

**WATER QUALITY ASSESSMENT OF LAKE HAWASSA,
ETHIOPIA, USING MACROINVERTEBRATE AND
DIATOM BASED MULTIMETRIC INDEX**

BY

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A Thesis Presented to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biology (Fisheries and Aquatic Science)


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Declaration

I, undersigned, declare that this thesis has been composed solely by myself and that it has not been submitted, in whole or in part, in any previous application for a degree. Except where states otherwise by reference or acknowledgment, the work presented is entirely my own.

Name: Tarekegn Wondmagegn Gelaw

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Dedication

This thesis is dedicated to my late father, **WONDMAGEGN GELAW**.

\\\\\\=+=/////

I also dedicated this thesis to my mother, **AYALNESH EWUNETU (MAMAYE)**. She represents all Ethiopian mothers who devoted themselves to the best of their children. She has keen interest to education, and sacrifices her life for my success. Thanks my hero and long live to you!

ABSTRACT

Lake Hawassa is one of the most threatened Rift Valley lakes because of human pressure like adverse land use in the watershed, urban development and rapid growth of industries. As a result, the social, economic and ecological functions of the lake are under question. Thus, there is a need for continuous follow-up and monitoring the lake ecology to maintain its water quality and biological integrity. Benthic macroinvertebrates and diatoms are often used for biological water quality assessment because they respond to different stressors and are sensitive to changes in aquatic environment. This study was conducted to assess ecological quality of Lake Hawassa by using diatoms and macroinvertebrate based multimetric index of biotic integrity. Both biological and the physicochemical data were collected from 12 sampling sites using standard procedures in the dry and wet seasons of the years 2015 and 2016. Data on selected physicochemical and habitat quality parameters [in-terms of percent disturbance score (PDS)] were used to categorize each sampling station.

Based on the PDS, sampling stations were classified ranging from as having no evident disturbance (0-25%) to highly disturbed status (75-100%). The PDS classified the 12 sites into three categories: four sites were categorized as having minimal disturbance (C1), five sites as moderate disturbance (C2) and three sites as high disturbance (C3). The physicochemical parameters showed a strong significant relationship along the three categorized sites (Kruskal-Wallis Test $P < 0.01$). Except for dissolved oxygen (DO) and nitrite (NO_2^-), the other physicochemical variables had the highest mean record at highly disturbed sites while DO was mostly higher in minimally disturbed sites. Cluster analysis based on some selected physicochemical variables and PDS also showed a clear distinction between the three-disturbance levels.

From the 12 sites, 43 families of macroinvertebrates which belonged-to 14 taxonomic groups were collected and identified. The Order Hemiptera was the most diverse group followed by Odonata and Coleoptera. The most abundant families were Coenagrionidae, Physidae and Pleuroceridae which made up 19.53, 15.29 and 10.72%, respectively. These

families and Chironomidae, Planorbidae, Piscicolidae, Lumbriculidae, Libellulidae and Corixidae were more abundant in highly disturbed sites and showed a positive association with SRP, NO_3^{2-} , NH_4^+ , TP, EC and PDS (Spearman correlation $P < 0.05$). Polymitarciidae, Baetidae, Hydrophilidae, Notonectidae, Naucoridae, Belostomatidae and Galastocoridae were inversely related to SRP, NO_3^{2-} , NH_4^+ , TP and EC and showed a negative but significant correlation with PDS ($P < 0.05$); and might be considered as indicators of minimally disturbed sites in the littoral area of Lake Hawassa.

A total of 105 diatoms species belonging to 39 genera were collected and identified from the 12 sites. The highest and the lowest species numbers were in minimally and highly disturbed sites, respectively, with 45 and 21 species recorded in the dry season. The genus *Nitzschia* was the most diverse group and contributed 15 species, followed by *Gomphonema* (8 species). *Cymbella*, *Epithemia* and *Navicula* genera comprised five species each and the other diatoms assemblages were represented by < 5 species. In terms of relative abundance *Nitzschia frustulum* Kützing (17.5%), *Achnantheidium minutissimum* Kutzing (9.4%), and *Gomphonema parvulum* Kutzing (8.4%) were the most abundant species. Most of the diatom species did not show significant temporal variation ($P > 0.05$). *Achnantheidium minutissimum*, *Aulacoseira muzzanensis*, *A. ambigua*, *Navicula tenella*, *Nitzschia frustulum*, *Staurosira brevistriata*, and *Staurosira construens* species showed significant spatial variation ($P < 0.05$). Diatom species *Fragilaria capucina*, *F. tenera*, *Gomphonema gracile*, *G. olivaceum*, *G. parvulum*, *G. pumilum* var. *rigidum*, *Nitzschia frustulum*, *N. kuetzingioides*, and *N. palea* were recorded from sites which had high concentration of nutrients and showed a strong significant association with them ($P < 0.01$). *Achnantheidium minutissimum*, *Aulacoseira ambigua*, *Cymbella cistula*, *C. kappii*, *C. turgidula*, *Encyonema muelleri*, *E. silesiacum*, *Encyonopsis microcephala*, *Navicula tenella* and *Ulnaria acus* were more related to sites with relatively good DO. They were also negatively associated with TP, SRP, NO_3^{2-} and NH_4^+ which indicates that they are good indicators of the minimally disturbed sites with low nutrient concentration. Analysis of similarities (ANOSIM) revealed significant variation on both diatom and macroinvertebrate assemblages among the three-clustered sites ($P < 0.01$) which was supported by high values of dissimilarity percentage.

Out of 57 macroinvertebrate and diatoms candidate metrics, only 18 were selected as core metrics based on redundancy analysis (redundant metrics were rejected), response to environmental variables (metrics having significant relationship were accepted), percent discriminatory efficiency (metric > 50% were selected) and evaluation of the sensitivity of the metric of interquartile ranges (metrics with sensitivity value 2 or 3 were nominated). These core metrics were used to develop the multi-assemblage multimetric index of biotic integrity of Lake Hawassa (MMIH). These MMIH index values were divided into quartile ranges of ecological quality classes such as 73-90, 55-72, 37-54 and 18-36, which were classified as very good, good, fair and poor quality, respectively. Based on these ranges, the MMIH index classified the sampling sites into three categories from very good (C1) to fair (C2) and poor (C3). The ecological quality ratio (EQR) further characterized the C1 sites as high quality, C2 sites as moderate quality and C3 sites as poor quality sites. Validation of the MMIH index using the independent data set of Lake Hawassa and Lake Ziway showed that the MMIH had good performance to discriminate between the reference and non-reference sites and evaluate the ecological quality of the clustered sites of both lakes. The MMIH index had inverse strong relationship with PDS which indicated that the MMIH index responded well to the habitat quality degradation of Lake Hawassa ($n=9$, $r^2=0.91$, $P=0.0003$). Generally, it is concluded from this study that the MMIH index is robust and can clearly discriminate between sites exposed to different stressors. Thus, it can be applied by end-users as a practical tool for measuring the ecological quality of different sections of lake littoral. Therefore, it can be recommended to the concerned environmental authorities to develop a management strategy to rehabilitate the disturbed sites and to undertake regular monitoring program to maintain good habitat integrity of the Lake Hawassa ecosystem.

Keywords/phrases: disturbance score; littoral zone; management; multi-assemblages, non-reference; reference sites; stressor; validation

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LISTS OF ABBREVIATIONS

ANOVA- Analysis of variance

APHA- American Public Health Association

Chl a - Chlorophyll a

DCA- Detrended Correspondence Analysis

DO- Dissolved Oxygen

EC- Electric conductivity

FDEPA- Florida Department Environmental Protection Authority

KDHEBW -Kansas Department of Health and Environment Bureau of Water

MEA -Millennium Ecosystem Assessment

NSWGOEH- New South Wales Government Office of Environment and Heritage

NTU- Nephelometric turbidity units

PCA- principal component analysis

PDS- percent disturbance score

RDA- redundancy analysis

SRP- Soluble reactive phosphorus

TP- Total phosphorus

TSS- Total suspended solids

WWAP- United Nations World Water Assessment Programme

CHAPTER 1: GENERAL INTRODUCTION

1.1. BACKGROUND INFORMATION

Water is essential to human life and it comprises marine, estuarine, groundwater and freshwater environments. From these, freshwater ecosystems cover the least proportion and are known by their richest species diversity and indigenous resource (Revenga *et al.*, 2005; Collen *et al.*, 2014). For many years, freshwater bodies have been used as indispensable resources to sustain human existence. Hence, most development activities and human settlements have been established around freshwater bodies. But, these activities have a negative impact on the nearby water bodies deteriorating their overall ecological functions (Revenga *et al.*, 2005) which leads to change in species abundance and diversity (Strayer, 2006).

Overexploitation, pollution, physical habitat modification and degradation, habitat loss, water abstraction and introduction of invasive species are the leading causes of freshwater species reduction and ecosystem degradation (Revenga *et al.*, 2005). These factors have been considered as important drivers that affect the global freshwater ecosystems for the next 50 years (MEA, 2005). Millennium Ecosystem Assessment (MEA, 2005) has reported that due to the above listed factors, aquatic ecosystems have faced a loss of almost 60% of their ecosystem services. These degradations of the ecosystem services are more pronounced in developing countries like Ethiopia. Because, most of sewage and industrial wastes in developing countries are discharged into streams, rivers, reservoirs and lakes without treatment and are responsible for impairments of these water bodies (WWAP, 2017). The economic loss due to inadequate

sanitation and water supply accounts for USD 260 billion annually in low-income countries which is 1.5% of their GDP (Hutton, 2013).

Thus, conservation, rehabilitation and sustainable use of the freshwater ecosystem have been getting better attention in order to reduce the loss and maintain all ecosystem functions. To realize these important achievements, the water quality of freshwater ecosystem should be monitored. In recent years, freshwater quality has become the main concern since it is scarce and has economic, social and ecological importance, which is expensive and often impossible to replace when these ecosystems are degraded. Its quality is commonly characterized by the physical, chemical, biological and aesthetic (appearance and smell) features (Chapman, 1996; NSWGOEH, 2017). A complete monitoring of these characteristics would show a good picture of water quality.

These water quality features are mainly affected by anthropogenic pressures which are responsible for increase of contaminants (like nutrients, heavy metals, pathogenic organisms etc.), physical disturbance (physical alteration, habitat loss and degradation, water withdrawal etc.) and change in biota (like reduction of diversity, species dominance, introduction of invasive species, etc.). To overcome these impairments, government and non-government organizations has started screening the water quality of freshwater ecosystem. In the 1970's, chemical monitoring was used to quantify the impairment level of water-bodies and then biomonitoring established as an alternative means for monitoring of the systems (Mandaville, 2002).

Although chemical monitoring is useful, fluctuations in water quality may be missed by irregular chemical sampling which can be reflected in biological assessments (Winter *et*

al., 2002). Moreover, chemical monitoring only record the contaminants that are measured and analyzed whereas the biota may respond to many other unmeasured alterations. Rosenberg (1998) summarizes this concept as "Chemical measurements are like taking snapshots of the ecosystem, whereas biological measurements are like making a videotape".

In addition, chemical analyses provide no indication of the physical nature (habitat complexity) of the aquatic ecosystem, just as habitat assessments do not address the chemical quality of the water body. But the biological community of the water body reflects the chemical and physical quality of the system. It is believed that characterizing the water body inhabitants' can provide a better overall view. It can be argued that if we wish to maintain healthy, diverse biological communities, it is more appropriate to monitor the aquatic community rather than chemical variables only (Winter *et al.*, 2002).

Therefore, biological monitoring provides a direct measure of ecological integrity by using the response of biota to environmental change (Karr, 1991; Dolédec and Statzner, 2010). Most of the biota used for water quality monitoring purpose are periphyton, macroinvertebrates, macrophytes and fish. Among them, the benthic macroinvertebrates and diatoms are popular and effective in response of different stressors in the water quality monitoring practice (Hering *et al.*, 2006b; Abebe Beyene *et al.*, 2009; Gabriels *et al.*, 2010; Wang *et al.*, 2015).

Both diatoms and macroinvertebrates are sensitive to changes in both the abiotic and biotic environment, and their responses could be predictable, enabling researchers to make reliable causal inferences (Reid *et al.*, 1995). For example, macro-invertebrates

have the following important advantages: they are large enough to be seen with the unaided eye. They are also relatively abundant, sensitive and respond to both natural and man-induced changes in their environment, taxa (family, genus or species) differ in their tolerance to pollutants. These organisms dwell more or less confined to a specific area and exhibit little movement out of the area, ability to retain (bio-accumulate) toxic substances, easy, requires few people and minimal equipment, low cost and do not hurt other organisms.

Similarly, diatoms have many advantages for biomonitoring such as ability to adapt in all aquatic environments in great number. They are also sensitive to changes in water chemistry (i.e. many taxa have well defined ecological optima and tolerances). Diatoms exhibit short cell cycles and rapid colonization of new habitats that do not appear to have strong habitat preferences (regarding substrate type, beyond broad distinctions between rocks, macrophytes, silt and sand). Permanent slides can be prepared for analysis and their frustules are readily preserved in lake sediments (because diatom taxonomy is based largely on the preserved silicon valve) (Reid *et al.*, 1995; Taylor *et al.*, 2007; Bere and Tundisi, 2010). Therefore, these biological parameters in collaboration with that of physicochemical parameters certainly provide useful information about pollution profile of lakes.

Since the 1990s, water quality bioassessment using single biotic index (organism) has been displaced by the integrated bioassessment based on multiple biotic indices, because the single assemblage base biometrics can only represent one community and functions or respond to limited stressors which may affect the accuracy/quality of assessment (Wang *et al.*, 2015). So, for more vigorous water quality assessment, using at least two

assemblages has been recommended which may provide different responses for potential stressors. The use of multi-assemblages water quality assessment has not been developed for lakes in general, and for this reason, there is a need to develop a multimetric index with a foundation of multi-assemblages to evaluate the water status of lakes in Ethiopia.

Lake Hawassa is one of the most threatened Rift Valley lakes in Ethiopia because of human interaction related to adverse watershed land use, rapid urban development and industrial expansion.

Several studies have recorded the pollution history of Lake Hawassa. A study conducted on its tributary (Tikur Wuha River) showed that huge amount of effluents are discharged especially from Hawassa Textile Factory into the river and entered the lake without efficient controlling system (Zerihun Desta, 2003). The impact of this phenomenon was observed in death of livestock and deterioration of quality of irrigated vegetables (Zerihun Desta, 2003; Birnesh Abay, 2007). Zinabu Gebre-Mariam and Zerihun Desta (2002) also observed that the effluent treatment ponds of the factory do not efficiently reduce the chemicals of the discharge which had adverse effect on the lake ecology. The bioaccumulation loads of trace metals of Lake Hawassa were higher in concentration than Lake Ziway which were taken from fish organs, sediment and water samples (Kebede Nigussie *et al.*, 2010). Important work of Amanuel Aklilu (2011) on water quality of the lake using benthic macroinvertebrates showed the overall macroinvertebrates assemblage was dominated by pollution tolerant families.

The research done by Yeman Gebre-Egziabher (2004) showed that because of increase in catchment runoff, lake level fluctuation was observed within the past 25 years. The

anthropogenic activities have been increasing occasionally in the lake catchment such as agricultural and urban expansion which probably increase impervious areas that contribute to have high runoff (Daniel Shewangizaw and Yonas Michael, 2010; Amanuel Aklilu, 2011). This indirectly indicates that the runoff from the lake catchment probably takes different contaminants into the lake. Besides this, the wastes from the surrounding hotels and resorts and traditional fisheries activities may also have an effect on the alteration of the lake ecosystem. The cumulative effect of these activities may have a negative influence on the biodiversity and the fishing activity through which the local peoples/ fishermen and the recreational sector rely on. Most of these human-induced changes affect the fish stock and health (Zeitoun and Mehana, 2014), human health (Anyanwu *et al.*, 2018), aesthetic quality and socioeconomic functions (WHO, 2003b) and the general overall ecosystem functions of the lake (Munir *et al.*, 2016).

Therefore, management of the lake water quality is crucial to preserve and use the resource in sustainable condition. As the stressors are many, their impacts on the lake ecology and the biotic community are expected to be many. Addressing various ecological impairments needs multi-perspective approach rather than using physicochemical methods which may be unrealistic, in view of the cost and time, but more so because such analyses will not give overall picture of the ecosystem health of the lake. Hence, it is deemed better to supplement or replace the physicochemical methods with other bioassessment tools, which are cheaper and more diagnostic of the water quality and biotic integrity of the lake. In this study, the multi-assemblage (benthic macroinvertebrates and diatoms) based multimetric index was used to assess the water quality status of the lake.

1.2. OBJECTIVES

1.2.1. General Objective

- To assess water quality and biotic integrity of Lake Hawassa using Diatoms and Macroinvertebrate indices with integration of physicochemical and habitat quality parameters

1.2.2. Specific Objectives

1. To characterize the shoreline habitat quality and identify representative reference and non-reference sites of Lake Hawassa
2. To assess the spatial and temporal distribution of physicochemical parameters of the littoral areas of the lake
3. To assess the relationship between abiotic factors and macroinvertebrate diversity and abundance in the littoral area of Lake Hawassa
4. To assess the abiotic factors associated with the spatial and temporal variation of attached diatoms in the littoral zone of Lake Hawassa
5. To identify potential macroinvertebrate and diatom metrics/indices that able to discriminate the reference and non-reference sites of the lake.
6. To develop a combined macroinvertebrate and diatom multimetric index of biotic integrity of L. Hawassa (MMIH) and validate its capability of discriminating reference from non-reference sites of the lake.

1.3. RESEARCH QUESTIONS

The following research questions were addressed:

- Do habitat quality and physicochemical parameters show significant variation between clustered sites in the lakeshores?
- Are macroinvertebrates and diatoms metrics/indices able to discriminate between reference and non-reference sites?
- Is the developed multimetric index biotic integrity of L. Hawassa (MMIH) able to discriminate between the lake reference and non-reference sites?

1.4. DESCRIPTION OF THE STUDY AREA

1.4.1. Morphometry and physicochemical features

Lake Hawassa lies 275 km south of Addis Ababa, in the Main Ethiopian Rift (MER), has surface elevation 1,680 m above sea level (6°33' - 7°33' N and 38°22' - 38°29' E) (Welcome, 1972). The surface area of the lake is about 90 km², 16 km long, up to 8 km wide, and it has estimated volume of 1.3 billion m³. The maximum and mean depth of the lake is about 21 m and 11 m, respectively (Elizabeth Kebede *et al.*, 1994; Yeman Gebre-Egziabher, 2004).

Tikur Wuha Rivers is the only perennial tributary river of Lake Hawassa that drains from the vast swamps of Wendo Genet area (highlands on the east). However, it has no visible outlet but there may be groundwater flow away from the lake on the south west and north sides, which may account for its relatively low alkalinity (**Table 1.1**), compared with either Lake Shala or Lake Abiyata, both of which are also terminal lakes (Makin *et al.*, 1975; Yeman Gebre-Egziabher, 2004).

Table 1. 1. Some limnological features of Lake Hawassa

Parameters	Records	References
Altitude (m)	1680	Makin <i>et al.</i> (1975)
Surface area (Km ²)	90	Makin <i>et al.</i> (1975)
Max. depth (m)	21	Yeman Gebre-Egziabher (2004)
Mean depth (m)	11	Elizabeth Kebede <i>et al.</i> (1994)
Catchment areas(km ²)	1436	Nigatu Wondrade <i>et al.</i> (2014)
Secchi depth (m)	0.70-0.8	Elizabeth Kebede <i>et al.</i> (1994)
Chl a(µg/L)	13.57-23.97	Girma Tilahun and Ahlgren (2010)
Conductivity (µs/cm)	780-965	Zinabu Gebre-Mariam <i>et al.</i> (2002)
Salinity (g/L)	0.8	Elizabeth Kebede <i>et al.</i> (1994)
pH	8.75	" "
Na ⁺ (meq/L)	5.96-8.20	Zinabu Gebre-Mariam <i>et al.</i> (2002)
K ⁺ (meq/L)	0.6-0.87	" "
Ca ²⁺ (meq/L)	0.43-0.60	" "
Mg ²⁺ (meq/L)	0.42-0.52	" "
HCO ₃ + CO ₃ (meq/L)	7.49-8.25	" "
Cl ⁻ (meq/L)	0.39-1.03	" "
SO ₄ ²⁺ (meq/L)	0.0-0.73	" "
SiO ₂ (mg/L)	26.0-59.2	Girma Tilahun and Ahlgren (2010)
NO ₃ -N (µg/L)	58	Elizabeth Kebede <i>et al.</i> (1994)
NO ₂ -N(µg/L)	34.9	Elizabeth Kebede <i>et al.</i> (1994)
PO ₄ -P(µg/L)	11.5-19.3	Girma Tilahun and Ahlgren (2010)
TP (µg/L)	30.0-36.2	Elizabeth Kebede <i>et al.</i> (1994)
Sum of cations (meq/L)	7.56-9.97	Zinabu Gebre-Mariam <i>et al.</i> (2002)
Sum of anions (meq/L)	8.4-9.4	" "

1.4.2. Land use land cover change and hydrology of the Lake Hawassa

Catchment

Lake Hawassa catchment is a closed-watershed in the Central Main Ethiopia Rift Valley with an area coverage of 1436 km² (**Table 1.1**). According to Nigatu Wondrade *et al.* (2014), the land cover pattern of the Lake Hawassa catchment was mainly categorized into main features such as Cropland (56.4%), Woody vegetation (13.6%), water (6.7%), Forest (5.6%), Grassland (4.6%), Swamp (5.01%), Scrub (4.2%), Bare-land (2.8%) and Built-up (1.7%) (**Fig. 1.1**). Since most of the catchment has been covered by cropland, much of the runoff which probably carries contaminants and sediments loads comes from

this land use feature. The increasing expansion of urban areas also contributes a significant amount of waste on riparian zone of the lake. Therefore, these land use classes had major effect on the habitat quality of the lake which in-turn affects the chemical and biological elements of the lake.

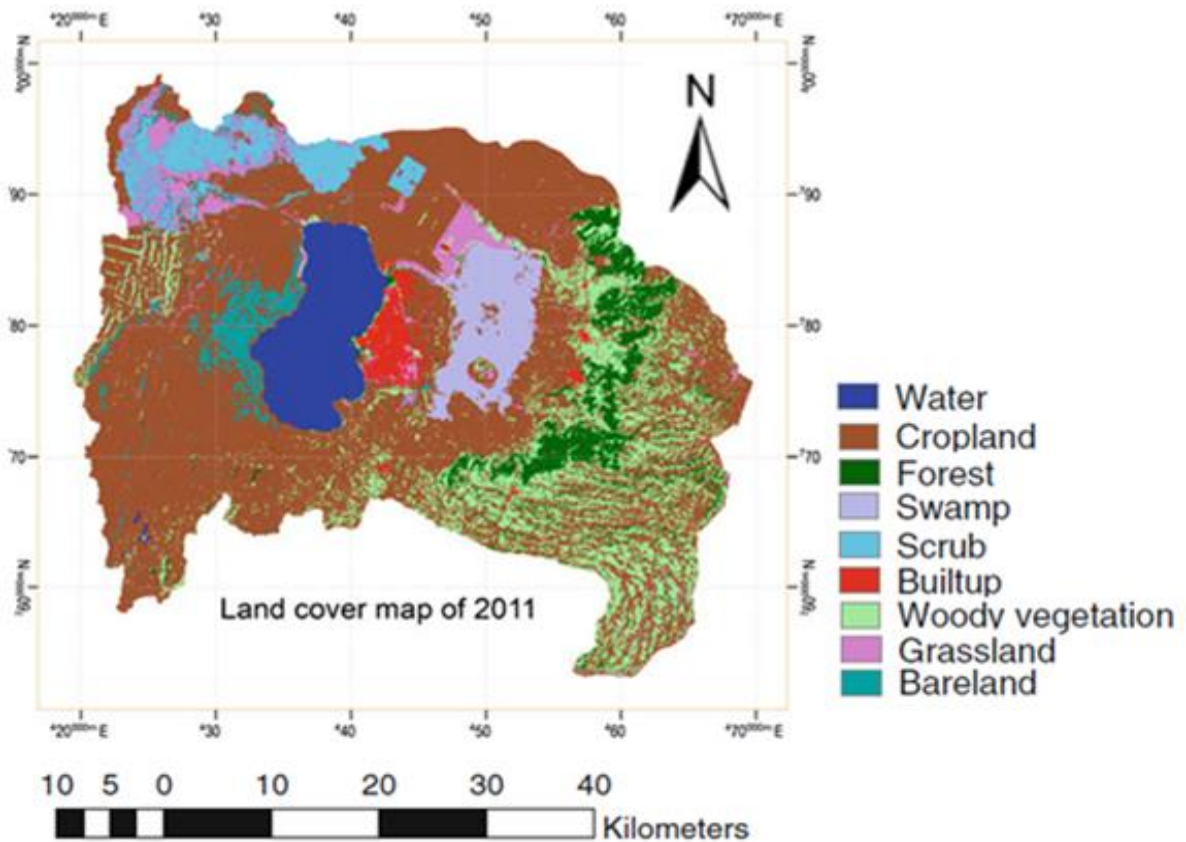


Figure 1. 1. Land use of Lake Hawassa catchment (Nigatu Wondrade *et al.*, 2014) .

The Rift valley where the study lake is located is characterized by moist sub-humid to semi-arid climate (Daniel Gamachu, 1977). Its mean monthly maximum atmospheric temperature varies between 24.5 and 30°C, while the mean minimum monthly air temperature ranges from 10.3 to 14.5 °C (**Fig. 1.2**). Its annual rainfall and annual temperature range, 1028mm, 9-29°C, respectively (Yeman Gebre-Egziabher, 2004). The

lake region has an extended rainy period from March to October, the maximum rainfall is usually 126.1 mm in May, and a minimum record is 24.5 mm in December (**Fig. 1.3**).

The developed water balance for Lake Hawassa suggests that direct precipitation and runoff accounts 56% and 44% of the total input whereas evaporation dominates losses from the lake and accounts 93% and continuous ground seepage contributes 7% as reported by Yeman Gebre-Egziabher (2004). The Tikur Wuha River, which comes from the eastern side of the catchment, contributes most of the water input to the lake that crosses the Lake Cheleleka since both lakes in Hawassa Caldera, were united as a single lake in the last century. The surface area of Lake Cheleleka was about 12 km² in 1972 but it has completely disappeared as a result of siltation (Nigatu Wondrade *et al.*, 2014). The lake floor which once was covered by water is now filled by sediment transported from the eastern highland because of deforestation that has taken place over the last 30 years (WWDSE, 2001). Lake Cheleleka served as sediment trap for Tikur Wuha River, which flows into Lake Hawassa before it filled up with silt. Thus, as Lake Cheleleka lost its function as a sediment trap, water and sediment load flowed directly to Lake Hawassa and high sediment load is observed on the river mouth region of the lake. The heavy metal concentration was higher in the sediments when compared with the lake water as reported by Tigist Ashagre *et al.* (2015).

There is also a noticeable lake level fluctuation of Lake Hawassa because of variations in rainfall and evaporation. In addition, the effect of land use changes like urban expansion and deforestation results an increase surface runoff, which has an effect of raising the lake level (Daniel Shewangizaw and Yonas Michael, 2010) and discharges contaminants to the lake. Over time, there tend to be periods of several years when rainfall or

evaporation is either above or below the long-term average which might cause lake habitat disturbance. Hence, besides the seasonal fluctuations in lake level, there are also cyclic or long-term variations (Makin *et al.*, 1975).

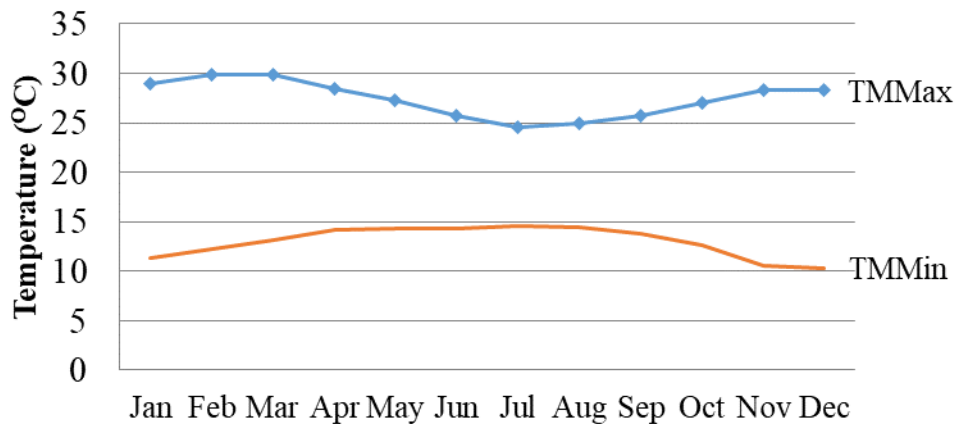


Figure 1. 2. Mean monthly maximum (TMMax) and minimum air temperature (TMmin) during 1985-2016 around Lake Hawassa (Data Source: Ethiopian National Meteorological Service Agency, ENMSA)

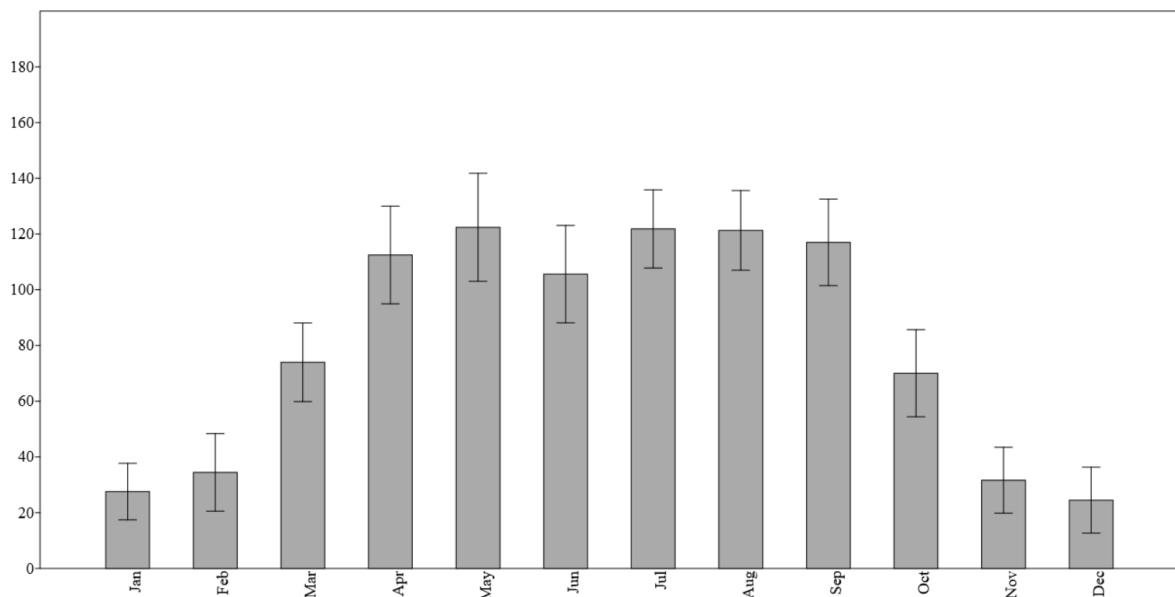


Figure 1. 3. Mean monthly rainfall during 1985-2016 around Lake Hawassa: the bar indicates deviation from the mean (Data Source: Ethiopian National Meteorological Service Agency, ENMSA)

1.5. SAMPLING TECHNIQUE AND DATA COLLECTION

Sampling sites were selected according to the degree exposure of the lake to different anthropogenic activities in the littoral zone of the lake segment of Lake Hawassa. Sampling for macroinvertebrates and diatoms was done up to maximum of 1 m depth contour as in Ontario benthos biomonitoring network (Jones *et al.*, 2007). The physicochemical and the biological data were sampled during February to November of the years, 2015 and 2016 in dry and wet months (**Fig. 1.4**). All the physicochemical and biological data were collected at the same site and time with three replicates.

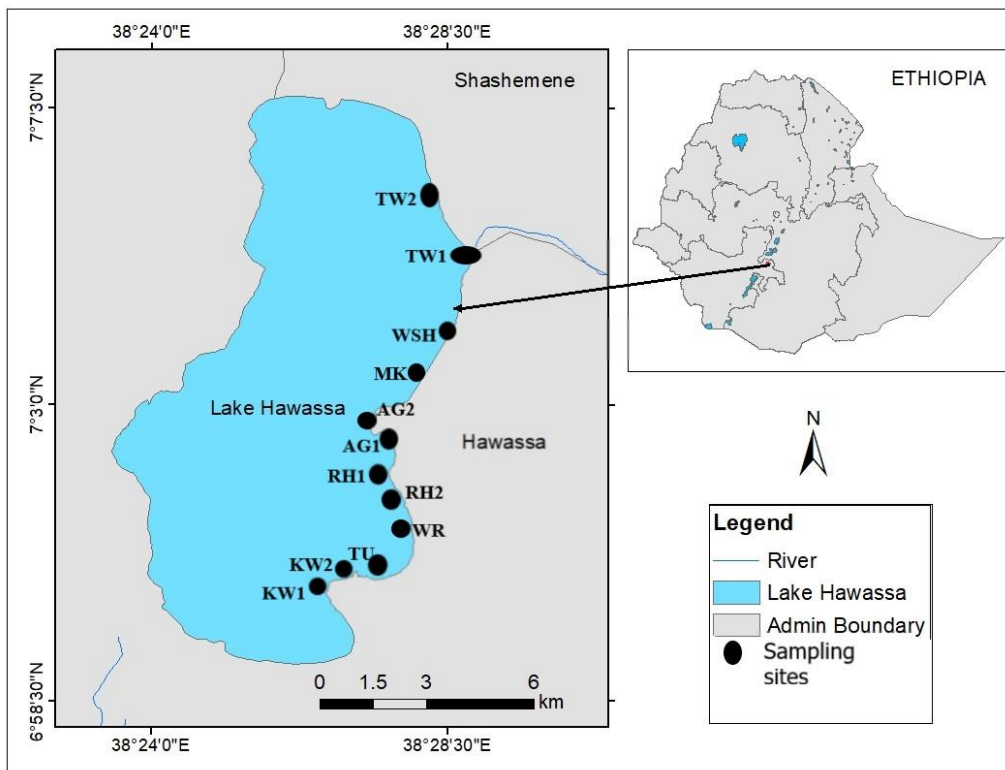


Figure 1.4. Location of L. Hawassa and the sampling sites in this study

1.6. SAMPLING SITES DESCRIPTION

The reference sites and impacted sites were selected according to the exposure of anthropogenic activities and wadable areas of the littoral zone of the lake (≤ 1 meter depth **Fig. 1.4**).

