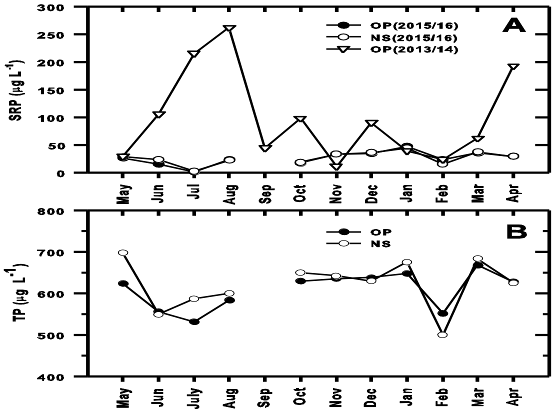


Fig. 4.6. Temporal variations in the levels of SRP(A) and TP(B) at the near-shore(NS) and open water(OP) sites of the present study on Koka Reservoir in comparison with SRP data reported previously for the same reservoir(Major, 2016).

The differences in the concentrations of SRP and TP between the two sampling sites were not statistically significant (Mann-Whitney Rank sum test, *p*=0.0937 and *p*= 0.974, respectively, Table 4.2). The concentration of silica (mg/l) varied temporally with annual mean values of 16.83±4.25 and 27.38±4.95 for the open water and near-shore sites, respectively (Table 4.2).

Table 4.2. Annual means and Ranges (in parentheses) of physico-chemical parameters recorded in the present study.

	Open water site		Near-shore sites		
Physico-chemical parameter	Mean ±Std. Error	Ranges of values	Mean ±Std. Error	Ranges of values	Test performed at *p* =0.05, n=22
DO (mg/l)	6.131±0.416	3.8 (May)-8.8(Mar)	6.147±0.419	3.7(May)-8.8(Mar)	(*p*=0.554)
pH-	7.123±0.205	6.05(Jun)-8.03 (Jan)	7.214±0.222	6.03 (Jun)-7.95(Jan)	(*p*=0.767)
Temp.(ºC)	25.868±0.915	22.4 (Aug)-31.3(Mar)	25.691±0.851	22.4(Au)-31.1(Mar)	(*p*=0.818)
Salinity (ppt)	0.217±0.0239	0.11(Aug)-0.34(Jan)	0.223±0.0228	0.13(Aug)-0.34(Jan)	(*p*= 0.862)
TDS(mg/l)	302.909±33.170	160(Aug)-473(Jan)	330.727.±0.0798	162(Aug)-473 (Jan)	(*p*=0.690)
TSS (g/l)	0.599±0.156	0.015(Jul)-1.672(Mar)	0.471±0.0798	0.029(Jul)-1.5(Mar)	(*p*=0.742)
Turbidity (NTU)	1027.945±276.536	48.39 (Jul)-2820(Mar)	1099.763±287.311	48.29(Jul)-2970(Mar)	(*p*= 0.844)
Z_{SD} (cm)	10.709±1.589	3(Mar)-17(Jul)	9.609±1.623	3.05(Mar)-17.01(Jul)	(*p*= 0.972)
TP (µg/l)	609±13.6	532(Jul)-668(Mar)	609±13.6	500(Jul)-698(Mar)	(*p*=0.568)
SRP (µg/l)	26.8±2.84	28(Feb)-48(Jan)	29.51±2.62	29.2(Feb)-45(Jan)	(*p*=0.937)
SRP:TP	46.1±0.0037	28.8(Jun) -74.1(Jan)	47.1±0.0034	29.1(Jun)-69.8(Jan)	(*p*= 0.849)
Nitrate (µg/l)	51.2±0.00975	16.5((Apri)-135(May)	51.4±0.0206	9.69(April) -250(May)	(*p*= 0.278)
Silica (mg/l)	22.648±2.893	7.456(Oct)-38.133(April)	26.45±3.6	5.432(Oct)-39.6225(April)	(*p*= 0.424)

Table: 4.3 Spearman Rank Order Correlation of limnological parameters measured during the present study on Koka reservoir

Parameters	DO	pH	Temp	Sal	TDS	TSS	Turb	Z_{SD}	TP	SRP	SRP:TP	Nitrate	Si	WL
DO	1.000													
pH	0.491	1.000												
Temp	0.582	0.318	1.000											
Sal	0.347	0.708^{*0}	**0.644***	1.000										
TDS	0.345	**0.645***	**0.682***	0.98**	1.000									
TSS	-0.291	0.427	0.345	0.594	0.555	1.000								
Turb	-0.291	0.427	0.345	0.594	0.555	**0.98****	1.000							
Z_{SD}	0.201	-0.539	0-.347	**-0.64***	-0.584	**-0.987****	-0.9**	1.000						
TP	**0.84****	**0.75****	0.455	0.484	0.400	.036	0.036	-0.146	1.000					
SRP	0.273	0.600	**0.682***	**0.81****	**0.83****	**0.627***	**0.627***	-0.63*	0.436	1.000				
Nitrate	-0.528	-0.569	0.036	-0.188	-0.073	0.068	0.068	0.062	**-0.71***	-0.087	-0.118	1.000		
Si	0.364	-0.055	0.191	0.050	0.009	-0.227	-0.227	0.164	0.355	0.027	0.036	-0.405	1.000	
WL	-0.336	0.200	-0.482	-0.009	-0.127	0.073	0.073	-0.114	0.045	-0.073	-0.036	-0.323	-0.118	1.000

*Correlation significant at *p*=0.05
**Correlation significant at *p*=0.01

The lowest and highest levels of dissolved oxygen occurred at both sampling sites during the months of May and March, respectively (Table 4.2). The annual means of dissolved oxygen (mg/l) for the open water (6.131±0.41) and near-shore (6.147±0.4) sites were similar (Table 4.2). The highly correlated salinity, conductivity, and TDS (Table 4.3), which varied temporally, reached their highest peaks during the dry months of the sampling period. Relatively higher salinity values (Table 4.2), compared to those reported previously for the same reservoir, were recorded. Earlier reports on the reservoir were based on one-time and/or short-term sampling (Elizabeth Kebede and Willén, 1996; Yeshiemebet Major et al., 2017; Willén et al., 2011). Thus, direct comparison with our study may not be appropriate. However, comparisons of observations documented during the sampling months of these reports with those of similar months of our investigation still show the difference. Elizabeth Kebede and Willén, (1996) for instance, reported a salinity of 0.2 g/l from one-time sampling during the short rainy season (March–May), while the salinity observed during the corresponding months of our sampling period ranged from 0.22 to 0.31 g/l. pH of the present study ranged from 6.03 to 8.06, with increasing trend towards the drier and warmer months. According to Melaku Mesfin et al., (1988), the pH of the reservoir during June–July, 1984 was 8.3, a higher pH recorded for the reservoir during similar months of the present sampling period (6.0–6.1). Elizabeth Kebede and Willén, (1996) also reported a pH of 8.3 for the minor rainy period (March–May), which is again considerably higher than those observed during the corresponding months of the study reported here (7.01–7.07).

4.3.2. Total Input of Phosphorus from Allochtonous Sources

The total annual input of TP from allochtonous sources (riverine and atmospheric) to the reservoir was 512012 $*10^6$ mg resulting in annual areal load to the reservoir, L (P)/m^2, of 2560 mg/m^2 (Chapter 3).

4.3.3. Trophic State Response

Both the model predicted and observed values surpassed the cutoff point of 100 mg m^{-3} for eutrophic state putting the reservoir under the category of hypereutrophic. However, the model predicted mean annual TP concentration from nutrient input from allochtonous sources (riverine and atmospheric sources), P_λ (156.6 mg/m^3) constitutes only 25.7 % of the observed mean annual TP concentration (609 mg/m^3).

4.4. DISCUSSION

4.4.1. Hydrological features

The differences observed in hydrological and physico-chemical features between 2015/16 and the earlier years can be attributed primarily to the El Niño-induced drought in Ethiopia. The distinct temporal pattern exhibited by monthly mean water level records of Koka Reservoir (**Fig. 4.3a**) is consistent with the contention that water level fluctuations are more frequent in regions where rainfall events are strongly seasonal (Coops et al., 2003; Geraldes and Boavida, 2005). The observed relatively low mean monthly water level compared to ealier years (Fig.4.3a) could be due to the reduction in the inflow of the rivers and complet dryup of Modjo River (Chapter 3) and possibly due to evaporative loss of reservoir water at a rate exceeding precipitation. The longer water residence time observed during the present study, compared to those of earlier years, could possibly be due to the lower outflow rate associated with low water level during the present study. However, the water residence time of the reservoir is likely to decrease in the future due to the expected reduction in volume caused by the progressive buildup of silt deposit **(Fig. 4.4)** unless offset by such climate anomal.

4.4.2. Physico-chemical features

The observed low water transparency, manifested in the high levels of TSS and turbidity and low Secchi disk depth (Z_{SD}, <17 cm), was probably due to the re-suspension of bottom sediments. This is in agreement with the contention that sediment re-suspension due to wind-driven mixing is quite common in shallow lakes and reservoirs (Sun et al., 2006). Some studies have shown that extreme water level reduction also affects the thermal regime of water bodies thereby converting monomictic lakes to polymictic ones (Jeppesen et al., 2015). However, such reduction in water level may not have had any effect on the mixing regime of Koka Reservoir as it was already polymictic even during earlier years when the reservoir was deeper (Elizabeth Kebede and Willén, 1996; Melaku Mesfin et al., 1988). The non-significant association of the water level with turbidity and Secchi disk transparency records of the present study (Table 4.3) also reflected this fact**.** The relatively higher water temperature recorded during our sampling period **(Table 4.2),** compared to that of an earlier report (Yeshiemebet Major et al., 2017), could be due to the drought-associated increased air temperature (**Fig. 3.3, Chapter 3**). This is consistent with the assertion of Whitehead et al., (2009) that the most immediate response of lakes and rivers to climate change is an increase in their water temperature**.** The considerably lower DO concentrations (3.75-8.85 mg/l) of the present study relative to those documented in an earlier report (4.48-9.61 mg/l) by Yeshiemebet Major et al., (2017) may have resulted from the high rate of mineralization processes and high water temprature. The consequent high rate of oxidative consumption of oxygen was possibly enhanced by the relatively high water temperature. The observed lower levels of oxygen in the reservoir may also be attributable to the presumably lower rates of its photosynthetic production. The previously recurrent thick *Microcystis* bloom did not occur during our sampling period probably due to severe nitrogen limitation (Samson Tilahun and Demeke Kifle, 2019, Chaper 5). However, there was no evidence of association between the concentration of dissolved oxygen and temperature **(p>0.05, Table 4.3)** in the present study. Other factors such as frequent mixing, which are evident in Koka Reservoir, may have also greatly influenced the level of oxygen. Relatively high values (Table -4.2) of the highly correlated salinity and TDS **(Table 4.3)** were observed. Earlier reports on the reservoir were based on one-time and/or short-term sampling

(Elizabeth Kebede and Willén, 1996; Yeshiemebet Major et al., 2017; Willén et al., 2011). Thus, direct comparison with our study may not be appropriate. However, comparison of observations documented during the sampling months of these reports with those of similar months of our investigation show that the salinity values of the present study are higher (Elizabeth Kebede and Willén, 1996). Elizabeth Kebede and Willén (1998) reported a salinity of 0.2 g/l from one-time sampling during the short rainy season (March–May), while the salinity observed during the corresponding months of our sampling period ranged from 0.22 to 0.31 g/L. Despite the reduction in riverine input of solutes that constitute salinity and TDS, the higher values recorded during the present study could be due to increased concentration caused by evaporative loss of water at a rate exceeding precipitation (Lewis Jr, 1996) reflected by a relatively lower water level. Lower pH values recorded during our sampling period may be associated with the presumably lower rates of photosynthetic activity (Samson Tilahun and Demeke Kifle, 2019, Chapter 5) and/or higher rates of mineralization of organic matter (Cunha-Santino et al., 2008). Therefore, the simultaneous high rate of mineralization favored by the prevailing high water temperature may have also contributed to the observed low pH in the reservoir during the present study.

The El Niño induced drought may partly explain the observed nutrient levels recorded during the present study. Thus, external inputs of both nitrogen and phosphorus were presumably reduced during our sampling period **(Chapter 3).** This is particularly evident in the relatively low availability of nitrogen during the present study **(Table-4.2)** compared to the ranges of NO_3 (0.07-1.14 mg/l), and NH_3 (0. 197-0.557mg/l) reported by Yeshiemebet Major et al., (2018). This may suggest that riverine input is the major source of nitrogen for Koka Reservoir. Loss of available nitrogen owing to higher water temperature (Downing et al., 1999; Lewis Jr, 1996; Lewis, 2000) and low oxygen concentration could also partly account for the severe nitrogen limitation during our sampling period. This severe nitrogen limitation in the reservoir may have caused disappearance of the recurrent cyanobacterial bloom almost exclusively dominated by *Microcystis*, which had been evident in the reservoir during several preceding years (Samson Tilahun and Demeke Kifle, 2019; Chapter 5). According to Samson Tilahun and Demeke Kifle (2019; Chapter 5), this condition also triggered the emergence and unusual dominance of nitrogen-fixing cyanobacterial genera, namely *Cylindrospermopsis* and *Dolichospermum* (Chapter 5**).** However, the concentrations of SRP and TP remained high at least to the needs of phytoplankton growth during most of the sampling occasions. This may signify the potential of internal loading as a source of phosphorus supply in the reservoir. Several investigators have studied recycling of phosphorus between the sediment and the water column in lakes and reservoirs (Sondergaard et al., 2001; Wang et al., 2009; Welch and Cooke, 1995, 2005; Yuanyuan and Feizhou, 2008). These studies revealed that the supply of phosphorus through internal loading is quite significant and may even constitute major source of phosphorus in some lakes. For example, (Steinman et al., 2004) and (Penn et al., 2000) reported that phosphorus input through internal loading constituted up to 66 and 80% of the total phosphorus budget of the studied lakes, respectively. This internal input of P has also been shown to be even more important in shallow lakes and reservoirs (Boers et al., 1998; Welch and Cooke, 2005). There are no reported studies on the level of sediment phosphorus and its role as a possible source for internal loading of phosphorus in Koka Reservoir. However, circumstantial evidences suggest that the sediment has accumulated a huge phosphorus reserve, which could play a major role in the dynamics of this nutrient in the reservoir. Earlier bathymetric surveys made on the reservoir have estimated that 10–25 Mm^3 of the sediment is retained annually (Michael Abebe, 2001). This has been well reflected by the progressive silt-up of the reservoir (Fig.4.5). As this sediment originates primarily from a highly degraded agricultural land, phosphorus may have been retained concurrently by the reservoir over the last several decades. The long water residence time of the reservoir **(Fig. 4.3 c),** may have facilitated the retention and progressive silting up of the reservoir and possibly concomitant accumulation of phosphorus in the sediment. The moderately high TP concentrations despite the presumably significant reduction in phosphorus input from allochthonous sources **(Chapter 3)** seem to suggest that internal loading could contribute to the dynamics of phosphorus concentrations in the reservoir. Furthermore, the discrepancy between the observed mean annual concentration of total phosphorus 609 mg/l and the model predicted mean annual in-Lake TP concentration from allochtonous sources (Atmospheric and Riverine sources combined), chapter 3 (P_λ) (156.6 mg/m^3) may also corroborate the suggested possibility of internal loading as the latter constituted only 25.7 % of the former. Several studies have shown that the release of Fe-bound phosphorus is the major route of internal P loading in lakes (Petticrew and Arocena, 2001). Circumstantial conditions in Koka Reservoir do not seem to support the release of Fe-bound phosphorus as a possible mechanism of internal recycling in the reservoir if at all Fe-bound phosphorus constitute significant fraction of phosphorus in the sediment. Release of Fe-bound phosphorus is greatly influenced by redox potential, temperature, and pH (Jiang et al., 2008; Smolders et al., 2001). Anaerobic and reducing conditions enhance the release of Fe-bound phosphorus due to reductive dissolution (Søndergaard et al., 2003). Such conditions may be common in deeper lakes that exhibit thermal stratification with hypolimnetic anoxia. Release of Fe-bound P does not seem to be a relevant mechanism in Koka Reservoir because it is fully exposed to wind action and hence remains well oxygenated with presumably high redox potential. Release of phosphorus in highly oxic shallow lakes reported by some investigators (Bostrom et al., 1982; Jensen and Andersen, 1992; Lee et al., 1977) seems to suggest that other mechanisms and factors are involved (Søndergaard et al., 2003). The high concentration of phosphorus observed during our sampling period, despite the prevailing oxidizing condition, may suggest mineralization of organic matter could be the possible mechanism of internal loading in Koka Reservoir. Nutrient input to Koka Reservoir through runoff during the rainy season used to result in gradual buildup of cyanobacterial blooms almost exclusively dominated by *Microcystis*, which reaches its peak during the warmer and drier months (November to February) **(Chapter 5).** The buildup of *Microcystis*s pp. bloom is accompanied by the high uptake of dissolved phosphorus, which constitutes the organic bound phosphorus in the sediment of the reservoir following the collapse of the bloom. The major form of organic-bound phosphorus of autochthonous origin in Koka Reservoir is, therefore, most probably the phosphorus in *Microcystis* biomass. Several authors (Jin et al., 2006; TEZUKA, 1989; Wang et al., 2016; Xie and Xie, 2002; Yuanyuan and Feizhou, 2008) reported phosphorus release by the decomposing *Microcystis* cells of a previous bloom as possible mechanism of internal loading. Wang et al., (2016), who also studied the effect of an algal bloom on phosphorus exchange at the sediment-water interface in Meiling Bay, Lake Tahiu, China, reported significantly high total phosphorus (TP), total dissolved phosphorus (TDP), and soluble reactive phosphate-phosphorus (SRP) observed during the first few days of their experiment on algal decomposition. From a similar study made on the role of cyanobacterial bloom in the phosphorus cycle between the sediment and overlying water column,(Chuai et al., 2011) reported that phosphorus release from cyanobacterial decomposition was quite high playing a major role in the phosphorus biogeochemical cycle of the studied lake. Decomposition of organic matter is enhanced by high water temperature (Jensen and Andersen, 1992). and high level of dissolved oxygen (Moore et al., 1992). Koka reservoir, a tropical freshwater, exhibited high levels of water temperature during our sampling period (22.4 to 31.3 ºC), which were even higher than those reported by (Yeshimebet Major et al., 2017; 22.6–26.2) owing to the higher maximum air temperature associated with the El Nino induced drought. The frequently and completely mixing Koka Reservoir (Elizabeth Kebede and Willén, 1996; Melaku Mesfin et al., 1988), which was reported to have supported abundant populations of benthic invertebrates (Melaku Mesfin et al., 1988) that may have increased the oxic layer of the sediment (Bostrom et al., 1982), was well oxygenated during our sampling period. Therefore, we contend that mineralization of organically bound phosphorus of both autochthonous (mainly *Microcystis* bound phosphorus) and allochthonous origin, which is favored by the prevailing high water temperature and dissolved oxygen, may be the major source of the phosphorus recycled in Koka Reservoir. Bottom sediment re-suspension due to wind-driven turbulence might also be an important factor influencing sediment phosphorus release in Koka Reservoir. Re-suspension of sediments due to wind-induced turbulence causes the redistribution of phosphorus at the water-sediment interface (Haggard et al., 2012). This may cause an equilibrium shift favoring either net sorption or release of phosphorus depending on several factors (Christophoridis and Fytianos, 2006; Søndergaard et al., 2003; Wang et al., 2009). Experimental studies by Hansen et al., (1997); Sun et al., (2006); Wang et al., (2009) have revealed that re-suspension of sediments in lakes and reservoirs causes a significantly higher rate of sediment phosphorus release compared to that from undisturbed sediments. Sediment re-suspension in the reservoir, as was reflected by high TSS, turbidity and low Secchi disk transparency values could be another factor promoting the release of sediment bound phosphorus in Koka Reservoir during the present study. Such phosphorus recycling between the sediment and the water column has been suggested as the reason for the failure of water quality improvement following reduction in the external input of nutrients (Jeppesen et al., 1999; Lauridsen, 1999; Welch and Cooke, 1995). According to a review by Sondergaard et al., (2001), several years, may be decades, are required before noticeable water quality improvement after reduction in the external input of nutrients is achieved. As a result, attempts have been made to combine reduction in external input with some measures of reducing internal loading (Welch and Cooke, 1995) to achieve fast recovery of lakes (Søndergaard et al. 2001). Sediment dredging and *in situ* immobilization are major approaches mentioned in the literature that have been applied for the control of phosphorus recycling (Quaak et al., 1993; Søndergaard et al., 2003; Wang and Jiang, 2016; Welch and Cooke, 2005). Although some success stories regarding control of internal loadings using sediment dredging have been reported, the extremely high cost (Ji-Cheng et al., 2008), and possible adverse effects on the flora and fauna of the sediment have been suggested as potential drawbacks. Due to the prohibitively high cost associated with the removal and disposal of the huge sediment (Quaak et al., 1993) that has been accumulated over the years **(Fig..4.4),** sediment dredging may not be a feasible measure for Koka Reservoir. *In situ* immobilization of sediment phosphorus using chemical treatment, particularly application of iron to increase the sorptive capacity (Quaak et al., 1993; Sondergaard et al., 2001) of the reservoir sediment could be a potential alternative. This measure could be effective given the high redox potential that allows permanent immobilization of the iron bound phosphorus. Its frequent mixing and oxygenation avoids the need for hypolimnetic aeration applied for deeper lakes that exhibit thermal stratification with hypolimnetic anoxia. However, detailed analysis of sediment characteristics such as Fe: P ratio, major form of phosphorus in the sediment and mechanism of phosphorus release should precede practical application of this control measure.

4.5. CONCLUSIONS

As the reservoir receives much of its annual water and nutrient input through its feeder rivers, the observed changes in hydrologic (water level, discharge rate and water residence time) and hydro-chemical parameters (TDS, salinity, conductivity and nutrients), are essentially the direct and/or indirect consequences of the recent incidence of El Niño-induced drought in Ethiopia. Despite the expected reduction in the external input of nutrients due to the El Niño-induced drought, concentrations of both TP and SRP were considerably high during our sampling period. This may suggest that phosphorus supply through internal loading is operating in Koka Reservoir.

Considering the long history of sediment deposition and the most likely concurrent nutrient loading since 1960 and the high retention efficiency associated with the prevailing long water residence time, the reservoir may have already accumulated a huge phosphorus reserve in the sediment. The huge phosphorus reserve in the sediment may serve as a source of this nutrient through internal loading for several years to come. This may challenge future restoration effort.

CHAPTER FIVE

5.0 THE INFLUENCE OF EL NIÑO-INDUCED DROUGHT ON CYANOBACTERIAL COMMUNITY STRUCTURE IN A SHALLOW TROPICAL RESERVOIR (KOKA RESERVOIR, ETHIOPIA)

5.1. INTRODUCTION

It is now evident that the expansion of toxic cyanbacteria has become a global concern (Fristachi et al., 2008; Zhang et al., 2012). Water quality deterioration and the consequent public health risk linked to harmful algal blooms are mainly associated with members of cyanobacteria such as *Aphanizomenon, Cylindrospermopsis, Dolichospermum* and *Microcystis* (Falconer and Humpage, 2005). The recent increase in the temporal and spatial recurrence of harmful algal bloom is often associated with climate change such as global warming and the subsequent changes in hydrological cycles or rain fall pattern (Zhang et al., 2012 and the references therein) and ever increasing eutrophication of fresh water bodies (O'Neil et al., 2011). This is attributed to the influence of climate changes on the physico-chemical environment such as water temperature (Whitehead et al., 2009), water level (Siderius et al., 2018) water residence time, thermal regime, nutrient availability and biological processes such as growth rate and bloom formation (Paerl and Paul, 2012). Members of cyanobacteria can take advantage of their adaptive strategy to deal with these limnological changes. For instance, water bodies with longer water residence tend to get warmer and stratify (Neil et al., 2010). In such cases, some members of cyanobacteria such as *Microcystis* may benefit from their capability to remain in the epiliminion (Visser et al., 2005) where sufficient light is available by virtue of their positive buoyancy as seen in *Microcystis* and *Anabaena* (Neil et al., 2010). Some members are adapted to low irradiance as seen in

Cylindrospermopsis (Padisák, 1997; Dokulil and Teubner, 2000; Fabbro and Duivenvoorden, 2000; Moustaka-Gouni et al., 2007). They can also benefit from the high water temperature as they have higher optimal growth temperature (Paerl and Huisman, 2009) compared to eukaryotic algae (Paerl and Paul 2012). The recent expansion of these harmful algal blooms in fresh water systems has also been associated with progressive enrichment of water bodies with macronutrients, particularly nitrogen and phosphorus (Zhang et al., 2012), which is greatly influenced by climatic conditions such as rainfall pattern, particularly in water bodies of the tropical region which are largely of riverine origin (Lewis Jr, 1996). Several reports show that future climate change scenarios such as global warming may favor dominance of cyanobacteria over other phytoplankton groups (Paerl and Huisman, 2009) through the promotion of their expansion in space and increasing the frequency of their occurrence in time (Zhang et al., 2012 and the references therein). For instance, one of the toxic cyanobacterial genera, *Cylindrospermopsis*, which was once thought to be restricted to the tropical region, has now been reported to have proliferated in temperate waters including northern Europe and America (Sinha et al., 2012 and the references therein) suggesting the ever increasing global expansion of cyanobacteria. The influence of meteorological events such as the El Niño-Southern Oscillation (ENSO) cycles, which play a role in global climate, on the structure of phytoplankton communities has recently been studied in marine ecosystems (e.g. (Barber et al., 1996; Blanchot and Rodier, 1996; Liu et al., 1998). Climate change predictions indicate that climate change anomalies such as El Niño induced drought and La Niña induced flooding are expected to increase in the future (Salinger, 2005). They may greatly modify the limnology of lentic systems such as Lakes and reservoirs in a way that it not only promotes the dominance of cyanobacteria but will also have great influence by fine-tuning cyanobacterial community structure. Determination of the specific environmental conditions that lead to cyanobacterial blooms is a crucial quest for all researchers of the world. Establishment of causality between physico-chemical, biological or climatological factors and cyanobacterial blooms is also an important precondition for the effective management of reservoirs. One of the most obvious effect of such climate change anomalies is their influence on rainfall pattern and hydrological cycles (Baker and Miller, 2013) and their consequent impact on the annual nutrient budget of riverine origin in such lentic systems. Therefore, changes in rainfall pattern and hydrological cycles due to changes in climatic conditions such as El Niño-induced drought may have a profound effect on nutrient availability and other limnological conditions of the lentic systems, which may have dictating roles in shaping cyanobacterial community structure. The dominant cyanobacteria not only determine the toxicity and healthy functioning of the aquatic system, but may also have management implication, which triggered a considerable debate with regards to which nutrient to target for their effective control (Schindler et al., 2008; Wang and Wang, 2009). Studies made on the impact of these El Niño episodes on phytoplankton dynamics in continental waters are, however, relatively few (e.g. (Harris and Baxter, 1996; Padisák, 1998). Research works on the impacts of droughts associated with the El Niño incidence on cyanobacterial blooms that occur in arid and semi-arid regions, particularly those of East Africa, are especially very scarce. Cyanobacterial blooms that occurred in tropical and subtropical waters were reported to have resulted from annual rainfall deficit linked to droughts associated with El Niño events (e.g. (Bouvy et al., 2003; Câmara et al., 2015;

Harris and Baxter, 1996; McGregor and Fabbro, 2000). In arid and semi-arid regions such as that of the present study, the climatic impact associated with such events on lakes and reservoirs is especially critical, as these water bodies are scarce and play an important role as a primary source of water supply. This is mainly because the majority of lakes and reservoirs in the tropics are of riverine origin and the greatest proportion of their annual nutrient budget is also expected to be of riverine sources (Lewis, 1996). The annual nutrient inputs to these water bodies are thus primarily influenced by the rainfall pattern and hydrological cycles. Several experimental and field studies have shown the important roles of nutrients in dictating cyanobacterial community structures and thier temporal dynamics (Schindler et al., 2008). Several of these studies have also demonstrated the key role played by nutrient availability in shifting cyanobacterial community structure (Schindler et al., 2008; Wang and Wang, 2009). As it is the case in other African rift valley lakes (Muvundja et al., 2009; Baker and Miller, 2013) of the tropics, the abundance/dominance of potentially toxic cyanobacteria is becoming increasingly common in many of the Ethiopian rift valley lakes (Melaku Mesfin et al., 1988; Elizabeth Kebede and Willén, 1998; Willén et al., 2011). Out of the 206 species of phytoplankton identified in samples collected from Ethiopian Rift valley lakes, 61 species belonged to cyanobacteria (Elizabeth Kebede and Willén, 1996) (see Chapter 2). *Microcystis* spp. are among the most important component species of the phytoplankton communities supported by the Ethiopian Rift Valley lakes (Elizabeth Kebede and Willén, 1996) possibly favored by high nutrient availability and other limnological conditions. Koka Reservoir is one of such water bodies in the Ethiopian rift valley that have been severely impacted by nutrient pollution through agricultural runoff and the consequent eutrophication (chapter 3 and 4)**.** Koka Reservoir presumably receives much of its annual nutrient budget from diffuse and point sources via Awash River and Modjo River respectively (chapter 3 and 4). Heavy rainfall mobilize nutrients from the agriculturally dominated upper Awash catchment and increase nutrient loading into the reservoir (Chapter 3) thereby inducing cyanobacterial bloom formation. Such hydrological processes are known to favor the proliferation of harmful algal blooms (Reichwaldt and Ghadouani, 2012). Long-term flow records at the most downstream gauging station located at Melka Hombole by the Ministry of Water Irrigation, and Electricity (MWIE) indicate that the highest inflow and the largest sediment yield occur during the major rainy season (June to September), with the highest peak in August (**Chapter 4**). Although there are no reports on water quality especially on nutrient levels, nutrient transport and load to the reservoir presumably follow that same temporal pattern (**Chapter 4).** It is also expected that those factors that were reported to have increased sediment yield in the runoff (Michael Abebe, 2001) may have had the same effect on nutrient transport **(Chapter 4).** The huge amount of nutrient input during the major rainy season initiates a gradual buildup of cyanobacteria in the reservoir. *Microcystis* was reported to be highly competent under conditions of such non-limiting nutrient supply (Nalewajko and Murphy, 2001), which may explain the dominance of this genus in the years prior to our sampling period. The bloom of *Microcystis*, which almost exclusively dominates the phytoplankton community of the reservoir, reaches its peak characterized by greenish surface scum during the drier and warmer months (late December-February). Such recurrent cyanobacterial bloom in which *Microcystis* was dominant has been evident over the last few decades (Amha Belay and Wood, 1982; Melaku Mesfin et al., 1988;

Elizabeth Kebede and Willén, 1996; Willén et al., 2011). As indicated above, changes in the availability of nutrients have been shown to affect the cyanobacterial community structure in lakes and reservoirs (Schindler et al., 2008; Wang and Wang, 2009). For instance, alteration of external input of nutrients has resulted in a shift in the cyanobacterial community structure from dominance of non-nitrogen fixing genera to nitrogen fixing ones (Schindler et al., 2008; Wang and Wang, 2009) (see Chapter 2). Therefore, changes in rainfall pattern and hydrological cycles due to changes in climatic conditions such as El Niño-induced drought may have a profound effect on nutrient avalability and other limnological conditions of the reservoir (Chapter 4). This may consequently influence the cyanobacterial community structure in the reservoir. The El Niño induced drought, which was reported to be one of the worst in decades (OCHA, 2016), occurred in Ethiopia in 2015 causing the failure of the main rainy season, *Kiremt* rainfall (Gleixner et al., 2016). Here, we report the effect of El Niño induced drought on the spatio-temporal dynamics of cyanobacteria in Koka Reservoir based on data documented over a one year sampling period (May 28 2015-April 28 2016), which coincided with the incidence of El Niño.