Kuyu Wata1(KW1: N: 07°00'22.600"; E: 38°27'01.800") and Kuyu Wata 2(KW2: N: 07°00'24.600"; E: 38°27'07.700") were selected as reference sites where there are few inhabitants around the area, and the catchment area has been protected from human and animal presence. There are no/ few fishing activities, recreational tours, resorts, industrial plants, and the shore area is more or less covered with macrophytes. The near reference sites have natural and stabilized shoreline, good macrophyte and riparian vegetation, low exposure to human intervention and good watershed management practice (**Plate 1.1**).

Tulu (TU: N: 07°00'21.933"; E: 38°27'20.410"): is the site surrounded by a few inhabitants, some farming practice and washing clothes activities. The inhabitants also used the lake water for drinking. This site is covered by emergent macrophytes (*Echinocloa* spp.) and has stabilized shoreline area with low erosion and sediment loads. Its catchment area has been protected from human and animal disturbance, especially in the mountainous region (**Plate 1.1**).

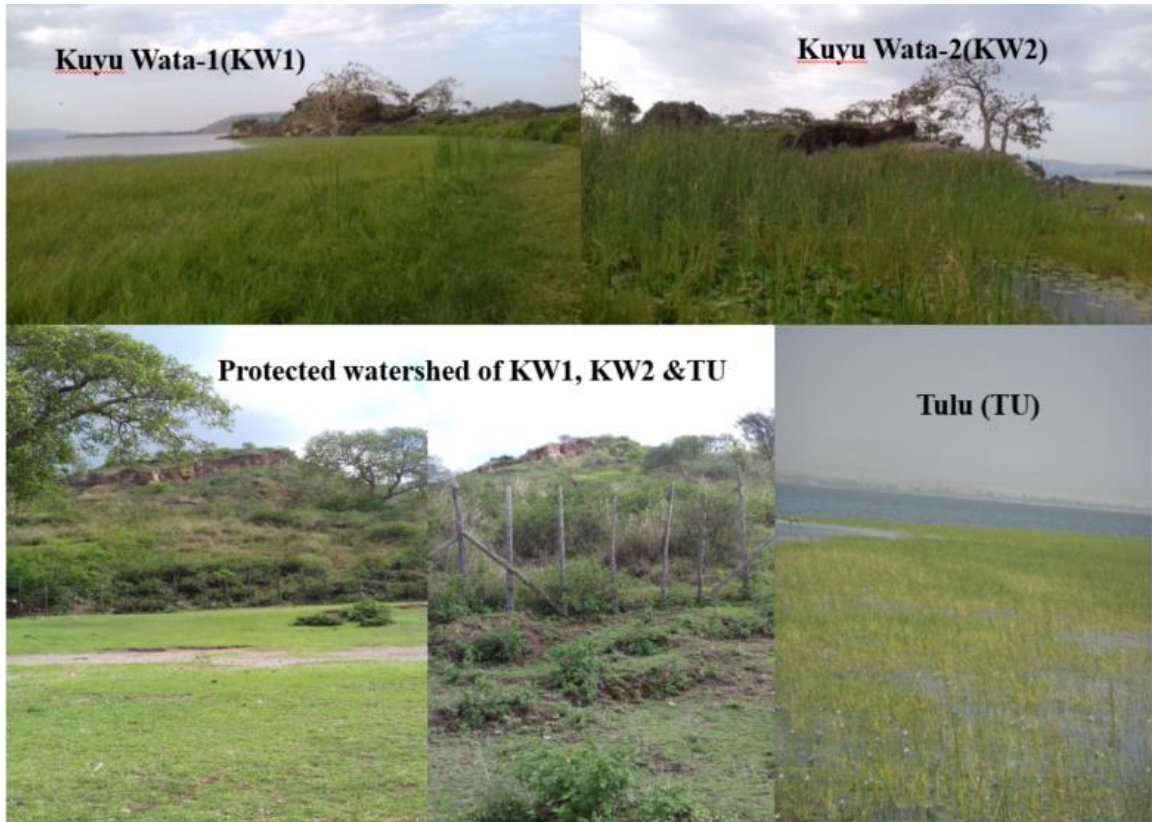


Plate 1. 1. Photos of Kuyu Wata-1(KW1), Kuyu Wata-2 (KW2), Tulu (TU) sites and their watershed

Woranch (WR: N: 07°00'52.900"; E: 38°27'58.900"): this site is exposed to recessionary farming on shore area of the lake which is dominated by vegetables using the lake water as irrigation source, and use of chemicals like pesticides is common. The shore area is highly modified and full of sediment which comes from degraded catchment, aftermath of road construction and agricultural practice. As a result, the site had a high sediment load, is muddy with high organic content and had bad odour/smell, less macrophyte and riparian vegetation cover and no buffer area. This sampling station would represent most of the lake boundary which had an experience of yearly round crop and vegetable farming activities (**Plate 1.2**) . There is also fishing activities, washing clothes, livestock watering etc.



Plate 1. 2. Photos of Worancha (WR) site

Referral Hospital-1 (RH1: N: 07°01'27.800"; E: 38°27'50.600"): situated near Hawassa Referral Hospital and is part of the Hawassa University was highly exposed to medical effluents and human sewerage. This site is also exposed to urban wastes like plastic bags which covered the shoreline area, electronic materials, and others. The failure of the stormwater treatment plant has resulted in all untreated waste water being directly disposed into this sampling station, hence the shoreline habitat is highly disturbed. Lack of riparian vegetation cover and highly modified shoreline habitat are the characteristics of this sampling station. During the study period, there was recession farming more of vegetables that use more of pesticides, and road and building construction (**Plate 1.3**).



Plate 1. 3. Photos of Referral Hospital-1(RH1) site

Referral Hospital-2 (RH2: N: 07°01'26.610"; E: 38°27'51.323"): is located at least 800m distance from RH1 and is somehow covered with macrophyte and good riparian vegetation cover, less modified shoreline habitat, and limited agricultural practice (i.e. mango and banana plantation). But, there is one cafeteria and some inhabitants which contribute certain burden on the disturbance of this sampling site.

Amora Gedel-1 (AG1: N: 07°02'31.000"; E: 38°27'31.500"): this site is the main fish landing and processing site and showed intensive fishing activities. Its surrounding degraded catchment was also source of different contaminants and siment (**Plate 1.4**). Due to the failure of the stormwater treatment plant, the liquid and the solid waste is directly dumped to this sampling site. Because of this, a lot of organic debris and non-

biodegradable wastes (plastic wastes, sediments) are evident at this site. Generally, this site has characteristic of high shoreline modification, low riparian vegetation cover, high sedimentation, high domestic wastes and stormwater disposal area etc. (**Plate 1.4**).



Plate 1. 4. Photos of Amora Gedel-1(AG1) site

Amora Gedel-2 (AG2: N: 07°02'34.641"; E: 38°27'26.494"): is subjected to impacts such as car washing and recreational wastes. It has stabilized shoreline area, good macrophyte and riparian vegetation cover and in some instances, it is protected at the entrance, which limits human activities, especially cattle intrusion.

Meklit (MK: N: 07°03'12.300"; E: 38°27'59.100"): is a site where recreational activities are intensive and more human contacts are observed. This site is characterized by

shoreline disturbance (artificial bedding), food leftover disposed directly to the lake, leakage of oil form motorised boats, plastic bottles and bags, etc. There is an artificial rock belt buffer which only controls the runoff from direct entry to the lake but it is narrowed and used as a walkway. Thus, this site can be expressed as having good riparian vegetation, narrow buffer and mismanaged wetlands, modified shoreline area (artificial bottom substrate, restaurants built in and around littoral area), highly exposed to recreational activities (boating, walking near the lake, bathing, dropping plastic caps, organic wastes, restaurant wastes, etc.) (Plate 1.5).



Plate 1. 5. Photos of Meklit (MK) site

Wabishebele Hotel (WSH: N: 07°03'27.911"; E: 38°28'08.80"): this site is characterized by having good riparian and macrophyte vegetation cover, protection wall built with gabion and fenced with mesh wire to limit human contact, stormwater runoff protection, a well-established wetland which treats the stormwater runoff before joining to the lake. The Hawassa City Environmental Authority has shown good management action which seems a pilot project work because this practice is more or less limited to this area. The Wabishebele Hotel Managers have also good participation in the lake management practice (**Plate 1.6**).



Plate 1. 6. Photos of Wabishebele Hotel (WSH) site

Tikur Wuha 1(TW1: N: 07°05'21.600"; E: 38°28'48.900"): this site is situated at the junction point of the Tikur Wuha River and the lake. The river carries sediments, contaminants from the degraded catchment and industrial effluents(from AwassaTextile factory) to the lake. It also acts as the main landing site through which fish harvesting, processing and marketing are practised. The shore area is covered with mud which has a high content of sand and organic matter. There are also pumice stones which float on the surface. It has been exposed to cattle grazing and harbours many birds (**Plate 1.7**).

Generally, the TW1 site is characterized by high shoreline modification, no buffer zone, low coverage of macrophytes and riparian vegetation, high fishing activities, high sedimentation and the existence of abundant Nile cabbage (water lettuce).



Plate 1. 7. Photos of Tikur Wuha-1(TW1)

Tikur Wuha 2 (TW2: N: 07°05'26.725"; E: 38°28'42.34"): is characterized by good coverage of macrophytes and less riparian vegetation. There is a settlement on the periphery of the lake and drainage canal which drains runoff into the lake. Over-grazing and livestock watering, washing clothes and irrigating crops were some of the additional activities which can threaten the lake ecosystem. TW2 can be characterized as having a long belt of emergent macrophytes, low coverage of riparian vegetation, modified shoreline area with agricultural activities, no established buffer area and can represent recessionary farming activities dominated by cereal crops production.

1.7. THESIS OUTLINE

This thesis compiles the research study done to develop a composite multimetric index based on benthic macroinvertebrates and diatoms diversity, composition, and distribution in the littoral area of Lake Hawassa. Biomonitoring on lakes started far behind that of rivers, and most of the bioassessment metrics and indices developed refer to lotic temperate systems. This study has attempted to extend the few studies on lake bioassessment (Phiri *et al.*, 2007; Wang *et al.*, 2015) and to adopt the methodologies to tropical lake condition.

The major challenge of lake bioassessment is that natural spatial heterogeneity and temporal variation may mask effect of stressors on biota. Despite this, there are some advantages of doing bioassessment on the same lake which were considered in this study.

1. Confounding spatial effects are reduced, except those due to identified stressors;
2. Close-range sampling of abiotic and biological parameters can easily extract factors and metrics that govern autecological and stressor traits;
3. Seasonal variances are easy to uncouple;
4. Ecoregional influences on indices are reduced.

This thesis is organized into six chapters. The first chapter is more of introductory and provides general information on using benthic macroinvertebrates and diatoms in water quality analysis and also reviews relevant literature dealing with the advancement of the science and also describes the sampling sites and methods briefly.

The second chapter deals with the importance of habitat quality assessment (in terms of percent disturbance score (PDS)) for discriminating the sampling sites as reference and non-reference sites which is important in the lake water quality analysis.

The third chapter deals with the composition (at family level), abundance and seasonal variation of the macroinvertebrates in relation to some physicochemical factors in the lake. The distribution of the macroinvertebrate families within clustered sites of the lake was discussed and major families able to characterize the reference and non-reference sites were sorted out. The fourth chapter also deals with the species composition, abundance and seasonal variation of the attached diatoms in relation to some physicochemical factors in the lake. The distribution of the diatom species along clustered sites of the lake was examined and major species able to characterize the reference and non-reference sites were picked out.

The fifth chapter deals with the development of multimetric index of biotic integrity of Lake Hawassa (MMIH) using the entire assemblages of benthic macroinvertebrates and diatoms. Various metrics were tested before selection for the final index development. After core metrics were selected based on well-established standard procedures, metrics were scored using discrete type of scaling. Clear picture about the quality of the water can be obtained if metrics are combined as an index than merely focusing on a single metrics. Then, an aggregated MMIH score was calculated and the discrimination potential of the index was evaluated and validated. The last chapter summarizes the core points addressed during the study period and also highlights research gaps and recommendations for further study.

CHAPTER 2: HABITAT QUALITY ASSESSMENT AND REFERENCE SITE SELECTION OF LITTORAL AREA OF LAKE HAWASSA, ETHIOPIA

2.1. INTRODUCTION

Aquatic ecosystems are exposed to a number of point and non-point sources of pollutants particularly from industries, sewage treatment plants, drainage from urban and agricultural areas (Wang *et al.*, 2016; USEPA, 2018). These anthropogenic activities have been increasingly affecting lentic ecosystem of the country. Among lentic systems, Lake Hawassa is a case as one highly threatened by human-induced changes on its physical, chemical and biological elements.

The catchment of Lake Hawassa has been subjected in a series of changes for more than three decades like transformation of Lake Cheleleka to grass covered swamp, ever green vegetation to cultivated lands and urban areas (Yeman Gebre-Egziabher, 2004; Nigatu Wondrade *et al.*, 2014). The deleterious anthropogenic activities such as construction of irrigation and drainage systems, clearing of forest, building of factories and use of fertilizers, herbicides and pesticides, which have been observed in the lake catchment, contribute a significant effect towards the damage of Lake Hawassa, Tikur Wuha and Shallo Swamp (Zerihun Desta, 2003). These might be the possible reasons through which high sediment load, water quality deterioration, shoreline modification and habitat degradation have been observed on the indispensable but fragile systems of Lake Hawassa.

The previous studies which were conducted on the lake tributary (Tikure Wuha River) showed that huge amount of effluents discharge especially from Hawassa Textile Factory into the River (Zerihun Desta, 2003) which goes to the lake without efficient treatment and causes social, ecological and economic impacts like skin rash, the death of livestock and deteriorated quality of irrigated vegetables (Zinabu Gebre-Mariam and Zerihun Desta, 2002; Zerihun Desta, 2003; Birnesh Abay, 2007). Yeman Gebre-Egziabher (2004) also showed the level of Lake Hawassa fluctuated within the past 25 years because of increment of catchment runoff which resulted from degradation of the watershed. Runoff from the lake catchment also probably took different contaminants, sediments and biodegradable and non-biodegradable wastes into the lake. Besides this, the wastes from the surrounding hotels and resorts and traditional fisheries activities may also have an effect on the alteration of the lake ecosystem.

In addition, there are also stormwater canals which are opened directly to the lake, recessional farming, disposal of institution, construction and household wastes, shoreline modification, clearing of riparian vegetation, mismanaged catchment sourced runoff and others which will have a decisive effect on the lake ecology in the near future. Consequently, there would be an increase in organic content, turbidity, nutrient, ionic composition, temperature and others which in-turn results in a change of trophic state, depletion of dissolved oxygen, deteriorated water quality, altered food chain, and micro and macro inhabitants like algae, macroinvertebrates, fish etc. severely affected (Zeitoun and Mehana, 2014; Munir *et al.*, 2016). Furthermore, the human health (Anyanwu *et al.*, 2018), aesthetic quality and socioeconomic functions (WHO, 2003b) of the lake would be

affected. Therefore, the social, economic and ecological functions of the lake through which the local communities rely on would be impaired.

The level of impairments to the lake ecology should be analyzed and a mitigation measure should be forwarded for sustainable utilization of this aquatic resource. But drawing effective management option needs a reliable information on the lake physical, chemical and biological changes. These changes would be diagnosed by dwellers organisms of the lake like benthic macroinvertebrates, periphyton diatoms and other communities structure because they would have their own signature when the physical, chemical and biological alterations occur (Dolédec and Stutzner, 2010). In addition, anthropogenic activities has an influence on the hydromorphological alteration of the lake which has an impact on the composition and abundance of the aquatic communities (McGoff and Irvine, 2009). Using habitat quality assessment for diagnosing the hydromorphological alteration of the lake is highly recommended (Rowan *et al.*, 2008).

Therefore, the need of assessing the lake habitat quality is a base to identify the main stressors that contribute major cause of degradation of the littoral area of the lake and to take possible management actions. It also provides reliable information to classify the reference and non-reference sites based on disturbance level of the sampling stations that helps to assess the current ecological condition of the sampling stations by comparing the measurements of “good” quality sites to the others. Since there are no historical estimates of the lake reference condition of L. Hawassa, as indeed for other lakes in the country, it is advisable to use “minimally-disturbed sites” as references. Hence, in this study, I characterized the shoreline habitat quality and identified representative reference sites of Lake Hawassa using habitat quality assessment and physicochemical parameters.

2.2. MATERIAL AND METHODS

2.2.1. Habitat quality assessment and reference site selection

To identify the main anthropogenic activities observed in riparian zone of Lake Hawassa, besides keen observation, the land use land cover change analysis was used. The general land use land cover of the riparian zone around 1000 m distance from the shoreline was assessed using geographic information system (GIS). The software Erdas Imagine 2016 version software and supervised, maximum likelihood classification method was used to classify the image of the riparian zone of Lake Hawassa. The image data were acquired at intervals between 1985 and 2015, which cover a time span of approximately 30 years.

Selection of reference and non-reference sites was done using habitat quality survey (Jones *et al.*, 2004; McGoff *et al.*, 2013; FDEPA, 2015). The sampling site selection was done based on habitat quality assessment against notable human activities within 300 m of the shoreline. It included assessment of vegetation quality (percentage nuisance macrophytes like Nile cabbage, water hyacinth, etc., algal mats, coverage of other vegetation). In addition, stormwater inputs (sources of stormwater and presence of management practice) and bottom substrate quality were assessed. The lakeside adverse human alternations (like presence of man-made structures, roads, or other disturbance adjacent), upland buffer zone (coverage of riparian vegetation and width of buffer zone) and adverse watershed land use were evaluated to categorize the sampling sites into reference and non-reference sites (**Appendix 1**). To estimate the relative disturbance among sites, the level of each activity at each site were scored qualitatively on a scale of 20-16 (no evident disturbance), 15-11(minimal disturbance), 10-6 (moderate

disturbance), and 5-1(high disturbance). An overall total disturbance score of each site were obtained by summing the score of each activities and converted into percentage. Since the disturbance score was assessed by providing high values for no human disturbance and low value for high human disturbance, it should be reversed by subtracting the calculated score from 100%. Therefore, the lowest index values (PDS) were assigned to no evident disturbance and the highest values for high disturbance values. Then, each site was assigned as belonging to one of four categories based on their percent disturbance score (PDS): no evident disturbance (00-25%), minimal disturbance (26-50%), moderate disturbance (51-75%), and high disturbance (76-100%).

Physicochemical parameters, nutrient and Chlorophyll-a analysis:

Physicochemical sampling was done on the selected sites during February to November 2015 and 2016. The *in-situ* physicochemical parameters such as temperature(T), pH, conductivity (EC) and dissolved oxygen(DO) were measured with a combined portable HQ40D Multimeter. Turbidity was measured by a OAKTON Turbidimeter(T-100).

Nitrite (NO_2^-), Nitrate (NO_3^{2-}), Amonium ion (NH_4^+), Soluble reactive phosphate (SRP) and Total phosphate (TP) were analysed spectrophotometrically in the Limnological laboratory of Addis Ababa University. Composite water samples (by integrating sample from the surface and 1 m depth) were taken from each sampling site in duplicates for nutrient analysis. Nitrate was analyzed with sodium salicylate method (Robarge *et al.*, 1983), ammonium with indo-phenol blue(APHA, 1995), and soluble reactive phosphate (SRP) with ascorbic acid method (APHA, 1999). Nitrite concentration was determined by the reaction between sulfanilamide and N-naphthyl-(1)-ethylenediamine dihydrochloride

(APHA, 1995). Total phosphorus (TP) and silica (SiO₂) were determined using persulfate digestion method and Molybdosilicate method, respectively (APHA, 1999).

Total suspended solids (TSS) samples were filtered using Whatman GF/C filters and analyzed according to Wetzel and Likens (2001). Chlorophyll a concentrations were measured from each site. 200 - 500 ml of the lake water was filtered through Whatman GF/C filters. The filters were folded with aluminium foil, labelled and transported to the laboratory in an ice box which was stored not more than one day. Pigments were ground and extracted in 90% acetone. After grinding, the algal material was centrifuged. Then, the extract was decanted into 5 ml cuvette and the absorbance of a chlorophyll a was measured spectrophotometrically at wavelengths 665 and 750 nm without phaeopigments correction according to Talling and Driver (1963).

2.2.2. Data analysis

The GIS software Erdas Imagine 2016 version and supervised, maximum likelihood classification method was used to classify the image land use land cover of the lake within riparian zone around 1000 m distance from the shore. Multivariate data analysis was used to show the mean spatial and temporal variation of the physicochemical analysis. The spatial and temporal variation of the environmental parameters were analyzed using Kruskal-Wallis Test and Mann-Whitney U Test, respectively. Data analysis was done with Statistical Package for Social Science Students (SPSS Inc., software version 20.0) and Microsoft Excel office version 2010.

Site clustering was done by Euclidean similarity measures of Ward's Method of PAST software using some selected physicochemical and percent disturbance score (PDS).

Principal components analysis (PCA) was employed to show the distribution of the physicochemical parameters along the study sites using PAST software version 3.15.

2.3. RESULT

2.3.1. Habitat quality assessment and reference site selection

The land use land cover change (LULC) analysis showed the major land use classes responsible for habitat quality deterioration of the lake. Among the land cover classes of the riparian area of the lake, cropland was the most dominant feature which occupied 17.6 % (2509.6 ha) and 21.1 % (3011.8 ha) in 1985 and 2015, respectively (**Table 2.1; Fig. 2.1**). Among the sampling sites, WR, RH1 and TW2 were representative sites where recessionary farming was dominant.

The LULC analysis also showed a major reduction of grassland-open area and riparian vegetation cover at the rate of 6.46 and 3.53 %, respectively, while 5.78 % increment was observed on the built-up areas (**Table 2.1; Fig. 2.1**). Generally, the expansion of cropland and built-up areas would have a direct and indirect effect on the habitat quality of Lake Hawassa resulting in deterioration of the vegetation quality, increased stormwater input, altered bottom substrate quality, reduced buffer zone areas and increased adversity of watershed land use. All these factors significantly contributed to increase the percent disturbance score (PDS) of the habitat quality assessment of the lake.

Table 2. 1. Spatial coverage and proportion of land cover classes resulted from classified images

Land cover (LC) class	Jan-1985		Mar-2015		% Overall change
	Ha	%	Ha	%	
Water body (Lake)	8801.73	61.62	8934.68	62.55	0.931
Cropland	2509.56	17.57	3011.83	21.09	3.52
Vegetation	1117.145	7.82	612.72	4.29	-3.53
Built-up	254.88	1.78	1080.74	7.57	5.78
Grass cover-Open area	1161.72	8.13	239.625	1.678	-6.46
Macrophyte	438.21	3.07	403.65	2.83	-0.24
Total area (ha)	14283.25	100	14283.25	100	0.00

Note: - sign showed a reduction of LC classes

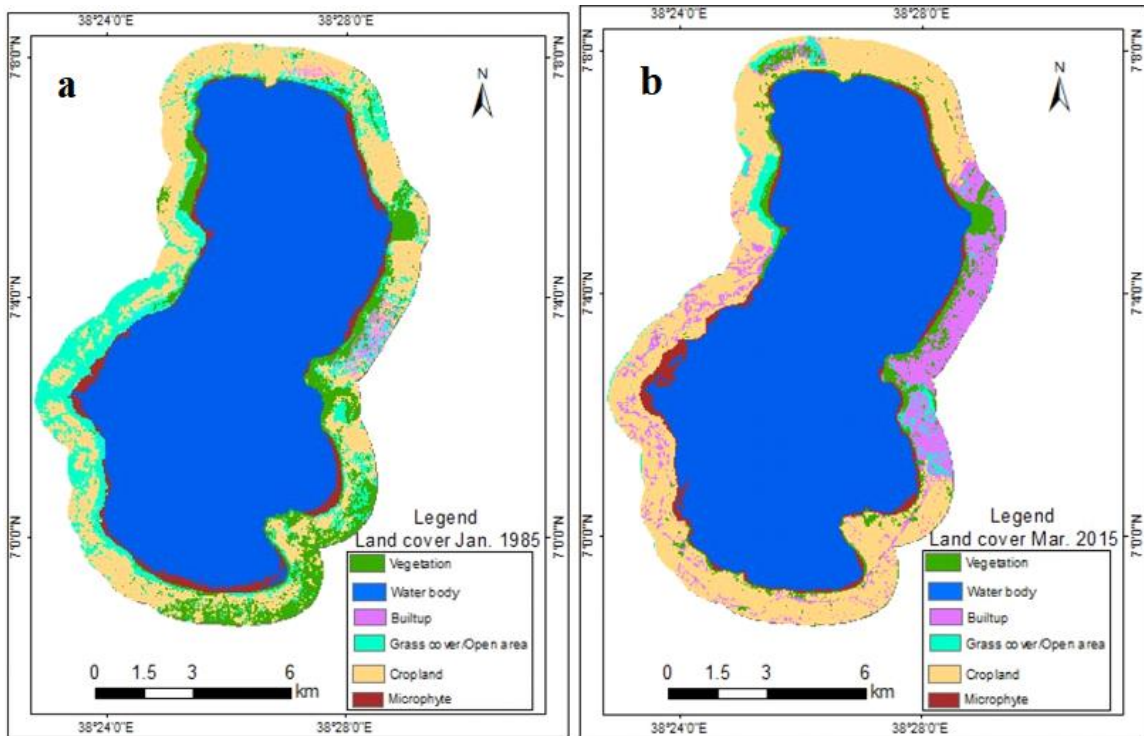


Figure 2. 1. Land use land cover change of Lake Hawassa and its riparian zone from 1985 (a) to 2015 (b)

From the habitat quality assessment, none of the sampling sites were categorized under no-evident disturbance (**Table 2.2**). But, site Kuyu Wata-1 (KW1), Kuyu Wata-2 (KW2), Tulu (TU) and Wabishebele Hotel (WSH) were classified under minimum disturbance category and coded as C1(**Table 2.2**). Sites such as Referral Hospital-2 (RH2), Amora Gedel-2 (AG2), Meklit (MK), Tikur Wuha-1 (TW1) and Tikur Wuha-2(TW2) were categorized under moderate disturbance and coded as C2 (**Plate 1.5 & 1.7**). Worancha (WR), Referral Hospital-1 (RH1) and Amora Gedel-1 (AG1) were grouped under high disturbance class and coded as C3 (**Table 2.2**).

Table 2. 2. Criteria for the human disturbance scores within 300 m of the shore zone of Lake Hawassa. Abbreviations: VQ= Vegetation Quality, SWI= Stormwater Inputs, BSQ= Bottom Substrate Quality, LAHA= Lakeside Adverse Human Alternations, UBZ=Upland Buffer Zone, AWLU= Adverse Watershed Land Use, THQS= Total habitat quality score from 120pts, HQ%= percent habitat quality, PDS= Disturbance score, CB= Class boundary, C= clusters, KW1= Kuyu Wata-1, KW2= Kuyu Wata-2, TU= Tulu, RH2= Referral Hospital, AG2= Amora Gedel-2, WSH= Wabishebelel Hotel, MK=Mekilt, TW1= Tikur Wuha-1, TW2= Tikur Wuha-2, WR= Worancha, RH1= Referral Hospital, AG1= Amora Gedel

Sites	VQ	SWI	BSQ	LAHA	UBZ	AWLU	THQS	HQ%	PDS	CB	C	Site status
KW1	18	19	15	16	10	10	88	73.33	26.67	26-50	C1	Minimally disturbed
KW2	18	19	15	16	11	10	89	74.17	25.83		C1	
TU	14	17	14	10	8	8	71	59.17	40.83		C1	
WSH	18	16	13	6	11	2	66	55	45		C1	
RH2	17	2	14	7	11	1	52	43.33	56.67	51-75	C2	Moderately disturbed
AG2	16	5	11	9	8	4	53	44.17	55.83		C2	
MK	12	14	4	1	7	1	39	32.5	67.5		C2	
TW1	12	4	4	8	11	1	40	33.33	66.67		C2	
TW2	14	5	9	12	11	6	57	47.5	52.5		C2	
RH1	5	2	4	1	7	1	20	16.67	83.33	76-100	C3	
WR	1	5	1	1	11	1	20	16.67	83.33		C3	Highly disturbed
AG1	3	5	1	1	11	1	22	18.33	81.67		C3	

2.3.2. Physicochemical parameters

Most of the parameters (water temperature (T), conductivity (EC), pH, Dissolved Oxygen (DO), Total phosphorus (TP), soluble reactive phosphate (SRP), Nitrite (NO_2^-), Nitrate(NO_3^{2-}), Turbidity and Total suspended solids (TSS)) showed highly significant variation between the sampling sites (Kruskal-Wallis Test $p < 0.01$). However, Ammonium (NH_4^+) and Silicate (SiO_2) did not show significant spatial variation (Kruskal-Wallis Test $p > 0.05$; **Table 2.3**). All physicochemical parameters, except T, SRP, and NO_3^{2-} , showed significant temporal variation (EC, TP, NH_4^+ , NO_2^- , SiO_2 , Turbidity and TSS Mann-Whitney U Test at $p < 0.01$; DO and pH Mann-Whitney U Test at $p < 0.05$). Chlorophyll a (Chl a) showed highly significant temporal and spatial variation ($p < 0.01$; **Table 2.3**).

Table 2. 3. Spatial and temporal mean and standard deviation of the physicochemical parameters of Lake Hawassa, 2015 and 2016. (Abbreviations: TW1-Tikur Wuha 1; TW2- Tikur Wuha2; WSH-Wabisheble Hotel; MK-Mekilit; AG1-Amora Gedel 1; AG2-Amora Gedel2; RH1- Referral Hospital 1; RH2- Referral Hospital 2; WR-Worancha; TU-Tulu; KUW1- Kuyuwata1; KUW2- Kuyuwata2; DR- Dry Season; WT-Wet Season; T-Temperature; EC- Conductivity; DO-Dissolved Oxygen; TP-Total Phosphorus; SRP-Soluble reactive phosphate; Chl a-Chlorophyll a; TSS- Total Soluble Solids; TUR- Turbidity; **P** and **P'**- spatial and temporal significant values, respectively)

Site	Season	T (°C)	EC (µs/cm)	pH	DO (mg/l)	TSS (mg/l)	Chl a (µg/l)	TUR (NTU)
KW1	DR	24.58±1.16	586.5±303	8.9±0.30	9.6±0.9	26.94±4.22	7.84±5.27	5.38±2.59
	WT	26.25±2.3	868.1±7.5	9.0±0.04	9.2±0.35	27.06±3.35	18.78±4.37	9.55±1.06
KW2	DR	25.6±1.1	589.9±302	8.95±0.2	9.6±1.45	31.44±13.32	5.28±3.83	5.39±2.14
	WT	25.74±0.9	865.9±3.8	9.09±0.0	9.5±0.64	32.98±3.07	14.74±6.42	8.36±0.62
TU	DR	26.64±1.4	598.1±301	8.8±0.2	6.3±1.2	29.69±3.02	6.64±3.49	6.60±2.06
	WT	25.00±1.2	209.5±1.9	9.00±0.2	6.2±4.2	4.25±3.12	7.38±1.62	7.90±2.72
WSH	DR	28.75±1.6	623.4±304	8.7±0.1	7.2±1.84	34.09±11.38	8.93±1.82	9.78±5.59
	WT	25.59±2.3	209.7±5	8.7±0.12	4.5±1.65	67.67±37.78	14.77±6.78	30.52±15.45
RH2	DR	29.65±1.6	604±287.6	8.8±0.44	7.9±0.47	25.17±4.47	13.96±3.79	8.08±3.86
	WT	26.03±0.18	208.6±1.5	9.05±0.1	7.5±1.8	36.67±9.23	10.55±6.45	19.69±10.85
AG2	DR	28.73±0.98	590.4±274	9.0±0.17	11.4±0.9	27.25±4.82	0.83±0.00	11.38±1.57
	WT	26.35±0.8	208.2±1.3	9.1±0.18	9.3±2.8	59.38±4.79	21.94±6.63	11.47±1.46
MK	DR	23.5±0.08	991.8±1.4	8.8±0.09	3.6±3.1	13.19±2.10	3.48±0.00	6.37±1.23
	WT	24.58±1.1	864.4±7.2	8.85±0.2	6.1±3.7	33.25±1.58	12.39±1.56	6.78±1.38
TW1	DR	23.13±0.5	977.3±7.5	8.9±0.03	5.1±0.06	49.19±28.53	31.75±15.22	14.76±11.91
	WT	27.76±1.4	756±116.1	8.7±0.32	6.8±0.53	15.23±5.79	9.49±1.58	11.84±2.06
TW2	DR	26.5±1.9	516.1±356	8.4±0.7	5.5±0.21	26.50±0.38	7.65±0.00	11.75±1.06
	WT	24.78±4.9	176.85	8.7±0.3	7.1±1.8	90.25±8.65	22.82±15.73	17.37±5.36
WR	DR	26.3±0.7	1099.9±38	8.8±0.06	2.84±0.45	67.88±0.52	8.34±0.00	22.75±0.38
	WT	26.8±0.8	877.4±4.7	8.9±0.02	4.8±1.48	227.50±0.65	10.85±1.86	37.15±15.28
RH1	DR	26.8±0.1	1045.1±8	8.95±0.2	5.9±0.48	126.00±4.17	9.04±0.00	33.86±14.29
	WT	28.9±1.4	865.5±7.9	9.1±0.02	7.99±0.32	158.51±9.20	19.74±2.61	35.41±14.93
AG1	DR	28.11±1.1	1087±24.2	9.2±0.03	7.7±1.21	37.21±17.06	12.16±1.16	15.43±4.21
	WT	27.95±4.4	872.1±10.6	9.1±0.03	6.9±1.2	32.80±11.81	5.01±1.92	11.22±1.37
P		0.000	0.000	0.000	0.000	0.000	0.008	0.000
P'		0.578	0.000	0.048	0.021	0.001	0.000	0.002

Table 2.3. (Continued)

Site	Season	TP (mg/l)	SRP (mg/l)	NH ₄ ⁺ (mg/l)	NO ₂ -N (mg/l)	NO ₃ -N (mg/l)	SiO ₂ (mg/l)
KW1	DR	0.8±0.19	0.61±0.32	0.13±0.10	0.17±0.10	0.55±0.21	1.03±1.02
	WT	0.56±0.2	0.24±0.14	0.06±0.05	0.16±0.12	0.17±0.02	1.78±0.15
KW2	DR	0.84±0.1	0.38±0.04	0.02±0.01	0.11±0.12	0.38±0.18	1.08±1.07
	WT	0.72±0.23	0.39±0.34	0.09±0.06	0.18±0.06	0.17±0.01	1.68±0.56
TU	DR	0.7±0.72	1.83±1.40	0.11±0.08	0.13±0.08	3.01±1.15	1.11±0.62
	WT	2.0±0.17	1.14±0.89	0.05±0.01	1.40±0.28	2.66±0.53	1.08±0.41
WSH	DR	1.6±0.99	0.86±0.52	0.06±0.02	0.34±0.32	2.31±1.07	1.01±0.91
	WT	2.9±0.7	1.31±0.07	0.06±0.02	1.66±0.78	1.91±0.46	1.29±0.65
RH2	DR	2.87±0.53	0.99±0.49	0.06±0.02	0.34±0.32	2.93±0.51	1.01±0.91
	WT	2.9±0.75	0.31±0.07	0.05±0.02	1.66±0.78	2.03±0.64	1.29±0.65
AG2	DR	2.1±0.36	0.55±0.04	0.06±0.03	0.23±0.16	3.57±0.46	1.18±0.81
	WT	2.6±0.58	0.53±0.18	0.05±0.02	1.51±0.24	3.41±0.89	1.18±0.42
MK	DR	1.3±0.11	1.03±0.02	0.03±0.01	0.02±0.02	2.89±0.89	1.23±0.7
	WT	1.9±0.07	1.23±0.09	0.24±0.16	0.25±0.18	2.55±0.53	1.62±0.11
TW1	DR	1.96±0.63	1.10±0.05	0.04±0.01	0.02±0.02	3.77±0.52	1.40±0.56
	WT	2.8±0.8	1.42±0.52	0.13±0.07	0.31±0.02	3.17±0.92	1.71±0.64
TW2	DR	2.0±0.19	1.64±0.21	0.08±0.05	0.35±0.24	3.85±0.63	1.06±0.94
	WT	2.1±0.22	1.61±0.06	0.06±0.02	1.18±0.32	3.66±0.52	0.92±0.20
WR	DR	3.8±0.5	1.66±0.51	0.02±0.02	0.04±0.04	3.14±0.76	1.35±0.80
	WT	4.1±0.4	1.88±0.25	0.28±0.09	0.25±0.05	3.67±0.52	1.88±0.55
RH1	DR	2.8±0.56	1.17±0.07	0.03±0.01	0.06±0.04	3.64±0.93	1.40±0.87
	WT	3.5±0.15	1.25±0.22	0.09±0.06	0.15±0.14	2.55±0.73	1.48±0.50
AG1	DR	3.8±0.5	1.92±0.07	0.06±0.03	0.13±0.03	4.44±0.92	1.11±0.47
	WT	4.2±1.4	1.58±0.53	0.39±0.26	0.37±0.16	2.67±0.52	1.84±0.43
P		0.000	0.000	0.299	0.000	0.000	0.130
P'		0.005	0.663	0.000	0.000	0.267	0.005

The principal components analysis (PCA) of Axis 1 and Axis 2 explained 58.56% of the total variance (**Fig. 2.2**) regarding the sites versus physicochemical association; with the first axis and second axis contributing 34.34% and 24.22% of the variations, respectively. The analysis showed that Worancha (WR), Referral Hospital (RH1), Amora Gedel-1 (AG1), Tikur Wuha-1(TW1), and Meklit (MK) sites were discriminated from other sites by axis 1, owing to higher values of Total Suspended Solids (TSS), Turbidity(TUR), Total Phosphorus (TP), Soluble Reactive Phosphate(SRP), Nitrate(NO_3^{2-}), Ammonium ion (NH_4^+), Conductivity (EC), and Silica(SiO_2) - which had highest loading factor and were positively correlated with this axis ($r=0.73, 0.72, 0.69, 0.70, 0.50, 0.66, 0.76$ and 0.69 , respectively). There were also high records of these parameters on sites Referral Hospital-1 (RH1) and Amora Gedel-1(AG1) whereas temperature (T), Chlorophyll a (Chl a) and pH had smaller loading factor with weak positive correlation with those sites and Axis 1(**Table 2.4**). But nitrite ($\text{NO}_2\text{-N}$) and dissolved oxygen (DO) were negatively correlated with the first axis ($r= -0.53$ and -0.62 , respectively; **Table 2.4** and **Fig. 2.2**).