Our report may provide a good insight into how climate change anomalies may shape the cyanobacteria community structure in tropical climatic conditions and elsewhere with similar environmental settings.

5.2. MATERIALS AND METHODS

5.2.1. Koka Reservoir

Complete description of Koka Reservoir is provided in Chapter 3.

5.2.2. Sampling Protocol and Analytical Methods

As indicated in earlier chapters, the reservoir is currently serving as a source of fresh water supply for thousands of local inhabitants, livestock watering and fisheries (Chapter 4 and 6). The selection of sampling points was made based on the likelihood of exposure and high-risk area to human and livestock (Chapter 4&6 and Fig. 4.1 and Fig. 6.1). For sampling frequency and sampling protocol, see chapter 4. Methods of *insitu* determination of physico-chemical parameters and laboratory analysis of inorganic nutrients are described in Chapter 4.

5.2.3. Identification and Enumeration of Phytoplankton taxa

From properly mixed composite samples, 250 ml aliquots were dispensed into brown bottles and immediately fixed with Lugol's iodine (0.01% v/v) and transported in cool iceboxes for identification and enumeration of phytoplankton. Properly fixed sample (100ml) was then transferred to a sedimentation chamber (100 ml measuring cylinder) and allowed to stand for 24 hours in the dark. Then, 90 ml of the sample (supernatant) was carefully siphoned off. The remaining 10 ml of the sample was then carefully mixed to homogenize the sample and 1 ml was then loaded to a Sedgewick-Rafter cell and allowed to settle. Identification of the major phytoplankton taxa (with $\geq$ 5% of total phytoplankton abundance) to the genus/species level was made using relevant taxonomic literature (e.g. Cronberg and Komárek, 2004; Komárek and Anagnostidis, 2005; Komárek and Kling, 1991). Counting was then carried out randomly in 30-50 grids and the actual cell number per ml in the original sample was then calculated using the equations given in Hötzel and Croome (1999). For the filamentous and colonial algae, the number of cells per filament/colony for the first 30-50 filaments/colonies observed was determined and the mean number of cells per filament/colony for each sample was calculated. The average number of cells per filament/colony was multiplied by the number of filaments/ colonies to estimate the abundance of filamentous or colonial taxa. Total phytoplankton abundance for each sampling month was determined by adding up the cell counts of all enumerated taxa. The equation proposed by Hötzel and Croome (1999) for calculating the cell number per ml of the original sample is given by

$$Cell\ mL^{-1} = \frac{N * 1000mm^3}{A * D * F}$$

Where: - N= is the number of cell count/unit counted
A= area of field (mm^2)
D= Depth of field Sedgwick-Rafter chamber (mm)
F= number of fields counted

5.2.4. Estimation of phytoplankton biomass as chlorophyll-a (Chl-a)

Phytoplankton biomass was estimated as Chl-a concentration. Samples were filtered onto GF/F in duplicates and kept frozen overnight. The filters were manually ground to facilitate the extraction of the pigments in 90% aqueous acetone and kept for 12 hours in the dark. Absorbance was measured at 665nm and 750nm with a spectrophotometer (Jenway 6300, UK).

Determination of chlorophyll-*a* concentration was made using the equation of Lorenzen (1967).

$$Chl - a\ (\mu g\ L^{-1}) = \frac{(K)(F)\left(E_{665_b} - E_{665_a}\right)(V_E)}{(V_{SF})(z)}$$

Where:-
E_{665b}=Turbidity-corrected aborption at 665nm before acidification
E_{665a}=Turbidity-corrected absorption after acidification=A665b-A750a where a is absorption values
K=Absorption coefficient of chlorophy a, 11
F= Factor to equate the reduction in the absorbance to the initial concentration of Chl-a, 1.7:0.7=2.43
R= Maximum ratio of E_{665b} to E_{665a} in the absence of phaeopigments= 1.7
V_E= Volume of extract in ml
Vsf= Volume of sample filtered
Z= Length of light path through a cuvate, 1cm

5.2.5. Statistical Analyses

Student t-test was employed to analyze the spatial difference in the annual mean of the environmental variables conforming to normal distribution (Chapter 4). However, several of the environmental variables were highly skewed and failed the Kolmogorove normality test at $p<0.05$ (Chapter 4). Therefore, nonparametric test statistics, namely Mann-Whitney was employed to examine the difference in the levels of environmental variables between sampling sites. Tests were considered significant at $p<0.05$ (see chapter 4). Analyses were carried out using SPSS ver.20.

Multivariate statistical analysis was employed to determine the relationships between phytoplankton composition and environmental variables. Appropriate ordination model was selected based on the beta characteristics of the species response (Lepš and Šmilauer, 2003). According to Lepš and Šmilauer (2003), species response with long gradient length suggests that species response follow nonlinear model and hence they recommend the use of unimodal ordination model. The unconstrained deterended correspondence analysis (DCA) of the data sets revealed that the largest gradient length was 0.936 suggesting that the species response follow a linear model. Redundant Analysis (RDA), a linear model was, therefore, employed for the analysis. Log-transformation of environmental data (except pH) to avoid the effect of outliers and standardization by Z-scoring to remove heterogeneity in the measurement scales of the environmental variables were also made (Kenkel, 2006). The selection of the environmental variables to be included in the analysis was carried out using forward manual selection method based on ecological significance. Inter-correlated variables responsible for large inflation factor were removed from the analysis iteratively so as to maintain the inflation factor as low as possible. Rare phytoplankton species constituting less than 5% of the total phytoplankton abundance were not included in the analysis. The significance of the relationships between the environmental variables and phytoplankton composition were tested using randomization technique, namely Monte Carlo permutation test with 499 unrestricted permutationat ($p< 0.05$). Ordination of sample scores in species vectors was carried out to analyze differences in the observed species composition based on Bray-Curtis distances among samples. The multivariate statistical analysis was carried out using Canoco ver. 4.5 and PAST.

5.2.6. Acquisition of Meteorological data

The weather conditions, namely the daily mean minimum and maximum air temperature and rainfall data during the sampling period were obtained from the National Meteorological Agency of Ethiopia (NMAE) (Chapter 4).

5.3. RESULTS

The physicochemical results have already been discussed in ealier chapter , chapter 4

5.3.1. Phytoplankton Community Composition and Abundance

The phytoplankton community of Koka Reservoir during the study period comprised primarily species belonging to the genera of Cyanophyceae or cyanonbacteria (*Dolichospermum, Cylindrospermopsis, Microcystis*), Bacillariophyceae (*Aulacoseira, Cyclotella*, *Navicula, Synedra*), Chlorophyceae (*Chlamydomonas*, *Scenedesmus*) and Euglenophyceae (*Euglena, Phacus, Trachelomonas* (Table 5.1.).

Table 5.1 List of phytoplankton species identified samples collected from Koka Reservoir during the study period

Cyanobacteria(Cyanophyceae)
Dolichospermum circinale (Raben. et al.) Wacklin et al.
*D. spiroides (*Kleb.) Wacklin et al.
Cylindrospermopsis africana Kom. & Kling
C. raciborskii (Wolosz.) Seenayya & Raju
C. curvispora M. Watanabe
Microcystis aeruginosa (Turp.) Kuetz.
M.panniformis Komár. et al.
Pseudoanabaena limnetica(Lemm.) Kom.

Chlorophyceae (Green algae)
Actinastrum sp.
Elaktothrix sp
Pediastrum simplex Meyen
Scenedesmus abundans (Kirchn.) Chod.
S. bijugatus (Turp.) Lagerh.
S. dimorphus (Turp.) Küetz.
S. quadricauda (Turp.) de Breb.

Bacillariophyceae (Diatoms)
Aulacoseira granulata (Ehr.) Simon.
Cyclotella meneghiana Kutz.
Cymbella sp.
Navicula cryptocephala Kutz.
Rhoicosphenia abbreviata (C. Agardh) Lange-Bert
Synedra ulna (Nitz.) Ehr.

Euglenophyceae (Euglenoids)
Euglena spirogyra Ehr.
P. acuminatus Stokes
P. longicauda (Ehr.) Dujardin
Trachelomonas sp.

Species of cyanobacteria were the most dominant phytoplankton taxa throughout our sampling period (**Fig. 5.1**), followed by those of Bacillariophyceae **(Fig. 5.2).** The relative abundance of Chlorophyceae and Euglenophyceae was low (<5%). *Microcystis* was the most dominant taxon during the earlier months of our sampling period, particularly at the near-shore sites **(Fig. 5.1)**. Patches of cyanobacterial scum in holes and pockets created by animal hooves were almost exclusively dominated by *Microcystis* spp. during the earlier months of the study period (May-Aug, 2015, **Plate 5.1).**


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Plate 5.1. Patches of cyanobacterial scum almost exclusively dominated by *Microcystis* spp. along the shore of Koka Reservoir (May28, 2015). Photo by Samson Tilahun.

However, a gradual buildup of heterocystous cyanobacterial species, namely *Cylindrospermopsis africana, Dolichospermum circinalis, D. spiroides, C. curvispora,* and *C. raciborskii* was observed during the rest of our sampling period. *C. africana* was the most dominant cyanobacterium during the months corresponding to the dry and minor rainy periods (**Figs. 5.1 & 5.3)**. Occurrences of heterocysts became increasingly common within the trichomes of *Cylindrospermopsis* and *Dolichospermum*. A related species, *C. curvispora*, with terminal heterocysts at both ends of the trichomes, also became common during the months corresponding to the dry and minor rainy periods (**Plates 5.3 & 5.4**.).

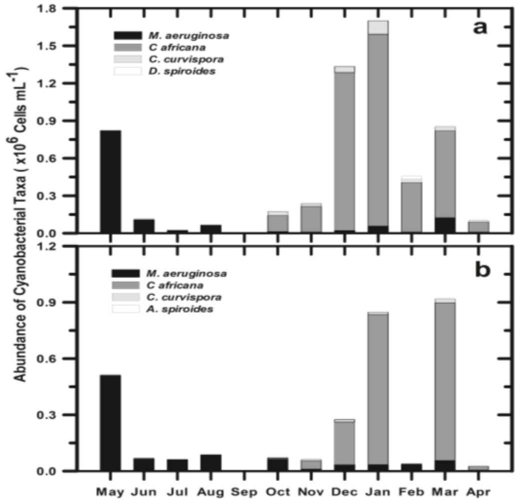

Figure 5.1 Temporal variations in the abundance of cyanobacterial taxa at the near shore (a) and open water (b) sites of the present study.

The other heterocystous cyanobacterial species that was frequently encountered during our sampling period was *Dolichospermum spiroides* with multiple intercalary heterocysts (**Fig. 5.1, Plate 5.4).** Members of Baclliriophyceae remained the second most dominant phytoplankton group during the entire period of our sampling **(Fig. 5.2)**

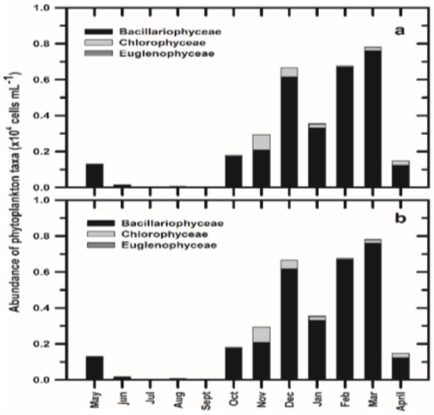

Figure 5.2 Temporal variations in the abundance of other phytoplankton taxa at the near shore (a) and open water (b) sites of the present study.

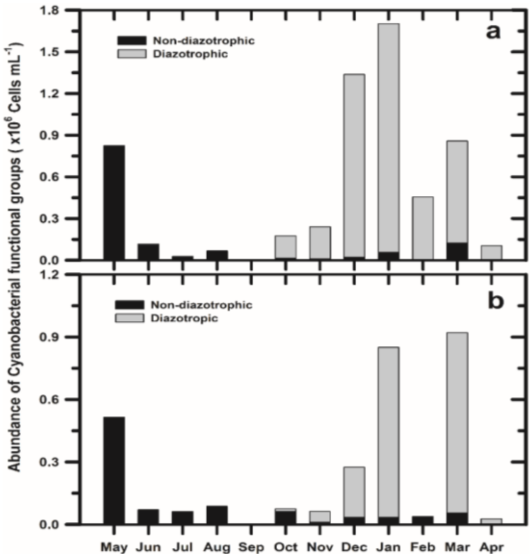

Figure 5.3 Temporal variations in the abundance of functional groups of cyanobacteria (Diazotrophic and Non-diazotrophic) at the near-shore (a) and open water (b) sites of the present study in Koka Reservoir**.**

5.3.2. Chlorophyll-a

Chlorophyll-*a* (Chl-*a*) concentrations ranged from 0.238 to 6.5 μg L^{-1} at the near-shore sites and from 0.182 to 4.9 μg L^{-1} at the open water sites. The annual means of Chl-*a* were 2.5 and 2.52 for the near-shore and open water sites, respectively. Peaks in Chl-*a* concentration were observed in May, 2015 and March, 2016, with the level recorded on the former date being slightly higher than that measured on the latter in the open water samples. Furthermore, both sampling sites generally had higher Chl-*a* concentration during all months corresponding to dry and minor rainy periods except February and April **(Fig. 5.4),** when considerably low phytoplankton abundance and chlorophyll-*a* concentrations were evident.

Chlorophyll-*a* showed positive correlation (r= 0.882, $p<0.05$) with total phytoplankton abundance.

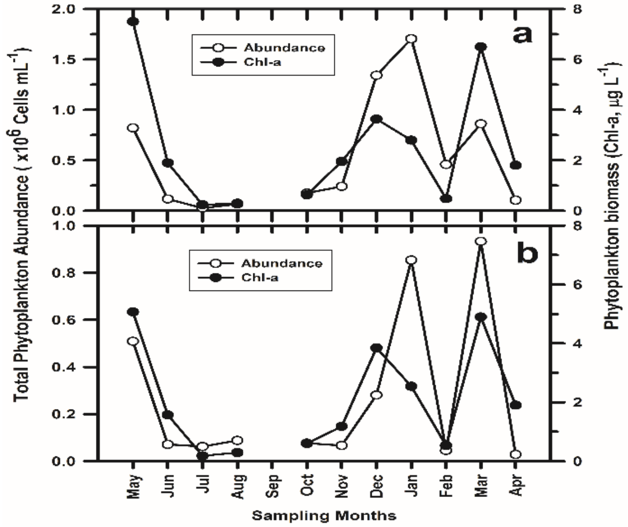

Figure 5.4 Temporal variations in phytoplankton biomass measured as Chl-a in relation to total phytoplankton abundance at the near-shore (a) and open water (b) sites of the present study on Koka Reservoir.

5.3.3. Species –environment relationship

Redendency analysis revealed that about 65 % of the total variance in species composition was explained by the selected environmental variables **(Table 5.2).** The first two axes accounted 100% of the species variability explained by the selected environmental variables.

Table 5.2. Summary of RDA results of species-environment relationships

Axes	1	2	3	4	Total variance
Eigenvalues	0.528	0.125	0.000	0.332	1.000
Species-environment correlations	0.792	0.884	0.000	0.000	
Cumulative percentage variance	52.8	65.2	65.2	98.5	
of species-environment relation:	80.9	100.0	0.0	0.0	
Sum of all eigenvalues					1.000
Sum of all canonical eigenvalues					0.652

Heterocystous cyanobacteria, namely *C. africana, C. curvispora,* and *D. spiroides* showed positive correlation with temperature, TP, and SRP and negative correlation with nitrate. *Microcystis* spp., however, showed positive correlation with nitrate and salinity **(Fig.5.5).** TP exhibited negative correlation with Z_{SD}.

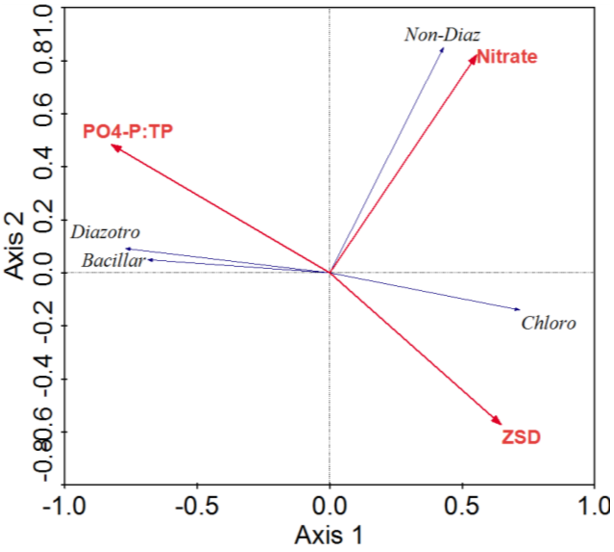

Figure 5.5 Redundancy analysis (RDA) biplot of species-environment relationship (Species: Diazotrophic and Non-diazotrophic cyanobacteria, Bacillario=Bacillariophyceae, Chloro= Chlorophyceae); Environmental parameters, Z_{SD}= Secchi disk depth, SRP: TP= Soluble Reactive Phosphate-phosphorus:Total Phosphorus, Nitrate**)**

5.3.4. Spatio-temporal Variations in Phytoplankton Species Composition

Ordination of sample scores in species vectors to analyze the spatial and temporal variability of samples in species composition revealed the absence of spatial variation in species composition and abundance between the two sampling sites. All pairs of monthly samples of the two sampling sites appeared in very close proximity to each other in the ordination diagram **(Fig. 5.6)** suggesting that they are similar in the observed species composition and abundance (Ter Braak, 1994).

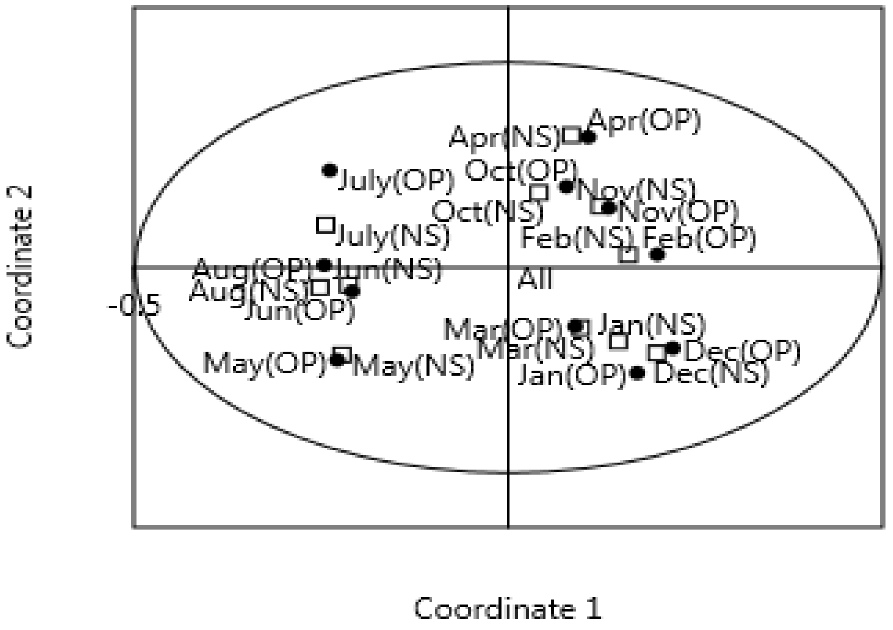

Figure 5.6 Ordination of sample scores (Bray-Curtis distances) in species vectors (Sampling sites: (NS), Open circle= Near-shore samples, (O) = Open water samples)

However, the ordination of monthly samples suggest clear temporal variations in the observed species composition. Samples of months corresponding to the rainy season (May-August, 2015) tended to cluster together with greater relative abundance of *Microcystis* spp. and Chlorophytes as opposed to those of the dry and minor rainy seasons (October-April), which were largely dominated by heterocystous cyanobacteria, namely *C africana*, *C. curvispora,* and *D. spiroides* **(Fig 5.3 and Fig. 5.6).**

5.4. DISCUSSION

Ordination of sample scores in species vectors to analyze the spatial and temporal variability in species composition revealed the absence of spatial variation in species composition and abundance between the two sampling sites. This was well reflected in their negligibly small Bray-Curtis distances **(Fig. 5.6)** whereby all pairs of monthly samples of the two sampling sites appeared in very close proximity to each other in the ordination diagram suggesting their similarity in their species turnover. Patches of cyanobacterial scum, which were almost exclusively dominated by *Microcystis* species, were observed at the shore in enclosures and holes created by animal hooves during the earlier months of sampling (May-Aug) Fig 5.3 and **(Plate 5.1),** particularly in May. The high abundance of *Microcystis* during the months of May, June and August may have resulted from the remnant of the previous blooming season. The progressive decline in the abundance of this genus during the subsequent months seems to suggest that there was no continued *in situ* growth. Koka Reservoir exhibits regular bloom episodes during the drier and warmer months (December-February) and collapse of the blooms during the subsequent months eventually disappearing during the rainy season. A new buildup of *Microcystis* populations starts again following the rainy season possibly favored by the improved underwater light climate and nutrient-rich conditions that result from input through runoff during the previous season. *Microcystis* was reported to be highly competent under conditions of such non-limiting nutrient supply (Nalewajko and Murphy, 2001), which may explain the almost year-round dominance of this genus manifested by thick greenish *Microcystis* bloom in the years prior to our sampling period (**Plate 5.2)**. This regular *Microcystis* bloom episode did not, however, show up during our sampling period **(Plate 5.3)** possibly due to the El Niño induced drought and the consequent reduction in the external input of nutrients and changes in other limnological conditions of the reservoir.

Plate 5.2 Cyanobacterial bloom almost exclusively dominated by *Microcystis* spp. in the earlier years prior to the present study in Koka Reservoir (Jan, 2007). Photo by Samson Tilahun

Plate 5.3. Koka reservoir during our sampling period (January 2016) (compare with plate-1. Both plate 5.2 and 5.3 were taken during the peak blooming month (January). Photo by Samson Tilahun.

The reduced nutrient condition (Chapter 4) of the reservoir was also reflected in the observed chlorophyll-*a* concentration, which was noticeably lower than those reported in earlier studies that were mostly based on one-time sampling (Melaku Mesfin et al., 1988; Elizabeth Kebede and Willén, 1996; Willén et al., 2011). The cyanobacterial community was rather dominated by the heterocystous genera particularly during the later months of sampling. Therefore, several features of the reservoir including water transparency, temperature, pH, aggregate chemical parameters like salinity and nutrient availability observed during our sampling period **(Table 4.2, Chapter 4)** may have favored the unusual dominance of *Cylindrospermopsis* in Koka Reservoir. Diatoms remained the second most dominant group of phytoplankton throughout our sampling period. The group might have been favored by the availability of silica **(Table 4.2. Chapter 4)** at high concentrations and possibly by the frequent mixing of the reservoir. RDA revealed the negative correlation of abundance of diatoms with Secchi disk levels **(Fig. 5.5)** suggesting that frequent mixing of the reservoir might have favored the dominance of this phytoplankton group. Frequent mixing re-suspendes bottom sediment thereby decreasing water clarity as reflected in the observed high TSS and turbidity levels and low Secchi disk depths, facilitates internal nutrient loading **(chapter 4).** It also promotes re-suspention of sinking diatoms to the surface thereby allowing them to remain in the part of the water column where sufficient light energy can be harvested for photosynthesis (Reynolds, 2006). Elizabeth Kebede and Willén (1996) also reported the occurrence of diatoms in large abundance in the reservoir owing to the frequent mixing of the reservoir, which was suggested to be one of the limnological conditions favoring their proliferation.

5.4.1. Effect of Water Transparency

Other physical conditions that prevailed during the sampling period may have also contributed to the observed dominance of *Cylindrospermopsis* in Koka Reservoir. *Cylindrospermopsis* is able to adapt to and even become dominant in turbulent and frequently mixing water bodies (Soares et al., 2013) owing to its greater tolerance to low irradiance than other phytoplankton taxa (Dokulil and Teubner, 2000; Fabbro and Duivenvoorden, 2000; Moustaka-Gouni et al., 2007; Padisák, 1997). This explains the unusual dominance of *Cylindrospermopsis* in Koka Reservoir, which was characterized by frequent mixing and low irradiance as suggested by the high turbidity levels of 1027±276 and 1099± 287 NTU recorded for the open water and near- shore sites, respectively and the noticeably low Secchi disk depths that remained below 20 cm **(Table 4.2 chapter 4).** The re-suspension of sediments due to the frequent mixing of the reservoir discouraged the growth of other phytoplankton taxa, while promoting the dominance of *Cylindrospermopsis* (Moustaka-Gouni et al., 2007).

5.4.2. Effect of Temperature

The observed water temperature ranged of 22.4-31.1°C at near-shore sites and of 19.2-31.3°C at the open water sites showed positive correlation with cyanobacterial abundance **(Table 4.2; chapter 4)**. High temperature generally favors cyanobacterial growth (Whitehead et al. 2009; Dokulil and Teubner, 2000; Kosten et al., 2012). Climate change also enhances the competitive advantage of cyanobacterial dominance partly by reducing water viscosity, which lessens resistance to vertical migration thereby allowing cyanobacteria to move up and down with relative ease to harness light from the surface and acquire nutrients from the deeper part of the water column (Paerl and Huisman, 2009). The expansion of cyanobacterial bloom with increasing temperature, but without any significant change in nutrient loading signifies the independent role played by temperature in bloom formation (Stüken et al., 2006). A survey conducted on 143 lakes of varying trophic states by Kosten et al. (2012) indicated that within total phytoplankton biomass, biomass values of members of cyanobacteria were found to increase with temperature. Robarts and Zohary (1987) also observed this positive correlation between cyanobacteria and temperature. The observed increase in the abundance of *Cylindrospermopsis* towards the latter months of our sampling period characterized by higher water temperature may, therefore, suggest that this genus is favored more by high water temperature. *C. raciborskii* was reported to have a more competitive advantage over other cyanobacterial species such as *M. aeruginosa* at higher temperature (Yamamoto and Shiah, 2012). This is consistent with our observation that the related species, *C. africana* was more abundant than *Microcystis* at the high temperature levels observed in the present study. Thus, temperature may also partly explain the dominance of *Cylindrospermopsis* during our sampling period.

5.4.3. Effect of Salinity, Conductivity and TDS

Salinity and TDS showed strong correlation and similar temporal trends as would be expected **(Table 4.3, Chapter 4).** The increasing trends observed during the drier and warmer months are attributable to increasing evaporative loss of water at a rate exceeding precipitation, which leads to evaporative concentration of solutes (Lewis Jr., 1996, Chapter 4). The slightly higher salinity values observed during the present study period relative to those of earlier reports (Elizabeth Kebede and Willén, 1996) may have resulted from the extended dry and warmer weather condition associated with the El Niño induced drought. The observed levels of salinity were (0.217±0.0239 and 0.224±0.0203) ppt for the open water and near-shore sites, respectively, (Table 4.2, Chapter 4). The salinity levels were not, however, high enough to result in a shift in cyanobacterial dominance from *Microcystis* to *Cylindrospermopsis* as the salinity levels were still well below that reported (> ≈ 0.5-2 g L^{-1}) by Pearl et al.(1983) to be inhibitory to *Microcystis aeruginosa*.

Earlier studies made on Koka Reservoir (Elizabeth Kebede and Willén, 1996; Willén et al., 2011) have also reported a salinity level of 0.2 g/l for the same reservoir, during which *Microcystis* was the most dominant cyanobacterium.

5.4.4. Effect of pH

Cylindrospermopsis remained dominant over the observed range of pH (6.4-8.83, **Table 4.2, Chapter 4)** in the reservoir, an observation, which is in agreement with the findings of Bonilla et al. (2012) who reported that *Cylindrospermopsis* grew within even wider pH range (5.49-9.91). Contrary to our observation, Padisák (1997) reported growth of *Cylindrospermopsis* within a narrow range of alkaline pH (8.0-8.7). Photosynthesis by cyanobacteria was reported to elevate pH significantly (Yamamoto and Nakahara, 2005). However, our observation is consistent with the results of the Spearman Rank Order Correlational analysis of the relationship between cyanobacterial photosynthesis and pH. The observed weak and non-significant correlation imparted by the afore-mentioned statistical analysis for the relationship between pH and Chl-*a* (correlation coefficient= 0.29 and p=0.188) seem to corroborate our argument.

5.4.5. Effect of Nutrient Availability

Nutrient availability has been implicated as one of the major environmental variables that control cyanobacterial dominance and their temporal dynamics (Liu et al., 2011). Several experimental and field studies have shown the important role of nutrients in dictating cyanobacterial community structure and its temporal dynamics (Schindler et al., 2008). As the great proportion of the annual nutrient budget of Koka Reservoir seems to be of riverine origin, it is largely influenced by rainfall pattern and hydrological cycles **(Chapters 3 & 4).** Therefore, changes in temperature, rainfall pattern and hydrological cycles due to changes in climatic conditions such as El Niño induced drought may have a profound effect on nutrient availability and other physico-chemical conditions of the reservoir **(Chapter 4),** which may consequently influence the cyanobacterial community structure in the reservoir (Figueredo and Giani, 2009b) 2009). Our sampling period (May 2015-April 2016) coincided with the El Niño induced drought, which caused failure of the main rainy season (*Kiremt* rainfall) (Gleixner et al., 2016, OCHA, 2016). It was characterized by intermittent rainfall, which was much lower in intensity compared to what is typical for the study area. The flow of Awash River was significantly reduced, while Modjo River completely dried up in comparison with their levels in the earlier years **(Chapter 4, Fig. 4.3 & Plate 4.1)**.

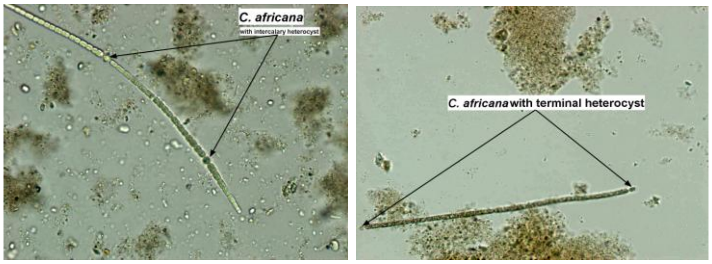

Plate 5.4 A microscope snap shot of *C. africana*, the dominant species with intercalary heterocyst (Left) and with terminal heterocyst at both ends (Right) 40 x. (Photo by Samson Tilahun*)*

Plate 5.5 A microscope snapshot of *C. curvispora* with terminal heterocyst 40x (Right) and *D. spiroides* (Left) (photo by Samson Tilahun, 2016).

This condition may have caused a significant reduction in the reservoir's annual nutrient input received through runoff **(Chapter 4).** The observed low nutrient availability, particularly nitrogen during most months of the sampling period might have led to the proliferation of heterocystous cyanobacteria (**Figs 5.1. &5.3).** Although the drought was expected to have caused a significant reduction in the external input of both nutrients (nitrogen and phosphorus), nitrogen was more likely to be a limiting nutrient in the reservoir compared to phosphorus during our sampling period **(Chapter 4).** While phosphorus could possibly be replenished from the sediment through internal loading, the available nitrogen was most probably lost through denitrification owing to the observed high annual mean water temperature **(Chapter 4, Table 4.2**) which promote bacterial metabolic process **(Chapter 4).** Ammonia concentration during most of the present sampling months was below the detection limit of the method of analysis employed (**Chapter 4 and Fig. 4.6)**. Sas (1989, cited in Bouvy et al., 2003) also argued that water bodies with nitrogen concentration below 0.1 mg/l are regarded as nitrogen-deficient. Several studies have reported that reduction in the external input of nitrogen induce the proliferation of diazotrophic cyanobacteria as they are able to survive nitrogen stress through nitrogen- fixation (Tilman et al., 1982; Howarth et al., 1988; Moustaka-Gouni et al., 2007; Schindler et al., 2008; Wang and Wang, 2009), an argument that seems to corroborate our observation in Koka Reservoir. The unusual dominance of the nitrogen-fixing cyanobacterial genus, namely *Cylindrospermopsis* and the co-occurrence of *Dolichospermum* in large abundance during our sampling period may be attributed to the severe nitrogen limitation in the reservoir. The dominance of the genus *Cylindrospermopsis* was not reported for the reservoir in most of the earlier studies

(Melaku Mesfin et al., 1988; Elizabeth Kebede and Willén, 1996; Willén et al., 2011). Experimental and field investigations (e.g. (Blomqvist et al., 1994; Bouvy et al., 2000; Briand et al., 2002; Crossetti and Bicudo, 2008; Marinho and Huszar, 2002) have demonstrated the association of the emergence of nitrogen-fixers such as *Anabaena* and *Cylindrospermopsis* with limiting levels of combined inorganic nitrogen sources (ammonia and nitrate). *Cylindrospermopsis raciborskii,* which responds to environmental variations in nitrogen levels by alternating between N-fixation and dissolved inorganic N-assimilation (Moisander et al., 2012), may be considered a generalist in terms of nitrogen (N) usage due to its facultative diazotrophy (Soares et al., 2013). Câmara et al., (2015), in their study on phytoplankton of a tropical reservoir, attributed the shift in dominance from *Microcystis* spp. to such heterocystous cyanobacteria as *Anabaena circinalis* and *Aphanizomenon flos-aquae* to lower concentration of ammonia-nitrogen. Similarly, Padisák and Istvánovics, (1997) have also reported negative correlation between ammonia concentration and abundance of heterocystous cyanobacteria in Mundaú Reservoir, in Brazil. The observed shift in cyanobacterial dominance from the persistently dominant *Microcystis* spp. over the last several years in the reservoir to the dominance of heterocystous cyanobacteria in this study may also indirectly suggest the effect of the El Niño induced drought on the biogeochemical condition of the reservoir in terms of nutrient availability. Thus, climate change associated with pluvial anomaly linked to El Niño can influence phytoplankton population dynamics in continental waters, as demonstrated frequently for oceanic ecosystems. As climatic factors like the El Niño event are associated with tremendously reduced precipitation and the consequent unusually low water input to freshwaters, particularly in semi-arid and arid regions, the resultant low levels of nutrients especially nitrogen lead to the emergence and dominance of noxious nitrogen-fixers such as *Cylindrospermopsis raciborskii*. *Cylindrospermopsis* spp. should be species of primary concern owing to lack of visual monitoring cues such as scum formation (Plate 5.3) and variation in the color of the water body, relative toxicity and persistence of this species year round in many areas. The absence of the greenish surface scum associated with *Microcystis* bloom, which was common in earlier years (Plate 5.3), also seems to reflect the replacement of *Microcystis* by *Cylindrospermopsis.* Some studies have, however, reported that *Microcystis* is favored by the availability of low levels of nitrogen (Paerl et al., 2011; Rückert and Giani, 2004), a finding, which contrasts with our observation in Koka Reservoir. We have, however, some doubt as to wether there was actually nitrogen limitation in the studied lakes. For instance, Paerl et al., (2011) reported the dominance of *Microcystis* over nitrogen-fixing cyanobacterial genera (*Anabaena and Aphanizomenon*) under condition of "chronic nitrogen limitation". Similarly, (Rückert and Giani, 2004) reported the dominance of non-nitrogen fixing cyanobacteria, namely *Microcystis viridis* under low NO_3-N. In both studies, the authors, however, reported the availability of ammonia, which was high particularly in the lake studied by (Rückert and Giani, 2004). If ammonia is available in the system, designating such a system as nitrogen-limited is puzzling. Ammonia has been reported to be the most preferred form of nitrogen for *Microcystis* and other cyanobacteria due to the less energy requirement for its assimilation (Donald et al., 2013). An experimental study by Rückert and Giani, (2004) on the effect of nitrate and ammonia on the growth and protein concentration of *Microcystis viridis* also revealed this fact. This genus grew faster in a medium rich in ammonia than in nitrate suggesting that ammonia is a more favorable form of nitrogen than nitrate. *Microcystis* spp. had been the most dominant cyanobacterial taxa in Koka Reservoir (Elizabeth Kebede and Willén, 1996; Major et al., 2017; Willén et al., 2011) as manifested in the regular episodes of heavy greenish surface blooms observed over several years prior to our sampling period **(Plate 5.2).** In the present study, this episode of greenish surface bloom almost exclusively dominated by *Microcystis* species did not occur during the dry months of our sampling period **(Plate 5.3**) possibly due to nitrogen-limitation associated with the drought. The Chl-*a* concentration observed in this study was low compared to those documented in earlier reports (Elizabeth Kebede and Willén, 1996; Willén et al., 2011, Yeshiemebet Major et al., 2017) and this could be associated with the presumably lower phytoplankton standing crop.

Experimental and field studies have shown that non-diazotrophic cyanobacteria are highly competent under nutrient-rich conditions and in fact dominate over diazotrophic cyanobacteria (Moisander et al., 2008). This could be particularly true for Koka Reservoir in which the non-diazotrophic cyanobacteria, *Microcystis* spp., had been the most dominant over several years in the reservoir prior to our sampling (Elizabeth Kebede and Willén, 1996; Yeshiemebet Major et al., 2017; Willén et al., 2011) possibly due to nutrient-rich conditions. The reservoir receives much of its annual nutrient budget from diffuse sources through Awash and Modjo rivers during the major rainy season ,June–September, **(Chapters 3 and 4).** The huge amount of nutrient input occurring during these months initiates a gradual buildup of *Microcystis* spp. populations in the reservoir with the highest peak of abundance occurring during the months of the dry and minor rainy periods (Dec-Mar). However, such cyanobacterial genera, unlike the nitrogen-fixers, do not have an alternative source of nitrogen, which can be utilized during the depletion of ambient combined nitrogen sources (Reynolds, 2006). The disappearance of *Microcystis* bloom in Koka Reservoir and the complete dominance of cyanobacteria by the heterocystous genus, namely *Cylindrospermopsis*, during the dry months of our sampling period seems to be in line with the above argument. Considering the presumed nitrogen-limitation in Koka Reservoir during our sampling period, the primary source of nitrogen for the dominant cyanobacteria, namely *Cylindrospermopsis* and other heterocystous genera, may have been atmospheric nitrogen. *Cylindrospermopsis* was reported to be an opportunistic cyanobacterial genus in relation to its source of nitrogen supply. It preferably uses dissolved combined inorganic nitrogen when it is available (Burford et al., 2006; Sprőber et al., 2003) and resorts to nitrogen-fixation when it is scarce (Moisander et al., 2008). This ability of shifting its nitrogen source provides *Cylindrospermopsis* a survival advantage (Moisander et al., 2008) and even the capacity to become dominant over other cyanobacteria, particularly such non-nitrogen fixing cyanobacteria as *Microcystis* (Hadas et al., 2012). The increasing occurrence of heterocystous cyanobacteria, *C. africana*, *C. curvispora*, *C. raciborskii* and *D. spiroides* in our samples seem to corroborate the contention that there was nitrogen-limitation in the reservoir and nitrogen-fixation may have been the primary source of nitrogen. Non-nitrogen fixing cyanobacterial genera like *Microcystis* were reported to survive short episodes of nitrogen-limitation through their ability to store surplus nitrogen as phycobillins (Allen, 1984,cited in (Kromkamp, 1987). This mechanism may not, however, serve for conditions of nitrogen-limitation extending over a longer period of time such as the one observed in Koka Reservoir during our sampling period. Our results show that *Microcystis* was persistently and overwhelmingly dominated by the nitrogen-fixing cyanobacteria, mainly *Cylindrospermopsis*, possibly due to nitrogen-limitation lasting for a longer period of time, which *Microcystis* spp. could not cope-up with. The observed unusual dominance of *Cylindrospermopsis* during our sampling period may, therefore, be at least partly attributed to nitrogen-limitation caused by the drought. Such shift in the dominance of cyanobacterial genera between diazotrophic and non-diazotrophic taxa depending on the availability of dissolved inorganic nitrogen has been reported previously for other aquatic systems (Schindler et al., 2008; Wang and Wang, 2009; Schindler, 2012). A cross-sectional survey in Jan 2019 revealed the dominance of *Microcystis* and the re-appearance of the thick *Microcystis* bloom in the reservoir (See Plate 5.6 and Plate 5.7).

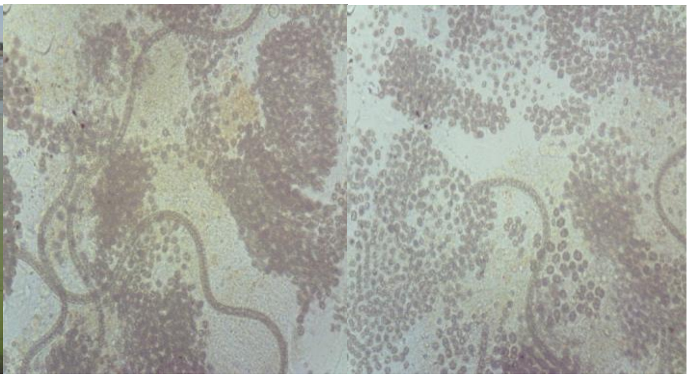

Plate 5.6 (Right) The re-appearance of the thick cyanobacterial bloom dominated by *Microcystis* spp. in Koka Reservoir (Jan, 2019), (Compare with Plates 5.2 and 5.3. Note that both plates 5.2 and 5.3 were taken during the peak blooming month (January). Photo by Samson Tilahun.

Plate 5.7 (Left) A microscope snapshot of the dominant *Microcystis* sp. and the co-occurring diazotrophic cyanobacterium (*Cylindrospermopsis*) from samples of Jan. 2019 at 40x (photo by Samson Tilahun, 2019). Compare with plate 5.4.

This is probably attributable to the restoration of the normal weather conditions and improved nutrient availability, particularly nitrogen. Although heterocystous cyanobacteria, namely *Dolichospermum circinalis*, and *Cylidrospermopsis* spp. still co-occurred in large abundance, the dominance of *Microcystis* during this period seems to support the claim that non-nitrogen fixing cyanobacteria are much more competent under nutrient-rich conditions (Moisander et al., 2008). Strong competition among members of cyanobacteria and competitive advantage were also another reported factors determining the cyanobacterial community structure. Faster growth, tolerance to adverse environmental conditions and use of allelochemicals against others are among the features associated with the competitive advantages that result in the dominance of some genera over others. The inhibitory effect of allelopathic chemicals released by *Cylindrospermopsis*, which may have suppressed *Microcystis* growth in the tropical Lake Lagua Santa, Brazil, was suggested by Figueredo et al. (2007) to partly explain the dominance of *Cylindrospermopsis* over *Microcystis*. Rzymstic et al. (2014) also carried out experimental study on allelopathic effect of cylindrospermopsin and *Cylindrospermopsis raciborskii* on the growth and metabolism of *Microcystis aeruginosa*. According to their report, cylindrospermopsin (CYN, 10-50 µg L^{-1}) caused both cell necrosis and growth inhibition of *Microcystis aeruginosa* cells. Furthermore, non-cylindrospermopsin-producing cells were also reported to produce chemicals other than CYN, but with similar allelopathic effect (Rzymski et al., 2014). According to Figueredo and Giani (2009), biological interactions such as allelopathy were more important than environmental variables to explain the stable dominance of *C. raciborskii* in the reservoir of their study despite changes in environmental conditions that could have sustained the co-existence of other phytoplankton species. Although experimental data that can confirm the involvement of such chemicals in Koka Reservoir are lacking, the possible role of allelopathic chemicals for the observed unusually low abundance of *Microcystis* and the dominance of *Cylindrospermopsis* during our sampling period cannot be ruled out.

5.4.6. Implication of the emergence and dominance of *Cylindrospermopsis* under nitrogen-deplete condition in Koka Reservoir.

Several experimental and field studies have been conducted on growth limiting nutrients and on possible intervention strategy to reverse eutrophication related water quality problems (Conley et al., 2009; Wang and Wang, 2009; Scott and McCarthy, 2010). Suggested intervention strategies based on these studies, however, triggered considerable debate among scientists. For instance, some argue that effective control of harmful algal blooms can only be achieved through reduction of external input of phosphorus only rather than nitrogen (Schindler et al., 2008; Wang and Wang, 2009). According to these authors, reducing external input of nitrogen is of no use as it simply induces the proliferation and dominance of nitrogen-fixing cyanobacterial genera. This is in agreement with our observation in Koka Reservoir that the low nitrogen condition was associated with the unusual dominance of heterocystous cyanobacteria, namely *C. africana, C. curvispora,* and *D. spiroides*. Conley et al. (2009) also indicated that the *phosphorus only* strategy is likely to fail in lakes in which phosphorus is rapidly recycled between the sediment and the water interface. This also seems to be the case for Koka Reservoir as the concentration of phosphorus remained high enough at least to support cyanobacterial growth despite the expected drought-associated reduction in its external input. This may imply the rapid recycling between the sediment and the water column (Chapter 4) as suggested by Conley et al. (2009). Effective control of both *Microcystis* and nitrogen fixers in such lakes, therefore, needs reduction of both nitrogen and phosphorus (Conley et al., 2009). The above claims and arguments are based on data generated from ecosystems and climatic conditions different from those of the rift valley lakes. Therefore, they may not represent the conditions of the rift valley lakes, particularly the Ethiopian rift valley lakes. Furthermore, there are no reports on limiting levels of nutrients based on data generated locally for the conditions of the Ethiopian rift valley lakes including Koka Reservoir. However, the recent incidence of El Niño induced drought may provide a good insight into the short-term response of Koka Reservoir to reduction in the external input of both nutrients (nitrogen and phosphorus). Despite the reduction in the external input of both nutrients, phosphorus was not a limiting nutrient in the reservoir possibly due to its recycling between the water column and the presumably huge phosphorus reserve in the sediment as suggested by Conley et al. (2009). On the other hand, nitrogen was at low concentrations (0.052±9.75 and 0.051±2.06 mg/l) at the open and near-shore sampling sites, respectively, which according to Sas (1989, cited in Bouvy et al., 2003) makes the reservoir a water body under severe nitrogen-limitation. The observed unusual dominance of heterocystous cyanobacteria in the reservoir may be attributable to the prevailing severe nitrogen-limitation as suggested by Wang and Wang (2009) and Schindler (2012). The limonological response of Koka Reservoir during the recent incidence of El Nino seems to suggest that there is a large phosphorus reserve in the sediment, which could sustain cyanobacterial growth through internal recycling. Such shallow and completely mixing water bodies are less likely to recover (Jones and

Poplawski, 1998; Londe et al., 2016). Therefore, reduction in the external inputs of phosphorus may not be an immediate solution for Koka Reservoir (Chapter 4). The presumed severe nitrogen-limitation in Koka Reservoir during the drought period implies that the reservoir's nitrogen source is agricultural runoff (Chapter 4). This means that reduction in the external input of nitrogen causes severe nitrogen-limitation. However, the emergence and unusual dominance of nitrogen-fixing cyanobacteria may complicate efforts aimed at the recovery of the reservoir through intervention strategy geared towards reducing the external input of nutrients. The nutrient data recorded during the incidence of El Niño also seem to suggest that reduction in the external input of nutrients may not enable Koka Reservoir to recover from eutrophication related water quality problems at least in the near future.

5.5. CONCLUSIONS

The drought associated with the incidence of El Niño seems to have resulted in severe nitrogen limitation in the reservoir. This has led to a phytoplankton community in which the previous exclusively bloom-forming *Microcystis* spp. occurred at unusually low abundance. The unusually low abundance of *Microcystsi*s during the present sampling period is attributable partly to the exteremly low of nitrogen level in the reservoir. This concurred with the overwhelming dominance of the diazotrophic cyanobacterial taxa, C. *africana, C. curvipora* and *D. spiroides* during our sampling period. Such a shift from *Microcystis*-dominated phytoplankton community to *Cylindrospermopsis*-dominated one may suggest that phosphorus could be the key controlling nutrient in the reservoir as nitrogen is probably never limiting for such cyanobacterial genera.

The emergence and unusual dominance of *C. africana* and the co-occurrence of other heterocystous cyanobacteria and the increase in the frequency of heterocysts in the trichomes of these genera suggest that there was severe nitrogen-limitation in the reservoir and nitrogen fixation was probably the primary source of nitrogen during our sampling period. Emergence of nitrogen fixing genera that are capable of satisfying their nitrogen requirement and the presumably huge phosphorus reserve already accumulated in the reservoir may imply that reduction in the external input of both nutrients can hardly help in reversing the current situation in Koka Reservoir at least in the near future.

CHAPTER SIX

6.0 TEMPORAL DYNAMICS OF INTRA-AND EXTRA-CELLULAR MICROCYSTINS CONCENTRATIONS IN KOKA RESERVOIR (ETHIOPIA): IMPLICATIONS FOR PUBLIC HEALTH THREAT

6.1. INTRODUCTION

Cyano-toxins in fresh water systems are becoming a serious threat to public health of global concern (Davis et al., 2009; Zhang et al., 2012). Several members of cyanobacteria including *Microcystis* are known to produce potent toxins, which cause high public health risk (Falconer and Humpage, 2005). Human illness and fatal poisoning in aquatic, wild and domestic animals linked to cyanotoxins have been reported by several authors (Backer, 2002; Carmichael et al., 1997). Microcystins, cylindrospermopsin, and anatoxins are among the cyanotoxins of high public health risk produced by cyanobacteria.

Several attempts have been made to remove these toxins from drinking water supply to minimize potential public health risk. Conventional methods such as flocculation, sand filtration, and sedimentation were reported as possible approaches for the removal of cyanotoxins (Jurczak et al., 2005). However, although cell-bound toxins can be removed using these methods, it is required to ensure that the procedures do not cause any cell disruptions thereby resulting in the eventual release of toxins into the aqueous phase (de Figueiredo et al., 2004; Fan et al., 2014), which may even exacerbate the condition (Hawkins et al., 1985). Furthermore, these approaches may not always be effective as the toxins from the aqueous phase, for instance, cannot be removed by these methods (de

Figueiredo et al., 2004). They may not also be helpful for protecting the public from the potential risk associated with ingesting food animals like fishes caught from lakes supporting cyanobacterial blooms (Ibelings and Chorus, 2007). This situation makes the potential public health risk associated with cyanobacteria and their toxins a complicated issue to address. As it is the case with water bodies found in other parts of the world, the dominance of potentially toxic cyanobacteria has been reported for many lakes of the Ethiopian Rift Valley (Elizabeth Kebede and Willén, 1998; Melaku Mesfin et al., 1988; Willén et al., 2011,). As most of these water bodies are currently serving as sources of freshwater used for drinking water supply, watering livestock, irrigation, fisheries, sanitation and recreation, the potential public health hazard and economic loss is immense. For instance, incidence of massive fish kills in Lake Chamo, one of the rift valley lakes in Ethiopia, and deaths of terrestrial wild animals around this lake in the year 1978 were linked to cyanotoxins (Amha Belay and Wood, 1982). A cross sectional survey carried out on seven of the Ethiopian rift valley lakes has also demonstrated the dominance of toxic cyanobacteria and presence of associated toxins at concentrations much higher than the permissible level in most of the water bodies (Willén et al., 2011). This suggests the potential public health hazard associated with the use of these water bodies. According to Willén et al. (2011), Koka Reservoir was reported to be in the worst situation, with *Microcystis aeruginosa* overwhelmingly dominating the cyanobacterial assemblage and with the level of total microcystins (MCs, 45–54 μg L−1) greatly surpassing the permissible level (1 μg/l) set by WHO (WHO, 1998). Such reports from cross sectional study, may provide an alarming signal inviting a detailed and more comprehensive study on the occurrence of cyano-toxins. Willén et al., (2011) also suggested the need for long-term sampling at different times of the year and identification of the major cyanobacterial genera related to toxin production. This will also help understand the link between the recurrence of cyanobacterial blooms and environmental variables to develop appropriate intervention strategies to mitigate blooms and their toxins. Protection of public health through establishing an early warning system also calls for such comprehensive study. Therefore, the present study is aimed at investigating temporal changes in *Microcystis* spp. and the concentrations of microcystins in both water and algal seston in Koka Reservoir through a long–term sampling program covering all the seasons. The study also addresses the physico-chemical conditions associated with the dynamics of *Microcystis* spp. bloom and their toxins.

6.2. Materials and methods

6.2.1. Selection of sampling sites and sampling frequency for toxin analysis

Detailed description of Koka Reservoir has been given in Chapter 4. As it is indicted in earlier chapters, (chapter 3), thousands of the local inhabitants use the reservoir as a source of fresh water supply and make their living through subsistence commercial fisheries. Sites along near-shore lines were considered as high risk areas as these sites are points where local people fetch water for household purposes, wash clothes, play, swim and provide water to the livestock **(chapters 4 and 5 See plate 3.1.).**

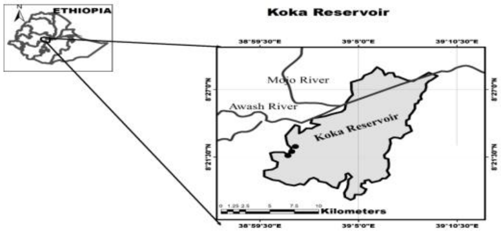

Figure 6.1 Koka Reservoir, sampling sites and its surrounding.

Sampling was carried out during seven months of the different seasons: major rainy season (June-September), dry season (October-February), and minor rainy season (March-May) at three points along the shore. Accordingly, samples were collected during the months of May, August, October, February, March, and April.

6.2.2. Sampling Protocol and determination of limnological parameters.

Procedures for sample collection, sample treatment, transportation are given in the preceding chapters, chapters 4 &5. On site measurements of physico-chemical parameters such as dissolved oxygen (DO), pH, water temperature (Temp), salinity (Sal) and turbidity (Turb.) were measured on using properly calibrated field meters as described in Chapter 4. Spectrophotometric analysis of inorganic nutrients was carried out following standard procedures outlined in (APHA, 1999; Wetzel and Likens, 2000) as described in chapter 4. Detailed procedures for identification and counting of major cyanobacterial taxa are given in Chapter 5.

6.2.3. Analysis of Microcystins

Analysis of microcystins in water and algal seston was carried out following the standard procedures outlined below.

6.2.3.1. Separation of intra-and extra-cellular microcystins

Lake water was filtered onto GF/F (pore size 0.7µm) using a vacuum pump under mild pressure to avoid cell breakage and separate the intracellular cyanotoxins from the extra-cellular ones as described in (Park et al., 1998). The intracellular toxins were extracted from the cyanobacterial cells retained on the GF/F filter papers (Whatman, UK). The filter papers with algal seston (for cell-bound toxins) as well as particle-bound toxins were dried at 50 °C overnight and immediately transferred to -20 °C for long-term storage until analysis. Similarly, the filtrate (the aqueous phase) was kept frozen at -20 °C until analysis for extra-cellular toxins.

6.2.3.2. Extractions and Analyses of Microcystins

All reagents used were of high-performance liquid chromatographic grade (HPLC grade). Methanol was obtained from sigma Aldrich. Solid phase extraction (SPE) system used for concentration and clean up of the water sample was purchased from Waters Corporation (USA). Microcystin-LR (5µg L^{-1}), -RR (5µg L^{-1}), and YR (5µg L^{-1}) standards were purchased from Sigma Aldrich (Germany).

6.2.3.3. Microcystins in Water Sample

Methanol of 0.5 ml volume was added to 50 ml water sample, which was previously filtered with 0.7µm pore size filter paper and then the mixture was sonicated for 10 minutes for degassing. The extraction (SPE) column was conditioned by rinsing with 10 mL methanol followed by 10 ml deionized water. The water sample was introduced into and passed through the conditioned SPE column under vacuum at a flow rate of 1 drop/sec. After being rinsed with 10 ml of 5% methanol, the column was dried for 20 min. The target analytes were eluted with 10 ml of 50% methanol. The elute was then dried up by a nitrogen stream and then re-dissolved by 300 µl of 50 % methanol and analyzed by liquid chromatography electro spray ionization high resonance mass spectrometry (LC-ESI-HRMS) method.

6.2.3.4. Microcystins in algal seston and on particulate matter

The extraction method was adapted from method by Lawton (Lawton et al., 1994) with a slight modification as was used for a study on a different lake (Tigist Zewde et al., 2018a). Prior to extraction, the filter paper, which contains the seston, was placed in a suitable container and freeze-thawed. Filter papers were placed in glass beakers containing 20 ml of methanol and allowed to extract for 1 h at room temperature. The liquor was then decanted into a pear-shaped rotary evaporation flask (50 ml) and the filter was gently squeezed with a spatula to ensure maximum transfer of the liquid. The extraction procedure was repeated twice. The sample was rotary evaporated at 40^{o}C *in vacuo* until dry. Then, the liquor from the second, and subsequently the third, extraction was added to the flask and dried as before. The residue was re-suspended in 500 µl of 50% aqueous methanol prior to analysis by the same LC-ESI-HRMS method used for the water samples.