Table 2. 4. Principal component analysis of correlation coefficient of the environmental variables used in this study with the first two principal component axes (strong correlations are marked **bold**; see abbreviations of Table 2.2)

Variables	Axis 1	Axis 2
T	0.25	0.40
EC	0.76	-0.63
pH	0.11	-0.58
DO	-0.62	-0.31
TP	0.69	0.43
SRP	0.70	0.39
NH ₄ ⁺	0.66	-0.32
NO ₂ ⁻	-0.53	0.77
NO ₃ ²⁻	0.46	0.70
SiO ₂	0.69	-0.65
TSS	0.73	0.17
Chl a	0.01	0.17
TUR	0.72	0.37

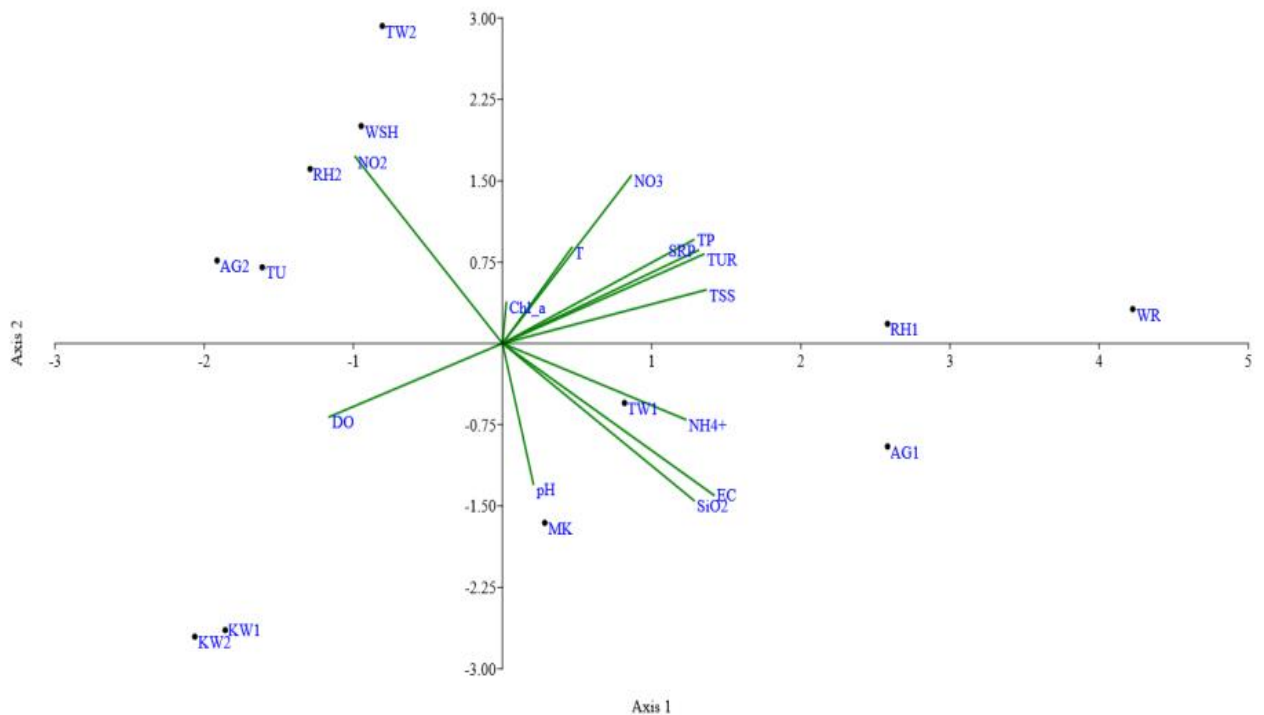


Figure 2.2. Principal component analysis (PCA) of the ordination diagram of the physicochemical factors at study sites (for abbreviations see Table 2.1)

2.3.3. Environmental variables within clustered sites

The physicochemical parameters in the three categories of the littoral zone of L. Hawassa are listed in **Table 2.5**. Water temperature (T) varied from 20.1 to 33.0 °C, and the highest mean record was observed at highly disturbed sites. The DO varied from 0.70 to 12.2 mg/L and it was mostly higher in minimally disturbed sites with mean value 7.76 and lowest in highly disturbed sites (**Table 2.5**). Most of the other parameters except NO_2^- and Chl a showed an increasing trend when there was an increase disturbance of the sampling sites. The environmental parameters such as T, DO, EC, pH, TP, SRP, NO_2^- , NO_3^{2-} , TSS and TUR showed a strong significant relationship along the three clustered sites ($P < 0.01$; **Table 2.5**). However, the NH_4^+ , Silica and Chl-a did not show a statistically significant variation along the three clustered sites ($P > 0.05$; **Table 2.5**).

Similarly, cluster analysis also showed a clear distinction between the three disturbance levels using some selected physicochemical variable and percent disturbance score (PDS; **Fig. 2.3**). Clustering (C) of the sampling sites with percent disturbance score (PDS) and the selected environmental parameters discriminated the reference sites from non-reference sites. Therefore, KW1, KW2, TU and WSH were categorized as reference sites (C1) with minimal disturbance. The non-reference sites were also classified into two categories such as sites with moderate disturbance like Referral Hospital-2 (RH2), Amora Gedel-2 (AG2), Tikur Wuha 1(TW1), Tikur Wuha-2(TW2) and Meklit (MK) categorized under C2 and sites with high disturbance like Worancha (WR), Referral Hospital-1(RH1) and Amora Gedel-1 (AG1) categorized under C3 (**Table 2.2; Fig. 2.3**).

Table 2.5. Mean and range values of measured physicochemical variables at the minimally disturbed(C1), moderately disturbed(C2) and highly disturbed(C3) sites of Lake Hawassa (P-value= significant values with Kruskal-Wallis Test; for other abbreviations see Table 2.1)

Variable	C1	C2	C3	P-value
T(°C)	26.02(23-32.5)	26.10(20.1-32.2)	27.48(23.2-33.00)	0.003
DO(mg/L)	7.76(2.17-10.98)	7.01(0.70-12.2)	6.02(2.1-8.8)	0.001
EC(µs/cm)	568.88(205.8-909)	589.35(154.9-993)	974.50(856-1170)	0.000
pH	8.89(8.47-9.18)	8.82(7.72-9.26)	9.00(8.54-9.24)	0.015
TP(mg/L)	1.28(0.01-3.65)	2.25(1.19-3.73)	3.70(2.22-5.95)	0.000
SRP(mg/L)	0.85(0.06-3.14)	1.04(0.22-1.93)	1.58(0.98-2.17)	0.000
NH₄⁺(mg/L)	0.07(0.01-0.22)	0.08(0.02-0.38)	0.15(0.00-0.66)	0.133
NO₂⁻(mg/L)	0.52(0.0-2.42)	0.59(0.0-2.42)	0.17(0.0-0.52)	0.005
NO₃²⁻(mg/L)	1.40(0.15-4.09)	3.18(1.16-4.46)	3.35(1.19-5.23)	0.000
Silica(mg/L)	1.25(0.07-2.21)	1.26(0.14-2.31)	1.51(0.58-2.41)	0.093
TSS(mg/L)	31.76(1.0-104.0)	37.61(9.33-99.0)	108.32(21.0-228.5)	0.000
Chla(µg/L)	10.54(1.46-26.27)	13.48(0.83-47.85)	10.86(2.50-23.04)	0.693
TUR(NTU)	10.43(2.46-47.5)	11.95(2.97-32.60)	25.97(9.13-52.70)	0.000

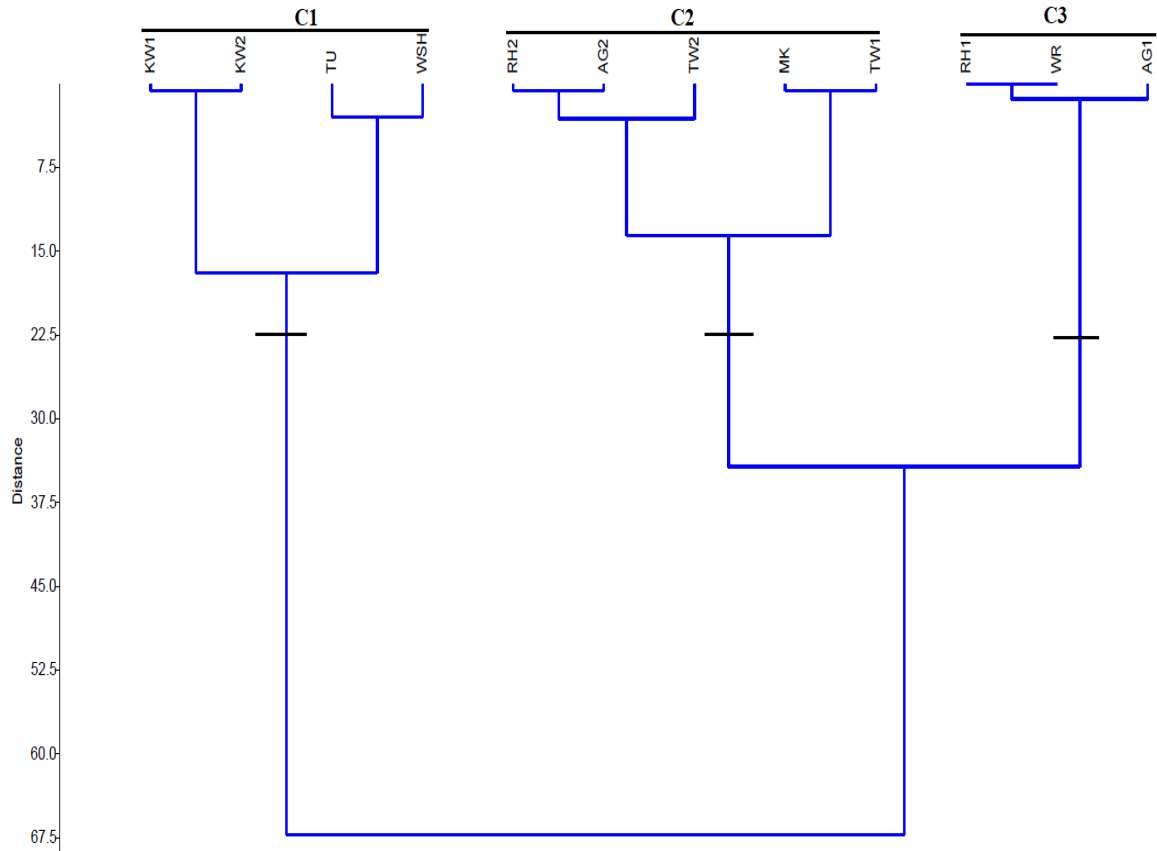


Figure 2. 3. Clustering of sampling sites using percent disturbance score (PDS) and some selected physicochemical variable of Lake Hawassa (for abbreviations see Table 2.1)

2.4. DISCUSSION

Aquatic ecosystem health has suffered from adverse land use land cover change (LULC) which is considered as a dominant stressor in the water quality and ecosystem deterioration of a watershed (Zhao *et al.*, 2012; Nigatu Wondrade *et al.*, 2014). The LULC change has been associated with rapid expansion of human population and their demands for goods and services that could increase pressure on the aquatic ecology. Classification of the LULC change could indicate the land use type that would be more responsible for the majority of the lake habitat quality degradation.

Geographical information systems (GIS) and remote sensing was used for the LULC classification of L. Hawassa and its riparian zone 1000m distance from the shoreline with a total area of 14, 283.25ha. This was done only to have general picture of how LULC changes might indicate the most responsible stressor type that threaten the lake ecosystem in general. The land cover classes were the water body (representing the lake which covers 62.6% of the total area), cropland, vegetation, built-up, grass cover-open areas and macrophytes (**Table 2.1; Fig. 2.1**). According to these classifications, in the riparian zone of the lake, cropland was the most dominant land cover and showed an increasing trend from 2509.56 ha (January, 1985) to 3011.83 ha (March, 2015) (**Table 2.1; Fig. 2.1**). Similar increasing trends of cropland expansion was observed in total area of the catchment of Lake Hawassa (Yeman Gebre-Egziabher, 2004; Nigatu Wondrade *et al.*, 2014). Among the sampling sites, WR, RH1, and TW2 were representative sites of agricultural activities where recession farming was dominant. The possible reason for the expansion of the cropland can be attributed to population pressure and migration in

search of farming lands, as noted by Nigatu Wondrade *et al.* (2014). This land cover feature might be one of the main stressor types that could cause high degradation level of the lake habitat quality.

The highest rate of increment was observed on built-up areas (**Table 2.1; Fig. 2.1**). This classification feature has also shown a rapid increasing rate within the overall watershed in the previous studies (Yeman Gebre-Egziabher, 2004; Nigatu Wondrade *et al.*, 2014). Except KW1, KW2 and TU sites, other sites were highly exposed to rapid urban development and more impervious surface area, which result in high surface runoff. AG1 and RH1 sites had high stormwater input that came directly from Hawassa City and disposed biodegradable (agricultural, human, restaurants, institution etc. wastes) and non-biodegradable (like plastics, electronic, construction, etc.) wastes to the lake.

Generally, the overall increasing trends of the cropland and built-up may have been accelerated at the expense of the reduction of other riparian features such as grassland-open area and riparian vegetation cover with a diminishing rate of 6.46 and 3.53 %, respectively (**Table 2.1**). Based on this LULC change analysis, expansion of the agricultural activities and urbanization and its attendant effect would be responsible for the disturbances in the sampling sites of L. Hawassa. Therefore, these land use features would have direct and indirect effects on the habitat quality of Lake Hawassa that have contributed for the deterioration of vegetation quality, increased stormwater input, altered bottom substrate quality and increased adversity of watershed land use. All these factors significantly contribute to increase the percent disturbance score (PDS) of the habitat quality assessment of the lake.

2.4.1. Habitat quality assessment and reference site selection

Biomonitoring is a key tool used for tracking and measuring anthropogenic impacts and providing feedback in order to sustainably manage aquatic ecosystems (Friberg *et al.*, 2011). To do this, there is a need of selecting reference sites to compare the current status of the site with the natural condition with/without human disturbance (Stoddard *et al.*, 2006); this requires habitat quality assessment expressed by the gap from minimally disturbed condition (Sánchez-Montoya *et al.*, 2009). Thus, in this study, vegetation quality, stormwater inputs, bottom substrate quality, lakeside adverse human alternations, upland buffer zone and adverse watershed land use were the most important variables used to measure the littoral habitat quality or disturbance level of Lake Hawassa to identify the representative reference sampling sites (**Table 2.2; Appendix 1**).

Lake Hawassa is one of the most threatened lakes in the main rift valley with different levels of anthropogenic activities (Amanuel Aklilu, 2011; Nigatu Wondrade *et al.*, 2014; Yonas Abebe *et al.*, 2018 and the current study). So, it is hard to find sites on the shoreline of the lake free of human intervention or “pristine” to take as reference sites. The habitat quality measured in terms of percent disturbance score (PDS) of this study revealed that there were no sampling sites categorized under no-evident disturbance (**Table 2.2**). The Ontario Benthos Biomonitoring Network (OBBN) Protocol Manual recommends to use minimally disturbed sites as reference sampling sites (Jones *et al.*, 2007). Thus, in the current study, there were minimally disturbed sampling stations that are considered as reference sites namely Kuyu Wata-1 (KW1), Kuyu Wata-2 (KW2), Tulu (TU) and Wabishebele Hotel (WSH). These sites had percent disturbance score

(PDS) within the range of minimal disturbance and had minimum disturbance score values when compared with non-reference sites (**Table 2.2; Plate 1.1 and 1.6**).

The features which most commonly characterized the minimally disturbed sites of Lake Hawassa include: naturalness of shoreline, few inhabitants, less fishing activities, good watershed management practice, no/few farming practice, good lake offshore management practices (especially WSH, **Plate 1.6**), no visible input of stormwater and no-evidence of point source pollution. Previous study of Amanuel Aklilu (2011) also selected the WSH site as reference site because of having less human and animal pressure, good macrophyte and riparian vegetation and good lake management practice.

The moderate disturbance sites were characterized by having some recreational activities (results in disposal of organic and inorganic wastes, linkage of oil from motorised boats, etc.), high shoreline disturbance/modification (like artificial substrate, narrowed artificial rock belt buffer, mismanaged wetland, restaurants built in and around littoral area, high fishing activities), but had good riparian vegetation cover. Referral Hospital-2 (RH2), Amora Gedel-2 (AG2), Meklit (MK), Tikur Wuha-1 (TW1) and Tikur Wuha-2 (TW2) were clustered under moderate disturbance and were coded as C2 (**Table 2.2; Plate 1.5 & 1.7**).

Worancha (WR), Referral Hospital (RH1) and Amora Gedel (AG1) were categorized under high disturbance class and coded as C3 (**Table 2.2; Plate 1.2, 1.3 & 1.4**). This category was most characterized by having multiple stressors such as recessional agriculture, building and road construction, input of stormwater, modified shoreline, lack of buffer zone, less riparian vegetation, fishing activities, bathing, washing clothes,

livestock watering, institutional waste discharge (like hospital waste, toilet and other household wastes), etc.

2.4.2. Physicochemical parameters

The environmental parameters are important describers of the water bodies in terms of either water quality or productivity, and are also associated with habitat condition.

a. Water temperature and Dissolved Oxygen:

One of the environmental parameters considered as controlling factors for aquatic life is water temperature. It affects the rate of metabolic activities, reproductive activities and life cycles (Carr and Neary, 2008). The water temperature record was higher where there is high intervention of human disturbance. Sites AG1, RH1, RH2 and AG2 had a records of greater than 30°C which were characterized by high level of human induced disturbance that also resulted in high records of EC, pH, TP and NH₄⁺. The lower temperature record was observed at minimally disturbed sites with a mean record of 26.02 °C which was characterized with few inhabitants and recessional farming practice, and good management activities (**Table 2.3 & 2.6; Plate 1.1 & 1.6**). They had also high shade by macrophytes (*Typha* and other emergent ones) which might contribute to reduction of the water temperature.

The mean water temperature records of Lake Hawassa, in this study was higher than in the previous studies which were done by different scholars (Zinabu Gebre-Mariam *et al.*, 2002; Girma Tilahun and Ahlgren, 2010; Adimasu Woldesenbet, 2015 ; **Table 2.6**). This might be due to an increment of disturbance of the Lake Habitat occasionally, and runoff carries different contaminants and sediments (Yonas Abebe *et al.*, 2018) which contribute

to an increase in water temperature. Lakeman-Fraser and Ewers (2014) also noted that habitat fragmentation and temperature variation affected the ecology of the lake system. Apparently, the sites which showed high records of water temperature were highly exposed to stormwater runoff, sedimentation from the degraded catchment, habitat degradation and shoreline modification and human intervention.

Dissolved Oxygen (DO) in the water column is one of the most important components of the aquatic system which is the product of photosynthesis besides diffusion from the atmospheric oxygen. It is required for the metabolism of aerobic organisms (decomposers), and it influences inorganic chemical reactions called decomposition reaction. Dissolved oxygen is often used as an indicator of water quality, so high concentrations of oxygen usually indicate good water quality. In this study, the mean DO was 7.07 mg/l (**Table 2.6**) which was lower than Adimasu Woldesenbet (2015). This may result from the increment of the water temperature in this study period. Sites with a high human intervention like Worancha and Meklit (mean value 3.8 and 4.8 mg/l, respectively), the dissolved oxygen was found lower when compared with minimally disturbed sites. However, in the protected and minimally disturbed sites, DO had higher values (eg. KW1 and KW2 with mean values of 9.4 and 9.5 mg/l, respectively). Generally, the DO concentration decreased as the disturbance of the Lake Habitat increased and water temperature increased (**Table 2.5**). Any gas, including oxygen, dissolved in water is inversely proportional to the temperature of the water; as temperature increases, dissolved oxygen (gas) decreases (Carr and Neary, 2008).

b. Nutrients

Nutrients are elements essential to life. In aquatic systems, nitrogen and phosphorus are the two nutrients that most commonly limit maximum biomass of algae and aquatic plants, which occurs when concentrations in the surrounding environment are below requirements for optimal growth of algae, plants and bacteria (Carr and Neary, 2008). The Lake Hawassa nutrients concentration have increased from time to time including the current study (Zinabu Gebre-Mariam *et al.*, 2002; Girma Tilahun and Ahlgren, 2010; Adimasu Woldesenbet, 2015 ; **Table 2.6**). In this study, the mean PO₄-P and NO₃-N concentration were 599.8µg/L with a range of 12.2-3144.88µg/L, and 705.7 8µg/L with a range of 68.97-7462.68µg/L, respectively.

Total Phosphorus (TP) and Soluble Reactive Phosphate (PO₄-P)

The total phosphorus (TP) concentration in this study showed higher record at highly disturbed sites such as Amora Gedel-1 (AG1), Worancha (WR) and Referral Hospital (RH1) with mean records of 3990.3, 3970.4 and 3129.6 µg/L, respectively. Soluble Reactive Phosphate (PO₄-P) had also a similar trend to TP having a high record on WR, AG1 and RH1 sites with mean values of 1773.1, 1748.0 and 1625.5 µg/L (**Table 2.3 & 2.5**). The reason behind of having high records of TP and PO₄-P might be the high intervention of human activities such as bathing, washing clothes, stormwater discharge, livestock watering, contamination with toilette and hospital wastes and detergents, etc. Besides this, recessional farming activities practised near the shore area cause release of excess fertilizer such as DAP (Di-Ammonium Phosphate) and to have a high load of phosphate. The lower record phosphate was observed at minimally disturbed sites of Kuyu Wata-2 (KW2 with mean records of 384.3µg/L) although this amount is greater

than the maximum permissible limit set by WHO (1984) and EEPA (2003) which is 5 to 20 $\mu\text{g/L}$. Generally, the total phosphorus (TP) concentration was higher in the current study and also had higher value in wet season than in dry season, even though there were some exceptions (**Table 2.3 and Table 2.6**).

Nitrate (NO_3^{2-}), Nitrite (NO_2^-) and Ammonium (NH_4^+)

The Nitrate ($\text{NO}_3\text{-N}$) concentration of Lake Hawassa had higher values at non-reference sites of Tikur Wuha-2 (TW2) with an overall mean of $3751.57\mu\text{g/L}$. This might be because of recessional agricultural activities such as livestock grazing and recessional farming which dominated in the area that may have a substantial contribution for the increments of nitrate. As noted by the European Commission (EUC, 2010) and observed during the present study, agricultural activities have been considered as the main responsible factor which results an increase in nitrate concentration. The other sites like Amora Gedel-2 (AG2), Amora Gedel-1 (AG1), Worancha (WR), Referral Hospital-1 (RH1) and Tikur Wuha-1 (TW1) also have considerably high records of nitrate (**Table 2.3; Fig. 2.2**). These sites have open drainage canals which receive household wastes, all the city runoff and agricultural wastes which feed into the lake. The RH1 and WR sites have year round recessional farming that might be responsible for leak of excess fertilizers into the lake. A lower concentration of nitrate was recorded at Kuyu Wata-1 (KW1) and Kuyu Wata-2 (KW2) with overall mean records of 357.9 and $274.2\mu\text{g/L}$, respectively. The higher concentration of nitrate was observed in the dry season than the wet season (**Table 2.3**) although it did not show temporal significant variation (Mann-Whitney U Test $P>0.05$). The nitrite (NO_2^-) concentration was higher at WSH, RH2, AG2, TW2 and TU in the increasing order (**Table 2.3**).

Ammonium ion (NH_4^+) concentration of Lake Hawassa was higher in more human-induced impacted sites, such as AG1, WR and MK with mean values of 221.2, 152.5 and 131.9 $\mu\text{g/l}$, respectively (**Table 2.3**) even though there are no maximum contaminant level (MCL) set by the USEPA (2013) or guideline values from the World Health Organization (WHO, 2003; 2011) and European Food Safety Authority (EFSA, 2012) because it has no health risk. But WHO did recognize odour effects of ammonium ion at a concentration of 1500 $\mu\text{g/L}$ and taste effects at 35000 $\mu\text{g/L}$ on alkaline pH (WHO, 2003a and 2011; USEPA, 2013). There are water quality criteria for aquatic life expressed as total ammonia nitrogen in $\mu\text{g/L}$ at pH 7 and 20°C for acute (1hr) and chronic (30 days) exposure of 17000 and 1900, respectively, as recommended by USEPA in 2013 (USEPA, 2013). Therefore, Lake Hawassa ammonia concentration was under the maximum permissible limit (**Table 2.6**).

Chlorophyll a, Total Suspended Solid (TSS) and Turbidity

The biomass of phytoplankton was measured in-terms of chlorophyll a (Chl a) during the study period. The maximum and minimum records of Chl a was at Tikur Wuha-1 (TW1) and Referral Hospital-1(RH1) with mean values of 47.85 and 0.83 $\mu\text{g/l}$, respectively, and these are disturbed sites (**Table 2.3**). The highest record at TW1 might be from the inflow of different nutrient contaminants through River Tikur Wuha. However, the overall mean of the lake Chl a was lower when compared with previous research results (Girma Tilahun and Ahlgren, 2010; Adimasu Woldesenbet, 2015; **Table 2.6**). This might be related to increasing lake turbidity with non-algal component which inhibits photosynthetic light penetration (Dzialowski *et al.*, 2011). Once chlorophyll-a values reach 10 $\mu\text{g/L}$, the lake water would have taste and odor problems (KDHEBW, 2011).

The TSS and turbidity of the lake was higher on sites with high human disturbance (i.e. WR= 147 mg/L and AG1=142.26 mg/L of TSS; AG1=34.63 NTU and WR1= 29.95 NTU of Turbidity) and had multiple stressors (**Table 2.3**). The minimum records were found at less disturbed sites (i.e. TU=16.97 mg/l and KW1=27.00 mg/l of TSS; KW1=7.47, TU=7.25 and KW2= 6.87 NTU of turbidity). According to WHO (1984 and 2011), the permissible limit of Turbidity (<5NTU and best <1NTU) of the lake water is not recommended for drinking which is beyond the limit (**Table 2.6**). In addition, the overall mean records of TSS and turbidity were higher in the current study than on the previous record (Adimasu Woldesenbet, 2015; **Table 2.6**) and they also showed strong correlation ($r^2=0.89$) and weak correlation with Chl a (TSS & Chl a $r^2= 0.011$ and TUR & Chl a $r^2= 0.046$). Thus, it can be concluded that the water turbidity of Lake Hawassa might not be sourced from phytoplankton origin.

Table 2. 6. Comparison of some physicochemical parameters of Lake Hawassa in previous and current studies

Variables	¹ Tilahun Kibret and Harrison (1989)	² Elizabeth Kebede <i>et al.</i> (1994)	³ Zinabu Gebre-Mariam <i>et al.</i> (2002)	⁴ Girma Tilahun and Ahlgren (2010)	⁵ Adimasu Woldesenbet (2015)	Current study
T(°C)	21-25	-	-	23.5±1.8	21.23	26.4±2.39
EC(µs/cm)	860	830	846(780-965)	844±6.5	750.1	678.81±334.6
pH	8.8	8.75	8.8(8.3-9.1)	8.6±0.33	7.5	8.89±0.28
NO₃-N(µg/L)		58.3	-	2.5±2.15	5270	705.7±1416.5
NH₄+N(µg/L)		5.7	-	118±59	-	93.4±112
NO₂-N(µg/L)		34.9	-	-	40	459.9± 602
TP(µg/L)		30.0-36.2	-	34.1±13.2	370	1898.7±1.42
PO₄-(µg/L)		12.4	16.9 (0-59)	15.4±3.9	1120	599.8±621
SiO₂(mg/L)		37.9-42.6	38.6(19-65)	37.6±11.6	-	1.3215±0.695
DO(mg/L)		-	-	-	17.85	7.07±2.61
Turbidity(NTU)		-	-	-	8.44	14.95±11.9
TSS(mg/l)		-	-	-	0.0184	53.3±51.67
Chl a(µg/l)		-	-	18.77±5.2	25.45	11.85±8.73

¹Collected in 1983/84, ²data collected on 1991, ³collected between 1990-2000 (mean with range) and ⁴the data were collected at 2005 (mean ± standard deviation), ⁵ collected in 2011/12

2.4.3. The distribution of physicochemical variables within clustered sites

The hydrological and morphological feature of surface waters, along with the water physical and chemical properties, supports all the life functions of the organisms within water bodies (Dalu *et al.*, 2016). The change in these features can result a change in life supporting function of water bodies. Most of the observed changes in hydrological, morphological, physical and chemical variables of surface waters come from anthropogenic activities. This phenomenon is more pronounced in Lake Hawassa which is highly threatened by human pressure. Because of this, there were noticeable changes in physicochemical and biological elements (for biological elements see Chapter 3 and 4).

The water temperature (T), conductivity (EC), pH, total phosphorus (TP), soluble reactive phosphate (SRP), ammonium (NH_4^+), nitrate (NO_3^{2-}), Silica, total suspended solids (TSS), and turbidity (TUR) had higher mean values at highly disturbed sites (C3) and low values at minimally disturbed (C1) sites (**Table 2.4**). Similarly, Amanuel Aklilu (2011) reported that the highly disturbed sites such as Amora Gedel (in the current study AG1) and Referral Hospital (in the current study RH1) sites had higher values of soluble reactive phosphate and nitrate. The shoreline modification, lack of riparian vegetation cover, stormwater input, sedimentation, road and building construction, recreational activities, direct discharge of household wastes including toilet wastes, and recessionary farming practice might be the main factors responsible for the increasing concentration of these physicochemical parameters in highly disturbed sites. However, DO showed high mean records at minimally disturbed sites and low values at highly disturbed sites (**Table 2.4**).

The environmental parameters such as T, DO, EC, pH, TP, SRP, NO_3^{2-} , TSS and TUR showed strong significant variation along the three clustered sites ($P < 0.01$; **Table 2.5**). However, the NO_2^- , NH_4^+ and Silica did not show a statistically significant variation along the three clustered sites ($P > 0.05$; **Table 2.5**).