6.2.3.5. LC-ESI-HRMS method

For the separation of toxins and background, 10.0 µl of each sample and standard was injected with an Accela auto sampler (Thermo Fisher Scientific) in no waste mode into a HPLC column Supelco Ascentis Express C18 , 5 cm x 2.1 mm, 2,7µm (Sigma-Aldrich/Merck). The column was maintained at 30 °C. Two solvents were used as mobile phase, A: acetonitrile with 0.1% formic acid LC-MS chromasolv (Fluka) and B: Milli-Q water from a Simplicity system (Millipore) with 0.1% formic acid for LC/MS (Fluka). The solvents were pumped through the column using an Accela pump (Thermo Fisher Scientific). The column was equilibrated with 80% A and 20% B at 500 µl/min before the first injection and eluted with the following procedure: 20% B for 30 seconds, then a linear gradient up to 60% B over 6 minutes 30 seconds, and finally 95% B for 1 minute. The column was then re-equilibrated at 20% B for 2 minutes. The flow rate was 500 µl/min for all steps. The eluted components from the column were detected using electro spray ionization and high-resolution MS on a Thermo Orbi trap XL (Thermo Fisher

Scientific). The electro spray setting was as follows: sheath gas flow rate: 70, aux gas flow rate: 10, sweep gas flow rate: 10, spray voltage: 4.5 kV, capillary temp: 330 °C, capillary voltage: 37V and tube lens: 80V. Detection parameters for the MS-analyzer was as follow: resolution: 30000 scan type: full, polarity: positive, mass range: 330-1200, micro scans 1 and max. Inject time: 250ms. Lock mass was enabled for correction of background ions from di butyl phthalate (m/z 279.159086), di-isooctyl phthalate (m/z 391.284286 , m/z 413.266231 and 803.543240) and irganox (m/z 553.459115).(Audrey Roy-Lachapelle et al., 2015; Semyalo et al., 2011) Ion chromatograms where extracted for the analytes at the following masses: MC-YR (m/z 523.1817, m/z 1045.5353), MC-LR (m/z 498.2817, m/z 995.5560), MC-RR (m/z 519.7902, m/z 1038.5731) using Thermo X calibur 2.1. The mass tolerance was set to 6 ppm. A calibration curve was constructed using standard concentrations produced by diluting the standards (Sigma-Aldrich) in Milli-Q water. The standard concentrations were as follows (µg L^{-1}): 0.5, 1, 2, 5, 10, 25 and 50. The limit of detection (LoD) and limit of quantitation (LoQ) where determined based on signal to noise of 3 and 9, respectively. LoD for all components was determined to be 0.5 µg $L^{-1}$and LoQ to be 2 µg L^{-1}.

Standards below 2 µg L^{-1} were excluded from the calibration curve. Calibration curves and regression coefficients were as follows:

MC-YR: $Y = -20995.2+35179.2X, R^2 = 0.9981,$

MC-LR: $Y = -33075.4+73869.2X, \quad R^2 = 0.9991$

MC-RR: $Y = -115052+139433X, \quad R^2 = 0.9938.$

Quantitation of the unknown samples was done using these calibration curves in Thermo X calibur Quan browser. Note that we have previously applied these procedures for measuring concentrations of microcystins in fish species collected from the same reservoir (Zewde et al., 2018b) as well as extra- and intracellular MCs concentrations in samples collected from Lake Hora-Arsedi, Ethiopia (Zewde et al., 2018a).

6.2.3.6. Statistical Analysis

Data of several of the variables including *Microcystis* cell abundance, major MC variants and some environmental parameters were highly skewed and hence failed to conform to the assumption of normality (Shapiro-Wilk, $p<0.05$,Chapter 4). Therefore, non-parametric statistics, Spearman Rank Order Correlation, was used to determine the possible associations among the variables. Test results were considered significant at $p<0.05$. SPSS ver. 20 was used for all statistical analyses. Graphs were plotted with Sigmaplot ver. 10.

6.3. RESULTS

All major MC variants, MC-LR, MC-YR, and MC-RR, were detected in algal seston and water samples during all months except August (**Figs. 6.3 and 6.4**). The concentrations of these toxins were higher than the permissible level established for drinking water 1 µg L^{-1} (WHO, 2004) on the majority of the samples (**Fig. 6.5**).

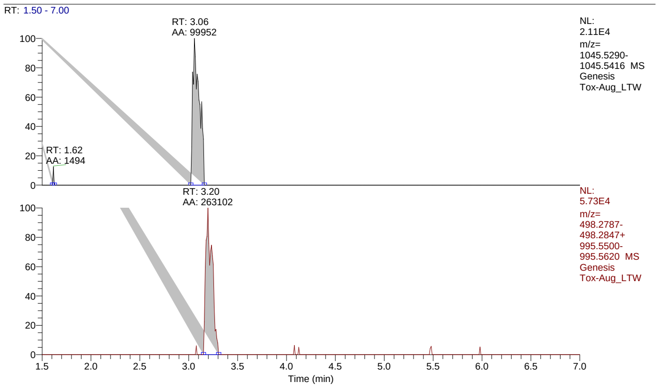

Figure 6.2. Chromatograms showing the presence of MC-YR (top) and -LR (bottom) in water samples collected during August (2015) from Koka Reservoir

Exceptionally high concentrations (µg L^{-1}) of all intra-cellular MC variants, MC-LR (815) MC-YR (466.6) and MC-RR (265 .68), were observed in May **(Fig.6.5),** which were respectively about 815, 467, and 266 times the permissible level.

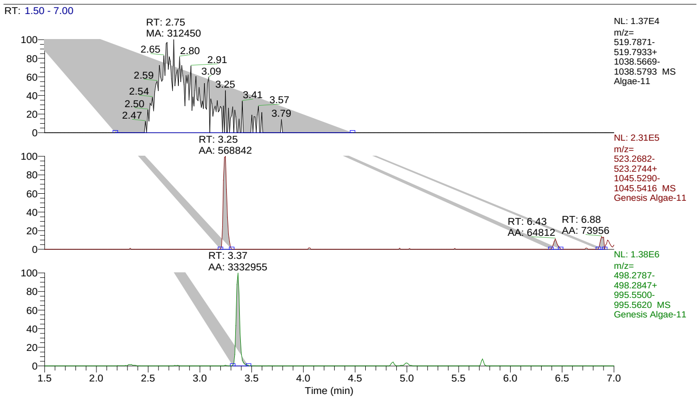

Figure 6.3. Chromatograms showing the presence of MC-RR (top), -YR (middle), and -LR (bottom) in algal samples collected during March (2016) from Koka Reservoir

Exceptionally high concentrations (µg L^{-1}) of all intra-cellular MC variants, MC-LR (815) MC-YR (466.6) and MC-RR (265 .68), were observed in May **(Fig.6.5),** which were respectively about 815, 467, and 266 times the permissible level. These concentrations also exceeded the maximum permissible level set for water bodies intended for recreational purposes (20 µg L^{-1}) (WHO, 1998).

A decrease in their concentrations occurred in October, December, February, and April before their increases in March was observed **(Fig. 6.5).**

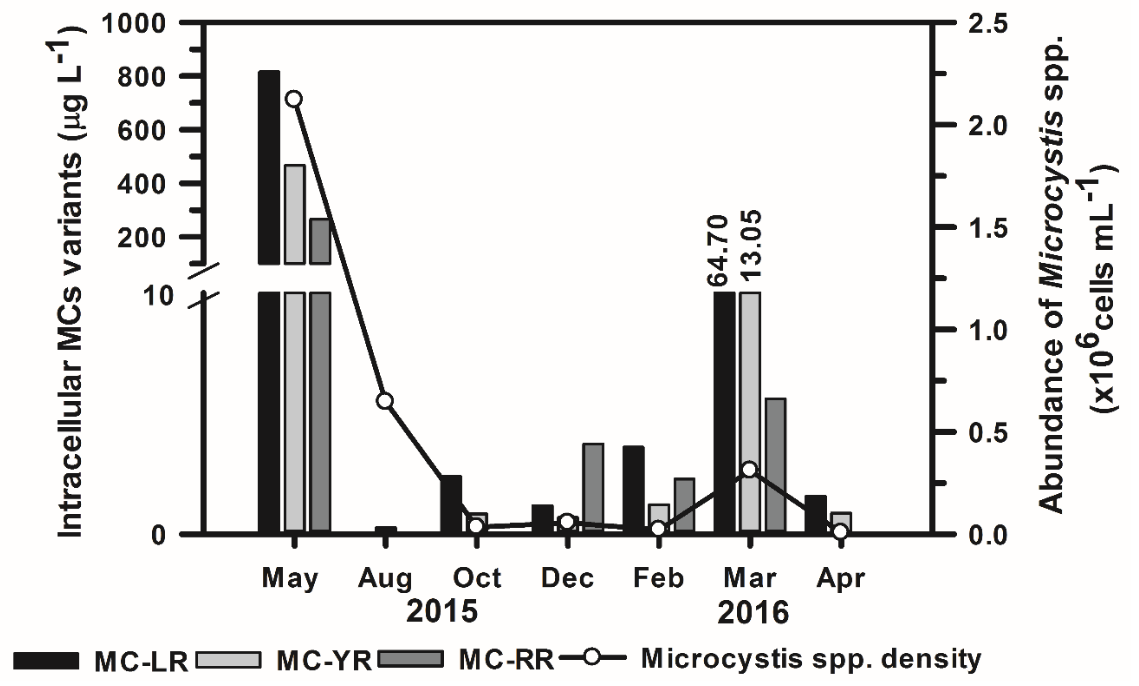

Figure 6.4. Temporal variations in the concentrations of major microcystin variants in relation to the total abundance of *Microcystis* spp. at a near-shore site in Koka Reservoir . Figures at the top of the bars refer to the maximum levels measured for the corresponding variants

However, their concentrations were still higher than the WHO's safety limit of (1 µg L^{-1}) and those reported for Lake Chivro in Zimbabwe (0-2.855 µg L^{-1}; Mwaura et al., 2004).

Among the major MC variants, MC-LR constituted the largest proportion of the total MC concentrations in all sampling months except December, when the concentration of MC-RR was the highest, while in August sample, only MC-YR was detected (**Fig. 6.6).**

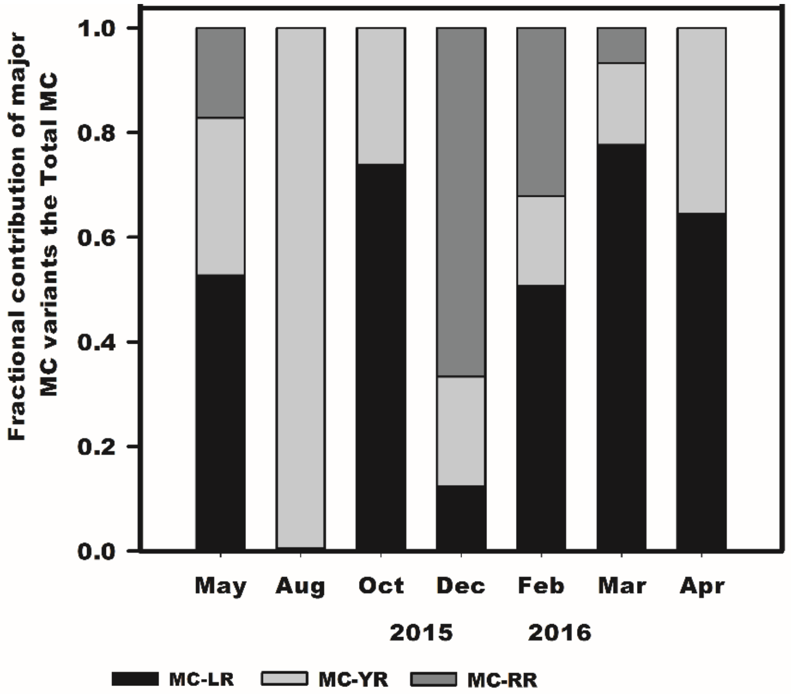

Figure 6.5 Temporal variations in the relative contributions of the major MC-variants to the total intra-cellular MC (May 2015- April 2016)

The extra-cellular toxins were relatively less concentrated and less frequently detected compared to the cell-bound ones (**Figs 6. 5 & 6.7**).

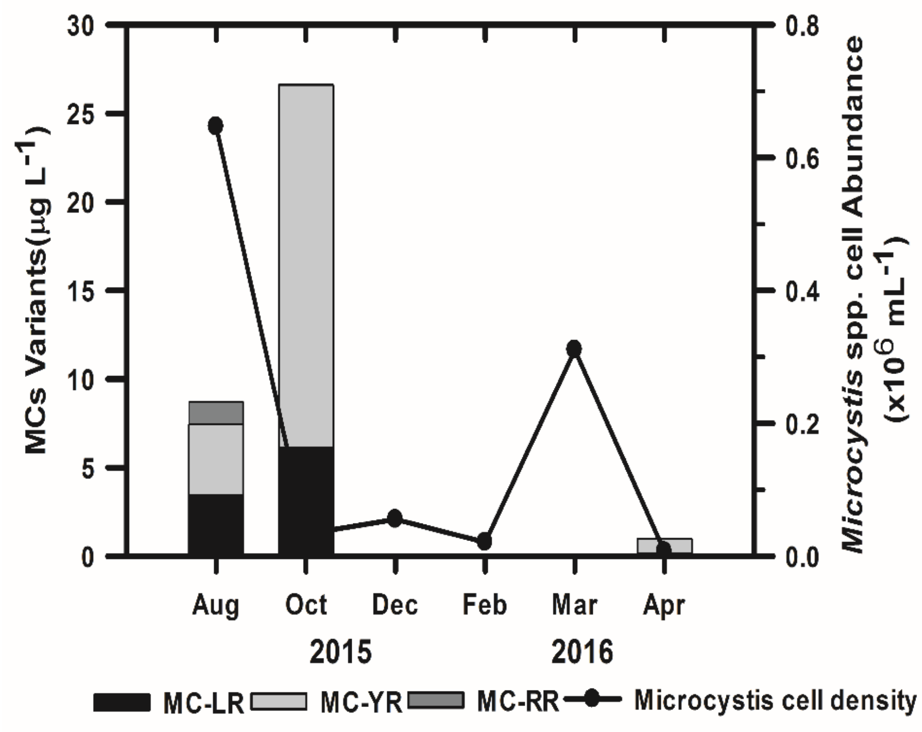

Fig. 6.6. Temporal variations in the concentrations of extracellular microcystins (MCs) variants in relation to cell densities of *Microcystis* spp. at near-shore site of the present study on Koka Reservoir.

However, the extra-cellular fraction of total MC was >43 and >14 times the total intracellular MC concentration measured in August and October, respectively **(Table 6.1).**

Table 6.1. Temporal changes in the relative contributions of the total intra-cellular and extra-cellular MCs in Koka reservoir during the present study

Month	Total MCs	Total MCs(in water)	Ratio (Total MCs (water):
May	1548.13825		
Aug	0.265	11.456	43.23018868
Oct	3.2215	47.057	14.60717057
Feb	7.1075	BD*	
Mar	83.3405	BD*	
Apr	2.4445	1.934	0.791163837

*BD Below detection

A strong positive correlation was observed among the extra-cellular microcystins (MC-LR, MC-YR, and Total MC) (Spearman Rank Order Correlation $p<0.05$) (Table 6.2).

Table 6.2 Spearman Rank Order Correlation among extra-cellular MC variants, Total MC and *Microcystis* cell abundance

	Microcystis	YR water	LR water	Total MC water
Microcystis	1			
YR water	0.112	1		
LR water	0.030	**0.919***	1	
Total MC water	0.030	**0.963****	**0.991****	1

**Correlation significant at the 0.05 level (2-tailed), **Correlation significant at the 0.001 level (2-tailed)*

Microcystis spp. were overwhelmingly dominated by the diazotrophic cyanobacterial genera Cylindrospermopsis spp. *and D. spiroides* **(Fig.6.7)** during the latter months of sampling (chapter 5).

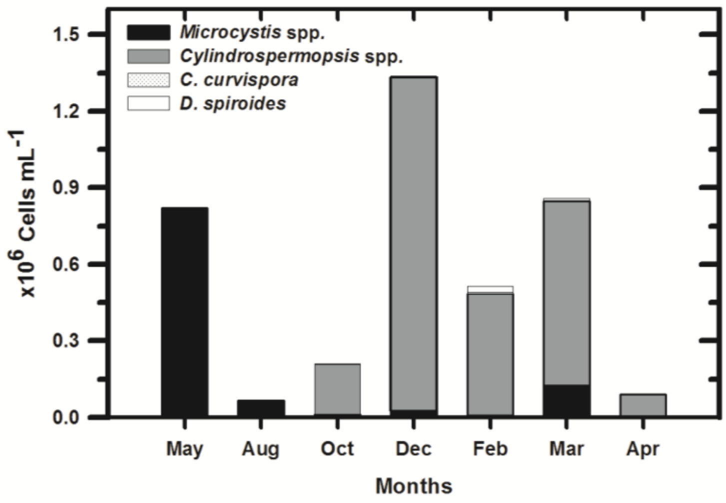

Figure 6.7 Temporal variations in the relative abundance of cyanobacterial taxa at the near-shore sites of the present study on Koka reservoir.

Although the concentrations of total intra-cellular MCs seem to follow similar temporal pattern **(Fig.6.9),** the positive association was not significant (Spearman Rank Order Correlation, *P*>0.05, Table 6.3).

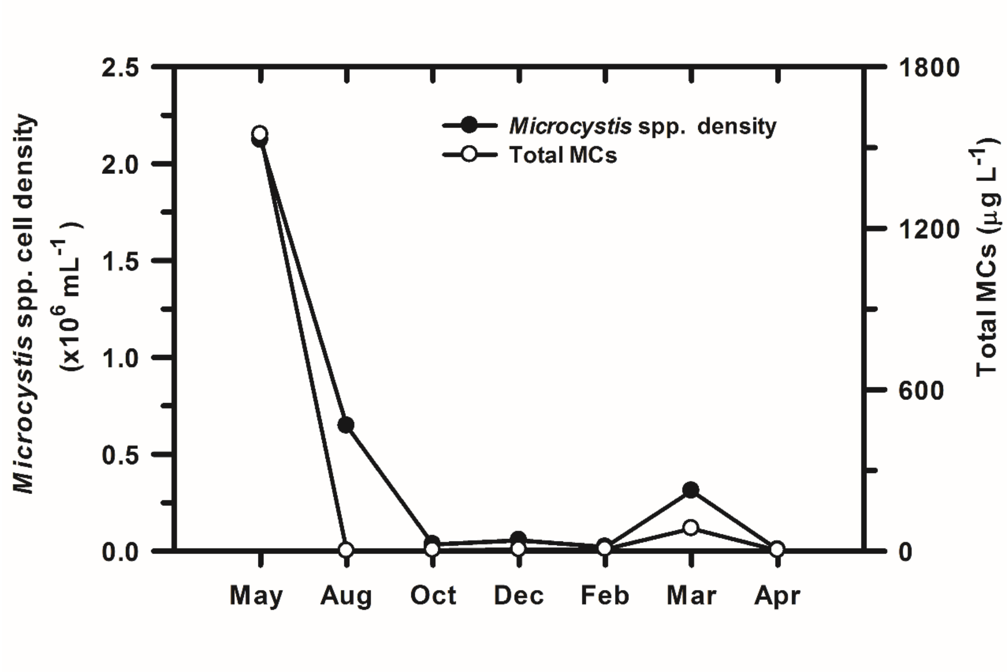

Figure 6.8 Temporal variations in total intra-cellular microcystins (MCs) concentrations in relation to total density of *Microcystis* spp. at near-shore site of the present study on Koka Reservoir.

Table 6.3 Spearman Rank Order correlations among *Microcystis* cell abundance, major intracellular MCvariants, Total MC and some environmental variables.

	Temp	SRP	Nitrate	*Microcystis*	MC-	MC-	MC-	Total	Sal
Temp	1.000								
SRP	**.857***	1.000							
Nitrate	-.090	.090	1.000						
Microcystis abunda	-.143	.214	**.793***	1.000					
MC-YR	.429	.143	.270	.143	1.000				
MC-LR	.321	.036	.306	.214	**.964****	1.000			
MC-RR	.408	.445	.243	.556	.704	.704	1.000		
Total MC	.357	.214	.162	.357	**.857***	**.893****	**.927****	1.000	
Sal	**.829***	**.811***	-.291	0.000	.342	.252	.654	.523	1.000

**Correlation significant at the 0.05 level (2-tailed),*
***Correlation significant at the 0.001 level (2-tailed)*

6.4. DISCUSSION

The concentrations of microcystins in the majority of the samples exceeded the safety WHO limit of 1 µg L^{-1} (WHO, 2004) suggesting the potentially high public health risk associated with the use of the reservoir water. Among the different MC variants, MC-LR is known to be the most potent toxin, and exposure to this toxin may lead to severe liver damage (Chorus et al., 2000), massive intra-hepatic hemorrhage, liver swelling and death (Weng et al., 2007), genotoxicity and carcinogenicity (Dittmann and Wiegand, 2006). The high levels of MC-LR detected in the majority of the samples collected from Koka Reservoir in this study, therefore, indicate extremely high potential public health risk associated with the use of the reservoir as a source of fresh water supply. Willén et al. (2011) also reported MC-LR as the variant constituting the greatest proportion of total MCs in the reservoir. Such incidence of occurrence of high levels of MC-LR was also reported for several other water bodies (Kotak and Zurawell, 2007). Some authors reported that microcystins are normally cell-bound toxins, which are released to the extra-cellular environment during cell lyses (Li et al., 2010; Tsuji et al., 2001) and this contention corroborates the findings of the present study in Koka Reservoir. It should be noted, however, that the fractionation procedure for intra-cellular and extra-cellular MCs does not allow us to accurately determine the true proportion of the intra/extra cellular MC fractions. MCs that are bound to algal surfaces and other particulate matter (with particle size larger than 0.7 µm) are retained by the filter paper and are not, therefore, included in the measurement of MCs in the filtrate. These particle bound MCs are actually part of the extra-cellular MCs. In this way, the procedure may overestimate the intra-cellular MCs and underestimate the extra-cellular MCs.

Table 6.4. Temporal changes in the relative contribution of the total intra-cellular and extra-cellular MCs in Koka reservoir during (May-2015-April 2016).

Month	Total MCs	Total MCs(in water)	Ratio (Total MCs (water):Total MCs(in algal seston)
May	1548.13825		
Aug	0.265	11.456	43.23018868
Oct	3.2215	47.057	14.60717057
Feb	7.1075	BD*	
Mar	83.3405	BD*	
Apr	2.4445	1.934	0.791163837

***BD** = Below Detection, Limit of detection (LOD) for all variants was 0.5µg/l

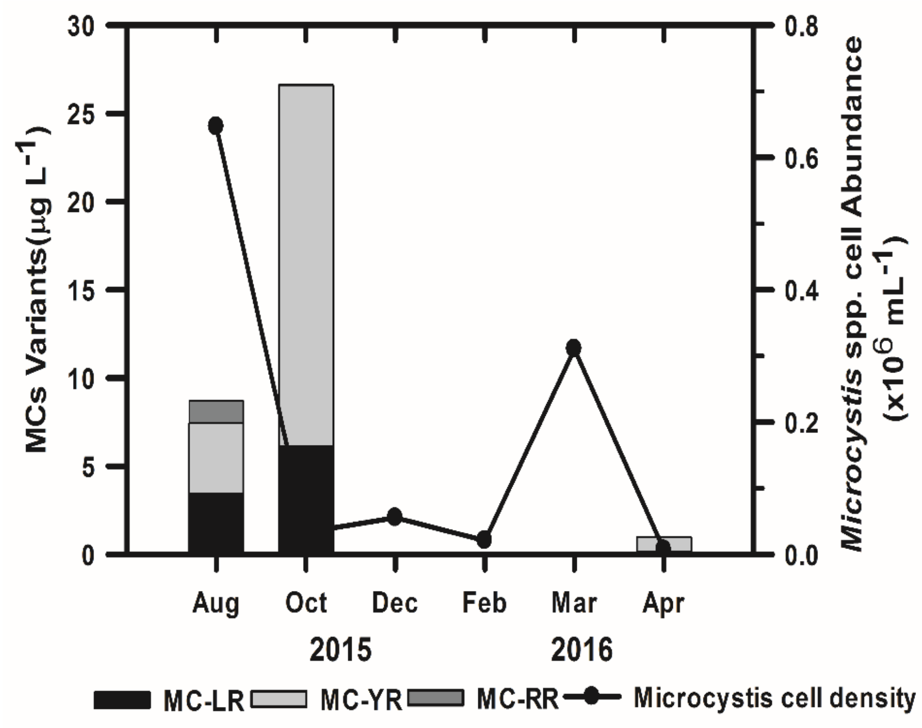

Figure 6.9 Temporal variations in the concentrations of extracellular microcystin (MCs) variants in relation to cell densities of Microcystis spp. at near-shore site of the present study on Koka reservoir during Aug. 2015-April 2016.

The relatively high concentrations of these extra-cellular MCs observed in August and October could, therefore, be partly attributed to the most likely release of MCs due to cell lyses (Park et al., 1998; Tsuji et al., 1994) following the collapse of the bloom after May, 2015, which corresponded to lower *Microcystis* cell abundance during these months. The release of cyanotoxins to the extra-cellular environment due to cell lyses during the collapse of a bloom has also been reported previously for other freshwaters (Fromme et al., 2000; Park et al., 1998). The strong, significant and positive correlations (**Table 6.2**) among the extra-cellular MC variants and total microcystins (MC-LR, MC-YR, and Total MC) may also suggest the involvement of a common and non-specific release mechanism, which could possibly be cell lyses, a condition by which all cellular contents are released at the same time. Concentrations (μg L^{-1}) of up to 20 of MC-LR, 6.13 of MC-YR, and 1.27 of MC-RR **(Fig. 6.7)** were found in the water samples, with all values greatly surpassing the WHO's (1996) safety limit for drinking water supply. Re-dissolution of the loosely bound MCs and their re-distribution during mixing has also been reported as a possible mechanism for their high concentrations in the water column long after the bloom had collapsed (Song et al., 2015). This is also possible in Koka Reservoir, which is a shallow and frequently mixed water body (Kebede and Willén, 1998; Mesfin et al., 1988; Willén et al., 2011). According to Song et al., (2015); Verspagen et al., (2005), the sediment may serve as a source for *Microcystis* and MCs and determine their fate (Wörmer et al., 2011; Wu et al., 2012). Persistent *Microcystis* cells in the sediment, for instance, have been reported to serve as inoculums for the next blooming season (Verspagen et al., 2005). Sediment of Koka reservoir might have also partly contributed to the total MC concentration in the water samples as sediment re-suspension due to frequent mixing is a common phenomenon in the reservoir. Re-suspension of bottom sediment in Koka Reservoir in the present study was reflected by the high values of turbidity (**Chapter 4)**. The sediment may also serve as a sink for the extra-cellular MCs as they tend to get adsorbed onto particulate materials (Song et al., 2015; Verspagen et al., 2005). As suggested earlier, the sediment bound MCs cannot be recovered in the filtrate following the procedure used in this study. This could partly explain the relatively low concentration of extra-cellular MCs and their less frequent occurrence in the water samples of the present study compared the cell bound ones. However, provision of definitive conclusion on the role of the sediment in the dynamics of *Microcystis* and MCs in the water/sediment interface of Koka Reservoir requires further study involving sediment sampling. Similar to the intracellular MCs, the total extra-cellular MC was constituted largely by MC-LR followed by MC-YR. MC-RR was detected in the filtrates of August sample only, constituting only a small fraction of the total extra-cellular toxins. The total intracellular MC concentration was higher than the concentration of total extra-cellular MC during all sampling months except August and October. The highest total MC concentration 83 (µg L^{-1}) was observed in March sample constituting MC- LR 64.7 (µg L^{-1}), MC- RR 5.6 (µg L^{-1})) and MC-YR 13.03, (µg L^{-1}) next to that of May sample. It coincided with the highest *Microcystis* abundance possibly favored by the relatively better availability of nitrogen due to the rainfall event that occurred in March. It is possible that the toxins were associated with the new *Microcystis* cells formed during the warmer months though at expectedly low rate of growth associated with nitrogen-limitation compared to the diazotrophic cyanobacteria (chapter 5). All extra-cellular MC variants were below the detection limit of the analytical method used (**Table-6.1**) in December, February and March. Photolysis and biodegradation of the extra-cellular toxins (Tsuji et al., 1994), owing to the intense solar radiation and high water temperature prevailing in semi-arid tropical region where Koka Reservoir is located, might have also been partly responsible for the presence of low and undetectable levels of extra-cellular toxins during the majority of the sampling months. Several field-based and experimental studies (Ballot et al., 2003; Davis et al., 2009; Jähnichen et al., 2011; Watanabe and Oishi, 1985) have been carried out to study the environmental factors dictating the dynamics of cyanobacterial bloom and toxin production. Water temperature, pH, nutrient availability, and transparency are among the several environmental factors regulating the spatio-temporal dynamics of cyanobacteria and their toxins. The majority of the limmnological parameters measured in Koka Reservoir during the present study period were within the ranges reported to be optimal for the proliferation of *Microcystis* and toxin production. For instance, temperature exceeding 20°c generally promotes the proliferation of cyanobacteria due to its direct effect on their metabolic activity and changes in the physical characteristics of the aquatic environment (O'neil et al., 2012). According to Watanabe and Oishi, (1985), growth of *Microcystis aeruginosa* increased considerably at a temperature of 32 °C under culture conditions. The water temperature of Koka reservoir during the study period ranged from 22.4 to 31.1°C **(Chapter 4,** a range of water temperature that is within the reported optimal range for *Microcystis* growth. The relatively higher water temperature of the present study period could be due to the higher air temperature associated with the drought (**Chapter 4).** The direct role of temperature in MC production was also reported by Davis et al., (2009). According to Davis et al. (2009), two- to threefold increase in MC levels with the increase in temperature from 26.9±1.7 to 30.6±1.4 was observed. The other limnological condition, which is believed to favor *Microcystis* growth and MC production, is a long water residence time **(Chapter 4).** Long water residence time was reported to favor both the proliferation of *Microcystis* and MC production. Extended dry condition generally increases water residence time by up to 45 % (Romo et al., 2013), which was particularly true for Koka Reservoir of the present study period. The estimated mean water residence time of the reservoir during our sampling period was 395±24.5 days, which was 35% longer than that of the preceding year (258± 16.1) (Chapter 4). According to Romo et al., (2013), as a result of the increase in water residence time due to drought, the size of *Microcystis aeruginosa* population and MCs concentration increased more than twice. The results of the present study, however, show to the contrary that *Microcystis* abundance was much lower than those of earlier reports (Elizabeth Kebede and Willén, 1996; Willén et al., 2011) despite the longer water residence time of the present study period. The majority of the observed limnological conditions are within the optimal range reported for the proliferation of *Microcystis* spp except the low availability of combined inorganic nitrogen. The unusual disappearance of the dense *Microcystis* bloom and *Microcystis* spp. occurrence at relatively low abundance during the present study may be associated with the severe nitrogen limitation (Samson Tilahun and Demeke Kifle, 2019; **Chapter 5**). According to Samson Tilahun and Demeke Kifle (2019), the observed low level of nitrogen could most probably be associated with a reduction in riverine input of nutrients due to the recent incidence of El Niño induced drought in Ethiopia (**Chapter 4)**. The nutrient limitation, which was more pronounced in nitrogen than phosphorus **(chapter 4**, Samson Tilahun and Demeke Kifle, 2019), suggested that, while the available nitrogen is lost through the denitrification process owing to the high water temperatures associated with the drought, phosphorus was possibly being replenished through internal recycling from the presumed huge phosphorus reserve in the sediment **(Chapter 4).** The significant positive association of

Microcystis cell abundance and nitrate concentration (Spearman Rank Order Correlation r =0.793, p<0.05) (Table-4) may suggest that nitrogen played a key role in *Microcystis* spp dynamics, which in turn influenced the MCs level in the reservoir in the present study. According to Wang et al., (2010), the effect of nitrogen on MC production is through its influence on *Microcystis* cell abundance. Furthermore, there is no evidence of association of intra-cellular MCs with *Microcystis* spp. cell abundance and other environmental variables (**Table 6.3**), which may suggest that nitrogen limitation was of overriding importance in influencing the proliferation of *Microcystis* spp. thereby possibly affecting its toxin production. This is possibly because, unlike diazotrophic cyanobacteria such as *Cylindrospermopsis*, the proliferation of bloom forming non-diazotrophic cyanobacteria such as *Microcystis* may be seriously impacted by severe nitrogen limitation. Members of this ecological group do not have an alternative source of nitrogen to survive nitrogen stress that prevails over a long period such as the one observed in Koka Reservoir (Samson Tilahun and Demeke Kifle, 2019, Chapter 5). The role of nitrogen in influencing *Microcystis* growth and toxin production was reported by (Park et al., 1993). According to their report, an increase in NO_3-N concentration favored the growth of *Microcystis* in Lake Sow, Japan. This is in agreement with our observation that *Microcystis* abundance was positively and significantly associated with nitrogen concentration (Spearman Rank Order Correlation, r =0.793, p<0.05) as indicated earlier (**Table 6.3**). According to a review by O'neil et al., (2012), MC+ strains are not competent under low-nitrogen stress because of the additional nitrogen requirement for the synthesis of microcystin, which is a nitrogen-rich compound, and the enzymes involved in the synthesis of the MCs. This may influence the relative abundance of toxic and nontoxic (MCY- and MCY+) strains of *Microcystis* thereby influencing MC concentration (Davis et al., 2009). For instance, Dai et al., (2008), reported substantial decrease in cellular MCs concentration immediately following the depletion of nitrogen suggesting the importance of nitrogen for the production of MCs. In the present study, however, there is no direct evidence for the association of intra-cellular MC with nitrogen (Spearman Rank Order Correlation r= 0.162, *p*>0.05,) (**Table-6.3**). This could be primarily due to the inherent problem of the procedure that does not allow accurate determination of the true intracellular MCs, which may significantly affect the possible association of MCs with nitrogen levels and other environmental variables. Furthermore, positive correlation between *Microcystis* biomass and total intracellular MCs concentration was reported by several authors including (Wang et al., 2010). Although *Microcystis* cell abundance and total intracellular MC concentration followed similar temporal pattern (**Fig. 6.8**), Spearman Rank Order Correlation analysis showed no evidence of significant association (r=,0.357 *p*>0.05). Lack of significant association between *Microcrystis* cell abundance and intra-and extra cellular MC in the present study (**Tables 6.4 and 6.5**) is plausible and may substantiate our claim regarding the inherent problem associated with determining the intra/and extra cellular MCs fractions using the conventional procedure. We, therefore, suggest that understanding the dynamics of intra-/extra cellular MCs in relation to environmental factors requires an accurate and exclusive determination of the intra- and extra-cellular MCs. Furthermore, *Dolichospermum,* a diazotrophic cyanobacterial genus, which is capable of producing MCs, was also encountered in the present study although with relatively lower abundance compared to *Cylindrospermopsis* (**Fig.6.7)**. Occurrence of *Dolichospermum* was reported in Koka Reservoir and other Ethiopian rift valley lakes by (Willén et al., 2011) and elsewhere in Africa by (Krienitz et al., 2002). It is a ubiquitous fresh water genus found throughout the world particularly in lentic water bodies such as lakes and reservoirs (O'neil et al., 2012). The genus was reported to possess the MCY encoding genes (Rouhiainen et al., 2004). A direct evidence for MC production by this genus was also reported for some water bodies (Halinen et al., 2007). However, the observed MCs were exclusively attributed to *Microcystis* spp. in a reservoir where *Dolichospermum spiroides* and *Microcystis* spp. co-existed (Li et al., 2010). Li et al., (2010) argued that MCY gene responsible for MC production was detected in *Microcystis* spp. only. However, the contribution of *D. spiroides* to the observed MCs levels in Koka Reservoir in the present study cannot be ruled out until such molecular analysis is carried out. Therefore, the co-occurring *Dolichospermum* might have been partly responsible for the observed MC concentration of the present study.

6.4.1. Cylindrospermopsis and Cylindrospermopsin

As indicated above and in chapter 5, *Cylindrospermopsis* was the most dominant cyanobacterial genus during the latter months of sampling (November to April) (Chapter 5, **Fig. 6.7**). As a diazotrophic cyanobacterial genus, it could be favored by the prevailing severe nitrogen limitation during those months (Chapter 5, Samson Tilahun and Demeke Kifle, 2019). Rukuer et al. (2007) also reported lack of correlation between levels of cylindrospermopsin (CYN) and nitrogen availability, while a significant correlation between total phosphorus and CYN was observed, which is in agrement with our report (Samson Tilahun and Demeke Kifle, 2019 and chapter 5). This may suggest that for members of the diazotrophic cyanobacteria, nitrogen is less likely to be limiting, while phosphorus plays a key role in influencing their dynamics and toxin production. In addition to producing a potent hepatotoxin like microcystin, cylindrospermopsin produced by this genus has been reported to cause damage to other organs and cells such as kidneys, and lymphoid cells (Hawkins et al., 1985). Signals that could possibly be of cylindrospermopsin were observed during the dry months corresponding to the dominance of *Cylindrospermopsis*. This, however, must be confirmed with further analysis. If future analysis confirms the presence of cylindrospermopsin, this will obviously, further exacerbate the already high public health risk associated with the MCs produced by *Microcystis*.

6.5. CONCLUSIONS

The study reveals extremely high potential public health threat associated with the use of Koka Reservoir as a source of water intended for drinking water supply, fisheries, livestock watering, and even for recreation. MC-LR, the most potent toxin constituted the greatest proportion compared to other variants in the majority of the samples.The fact that several of the algal samples and some of the water samples contained MCs above the permissible level set by WHO, indicates that the water of Koka Reservoir is unsafe for house hold purposes throughout the year. The reservoir is unsafe also for recreational purposes due to the high levels of MCs, and possibly CYN, found in both water and algal seston considering the abundance of *Cylindrospermopsis* spp. The results of the study suggest that nitrogen might be the key regulating factor for the dynamics of *Microcystis* and possibly MCs production in Koka Reservoir during the study period.

The high level of MCs despite the unusually low abundance of *Microcystis* during the present study (compared to its bloom forming and exclusive dominance in the earlier years) also implies that the MCs could have been much higher had it not been due to nitrogen-depletion that limited the proliferation of this genus. *Dolichospermum*, the co-occurring cyanobacterium, might have also been responsible for the observed level of MCs although its confirmation requires further study.

7.0 CHAPTER SEVEN

SYNTHESIS AND SUMMARY

7.1. Introduction

The influence of climate change such as El Niño induced drought and La Nina induced flooding, and global warming has been linked to the increase in the recurrence and global expansion of harmful algal bloom possibly due to its direct effect on nutrient availability and other limnological conditions in lentic systems (Paerl and Paul, 2012). Lakes and reservoirs located in tropical regions such as Koka Reservoir are particularly affected as they are largely of riverine origin (Lewis Jr, 1996) whose hydrology, limnology and nutrient input are directly influenced by climatic conditions. The present study addressed the impact of the recent incidence of climate change anomaly, namely El Niño induced drought, which occurred in 2015/16 in Ethiopia, on the limnology of Koka Reservoir. Particular attention was given to nutrient availability, hydrologic and hydro-chemical responses of the reservoir and their consequence on the dynamics of cyanobacteria community structure and their toxins. The study also attempted to assess riverine and atmospheric inputs of nutrients their potentials as sources of eutrophication on the reservoir. Based on the results of the study, possible implications for potential public health risk associated with the use of the reservoir as a source of fresh water supply and possible intervention strategy are discussed. Much of the observed conditions of the reservoir seem to be a direct reflection of the drought. Our results revealed that the drought, among others, affected the mean annual water level, water residence time, water temperature, nutrient availability and cyanobacterial community structure. For instance, the relatively low flow rate and sediment transport observed during the present study, compared to long-term average, are most probably attributable to the El Niño induced drought. There are no historical data on nutrient flux to the reservoir. However, the observed relatively low flow rate of Awash River and sediment transport may suggest that there was a concomitant reduction in the annual nutrient input to the reservoir. Mojo River, which receives untreatreated wastewater from several tanning industries, located both sides of its course, carries nutrients originating from both diffuse and point sources thereby contributing a significant fraction of the total nutrient input to the reservoir. The river, however, dried up completely during the present study possibly associated with the drought. The extended dry weather condition during the present study may, on the other hand, enhance wind-induced erosion from the agriculturally-dominated Awash basin, which could be an additional source of nutrients for the reservoir through dry fall. The results, however, revealed that atmospheric input of nutrients to the reservoir was relatively low compared to riverine input except for ammonia. Furthermore, the present low nutrient concentrations in the reservoir compared to those reported in earlier studies may suggest that riverine nutrient input could be the major source of nutrient pollution of the reservoir. The study shows that although both nitrogen and phosphorus are expected to have common riverine sources, nitrogen limitation was much more severe than phosphorus. There could be several explanations for this including loss of the available nitrogen through the denitrification process owing the prevailing high water temperature that promotes bacterial metabolism. The relatively high concentration of phosphorus in the reservoir, despite the reduction of nutrient input from allochtonous sources, may suggest that phosphorus could possibly be replenished from autochthonous sources through internal loading from the presumably huge phosphorus reserve in the sediment. Our study also revealed the overwhelming dominance of diazotrophic cyanobacteria over *Microcystis* rather unusually for an extended period of time. This is the first report on the persistent dominance of diazotrophic cyanobacteria over *Microcystis,* which had been the dominant genus over the last several years in the reservoir. The emergence and unusual dominance of diazotrophic cyanobacteria might have taken advantage of their capability of surviving the prevailing nitrogen stress during the present study. This group of organisms is capable of satisfying its nitrogen requirement through nitrogen fixation from the abundantly available atmospheric nitrogen. The emergence of this group of organisms possibly due to the supply of phosphorus from autochthones sources through internal loading from the sediment may have serious management implications and cyanotoxin related public health risks as described below.

7.2. Management Implication

Several experimental and field studies have been conducted on growth limiting nutrients and on possible intervention strategy to reverse eutrophication related water quality problems (Conley et al., 2009; Wang and Wang, 2009; Scott and McCarthy, 2010). Suggested intervention strategies based on these studies, however, triggered considerable debate among scientists. For instance, effective control of harmful algal blooms can only be achieved through reduction of external input of phosphorus only rather than nitrogen (Schindler et al., 2008; Wang and Wang, 2009). According to these authors, reducing external input of nitrogen is of no use as an intervention strategy because it simply induces the proliferation and dominance of nitrogen-fixing cyanobacterial genera. This is in agreement with our observation in Koka Reservoir that the low nitrogen condition was associated with the unusual dominance of heterocystous cyanobacteria, namely *C. africana, C. curvispora,* and *D. spiroides*. Conley et al. (2009) also indicated that the *phosphorus only* strategy is likely to fail in lakes in which phosphorus is rapidly recycled between the sediment and the water interface. This seems to be the case for Koka Reservoir as the concentration of phosphorus remained high enough at least to support cyanobacterial growth despite the drought-associated reduction in its external input possibly due to the rapid recycling between the sediment and the water column as suggested by Conley et al. (2009). Effective control of both *Microcystis* and nitrogen fixers in such lakes, therefore, needs reduction of both nitrogen and phosphorus (Conley et al., 2009).

The above claims and arguments are based on data generated from ecosystems and climatic conditions different from those of the rift valley lakes. Therefore, they may not represent the conditions of the rift valley lakes, particularly the Ethiopian rift valley lakes. Furthermore, there are no reports on limiting levels of nutrients based on data generated locally for the conditions of the Ethiopian rift valley lakes including Koka Reservoir. However, the recent incidence of El Niño induced drought may provide a good insight into the short-term response of Koka Reservoir to reduction in the external input of both nutrients (nitrogen and phosphorus). Despite the reduction in the external input of both nutrients, phosphorus was not a limiting nutrient in the reservoir possibly due to its rapid recycling between the water column and the presumably huge phosphorus reserve in the sediment as suggested by Conley et al. (2009). On the other hand, nitrogen was at low concentrations (51.2±0.00975) and (51.4±0.0206) µg/l at the open and near-shore sampling sites, respectively, which according to Sas (1989, cited in Bouvy et al.,

2003) makes the reservoir a water body under severe nitrogen-limitation. The observed unusual dominance of heterocystous cyanobacteria in the reservoir may be attributable to the prevailing severe nitrogen-limitation as suggested by Wang and Wang (2009) and Schindler (2012). The limonological response of Koka Reservoir during the recent incidence of El Nino seems to suggest that there is a large phosphorus reserve in the sediment, which could sustain cyanobacterial growth through internal recycling. Such shallow and completely mixing water bodies are less likely to recover (Londe et al., 2016). Therefore, reduction in the external inputs of phosphorus may not be an immediate solution (Jones and Poplawski, 1998). The presumed sever nitrogen-limitation in Koka Reservoir during the drought period implies that the reservoir's nitrogen source is agricultural runoff. This means that reduction in the external input of nitrogen causes severe nitrogen-limitation. This condition was well reflected by the emergence and unusual dominance of nitrogen-fixing cyanobacteria. This scenario may complicate efforts aimed at the recovery of the reservoir through intervention strategy geared towards reducing the external input of nutrients. The nutrient data recorded during the incidence of El Niño also seem to suggest that reduction in the external input of nutrients may not enable Koka Reservoir to recover from eutrophication related water quality problems at least in the near future. One of reported strategies to reverse such situation is sediment dredging, which is prohibitively expensive for countries like Ethiopia. *In situ* immobilization of sediment phosphorus using chemical treatment, particularly application of iron to increase the sorptive capacity (Quaak et al., 1993; Sondergaard et al., 2001) of the reservoir sediment could be a potential alternative. This measure could be effective given the high redox potential that allows permanent immobilization of the iron bound phosphorus. Its frequent mixing and oxygenation avoids the need for hypolimnetic aeration applied for deeper lakes that exhibit thermal stratification with hypolimnetic anoxia. Detailed analysis of sediment characteristics such as Fe:P ratio, major form of phosphorus in the sediment and mechanism of phosphorus release should, however, precede practical application of this control measure.

7.3. Public Health Implication

The fact that several of the algal samples and some of the water samples contained MCs above the permissible level set by WHO indicates that the water of Koka Reservoir is unsafe for household purposes, livestock watering fisheries and recreation throughout the year. Furthermore, MC-LR, the most potent toxin constituted the greatest proportion compared to other variants in the majority of the samples, putting public health at high risk. The emergence of diazotrophic cyanobacterial genera, namely *Cylindrospermopsis*, and *Dolichospermum* may also imply worsening situation of cyanotoxins-related public health risk. The genus *Cylindrospermopsis* is known to produce potent hepatotoxin like microcystins. For instance, cylindrospermopsin produced by this genus has been reported to cause damage to organs and cells such as kidneys, and lymphoid cells (Hawkins et al., 1985). Signals that could possibly be of cylindrospermopsin were observed during the dry months corresponding to the dominance of *Cylindrospermopsis (*data not shown). This, however, must be confirmed with further analysis. If future analysis confirms the presence of cylindrospermopsin, this will obviously, further exacerbate the already high public health risk associated with the MCs produced by *Microcystis*. The high level of MCs despite the low abundance of *Microcystis* in the present study also implies that the levels of MCs could have been much higher had it not been due to nitrogen limitation that limited the proliferation this genus. *Dolichospermum*, the co-occurring cyanobacterium, could also be responsible for the observed high concentration of MCs despite the low abundance of ***Microcystis*.**

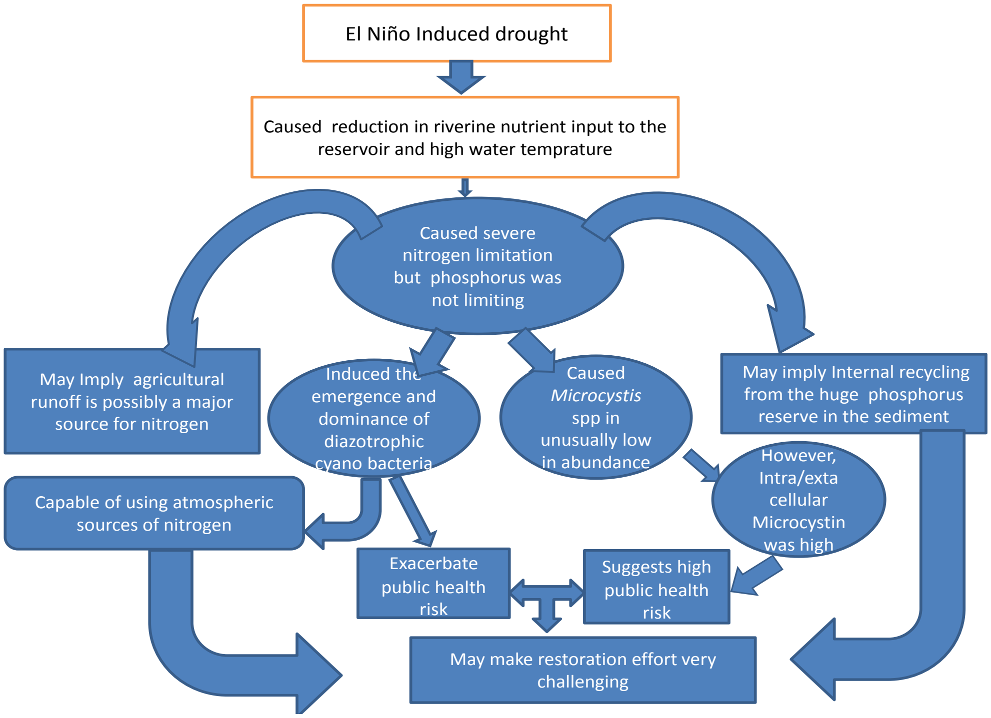

Fig. 7.1. Pictorial summary of the influence of the El Niño induced drought and its implication for public health threat and management of Koka reservoir

Limitation of the study and Recommendations

Some of the implications of this study are based on circumstantial evidences. Therefore, We recommend addressing the following issues in future studies to be made by the scientifc community.

- Similar future studies on the reservoir should address the potential public health risk associated with the newly emerging cyanobacterial genera and their toxins, namely *Cylindrospermopsis* and cylindrospermopsin, and *Dolichospermum* and MCs. Direct evidence for the possible role of

Dolichospermum in the production of the observed MCs in the reservoir requires molecular analysis. We, therefore, recommend that future studies in the reservoir should involve detection of the MC encoding genes in this genus.

- The sediment may serve as a sink for MCs adsorbed onto particulate materials. It may also serve as a source through re-dissolution of the loosely bound MCs and their re-distribution during mixing. Thus, future research on the reservoir should also target the sediment as a possible source of MC in the water column, which may be responsible for the high concentration of MC even in the absence of visible bloom.
- As internal phosphorus recycling has a serious management implication, future studies on the reservoir should involve *in situ* enclosure experiment and/ or sediment sampling to confirm the possible role of autochthonous source of phosphorus in supporting cyanobacteria in the reservoir.

The results of the present study however, still revealed that Koka Reservoir is unsafe and unsuitable through out the year for any household purposes, liviestock watering and even for recreation due to the extremely high potential public health risk associated with cyanotoxins. It is thus, strongly recommended the local community be provided an alternative sources freash water supply accompanied by awareness creation to the local community on the potential risk associated with the use of the reservoir water.

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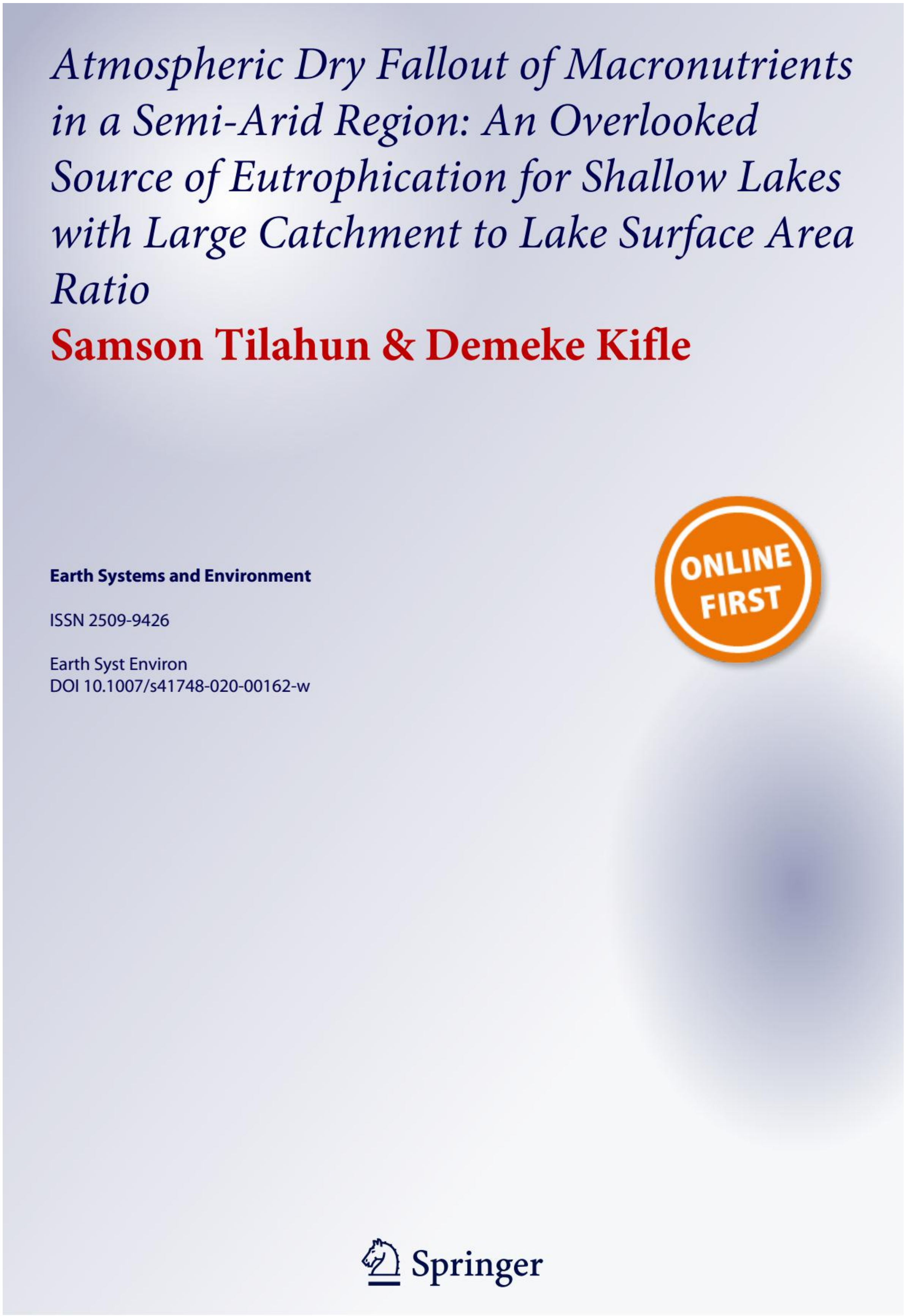
APPENDICES

Appendix 1

Journal articles, conference papers



Atmospheric Dry Fallout of Macronutrients in a Semi-Arid Region: An Overlooked Source of Eutrophication for Shallow Lakes with Large Catchment to Lake Surface Area Ratio

Samson Tilahun & Demeke Kifle

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ORIGINAL ARTICLE

Atmospheric Dry Fallout of Macronutrients in a Semi-Arid Region: An Overlooked Source of Eutrophication for Shallow Lakes with Large Catchment to Lake Surface Area Ratio

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Abstract

Compared to riverine input of nutrients, atmospheric input of nutrients seems to have been given less attention, particularly for lakes and reservoirs with large catchment to lake/reservoir surface area ratio. This is possibly due to the general notion that this nutrient source is not quantitatively important compared to riverine sources considering the fact that the absolute load of atmospheric inputs is a function of the collecting surface area of the lakes. However, it should be noted that nutrient input relative to the size of the lake is more relevant than the absolute load for eutrophication-related water quality problems. Furthermore, for lakes located in arid and semi-arid regions where dry weather conditions prevail during most of the times of the year, riverine inputs of nutrients are greatly reduced. On the other hand, atmospheric input of nutrients through dry fall could be considerable. The results of the eight-month sampling of the present study show that the areal total phosphorus load originating exclusively from atmospheric source, L (P), was 195.05 mg m^{-2} making the model-predicted in-lake total phosphorus (TP) concentration, P_{λ}, to be 11.84 mg m^{-3}. This value surpassed the cutoff point of 10 mg m^{-3} for oligotrophic state making the reservoir mesotrophic based on the probabilistic distribution of trophic state. This suggests that atmospheric sources could sustain the supply of phosphorus during the dry period when riverine input is greatly reduced or even after complete control of riverine sources is achieved. Our results also show that atmospheric input of other nutrients, particularly ammonia, which may trigger the emergence of potentially toxic cyanobacteria, was considerable.

Keywords Atmospheric deposition · Dry fall · Koka reservoir · Nutrients · Semi-arid

1 Introduction

Atmospheric deposition is one of the major pathways of nutrient inputs to inland waters particularly in areas where extreme land degradation associated with intense agricultural operations has occurred (Ahn and James 2001; Baker and Miller 2013). Atmospheric deposition of nutrients onto lakes and reservoirs may take place through snow fall or rain fall, which are collectively termed wet deposition, while that, which takes place through gaseous phases and/or as particle-bound, is known as dry deposition (Zhai et al. 2009). In lakes located in tropical climate such as Koka Reservoir, the subject of the present study, deposition through rainfall and deposition through gaseous phases and as particle-bound are the relevant mechanisms of nutrient inputs of wet and dry depositions, respectively. Atmospheric deposition may have significant contribution to total nutrient inputs to lakes and reservoirs (Anderson and Downing 2006), with its contribution sometimes exceeding that of riverine loading (Jassby et al. 1994; Muvundja et al. 2009; Tamatamah et al. 2005). This could be because atmospheric nutrients may also originate from sources other than agricultural practices such as automobile emission exhaust biomass burning (Londe et al. 2016; Tamatamah et al. 2005), and unpaved roads (Herut et al. 1999). For instance, large-scale deforestation and biomass burning have been suggested as sources of atmospheric deposition of nutrients in African great lakes such as Lake Victoria (Tamatamah et al. 2005). This could also be true for Koka Reservoir as such practices are becoming

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Hydrologic and Hydro-Chemical Responses of Koka Reservoir to Extreme Hydro-Climatic Conditions: Implications for Phosphorus Recycling and Management of the Reservoir

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ABSTRACT

The observed hydro-chemical and hydrologic conditions during the recent incidence of El Niño induced drought (2015/ 16) seem to suggest the potential challenge to efforts that may be made to restore Koka Reservoir. The impact of this extreme weather condition on the limnology of Koka Reservoir was studied through a one-year sampling with emphasis on nutrient availability. Our study shows that, despite the expected reduction in the external input of nutrients due to the El Niño induced drought, concentrations of both TP and SRP were considerably high. This suggests that phosphorus supply through internal loading is operating in Koka Reservoir. This was also well reflected by the large discrepancy between the model predicted total phosphorus concentrations and the observed values. The prevailing limnological conditions of the reservoir including high water temperature, and frequent mixing may have also promoted the recycling of phosphorus from autochthonous sources. Phosphorus recycling in the reservoir implies that future efforts to restore the reservoir through reduction of nutrients from allochthonous sources may not be effective. The recent report on the emergence of diazotrophic *cyanobacteria* that can fulfill their nitrogen requirement from the abundantly available atmospheric source and the presumably huge phosphorus reserve in the sediment are among the factors that possibly challenge the restoration effort.