Generally, this study showed that there was deterioration of littoral habitat quality of the lake because of low score in vegetation quality, high stormwater inputs, low bottom substrate quality, high lakeside adverse human alternations, lack of upland buffer zone and adverse watershed land use effect. These factors directly or indirectly influence nutrient concentration of the lake. Partly because of this, the lake nutrient concentration showed an increasing trend for over two decades (**Table 2.6**). On the other hand, human pressure has been increasing at alarming rate but mitigation measures to reduce the deterioration of the lake habitat quality were limited. This may create more challenging conditions for the sustainable ecosystem functioning of the lake. Except the Hawassa Textile and Hawassa industrial park, other private or state-owned industries, resorts, hotels, institutions (like Referral Hospital of Hawassa University) and other sectors should install treatment plants to treat the wastes they generate. Besides this, there should be integrated management approach to conserve and rehabilitate the littoral side of the lake which has high human pressures from multiple stressor types (except Wabishebele Hotel site, WSH). The lake management practice on Wabishebele Hotel made some improvement on habitat quality and rehabilitated some lake segments, when compared to other sites. The practice limited human entrance, prevented stormwater discharge, practiced good wetland management that strengthened buffering capacity, stabilized shoreline, maintained good riparian vegetation cover, prevented solid wastes and

sediment entrance to the lake. As a result, the WSH site was categorized as a reference site.

Such lake management activities should be practiced in all over the littoral area of the lake by prioritizing poor habitat quality sites which are recognized as highly disturbed sites (WR, RH1 and AG1). The presence of sufficient habitats of adequate quality will have successful conservation of aquatic life, sustainable social and economic functions of the lake (Michiel, 2001). Further, it is recommended that the habitat quality and physicochemical assessment monitoring practice will have lasting results if biological elements like macroinvertebrates and diatoms, which can amplify long time changes in water quality of the lake, are included.

CHAPTER 3: ABIOTIC FACTORS AND MACROINVERTEBRATE DIVERSITY AND ABUNDANCE IN THE LITTORAL AREA OF LAKE HAWASSA, ETHIOPIA

3.1. INTRODUCTION

Because of their abundance and position as “middleman” in the aquatic food chain, macroinvertebrates play a critical role in the balance and natural flow of energy and nutrients. Macroinvertebrates are an important part of the food chain, especially for fish. Many of them feed on plankton and bacteria which are on the lower end of the food chain. They have different feeding habits some shred and eat leaves and others depend on organic matter that enters the water. As macroinvertebrates die, they decay, leaving behind nutrients that are reused by aquatic plants and other animals in the food chain. Macroinvertebrates regulate rates of primary production, decomposition, water clarity, thermal stratification, and nutrient cycling in lakes (Strayer, 2006). Moreover, they are also widely used in bio-monitoring (Sandin and Hering, 2004; Jun *et al.*, 2012; Wang *et al.*, 2015; Poikane *et al.*, 2016; Ayenew Gezie *et al.*, 2017).

Numerous macroinvertebrate species occupy particular microhabitats at various depths in lakes and at various times of the years. These spatial and temporal distributions suggest that benthic species have different preferences for particular ranges of physicochemical parameters like temperature, pH, current velocity, and types of substrate (Covich *et al.*, 1999). The microhabitats which are placed at littoral zone and benthic habitats of lentic water bodies are functionally important, providing shelter against predation and wave action, feeding zones and habitat (McGoff and Irvine, 2009) and also act as a breeding

ground for macroinvertebrates. Benthic habitats of lakes are associated with the bottom substrate and can be substantially heterogeneous in both vertical and horizontal dimensions. This heterogeneity is associated with the physical structure created by sediments, rocks, aquatic plants and woody debris from riparian habitat and steep chemical gradients within the sediments and the water column (Covich *et al.*, 1999; Rasmussen *et al.*, 2012). Therefore, abiotic factors such as physicochemical parameters have a great role in structuring the composition and abundance of the macroinvertebrates.

Physical and chemical changes are mostly accelerated by anthropogenic activities in the surrounding of the lake catchment which affect the abundance and diversity of the macroinvertebrate. Strayer (2006) maintained that many freshwater habitats have been seriously degraded by human activities and consequently, there has to be a change in the dynamism of the physicochemical parameters and on the macroinvertebrates and other biota.

Generally, understanding the ecological relationship between abiotic factors and macroinvertebrates is required to guide scientific strategies for lake ecosystem management. The littoral area of lakes is the most highly impacted region where protection and restoration actions are highly needed. This research aimed to add to the scientific knowledge about the spatial and temporal distribution of macroinvertebrate and their relationship with physicochemical in the littoral areas of Lake Hawassa, to use this information for lake management.

3.2. MATERIALS AND METHODS

3.2.1. Macroinvertebrate sampling and diversity indices

Macroinvertebrate sampling was done along a three representative lake segment with transects running from the shoreline of Lake Hawassa to 1 m depth contour (**Fig. 3.1**). Travelling Kick and Sweep along the transects with a D-Frame Net of 500 μm mesh size was employed to collect the macroinvertebrates. The bottom substrate was vigorously kicked off (~for 10 minute) to disturb it to a depth of ~5 cm and the net swept back and forth and up and down until at least 100 macroinvertebrates were collected and triplicated following OBBN procedure (Jones *et al.*, 2004). Then, the collected sample was sieved, and rinsed off to remove large rocks, plant materials and other large substrate features. The net contents were transferred to a bucket and preserved in 5% formalin and 70% ethanol for later identification. Identification and counting were done using a dissecting microscope. Taxonomic identification was made to family level using standard keys (Gerber and Gabriel, 2002; Bouchard *et al.*, 2004; Birmingham *et al.*, 2005; Oscoz *et al.*, 2011; Mbogho and Sites, 2013) in the Limnological laboratory of Addis Ababa University. The diversity indices such as taxa richness(**TaxaS**), Shannon-Weaver Index (**H'**) (Shannon and Weiner, 1963), Dominance index (**D**) and Simpson's diversity index(**SDI**), evenness (**E**), equitability_J (**J**), were calculated using PAST software. At the same time, physicochemical parameters were sampled along with the macroinvertebrate sampling (see Chapter 2 of the materials and methods section).

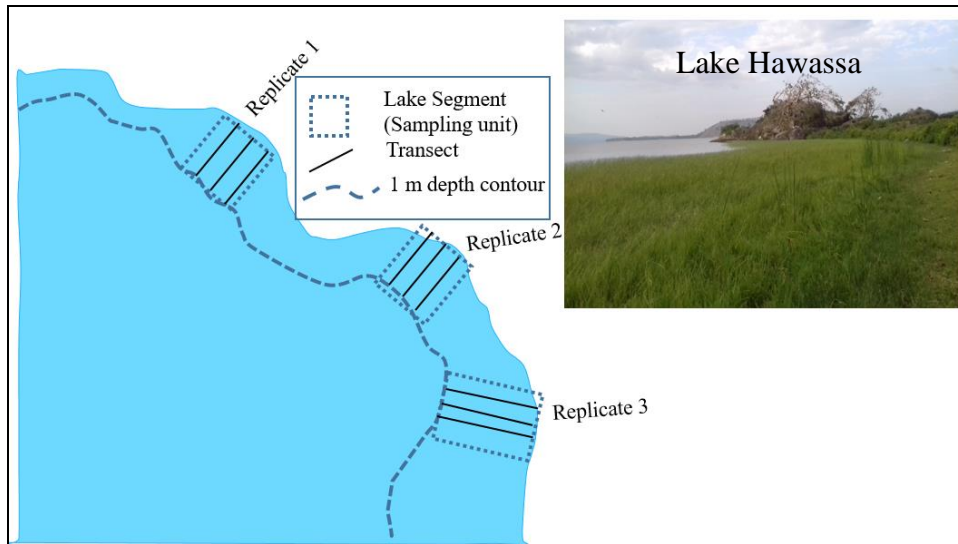


Figure 3. 1. Traveling kick and sweep method for Lake Hawassa

3.2.2. Data analysis

Spearman correlation was used to analyze the relationship between the macroinvertebrate and the physicochemical parameters. The spatial and temporal variations among the diversity indices were tested using Kruskal Wallis and Mann Whitney U tests. The relationship between the diversity indices and physicochemical was analyzed by Spearman correlation in IBM SPSS Statistical software for Windows Version 20.0 (Ho, 2013). The relationship between macroinvertebrate abundance and physicochemical variables was evaluated by redundancy analysis (RDA) using CANOCO for windows 4.5 version Software (Ter-Braak and Smilauer, 2002). RDA was used because the species data showed a linear response to the environmental variables and the longest gradient length was less than 3. For this analysis, the macroinvertebrate families which had less than 1% of total contribution were excluded. Analysis of similarity (ANOSIM, permutation N: 9999) and Similarity Percentages (SIMPER) analysis were employed to

show significant variation and quantify taxa contributing to the average Bray-Curtis similarity/dissimilarity among clustered groups (C1, C2 and C3).

3.3. RESULT

3.3.1. Spatial variation in macroinvertebrate composition, abundance and distribution

A total of 23050 individual macroinvertebrates were collected and identified. They belonged to 43 families and 14 taxonomic groups (**Table 3.1; Appendix 2**). Among these, the highest diversity record was observed in Order Hemiptera which had 10 families, followed by Odonata and Coleoptera, each contributing 5 families. However, in terms of abundance, the Odonata family of Coenagrionidae had the highest percentage of occurrence (19.53%). Next to Coenagrionidae, the Gastropoda families of Physidae and Pleuroceridae were more abundant and contributed 15.29 and 10.72 % of relative abundance, respectively (**Table 3.1 & 3.2**).

Highest macroinvertebrate family diversity was recorded at minimally disturbed (WSH, TU, KW1, and KW2) sites whereas the lowest families were found at highly disturbed sites with multiple stressors (**Fig. 3.2**). In terms of abundance, the highest record was found at reference sites of KW1 and TU with 10.33 % (2380 number of individuals) and 9.56 % (2203 number of individuals) relative abundance, respectively, whereas the minimum abundance was at highly disturbed sites of RH1 with 7.04% of occurrence.

The distribution of invertebrate taxa between the three-clustered sites had significant variation (ANOSIM $P= 0.004$, $R=0.5476$). From SIMPER analysis, the overall average dissimilarity between C1 and C2, C1 and C3, and C2 and C3 were 57.3, 68.8 and 40.69,

respectively. Over 50% variability between C1 and C2 came from Coenagrionidae, Belostomatidae, Pleuroceridae, Naucoridae and Physidae, families and between C1 and C3 was Coenagrionidae, Belostomatidae, Naucoridae and Physidae, and between C2 and C3 was Coenagrionidae, Pleuroceridae and Physidae.

Table 3. 1. Lists of identified macroinvertebrate families on littoral areas of L. Hawassa

Taxa	Family	Taxa	Family/Taxa
Odonata	Coenagrionidae(Larva)	Diptera	Chironomidae
	Aeshnidae		Tabanidae
	Cordulegastridae		Psychodidae
	Libellulidae		Culicidae
	Gomphidae		Ephemeroptera
Hemiptera	Corixidae		Caenidae
	Notonectidae		Polymitarcyidae
	Belostomatidae	Hirudinae	Glossiphoniidae
	Naucoridae		Piscicolidae
	Gelastocoridae	Gastropoda	Physidae
	Nepidae		Planorbidae
	Veliidae		Pleuroceridae
	Mesoveliidae	Lepidoptera	Pyralidae
Gerridae	Megaloptera	Corydalidae	
Coleoptera	Cicadellidae	Trichoptera	Sericostomatidae
	Curculionidae		Leptoceridae
	Hydrophilidae	Oligochaeta	Lumbriculidae
	Hydrophilidae(larvae)		Naididae
	Dytiscidae	Argulioda	Argulidae
	Dytiscidae larvae	Arachnida(class)	Tetragnathidae
	Noteridae		Pisauridae
	Noteridae larvae		Hydracarina
	Scirtidae	Dermaptera	Labiduridae

Table 3. 2. Total % occurrence of macroinvertebrate distribution in the littoral areas of L. Hawassa

Taxa	Family/ Taxa	No. id.	%
Odonata	Coenagrionidae	4502	19.53
	Aeshnidae	74	0.32
	Cordulegastridae	4	0.02
	Libellulidae	281	1.22
	Gomphidae	4	0.02
Diptera	Chironomidae	1218	5.28
	Tabanidae	31	0.13
	Psychodidae	8	0.03
	Culicidae	18	0.08
Hemiptera	Corixidae	862	3.74
	Notonectidae	851	3.69
	Belostomatidae	1850	8.03
	Naucoridae	2071	8.98
	Gelastocoridae	314	1.36
	Nepidae	65	0.28
	Veliidae	38	0.16
	Mesoveliidae	55	0.24
	Gerridae	61	0.26
	Cicadellidae	62	0.27
Coleoptera	Curculionidae	1	0.00
	Hydrophilidae	269	1.17
	Hydrophilidae (larvae)	102	0.44
	Dytiscidae	42	0.18
	Dytiscidae larvae	71	0.31
	Noteridae	161	0.70
	Noteridae larvae	6	0.03
	Scirtidae	1	0.00
	Ephemeroptera	Baetidae	1857
Caenidae		227	0.98
Polymitarcyidae		630	2.73
Hirudinae	Glossiphoniidae	70	0.30
	Piscicolidae	440	1.91
Gastropoda	Physidae	3525	15.29
	Planorbidae	203	0.88
	Pleuroceridae	2471	10.72
Lepidoptera	Pyralidae	27	0.12
Megaloptera	Corydalidae	15	0.07

Table 3. 2. Continued

Taxa	Family/ Taxa	No.Id.	%
Trichoptera	Sericostomatidae	106	0.46
	Leptoceridae	40	0.17
Oligochaeta	Lumbriculidae	245	1.06
	Naididae	12	0.05
Argulioda	Argulidae	23	0.10
Arachnida(class)	Tetragnathidae	35	0.15
	Pisauridae	51	0.22
	Hydracarina	42	0.18
Dermaptera	Labiduridae	9	0.04

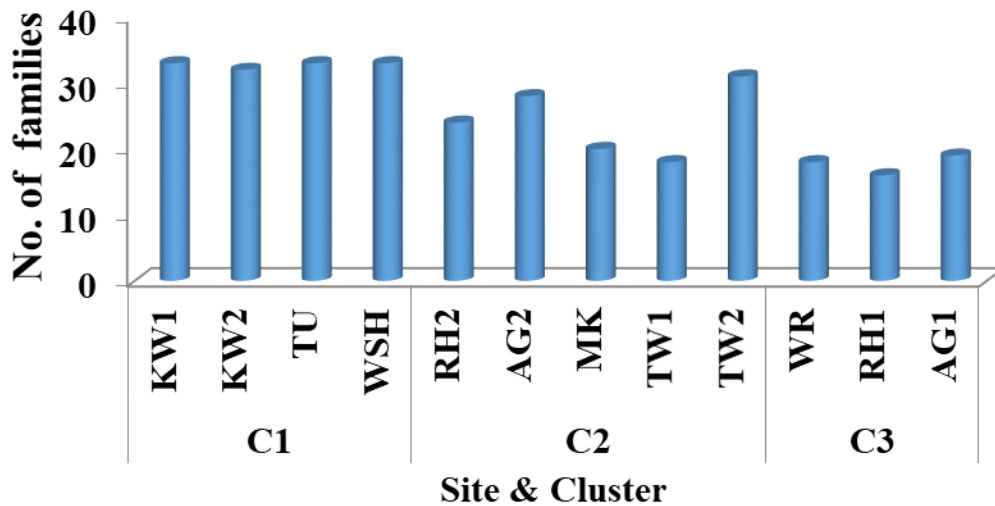


Figure 3. 2. Spatial distribution of Macroinvertebrate of littoral areas of L. Hawassa.

(KW1= Kuyu Wata-1, KW2=Kuyu Wata-2, TU= Tulu, RH2= Referral Hospital, AG2= Amora Gedel-2, WSH= Wabishebelel Hotel, MK=Mekilt, TW1= Tikur Wuha-1, TW2= Tikur Wuha-2, WR= Worancha, RH1= Referral Hospital, AG1= Amora Gedel, C1= minimally disturbed sites, C2= moderately disturbed sites, C3= highly disturbed sites)

The Shannon diversity index(H') indicated that the sites WSH and KW1 had highest diversity records with an index value of $H'= 2.5$ whereas MK showed the lowest diversity value ($H'=1.6$) (**Table 3.3**). Generally, most of the highest values were observed in minimally disturbed sites and the lowest values were in moderately and highly disturbed. The other diversity indices including H' showed a significant spatial variation (Kruskal Wallis Test $P<0.01$) except equitability (**J**) index. However, the equitability index showed the highest index values on RH2, KW1, and WSH which had values of 0.74, 0.72, 0.71 and 0.71, respectively (**Table 3.3**). All the diversity indices did not show a statistically significant temporal variation(Mann Whitney U Test $P>0.05$).

Table 3. 3. The Distribution of some macroinvertebrate diversity indices along with sampling sites of L. Hawassa (Taxa_S= taxa richness, NID=Number of individuals, D= Dominance, H'= Shannon, SDI=Simpson's diversity index, E= Evenness, J= Equitability, for other abbreviations see on **Fig 3.2**)

Cluster	Sites	Taxa_S	NID	D	SDI	H'	E	J
C1	KW1	33	2380	0.1062	0.8938	2.507	0.3717	0.717
	KW2	30	2116	0.1282	0.8718	2.343	0.347	0.6888
	TU	33	2203	0.1653	0.8347	2.155	0.2696	0.6217
	WSH	33	2012	0.1225	0.8775	2.486	0.3639	0.7109
C2	RH2	24	1902	0.1179	0.8821	2.337	0.4312	0.7353
	AG2	29	1802	0.1879	0.8121	2.14	0.2931	0.6356
	MK	19	1756	0.3519	0.6481	1.623	0.2668	0.5512
	TW1	18	1855	0.236	0.764	1.958	0.3938	0.6776
	TW2	32	1910	0.1542	0.8458	2.457	0.3647	0.709
	WR	20	1712	0.172	0.828	2.111	0.413	0.7048
C3	RH1	16	1624	0.1842	0.8158	1.908	0.4211	0.6881
	AG1	19	1778	0.2631	0.7369	1.759	0.3057	0.5975

3.3.2. Seasonal variation in macroinvertebrate diversity and abundance of L.

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10536 and 12514 individuals were recorded, with 45.71 and 54.29% of occurrence in wet and dry season, respectively. However, when the cumulative diversity records between dry and wet seasons are compared, the highest record was observed in the wet season (42 families in wet and 38 families in dry). The highest and the lowest record of the seasonal composition of macroinvertebrates ranged from 32 families at Tulu (TU) and 10 families at Referral Hospital 1(RH1) site, respectively, both in the dry season. In wet season, the highest diversity was recorded at Wabishebele Hotel (WSH) with 31 families and the lowest was RH1 which had 15 families (**Fig 3.3**).

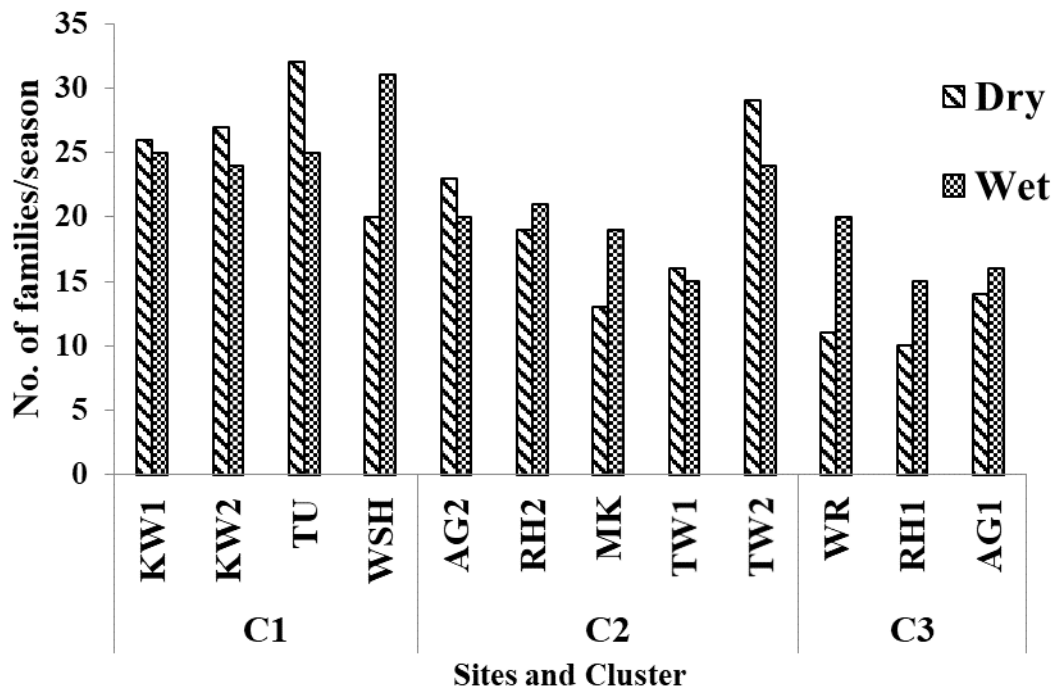


Figure 3.3. Seasonal distribution of macroinvertebrate along the study sites (see Abbreviations on Fig 3.2)

3.3.3. The relationship between the macroinvertebrate and physicochemical parameters

Results of redundancy analysis (RDA) indicated that the first axis and the second axis accounted for 62.2% and 11.8 % of the cumulative percentage of variance in species–environmental relationship, respectively (**Table 3.4**). The first axis showed a positive correlation with Soluble reactive phosphate (SRP), Nitrate (NO₃-N), Ammonium ion (NH₄⁺), Total phosphorus (TP), conductivity (EC), Turbidity (TUR), Total suspended solids (TSS), pH, and water temperature (T). Except for water temperature and pH, the other environmental parameters were strongly correlated with the axis but negatively correlated with DO (**Table 3.4; Fig. 3.4**).

Macroinvertebrate families such as Chironomidae (CHIR), Coenagrionidae (COEN), Planorbidae (PLAN), Physidae (PHYS), Pleuroceridae (PLEU), Piscicolidae (PISC), Lumbriculidae (LUMB), Libellulidae (LIBE), Corixidae (CORI) were positively correlated with soluble reactive phosphate (SRP), nitrate (NO₃-N), ammonium ion (NH₄⁺), total phosphorus (TP), conductivity (EC), turbidity (TUR) and total suspended solids (TSS). They were also more abundant and characterized sites such as AG1, RH1, WR, MK, TW1 and TW2 which had high concentrations of these nutrients and ions (EC). The other families had a negative association with these environmental variables (**Fig. 3.4**).

On the other hand, Polymitarcyidae, Hydrophilidae and Notonectidae were inversely related to SRP, NO₃, TP and EC. The second axis was positively correlated with all parameters except DO, TSS and TUR (**Table 3.4 and Fig. 3.4**). These and Baetidae,

Naucoridae, Belostomatidae and Galastocoridae families of macroinvertebrate were abundant in minimally disturbed sites when compared with others (Fig. 3.4).

Table 3. 4. The result of redundancy analysis (RDA) of macroinvertebrate versus physicochemical variables relationship including eigen-values and cumulative percentage variance of species-environment relation explained by the first two axes (**bold** correlation values are significant at **P<0.05**)

Variables	Axis 1	Axis 2
Eigenvalues	0.622	0.118
Cumulative percentage variance of species-environment relation	62.2	74
Temperature(T, °C)	0.1551	0.0766
Conductivity(EC, µs/cm)	0.762	0.0005
pH	0.1685	0.2399
Dissolved oxygen (DO, mg/l)	-0.4994	-0.1583
Total phosphorus (TP, mg/l)	0.5648	0.0742
Soluble reactive phosphate(SRP, mg/l)	0.6592	0.2884
Ammonium (NH₄⁺, mg/l)	0.7171	0.236
Nitrate (NO₃-N, mg/l)	0.527	0.1586
Total suspended solids(TSS, mg/l)	0.4974	-0.1171
Turbidity (TUR, NTU)	0.4982	-0.059

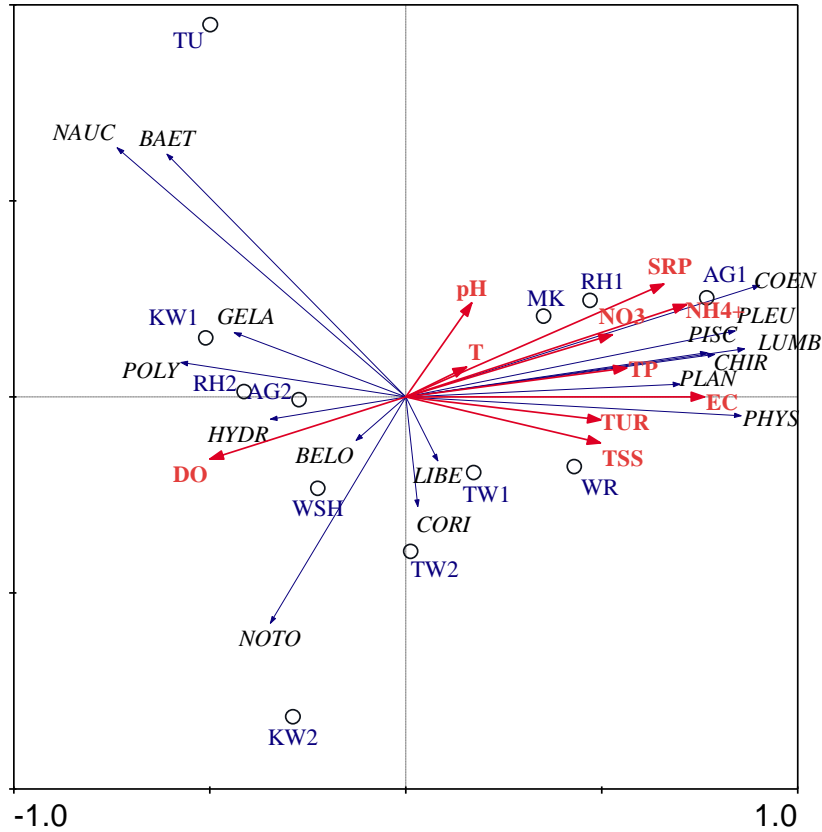


Figure 3. 4. Tri-plot of the first two axes of redundancy analysis (RDA) for macroinvertebrate and physicochemical parameters. (Abbreviations: DO- Dissolved Oxygen; EC- conductivity, TP- Total phosphorus, SRP- Soluble Reactive phosphate and Temp- Temperature, CHIR- Chironomidae, COEN- Coenagrionidae, PLAN- Planorbidae, PHYS- Physidae, PLEU- Pleuroceridae, PISC- Piscicolidae, LUMB- Lumbriculidae, LIBE- Libellulidae, CORI-Corixidae, NOTO-Notonectidae, BELO-Belostomatidae, HYDR-Hydrophilidae, POLY-Polymitarciidae, GELA-Gelastocoridae, NAUC-Naucoridae and BAET-Baetidae; and see other abbreviations on Fig 3.2).

Conductivity (EC), Total Suspended Solids (TSS) and Turbidity showed negatively correlated and significantly varied with the taxa richness (Taxa S) and Shannon diversity index (H'), whereas Nitrate (NO_3^-) had positively correlated and significant variation with Taxa S and H' (Table 3.5).

Table 3. 5. Spearman's correlation coefficient between selected physicochemical factors and macroinvertebrates diversity and richness (Taxa richness =Taxa S; Shannon diversity index= H' ; Simpson's diversity index= **SDI**, Evenness=E, Equitability =J)

Parameters	Taxa S	H'	SDI	E	J
Temperature (T, °C)	-0.257	-0.203	-0.176	-0.18	0.014
Conductivity (EC, μs/cm)	-0.650*	-0.622*	-0.256*	0.124	-0.287
pH	-0.309	-0.301	-0.045	0.065	-0.280
Dissolved Oxygen(DO, mg/l)	0.120	0.159	-0.015	0.168	0.168
Total Phosphorus (TP, mg/l)	0.428*	0.410**	-0.319	-0.289	0.392
Soluble reactive phosphate (SRP, mg/l)	0.558*	0.459	-0.043	-0.025	0.140
Ammonium ion (NH_4^+, mg/l)	-0.336	-0.501	0.040	0.032	0.476
Nitrite(NO_2^-, mg/l)	0.593*	0.557	0.095	-0.02	0.392
Nitrate(NO_3^-, mg/l)	0.745**	0.641*	-0.084	-0.114	0.245
TSS (mg/l)	-0.815**	-0.718**	-0.116	-0.509*	-0.260
Turbidity (NTU)	-0.668*	-0.734**	-0.264**	-0.153	-0.441

* Significant at 0.05 & **Significant at 0.01 level (2-tailed)

3.4. DISCUSSION

3.4.1. Spatial variation in macroinvertebrate diversity and abundance in L.

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43 families belonging to 14 taxonomic groups of macroinvertebrates were recorded during the sampling period (**Table 3.1, Appendix 2**). When compared with Tilahun Kibret and Harrison (1989) and Amanuel Aklilu (2011) records (around 27 and 38 families, respectively), there was higher diversity observed in the present study. However, there were records of different orders and families not observed in the current study like the Ostracoda in Tilahun Kibret and Harrison (1989) and Plecoptera families in Amanuel Aklilu (2011). The Trichoptera families were also missed in the report of Amanuel Aklilu (2011) while recorded in the Tilahun Kibret and Harrison (1989) and in the current study.

The order Hemiptera had highest diversity record observed in current study and Tilahun Kibret and Harrison (1989) although there was a difference in number and types of recorded families (i.e. 10 and 8 families in the current study and Tilahun Kibret and Harrison (1989), respectively; **Table 3. 6**). The Ostracoda were not found in the current study but were abundant in the earlier records. But in the current study, the Odonata family of Coenagrionidae had the highest abundance and contributed over 19 % relative occurrence in sites having high coverage of submerged macrophytes (**Table 3.2**). The abundance of Coenagrionidae (Zygoptera) in the present study could be due to their shorter life cycle and widespread distribution and tolerant to wide range of habitat (Narender *et al.*, 2016) and also favored by the macrophyte coverage of the lake due to

their endophytic oviposition habit, food source and concealment from predators as reported in Osborn (2005) and Iwata and Watanabe (2009). In addition, they can tolerate fairly anoxic conditions if there is vegetation cover (Osborn, 2005). The findings of Beteal Assefa (2010) on the macroinvertebrate of Lake Hayq supports the above idea that showed the macroinvertebrate diversity and abundance of the lake had a positive association with the macrophyte vegetation cover. The highest abundance of Coenagrionidae was also observed in Lake Kariba of Zimbabwe (Phiri *et al.*, 2012).

The second dominant groups were the Gastropod families of Physidae and Pleuroceridae which were more dominant in disturbed sites of the lake. The previous study of Amanuel Aklilu (2011) also reported that these two families of Gastropod groups were dominant in his collection of macroinvertebrates of the lake next to Hemipteran families of Naucoridae and Notonectidae.

Higher spatial distribution of macroinvertebrate family diversity was observed at minimally (C1) disturbed sites and lower at moderately (C2) and highly disturbed (C3) sites with some irregular trends (**Fig 3.2**). Generally, as the disturbance increased, the diversity of the macroinvertebrates decreased. Similar trends were also observed from wetlands of Lake Tana (Ayenew Gezie *et al.*, 2017), head of Blue Nile River (Assefa Wosnie and Ayalew Wondie, 2014), Kebena and Akaki Rivers (Abebe Beyene *et al.*, 2009), Upper Awash River (Fasil Degefu *et al.*, 2013) and Tikur Wuha River (Birnes Abay, 2007). The lowest diversity was recorded at Referral Hospital 1 (RH1 family number =16; **Fig. 3.2**) which is highly disturbed littoral area (i.e. high construction activities, wastes from the hospital, stormwater drains, recession farming, high shoreline disturbance, most of the shore area has been littered by non-biodegradable

wastes/plastics etc.). Similarly, the other disturbed sites such as Tikur Wuha 1(TW1), Meklit (MK), Amora Gedel (AG1) and Worancha (WR) had lower diversity records as well.

The SIMPER result showed that the reference group of macroinvertebrate showed more than 50% similarity and the non-reference (moderate and high disturbed) groups had also >60% similarity. However, there was highest dissimilarity percentage (68.8%) between the reference and highly disturbed sites. The environmental variation caused by human pressure might be the responsible factor for the major dissimilarity of these groups. Similar findings also observed in Lake Tailu of China (Cai *et al.*, 2011). Hence, this may lead to variation on the distribution of macroinvertebrate families between the three-disturbance levels. As a result, in this study, the distribution of macroinvertebrate assemblage between the three-clustered sites had significant variation (ANOSIM P= 0.004, R=0.5476). Besides this, the SIMPER analysis showed the overall average dissimilarity of macroinvertebrate taxa between reference and moderate disturbance, reference and high disturbance, and moderately and highly disturbed sites were 57.3, 68.8 and 40.69, respectively. The macroinvertebrate families such as Coenagrionidae, Belostomatidae, Naucoridae and Physidae were responsible for the highest dissimilarity percentage observed between reference and highly disturbed groups, and these can be incorporated as metrics in development of multimetric index.

The Shannon diversity index(H') result indicated that the minimally disturbed sites had the highest diversity records whereas the disturbed site showed the lowest diversity value (**MK: H' =1.6; Table 3.3**). It is mostly common when there is anthropogenic activities in littoral and riparian zones of a lake which would probably increase habitat disturbance

and degradation that results in reduction of family diversity and also extinction of families in severe degradation (Strayer, 2006). Based on H' index classification of Staub *et al.* (1970), the pollution status of the sampling stations ranged from light pollution to moderate pollution since the H' value was found between 1.6 to 2.5 (**Table 3.7**). Therefore, all minimally disturbed sites, 3 of moderately disturbed (RH2, AG2 and TW2) and WR of highly disturbed sites were categorized under light pollution while MK and TW1 of moderately and AG1 and RH1 of highly disturbed sites were classified under moderate pollution (**Table 3.7**). Generally, the richness, Simpson index, Shanon diversity index and Equitablity_J index decreased with increasing disturbance whereas dominance (D) index increased with increasing disturbance except for a few observations (**Table 3.3**). Similar responses were observed in different studies where, as the degree of disturbance increased, there was decrease in the above indices except for the dominance index (Trigal-Dominguez *et al.*, 2010; Fasil Degefu *et al.*, 2013; Aschonitis *et al.*, 2016).

Table 3. 6. The macroinvertebrate diversity of L. Hawassa at 1989 and current study (+=presence and -= absence).