Keywords: Dry Weather, Eutrophication, Nutrient Recycling, Phosphorus, Trophic State.

The influence of El Niño-induced drought on cyanobacterial community structure in a shallow tropical reservoir (Koka Reservoir, Ethiopia)

Samson Tilahun & Demeke Kifle

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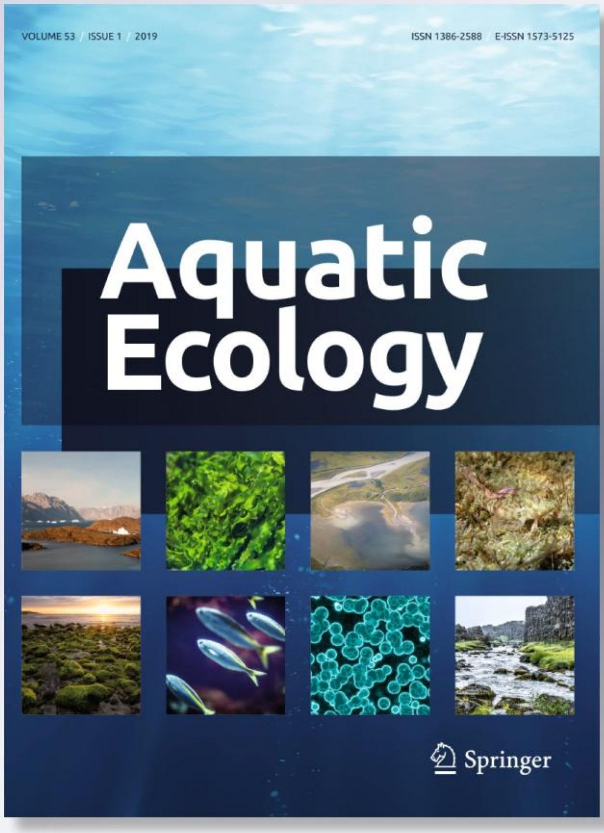

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The influence of El Niño-induced drought on cyanobacterial community structure in a shallow tropical reservoir (Koka Reservoir, Ethiopia)

Samson Tilahun · Demeke Kifle

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Abstract Koka Reservoir in Ethiopia has been severely impacted by water quality deterioration associated with harmful algal blooms. As a large proportion of the annual nutrient budget of the reservoir is believed to be of riverine origin, nutrient availability is presumably largely influenced by rainfall pattern and hydrological cycles, which are related to changes in climatic conditions such as El Niño-induced drought. The effect of El Niño-induced drought, which occurred in 2015, on the cyanobacterial community structure of Koka Reservoir was, therefore, investigated from May 28, 2015 to April 28 2016. The drought caused the failure of the main rainy season, which expectedly caused reduction in the external input of nutrients and changes in other limnological conditions of the reservoir. These changes, particularly nitrogen limitation, triggered the unusual dominance of the diazotrophic cyanobacterial genus *Cylindrospermopsis* over the previously persistently dominant nondiazotrophic genus, *Microcystis*, in the reservoir.

Keywords Climate change · *Cylindrospermopsis* · Diazotrophic · *Microcystis* · Nutrient limitation

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Introduction

It is now evident that the expansion of toxic cyanobacteria has become a global concern (Fristachi et al. 2008; Zhang et al. 2012). Water quality deterioration and the consequent public health risk linked to harmful algal blooms are mainly associated with members of cyanobacteria such as *Aphanizomenon*, *Cylindrospermopsis*, *Dolichospermum* and *Microcystis* (Falconer and Humpage 2005). These cyanobacterial genera are known to produce a variety of toxins that cause acute and possibly chronic public health problems and fatal poisoning in aquatic and domestic animals (Backer 2002; Carmichael et al. 1997). Public health risk associated with cyanobacterial toxins also applies to consumption of sea food (Ibelings and Chorus 2007) such as fishes grown in water bodies with harmful algal blooms (Watkinson et al. 2005). Several reports show that future climate change scenarios such as global warming may favor dominance of

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Temporal dynamics of intra-and extra-cellular microcystins concentrations in Koka reservoir (Ethiopia): Implications for public health risk

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ARTICLE INFO

Keywords:
Cyanobacteria
Intra-cellular
Extra-cellular
Eutrophication
Microcystis, microcystin nitrogen
Tropical

ABSTRACT

This study was carried out with a view of understanding the temporal dynamics of microcystin concentrations in both algal seston and water samples and the associated public health risk. All the major MC variants, namely MC-LR, MC-YR, and MC-RR, were detected in both the algal seston and water samples. In the majority of the samples, the most potent variant, MC-LR, constituted the greatest proportion of the total MC concentration suggesting extremely high potential public health risk. The exceptionally high concentrations (μg L^{-1}) of all the variants, MC-LR (815), MC-YR (466.6) and MC-RR (265.68), were observed in May. Although the extracellular MCs were relatively less concentrated and less frequently detected, concentrations (μg L^{-1}) of up to 20 of MC-LR, 6.13 of MC-YR, and 1.27 MC-RR were encountered. The strong and significant association between *Microcystis* abundance and concentration of nitrate (Spearman Rank Order Correlation r = 0.793, $p < 0.001$) may suggest that nitrate is the key dictating factor in the dynamics of *Microcystis*, and may have consequently influenced the MC levels in the reservoir.

1. Introduction

The emergence and expansion of harmful algal blooms and their associated toxins in fresh water system is becoming a serious threat to public health of global concern (Davis et al., 2009; Zhang et al., 2012). Several members of cyanobacteria including *Microcystis* are known to produce potent toxins, which are of high public health risk (Falconer and Humpage, 2005). Cyanotoxins associated human illness and fatal poisoning in aquatic, wild and domestic animals have been reported by several authors (Backer, 2002; Carmichael et al., 1997). Several attempts have been made to remove cyanotoxin from drinking water supply to minimize potential public health risk. Conventional methods such as flocculation, sand filtration, and sedimentation were reported as possible approaches for the removal of cyanotoxins (Jurczak et al., 2005). However, although cell-bound toxins can be removed using these methods, it is required to ensure that the procedures do not cause any cell disruptions thereby resulting in the eventual release of toxins into the aqueous phase (de Figueiredo et al., 2004; Fan et al., 2014), which may even exacerbate the condition (Hawkins et al., 1985). Furthermore, these approaches may not always be effective as the toxins from the aqueous phase, for instance, cannot be removed by these methods (de Figueiredo et al., 2004). They may not also be helpful for protecting the public from the potential risk associated with ingesting food animals like fishes caught from lakes supporting cyanobacterial blooms (Ibelings and Chorus, 2007). This situation makes the potential public health risk associated with cyanobacteria and their toxins a complicated issue to address. As it is the case with water bodies found in other parts of the world, the dominance of potentially toxic cyanobacteria has been reported for many lakes of the Ethiopian Rift Valley (Kebede and Willén, 1998; Mesfin et al., 1988; Willén et al., 2011). As most of these water bodies are currently serving as sources of freshwater used for drinking water supply, watering livestock, irrigation, fisheries, sanitation and recreation, the potential public health hazard and economic loss is immense. For instance, incidence of massive fish kills in Lake Chamo, one of the rift valley lakes in Ethiopia, and deaths of terrestrial wild animals around this lake in the year 1978 were linked to cyanotoxins (Amha and Wood, 1982). A cross sectional survey carried out on seven of the Ethiopian rift valley lakes has also demonstrated the dominance of toxic cyanobacteria and presence of associated toxins at concentrations much higher than the permissible level in most

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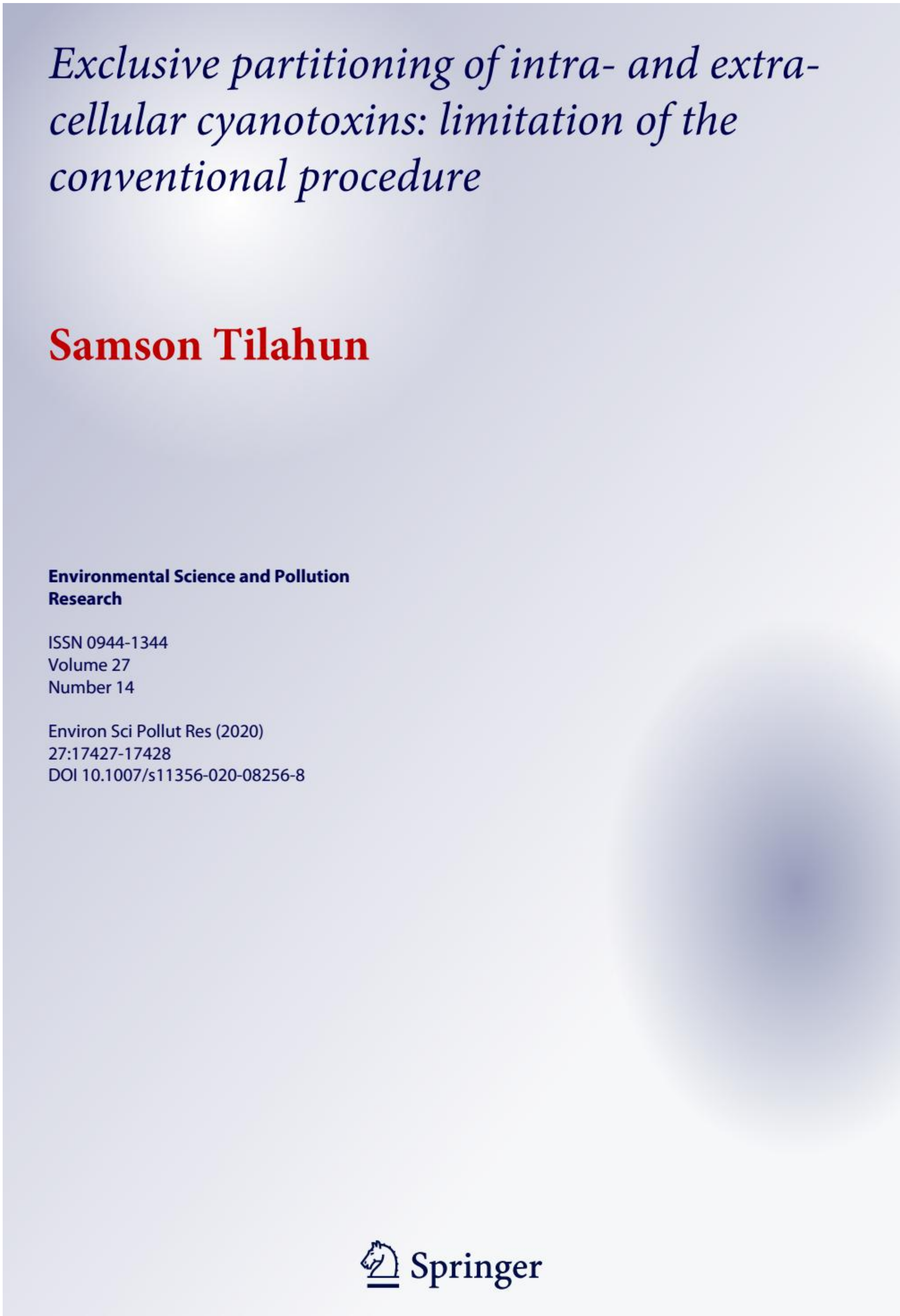

Exclusive partitioning of intra- and extra-cellular cyanotoxins: limitation of the conventional procedure

Samson Tilahun

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Exclusive partitioning of intra- and extra-cellular cyanotoxins: limitation of the conventional procedure

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Reports show that the presence of cyanotoxins in drinking water supply lakes and reservoirs causes serious public health impact (Hawkins et al. 1985; Falconer et al. 1983; Carmichael et al. 2001; Jochimsen et al. 1998). Reliable public health risk assessment associated with cyanotoxins requires accurate and exclusive determination of both the cell-bound toxins (intra-cellular) and those dissolved in water (extra-cellular) fractions. A technically sound procedure for exclusive determination of intra- and extra-cellular cyanotoxins is a key step also for any scientific study related to the dynamics of synthesis and release of cyanotoxins. This important issue, however, seems to be given little attention in many of the reported studies. The conventional procedure currently available for partitioning the intra- and extra-cellular toxins has inherent limitation particularly from field samples collected from highly turbid water bodies. The conventional procedure for separation of the two fractions currently in use involves the filtration of the samples onto 0.7 μm GF/C filter paper (Lawton et al. 1994; Merel et al. 2013; Park et al. 1998; Zheng et al. 2004). In this procedure, it is assumed that the cell-bound toxins (intra-cellular) are retained in the filter paper and the soluble cyanotoxins (extra-cellular toxin) are recovered in the filtrate. Reports show that cyanotoxins have high tendency of binding to particulate matter (Wu et al. 2012). This implies that the extra-cellular toxins bound to particulate matter above particle size of 0.7 μm including those bound to cyanobacterial cell surfaces are retained on the GF/C not recovered in the filtrate. Tilahun et al. (2019) also noted that the procedure might overestimate the intra-cellular toxins and underestimate the extra-cellular fraction. One of the notions regarding cyanotoxins repeatedly indicated in the literature is that cyanotoxins normally remain intra-cellular unless released during cell lysis and decay (Park et al. 1998; Tsuji et al. 1994). This idea does not seem to survive the scientific scrutiny given the inherent problem of the conventional procedure for separation of the two fractions. The view that cyanotoxins normally remain intra-cellular might have resulted from a faulty procedure that caused infrequent detection of the extra-cellular toxins in the filtrate. This procedural limitation may lead to inaccurate public health risk assessment especially in thermally stratified lakes and in absence of visual cue of harmful algal bloom. This is particularly true as particulate-bound toxins may also serve as source of the toxins to the water column (Wörmer et al. 2011; Wu et al. 2012) later during mixing. Future climate scenario not only promotes the dominance of potentially toxic cyanobacteria (Paerl and Huisman 2009; Paerl and Paul 2012) but also may influence their community structure (Tilahun and Kifle 2019) which may challenge protection of public health through restoration efforts. In such situation, reliable public health risk assessment and establishing an early warning system could be one possible option which demands accurate determination of the two fractions. Furthermore, lack of accurate and exclusive partitioning procedure possibly partly contributed to the challenges for studies on the dynamics of the two fractions in relation to the environmental factors and for lack of conclusive

Appendix -2

DAILY WATER LEVEL AND OUTFLOW DATA OF KOKA RESERVOIR

FOR THE YEARS, 2013/14, 2014/2015, 2015/16

Source: Ministry of Water Irrigation and Electricity of Ethiopia.

Date year 2013/14	Water Level 2013/14	Outflow 2013/14	Date /year (2014/15)	Water Level (2014/15)	Out flow 2014/15)	Date year 2015/16	Water level (2015/16)	Out flow (2015/16)
5/28/2013	105.27	5127388	5/28/2014	104.82	3626322	5/28/2015	103.49	1644020
5/29/2013	105.24	4904116	5/29/2014	104.8	3811710	5/29/2015	103.47	1635564
5/30/2013	105.2	5235048	5/30/2014	104.78	4110624	5/30/2015	103.45	1618134
5/31/2013	105.16	5038586	5/31/2014	104.76	3414460	5/31/2015	103.45	1644860
6/1/2013	105.12	5280576	6/1/2014	104.74	3265626	6/1/2015	103.42	1572298
6/2/2013	105.08	5148514	6/2/2014	104.72	3904922	6/2/2015	103.46	1651244
6/3/2013	105.04	4759692	6/3/2014	104.7	4447016	6/3/2015	103.47	1737778
6/4/2013	105.01	4758754	6/4/2014	104.68	4175234	6/4/2015	103.47	1548470
6/5/2013	104.97	4728514	6/5/2014	104.64	3727486	6/5/2015	103.44	1750252
6/6/2013	104.94	5095272	6/6/2014	104.61	2917082	6/6/2015	103.41	1790628
6/7/2013	104.9	4817022	6/7/2014	104.56	3876264	6/7/2015	103.4	1855868
6/8/2013	104.88	5409250	6/8/2014	104.52	4567822	6/8/2015	103.38	1859424
6/9/2013	104.85	4723614	6/9/2014	104.48	5776638	6/9/2015	103.36	1972152
6/10/2013	104.81	5360796	6/10/2014	104.44	4315290	6/10/2015	103.33	1759912
6/11/2013	104.76	5075168	6/11/2014	104.38	4348862	6/11/2015	103.3	1756510
6/12/2013	104.72	4886966	6/12/2014	104.32	5407710	6/12/2015	103.27	1846824
6/13/2013	104.67	4583838	6/13/2014	104.26	3709272	6/13/2015	103.24	1828428
6/14/2013	104.66	4837616	6/14/2014	104.23	4761498	6/14/2015	103.2	1724226
6/15/2013	104.62	5378982	6/15/2014	104.18	5102454	6/15/2015	103.17	1887256
6/16/2013	104.59	4847990	6/16/2014	104.13	4781098	6/16/2015	103.14	1886542
6/17/2013	104.6	4978078	6/17/2014	104.08	4247936	6/17/2015	103.11	1813364
6/18/2013	104.6	5941068	6/18/2014	104.03	3623578	6/18/2015	103.09	1850086
6/19/2013	104.6	4940502	6/19/2014	103.98	4177614	6/19/2015	103.08	1761620
6/20/2013	104.61	5599272	6/20/2014	103.93	4466392	6/20/2015	103.12	1808562
6/21/2013	104.62	5184130	6/21/2014	103.87	2916480	6/21/2015	103.05	1703324
6/22/2013	104.61	4749710	6/22/2014	103.83	2979214	6/22/2015	102.98	1524194

6/23/2013	104.6	4899510	6/23/2014	103.8	3737804	6/23/2015	102.93	1292942
6/24/2013	104.58	5111638	6/24/2014	103.78	3511340	6/24/2015	102.9	1743700
6/25/2013	104.56	4786404	6/25/2014	103.73	3279976	6/25/2015	102.9	1813336
6/26/2013	104.6	4905866	6/26/2014	103.71	3568278	6/26/2015	102.88	1724604
6/27/2013	104.62	5352648	6/27/2014	103.7	3259144	6/27/2015	102.92	1890742
6/28/2013	104.64	4875206	6/28/2014	103.7	3127530	6/28/2015	102.98	1752338
6/29/2013	104.63	4904816	6/29/2014	103.7	3510584	6/29/2015	102.98	1646302
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1/4/2014	108.59	3994522	1/4/2015	107.45	2859640	1/4/2016	105.6	1531404
1/5/2014	108.57	3080294	1/5/2015	107.43	3494722	1/5/2016	105.57	1542506
1/6/2014	108.55	3908016	1/6/2015	107.4	3602536	1/6/2016	105.55	3006514
1/7/2014	108.52	2297876	1/7/2015	107.38	2764510	1/7/2016	105.51	1492596

1/8/2014	108.5	3554306	1/8/2015	107.36	3260320	1/8/2016	105.49	1338680
1/9/2014	108.47	3162236	1/9/2015	107.33	3246894	1/9/2016	105.47	1509382
1/10/2014	108.45	3426934	1/10/2015	107.31	3648148	1/10/2016	105.45	1356418
1/11/2014	108.43	3227574	1/11/2015	107.29	1772190	1/11/2016	105.43	1574734
1/12/2014	108.4	4072040	1/12/2015	107.27	3392522	1/12/2016	105.41	1453396
1/13/2014	108.38	3147438	1/13/2015	107.24	2978584	1/13/2016	105.39	1472604
1/14/2014	108.35	2894276	1/14/2015	107.22	3342556	1/14/2016	105.38	1469664
1/15/2014	108.34	3203900	1/15/2015	107.2	3790080	1/15/2016	105.36	1359862
1/16/2014	108.33	3792166	1/16/2015	107.18	3765720	1/16/2016	105.34	1421182
1/17/2014	108.31	3278576	1/17/2015	107.15	5781146	1/17/2016	105.32	1226456
1/18/2014	108.29	3727738	1/18/2015	107.11	4070640	1/18/2016	105.31	1537830
1/19/2014	108.27	3073910	1/19/2015	107.07	2790592	1/19/2016	105.3	1623790
1/20/2014	108.25	2912490	1/20/2015	107.05	2940392	1/20/2016	105.29	1347360
1/21/2014	108.23	3788946	1/21/2015	107.03	3044244	1/21/2016	105.27	1415806
1/22/2014	108.2	3944808	1/22/2015	107.01	2748144	1/22/2016	105.26	1558956
1/23/2014	108.18	3385564	1/23/2015	106.99	3244836	1/23/2016	105.25	1557010
1/24/2014	108.16	4966570	1/24/2015	106.97	3138156	1/24/2016	105.24	1455762
1/25/2014	108.13	3480708	1/25/2015	106.95	2661750	1/25/2016	105.23	1473416
1/26/2014	108.1	3414810	1/26/2015	106.94	3219384	1/26/2016	105.22	1491462
1/27/2014	108.08	4727688	1/27/2015	106.92	3250716	1/27/2016	105.21	1542520
1/28/2014	108.07	3821706	1/28/2015	106.9	3020598	1/28/2016	105.19	1454306
1/29/2014	108.05	3563938	1/29/2015	106.87	3816484	1/29/2016	105.17	1559530
1/30/2014	108.01	3331132	1/30/2015	106.84	1215466	1/30/2016	105.15	1422022
1/31/2014	108	3020976	1/31/2015	106.83	3781022	1/31/2016	105.13	1347836
2/1/2014	107.98	2995048	2/1/2015	106.8	2876174	2/1/2016	105.11	1446970
2/2/2014	107.96	2092608	2/2/2015	106.78	2933420	2/2/2016	105.09	1748124
2/3/2014	107.94	2470188	2/3/2015	106.76	3030510	2/3/2016	105.07	1729042
2/4/2014	107.9	1875748	2/4/2015	106.74	3253768	2/4/2016	105.05	1628606
2/5/2014	107.86	2188438	2/5/2015	106.71	3049928	2/5/2016	105.03	1564206
2/6/2014	107.82	2075598	2/6/2015	106.7	2958746	2/6/2016	105.01	1659476
2/7/2014	107.8	523208	2/7/2015	106.67	2939748	2/7/2016	104.99	1801618
2/8/2014	107.79	0	2/8/2015	106.64	2779700	2/8/2016	104.97	2004324
2/9/2014	107.78	520226	2/9/2015	106.61	2941204	2/9/2016	104.95	1960476
2/10/2014	107.77	2254756	2/10/2015	106.59	2950808	2/10/2016	104.93	1978634
2/11/2014	107.75	2535288	2/11/2015	106.56	2921968	2/11/2016	104.91	2080442
2/12/2014	107.73	2637530	2/12/2015	106.54	2874102	2/12/2016	104.89	2036678
2/13/2014	107.73	3061772	2/13/2015	106.51	3051440	2/13/2016	104.87	2021320
2/14/2014	107.7	2676282	2/14/2015	106.49	3360322	2/14/2016	104.85	2080652
2/15/2014	107.68	2868026	2/15/2015	106.46	3466204	2/15/2016	104.83	1952160
2/16/2014	107.66	2148916	2/16/2015	106.44	3484642	2/16/2016	104.81	1983044

2/17/2014	107.63	2384620	2/17/2015	106.42	3887786	2/17/2016	104.79	2352532
2/18/2014	107.61	2374610	2/18/2015	106.39	3442012	2/18/2016	104.76	2049866
2/19/2014	107.58	2305968	2/19/2015	106.36	3317790	2/19/2016	104.73	2027788
2/20/2014	107.56	2364390	2/20/2015	106.33	4048310	2/20/2016	104.7	2697688
2/21/2014	107.54	2702770	2/21/2015	106.3	3467772	2/21/2016	104.65	3327716
2/22/2014	107.52	2471210	2/22/2015	106.27	3438428	2/22/2016	104.61	3283168
2/23/2014	107.5	2612358	2/23/2015	106.24	3413340	2/23/2016	104.58	3309320
2/24/2014	107.48	2903600	2/24/2015	106.21	3761674	2/24/2016	104.54	3220714
2/25/2014	107.45	3539116	2/25/2015	106.18	3365082	2/25/2016	104.5	1295462
2/26/2014	107.42	3520846	2/26/2015	106.16	3393656	2/26/2016	104.49	1392594
2/27/2014	107.4	3339378	2/27/2015	106.13	3392676	2/27/2016	104.47	1561364
2/28/2014	107.38	3316236	2/28/2015	106.1	3380314	2/28/2016	104.45	2050370
3/1/2014	107.35	3146248	3/1/2015	106.08	2799706	2/29/2016	104.44	2113258
3/2/2014	107.33	3266256	3/2/2015	106.06	3984022	3/1/2016	104.43	1965096
3/3/2014	107.3	2724778	3/3/2015	106.03	3413214	3/2/2016	104.41	2027634
3/4/2014	107.28	3117870	3/4/2015	106	3405486	3/3/2016	104.39	1989344
3/5/2014	107.25	4242602	3/5/2015	105.98	3533670	3/4/2016	104.37	2040318
3/6/2014	107.22	4164692	3/6/2015	105.95	3485762	3/5/2016	104.35	2123296
3/7/2014	107.19	4100628	3/7/2015	105.92	3389890	3/6/2016	104.33	2136456
3/8/2014	107.17	2803878	3/8/2015	105.89	3471244	3/7/2016	104.31	2000376
3/9/2014	107.14	2828154	3/9/2015	105.87	3283882	3/8/2016	104.29	1922256
3/10/2014	107.11	6088320	3/10/2015	105.85	3199000	3/9/2016	104.27	1748670
3/11/2014	107.09	5772956	3/11/2015	105.82	3192322	3/10/2016	104.25	2060674
3/12/2014	107.07	5452804	3/12/2015	105.78	3157770	3/11/2016	104.23	1887116
3/13/2014	107.03	5853386	3/13/2015	105.74	3197166	3/12/2016	104.21	1629712
3/14/2014	107	4271834	3/14/2015	105.7	3191972	3/13/2016	104.19	1663844
3/15/2014	106.98	4945612	3/15/2015	105.66	3454948	3/14/2016	104.17	1595692
3/16/2014	106.95	3820992	3/16/2015	106.6	3201352	3/15/2016	104.15	1717408
3/17/2014	106.88	3910410	3/17/2015	106.58	3200596	3/16/2016	104.12	1619422
3/18/2014	106.9	3750082	3/18/2015	105.55	3219664	3/17/2016	104.1	1771966
3/19/2014	106.89	3964772	3/19/2015	105.51	3200218	3/18/2016	104.07	1748348
3/20/2014	106.86	4670680	3/20/2015	105.48	3147074	3/19/2016	104.04	1619226
3/21/2014	106.85	3409392	3/21/2015	105.45	5610080	3/20/2016	104.01	1714566
3/22/2014	106.83	4288774	3/22/2015	105.4	6515586	3/21/2016	104	1692894
3/23/2014	106.81	4529980	3/23/2015	105.35	4680354	3/22/2016	103.95	1788752
3/24/2014	106.79	4132338	3/24/2015	105.3	4901694	3/23/2016	103.93	1718570
3/25/2014	106.75	4743298	3/25/2015	105.26	3433024	3/24/2016	103.92	1677242
3/26/2014	106.72	4206062	3/26/2015	105.22	4637808	3/25/2016	103.9	1771952
3/27/2014	106.7	4045706	3/27/2015	105.19	4495330	3/26/2016	103.87	1700650
3/28/2014	106.68	3311672	3/28/2015	105.17	3176558	3/27/2016	103.84	1729686

3/29/2014	106.65	5581170	3/29/2015	105.14	2901150	3/28/2016	103.81	1718962
3/30/2014	106.63	3396764	3/30/2015	105.12	3671038	3/29/2016	103.79	1774752
3/31/2014	106.61	3992422	3/31/2015	105.08	3560900	3/30/2016	103.76	1695246
4/1/2014	106.58	3566654	4/1/2015	105.05	3186610	3/31/2016	103.73	1776516
4/2/2014	106.55	4305728	4/2/2015	105.02	3204684	4/1/2016	103.7	1702778
4/3/2014	106.52	4640230	4/3/2015	104.99	3346224	4/2/2016	103.68	995750
4/4/2014	106.5	4060266	4/4/2015	104.96	2989154	4/3/2016	103.67	666736
4/5/2014	106.47	4371598	4/5/2015	104.93	3112704	4/4/2016	103.66	629972
4/6/2014	106.44	3829770	4/6/2015	104.9	4427808	4/5/2016	103.64	644252
4/7/2014	106.42	5806640	4/7/2015	104.86	3242134	4/6/2016	103.63	623294
4/8/2014	106.39	5933116	4/8/2015	104.83	3180184	4/7/2016	103.62	637616
4/9/2014	106.36	5041036	4/9/2015	104.79	3214610	4/8/2016	103.64	629230
4/10/2014	106.33	5794754	4/10/2015	104.75	3211026	4/9/2016	103.65	630182
4/11/2014	106.3	4689370	4/11/2015	104.71	3225012	4/10/2016	103.68	708946
4/12/2014	106.27	5129754	4/12/2015	104.68	2843190	4/11/2016	103.68	710010
4/13/2014	106.23	3938578	4/13/2015	104.64	2845304	4/12/2016	103.68	692370
4/14/2014	106.2	3605000	4/14/2015	104.61	2894514	4/13/2016	103.68	725508
4/15/2014	106.18	3514518	4/15/2015	104.58	2894220	4/14/2016	103.7	703990
4/16/2014	106.15	3672676	4/16/2015	104.54	2834328	4/15/2016	103.72	692552
4/17/2014	106.11	3892952	4/17/2015	104.5	2986424	4/16/2016	103.72	704900
4/18/2014	106.09	3415986	4/18/2015	104.46	2962904	4/17/2016	103.75	740474
4/19/2014	106.07	3479294	4/19/2015	104.42	3414054	4/18/2016	103.93	692860
4/20/2014	106.04	3121832	4/20/2015	104.38	2865016	4/19/2016	103.96	760074
4/21/2014	106.02	3125990	4/21/2015	104.34	2936374	4/20/2016	104	729554
4/22/2014	105.99	3951038	4/22/2015	104.31	2677416	4/21/2016	104.04	691572
4/23/2014	105.96	3843028	4/23/2015	104.27	2937732	4/22/2016	104.07	706566
4/24/2014	105.92	3907918	4/24/2015	104.23	3044720	4/23/2016	104.04	740698
4/25/2014	105.9	3742046	4/25/2015	104.19	2617538	4/24/2016	104.04	723506
4/26/2014	105.87	4703678	4/26/2015	104.16	2226406	4/25/2016	104.05	737478
4/27/2014	105.84	3563238	4/27/2015	104.1	2674504	4/26/2016	104.07	568050
4/28/2014	105.81	4093460	4/28/2015	104.04	2538228	4/27/2016	104.09	598444
4/29/2014	105.77	4228294	4/29/2015	104	2356508	4/28/2016	104.07	614334
4/30/2014	105.74	4152820	4/30/2015	103.98	3072748	4/29/2016	104.09	550060
5/1/2014	105.7	3701922	5/1/2015	103.94	1682856	4/30/2016	104.13	1604918
5/2/2014	105.67	4817162	5/2/2015	103.91	1675072	5/1/2016	104.16	531342
5/3/2014	105.63	5891494	5/3/2015	103.88	1689240	5/2/2016	104.26	506100
5/4/2014	105.6	4740610	5/4/2015	103.85	1434678	5/3/2016	104.36	603428
5/5/2014	105.57	3710952	5/5/2015	103.82	1728440	5/4/2016	104.44	609266
5/6/2014	105.54	5816272	5/6/2015	103.8	1698564	5/5/2016	104.49	688156
5/7/2014	105.5	5549488	5/7/2015	103.79	1744470	5/6/2016	104.54	656698