Taxa	Family/ Taxa	Tilahun Kibret and Harrison (1989)	Remark	Not found in the current study	
Odonata	Coenagrionidae	+	Reported as Zygoptera	Ostracoda	
	Aeshnidae	+			
	Cordulegastridae	+	Reported as Anisoptera		
	Libellulidae	+			
	Gomphidae	+			
Diptera	Chironomidae	+	Ceratopogonidae/Diptera Stratiomyidae/Diptera		
	Tabanidae	-			
	Psychodidae	-			
	Culicidae	+			
Hemiptera	Corixidae	+			
	Notonectidae	+			
	Belostomatidae	-			
	Naucoridae	+			
	Gelastocoridae	-			
	Nepidae	+			
	Veliidae	+			
	Mesoveliidae	+			
	Gerridae	+			
	Cicadellidae	-			
	Coleoptera	Curculionidae		-	
		Hydrophilidae		+	
		Hydrophilidae(larvae)		-	
Dytiscidae		+			
Dytiscidae(larvae)		+			
Noteridae		-			
Noteridae(larvae)		-			
Scirtidae		-			
		-			
Ephemeroptera	Baetidae	-			
	Caenidae	+			
	Polymitarcyidae	-			
Hirudinae	Glossiphoniidae	+			
	Piscicolidae	-			
Gastropoda	Physidae	-			
	Planorbidae	+			
	Pleuroceridae	-			
Lepidoptera	Pyralidae	-			
Megaloptera	Corydalidae	-			
Trichoptera	Sericostomatidae	+	Reported as Trichoptera		
	Leptoceridae	+			
Oligochaeta	Lumbriculidae	-			
	Naididae	+			
Argulioda	Argulidae	+			
Arachnida	Tetragnathidae	-			
	Pisauridae	-			
	Hydracarina	+			
Dermoptera	Labiduridae	-		Oribatidae/Acari	

Table 3. 7. Relationships between species diversity and pollution status according to Staub *et al.* (1970)

Diversity(H')	Pollution status	Sampling sites
3.5-4.5	Slight pollution	
2.0-3.0	Light pollution	KW1, KW2, TU, WSH, RH2, AG2, TW2 & WR
1.0-2.0	Moderate pollution	MK, TW1, RH1 & AG1
0.0-1.0	Heavy pollution	

3.4.2. Seasonal effects in macroinvertebrate diversity, abundance and distribution

There was a slight reduction in the number of individuals of macroinvertebrate in the wet season. This might be because of the runoff from the lake catchment and fluctuation of the water level (Yeman Gebre-Egziabher, 2004) which may lead to habitat destabilization. As a result, macroinvertebrate colonization may be difficult. Abundance of macroinvertebrates was augmented in the dry season because of food availability and shelter (Righi-Cavallaro *et al.*, 2010).

In contrast to abundance of macroinvertebrates, the family composition of the wet season was slightly more diverse than the dry season (42 families in wet and 38 families in dry). This is most likely due to the increase in habitat heterogeneity and input of allochthonous material during this period (Santana *et al.*, 2015; Kaboré *et al.*, 2016b). The composition of macroinvertebrate was generally higher at the sites which have been minimally disturbed (**Fig. 3.3**) in both seasons. As generally accepted, the impacts of anthropogenic activities have effects on the habitat quality and water chemistry of lakes which in turn

has an effect on the invertebrate communities. Impairment of habitat and water chemistry can lead to a reduced diversity of aquatic macroinvertebrates (Hepp *et al.*, 2013). Coenagrionidae, Chironomidae, Corixidae, Notonectidae, Belestomatidae, Baetidae, Physidae and Pleuroceridae families were the most persistent during the sampling period (Table 3.2).

3.4.3. Relation of macroinvertebrate distribution to physicochemical parameters

The distribution and abundance of macroinvertebrates are directly related to different environmental factors such as food availability and quantity, sediment type, substrate and water quality (Cai *et al.*, 2011; Virbickas *et al.*, 2011; Trichkova *et al.*, 2013). In this study, it was found that Chironomidae (CHIR), Coenagrionidae (COEN), Planorbidae (PLAN), Physidae (PHYS), Pleuroceridae (PLEU), Piscicolidae (PISC), Lumbriculidae (LUMB), Libellulidae (LIBE), Corixidae (CORI) had a positive association with soluble reactive phosphate (SRP), Nitrate (NO₃-N), Ammonium ion (NH₄⁺), total phosphorus (TP), conductivity (EC), Turbidity (TUR) and Total suspended solids (TSS). They were more abundant to sites which had moderate and high disturbance (AG1, RH1, WR, MK and TW1; Fig. 3.4). Most of these sites had a high concentration of organic matter, nutrients and ions (EC) that come from agricultural, industrial, domestic wastes, stormwater inputs etc. These allochthonous sources of organic matters and nutrients may increase the nutrient load of the lake water and would cause to increase the algal biomass which in turn increases the herbivore macroinvertebrates (Tank *et al.*, 2010). As a result, the tolerant macroinvertebrates which need higher concentration of nutrients could have a chance to increase in abundance.

Water temperature influences on both the chemical and biological characteristics of surface water. In this study, the abundance of macroinvertebrate families such as Chironomidae (CHIR), Coenagrionidae (COEN), Planorbidae (PLAN), Physidae (PHYS), Pleuroceridae (PLEU), Piscicolidae (PISC), Lumbriculidae (LUMB), Libellulidae (LIBE), Corixidae (CORI) had positive association with the water temperature (T) however they did not show a strong correlation (**Fig. 3.4**). Haidekker and Hering (2008) found that water temperature could somehow explain the quantitative differences of the benthic macroinvertebrate community.

As stated by Gerber and Gabriel (2002) and Bouchard *et al.* (2004), these macroinvertebrate families are considered as pollution tolerant (with a range of moderate to high tolerant families) and are common in disturbed environment. In this study, the above macroinvertebrates showed increase with the increase of nutrients and disturbance. However, many authors have argued that the Dipteran Chironomidae families cannot be considered as pollution tolerant because there are some species which are less tolerant to pollution (Eggermont and Verschuren, 2004; Getachew Beneberu *et al.*, 2014). However, in this study, relative abundance of Chironomidae showed an increasing trend as the disturbance of the sampling station increased. Similar finding also observed in previous study of the lake (Amanuel Aklilu, 2011) and shallow subtropical lake (Lake Taihu) (Cai *et al.*, 2011) and wetlands (Lu *et al.*, 2018) of China and small water bodies of Lake Victoria Basin, Kenya (Ngodhe *et al.*, 2014).

The freshwater Gastropoda families of Planorbidae, Physidae and Pleuroceridae are known for their tolerance to pollution and habitat disturbance (Mandaville, 2002; Bouchard *et al.*, 2004; Birmingham *et al.*, 2005; Strong *et al.*, 2008). Similarly, in the

current study, these non-insect families were found more dominant in sites with high human disturbance and relatively high concentration of nutrients (**Fig 3.4**). Ngodhe *et al.* (2014) conducted a research on the relationship between water quality parameters and macroinvertebrates diversity. They found that high periphyton biomass due to nutrient enrichment and sedimentation in small water bodies (Dams) of Lake Victoria Basin, Kenya, may favour some taxa such as Gastropoda (Snails), Diptera and Oligochaetes.

On the other hand, Polymitarcyidae, Hydrophilidae and Notonectidae were inversely related to SRP, NO₃, TP and EC; however, they showed a positive association with DO (**Table 3.4 and Fig. 3.4**). As noted by Mandaville (2002) and Bouchard *et al.* (2004), these organisms inhabit areas which are relatively less disturbed and with less polluted characteristics. They showed a positive relationship with DO even though they did not show a strong correlation in the present study. They also potentially characterized sites with minimally disturbed with a low concentration of nutrients and ions (**Fig. 3.4**). This is also supported by other researchers such as Hepp *et al.* (2013) and Kaboré *et al.* (2016a) who reported that the Ephemeroptera (Polymitarcyidae) and Coleoptera (Hydrophilidae) groups were less tolerant to pollution and habitat disturbance. Besides these families, Baetidae, Notonectidae, Naucoridae, Belostomatidae and Galastocoridae families were also abundant sites with minimal disturbance. They were also abundant with a relatively high concentration of DO as illustrated in **Fig. 3.4**.

Conductivity (EC), Total suspended solids (TSS) and Turbidity were negatively correlated and significantly varied with the taxa richness (TaxaS) and Shannon diversity index (**H'**; **Table 3.5**). Rashid and Pandit (2014) and Yazdian *et al.* (2014) also found that the turbidity and TSS had a negative relation with Taxa S and **H'**. However, Nitrate

(NO₃-N, mg/l) had positively correlated and significant variation with Taxa S and **H'** ($r^2=0.745$, $p<0.001$ and $r^2=0.641$, $p<0.05$, respectively; **Table 3.5**).

Generally, the current study demonstrated that macroinvertebrate abundance and diversity were affected by water chemistry and the habitat quality of the sampling stations (**Fig. 3.4**). The sites exposed to high intensity of human disturbance demonstrated less diversity and abundance of macroinvertebrates than those of the minimally disturbed sites. In addition, Polymitarcyidae, Baetidae, Hydrophilidae, Notonectidae, Naucoridae, Belostomatidae and Galastocoridae families can be considered as indicator organisms as they were abundant in minimally disturbed sites and can be used as metrics for further biomonitoring of Lake Hawassa water quality, although further study is recommended. Similarly, the macroinvertebrate assemblages which were more abundant in non-reference (moderately and highly disturbed) sites can be considered for bioassessment study in the lake. Since Taxa_S and **H'** were significantly correlated with ecologically important physicochemical parameters, they might be considered as important metrics for biomonitoring activity of Lake Hawassa.

CHAPTER 4: BENTHIC DIATOMS DIVERSITY, ABUNDANCE, AND DISTRIBUTION IN THE LITTORAL AREA OF LAKE HAWASSA, ETHIOPIA

4.1. INTRODUCTION

Diatoms have been playing a major role in chemical and biological processes for millions of years. One-fifth to one-fourth of the organic carbon has been fixed with these tiny organisms which should also be advocated for their reduction of greenhouse gas emission. They produce up to a quarter of the total oxygen production on earth and are a major food source for aquatic microorganisms and insect larva (Round *et al.*, 1990). They also contribute up to 40% of primary production in lakes and oceans, which is more than that of all the tropical rainforests on earth (Kiran *et al.*, 2016). They are also the best sequesterers of CO₂ and release more oxygen to the system (Benoiston *et al.*, 2017).

Moreover, diatoms have been used for water quality testing. Research done by Dixit *et al.* (1999) and Stenger-Kovacs *et al.* (2007) showed that diatoms which inhabit littoral areas of lakes and have periphyton behaviour can be used for determining long-term environmental conditions for the particular area in which they are collected. Fortunately, diatoms are ecologically diverse (in almost every freshwater habitat, the dead and living diatoms can be found in the substrate), have high reproduction rate which makes them respond quickly to environmental changes, and have specific sensitive values for the various levels of pollution (Dixit *et al.*, 1999).

Diatoms have also been used for the phycoremediation to remove nutrients which is a novel and cost-effective method of water treatment (Kiran *et al.*, 2016) and this is the most promising benefit of diatoms in bioremediation. They can consume all forms of nitrogen (nitrate, nitrite, urea, and ammonia) and phosphorus faster than other algae. The efficient removal ability of diatoms has challenged the growth of other algae and dominated phytoplankton dynamics in the system (Kiran *et al.*, 2016).

Diatoms have also huge economic and industrial importance. Millions of years of diatom frustules' being naturally fossilized have created huge deposits of these shells or diatomaceous earth. Some industry products such as abrasives, paints, filtering aids and cleaners are constituted from these deposits. It is estimated that a significant portion of the world's oil supply comes from diatom fossil beds (Vrieling *et al.*, 2003; Boittelle *et al.*, 2009).

Generally, diatoms have enormous uses in addition to the above-stated issues. It is advisable to have record lists of diatoms to the lowest possible taxa in order to harvest those functions of diatoms although identification and recording of their diversity remain challenging.

In previous studies of L. Hawassa, most of the diatoms studied were the planktonic diatoms (Elizabeth Kebede and Amha Belay, 1994; Girma Tilahun, 2006), and epissamic diatoms (Gasse, 1986; Telford *et al.*, 1999). But, the information on attached diatoms have been still limited and not independently studied. The objective of this study was, therefore, to fulfill this gap and add up to the scientific knowledge about these diatom assemblages, and assess the abiotic factors associated with the spatial and temporal

variation of attached diatoms in the littoral zone of Lake Hawassa, to use this information for biomonitoring purpose.

4.2. MATERIALS AND METHODS

4.2.1. Sampling of benthic diatoms

The physicochemical and diatoms sampling was done on 12 sampling station at the same time (see chapter 2 for physicochemical sampling procedure). Diatom sampling was done from cobbles and macrophyte from the littoral areas of Lake Hawassa at the maximum depth of 1m. Diatom sampling was done by taking cobbles and macrophyte substrates from the bottom of the lakeshore side of all sites randomly. Diatom samples were a composite of each site obtained pooled from a number of stones (typically a minimum of five stones with upper surface areas of cobble~25cm²) and macrophytes submerged stems (Five stems of macrophytes with 5cm length) usually with a minimum water depth requirement of 40-50 cm (King *et al.*, 2005; Martin and Fernandez, 2012).

About 50 ml of lake water poured into a tray and then remove the biofilm from the upper surface of the cobble and macrophyte by scrubbing vigorously with the toothbrush. Rinsing the toothbrush was done with lake water regularly. The water in the tray had a brown or dark brown hue as the biofilm is washed into it. Finally, the suspension was swirled in white-tray so any settled diatoms were re-suspended and all contents poured into a well-labelled 150ml plastic bottle. Then 70% ethanol was added to preserve the diatoms.

Diatoms were identified to species level through morphological features of their frustules by removing their organic carbon content. 5-10ml of the sample was taken from the

sample bottle to a beaker and homogenized by shaking. Concentrated sulfuric acid and potassium dichromate were added to remove the organic portion of the diatom frustule. Then, the acid in the sample was continually cleaned with distilled water and the supernatant was poured after the suspended diatom cells settled down. Then, a drop of cleaned diatom frustules sample was taken on a microscopic slide and placed on the hot plate. After it dried, the permanent slide was prepared by adding Naphrax mountant (refractive index of 1.73) on the coverslip and put into the dried slide. The bubbles were removed by pressing the coverslip with a great care.

The permanently prepared slide was taken to Environment Center of Lancaster University, Lancaster City, UK, for identification. Diatom frustules were examined with Carl Zeiss Axioskop light microscope at 1000x magnification, under oil immersion objective using bright-field illumination with a green filter to increase contrast.

The identification of diatoms was done morphologically using the shape, length and breadth of diatom frustules, raphe, number of striae and their arrangement. The identification was assisted by standard identification keys, manuals and publications (Gasse, 1986; Krammer and Lange-Bertalot, 1986; Krammer and Lange-Bertalot, 1988 and 1991a and b; Kelly, 2000; Krammer, 2000; Sonneman *et al.*, 2000; Taylor *et al.*, 2007; Taylor and Cocquyt, 2016). In addition, the expertise judgment and guidance and ample experience of Prof. Philip Barker from Environment Center of Lancaster University was very crucial in the identification of diatoms at the species level. Diatom valves were counted using a Carl Zeiss Axioskop light microscope at 1000x magnification. 500 valves per slide were counted and relative abundance in percentage was calculated for each species.

4.2.2. Data analysis

The Kruskal-Wallis and Mann-Whitney U tests (SPSS version 20) were employed to show the spatial and temporal variation of diatoms species abundance. The relationship between diatom abundance and physicochemical variables was evaluated by Redundancy analysis (RDA) using CANOCO for windows 4.5 version Software (Ter-Braak and Smilauer, 2002). The response of species data to environmental variables was checked by detrended correspondence analysis (DCA) which showed the species data had linear responses to environmental variables and the range of variation of the environmental variables was narrow, as observed from the length of longest gradient (1.626) which was less than 3 (Lepš and Šmilauer, 2003). Therefore, it is recommended to use RDA ordination diagram over others (Ter-Braak and Smilauer, 2002; Lepš and Šmilauer, 2003). Diatom diversity and richness indices such as Taxa richness (Taxa S), Shannon diversity index (H'), Evenness (E) and Equitability (J) indices were analysed using the Past software 3.15. One way ANOVA was employed to show spatial variation and t-test was used to test temporal variation of these diversity indices. The diatom species which contributed <1% relative abundance were not included in the RDA analysis for ease of handling of the data sets. Analysis of similarity (ANOSIM, permutation N: 9999) and Similarity Percentages (SIMPER) analysis were employed to show significant variation and quantify taxa contributing to the average Bray-Curtis similarity/dissimilarity among clustered groups (C1, C2 and C3).

4.3. RESULT

4.3.1. Temporal and spatial distribution of diatoms in Lake Hawassa

500 diatom valves were counted from each slide which was sampled from 12 sites within two seasons (dry and wet). A total of 105 species of diatoms belonging to 39 genera were identified (**Table 4.1; Appendix 3**). The highest (at KW2 =45) and the lowest (at RH1= 21) species record were observed both in the dry season (**Fig. 4.1**) of the minimally and highly disturbed sites of the lake, respectively. The species composition in the wet season was highest at KW2 and lowest at AG1 with records of 42 and 24, respectively. In general, the diversity trend was higher in minimally disturbed (C1) sites as compared with the moderately (C2) and highly disturbed (C3) sites (**Fig. 4.1**). Among the 39 genera *Nitzschia* spp. the most diverse groups which accounted for 15 species and followed by *Gomphonema* spp. which had 8 species. *Cymbella* spp., *Epithemia* spp. and *Navicula* spp. were the third diverse groups which had 5 species each. The other diatoms genera were represented by less than five species. In terms of species relative abundance, *Nitzschia frustulum* Kützing was the most dominant with relative occurrence of 17.5 %. *Achnantheidium minutissimum* Kutzing (9.4%), and *Gomphonema parvulum* Kutzing (8.4%) were the second and the third in their relative abundance, respectively (**Table 4.5**). However, most of the other species recorded were rare in their relative occurrence.

Achnantheidium exiguum (Grunow) Czarnecki, *Gomphonema gracile* Ehrenberg, *Navicula phyllepta* Kutzing, *Nitzschia etoshensis* Cholnoky showed significant temporal variation ($p < 0.050$). Similarly, from all diatom records only *Achnantheidium minutissimum* (Kutzing) Czarnecki 1994, *Aulacoseira muzzanensis* (F. Meister) Krammer, *A. ambigua*

(Grunow) Simonsen, *Navicula tenella* Brebisson, *Nitzschia frustulum* Kützing, *Staurosira brevistriata* Grunow, and *Staurosira construens* Ehrenberg species showed significant spatial variation ($P < 0.05$).

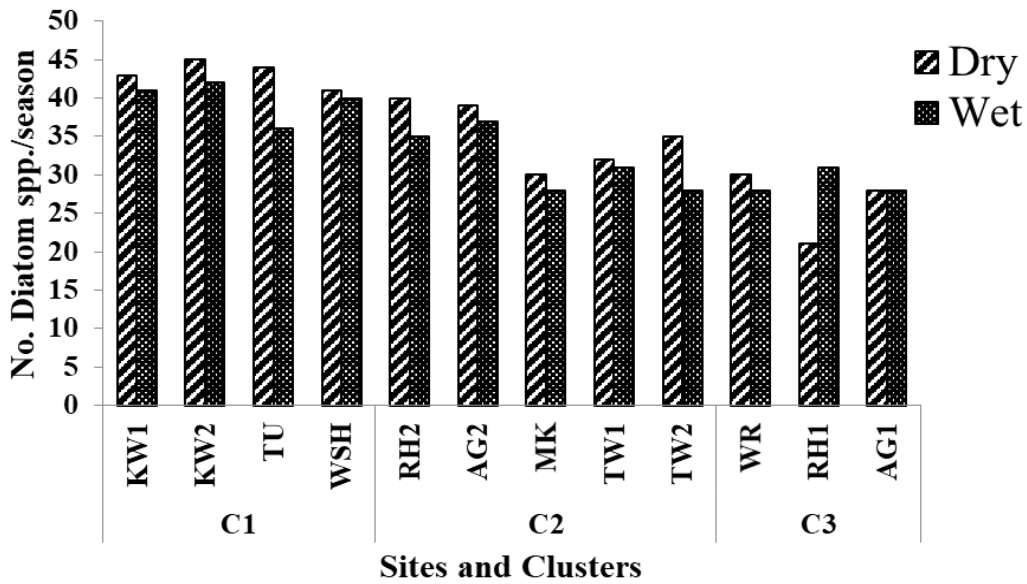


Figure 4. 1. Temporal and spatial distribution of diatom species of littoral areas of L. Hawassa. Abbreviations: KW1= Kuyu Wata-1, KW2= Kuyu Wata-2, TU= Tulu, RH2= Referral Hospital, AG2= Amora Gedel-2, WSH= Wabishebelel Hotel, MK=Mekilt, TW1= Tikur Wuha-1, TW2= Tikur Wuha-2, WR= Worancha, RH1= Referral Hospital, AG1= Amora Gedel

The distribution of diatom spp. between the three-clustered sites had significant variation (ANOSIM $P=0.0001$, $R=0.62$). From SIMPER analysis, the overall average dissimilarity diatom species between C1 and C2 was 47.37, in which *Achnantheidium minutissimum* (AMIT), *Nitzschia frustulum* (NIFR), *Navicula tenella* (NTNL), *Encyonopsis microcephala* (ENCM) and *Aulacoseira ambigua* (AAMB) contributed 51.5% for their difference. The average dissimilarity C1 and C3 was 53.27%, in which 50.6% of their difference came from AMIT, NIFR, *Gomphonema parvulum* (GPAR) and NTNL. The average dissimilarity between C2 and C3 was 48%, in which GPAR, NIFR, *Cymbella kappii* (CKPP), AAMB, *Nitzschia palea* (NPAL) and *Fragilaria capucina* (FCAP) accounted 49.9% for their difference.

Table 4. 1. Lists of identified diatom species from littoral areas of L. Hawassa

Codes	List of Diatom spp.
AIPX	<i>Achnanthes impexa</i> Lange-Bertalot 1989
AEXG	<i>Achnanthidium exiguum</i> (Grunow) Czarnecki 1994
AMIT	<i>Achnanthidium minutissimum</i> (Kutzing) Czarnecki 1994
AFCR	<i>Afrocymbella reichardtii</i> Krammer 2003
ALIB	<i>Amphora libyca</i> Ehrenberg 1841
AOAF	<i>Amphora ovalis</i> var. <i>affinis</i> (Kutzing) Van Heurck
AVEN	<i>Amphora veneta</i> Kutzing 1844
ASPH	<i>Anomoeneis sphaerophora</i> Pfitzer
AMUZ	<i>Aulacoseira muzzanensis</i> (F.Meister) Krammer 1991
AAMB	<i>Aulacoseira ambigua</i> (Grunow) Simonsen
AUGR	<i>Aulacoseira granulate</i> (Ehrenberg) Simonsen 1979
AGVA	<i>Aulacoseira granulata</i> var. <i>valida</i> (Hustedt) Simonsen 1979
CBAC	<i>Caloneis bacillum</i> (Grunow) Cleve 1894
CPLA	<i>Cocconeis placentula</i> Ehrenberg 1838
CPLI	<i>Cocconeis placentula</i> var. <i>lineata</i> (Ehrenberg) Van Heurck 1885
CAMB	<i>Craticula ambigua</i> (Ehrenberg) DG Mann in Round, 1990
NPSH	<i>Craticula buderi</i> (Hustedt) Lange-Bertalot in U.Rumrich, 2000
CRCU	<i>Craticula cuspidata</i> (Kutzing) D.G.Mann
CDUB	<i>Cyclostephanos dubius</i> (Fricke) Round
CKRM	<i>Cyclotella krammeri</i> Hakansson 1990
CMEN	<i>Cyclotella meneghiniana</i> Kutzing 1844
COCE	<i>Cyclotella ocellata</i> Pantocsek 1901
CCIS	<i>Cymbella cistula</i> (Ehrenberg) O. Kirchner 1878
CKPP	<i>Cymbella kappii</i> (Cholnoky) Cholnoky 1956
CLEP	<i>Cymbella leptoceros</i> (Ehrenberg) Kutzing
CNIS	<i>Cymbella neocistula</i> var. <i>islandica</i> Krammer 2002
CTGL	<i>Cymbella turgidula</i> Grunow in A. Schmidt et al. 1875
DSMI	<i>Diploneis smithii</i> (Brebisson) Cleve 1894
ECAE	<i>Encyonema caespitosum</i> Kutzing 1849
ENMU	<i>Encyonema muelleri</i> (Hustedt) D.G. Mann in Round,
ESLE	<i>Encyonema silesiacum</i> (Bleisch) D.G.Mann in Round
ENCM	<i>Encyonopsis microcephala</i> (Grunow) Krammer 1997
EADN	<i>Epithemia adnata</i> (Kutzing) Brebisson 1838
EAAL	<i>Epithemia argus</i> var. <i>alpestris</i> (W. Smith) Grunow 1860
EFRI	<i>Epithemia frickei</i> Krammer 1987
EHYN	<i>Epithemia hyndmanii</i> W. smith 1850
ESOR	<i>Epithemia sorex</i> Kutzing
EBLL	<i>Eunotia bilunaris</i> var. <i>linearis</i> (Okuno) Lange-Bertalot & Norpel
EFOR	<i>Eunotia formica</i> Ehrenberg 1843

Table 4.1. (Continued)

Codes	List of Diatom spp.
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EINC	<i>Eunotia incisa</i> W. Smith ex Gregory 1854
EMON	<i>Eunotia monodon</i> Ehrenberg var. <i>monodon</i>
FCAP	<i>Fragilaria capucina</i> Desmazieres 1830
FTEN	<i>Fragilaria tenera</i> (W. Smith) Lange-Bertalot 1980
GANT	<i>Gomphonema angustum</i> C. Agardh 1831
GAUG	<i>Gomphonema augur</i> Ehrenberg 1841
GGRA	<i>Gomphonema gracile</i> Ehrenberg 1838
GIPL	<i>Gomphonema intricatum</i> var. <i>pulvinata</i> (Braun) Grunow
GOLI	<i>Gomphonema olivaceum</i> (Hornemann) Brebisson 1838
GPAR	<i>Gomphonema parvulum</i> Kutzing 1849
GPRI	<i>Gomphonema pumilum</i> var. <i>rigidum</i> Reichardt & Lange-Bertalot 1997
GVIB	<i>Gomphonema vibrio</i> Ehrenberg 1843
AMMO	<i>Halamphora montana</i> (Krasske) Levkov 2009
AHUN	<i>Lemnicola hungarica</i> (Grunow) Round & Basson 1997
MBRA	<i>Mastogloia braunii</i> Grunow 1863
MELL	<i>Mastogloia elliptica</i> (Agardh) Cleve in Schmidt et al. 1893
MSLA	<i>Mastogloia smith</i> var. <i>lacustris</i> Grunow
MSMI	<i>Mastogloia smithii</i> Thwaites ex W. Smith 1856
NCPR	<i>Navicula capitoradiata</i> H. Germain 1981
NDEC	<i>Navicula decussis</i> Østrup 1910
NPHY	<i>Navicula phyllepta</i> Kutzing 1844
NRAD	<i>Navicula radiosa</i> Kutzing 1844
NRCH	<i>Navicula reichardtiana</i> Lange-Bertalot
NTNL	<i>Navicula tenella</i> Brebisson
NZCL	<i>Nitzschia</i> cf. <i>closterium</i> (Ehrenberg) W. Smith
NAFR	<i>Nitzschia amphibia</i> f. <i>frauenfeldii</i> (Grunow) Lange-bertalot 1987
NCLA	<i>Nitzschia clausii</i> Hantzsch 1860
NDEN	<i>Nitzschia denticula</i> Grunow in Cleve & Grunow 1880
NDES	<i>Nitzschia desertorum</i> Hustedt 1949
NDRA	<i>Nitzschia draveillensis</i> Coste & Ricard 1980
NETO	<i>Nitzschia etoshensis</i> Cholnoky
NIFR	<i>Nitzschia frustulum</i> (Kützing) Grunow in Cleve & Grunow 1880
NIGR	<i>Nitzschia gracilis</i> Hantzsch 1860
NKTG	<i>Nitzschia kuetzingioides</i> Hustedt 1959
NLTE	<i>Nitzschia latens</i> Hustedt 1949
NPAL	<i>Nitzschia palea</i> (Kutzing) W. Smith 1856
NPAD	<i>Nitzschia palea</i> var. <i>debilis</i> (Kutzing) Grunow, 1880
NTER	<i>Nitzschia terrestris</i> (J.B. Petersen) Hustedt 1934
NVER	<i>Nitzschia vermicularis</i> (Kutzing) Hantzsch in Rabenhorst 1860
PACR	<i>Pinnularia acrosphaeria</i> W. Smith 1853
PGSC	<i>Pinnularia gibba</i> var. <i>sancta</i> Grunow (Grunow ex Cleve)

Table 4.1. Continued

Codes	List of Diatom spp.
PVIR	<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg 1843
NGAS	<i>Placoneis gastrum</i> (Ehrenberg) Mereschkowsky 1903
ALVD	<i>Psammothidium levanderi</i> (Hustedt) Bukhtiyarova & Round 1996
SELI	<i>Pseudostaurosira elliptica</i> (Schumann) Edlund, Morales & Spaulding 2006
RABB	<i>Rhoicosphenia abbreviate</i> (Agardh) Lange-Bertalot 1980
RGIB	<i>Rhopalodia gibba</i> (Ehrenberg) Otto Müller 1895
RGBL	<i>Rhopalodia gibberula</i> (Ehrenberg) O Müller 1895
RMUS	<i>Rhopalodia musculus</i> (Kutzing) Otto Muller 1900
RRUP	<i>Rhopalodia rupestris</i> (W.Smith) Krammer in Lange-Bertalot & Krammer 1987
SPUP	<i>Sellaphora pupula</i> (Kutzing) Mereschkowsky 1900
SPHO	<i>Stauroneis sphoenicenteron</i> (Nitzsch) Ehrenberg 1843
PBTG	<i>Staurosira abrevistriata</i> Grunow
SCON	<i>Staurosira construens</i> Ehrenberg 1843
SPIN	<i>Staurosirella pinnata</i> (Ehrenberg) D.M. Williams & Round 1988
STMI	<i>Stephanodiscus minutulus</i> (Kutzing) Cleve & Moller 1882
SENG	<i>Surirella engleri</i> O.Muller 1903
SLIN	<i>Surirella linearis</i> W.Smith 1853
SOVI	<i>Surirella ovalis</i> Brebisson 1838
STAB	<i>Tabularia fasciculata</i> (C.Agardh) D.M. Williams & Round 1986
TFAU	<i>Thalassiosira faurii</i> (Gasse) Hasle 1978
TRUD	<i>Thalassiosira rudolfi</i> (Bachmann) Hasle 1978
NCOT	<i>Tryblionella apiculata</i> W.Gregory
NUBL	<i>Tryblionella umbilicata</i> (Hustedt) D.G.Mann 1990
FUAC	<i>Ulnaria acus</i> (Kutzing) Aboal in Aboal <i>et al.</i> 2003
SULC	<i>Ulnaria contracta</i> (Ostrup) E.A. Morales & M.L. Vis 2007

4.3.2. Diversity indices

The relationship between the diversity indices and environmental variable was examined to see their response to the physicochemical parameters. Taxa richness (Taxa S) index showed positive significant correlation with water temperature (T), dissolved oxygen (DO) and total suspended solids (TSS; $P < 0.01$; **Table 4.2**) whereas it had a negative significant correlation and response with that of TP, SRP, NH_4^+ , NO_3 and Turbidity ($P < 0.05$; **Table 4.2**). It showed a significant variation among sampling sites of the lake

Hawassa ($P=0.003$) and did not show significant variation among the two seasons such as dry and wet ($P=0.912$). The highest and lowest Shannon-Weiner Diversity Index (H') index was recorded at KW2 and AG1 with a value of 3.069 and 2.136 both in the dry season, respectively. The distribution of the H' was higher and lower on the minimally disturbed and highly disturbed sites. There was a significant spatial variation of H' index between clustered sites ($p=0.000$). The H' was shown significant correlation with Temperature (T), pH, total phosphorus (TP) and total suspended solids (TSS), dissolved oxygen (DO), nitrate (NO_3) and turbidity ($p<0.05$; **Table 4.2**).

The evenness and equitability indices had maximum-minimum value ranges from 0.54 to 0.24, and 0.81 to 0.60, respectively. However, they did not show statistically seasonal and spatial significant variation (Evenness $P>0.05$ and Equitability $P>0.05$). The maximum values of both indices were recorded on minimally disturbed sites and the minimum values were on highly disturbed sites. Evenness showed a negative significant variation with environmental variables such as TP, SRP, NO_3 and turbidity (**Table 4.2**).

Table 4. 2. Spearman’s correlation coefficient between selected physicochemical factors and diatom diversity and richness indices (Taxa S =Taxa richness; H’= Shannon diversity index; E=Evenness; J=Equitability, T=Temperature, pH, EC= Conductivity and DO= Dissolved Oxygen, TP=total phosphate, SRP= soluble reactive phosphate, NH₄⁺=Ammonium ion, NO₃= Nitrate, TSS= total suspended solids; ** & * = significant at 0.01 & 0.05, respectively)

Physicochemical parameters	Taxa S	H'	E	J
T(°C)	0.224*	0.145*	-0.137	-0.066
EC (µs/cm)	-0.029	-0.086	-0.131	-0.145*
DO(mg/L)	0.292**	0.216**	0.133	0.053
TP(mg/L)	-0.426**	-0.177*	-0.391**	-0.248**
SRP(mg/L)	-0.317**	0.119	-0.211**	-0.124
NH ₄ ⁺ (mg/L)	-0.149*	-0.100	0.016	0.018
NO ₃ (mg/L)	-0.509**	0.321**	-0.147*	-0.009
TSS(mg/L)	0.195**	0.185*	-0.043	0.077
Turbidity(NTU)	-0.464**	-0.319**	-0.184*	-0.034

4.3.3. Relation of diatoms distribution to physicochemical parameters

The first and the second axis of RDA explained 43.5 and 26.8% of the species-environment variance, respectively (**Table 4.3; Fig. 4.2**). The RDA ordination of the species-environmental relationship showed that pH, NH₄⁺, T, TP, SRP, EC, SiO₂,TUR, TSS, and NO₃ were positively correlated with the first axis (**Table 4.3**). The other

environmental variables such as Chl a, DO and NO₂ were showed a negative correlation with the first axis (**Table 4.3; Fig.4.2**). Axis-1 was also positively characterized by the following diatom species *Fragilaria capucina* (FCAP), *F. tenera* (FTEN), *Gomphonema gracile* (GGRA), *G. olivaceum* (GOLI), *G. parvulum* (GPAR), *G. pumilum* var. *rigidum* (GPRI), *Nitzschia frustulum*(NIFR), *N. kuetzingioides* (NKTG), and *N. palea* (NPAL). These diatom species characterized non-reference sites (TW1, RH2, MK, AG2, RH1, WR, and AG1) which had a relatively high concentration of NO₃, SRP, TP, TSS, TUR, EC, SiO₂, NH₄⁺, T and pH and high human intervention. They had also shown a positive correlation between these environmental variables (p<0.05, **Fig. 4.3**).

In addition, axis-1 was negatively associated with *Achnanthis minutissimum* (AMIT), *Aulacoseira ambigua* (AAMB), *Cymbella cistula*(CCIS), *C. kappii*(CKPP), *C. turgidula*(CTGL), *Encyonema muelleri* (ENMU), *E. silesiacum* (ELSE), *Encyonopsis microcephala* (ENCM), *Navicula tenella* (NTNL) and *Ulnaria acus* (FUAC). These diatom species were more abundant in KW1, KW2 WSH, TU, and TW2 sites with relatively good dissolved oxygen (DO) and nitrite (NO₂) when compared with the other sites (**Fig. 4.2**). The other important characterization was TW2 with the diatom species of AAMB, CCIS, CKPP, and FUAC with a high record of Chl a (**Fig. 4.2**).

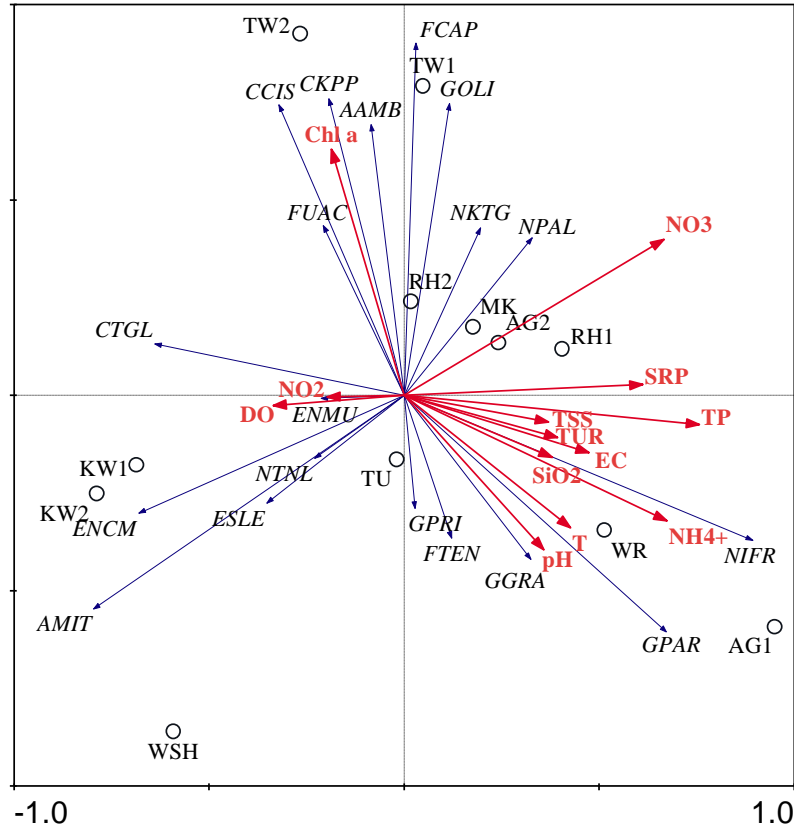


Figure 4. 2. Tri-plots of the first two axes of the redundancy analysis (RDA) showing the association of diatom species biomass and environmental variables

(Abbreviations: FCAP=*Fragilaria capucina*, FTEN= *F. tenera*, GGRA= *Gomphonema gracile*, GOLI= *G. olivaceum*, GPAR= *G. parvulum*, GPRI= *G. pumilum* var. *rigidum*, NIFR=*Nitzschia frustulum*, NKTG=*N. kuetzingioides*, NPAL= *N. palea*, AMIT= *Achnantheidium minutissimum*, AAMB=*Aulacoseira ambigua*, CCIS= *Cymbella cistula*, CKPP= *C. kappii*, CTGL= *C. turgidula*, ENMU= *Encyonema muelleri*, ELSE= *E. silesiacum*, ENCM= *Encyonopsis microcephala*, NTNL= *Navicula tenella* and FUAC= *Ulnaria acus*, for other abbreviations see Fig. 4.1 and Table 4.2)

Table 4. 3. The result of the redundancy analysis (RDA) of Diatom spp. versus environmental variables relationship including eigenvalues and percentage of variance explained by the first two axes (Strong correlations are marked as **bold** at $p < 0.05$).

Variables	Axis 1	Axis 2
Eigenvalues	0.435	0.268
Cumulative percentage variance of species-environment relation	43.5	70.3
T	0.4263	-0.3385
EC	0.4742	-0.1466
pH	0.3579	-0.3954
DO	-0.3359	-0.0259
TP	0.7574	-0.0751
SRP	0.6119	0.0275
NH ₄ ⁺	0.6739	-0.3213
NO ₂	-0.1979	-0.004
NO ₃	0.6661	0.3995
SiO ₂	0.3799	-0.1582
TSS	0.3707	-0.0678
Chl a	-0.1861	0.6296
TUR	0.3941	-0.1079

4.4. DISCUSSION

4.4.1. Spatial and seasonal diatoms diversity and abundance in Lake

Hawassa

In this study, 105 species of diatoms belonging to 39 genera were identified (**Table 4.1; Appendix 3**). The highest diversity of littoral areas of Lake Hawassa was found at KW2 which was characterized as a minimally disturbed site in dry and wet seasons (**Fig. 4.1**). This is a common phenomenon where minimally disturbed sites show high number of diatom species, which indicates relatively good quality of that site (Khan, 1990; Ghosh, 2015). The lowest record was observed at highly disturbed sites of RH1 and AG1 of the wet seasons with 21 and 24 individual species, respectively (**Fig. 4.1**). Among the 39 genera identified, *Nitzschia* spp were the most diverse groups (with 15 genera) followed by *Gomphonema* spp. with 8 species. *Nitzschia* is the most widely distributed diatom genus in inland waters with many species and most abundant taxon which is known by its ecological importance (Alakananda *et al.*, 2012; Trobajo *et al.*, 2012). *Cymbella* spp., *Epithemia* spp. and *Navicula* spp. were also have five-five species each. The other genera were represented by less than 5 species in terms of their diversity.

From the total diatom assemblages, *Nitzschia frustulum* was the most abundant which contributed 17.5%. Among all contributions, greater than 57% of this species relative occurrence was observed in moderately and highly disturbed sites. This might be related to ecological and tolerance capability of *Nitzschia* spp. in a human-disturbed environment with possible organic pollution (Delgado *et al.*, 2012). The research conducted on Kebena, Akaki and Modjjo rivers of Ethiopia by Abebe Beyene *et al.* (2009) and Tigist

Fantu (2016) also showed the dominance of *Nitzschia* spp. over others in highly disturbed sites.

There was also a huge dominance of *Achnantheidium minutissimum* Kutzing which was observed in minimally disturbed sites and contributed greater than 80% of the total diatom assemblage of the minimally disturbed sites. This diatom species seems to be relatively sensitive to human disturbance. A research conducted on Lake Kariba of Zambia and Zimbabwe showed that high dominance of *Achnantheidium* spp. on sites where there was minimal human disturbance (Phiriet *et al.*, 2007). Many biomonitoring research conducted in all over the world showed upstream rivers and/or minimally impacted sites were dominated by this species (Kelly *et al.*, 1995; Abebe Beyene *et al.*, 2009; Jamali *et al.*, 2012); even though there is discrepancy on its ecological distribution between scholars. The research conducted on 15 lakes of Bangalore, India, revealed that *Achnantheidium* was common and abundant from slightly to moderate eutrophic lakes (Alakananda *et al.*, 2010). It was also abundant with lakes having Na⁺ ions of tropical volcanic lakes of eastern Mexico (Vázquez and Caballero, 2013) which has similar ionic dominance with L. Hawassa (Zinabu Gebre-Mariam *et al.*, 2002). There was no significant variation of *A. minutissimum* distribution between dry and wet season ($P > 0.05$). *Gomphonema parvulum* Kutzing (8.4%) was the third in its relative abundance. However, most of the other species recorded were rare in their relative abundance. It was also observed that from the total identified diatoms species from Lake Kyaninga (Uganda), around 85 % were rare in their relative occurrence (Cocquyt *et al.*, 2010).

Even though, it is difficult to compare the diatom species diversity records of the present work with the previous studies, it can be fairly generalized that most of the diatom species listed in this study were new records for the lake (**Table 4.5**); this adds new the data sets to the diatom community of L. Hawassa (Gasse, 1986; Elizabeth Kebede and Amha Belay, 1994; Telford *et al.*, 1999).

The SIMPER result showed that the reference group of diatoms showed over 60% similarity and the non-reference (moderate and high disturbed) groups had >50% similarity. However, there was highest dissimilarity percentage (53.3%) between the reference and highly disturbed sites. The environmental variation caused by human pressure might be the responsible factor for the major difference of these groups as noted by Delgado *et al.* (2010). Hence, this may lead variation on the distribution of diatom species between disturbance levels. As a result, in this study, the distribution of diatom spp. between the three-clustered sites had significant variation (ANOSIM $P= 0.0001$, $R=0.62$). Besides this, the SIMPER analysis showed the overall average dissimilarity of diatom species between reference and moderate disturbance, reference and high disturbance, and moderately and highly disturbed sites 47.37, 53.27 and 48%, respectively. The diatom species such as AMIT, NIFR, GPAR and NTNL were responsible for the highest dissimilarity percentage observed between reference and highly disturbed groups. These diatom species can be incorporated as metrics in development of a multimetric index.

4.4.2. Diversity indices

Taxa richness (Taxa S) index showed positive significant correlation with water temperature (T), dissolved oxygen (DO) and total suspended solids (TSS; $P<0.01$; **Table**

4.2) whereas it had a negative significant correlation with that of TP, SRP, NH_4^+ , NO_3 and Turbidity ($P < 0.05$; **Table 4.2**). This indicates that the nutrient availability may regulate the composition of diatom assemblages. It showed a significant variation among sampling sites of the lake Hawassa ($P=0.003$) and did not show a significant seasonal variation ($P= 0.437$).

One of the most commonly used diversity indices is Shannon-Weiner Diversity index (**H'**) which is applied to different types of identified groups of organisms like diatoms to measure species richness and evenness within communities variable between species in their relative frequency (Martensson, 2016). In this research, **H'** was used to quantify the diatoms diversity on L. Hawassa. The highest and the lowest range of **H'** index was 3.1 and 2.1 at minimally disturbed and highly disturbed sites both in the dry season, respectively. The highest value of **H'** on minimally disturbed sites might be related to the high diversity records of diatom species (**Fig. 4.1**). According to Staub *et al.* (1970) ecological classification, all of sampling sites except KW2 (which had **H'** index value 3.1) categorized into light pollution since their diversity values were in the range of 2.00-3.00 (**Table 4.4**). This classification would not coincide with the prior classification of those sites which were listed under moderate and highly polluted/ disturbed sites. This is the limitation of the diversity indices which is insufficient to discriminate between various ranges and types of pollution including nutrient enrichment. In addition, they have limited use in a complex system which is affected by multiple stressors and basin-wide non-point source pollution (Jones, 1981; Metcalfe-Smith, 1994). There was a significant spatial variation of **H'** index values ($P=0.000$) but it did not show a significant statistical temporal variation ($P=0.912$). The **H'** showed significant correlation with

Temperature (T), pH, total phosphorus (TP) and total suspended solids (TSS) with $p < 0.05$ (**Table 4.2**), whereas dissolved oxygen (DO), Nitrate (NO_3), and Turbidity had a highly significant correlation ($P < 0.01$).

The evenness and equitability indices had maximum-minimum value ranges from 0.54 to 0.24, and 0.81 to 0.60, respectively. However, they did not show significant statistically seasonal and spatial variation ($P > 0.05$). The maximum values of both indices were recorded on minimally disturbed sites and the minimum values were on highly disturbed sites. Evenness showed a significant variation to environmental variables such as TP, SRP, NO_3 and Turbidity (**Table 4.2**).

Table 4. 4. Relationships between species diversity and pollution status according to Staub *et al.* (1970)

Diversity (H')	Pollution status
3.5-4.5	Slight pollution
2.0-3.0	Light pollution
1.0-2.0	Moderate pollution
0.0-1.0	Heavy pollution

Table 4. 5. Relative Abundance (%RA which rounded to 2 decimal places) of identified diatom spp. and their presence (+) and absence (-) in previous studies of L. Hawassa

Codes	List of Diatom spp.	%RA	Gasse (1986)	Telford <i>et al.</i> (1999)
AIPX	<i>Achnanthes impexa</i>	0.56	+	+
AEXG	<i>Achnanthidium exiguum</i>	0.59	-	-
AMIT	<i>Achnanthidium minutissimum</i>	9.42	+	+
AFCR	<i>Afrocybella reichardtii</i>	0.00	-	-
ALIB	<i>Amphora libyca</i>	0.22	-	-
AOAF	<i>Amphora ovalis</i> var. <i>affinis</i>	0.03	-	-
AVEN	<i>Amphora veneta</i>	0.33	-	-
ASPH	<i>Anomoeneis sphaerophora</i>	0.5	-	-
AMUZ	<i>Aulacoseira muzzanensis</i>	0.52	-	-
AAMB	<i>Aulacoseira ambigua</i>	2.92	+	+
AUGR	<i>Aulacoseira granulata</i>	0.42	-	-
AGVA	<i>Aulacoseira granulata</i> var. <i>valida</i>	0.01	-	-
CBAC	<i>Caloneis bacillum</i>	0.33	-	-
CPLA	<i>Cocconeis placentula</i>	0.18	-	-
CPLI	<i>Cocconeis placentula</i> var. <i>lineata</i>	0.28	-	-
CAMB	<i>Craticula ambigua</i>	0.02	-	-
NPSH	<i>Craticula buderii</i>	0.00	-	-
CRCU	<i>Craticula cuspidata</i>	0.02	-	-
CDUB	<i>Cyclostephanos dubius</i>	0.11	-	-
CKRM	<i>Cyclotella krammeri</i>	0.03	-	-
CMEN	<i>Cyclotella meneghiniana</i>	0.34	-	-
COCE	<i>Cyclotella ocellata</i>	0.14	-	-
CCIS	<i>Cymbella cistula</i>	2.73	-	-
CKPP	<i>Cymbella kappii</i>	6.18	-	-
CLEP	<i>Cymbella leptoceros</i>	0.1	-	-
CNIS	<i>Cymbella neocistula</i> var. <i>islandica</i>	0.00	-	-
CTGL	<i>Cymbella turgidula</i> Grunow	1.73	-	-
DSMI	<i>Diploneis smithii</i>	0.21	-	-

Table 4.5. (Continued)

Codes	List of Diatom spp.	%RA	Gasse (1986)	Talford <i>et al.</i>, 1999
ECAE	<i>Encyonema caespitosum</i>	0.03	-	-
ENMU	<i>Encyonema muelleri</i>	2.38	+	-
ESLE	<i>Encyonema silesiacum</i>	4.46	-	-
ENCM	<i>Encyonopsis microcephala</i>	5.93	-	+
EADN	<i>Epithemia adnata</i>	0.07	-	-
EAAL	<i>Epithemia argus</i> var. <i>alpestris</i>	0.00	-	-
EFRI	<i>Epithemia frickei</i>	0.07	-	-
EHYN	<i>Epithemia hyndmanii</i>	0.00	-	-
ESOR	<i>Epithemia sores</i>	0.00	-	-
EBLL	<i>Eunotia bilunaris</i> var. <i>linearis</i>	0.00	-	-
EFOR	<i>Eunotia formica</i>	0.00	-	-
EINC	<i>Eunotia incisa</i>	0.00	-	-
EMON	<i>Eunotia monodon</i>	0.00	-	-
FCAP	<i>Fragilaria capucina</i>	4.75	-	-
FTEN	<i>Fragilaria tenera</i>	1.03	-	-
GANT	<i>Gomphonema angustum</i>	0.09	-	-
GAUG	<i>Gomphonema augur</i>	0.05	-	-
GGRA	<i>Gomphonema gracile</i>	1.56	-	-
GIPL	<i>Gomphonema intricatum</i> var. <i>pulvinata</i>	0.25	-	-
GOLI	<i>Gomphonema olivaceum</i>	2.18	-	-
GPAR	<i>Gomphonema parvulum</i>	8.43	-	-
GPRI	<i>Gomphonema pumilum</i> var. <i>rigidum</i>	1.27	-	-
GVIB	<i>Gomphonema vibrio</i>	0.00	-	-
AMMO	<i>Halamphora montana</i>	0.01	-	-
AHUN	<i>Lemnicola hungarica</i>	0.02	-	-

Table 4.5. (Continued)

Codes	List of Diatom spp.	%RA	Gasse (1986)	Talford <i>et al.</i>, 1999
MBRA	<i>Mastogloia braunii</i>	0.01	-	-
MELL	<i>Mastogloia elliptica</i>	0.08	-	-
MSLA	<i>Mastogloia smith</i> var. <i>lacustris</i>	0.02	-	-
MSMI	<i>Mastogloia smithii</i>	0.00	-	-
NCPR	<i>Navicula capitoradiata</i>	0.3	-	-
NDEC	<i>Navicula decussis</i>	0.00	-	-
NPHY	<i>Navicula phyllepta</i>	0.84	-	-
NRAD	<i>Navicula radiosa</i>	0.72	-	-
NRCH	<i>Navicula reichardtiana</i>	0.03	-	-
NTNL	<i>Navicula tenella</i>	6.32	-	-
NZCL	<i>Nitzschia</i> cf. <i>Closterium</i>	0.07	-	-
NAFR	<i>Nitzschia amphibia</i> f. <i>frauenfeldii</i>	0.43	-	-
NCLA	<i>Nitzschia clausii</i>	0.00	-	-
NDEN	<i>Nitzschia denticula</i>	0.00	-	-
NDES	<i>Nitzschia desertorum</i>	0.1	-	-
NDRA	<i>Nitzschia draveillensis</i>	0.00	-	-
NETO	<i>Nitzschia etoshensis</i>	0.13	-	-
NIFR	<i>Nitzschia frustulum</i>	17.53	-	-
NIGR	<i>Nitzschia gracilis</i>	0.06	-	-
NKTG	<i>Nitzschia kuetzingioides</i>	1.58	+	-
NLTE	<i>Nitzschia latens</i>	0.00	-	-
NPAL	<i>Nitzschia palea</i>	4.8	-	+
NPAD	<i>Nitzschia palea</i> var. <i>debilis</i>	0.12	-	+
NTER	<i>Nitzschia terrestris</i>	0.00	-	-
NVER	<i>Nitzschia vermicularis</i>	0.00	-	-

Table 4.5. (Continued)

Codes	List of Diatom spp.	%RA	Gasse (1986)	Talford <i>et al.</i>, 1999
PACR	<i>Pinnularia acrosphaeria</i>	0.00	-	-
PGSC	<i>Pinnularia gibba</i> var. <i>sancta</i>	0.07	-	-
PVIR	<i>Pinnularia viridis</i>	0.00	-	-
NGAS	<i>Placoneis gastrum</i>	0.00	-	-
ALVD	<i>Psammothidium levanderi</i>	0.02	-	-
SELI	<i>Pseudostaurosira elliptica</i>	0.09	-	-
RABB	<i>Rhoicosphenia abbreviate</i>	0.00	-	-
RGIB	<i>Rhopalodia gibba</i>	0.01	-	-
RGBL	<i>Rhopalodia gibberula</i>	0.08	-	-
RMUS	<i>Rhopalodia musculus</i>	0.00	-	-
RRUP	<i>Rhopalodia rupestris</i>	0.00	-	-
SPUP	<i>Sellaphora pupula</i>	0.88	-	-
SPHO	<i>Stauroneis phoenicenteron</i>	0.00	-	-
PBTG	<i>Staurosira brevistriata</i>	0.13	-	+
SCON	<i>Staurosira construens</i>	0.42	-	-
SPIN	<i>Staurosirella pinnata</i>	0.33	-	-
STMI	<i>Stephanodiscus minutulus</i>	0.00	-	-
SENG	<i>Surirella engleri</i>	0.00	-	-
SLIN	<i>Surirella linearis</i>	0.03	-	-
SOVI	<i>Surirella ovalis</i>	0.00	-	-
STAB	<i>Tabularia fasciculata</i>	0.04	-	-
TFAU	<i>Thalassiosira faurii</i>	0.09	+	+
TRUD	<i>Thalassiosira rudolfi</i>	0.00	-	-
NCOT	<i>Tryblionella apiculata</i>	0.00	-	-
NUBL	<i>Tryblionella umbilicata</i>	0.10	-	-
FUAC	<i>Ulnaria acus</i>	3.92	-	-
SULC	<i>Ulnaria contracta</i>	0.69	-	-

4.3.3. Relation of diatoms distribution to physicochemical parameters

The first two axes of RDA explained 70.3 % of the species-environment variance (**Table 4.2**) while 30% unexplained variance might be due to variable not measured in this analysis (cf. Lepš and Šmilauer, 2003). The first and the second axis of RDA explained 43.5 and 26.8% of the species-environment variance, respectively (**Table 4.3; Fig. 4.2**). Axis-1 was positively characterized by the following diatom species such as *Fragilaria capucina* (FCAP), *F. tenera* (FTEN), *Gomphonema gracile* (GGRA), *G. olivaceum* (GOLI), *G. parvulum* (GPAR), *G. pumilum* var. *rigidum* (GPRI), *Nitzschia frustulum* (NIFR), *N. kuetzingioides* (NKTG), and *N. palea* (NPAL). These diatom species were distributed from moderately to highly disturbed sites (TW1, RH2, MK, AG2, RH1, WR, and AG1) which had a relatively high concentration of NO₃, SRP, TP, TSS, TUR, EC, SiO₂, NH₄⁺, T and pH (**Table 4.3; Fig. 4.2**).

Generally, diatom species such as *Fragilaria capucina* (FCAP), *F. tenera* (FTEN), *Gomphonema gracile* (GGRA), *G. olivaceum* (GOLI), *G. parvulum* (GPAR), *G. pumilum* var. *rigidum* (GPRI), *Nitzschia frustulum* (NIFR), *N. kuetzingioides* (NKTG), and *N. palea* (NPAL) had a strong significant correlation with nutrients (SRP, NH₄⁺, NO₃ and TP; **Table 4.3**). The research conducted on lakes and wetlands by Karthick *et al.* (2009) and Alakananda *et al.* (2010) also reported that *Gomphonema parvulum*, and *Nitzschia* spp. were more dominant in association with high nutrient and ionic contents. The genera *Gomphonema* was also abundant on highly disturbed sites of Lake Kariba (Phiri *et al.*, 2007). *Gomphonema parvulum* and *Nitzschia palea* were considered as pollution tolerant species in most of African, European and Asian rivers and lakes and had positive relation

with rising of nutrients (Ndiritu *et al.*, 2003; Ndiritu *et al.*, 2006; Phiri *et al.*, 2007; Stenger-Kovacs *et al.*, 2007; Blanco *et al.*, 2013; He *et al.*, 2016).

In addition, axis-1 was negatively associated with *Achnantheidium minutissimum* (AMIT), *Aulacoseira ambigua* (AAMB), *Cymbella cistula* (CCIS), *C. kappii* (CKPP), *C. turgidula* (CTGL), *Encyonema muelleri* (ENMU), *E. silesiacum* (ELSE), *Encyonopsis microcephala* (ENCM), *Navicula tenella* (NTNL) and *Ulnaria acus* (FUAC). These diatom species were abundant in KW1, KW2 WSH, TU, and TW2 sites with relatively good dissolved oxygen (DO) and nitrite (NO₂) and negatively associated with TP, SRP, NO₃ and NH₄⁺ (**Fig. 4.3**) when compared with the other sites. This might indicate they are a good indicator of the minimally disturbed sites with a low concentration of nutrients. Several studies showed that AMIT, ENCM and ELSE were abundant and common in sites where there are minimal human disturbance (Phiri *et al.*, 2007; Abebe Beyene *et al.*, 2009; Omar, 2010; Blanco *et al.*, 2013; Wang *et al.*, 2014; He *et al.*, 2016). This might strengthen the general conclusion that the diatom communities are affected by the change in physical and chemical variables including a change in the concentration of nutrients (Phiri *et al.*, 2007). The other important characterization was TW2 with the diatom species of AAMB, CCIS, CKPP, and FUAC that had a high record of Chl a (**Fig. 4.3**).

Generally, the RDA analysis clustered the diatom species into right and left where the former species included more of in the reference taxa whereas the latter incorporates the test taxa with relative proportion. Therefore, the reference taxa such as *Achnantheidium minutissimum*, *Aulacoseira ambigua*, *Cymbella cistula*, *C. kappii*, *C. turgidula*, *Encyonema muelleri*, *E. silesiacum*, *Encyonopsis microcephala*, *Navicula tenella* and

Ulnaria acus can be used for biomonitoring activities of Lake Hawassa as they were more abundant in sites which had relatively low concentration of nutrients and minimal human disturbance. These diatom species can be considered as good indicators of lake water quality and habitat integrity even though further study is needed to check their distribution through time and routine data set are needed to monitor water quality. Similarly, the non-reference taxa, which included *Fragilaria capucina*, *F. tenera*, *Gomphonema gracile*, *G. olivaceum*, *G. parvulum*, *G. pumilum* var. *rigidum*, *Nitzschia frustulum*, *N. kuetzingioides*, and *N. palea*, can also be used for bioassessment of the lake as they were more abundant in sites which had relatively high concentration of nutrients and high human disturbance.

CHAPTER 5: MULTIMETRIC INDEX DEVELOPMENT FOR LAKE HAWASSA (MMIH)

5.1. INTRODUCTION

Lake Hawassa is one of the most threatened Rift Valley Lake because of human pressure related to adverse watershed land use, urban development and expansion of industries. For example, around 1980s, there was no observed pollution signs on adjacent sites of the lake to Hawassa City since there was little recreational activities and urban development (Tilahun Kibret, 1985). Nowadays, recessional farming, deforestation, urbanization, recreational activities and industrial expansion are among the stressors type that significantly contribute for the observed change of the lake water quality. As a result of the effect of these multiple stressors, the lake ecology has degraded in terms of its physical, chemical and biological quality. For instance, the Hawassa Textile factory contributes to increased deterioration of the lake water quality because it releases its effluent to the tributary river without efficient treatment (Zinabu Gebre-Mariam and Zerihun Desta, 2002; Birnesh Abay, 2007).

Moreover, the water chemistry of the lake has been changing and nutrient (phosphorus and nitrogen) content of the lake has been on increasing trend for more than two decades (**Table 2.6**). The trace metal load analyzed from fish organs, sediments and water samples taken from Lake Hawassa and L. Ziway showed that there were high bioaccumulation load of trace metals from Lake Hawassa than L. Ziway (Kebede Nigussie *et al.*, 2010). Amanuel Aklilu (2011) also found that most of the macroinvertebrate assemblages of the lake in his collection were dominated by pollution

tolerant families. Generally, as consequences of overall human pressure that have been exerted on the lake and its watershed, the ecological changes may lead to overall impairments on the function of the lake ecosystem. Therefore, to have best management practice and to assure the sustainable function of the lake ecosystem, assessments of the lake water quality is important that can provide basic information about physical, chemical and biological features of the ecosystem.

Water quality assessment has been routinely done using the chemical and/or biological monitoring of the lake ecosystem.

Chemical monitoring provides information on the immediate water quality of the lake which could miss long term changes in water quality and physical nature of the aquatic ecosystem. Moreover, it may also lack accuracy that comes from irregular chemical sampling and inability to measure some constituents at low concentration (Masese *et al.*, 2013). In contrast to this, the biological monitoring uses aquatic organisms that helps to monitor changes in chemical and physical components of the aquatic environment and can enhance the ability to identify the level of degradation as well as the actions to take (Masese *et al.*, 2013; Stribling and Dressing, 2015).

In earlier times, indicator organisms (metrics) were used for biomonitoring practice based on their response to human perturbation in their community structure. Later, ecologists developed single biotic index to get better description on the human disturbance to aquatic ecology than the indicator organisms (Barbour *et al.*, 1996). In recent years, using single biotic index (single assemblage) is being replaced by multimetric biotic index. Single biotic index can only stand for one of the communities function and responds to

limited stressors which may affect the accuracy of the assessment (Wang *et al.*, 2015). Thus, the multi-metric approach which is based on multiple assemblages is more robust water quality assessor and indicates ecosystem integrity, and provide opportunity to respond for different stressors type at a time (Schoolmaster *et al.*, 2012; Wang *et al.*, 2015). The method uses an aggregation of individual community metrics that comprise benthic biological elements (like benthic diatoms and macroinvertebrate metrics) for the development of a single composite multimetric index. This can potentially reflect multiple effects of human impact on the structure and function of aquatic ecosystem (Barbour *et al.*, 1999; Menetrey *et al.*, 2011) and is based on comparing the biological metrics form minimally disturbed to highly disturbed sites of the water body (Stoddard *et al.*, 2006; Whittier *et al.*, 2007).

The application of multi-assemblage base multimetric index is quite recent and only few studies have been recorded from temperate (Clapcott *et al.*, 2014; Mendes *et al.*, 2014) and tropical regions (Wang *et al.*, 2015; Chen *et al.*, 2017) but not Ethiopian lakes, including Lake Hawassa. Since the water quality of Lake Hawassa has been impacted by a number of stressors, a multimetric approach is highly recommended to obtain accurate results of the response of macroinvertebrates and diatoms to the potential stressors of the littoral regions of the lake. Therefore, the objective of this study was to develop a combined macroinvertebrate and diatom multimetric index of biotic integrity of L. Hawassa (MMIH) and validate its capability of discriminating reference from non-reference sites of the lake.

5.2. MATERIALS AND METHODS

5.2.1. Benthic macroinvertebrate and diatom indices/ metric calculation

The list of identified macroinvertebrate and diatoms species were documented in chapter 3 and 4, respectively, and abundance and richness values were used in metrics calculation (**Table 5.1**). Most of the macroinvertebrate metrics were calculated using Microsoft excel and Past software. OMNIDIA software version 5.3 with a species list of version 2009 was employed to calculate the diatom indices/metrics listed in **Table 5.1** (Lecointe *et al.*, 1993). Four macroinvertebrate (i.e. RATM, RRTM, PRATM and PRRTM; **Table 5.1**; **Appendix 4**) and four diatom (i.e. RATD, RRTD, PRATD and PRRTD; **Table 5.1**; **Appendix 5**) reference based metrics were included as candidate metrics. The development of the MMIH index was based on three *a priori* clustered sites, namely, C1- minimally disturbed sites (KW1, KW2 and WSH), C2- moderately disturbed sites (MK, TW1 and TW2) and C3-highly disturbed sites (WR, RH1 and AG1). The remaining sites (TU of minimally disturbed, and RH2 and AG2 of moderately disturbed sites), which were selected randomly from three-clustered sites, were taken as independent data set and were not incorporated in the multimetric index development (MMIH).

5.2.2. Selection and removal of redundant metrics

A total of 57 macroinvertebrate and diatom candidate metrics (**Table 5.1**) representing various aspects of the diatom and macroinvertebrate communities related to family richness, taxonomic composition, tolerance measures, biotic indices and others (Kelly and Whitton, 1995; Barbour *et al.*, 1999; Rott *et al.*, 1999; Lecointe *et al.*, 2003; Bouchard *et al.*, 2004) were compiled.

The listed candidate metrics/indices were used for the selection of the potential/core metric which were incorporated in the development of multimetric index of biotic integrity of Lake Hawassa (MMIH). The procedure for selecting the potential metric was done according to Barbour *et al.* (1999), with some modification. The selection was done based on the metrics response to physicochemical parameters and with the relationship between themselves and their ability to characterize the reference and non-reference sites. Metrics which significantly correlated with physicochemical variables were selected for the next analysis. Redundancy analysis was employed to identify the pair of metrics with a significant correlation (i.e. correlation coefficient ≥ 0.7) (Ofenböck *et al.*, 2004; Hering *et al.*, 2006a). From the redundant metrics, the one which strongly correlated with the physicochemical variables was considered for further analysis and the others were rejected.

Percent discriminatory efficiency (%DE) of each metric was calculated to identify the most suitable metrics having high % DE (usually greater than or equal to 50%). Besides this, for diatom indices selection, percent inclusion of the diatom species in index calculation was also considered as an important criterion in which <50% inclusion was excluded for further analysis. The distribution of each metrics between the reference and non-reference sites was visualized using Box and Whisker plots. The sensitivity of the metrics was examined based on their interquartile overlap degree according to the method outlined in Barbour *et al.* (1996). Sensitivity values of the metrics were characterized as having 3, 2, 1 and 0 values based on the interquartile overlap degree. Hence, a sensitivity of 3 point was provided when there was no overlap. A sensitivity value of 2 was given when there was some overlap (but both medians are outside the interquartile range) and 1

if there was a moderate overlap but one median is outside the interquartile overlap. A value of 0 was provided when extensive overlap of interquartile range (both medians within the overlap) existed. Finally, only metrics with 3 and 2 sensitivity values were selected and incorporated in the MMIH index development.

5.2.3. Scoring of metrics

Using the discrete type of scoring system, calculated metric values were converted (normalized) to metric scores of 5, 3 or 1 depending on their proximity to the optimal values. Metrics whose values decreased with the increase of disturbance (positive metric) 5, 3 and 1 scoring was used, and the reverse scoring for negative metrics (metrics values increase with increasing disturbance) was used. For instance, positive metrics values above 75th percentiles were scored as 5. Metric values between and including the 75th and 25th percentiles were scored as 3, and all metric values below the 25th percentile were scored as 1 (Barbour *et al.*, 1996; Wang *et al.*, 2005).