5/8/2014	105.47	4132170	5/8/2015	103.76	2239286	5/7/2016	104.57	512134
5/9/2014	105.44	5560772	5/9/2015	103.74	1896524	5/8/2016	104.64	654990
5/10/2014	105.41	4367272	5/10/2015	103.74	1562806	5/9/2016	104.7	699860
5/11/2014	105.39	3628212	5/11/2015	103.81	1740410	5/10/2016	104.8	682892
5/12/2014	105.33	4139002	5/12/2015	103.84	1740914	5/11/2016	104.84	708806
5/13/2014	105.3	5297418	5/13/2015	103.84	1821834	5/12/2016	104.9	1471792
5/14/2014	105.29	4085340	5/14/2015	103.82	1649116	5/13/2016	104.95	1966314
5/15/2014	105.27	3973410	5/15/2015	103.8	1456518	5/14/2016	105	603750
5/16/2014	105.24	4472510	5/16/2015	103.78	1669836	5/15/2016	105.05	648102
5/17/2014	105.21	3641540	5/17/2015	103.76	1753332	5/16/2016	105.07	596792
5/18/2014	105.19	5078962	5/18/2015	103.76	1730610	5/17/2016	105.13	719670
5/19/2014	105.16	6392862	5/19/2015	103.73	1714678	5/18/2016	105.15	715442
5/20/2014	105.09	6376594	5/20/2015	103.7	1587474	5/19/2016	105.16	715512
5/21/2014	105.04	4864188	5/21/2015	103.67	1711234	5/20/2016	105.18	1201592
5/22/2014	105	4696552	5/22/2015	103.64	1703100	5/21/2016	105.2	1770566
5/23/2014	104.96	4616668	5/23/2015	103.61	1740970	5/22/2016	105.2	1698508
5/24/2014	104.92	4737852	5/24/2015	103.57	1511972	5/23/2016	105.21	2034956
5/25/2014	104.9	3819368	5/25/2015	103.54	1744596	5/24/2016	105.18	2606352
5/26/2014	104.88	5890108	5/26/2015	103.5	1400938	5/25/2016	105.16	3343228
5/27/2014	104.84	3488884	5/27/2015	103.47	1666812	5/26/2016	105.15	2868474
						5/27/2016	105.14	3144036

Appendix: - 3

Calculations of daily volume and HRT

Daily volume of Koka reservoir was calculated as

$$d_v = {990\text{Mm}^3 * d_{wl}(\text{m})}\Big/{110(\text{m})}$$

Where:- D_v= Daily volume (Mm^3) and D_{wl}= Daily water level (m).

The daily volume of the reservoir is based on the estimated volume of the reservoir as ($990Mm^3$) at full storage capacity of 110 m water level during our sampling year , 2015) see chapter 4.

And HRT was calculated as

$$HRT_{dly} = {V_d(Mm^3)}\Big/{Q_{dly}\, m^3\, d^{-1}}$$

Where:- HRT_{dly} = water residence time on a daily basis (days), V_d= daily volume (Mm^3), and Q_{dly}= daily discharge (m^3/d). see chapter 4

2013/14	**Daily Volume(Mm3)**	**Out flow 2013/14**	**Daily HRT**	**Date /year 2014/15**	**Daily Volume(Mm3)**	**Out flow (2014/15**	**Daily HRT**	**Date year**	**Daily Volume**	**Out flow 2015/16**	**Daily HRT**
5/28/2013	947.43	5127388	184.7783	5/28/2014	943.38	3626322	260.1479	5/28/2015	931.41	1644020	566.5442
5/29/2013	947.16	4904116	193.1357	5/29/2014	943.2	3811710	247.448	5/29/2015	931.23	1635564	569.3632
5/30/2013	946.8	5235048	180.8579	5/30/2014	943.02	4110624	229.4104	5/30/2015	931.05	1618134	575.385
5/31/2013	946.44	5038586	187.8384	5/31/2014	942.84	3414460	276.1315	5/31/2015	931.05	1644860	566.036
6/1/2	946.08	5280576	179.1	6/1/2014	942.66	3265626	288.6	6/1/2	930.78	1572298	591.9

013			623				613	015			87
6/2/2013	945.72	5148514	183.688	6/2/2014	942.48	3904922	241.3569	6/2/2015	931.14	1651244	563.9021
6/3/2013	945.36	4759692	198.6179	6/3/2014	942.3	4447016	211.8949	6/3/2015	931.23	1737778	535.874
6/4/2013	945.09	4758754	198.6003	6/4/2014	942.12	4175234	225.6448	6/4/2015	931.23	1548470	601.3872
6/5/2013	944.73	4728514	199.7943	6/5/2014	941.76	3727486	252.6529	6/5/2015	930.96	1750252	531.9005
6/6/2013	944.46	5095272	185.3601	6/6/2014	941.49	2917082	322.7506	6/6/2015	930.69	1790628	519.7562
6/7/2013	944.1	4817022	195.9925	6/7/2014	941.04	3876264	242.7698	6/7/2015	930.6	1855868	501.4365
6/8/2013	943.92	5409250	174.5011	6/8/2014	940.68	4567822	205.9362	6/8/2015	930.42	1859424	500.3808
6/9/2013	943.65	4723614	199.7729	6/9/2014	940.32	5776638	162.7798	6/9/2015	930.24	1972152	471.6878
6/10/2013	943.29	5360796	175.9608	6/10/2014	939.96	4315290	217.8208	6/10/2015	929.97	1759912	528.4185
6/11/2013	942.84	5075168	185.7751	6/11/2014	939.42	4348862	216.0151	6/11/2015	929.7	1756510	529.2882
6/12/2013	942.48	4886966	192.8559	6/12/2014	938.88	5407710	173.6188	6/12/2015	929.43	1846824	503.2586
6/13/2013	942.03	4583838	205.5112	6/13/2014	938.34	3709272	252.9715	6/13/2015	929.16	1828428	508.1742
6/14/2013	941.94	4837616	194.7116	6/14/2014	938.07	4761498	197.0115	6/14/2015	928.8	1724226	538.6765
6/15/2013	941.58	5378982	175.048	6/15/2014	937.62	5102454	183.7586	6/15/2015	928.53	1887256	492
6/16/2013	941.31	4847990	194.165	6/16/2014	937.17	4781098	196.0156	6/16/2015	928.26	1886542	492.0431
6/17/2013	941.4	4978078	189.1091	6/17/2014	936.72	4247936	220.5118	6/17/2015	927.99	1813364	511.7505
6/18/2013	941.4	5941068	158.4564	6/18/2014	936.27	3623578	258.3827	6/18/2015	927.81	1850086	501.4956
6/19/2013	941.4	4940502	190.5474	6/19/2014	935.82	4177614	224.0082	6/19/2015	927.72	1761620	526.6289
6/20/2013	941.49	5599272	168.1451	6/20/2014	935.37	4466392	209.4241	6/20/2015	928.08	1808562	513.1591
6/21/	941.58	5184130	181.6	6/21/201	934.83	2916480	320.5	6/21/	927.45	1703324	544.4

2013			274	4			337	2015			942
6/22/2013	941.49	4749710	198.2205	6/22/2014	934.47	2979214	313.6633	6/22/2015	926.82	1524194	608.0722
6/23/2013	941.4	4899510	192.1417	6/23/2014	934.2	3737804	249.9328	6/23/2015	926.37	1292942	716.4823
6/24/2013	941.22	5111638	184.1328	6/24/2014	934.02	3511340	266.001	6/24/2015	926.1	1743700	531.112
6/25/2013	941.04	4786404	196.6069	6/25/2014	933.57	3279976	284.6271	6/25/2015	926.1	1813336	510.7162
6/26/2013	941.4	4905866	191.8927	6/26/2014	933.39	3568278	261.58	6/26/2015	925.92	1724604	536.8885
6/27/2013	941.58	5352648	175.9092	6/27/2014	933.3	3259144	286.3635	6/27/2015	926.28	1890742	489.9029
6/28/2013	941.76	4875206	193.1734	6/28/2014	933.3	3127530	298.4144	6/28/2015	926.82	1752338	528.9048
6/29/2013	941.67	4904816	191.9889	6/29/2014	933.3	3510584	265.8532	6/29/2015	926.82	1646302	562.9708
6/30/2013	941.67	5061644	186.0403	6/30/2014	933.21	2999934	311.0768	6/30/2015	927	1556618	595.5218
7/1/2013	941.58	4954670	190.0389	7/1/2014	932.85	5361468	173.9915	7/1/2015	927.72	1417794	654.3405
7/2/2013	941.58	4914910	191.5762	7/2/2014	932.49	4946046	188.5324	7/2/2015	927.9	1591716	582.9558
7/3/2013	942.3	4802924	196.193	7/3/2014	932.4	3688650	252.7754	7/3/2015	927.9	829822	1118.192
7/4/2013	943.2	4585882	205.6747	7/4/2014	932.22	2974118	313.4442	7/4/2015	928.17	500332	1855.108
7/5/2013	943.92	4841046	194.9827	7/5/2014	932.04	2984254	312.3193	7/5/2015	928.26	726292	1278.081
7/6/2013	944.46	4719932	200.1003	7/6/2014	931.59	3704498	251.4754	7/6/2015	928.26	1270416	730.674
7/7/2013	945	0	#DIV/0!	7/7/2014	931.32	2557352	364.1736	7/7/2015	928.26	1444954	642.4149
7/8/2013	945.72	4530932	208.7253	7/8/2014	931.68	3510990	265.3611	7/8/2015	927.99	1594852	581.8659
7/9/2013	946.17	4641924	203.8314	7/9/2014	933.66	3358390	278.0082	7/9/2015	928.08	1423408	652.0126
7/10/2013	946.8	4506432	210.0997	7/10/2014	933.66	3186974	292.9613	7/10/2015	928.26	1434034	647.3068
7/11/	946.98	4457404	212.4	7/11/201	934.2	3005730	310.8	7/11/	928.26	1502200	617.9

2013			51	4			064	2015			337
7/12/2013	947.43	4655154	203.5228	7/12/2014	935.1	2384018	392.237	7/12/2015	928.26	1257074	738.4291
7/13/2013	947.7	3220812	294.2426	7/13/2014	935.64	1745114	536.1484	7/13/2015	928.08	1245580	745.0987
7/14/2013	948.06	2086756	454.3224	7/14/2014	936.36	1763902	530.8458	7/14/2015	927.99	1283856	722.8147
7/15/2013	948.15	1901522	498.6269	7/15/2014	937.08	1845144	507.8628	7/15/2015	928.26	1191148	779.2986
7/16/2013	948.42	3193330	297.0003	7/16/2014	936.9	2643242	354.4511	7/16/2015	928.26	1437562	645.7182
7/17/2013	948.51	4471208	212.1373	7/17/2014	937.08	3010686	311.2513	7/17/2015	928.26	1328026	698.9773
7/18/2013	949.32	4785830	198.3606	7/18/2014	937.8	3078054	304.673	7/18/2015	928.62	1347738	689.0212
7/19/2013	949.68	4451986	213.316	7/19/2014	938.16	3171154	295.8418	7/19/2015	928.8	1426810	650.9626
7/20/2013	949.77	4628848	205.185	7/20/2014	937.98	3091550	303.4012	7/20/2015	929.52	1621018	573.4174
7/21/2013	949.95	4568410	207.9389	7/21/2014	938.34	2170476	432.3199	7/21/2015	930.06	1450988	640.9839
7/22/2013	950.58	4499838	211.2476	7/22/2014	939.24	2807434	334.5546	7/22/2015	930.6	1628270	571.5268
7/23/2013	950.85	4555278	208.7359	7/23/2014	939.6	3320954	282.9307	7/23/2015	930.78	1416548	657.0762
7/24/2013	951.3	4585644	207.4518	7/24/2014	939.96	3199784	293.7573	7/24/2015	931.5	1417990	656.9158
7/25/2013	952.2	4402034	216.3091	7/25/2014	940.5	3208282	293.1475	7/25/2015	932.4	1443764	645.8119
7/26/2013	953.1	4364388	218.3811	7/26/2014	940.68	3011106	312.4035	7/26/2015	932.94	1673070	557.6216
7/27/2013	953.64	3972402	240.0663	7/27/2014	940.68	3058692	307.5432	7/27/2015	933.48	1393028	670.1086
7/28/2013	954.36	4133920	230.8608	7/28/2014	940.86	3362072	279.8453	7/28/2015	934.02	1349124	692.3159
7/29/2013	954.72	4777038	199.8561	7/29/2014	941.22	2647512	355.5111	7/29/2015	934.2	1463714	638.2394
7/30/2013	955.8	4389406	217.7516	7/30/2014	942.3	2281076	413.0945	7/30/2015	935.28	1467228	637.4469
7/31/	956.52	3446926	277.4	7/31/201	942.84	2514302	374.9	7/31/	935.28	1398096	668.9

2013			994	4			908	2015			669
8/1/2013	957.42	3526474	271.495	8/1/2014	943.47	2341710	402.8979	8/1/2015	936.36	1444408	648.2656
8/2/2013	958.14	3156020	303.5912	8/2/2014	943.92	2474346	381.4826	8/2/2015	936.9	1522598	615.3299
8/3/2013	958.5	2013914	475.9389	8/3/2014	944.64	765772	1233.579	8/3/2015	937.8	1662108	564.2233
8/4/2013	959.4	2282994	420.2376	8/4/2014	944.64	1423940	663.3987	8/4/2015	938.16	1727012	543.2273
8/5/2013	960.03	2135896	449.4741	8/5/2014	944.82	1413608	668.3748	8/5/2015	938.7	1652952	567.8931
8/6/2013	960.66	3134908	306.4396	8/6/2014	945.36	2663864	354.883	8/6/2015	939.6	1632960	575.3968
8/7/2013	961.47	4223044	227.6723	8/7/2014	945.9	2059680	459.2461	8/7/2015	940.14	1594054	589.7793
8/8/2013	962.28	3612014	266.4109	8/8/2014	946.98	1929928	490.6815	8/8/2015	940.23	1562092	601.9044
8/9/2013	963.18	4935504	195.1533	8/9/2014	948.6	2901878	326.8918	8/9/2015	941.85	1592920	591.2726
8/10/2013	964.08	4809238	200.4642	8/10/2014	949.86	1547476	613.8124	8/10/2015	942.66	1251922	752.9702
8/11/2013	964.53	4737992	203.5736	8/11/2014	951.66	2226238	427.4745	8/11/2015	943.38	1428714	660.3001
8/12/2013	966.06	4724300	204.4874	8/12/2014	952.56	2386202	399.195	8/12/2015	944.64	1399188	675.1344
8/13/2013	966.42	4232872	228.3131	8/13/2014	954	1574076	606.0698	8/13/2015	945.72	1308524	722.738
8/14/2013	967.05	4452924	217.1719	8/14/2014	955.44	2403422	397.5332	8/14/2015	946.62	1337252	707.8845
8/15/2013	968.22	4161822	232.6433	8/15/2014	957.24	2632154	363.6717	8/15/2015	947.25	1410542	671.5504
8/16/2013	969.12	3953040	245.1582	8/16/2014	957.42	4439568	215.6561	8/16/2015	948.06	1485974	638.0058
8/17/2013	970.02	4869830	199.1897	8/17/2014	957.96	2287810	418.7236	8/17/2015	948.96	1460564	649.7216
8/18/2013	970.74	5188582	187.0916	8/18/2014	958.86	1978984	484.5214	8/18/2015	949.32	1175258	807.7546
8/19/2013	971.64	4803596	202.2735	8/19/2014	959.76	2447970	392.0636	8/19/2015	949.95	2407202	394.6283
8/20/	972.36	4834914	201.1	8/20/201	960.48	2900506	331.1	8/20/	950.76	1747466	544.0

2013			122	4			422	2015			793
8/21/2013	972.9	5574184	174.5368	8/21/2014	961.2	1683794	570.8537	8/21/2015	951.48	1714734	554.8849
8/22/2013	973.53	3758944	258.9903	8/22/2014	962.28	2292556	419.7411	8/22/2015	952.92	1677648	568.0095
8/23/2013	974.34	3257380	299.1177	8/23/2014	962.82	4583278	210.0724	8/23/2015	954.18	1721174	554.3774
8/24/2013	975.06	3462046	281.6427	8/24/2014	963.72	2255050	427.3608	8/24/2015	955.62	1743448	548.1207
8/25/2013	975.78	4834270	201.8464	8/25/2014	964.8	3573528	269.9853	8/25/2015	956.52	1285914	743.8445
8/26/2013	976.5	4127060	236.6091	8/26/2014	965.52	2168474	445.2532	8/26/2015	956.7	1240288	771.3531
8/27/2013	977.22	3807300	256.6701	8/27/2014	966.42	2743594	352.246	8/27/2015	957.96	1331498	719.4603
8/28/2013	978.3	3346882	292.3019	8/28/2014	967.32	3404884	284.0978	8/28/2015	958.86	1385034	692.3007
8/29/2013	979.2	3120404	313.8055	8/29/2014	968.4	3481142	278.1846	8/29/2015	959.4	1204504	796.5104
8/30/2013	979.65	3531500	277.4034	8/30/2014	968.67	1155056	838.6347	8/30/2015	959.76	1724044	556.6911
8/31/2013	980.55	3434270	285.5192	8/31/2014	969.48	2295342	422.3684	8/31/2015	960.3	1739332	552.1085
9/1/2013	981.36	3064572	320.2274	9/1/2014	970.2	3735102	259.7519	9/1/2015	960.3	1743126	550.9068
9/2/2013	982.44	3049354	322.1797	9/2/2014	970.92	2393356	405.673	9/2/2015	960.66	1789130	536.9425
9/3/2013	983.34	3863272	254.5355	9/3/2014	972	3379138	287.6473	9/3/2015	960.84	2515366	381.9881
9/4/2013	984.24	4129692	238.3325	9/4/2014	972.72	3664360	265.4543	9/4/2015	961.2	1978284	485.8756
9/5/2013	985.5	4186126	235.4205	9/5/2014	972.9	3805550	255.6529	9/5/2015	961.2	1900556	505.7467
9/6/2013	987.3	4421424	223.2991	9/6/2014	973.26	1056062	921.5936	9/6/2015	961.47	1907192	504.1286
9/7/2013	988.47	4412128	224.0348	9/7/2014	973.8	1423870	683.9108	9/7/2015	961.65	1947288	493.8407
9/8/2013	989.46	5050864	195.8992	9/8/2014	974.52	2735628	356.2326	9/8/2015	962.1	1769502	543.7123
9/9/2	990	5000058	197.9	9/9/2014	974.88	2937830	331.8	9/9/2	962.37	1842708	522.2

013			977				368	015			585
9/10/2013	990.36	5757290	172.0184	9/10/2014	975.6	3942442	247.4608	9/10/2015	962.37	1785966	538.8512
9/11/2013	990.72	5883668	168.3848	9/11/2014	976.14	4035262	241.9025	9/11/2015	962.37	1826678	526.8416
9/12/2013	991.08	6043744	163.9844	9/12/2014	976.32	2202844	443.2089	9/12/2015	962.46	1725430	557.8088
9/13/2013	991.26	5685022	174.3634	9/13/2014	976.5	2166864	450.6513	9/13/2015	963.9	1656606	581.8523
9/14/2013	991.53	5824826	170.2248	9/14/2014	977.04	2045904	477.5591	9/14/2015	964.26	2030672	474.8477
9/15/2013	991.8	6000190	165.2948	9/15/2014	977.4	2033024	480.7617	9/15/2015	964.62	1703730	566.1813
9/16/2013	991.8	5971714	166.083	9/16/2014	977.94	2532880	386.098	9/16/2015	964.98	1867656	516.6797
9/17/2013	991.98	6010718	165.0352	9/17/2014	978.3	2350530	416.204	9/17/2015	965.16	1766170	546.4706
9/18/2013	992.16	6083378	163.0936	9/18/2014	978.48	2616516	373.9629	9/18/2015	965.7	1630272	592.3551
9/19/2013	992.52	4983888	199.1457	9/19/2014	978.48	1921794	509.1493	9/19/2015	966.06	1502102	643.1387
9/20/2013	992.43	5547430	178.8991	9/20/2014	978.57	2041116	479.4289	9/20/2015	966.06	1674274	577.0023
9/21/2013	992.34	5487356	180.8412	9/21/2014	978.84	3051468	320.7768	9/21/2015	966.06	1740088	555.1788
9/22/2013	992.16	5424860	182.8914	9/22/2014	978.84	3109246	314.8159	9/22/2015	966.06	1695134	569.9018
9/23/2013	992.25	6098540	162.7029	9/23/2014	979.02	2233672	438.3007	9/23/2015	965.97	1797670	537.3456
9/24/2013	992.16	5686912	174.4638	9/24/2014	979.38	2642738	370.5929	9/24/2015	965.97	1701028	567.8743
9/25/2013	992.16	6210974	159.7431	9/25/2014	979.65	2828714	346.3235	9/25/2015	965.97	1808240	534.2045
9/26/2013	992.07	5563950	178.3032	9/26/2014	979.65	3313968	295.6124	9/26/2015	965.97	1769418	545.9253
9/27/2013	992.07	5869766	169.0136	9/27/2014	979.92	1187382	825.2778	9/27/2015	965.97	1863582	518.3405
9/28/2013	991.98	5193342	191.01	9/28/2014	980.01	2062284	475.2061	9/28/2015	965.97	1722266	560.8715
9/29/	991.8	5897612	168.1	9/29/201	980.1	2725310	359.6	9/29/	966.06	1774808	544.3

2013			698	4			288	2015			18
9/30/			164.7	9/30/201			449.1	9/30/			546.4
2013	991.71	6018978	639	4	980.1	2181998	755	2015	965.97	1767724	484
10/1/			148.0	10/1/201			411.9	10/1/			552.2
2013	991.71	6698636	466	4	980.1	2379398	109	2015	965.97	1749062	789
10/2/			142.3	10/2/201			405.6	10/2/			581.8
2013	991.62	6964804	759	4	980.1	2416302	198	2015	965.88	1659896	919
10/3/			144.8	10/3/201			595.3	10/3/			577.3
2013	991.35	6843298	644	4	980.1	1646204	697	2015	965.79	1672720	77
10/4/			154.7	10/4/201			795.1	10/4/			586.4
2013	991.26	6407128	121	4	980.1	1232630	291	2015	965.79	1646764	775
10/5/			143.1	10/5/201			535.9	10/5/			562.7
2013	991.17	6926122	061	4	980.1	1828876	029	2015	965.7	1716134	183
10/6/			187.6	10/6/201			644.0	10/6/			555.6
2013	990.99	5280212	8	4	980.1	1521828	281	2015	965.7	1738002	38
10/7/			161.8	10/7/201			435.2	10/7/			543.2
2013	990.9	6121612	691	4	980.1	2251928	271	2015	965.61	1777566	203
10/8/			180.8	10/8/201			533.0	10/8/			463.3
2013	991.26	5480930	562	4	981	1840384	409	2015	965.52	2083788	485
10/9/			167.1	10/9/201			593.3	10/9/			281.8
2013	991.71	5933200	459	4	981.27	1653834	304	2015	965.43	3425282	542
10/10			159.4	10/10/20			418.0	10/10			299.8
/2013	991.8	6220088	511	14	981.81	2348374	808	/2015	965.25	3218866	727
10/11			148.2	10/11/20			422.6	10/11			282.3
/2013	992.07	6693652	106	14	981.99	2323496	347	/2015	965.07	3418506	075
10/12			148.7	10/12/20			559.1	10/12			281.6
/2013	992.16	6669040	71	14	982.08	1756356	577	/2015	964.89	3425800	539
10/13			159.4	10/13/20			345.3	10/13			286.1
/2013	992.25	6224008	23	14	982.17	2843582	989	/2015	964.8	3371522	616
10/14			148.3	10/14/20			395.9	10/14			280.0
/2013	992.25	6687282	787	14	982.17	2480716	22	/2015	964.62	3444966	086
10/15			162.5	10/15/20			356.9	10/15			284.0
/2013	992.07	6104882	044	14	982.17	2751490	593	/2015	964.44	3394790	942
10/16			177.7	10/16/20			372.0	10/16			383.9
/2013	991.98	5580876	463	14	981.99	2639140	871	/2015	964.26	2511250	761
10/17			166.2	10/17/20			674.4	10/17			536.6
/2013	991.8	5965176	65	14	981.9	1455846	532	/2015	964.08	1796550	285
10/18			160.5	10/18/20			448.7	10/18			893.3
/2013	991.53	6175008	715	14	981.9	2188074	508	/2015	963.9	1078980	437
10/19	991.71	6129634	161.7	10/19/20	981.81	2414916	406.5	10/19	963.81	1871324	515.0

/2013			894	14			607	/2015			418
10/20/2013	991.26	6186600	160.2269	10/20/2014	981.63	2873472	341.6181	10/20/2015	963.72	1746416	551.8273
10/21/2013	991.08	5782728	171.3862	10/21/2014	981.45	2896726	338.8135	10/21/2015	963.63	1736224	555.0148
10/22/2013	990.99	6082132	162.9346	10/22/2014	981.27	2751910	356.5778	10/22/2015	963.54	1800176	535.2477
10/23/2013	990.81	6368446	155.5811	10/23/2014	981.09	2624650	373.7984	10/23/2015	963.45	2091418	460.6683
10/24/2013	990.72	5842648	169.5669	10/24/2014	981.09	2099202	467.3633	10/24/2015	963.27	3247692	296.6014
10/25/2013	990.72	4320092	229.3284	10/25/2014	981	3368876	291.195	10/25/2015	963.09	3021578	318.7374
10/26/2013	990.36	6355146	155.8359	10/26/2014	980.82	4796204	204.4992	10/26/2015	962.91	3172288	303.538
10/27/2013	990	4739210	208.8956	10/27/2014	980.55	6094312	160.8959	10/27/2015	962.73	3326680	289.3966
10/28/2013	989.82	3395798	291.4838	10/28/2014	980.37	5494300	178.434	10/28/2015	962.55	3219146	299.0079
10/29/2013	989.82	3303986	299.5836	10/29/2014	980.1	2921968	335.4246	10/29/2015	962.37	3218572	299.0053
10/30/2013	989.73	3406284	290.56	10/30/2014	979.92	2789976	351.2288	10/30/2015	962.19	3165470	303.9643
10/31/2013	989.64	3331034	297.0969	10/31/2014	979.83	2028040	483.1414	10/31/2015	961.92	3147452	305.6186
11/1/2013	989.46	3191664	310.0138	11/1/2014	979.74	2459660	398.3233	11/1/2015	961.74	3151400	305.1787
11/2/2013	989.28	2689554	367.8231	11/2/2014	979.56	1474004	664.5572	11/2/2015	961.56	2873038	334.6841
11/3/2013	989.1	3377794	292.8243	11/3/2014	979.47	2625014	373.1294	11/3/2015	961.38	2653742	362.2733
11/4/2013	989.01	3372404	293.2656	11/4/2014	979.38	2954308	331.5091	11/4/2015	961.2	2597098	370.1054
11/5/2013	988.92	3395756	291.2223	11/5/2014	979.2	3950520	247.8661	11/5/2015	961.11	2405802	399.4967
11/6/2013	988.74	3452974	286.3445	11/6/2014	979.02	6582506	148.7306	11/6/2015	960.84	2431660	395.1375
11/7/2013	988.65	3336340	296.3277	11/7/2014	978.66	3962182	247.0003	11/7/2015	960.57	2386734	402.4621
11/8/	988.47	4706058	210.0	11/8/201	978.48	3668700	266.7	11/8/	960.39	2276596	421.8