Table 5. 1. List of candidate indices/metrics of macroinvertebrate and diatoms tested in this study (*Expected response to increasing disturbance)

Metrics		*Response
NFA	Number of families	Decrease
TABT	Total abundance taxa	Decrease
NEP	Number of Ephemeroptera	Decrease
NTR	Number of Trichoptera	Decrease
NOD	Number of Odonata	Decrease
NET	Number of Ephemeroptera and Trichoptera	Decrease
NOL	Number of Oligochaeta	Increase
NTT	Number of Tolerant taxa	Increase
NIT	Number of Intolerant taxa	Decrease
NETO	Number of Ephemeroptera, Trichoptera and Odonata	Decrease
NDI	Number of Diptera	Increase
NCH	Number of Chironomidae	Increase
NCHOL	Number of Chironomidae and Oligochaeta	Increase
NNIT	Number of non-insect taxa	Increase
RATM	Reference Abundance taxa of macroinvertebrate taxa	Decrease
RRTM	Reference richness taxa of macroinvertebrate taxa	Decrease
PABT	Percent abundance taxa	Decrease
PET	Percent Ephemeroptera and Trichoptera	Decrease
POD	Percent Odonata	Decrease
POL	Percent Oligochaeta	Increase
PTT	Percent tolerant taxa	Increase
PIT	Percent intolerant taxa	Decrease
PEP	Percent Ephemeroptera	Decrease
PTR	Percent Trichoptera	Decrease
PETO	Percent Ephemeroptera, Trichoptera and Odonata	Decrease
PDI	Percent Diptera	Increase
PCH	Percent Chironomidae	Increase
PCHOL	Percent Chironomidae and Oligochaeta	Increase
PNIT	Percent non-insect taxa	Increase
PRATM	Percent Reference Abundance of macroinvertebrate taxa	Decrease
PRRTM	Percent Reference Richness of macroinvertebrate taxa	Decrease
RETC	Ratio of Ephemeroptera and Trichoptera to Chironomidae taxa	Decrease
RITT	Ratio of Intolerant to Tolerant taxa	Decrease
HBI	Modified Hilsenhoff Biotic Index	Increase
H'	Shannon diversity index	Decrease

Table 5.1. List of candidate indices/metrics (**Continued**)

Metrics		*Response
CEE	Commission for Economical Community metric(Coste and Ayphassorho, 1991)	Decrease
DES	Descy's pollution metric (Descy, 1979)	Decrease
EPID	Pollution metric based on diatoms (Dell'Uomo, 1996)	Decrease
IBD	Biological Diatom Index (Prygiel and Coste, 1993)	Decrease
IDG	Generic Diatom Index (Coste and Ayphassorho, 1991)	Decrease
IDAP	Indice Diatomique Artois Pircardie (Prygiel <i>et al.</i> , 1996)	Decrease
IPS	Specific pollution sensitivity index (Cemagref, 1982)	Decrease
ROTT	Trophic metric (Rott <i>et al.</i> , 1999)	Decrease
SHE	Steinber & Schiefele trophic metric (Steinberg, 1987)	Decrease
SID	Indice saprobique, (Rott <i>et al.</i> ,1999)	Decrease
TID	IndiceTrophique (Rott <i>et al.</i> , 1999)	Decrease
SLA	Sladeczek's pollution index (Sládeček, 1986)	Decrease
LOBO	Lobo and Bresil (Lecointe <i>et al.</i> , 2003)	Decrease
IDP	Pampean Diatom Index (Gómez and Licursi, 2001)	Decrease
WAT	Watanabe pollution metric (Lecointe <i>et al.</i> , 2003)	Decrease
DI-CH	Swiss Diatom Index (Lecointe <i>et al.</i> , 2003)	Decrease
TDI	Trophic Diatom Index (Kelly and Whitton, 1995)	Increase
%PTV	Percent pollution tolerant valves of diatoms	Increase
RATD	Reference Abundance taxa of Diatoms (Present study)	Decrease
RRTD	Richness of reference taxa of Diatoms (Present study)	Decrease
PRATD	Percent Reference Abundance Taxa of Diatoms (Present study)	Decrease
PRRTD	Percent richness of reference taxa of Diatoms (Present study)	Decrease

5.2.4. Development of multimetric index of biotic integrity for Lake Hawassa

(MMIH)

The scored values of each potential metric value were combined into a multimetric macroinvertebrate and diatom index (MMIH) by summing up the score of each individual metric. The possible maximum and minimum MMIH index values were calculated (maximum value= total number of selected metric multiplied by 5 and minimum value= total number of selected metric multiplied by 1) and divided into quartile ranges to have four quality classes. Thus, the highest score classified as very good quality and the lowest score as poor quality. Box and Whisker plots were also used to visualize MMIH index's

distribution between the reference and non-reference sites and to test its potential to discriminate the minimally disturbed sites from the moderately and highly disturbed sites of Lake Hawassa.

5.2.5. Ecological quality ratio (EQR)

The ecological quality ratio (EQR) of each sampling station was used for the purpose of classification of ecological status. EQR was calculated by dividing MMIH values of each site with the median MMIH values of reference sites. The ratio is expressed as a numerical value usually between zero and one. Then the 90th percentile of the reference site of the EQR values was used to classify into five ecological classes. Generally, as the value of EQR becomes close to one, it is considered as high ecological status and as its values approaches to zero, it is considered as bad ecological status (ECEQR, 2007; Delgado *et al.*, 2010).

5.2.6. Validation of the MMIH

The validation of MMIH index was conducted using independent data sets which were not incorporated in the MMIH index development of L. Hawassa. One of the independent data set was taken from the reference and non-reference sites of Lake Hawassa. In addition, it was also validated using the independent data set from L. Ziway with box and whisker plot of Sigma Software version 10.0. The independent data set was taken from unpublished thesis of Abnet Woldesenbet (2019). Two-stage Least Squares (TSLS) regression analysis was also employed to test the relationship between the MMIH index and percent disturbance score (PDS) in the SPSS package version 20. Principal

component analysis (PCA) was also used to visualize the capability of MMIH index's distribution between the reference and non-reference sites of Lake Hawassa.

5.2.7. Data analysis

OMNIDIA software version 5.3 with a species list of version 2009 was employed to calculate the diatom indices/metric. Spearman rank correlation was used to check relationships of candidate metrics with themselves and with environmental parameters in order to select the core metrics. Two-stage least squares (TSLS) regression analysis was also employed to test relationship between the MMIH index and percent disturbance score (PDS). The above analyses were done with Statistical Package for Social Science Students (SPSS Inc., software version 20.0). Box and Whisker plot and Principal components analysis (PCA) were employed to show the discriminatory potential of the multimetric index (MMIH) among the reference and test sites of Lake Hawassa using Sigma-plot version 10.0 and PAST 3.15 software programmes, respectively. The validation of the potential of MMIH index with independent data set of L. Ziway was checked with Box and Whisker figures using Sigma-plot version 10.0.

5.3. RESULT

5.3.1. Metric Selection

5.3.1.1. Diatom metric selection

Metric selection was done based on percent inclusion of the diatom species in index calculation (the more species are included in index calculation, the more efficient index is to explain the ecology). In addition, their discrimination efficiency, correlation, and response to environmental parameter were checked (**Table 5.1& 5.2**). Therefore, IDAP, SID, WAT, DES, LOBO, IDP and DICH indices were excluded from selection due to <50% inclusion of the diatom species in the index calculation using OMNIDIA software. These indices did not also exhibit a significant correlation with ecologically important physicochemical variables such as TP, SRP and Nitrate (**Table 5.2**). IBD, IPS, TID, CEE and %PTV were not also included in metrics selection because of having a high correlation with SHE, IDG and TDI.

Thus, indices those which had greater than 50% discrimination efficiencies, $\geq 50\%$ species inclusion, lack redundancy and showed a significant correlation with most of physicochemical variables (EPID, SHE, SLA, IDG, TDI, and ROTTt) were included in metrics selection (**Table 5.2**). From the four reference taxa based metrics on, RATD and PRATD were selected due to their high discriminatory efficiency for the multimetric index (MMIH) development (**Table 5.1& 5.2**). These indices/metrics did not exhibit a complete overlap on their interquartile and had a potential to characterize the reference sites and non-reference sites as illustrated on **Fig. 5.1**.

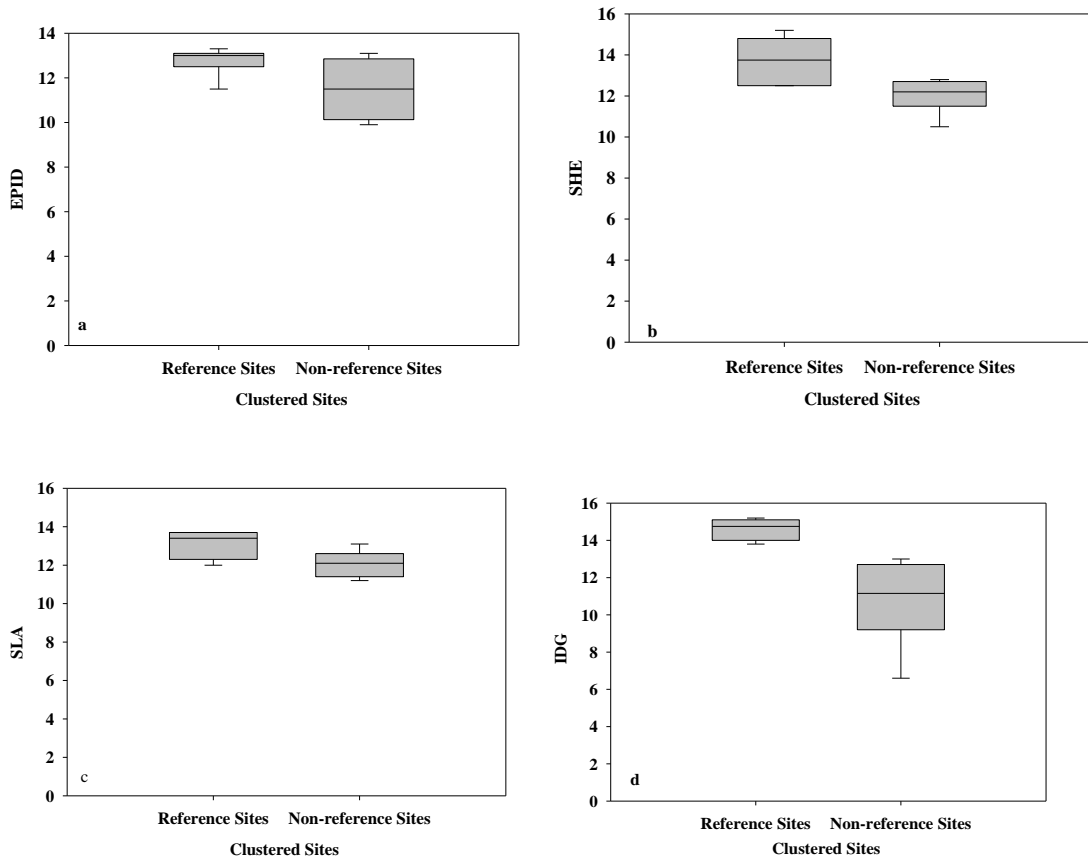


Figure 5. 1. Box-plots of the potentially selected indices and their distribution along the clustered sites (a-f) of Lake Hawassa: the line divides the box represent median, boxes represent interquartile ranges (25–75% percentiles) and range bars show maximum and minimum values; Note: all values used were standardized with quality 20 (for abbreviation see Table 5.1).

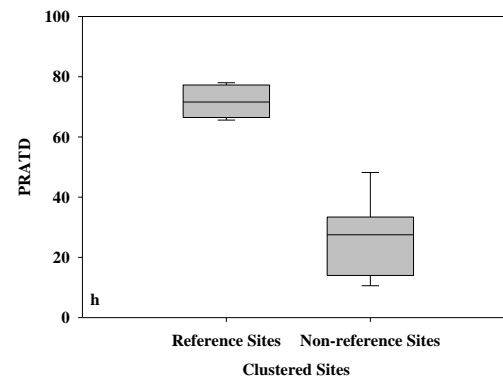
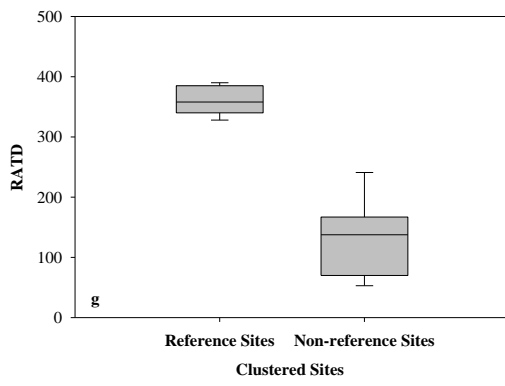
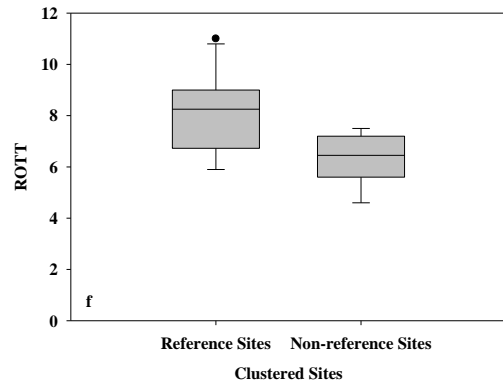
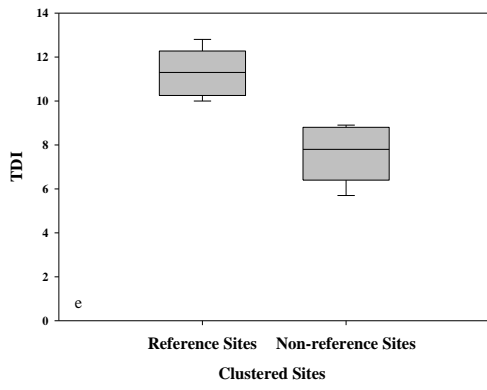


Figure 5.1. (Continued)

Table 5. 2. Spearman correlation of diatom indices/ metrics correlation with selected physicochemical variables, discrimination efficiency percentage (%DE) and average percent inclusion (%AI) of diatom species to the calculation of the indices and metrics in OMNIDIA software. (Abbreviations: T= temperature, EC=water conductivity, TP=total phosphorus, SRP=soluble reactive phosphate, Chl a= chlorophyll a and Tur= turbidity; NA: Not available; for other abbreviations see Table 5.1)

Metrics	T	EC	TP	SRP	NO3	Chl a	Tur	%DE	%AI
IDAP	-0.003	0.320**	0.117	0.182	0.146	-0.042	0.576**	17.1	21.22
EPID	-0.556**	-0.343**	-0.433**	-0.203*	-0.138	0.066	-0.226*	57.4	62.19
IBD	-0.176	-0.200	-0.097	0.202*	0.392**	-0.044	0.079	10.4	78.49
SHE	-0.661**	-0.170	-0.667**	-0.321**	-0.570**	-0.075	-0.486**	62.6	61.00
SID	-0.595**	-0.132	-0.108	-0.155	-0.17	-0.158	-0.466**	10.1	49.36
TID	-0.577**	-0.433**	-0.622**	-0.339**	-0.422**	0.035	-0.433**	93.3	56.94
WAT	-0.028	-0.375**	-0.123	-0.119	-0.192	-0.356**	-0.547**	42.6	38.43
IPS	-0.401**	-0.045	-0.222*	-0.062	0.282**	-0.149	-0.051	56.9	89.99
SLA	-0.399**	0.043	-0.454**	-0.354**	-0.462**	-0.240*	-0.385**	75.9	62.11
DES	-0.226*	0.109	-0.070	0.105	0.068	-0.355**	0.043	35.2	39.39
IDG	-0.406**	-0.545**	-0.762**	-0.481**	-0.559**	-0.157	-0.570**	83.3	99.82
CEE	-0.481**	-0.174	-0.581**	-0.318**	-0.555**	0.062	-0.424**	61.2	67.78
LOBO	0.465**	-0.147	0.158	-0.144	-0.168	-0.019	0.119	15.9	35.47
IDP	-0.806**	0.151	-0.139	-0.049	-0.133	-0.034	-0.170	89.9	46.38
DICH	-0.803**	0.048	-0.085	-0.116	-0.145	0.119	-0.266**	6.7	54.67
TDI	-0.442**	-0.371**	-0.762**	-0.532**	-0.628**	-0.262**	-0.537**	68.8	87.05
ROTT	-0.578**	-.433**	-0.624**	-0.340**	-0.425**	0.033	-0.435**	93.3	NA
%PTV	0.557**	0.515**	0.655**	0.372**	0.473**	0.059	0.558**	60.9	NA
RATD	0.089	-0.495**	-0.617**	-0.599**	-0.641**	-0.450**	-0.506**	77.4	NA
RRTD	0.335**	-0.282**	-0.162	-0.212*	-0.247*	-0.233*	-0.086	32.7	NA
PRATD	0.089	-0.495**	-0.617**	-0.599**	-0.641**	-0.450**	-0.506**	77.4	NA
PRRTD	-0.189	-0.054	-0.389**	-0.334**	-0.542**	-0.239*	-0.275**	14.2	NA

* & ** Correlation is significant at the 0.05 & 0.01 level (2-tailed)

5.3.1.2. Macroinvertebrate metric selection

The macroinvertebrate metrics such as NFA, TABT, NOD, NOL, NTT, NIT, NETO, NCHOL, NNIT, RAT, RRT, PABT, PEP, POD, POL, PTT, PETO, PCHOL and PNIT were excluded from selection because they did not show a significant relationship with most of the environmental parameters ($P > 0.05$; **Table 5.3**). NEP, NTR, NET, NDI, H' and NCH were excluded for metrics selection because of having significant correlation with PTR, PET, PIT, PDI and PCH ($P < 0.01$).

Therefore, those indices which had a high and significant correlation with most of the physicochemical parameters ($P < 0.05$; **Table 5.3**) and greater than 50% discrimination efficiencies (PET, PIT, PTR, PDI, PCH, RETCH, RITT, and HBI) were included in metrics selection. From the four metrics based on reference macroinvertebrate taxa, PARTM and PRRTM were selected due to their high discriminatory efficiency (**Table 5.1, 5.3 & 5.4**). These metrics did not exhibit a complete overlap on their interquartile and had a potential to characterize the reference sites and non-reference sites (**Fig 5.2**).

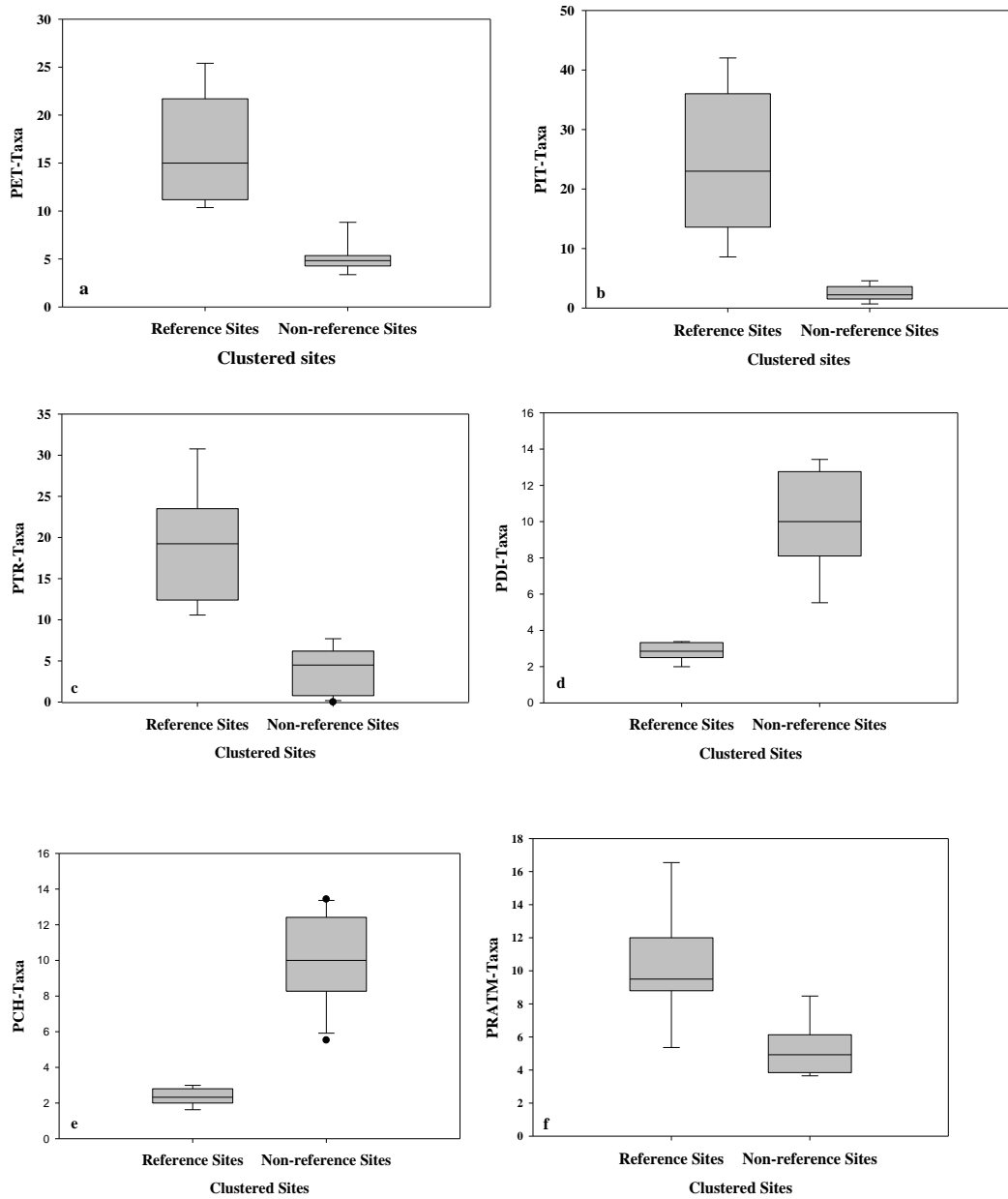


Figure 5. 2. Box-plots of the selected metrics of macroinvertebrate and their distribution along the clustered sites (a-j) of Lake Hawassa: the line divides the box represent median, boxes represent interquartile ranges (25–75% percentiles) and range bars show maximum and minimum values (for other abbreviations see Table 5.1).

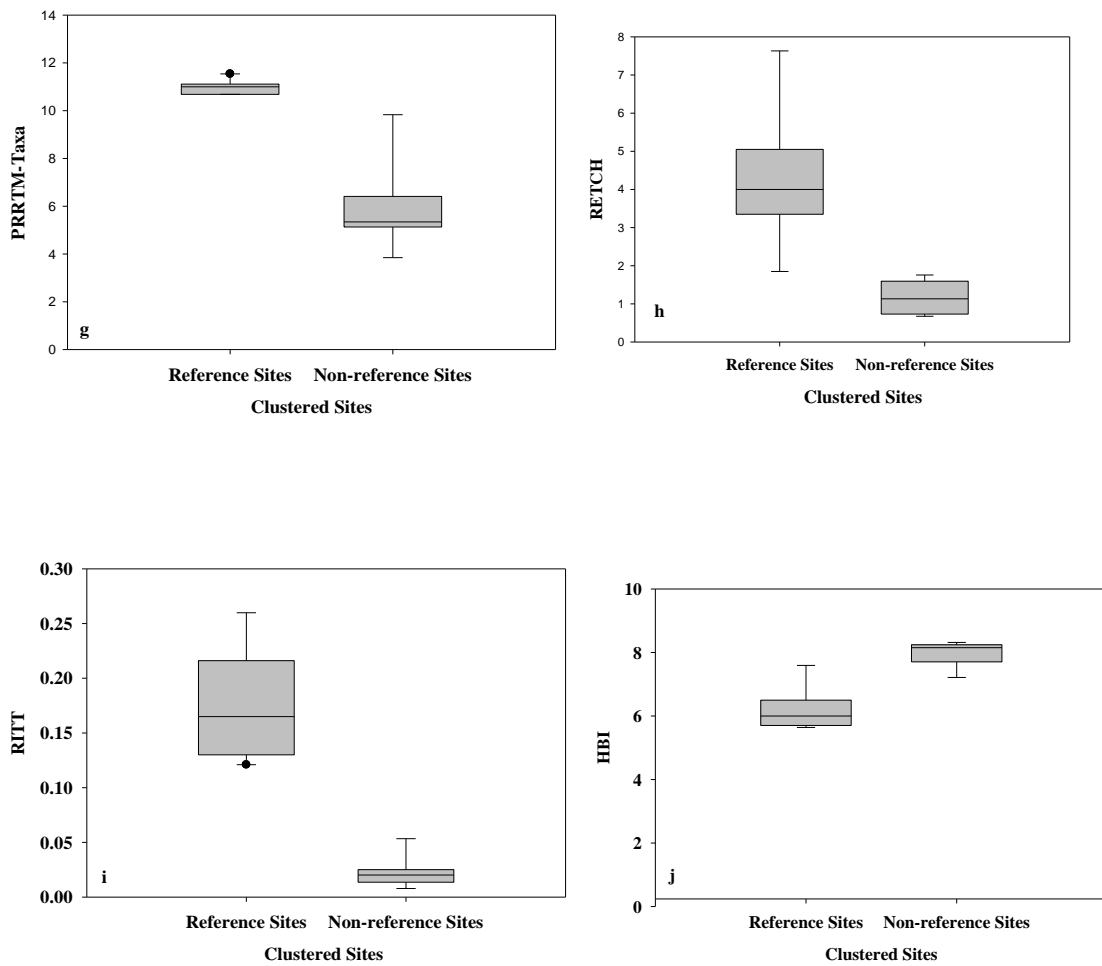


Figure 5.2. (Continued)

Table 5. 3. Spearman correlation of macroinvertebrate metric with some selected physicochemical variables and discrimination efficiency percentage (%DE) (Abbreviations: T= temperature, EC=conductivity, DO= dissolved oxygen, TP=total phosphorus, SRP=soluble reactive phosphate and TSS= total suspended solids; for other abbreviations see Table 5.1).

Metrics	T	EC	DO	TP	SRP	NO₂	NO₃	TSS	Chla	Turbidity	%DE
NFA	-0.10	-0.11	-0.19	-0.09	0.12	0.05	0.18	0.07	0.24*	-0.03	38.6
TABT	0.05	-0.09	-0.10	-0.03	0.16	0.207*	0.15	0.249*	0.303**	0.16	43.9
NEP	0.510**	-0.30**	0.17	0.485**	0.216*	0.08	0.251*	0.13	0.14	0.07	48.3
NTR	-0.18	0.19	-0.41**	0.10	0.439**	-0.08	0.332**	0.368**	0.344**	0.242*	60.9
NOD	0.15	-0.01	0.109	0.07	-0.20-	-0.31**	-0.08	0.306**	-0.35**	-0.13	36.9
NET	0.459**	-0.236*	0.05	0.347**	0.266**	0.397**	0.208*	0.15	0.13	0.10	18.9
NOL	0.228*	-0.12	0.455**	-0.10	-0.15	0.07	-0.11	-0.32**	-0.17	-0.20	72.8
NTT	0.10	-0.10	0.225*	-0.03	-0.01	0.243*	-0.03	-0.08	0.258*	-0.05	47.6
NIT	-0.07	0.05	-0.51**	0.08	0.125	0.00	0.07	0.355**	0.402**	0.222*	56.7
NETO	0.344**	-0.28**	0.16	0.15	-0.08	0.247*	-0.10	-0.12	-0.44**	0.02	14.4
NDI	0.379**	-0.37**	-0.70**	-0.27**	0.523**	0.240*	0.263**	-0.38**	-0.11	-0.274**	50.4
NCH	0.331**	-0.36**	-0.70**	-0.29**	0.534**	0.222*	0.273**	-0.40**	-0.12	-0.291**	45.9
NCHOL	0.305**	0.02	0.16	-0.09	0.16	-0.48**	-0.20	-0.41**	-0.31**	-0.239*	52.7
NNIT	0.00	0.10	0.19	-0.03	-0.05	0.11	-0.13	0.09	0.17	0.06	35.7
RAT	0.17	-0.04	0.00	0.10	0.232*	0.250*	0.17	0.223*	0.407**	0.17	46.2
RRT	-0.01	0.10	0.05	-0.13	0.02	0.05	0.20	-0.232*	0.19	0.296**	58.3
PABT	0.05	-0.09	-0.10	-0.03	0.266**	0.207*	0.15	0.249*	0.303**	0.16	44
PET	0.459**	-0.236*	0.05	-0.232*	0.266**	0.397**	0.208*	0.15	0.347**	0.218*	73.9
POD	0.15	-0.01	0.209*	0.07	-0.06	-0.08	-0.11	-0.20	-0.35**	-0.13	37.1
POL	0.228*	-0.212*	0.16	-0.10	0.07	-0.45**	-0.11	-0.32**	-0.17	-0.20	73
PTT	0.11	-0.02	0.08	0.03	0.01	-0.35**	-0.12	-0.44**	-0.16	-0.273**	31.6
PIT	-0.07	0.05	-0.51**	-0.208*	0.425**	0.00	0.274**	0.355**	0.402**	0.222*	74.7

* & ** Correlation is significant at the 0.05 & 0.01 level (2-tailed)

Table 5.3. (Continued)

Metrics	T	EC	DO	TP	SRP	NO₂	NO₃	TSS	Chl-a	Turbidity	%DE
PEP	0.510**	-0.30**	0.17	0.08	0.13	0.485**	0.07	0.216*	0.251*	0.259*	50
PTR	-0.18	0.19	-0.41**	-0.26**	-0.44**	-0.08	-0.33**	-0.37**	0.34**	-0.242*	70.9
PETO	0.344**	-0.28**	0.16	0.15	-0.08	0.247*	-0.10	-0.12	-0.44**	0.02	14
PDI	0.317**	-0.210*	-0.46**	0.266**	0.415**	0.203*	0.246*	0.288**	-0.38**	0.311*	68.9
PCH	0.317**	-0.41**	-0.46**	0.266**	0.415**	0.303*	0.246*	0.288**	-0.38**	0.36**	62.1
PCHOL	0.14	-0.10	0.14	0.02	-0.02	-0.47**	-0.14	-0.28**	-0.35**	-0.29**	40.6
PNIT	-0.02	0.11	0.402**	-0.13	0.00	-0.33**	-0.07	-0.13	-0.45**	-0.15	27.7
PRATM	0.17	-0.04	0.295**	0.310*	0.232*	0.250*	0.317**	0.223*	0.407**	0.17	79
PRRTM	-0.01	0.10	-0.232*	-0.13	0.296**	0.405*	0.32*	0.405*	0.19	0.202**	78.2
RETC	0.19	0.00	-0.33**	-0.38**	-0.49**	0.20	-0.31**	0.300**	0.362**	0.202*	79.5
RITT	-0.06	0.12	-0.63**	-0.25**	-0.54**	-0.04	0.328**	0.337**	0.347**	0.250*	72.7
HBI	-0.10	0.231*	-0.204*	0.231*	0.228*	0.14	0.275**	-0.13	-0.18	0.303**	57
H'	-0.257	-0.622*	-0.159	-0.41**	0.459	0.557	0.641*	-0.72**	0.117	-0.734**	53

* & ** Correlation is significant at the 0.05 & 0.01 level (2-tailed)

5.3.2. Developments of multi-metric index of L. Hawassa (MMIH)

From the list of 35 macroinvertebrate and 22 diatom candidate metrics, 10 and 8 macroinvertebrates and diatom metrics were selected, respectively, based on their response to the different level of disturbances, discriminatory power between the reference and test sites, and correlation with physicochemical parameters. The selected metrics were used for the development of multimetric index of biotic integrity of L. Hawassa (MMIH; **Table 5.4**). The MMIH used a 5, 3 or 1 discrete type scoring system to normalize the metric value for positive metric using 75th and 25th percentiles of the metric values and the reverse for negative metrics. For positive metrics, metric values above 75th percentiles were scored as 5. Metric values between and including the 25th and 75th percentiles were scored as 3, and all metric values below the 25th percentile were scored as 1(**Table 5.5**). The sum of the total score of each site was considered as the MMIH values. The maximum and minimum possible ranges of the MMIH values were 90 and 18, respectively. These MMIH values were divided into quartile ranges. So, the MMIH range values 73-90, 55-72, 37-54 and 18-36 were classified as very good, good, fair and poor quality, respectively.

Therefore, the current study showed that the multimetric index (MMIH) had a potential to discriminate clearly the reference site and the non-reference and between the three-disturbance level (minimal, moderate and high disturbance level) of the clustered sampling stations of the lake (**Fig 5. 3**). It put the minimally disturbed sites (C1) into very good categories (KW1, KW2, and WSH), moderately disturbed sites (C2) into fair (MK, TW1 and TW2) and highly disturbed sites (C3) into poor categories (**Table 5.6**).

Table 5. 4. The Selected potential macroinvertebrate and diatom metrics in this study

Metrics		
Macroinvertebrate Metrics	PET	Percent Ephemeroptera-Trichoptera taxa
	PIT	Percent Intolerant taxa
	PTR	Percent Trichoptera
	PDI	Percent Diptera
	PCH	Percent Chironomidae
	PRATM	Percent reference abundance of macroinvertebrate taxa
	PRRTM	Percent reference richness of macroinvertebrate taxa
	RETC	Ratio of Ephemeroptera and Trichoptera to Chironomidae taxa
	RITT	Ratio of intolerant to tolerant taxa
	HBI	Modified Hilsenhoff Biotic Index
Diatom Metrics	EPI-D	Pollution metric based on diatoms (Dell’Uomo, 1996)
	SHE	Steinber& Schiefele trophic metric (Steinberg, 1987)
	SLA	Sladeczek’s pollution index (Sládeček, 1986)
	IDG	Generic Diatom Index (Coste and Ayphassorho, 1991)
	TDI	Trophic Diatom Index (Kelly and Whitton, 1995)
	ROTTt	Trophic metric (Rott <i>et al.</i> , 1999)
	RATD	Reference Abundance Taxa of Diatoms (Present study)
	PRATD	Percent Reference Abundance Taxa of Diatoms (Present study)

Table 5. 5. Frequency distribution statistics of the final metrics and its scoring criteria

Metrics	Frequency distribution					Score		
	Min.	25th percentile	Median	75th percentile	Max.	5	3	1
PET	3.36	4.66	5.698	8.48	25.4	>8.48	4.66-8.48	<4.66
PIT	0.69	2.01	4.5	8.92	42.03	>8.92	2.01-8.92	<2.01
PTR	0	0.96	6.73	10.58	30.77	>10.58	0.96-10.58	<0.96
PDI	1.99	3.6	5.22	11.38	13.43	<3.6	3.6-11.38	>11.38
PCH	1.62	3.29	5.13	11.38	13.43	<3.29	3.29-11.38	>11.38
PRATM	3.65	4.53	7.3	11.02	16.55	>11.02	4.53-11.02	<4.53
PRRTM	3.85	5.24	8.12	11.43	12.82	>11.43	5.24-11.43	<5.24
RETC	0.67	1.13	1.8	3.25	7.63	>3.25	1.13-3.25	<1.13
RITT	0.01	0.02	0.037	0.1	0.26	>0.1	0.02-0.1	<0.02
HBI	5.64	6.3	7.4	8.18	8.32	<6.3	6.3-8.18	>8.18
EPID	9.9	11.6	12.55	13.25	13.6	>13.25	11.6-13.25	<11.6
SHE	10.5	10.9	12.55	12.875	15.2	>12.875	10.9-12.875	<10.9
SLA	11.2	11.55	12.35	12.975	13.7	>12.975	11.5-12.975	<11.5
IDG	6.6	9.75	12.55	13.6	15.2	>13.6	9.75-13.6	<9.75
TDI	5.7	6.575	8.65	9.875	12.8	>9.875	6.575-9.875	<6.575
ROTT	4.6	5.675	6.45	7.425	11	>7.425	5.675-7.425	<5.675
RATD	53	132.25	233	327	390	>327	132.25-327	<132.25
PRATD	10.6	26.45	46.6	65.4	78	>65.4	26.45-65.4	<26.45

Table 5. 6. The MMIH and their ecological interpretation. Abbreviations: WQC= water quality class, C1, C2 and C3= Cluster 1, 2 and 3, respectively

Clustered sites	Sites	Ecological interpretation of the MMIH			
		MMIH	WQC	Ecological Status	
C1	Minimally disturbed/ Reference	KW1	84	I	Very good
		KW2	80	I	Very good
		WSH	74	I	Very good
C2	Moderately disturbed	MK	50	III	Fair
		TW1	48	III	Fair
		TW2	50	III	Fair
C3	Highly Disturbed	WR	34	IV	Poor
		RH1	36	IV	Poor
		AG1	22	IV	Poor

The discrimination capacity of the MMIH index between reference and non-reference sites was tested by Box and Whisker plots within the comparison of the previously classification of sampling stations of L. Hawassa. Thus, the MMIH clearly discriminated the reference sites from the non-reference (test) sites (**Fig 5.3a**). It also showed a demarcation potential between the three clusters such as C1 (minimally disturbed), C2 (moderately disturbed) and C3 (highly disturbed sites) of the lake (**Fig 5.3b**).