2013			42	4			103	2015			535
11/9/2013	988.38	6661354	148.3752	11/9/2014	978.39	1608852	608.1293	11/9/2015	960.21	2433928	394.5104
11/10/2013	988.2	6785156	145.6415	11/10/2014	978.3	2269218	431.1177	11/10/2015	960.03	2401392	399.7806
11/11/2013	987.93	6115088	161.5561	11/11/2014	978.12	2915374	335.5041	11/11/2015	959.85	2416106	397.2715
11/12/2013	987.66	5087180	194.1469	11/12/2014	977.94	3091816	316.2995	11/12/2015	959.67	2527084	379.7539
11/13/2013	987.39	3755122	262.9449	11/13/2014	977.76	3097276	315.6838	11/13/2015	959.49	2449398	391.7248
11/14/2013	987.12	3113642	317.0307	11/14/2014	977.58	3117100	313.6184	11/14/2015	959.31	2397962	400.0522
11/15/2013	986.85	3506776	281.4123	11/15/2014	977.4	3033352	322.2178	11/15/2015	959.13	2408140	398.2866
11/16/2013	986.76	5023886	196.4137	11/16/2014	977.31	3883866	251.6333	11/16/2015	958.95	2362626	405.8831
11/17/2013	986.67	3389820	291.0686	11/17/2014	977.22	3477712	280.9951	11/17/2015	958.77	2354436	407.2185
11/18/2013	986.49	3575558	275.8982	11/18/2014	976.77	2610034	374.2365	11/18/2015	958.59	2274986	421.3608
11/19/2013	986.31	3486238	282.9153	11/19/2014	976.5	3621366	269.6496	11/19/2015	958.41	2208934	433.879
11/20/2013	986.04	3410722	289.1001	11/20/2014	976.41	3140732	310.8861	11/20/2015	958.23	2124948	450.9428
11/21/2013	985.86	3509212	280.9349	11/21/2014	976.41	3331076	293.1215	11/21/2015	958.05	2035740	470.6151
11/22/2013	985.77	3496878	281.9	11/22/2014	976.14	3627302	269.1091	11/22/2015	957.87	1802584	531.3872
11/23/2013	985.59	3127768	315.1097	11/23/2014	975.96	3661140	266.5727	11/23/2015	957.69	1450316	660.332
11/24/2013	985.5	3097864	318.1224	11/24/2014	975.78	3631558	268.6946	11/24/2015	957.51	1883630	508.3323
11/25/2013	985.41	3235988	304.516	11/25/2014	975.6	2947644	330.9762	11/25/2015	957.33	2049222	467.1675
11/26/2013	985.23	3940356	250.0358	11/26/2014	975.42	3246656	300.4384	11/26/2015	957.15	2045820	467.8564
11/27/2013	984.96	3346000	294.3694	11/27/2014	975.24	3136406	310.9419	11/27/2015	956.97	1815072	527.2353
11/28	984.78	3444476	285.9	11/28/20	975.06	2554902	381.6	11/28	956.79	2062606	463.8

/2013			013	14			428	/2015			743
11/29			293.8	11/29/20			276.4	11/29			461.0
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11/30			270.5	11/30/20			246.5	11/30			504.2
/2013	984.42	3638824	325	14	974.7	3952914	776	/2015	956.52	1896804	798
12/1/			265.0	12/1/201			312.8	12/1/			562.4
2013	984.24	3713920	138	4	974.43	3114258	932	2015	956.34	1700174	954
12/2/			325.8	12/2/201			323.0	12/2/			527.3
2013	984.06	3020374	073	4	974.16	3015936	042	2015	956.16	1813238	218
12/3/			280.6	12/3/201			191.7	12/3/			521.9
2013	983.88	3505152	954	4	973.8	5077982	691	2015	955.89	1831452	301
12/4/			254.7	12/4/201			242.8	12/4/			554.0
2013	983.7	3861564	413	4	973.53	4008032	948	2015	955.71	1724814	945
12/5/			309.7	12/5/201			230.9	12/5/			508.8
2013	983.52	3175690	028	4	973.35	4214560	494	2015	955.53	1877666	924
12/6/			218.2	12/6/201			233.7	12/6/			571.4
2013	983.34	4504794	875	4	973.08	4162396	788	2015	955.35	1671782	561
12/7/			189.6	12/7/201			240.7	12/7/			558.7
2013	983.07	5184914	02	4	972.9	4041114	504	2015	955.26	1709778	041
12/8/			253.5	12/8/201			228.1	12/8/			512.6
2013	982.89	3876726	361	4	972.72	4263700	399	2015	955.08	1862994	587
12/9/			310.3	12/9/201			236.0	12/9/			550.6
2013	982.71	3166324	631	4	972.54	4119626	748	2015	954.9	1734110	571
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/2013	982.53	3149090	044	14	972.27	4142824	877	/2015	954.63	1801758	325
12/11			332.3	12/11/20			233.1	12/11			588.3
/2013	982.35	2956156	065	14	972.09	4168500	99	/2015	954.45	1622334	191
12/12			313.2	12/12/20			235.0	12/12			627.4
/2013	981.9	3134642	415	14	972	4134802	778	/2015	954.27	1520834	649
12/13			318.4	12/13/20			227.6	12/13			677.5
/2013	981.72	3082674	638	14	971.82	4269692	089	/2015	954.18	1408316	326
12/14			291.8	12/14/20			254.5	12/14			841.9
/2013	981.54	3363724	016	14	971.46	3816036	731	/2015	954.09	1133188	521
12/15			295.3	12/15/20			214.8	12/15			898.7
/2013	981.18	3321738	815	14	971.19	4521216	073	/2015	954	1061466	57
12/16			272.6	12/16/20			317.5	12/16			821.4
/2013	981	3598462	165	14	971.01	3057642	682	/2015	953.91	1161286	256
12/17			206.1	12/17/20			320.3	12/17			922.1
/2013	980.91	4758628	329	14	970.83	3030636	387	/2015	953.82	1034390	087
12/18	980.73	4794860	204.5	12/18/20	970.65	4135544	234.7	12/18	953.73	1070776	890.6

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12/19/2013	980.46	3690022	265.7057	12/19/2014	970.47	4077780	237.9898	12/19/2015	953.64	1232700	773.6189
12/20/2013	980.37	3552514	275.9651	12/20/2014	970.29	4054498	239.312	12/20/2015	953.55	1124928	847.6542
12/21/2013	980.1	3605560	271.8302	12/21/2014	970.02	4162704	233.0264	12/21/2015	953.46	1428266	667.5647
12/22/2013	979.92	4120046	237.842	12/22/2014	969.84	4159680	233.1526	12/22/2015	953.37	1411466	675.4467
12/23/2013	979.74	3392200	288.8214	12/23/2014	969.66	4179084	232.0269	12/23/2015	953.19	1415876	673.2157
12/24/2013	979.56	3300738	296.77	12/24/2014	969.48	3973872	243.9636	12/24/2015	953.1	1568574	607.622
12/25/2013	979.29	3309908	295.8662	12/25/2014	969.3	3396330	285.3963	12/25/2015	952.92	1502550	634.2019
12/26/2013	979.2	3430770	285.417	12/26/2014	969.12	5245240	184.7618	12/26/2015	952.74	1265754	752.7055
12/27/2013	979.02	3712884	263.6818	12/27/2014	968.76	4132128	234.4458	12/27/2015	952.56	1112342	856.3553
12/28/2013	978.84	3380118	289.5875	12/28/2014	968.49	2804872	345.2885	12/28/2015	952.29	1319864	721.5062
12/29/2013	978.66	3137974	311.8764	12/29/2014	968.31	2861460	338.3972	12/29/2015	952.02	1411676	674.3899
12/30/2013	978.3	3570182	274.0196	12/30/2014	968.22	4191054	231.0206	12/30/2015	951.75	1556674	611.3997
12/31/2013	978.12	4658990	209.9425	12/31/2014	967.86	5024446	192.6302	12/31/2015	951.48	1671852	569.1174
1/1/2014	977.85	3677352	265.9114	1/1/2015	967.5	3604762	268.395	1/1/2016	951.21	1610182	590.7469
1/2/2014	977.67	4697406	208.1298	1/2/2015	967.32	3674230	263.2715	1/2/2016	950.94	1459164	651.7019
1/3/2014	977.49	3529512	276.9476	1/3/2015	967.14	2492910	387.9562	1/3/2016	950.67	1420230	669.3775
1/4/2014	977.31	3994522	244.6626	1/4/2015	967.05	2859640	338.1719	1/4/2016	950.4	1531404	620.607
1/5/2014	977.13	3080294	317.2197	1/5/2015	966.87	3494722	276.6658	1/5/2016	950.13	1542506	615.9652
1/6/2014	976.95	3908016	249.9862	1/6/2015	966.6	3602536	268.311	1/6/2016	949.95	3006514	315.9639
1/7/2	976.68	2297876	425.0	1/7/2015	966.42	2764510	349.5	1/7/2	949.59	1492596	636.2

014			36				809	016			003
1/8/2			274.7				296.3	1/8/2			709.2
014	976.5	3554306	372	1/8/2015	966.24	3260320	635	016	949.41	1338680	136
1/9/2			308.7				297.5	1/9/2			628.8
014	976.23	3162236	151	1/9/2015	965.97	3246894	059	016	949.23	1509382	865
1/10/			284.8	1/10/201			264.7	1/10/			699.6
2014	976.05	3426934	173	5	965.79	3648148	343	2016	949.05	1356418	737
1/11/			302.3	1/11/201			544.8	1/11/			602.5
2014	975.87	3227574	54	5	965.61	1772190	682	2016	948.87	1574734	589
1/12/			239.5	1/12/201			284.5	1/12/			652.7
2014	975.6	4072040	851	5	965.43	3392522	759	2016	948.69	1453396	402
1/13/			309.9	1/13/201			324.0	1/13/			644.1
2014	975.42	3147438	092	5	965.16	2978584	332	2016	948.51	1472604	039
1/14/			336.9	1/14/201			288.6	1/14/			645.3
2014	975.15	2894276	236	5	964.98	3342556	952	2016	948.42	1469664	312
1/15/			304.3	1/15/201			254.5	1/15/			697.3
2014	975.06	3203900	353	5	964.8	3790080	593	2016	948.24	1359862	061
1/16/			257.1	1/16/201			256.1	1/16/			667.0
2014	974.97	3792166	011	5	964.62	3765720	582	2016	948.06	1421182	926
1/17/			297.3	1/17/201			166.8	1/17/			772.8
2014	974.79	3278576	212	5	964.35	5781146	095	2016	947.88	1226456	61
1/18/			261.4	1/18/201			236.8	1/18/			616.3
2014	974.61	3727738	481	5	963.99	4070640	153	2016	947.79	1537830	165
1/19/			317.0	1/19/201			345.3	1/19/			583.6
2014	974.43	3073910	002	5	963.63	2790592	138	2016	947.7	1623790	346
1/20/			334.5	1/20/201			327.6	1/20/			703.3
2014	974.25	2912490	076	5	963.45	2940392	604	2016	947.61	1347360	087
1/21/			257.0	1/21/201			316.4	1/21/			669.1
2014	974.07	3788946	82	5	963.27	3044244	234	2016	947.43	1415806	807
1/22/			246.8	1/22/201			350.4	1/22/			607.6
2014	973.8	3944808	561	5	963.09	2748144	511	2016	947.34	1558956	759
1/23/			287.5	1/23/201			296.7	1/23/			608.3
2014	973.62	3385564	799	5	962.91	3244836	515	2016	947.25	1557010	776
1/24/			195.9	1/24/201			306.7	1/24/			650.6
2014	973.44	4966570	984	5	962.73	3138156	821	2016	947.16	1455762	283
1/25/			279.5	1/25/201			361.6	1/25/			642.7
2014	973.17	3480708	897	5	962.55	2661750	23	2016	947.07	1473416	716
1/26/			284.9	1/26/201			298.9	1/26/			634.9
2014	972.9	3414810	06	5	962.46	3219384	578	2016	946.98	1491462	34
1/27/	972.72	4727688	205.7	1/27/201	962.28	3250716	296.0	1/27/	946.89	1542520	613.8

2014			496	5			209	2016			591
1/28/2014	972.63	3821706	254.5015	1/28/2015	962.1	3020598	318.5131	1/28/2016	946.71	1454306	650.9703
1/29/2014	972.45	3563938	272.8583	1/29/2015	961.83	3816484	252.0199	1/29/2016	946.53	1559530	606.9329
1/30/2014	972.09	3331132	291.8197	1/30/2015	961.56	1215466	791.104	1/30/2016	946.35	1422022	665.496
1/31/2014	972	3020976	321.7503	1/31/2015	961.47	3781022	254.2884	1/31/2016	946.17	1347836	701.9919
2/1/2014	971.82	2995048	324.4756	2/1/2015	961.2	2876174	334.194	2/1/2016	945.99	1446970	653.7731
2/2/2014	971.64	2092608	464.3201	2/2/2015	961.02	2933420	327.6108	2/2/2016	945.81	1748124	541.0429
2/3/2014	971.46	2470188	393.2737	2/3/2015	960.84	3030510	317.0555	2/3/2016	945.63	1729042	546.9098
2/4/2014	971.1	1875748	517.7135	2/4/2015	960.66	3253768	295.2454	2/4/2016	945.45	1628606	580.5272
2/5/2014	970.74	2188438	443.5767	2/5/2015	960.39	3049928	314.8894	2/5/2016	945.27	1564206	604.313
2/6/2014	970.38	2075598	467.5183	2/6/2015	960.3	2958746	324.5632	2/6/2016	945.09	1659476	569.5111
2/7/2014	970.2	523208	1854.329	2/7/2015	960.03	2939748	326.5688	2/7/2016	944.91	1801618	524.4786
2/8/2014	970.11	0		2/8/2015	959.76	2779700	345.2747	2/8/2016	944.73	2004324	471.346
2/9/2014	970.02	520226	1864.613	2/9/2015	959.49	2941204	326.2235	2/9/2016	944.55	1960476	481.7963
2/10/2014	969.93	2254756	430.1707	2/10/2015	959.31	2950808	325.1008	2/10/2016	944.37	1978634	477.2838
2/11/2014	969.75	2535288	382.5009	2/11/2015	959.04	2921968	328.2171	2/11/2016	944.19	2080442	453.8411
2/12/2014	969.57	2637530	367.6053	2/12/2015	958.86	2874102	333.6207	2/12/2016	944.01	2036678	463.5048
2/13/2014	969.57	3061772	316.6696	2/13/2015	958.59	3051440	314.1435	2/13/2016	943.83	2021320	466.9374
2/14/2014	969.3	2676282	362.1816	2/14/2015	958.41	3360322	285.2137	2/14/2016	943.65	2080652	453.5357
2/15/2014	969.12	2868026	337.9049	2/15/2015	958.14	3466204	276.4234	2/15/2016	943.47	1952160	483.2954
2/16/	968.94	2148916	450.8	2/16/201	957.96	3484642	274.9	2/16/	943.29	1983044	475.6

2014			971	5			092	2016			778
2/17/			406.2	2/17/201			246.3	2/17/			400.8
2014	968.67	2384620	157	5	957.78	3887786	562	2016	943.11	2352532	915
2/18/			407.8	2/18/201			278.1	2/18/			459.9
2014	968.49	2374610	522	5	957.51	3442012	832	2016	942.84	2049866	52
2/19/			419.8	2/19/201			288.5	2/19/			464.8
2014	968.22	2305968	757	5	957.24	3317790	174	2016	942.57	2027788	267
2/20/			409.4	2/20/201			236.3	2/20/			349.2
2014	968.04	2364390	248	5	956.97	4048310	875	2016	942.3	2697688	991
2/21/			358.0	2/21/201			275.8	2/21/			283.0
2014	967.86	2702770	993	5	956.7	3467772	832	2016	941.85	3327716	32
2/22/			391.5	2/22/201			278.1	2/22/			286.7
2014	967.68	2471210	815	5	956.43	3438428	591	2016	941.49	3283168	627
2/23/			370.3	2/23/201			280.1	2/23/			284.4
2014	967.5	2612358	551	5	956.16	3413340	245	2016	941.22	3309320	149
2/24/			333.1	2/24/201			254.1	2/24/			292.1
2014	967.32	2903600	451	5	955.89	3761674	129	2016	940.86	3220714	278
2/25/			273.2	2/25/201			283.9	2/25/			725.9
2014	967.05	3539116	462	5	955.62	3365082	812	2016	940.5	1295462	958
2/26/			274.5	2/26/201			281.5	2/26/			675.2
2014	966.78	3520846	874	5	955.44	3393656	371	2016	940.41	1392594	937
2/27/			289.4	2/27/201			281.5	2/27/			602.1
2014	966.6	3339378	551	5	955.17	3392676	388	2016	940.23	1561364	85
2/28/			291.4	2/28/201			282.4	2/28/			458.4
2014	966.42	3316236	208	5	954.9	3380314	885	2016	940.05	2050370	782
3/1/2			307.0				341.0	2/29/			444.7
014	966.15	3146248	801	3/1/2015	954.72	2799706	072	2016	939.96	2113258	919
3/2/2			295.7				239.5	3/1/2			478.2
014	965.97	3266256	423	3/2/2015	954.54	3984022	921	016	939.87	1965096	82
3/3/2			354.4				279.5	3/2/2			463.4
014	965.7	2724778	142	3/3/2015	954.27	3413214	811	016	939.69	2027634	416
3/4/2			309.6				280.1	3/3/2			472.2
014	965.52	3117870	729	3/4/2015	954	3405486	362	016	939.51	1989344	713
3/5/2			227.5				269.9	3/4/2			460.3
014	965.25	4242602	137	3/5/2015	953.82	3533670	233	016	939.33	2040318	841
3/6/2			231.7				273.5	3/5/2			442.3
014	964.98	4164692	05	3/6/2015	953.55	3485762	557	016	939.15	2123296	076
3/7/2			235.2				281.2	3/6/2			439.4
014	964.71	4100628	591	3/7/2015	953.28	3389890	127	016	938.97	2136456	989
3/8/2	964.53	2803878	343.9	3/8/2015	953.01	3471244	274.5	3/7/2	938.79	2000376	469.3

014			986				442	016			068
3/9/2014	964.26	2828154	340.9503	3/9/2015	952.83	3283882	290.1535	3/8/2016	938.61	1922256	488.2856
3/10/2014	963.99	6088320	158.3343	3/10/2015	952.65	3199000	297.7962	3/9/2016	938.43	1748670	536.6536
3/11/2014	963.81	5772956	166.9526	3/11/2015	952.38	3192322	298.3346	3/10/2016	938.25	2060674	455.3122
3/12/2014	963.63	5452804	176.7219	3/12/2015	952.02	3157770	301.4849	3/11/2016	938.07	1887116	497.0919
3/13/2014	963.27	5853386	164.5663	3/13/2015	951.66	3197166	297.6574	3/12/2016	937.89	1629712	575.4943
3/14/2014	963	4271834	225.4301	3/14/2015	951.3	3191972	298.0289	3/13/2016	937.71	1663844	563.5805
3/15/2014	962.82	4945612	194.6817	3/15/2015	950.94	3454948	275.24	3/14/2016	937.53	1595692	587.5382
3/16/2014	962.55	3820992	251.911	3/16/2015	959.4	3201352	299.6859	3/15/2016	937.35	1717408	545.7934
3/17/2014	961.92	3910410	245.9896	3/17/2015	959.22	3200596	299.7004	3/16/2016	937.08	1619422	578.6509
3/18/2014	962.1	3750082	256.5544	3/18/2015	949.95	3219664	295.0463	3/17/2016	936.9	1771966	528.7347
3/19/2014	962.01	3964772	242.6394	3/19/2015	949.59	3200218	296.7267	3/18/2016	936.63	1748348	535.7229
3/20/2014	961.74	4670680	205.9101	3/20/2015	949.32	3147074	301.6516	3/19/2016	936.36	1619226	578.2763
3/21/2014	961.65	3409392	282.0591	3/21/2015	949.05	5610080	169.1687	3/20/2016	936.09	1714566	545.9632
3/22/2014	961.47	4288774	224.1829	3/22/2015	948.6	6515586	145.5894	3/21/2016	936	1692894	552.8994
3/23/2014	961.29	4529980	212.2062	3/23/2015	948.15	4680354	202.5808	3/22/2016	935.55	1788752	523.0183
3/24/2014	961.11	4132338	232.5826	3/24/2015	947.7	4901694	193.3413	3/23/2016	935.37	1718570	544.2723
3/25/2014	960.75	4743298	202.5489	3/25/2015	947.34	3433024	275.9491	3/24/2016	935.28	1677242	557.6297
3/26/2014	960.48	4206062	228.3561	3/26/2015	946.98	4637808	204.187	3/25/2016	935.1	1771952	527.7231
3/27/2014	960.3	4045706	237.3628	3/27/2015	946.71	4495330	210.5986	3/26/2016	934.83	1700650	549.6898
3/28/	960.12	3311672	289.9	3/28/201	946.53	3176558	297.9	3/27/	934.56	1729686	540.3

2014			2	5			735	2016			062
3/29/2014	959.85	5581170	171.9801	3/29/2015	946.26	2901150	326.1672	3/28/2016	934.29	1718962	543.5199
3/30/2014	959.67	3396764	282.5248	3/30/2015	946.08	3671038	257.7146	3/29/2016	934.11	1774752	526.3327
3/31/2014	959.49	3992422	240.3278	3/31/2015	945.72	3560900	265.5845	3/30/2016	933.84	1695246	550.8581
4/1/2014	959.22	3566654	268.9411	4/1/2015	945.45	3186610	296.6946	3/31/2016	933.57	1776516	525.5061
4/2/2014	958.95	4305728	222.715	4/2/2015	945.18	3204684	294.937	4/1/2016	933.3	1702778	548.1043
4/3/2014	958.68	4640230	206.6018	4/3/2015	944.91	3346224	282.381	4/2/2016	933.12	995750	937.1027
4/4/2014	958.5	4060266	236.0683	4/4/2015	944.64	2989154	316.0225	4/3/2016	933.03	666736	1399.399
4/5/2014	958.23	4371598	219.1944	4/5/2015	944.37	3112704	303.3922	4/4/2016	932.94	629972	1480.923
4/6/2014	957.96	3829770	250.1351	4/6/2015	944.1	4427808	213.2206	4/5/2016	932.76	644252	1447.819
4/7/2014	957.78	5806640	164.9456	4/7/2015	943.74	3242134	291.0861	4/6/2016	932.67	623294	1496.356
4/8/2014	957.51	5933116	161.384	4/8/2015	943.47	3180184	296.6715	4/7/2016	932.58	637616	1462.604
4/9/2014	957.24	5041036	189.8895	4/9/2015	943.11	3214610	293.3824	4/8/2016	932.76	629230	1482.383
4/10/2014	956.97	5794754	165.1442	4/10/2015	942.75	3211026	293.5977	4/9/2016	932.85	630182	1480.287
4/11/2014	956.7	4689370	204.0146	4/11/2015	942.39	3225012	292.2129	4/10/2016	933.12	708946	1316.207
4/12/2014	956.43	5129754	186.4475	4/12/2015	942.12	2843190	331.3602	4/11/2016	933.12	710010	1314.235
4/13/2014	956.07	3938578	242.745	4/13/2015	941.76	2845304	330.9875	4/12/2016	933.12	692370	1347.719
4/14/2014	955.8	3605000	265.1318	4/14/2015	941.49	2894514	325.267	4/13/2016	933.12	725508	1286.161
4/15/2014	955.62	3514518	271.9064	4/15/2015	941.22	2894220	325.2068	4/14/2016	933.3	703990	1325.729
4/16/2014	955.35	3672676	260.1237	4/16/2015	940.86	2834328	331.9517	4/15/2016	933.48	692552	1347.884
4/17/	954.99	3892952	245.3	4/17/201	940.5	2986424	314.9	4/16/	933.48	704900	1324.

2014			126	5			251	2016			273
4/18/2014	954.81	3415986	279.5123	4/18/2015	940.14	2962904	317.3036	4/17/2016	933.75	740474	1261.017
4/19/2014	954.63	3479294	274.3746	4/19/2015	939.78	3414054	275.2681	4/18/2016	935.37	692860	1350.013
4/20/2014	954.36	3121832	305.7051	4/20/2015	939.42	2865016	327.8935	4/19/2016	935.64	760074	1230.985
4/21/2014	954.18	3125990	305.2409	4/21/2015	939.06	2936374	319.8026	4/20/2016	936	729554	1282.976
4/22/2014	953.91	3951038	241.4328	4/22/2015	938.79	2677416	350.6328	4/21/2016	936.36	691572	1353.959
4/23/2014	953.64	3843028	248.1481	4/23/2015	938.43	2937732	319.4403	4/22/2016	936.63	706566	1325.609
4/24/2014	953.28	3907918	243.9355	4/24/2015	938.07	3044720	308.0973	4/23/2016	936.36	740698	1264.159
4/25/2014	953.1	3742046	254.7002	4/25/2015	937.71	2617538	358.2412	4/24/2016	936.36	723506	1294.198
4/26/2014	952.83	4703678	202.5713	4/26/2015	937.44	2226406	421.0553	4/25/2016	936.45	737478	1269.801
4/27/2014	952.56	3563238	267.3299	4/27/2015	936.9	2674504	350.3079	4/26/2016	936.63	568050	1648.851
4/28/2014	952.29	4093460	232.6369	4/28/2015	936.36	2538228	368.903	4/27/2016	936.81	598444	1565.41
4/29/2014	951.93	4228294	225.1334	4/29/2015	936	2356508	397.1979	4/28/2016	936.63	614334	1524.627
4/30/2014	951.66	4152820	229.1599	4/30/2015	935.82	3072748	304.5548	4/29/2016	936.81	550060	1703.105
5/1/2014	951.3	3701922	256.9746	5/1/2015	935.46	1682856	555.8764	4/30/2016	937.17	1604918	583.9364
5/2/2014	951.03	4817162	197.4254	5/2/2015	935.19	1675072	558.2984	5/1/2016	937.44	531342	1764.287
5/3/2014	950.67	5891494	161.3631	5/3/2015	934.92	1689240	553.456	5/2/2016	938.34	506100	1854.06
5/4/2014	950.4	4740610	200.4805	5/4/2015	934.65	1434678	651.4702	5/3/2016	939.24	603428	1556.507
5/5/2014	950.13	3710952	256.034	5/5/2015	934.38	1728440	540.5915	5/4/2016	939.96	609266	1542.774
5/6/2014	949.86	5816272	163.3108	5/6/2015	934.2	1698564	549.994	5/5/2016	940.41	688156	1366.565
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014			969				692	016			713
5/8/2014	949.23	4132170	229.7171	5/8/2015	933.84	2239286	417.0258	5/7/2016	941.13	512134	1837.664
5/9/2014	948.96	5560772	170.6526	5/9/2015	933.66	1896524	492.3007	5/8/2016	941.76	654990	1437.823
5/10/2014	948.69	4367272	217.2271	5/10/2015	933.66	1562806	597.4254	5/9/2016	942.3	699860	1346.412
5/11/2014	948.51	3628212	261.4263	5/11/2015	934.29	1740410	536.8218	5/10/2016	943.2	682892	1381.185
5/12/2014	947.97	4139002	229.0335	5/12/2015	934.56	1740914	536.8215	5/11/2016	943.56	708806	1331.196
5/13/2014	947.7	5297418	178.8985	5/13/2015	934.56	1821834	512.9776	5/12/2016	944.1	1471792	641.4629
5/14/2014	947.61	4085340	231.9538	5/14/2015	934.38	1649116	566.5945	5/13/2016	944.55	1966314	480.3658
5/15/2014	947.43	3973410	238.4425	5/15/2015	934.2	1456518	641.3927	5/14/2016	945	603750	1565.217
5/16/2014	947.16	4472510	211.7737	5/16/2015	934.02	1669836	559.3483	5/15/2016	945.45	648102	1458.798
5/17/2014	946.89	3641540	260.0246	5/17/2015	933.84	1753332	532.6088	5/16/2016	945.63	596792	1584.522
5/18/2014	946.71	5078962	186.3983	5/18/2015	933.84	1730610	539.6016	5/17/2016	946.17	719670	1314.728
5/19/2014	946.44	6392862	148.0464	5/19/2015	933.57	1714678	544.4579	5/18/2016	946.35	715442	1322.749
5/20/2014	945.81	6376594	148.3253	5/20/2015	933.3	1587474	587.9151	5/19/2016	946.44	715512	1322.745
5/21/2014	945.36	4864188	194.351	5/21/2015	933.03	1711234	545.2381	5/20/2016	946.62	1201592	787.8048
5/22/2014	945	4696552	201.2114	5/22/2015	932.76	1703100	547.6836	5/21/2016	946.8	1770566	534.7443
5/23/2014	944.64	4616668	204.6151	5/23/2015	932.49	1740970	535.6152	5/22/2016	946.8	1698508	557.4304
5/24/2014	944.28	4737852	199.3055	5/24/2015	932.13	1511972	616.4995	5/23/2016	946.89	2034956	465.3123
5/25/2014	944.1	3819368	247.1875	5/25/2015	931.86	1744596	534.1409	5/24/2016	946.62	2606352	363.1973
5/26/2014	943.92	5890108	160.2551	5/26/2015	931.5	1400938	664.9117	5/25/2016	946.44	3343228	283.0917
5/27/	943.56	3488884	270.4	5/27/201	931.23	1666812	558.6	5/26/	946.35	2868474	329.9

2014		475	5		893 348.0 82 348.0 82	2016 5/27/ 2016	946.26	3144036	141 300.9 698 665.0 356

Declaration

I, the undersigned declare that this dissertation is my original works and it has not been presented in other universities for a degree or other purposes. All sources of the materials used have been duly acknowledged.

Name: Samson Tilahun Waktola Signature ____________________

Date__________

The thesis has been done under my supervision

Name: Dr. Demeke Kifle_____ Signature ____________________

Date_________