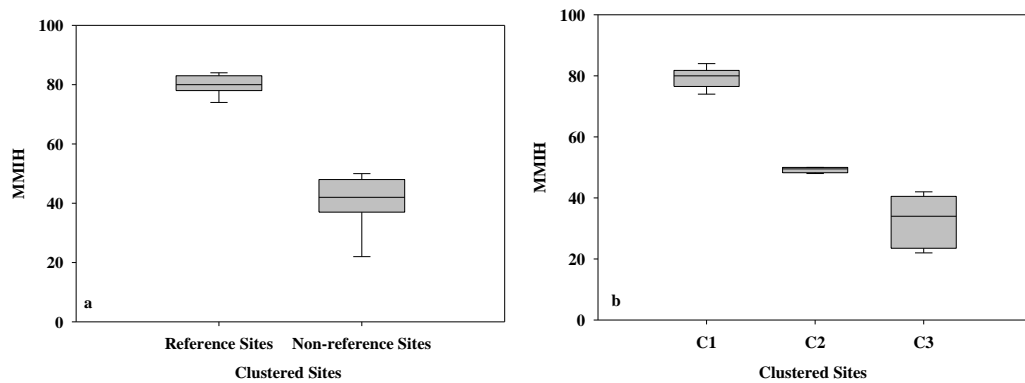


Figure 5. 3. Box-plots of the Multimetric Index (MMIH) and their distribution along the clustered sites: a) reference and non-reference sites, b) three clustered sites (C1= minimally disturbed sites, C2= moderately disturbed sites and C3= highly disturbed sites); the line divides the box represent median, boxes represent interquartile ranges (25–75% percentiles) and range bars show maximum and minimum values.

5.3.3. Ecological quality ratio (EQR)

The ecological quality ratio (EQR) was calculated and its range was between one and zero, but there were values greater than one which were considered as one. The 90th percentile of the reference site of the EQR values was 1. The sampling sites ecological status was classified based on the EQR values with its range (**Table 5.7**).

Table 5. 7. The classification of ecological status of Lake Hawassa with its values of Ecological Quality Ratio (EQR)

No.	Values of Ecological quality ratio(EQR)	Ecological status
1	≥ 1	High quality (reference site)(I)
2	$1 < \text{EQR} \leq 0.75$	Good quality(II)
3	$0.75 < \text{EQR} \leq 0.5$	Moderate quality(III)
4	$0.5 < \text{EQR} \leq 0.25$	Poor (IV)
5	$0.25 < \text{EQR} \leq 0$	Bad (V)

The Ecological Quality Ratio potentially discriminated the reference and non-reference sites. It characterized the minimally disturbed (C1) sites into high quality or reference condition (KW1, KW2, and WSH), moderately disturbed sites into moderate quality and highly disturbed sites into poor quality sites (**Table 5.8**). The Box and Whisker plots also showed that the EQR had the efficiency to discriminate between reference and non-reference sites and the three clustered sites (C1, C2 and C3; **Fig 5.4**).

Table 5. 8. The Ecological Quality Ratio (EQR) and their ecological interpretation:
Abbreviations: WQC= water quality class, C1, C2 and C3= Cluster 1, 2 and 3,
respectively

Clustered sites		Sites	Ecological interpretation of the EQR		
			EQR	WQC	Ecological Status
C1	Minimally disturbed/ Reference	KW1	1	I	High quality
		KW2	1	I	High quality
		WSH	1	I	High quality
C2	Moderately disturbed	MK	0.68	III	Moderate quality
		TW1	0.66	III	Moderate quality
		TW2	0.68	III	Moderate quality
C3	Highly Disturbed	WR	0.47	IV	Poor quality
		RH1	0.49	IV	Poor quality
		AG1	0.3	IV	Poor quality

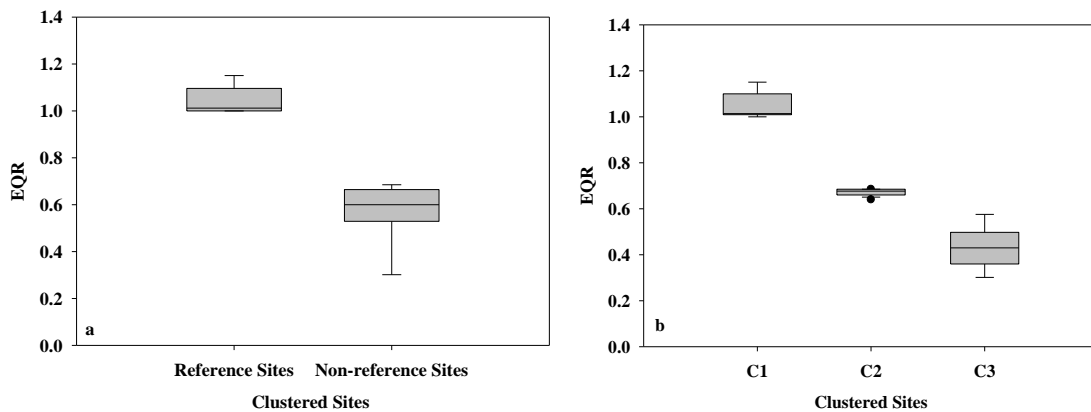


Figure 5. 4. Box-plots of the Ecological Quality Ratio (EQR) and their distribution along the clustered sites: the line divides the box represent median, boxes represent interquartile ranges (25–75% percentiles) and range bars show maximum and minimum values; C1= minimally disturbed sites; C2= moderately disturbed sites and C3= highly disturbed sites

5.3.4. Validation of the multi-metric index

The efficiency of the MMIH index to discriminate between reference and non-reference sites, and between *a priori* classification of the sampling station (i.e. minimal, moderate and high disturbance levels) were tested by Box and Whisker plots in previous section. Furthermore, to confirm the suitability and robustness of this newly developed index (MMIH index) it requires validation. The validation of the MMIH index was performed using independent data sets which were not used for the MMIH index. Thus, the performance of the MMIH index was tested in the new data sets of Lake Hawassa and L. Ziway. Three sites were taken from Lake Hawassa and subjected to validation using the MMIH index. Thus, the MMIH classified one sites as very good (MMIH value= 73) and two sites as fair (MMIH value= 46 and 52); which coincided with prior classification of these sites as in chapter 2.

The developed MMIH also discriminated L. Ziway's reference and test sites as shown in **Fig 5.5**. It showed good potential of characterizing the L. Ziway clustered sites. For example, from three reference sites (C1) two of them were classified as very good to good quality; from three moderately disturbed sites (C2) only one site was considered good whereas the rest were characterized as fair. The three highly disturbed sites (C3) of the lake were also classified as fair and poor. The principal component analysis (PCA) also clearly explained 97% of the variation between the reference site and non-reference/test site of Lake Hawassa with both the first and the second axis. This confirms the potential of the MMIH index to discriminate between reference and non-reference sites of Lake Hawassa (**Fig. 5.6**).

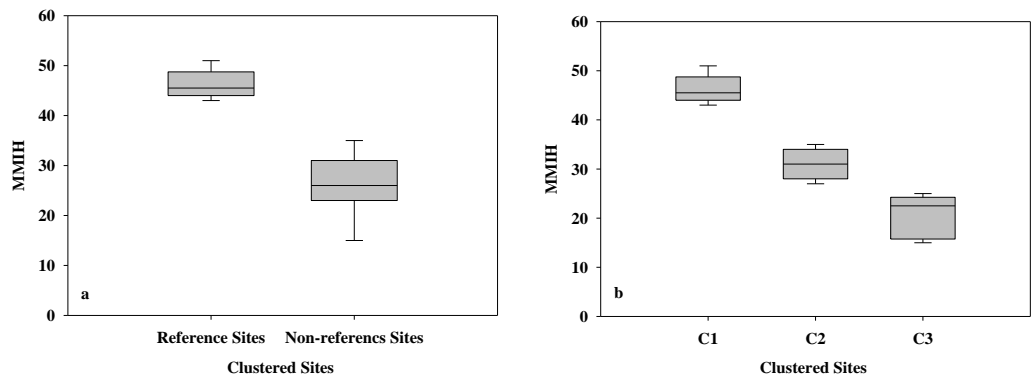


Figure 5. 5. Validation of the MMIH using Box and Whiskers plots along the clustered sites of (a) reference and non-reference sites, and (b) C1, C2 and C3 of L. Ziway: the line divides the box represent median, boxes represent interquartile ranges (25–75% percentiles) and range bars show maximum and minimum values

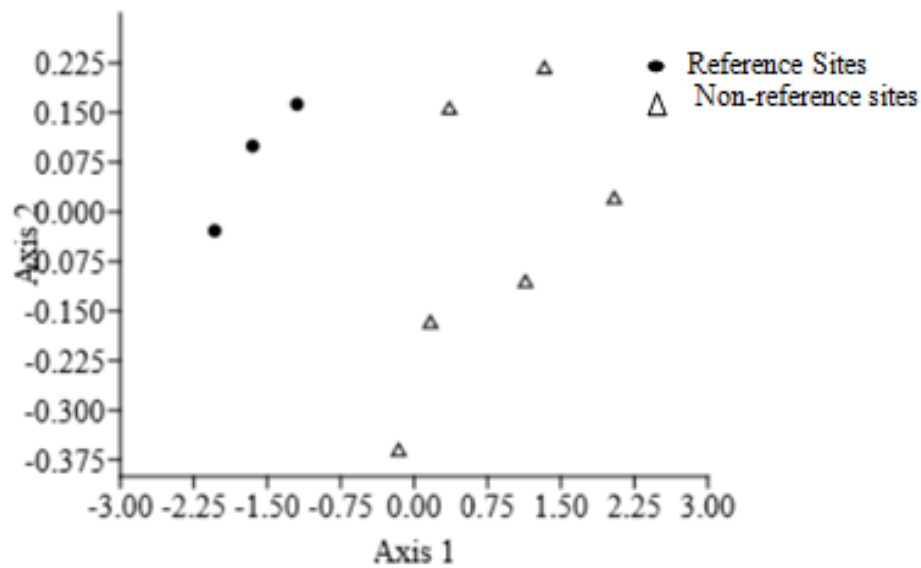


Figure 5. 6. Principal component analysis (PCA) showed the efficiency of MMIH to discriminate the reference and non-reference sites

The two-stage least squares (TSLS) regression analysis of MMIH showed an inverse but strong relationship with percent disturbance score (PDS) which demonstrates the MMIH index responsiveness to the habitat quality degradation of Lake Hawassa (n=9, R²=0.91, P=0.0003; **Fig. 5.7**). The MMIH index also showed significant positive response to DO, and negative but significant correlation with temperature, nutrients, TSS and turbidity and had high percent discriminatory efficiency (%DE; **Table 5.9**).

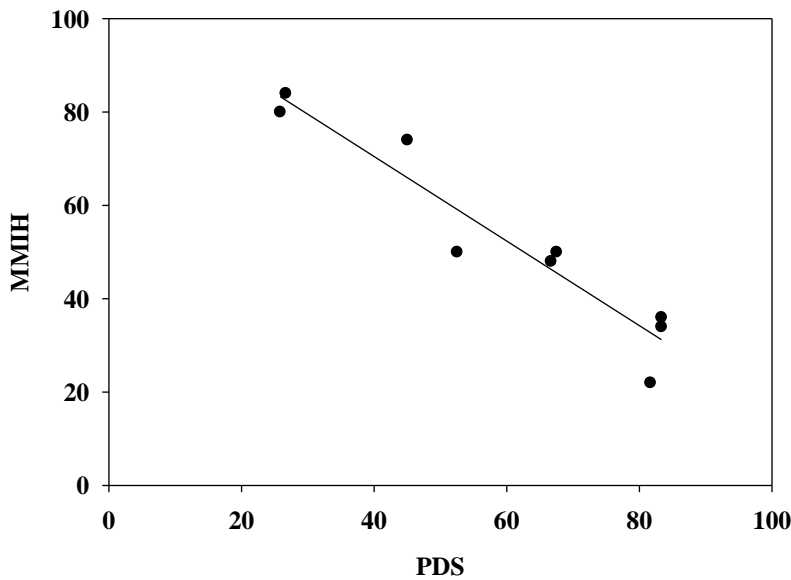


Figure 5. 7. Two-stage least squares (TSLS) regression Analysis of MMIH values against percent disturbance score (PDS; n=9, R²=0.91, P=0.0003)

Table 5. 9. Spearman correlation of MMIH index with some selected physicochemical variables (for abbreviations see Table 5.2)

	T	DO	TP	SRP	Nitrate	TSS	Tur	%DE
MMIH	-0.45**	0.47**	-0.75**	-0.56**	-0.56**	-0.678*	-0.72**	94

5.4. DISCUSSION

5.4.1. Metric Selection

The development of multimetric index involves a number of metrics from which potential metrics would be examined and evaluated to make a single composite index. The metrics incorporated into the multimetric development should be selected based on their responsiveness to the degradation level of the sampling stations (Hering *et al.*, 2006a). In addition, for the sake of data handling and statistical management, a practical number of potential metric should be incorporated in the development of multimetric index. From the candidate metrics, the selection of potential metrics was conducted based on their discriminatory power between the reference and non-reference sites, redundancy analysis and correlation with physicochemical parameters (Phiri *et al.*, 2007; Baptista *et al.*, 2011; Jun *et al.*, 2012; Wang *et al.*, 2015). So, the metrics that passed those criteria were considered as potential metrics and incorporated in the multimetric development.

The diatom metrics such as IDAP, SID, WAT, DES, LOBO, IDP and DICH were excluded from selection due to <50% inclusion of the diatom species. Generally, it is accepted that the more the species included in index calculation, the more efficiently index can explain the ecology. These indices did not also show a significant correlation with physicochemical parameters with ecological implication (i.e. TP, SRP and NO₃-N; $P > 0.05$; **Table 5.2**). The IBD, IPS, TID, CEE and %PTV indices which highly correlated with SHE, IDG and TDI were not also included in metrics selection (**Table 5.1 & 5.2**). Since a metric which is highly correlated with another metric would not contribute new information to the water quality assessment, it should be excluded from the collection of

core metrics (Barbour *et al.*, 1996; Hering *et al.*, 2006a). As the metric EPID and SLA were not redundant to any metrics, they were included in final selection. Similar procedures was also done on a metric selection which was conducted on Lake Dongting of China (Wang *et al.*, 2015) and 1031 lakes of the USA (Stevenson *et al.*, 2013). Thus, diatom indices those which had greater than 50% discrimination efficiencies and species inclusion during indices calculation, and showed a significant correlation within most of physicochemical variables (EPID, SHE, SLA, IDG, TDI and ROTT) were included in metrics selection.

Besides these potential indices, it was necessary to develop new metrics based on diatom reference community for Lake Hawassa to support the discrimination power of the developed multimetric index as stated in Delgado *et al.* (2010). Therefore, from the four candidate metrics based on reference diatom taxa (**Table 5.1**), RATD and PRATD were selected due to their high discriminatory efficiency (**Table 5.1&5.2**) which clearly discriminated the reference sites and non-reference sites (**Fig 5.1**).

In similar way, NFA, TABT, NOD, NOL, NTT, NIT, NETO, NCHOL, NNIT, RAT, RRT, PABT, PEP, POD, POL, PTT, PETO, PCHOL and PNIT of macroinvertebrate metrics were excluded from the selection because they didn't show a significant relationship with most of the environmental parameters (**P > 0.05; Table 5.3**). NEP, NTR, NET, NDI, and NCH were not also included within metrics selection because of having a significant correlation with PTR, PET, PIT, PDI and PCH (**P < 0.01**) which was considered as redundant. The discriminatory power of core metrics between the reference and non-reference sites is commonly practiced in most of water quality assessment which is usually based on redundancy analysis and correlation with physicochemical parameters

(Jun *et al.*, 2012; Wang *et al.*, 2015). Finally, a total of 18 macroinvertebrate and diatom metrics (32% of the tested metrics) were selected and incorporated for the multimetric development of L. Hawassa (MMIH; **Table 5.4**). All of the selected macroinvertebrate and diatoms metrics did not show complete overlaps in their interquartile ranges and had a sensitive value of 2 and 3 (**Fig. 5.1 and 5.2**), as recommended in Barbour *et al.* (1996).

5.4.2. Multi-metric index of L. Hawassa (MMIH), Ecological Quality Ratio (EQR) and their ecological interpretation

Habitat disturbance and nonpoint sources of pollution, which range from habitat alteration to chemical contamination, are accountable for most of the lake water quality degradation. Thus to determine these pollution levels, it is recommended to establish a multimetric index rather than a single metrics. Because the multimetric index serves as an integrated analysis as individual metrics may differ in their relative sensitivity to various levels of biological condition (Barbour *et al.*, 1999; Hering *et al.*, 2006a). Multimetric index often puts together metrics and uses biological measurements to quantify the degree to which human disturbance has influence on biological communities (Masese *et al.*, 2013; Schoolmaster *et al.*, 2013).

In this study, the MMIH index had a holistic characteristic which involved the integration of many candidate metrics of macroinvertebrates and diatoms into a composite index. Such a multimetric approach was conducted elsewhere and got better acceptance due to its effectiveness in lake-biomonitoring assessment (Barbour and Gerritsen, 1996; Hering *et al.*, 2006a). A multimetric index of Dongting Lake, China, which was established based on multi-assemblage communities such as macroinvertebrates and algae is a typical

example (Wang *et al.*, 2015). However, most of the bioassessment multimetric indices have been developed based on one community aggregation either macroinvertebrates (Seid Tiku *et al.*, 2013) or diatoms (Stevenson *et al.*, 2013) or phytoplankton (Lacouture *et al.*, 2006) or others. Consequently, these single-assemblage based indices can only stand for one of the communities function and respond to a limited number of stressors which may affect the accuracy of the assessment although they are efficient when compared to a single metrics (Barbour *et al.*, 1996). So, it is better to increase the precession of the single community based multimetric index by adding another additional assimblege of the community, as was done on one of the tropical lakes of China (Wang *et al.*, 2015). Since Lake Hawassa has been affected by multi-type stressors which come from different sources (more often from non-point sources), the need of multimetric index development is a mandatory practice to quantify the ecological status of the sampling stations that have suffered from human induced level of disturbances. The multimetric index of biotic integrity of L. Hawassa (MMIH) was developed in order to harvest these benefits.

The multimetric index of biotic integrity of L. Hawassa (MMIH) showed a clear demarcation between the reference and non-reference/ test sites (**Table 5.6; Fig. 5.3**). Similar discrimination potential of macroinvertebrate MMI was observed at natural wetlands of Southwest Ethiopia (Seid Tiku *et al.*, 2013). The multimetric index discriminatory power has been considered as an evaluation of the suitability of the index as Aschalew Lakew and Moog (2015) did in central and southeast highlands of Ethiopian streams and rivers. It also placed the minimally disturbed sites (C1) as very good categories (KW1, KW2 and WSH), moderately disturbed (MK, TW1 and TW2)

sites into fair and highly disturbed (WR, RH1 and AG1) sites into poor quality (**Table 5.6**). Hence, the MMIH index showed a robust result able to discriminate the three clusters of the sampling stations of the lake as previously classified (See Chapter 2). The result from a single macroinvertebrate metric applied to Lake Hawassa by Amanuel Aklilu (2011) did not clearly discriminate the reference and non-reference sites, but the present study MMIH index with multi-assemblage organisms did (**Fig 5.3**).

The ecological condition of the reference and non-reference sites of L. Hawassa was assessed by ecological quality ratio (EQR). EQR provides reliable and easily understandable information to the concerned body to take action and/or formulate possible management options, as documented by many scholars (Lepistö *et al.*, 2004; Wells *et al.*, 2007), and recommended by the European Commission Water Framework Directives (ECEQR, 2007). It was applied in lakes Flanders, Belgium (Gabriels *et al.*, 2010). Since the ecological quality ratio (EQR) is an ecological expression of the MMIH index, it can explain the status of the sampling sites in-terms of their ecological condition. Thus, in this study, the Ecological Quality Ratio of the lake clearly discriminated the reference and non-reference sites (**Fig. 5.4**) and characterized the minimally disturbed (KW1, KW2, and WSH) sites into high ecological quality or reference condition. Similarly, the moderately disturbed (MK, TW1, and TW2) sites categorized as moderate ecological quality and the highly disturbed (WR, RH1 and AG1) sites into poor ecological quality (**Table 5.8 & Fig. 5.4**).

Generally, the MMIH index and the EQR showed robust results that clearly discriminate sampling stations into their ecological quality level as very good, fair and poor quality.

Hence, conservation of very good quality sites and rehabilitation of the fair and poor quality sites are important recommendations that can be drawn from the current result.

5.4.3. Validation of the MMIH

The suitability and robustness of a newly developed multimetric index (MMI) requires validation which is an effective method for evaluating the lake condition to recommend future use of the MMI, effective restoration and conservation methods and research gaps (Jun *et al.*, 2012). As the MMIH is a newly developed index of L. Hawassa, its discriminatory efficiency was evaluated by Box and Whisker plots between the reference and non-reference sites, and it also showed its discriminating efficiency between the clustered groups. Moreover, to corroborate its validation, there is a need to test its potential using independent data sets of Lake Hawassa and Lake Ziway (not included in the MMIH index development), as was explained in many biomonitoring programs (Menetrey *et al.*, 2011; Lunde and Resh, 2012; Villamarín *et al.*, 2013).

Validation of multimetric index using independent data sets is an appreciable method that indicates how well the multimetric index would be expected to work with sampling sites and gives good insight to scale up and use for another lakes in the ecoregion (Lunde and Resh, 2012; Villamarín *et al.*, 2013). Three sites were taken from Lake Hawassa and subjected to validation using the MMIH index. Thus, the MMIH classified one site as very good (MMIH value= 73) and two sites as fair (MMIH value= 46 and 52), which coincided with prior classification of these sites in the previous section.

The MMIH index was also validated using the Lake Ziway independent data sets since L. Hawassa and L. Ziway are situated in the same ecoregion, namely Northern Eastern Rift

(Thieme *et al.*, 2005). Hence, for the validation the pre-classified sampling sites of Lake Ziway such as 3 reference, 3 moderately disturbed and 3 highly disturbed sites were used which were not used for the development of the MMIH index. This validation method showed that the MMIH was able to discriminate L. Ziway's reference and test sites as shown in **Fig. 5.5**. It also showed good potential of characterizing the L. Ziway clustered sites; such as from three reference sites two of them were classified as very good (67%) and one as good quality(33%), from three moderately disturbed sites only one site was considered good whereas the rest characterized as fair. The three highly disturbed sites of the lake were also classified as fair and poor. Thus, the validation of the MMIH index showed its potential to be used in the biomonitoring of the initial lake and lakes which are situated in the same ecoregion of Lake Hawassa.

Furthermore, the PCA also clearly discriminated the reference and non-reference sites of Lake Hawassa. This confirmed the responsiveness of the MMIH with different levels of degradation which were already observed in the reference and non-reference sites. It properly validated the demarcation potential of MMIH among reference and non-reference sites and most of their variations (97%) were explained by Axis 1 and Axis 2 (**Fig. 5.6**).

The MMIH index also showed an inverse significant relationship with the disturbance score (PDS: $n=9$, $R^2=0.91$, $P<0.01$, **Fig. 5.7**). As the disturbance increased, the MMIH index score values decreased. Thus, the significant inverse relationship between MMIH and PDS represents a strong responsiveness of the MMIH index to the lake habitat quality levels. Similar phenomenon was observed on the study of multimetric index of Lake Kariba (Phiri *et al.*, 2007), lakes of USA (Stevenson *et al.*, 2013) and Lake

Dongting (Wang *et al.*, 2015). Since the MMIH index is considered as a positive metric, its value decreased against the habitat disturbance (**Fig. 5.7**). Similar result was also observed on wetlands of California (Lunde and Resh, 2012). This wetland report showed that a higher multimetric index scores was considered as signal of a less disturbed environment, while lower multimetric index scores indicated highly disturbed habitat. MMIH index showed a better potential than that of the single metrics of diatoms and macroinvertebrates. Similar potential was also observed on the multimetric developments of Alaska's biomonitoring practice with high precision and high discrimination efficiency (Bouchard *et al.*, 2004).

In addition, the MMIH index also showed significant positive response to DO, and negative but significant correlation with temperature, nutrients, TSS and turbidity (**Table 5.9**). Similar response of the MMI index to nutrient concentration was observed in lakes of USA (Stevenson *et al.*, 2013), Lithuanian lakes (Šidagytė *et al.*, 2013) and lakes in Flanders, Belgium (Gabriels *et al.*, 2010). The negative response of the MMIH index to nutrients, TSS and turbidity corresponds with what can be expected for stress-related variables (Gabriels *et al.*, 2010). This indicates that the MMIH index was suitable for water quality assessment of Lake Hawassa.

The overall finding of the current research indicated that such multimetric approach which encompasses multi-taxa in the index development, has significant advantage when the water body is exposed to multiple stressor types not usually counted (Everard *et al.*, 2011). The index can also reduce the independent metric prediction problem and maximize the efficiency to express the not-visualized and unmeasured disturbance of segments of the lake ecosystem. As noted by Schoolmaster *et al.* (2012), when there is a

problem of understanding the exact cause of degradation of water quality, the multimetric approach is most effective. The discrimination power of the MMIH index between different levels of human disturbance showed the effectiveness of multimetric approach.

In addition, the benefit of such MMIH development to specific lakes is that it would also increase the performance of the multimetric index by reducing natural variability (like in geology, soils, landscapes, climate, and water chemistry) among sites since it is difficult to distinguish the effect of natural variability from human induced variability if it is applied across large spatial scales (Stevenson *et al.*, 2013). Furthermore, the MMIH index clearly discriminated the reference and non-reference sites of Lake Hawassa and showed similar performance on Lake Ziway (within the same ecoregion); thus, it can be recommended for application to biomonitoring activity in other lakes of the same ecoregion.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

The habitat quality of Lake Hawassa was deteriorated because of human pressure related to expansion of cropland and built-up land use features in its littoral and riparian zone. The percent disturbance score (PDS) and physicochemical variables were found useful to characterize habitat quality and classify the reference (minimally disturbed) and non-reference sites of Lake Hawassa. As a result, the cluster analysis which was based on PDS and physicochemical (PC) parameters categorized the littoral into three-disturbance groups or clusters (C) such as C1-consist of minimally disturbed sites (KW1, KW2, TU and WSH), C2- moderately disturbed sites (AG2, RH2, MK, TW1 and TW2) and C3- highly disturbed sites (WR, RH1 and AG1). Except for DO and NO_2^- , all physicochemical variables had the highest mean record in highly disturbed sites while DO was mostly higher in minimally disturbed sites. From this result, it can be generalized that the disturbance of the lake habitat might be the major factor to increase the deterioration of water quality of the lake since highly disturbed sites had high concentration of nutrients and other biodegradable and non-biodegradable wastes.

Order Hemiptera, Odonata and Coleoptera were the most diverse groups and Coenagrionidae, Physidae and Pleuroceridae were the most abundant families within respective decreasing order. Coenagrionidae, Physidae, Pleuroceridae, Chironomidae, Planorbidae, Piscicolidae, Lumbriculidae, Libellulidae and Corixidae families were non-reference taxa and can be considered as tolerant to human disturbance in littoral habitat of the lake. But Polymitarciidae, Baetidae, Hydrophilidae, Notonectidae, Naucoridae,

Belostomatidae and Galastocoridae were reference taxa which might be considered as less tolerant families to human disturbance since they were abundant in reference sites of littoral area of the lake.

Similarly, the species composition of diatoms were also affected by the disturbance level of littoral zone of the lake since the highest and lowest diversity record were observed in minimally and highly disturbed sites, respectively. The genus *Nitzschia* was the most diverse group; and *Nitzschia frustulum*, *Achnantheidium minutissimum*, and *Gomphonema parvulum* were the most abundant species. Diatom species *Fragilaria capucina*, *F. tenera*, *Gomphonema gracile*, *G. olivaceum*, *G. parvulum*, *G. pumilum* var. *rigidum*, *Nitzschia frustulum*, *N. kuetzingioides*, and *N. palea* were non-reference taxa as they were abundant at disturbed sites which had high concentration of nutrients. On the other hand, species such as *Achnantheidium minutissimum*, *Aulacoseira ambigua*, *Cymbella cistula*, *C. kappii*, *C. turgidula*, *Encyonema muelleri*, *E. silesiacum*, *Encyonopsis microcephala*, *Navicula tenella* and *Ulnaria acus* were reference taxa as they were abundant in minimally disturbed sites.

Generally, the diversity and abundance of the macroinvertebrate families and diatom species decreased as the disturbance of the littoral areas of the lake increased. In addition, the reference taxa of both macroinvertebrate and diatoms were considered as good indicators of minimally disturbed sites with low nutrient concentration and EC.

The selected potential metrics showed an ability of discriminating the reference from non-reference sites and they were used for the multimetric index of biotic integrity of L. Hawassa (MMIH) development. The developed MMIH index and Ecological Quality

Ratio (EQR) categorized efficiently the three-clustered sites of the lake which were coincides with the previous classification. This study clearly indicated that the MMIH index clearly responded in the predictive way to the level of environmental degradation. It showed the potential of the MMIH index to clearly discriminate between the reference and non-reference sites with multiple stressors, and this responsiveness is mainly because the MMIH was a multi-assemblage index. As the MMIH was developed within the same locality which may reduce the natural variability and account for the increased overall discrimination efficiency of the index. Generally, it can be concluded that the MMIH index is a robust index that could assist end-users by providing a practical tool for measuring the ecological quality of L. Hawassa.

6.2. RECOMMENDATIONS

Based on the result from this research work, the following recommendations were pointed out:

1. The highest nutrient and ionic (EC) concentrations were recorded from highly disturbed sites, due to high human intervention like recessional farming, high input of storm water, household wastes discharges including human waste, etc. Hence, environment authorities should formulate possible management options (such as buffer zone around lake shores) to reduce the human pressures and influx of wastes that cause water quality impairments. In addition, the lake management program that has been practice on the Wabishebele Hotel (WSH) site should be scaled up to other shoreline segments of the lake. Further research work is needed on other chemical

- contaminants such as heavy metals, pesticides analysis to have clear picture of overall pollution levels.
2. The macroinvertebrate families such as Polymitarcyidae, Baetidae, Hydrophilidae, Notonectidae, Naucoridae, Belostomatidae and Galastocoridae were more abundant in minimally disturbed sites of littoral area of Lake Hawassa. Similarly, the diatom species *Achnanthydium minutissimum*, *Aulacoseira ambigua*, *Cymbella cistula*, *C. kappii*, *C. turgidula*, *Encyonema muelleri*, *E. silesiacum*, *Encyonopsis microcephala*, *Navicula tenella* and *Ulnaria acus* had also a potential to express the minimally disturbed sites of the lake. These macroinvertebrate families and diatom species can be considered as good indicators of lake water quality and habitat integrity, even though further study is needed to check their distribution through time and routine data set are needed to monitor water quality. Assignments of the sensitive/ tolerant values both diatom species and macroinvertebrate families is mandatory to increase the use of these assemblages in biomonitoring of the lake.
 3. The MMIH index of Lake Hawassa demarcated the reference (minimally disturbed) from non-reference sites (moderate and highly disturbed) and hence can be gauged to identify impacted sites for restoration priorities. Protection and conservation of very good quality sites and rehabilitation of the fair and poor quality sites can be recommended in similar Rift Valley lakes based on this index.
 4. The validation which was done using independent data sets of Lake Hawassa revealed that the MMIH index had a potential to discriminate the reference and non-reference sites of the lake. It also discriminated ecological degradation between the reference and non-reference sites of L. Ziway. Therefore, MMIH can contribute for the

development of the ecoregional biocriteria practice since it showed good response to L. Ziway that is situated in the same ecoregion, Northern Eastern Rift.

5. Such ecological assessment work should be continued at concurrent sites as a regular biomonitoring program to understand the trend of water quality in the lake and to facilitate management measures. This study has confirmed that such bioassessment approach is transferable to other lakes in the region and is a sufficient tool to evaluate the ecological condition of sites affected by several human perturbations at ecoregional scales.

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Appendix 1. Physical Habitat quality assessment data recording field sheet for Lake Hawassa: Modified after (Jones *et al.*, 2004; FDEPA, 2015)

LAKE NAME	DATE (D/ M/ Y)	SAMPLING SITE NUMBER:			FIELD ID / NAME:
LATITUDE _____ LONGITUDE _____	INVESTIGATORS	SAMPLING LOCATION/DESCRIPTION:			LAKE SEGMENT SIZE:
PARAMETER	No surface inflow or outflow present, very long water residence time, groundwater seepage dominates <input type="checkbox"/>	Surface water inflow present, but outflow is rare, moderate to long water residence time <input type="checkbox"/>	Surface water inflow and outflow present sometimes with visible flow, short water residence time <input type="checkbox"/>	Impounded, hydrology of system artificially controlled <input type="checkbox"/>	
HYDROLOGY					
Color	Very clear, uncolored water (benthic sampling appropriate) <input type="checkbox"/>	Water somewhat tannin stained (benthic sampling appropriate) <input type="checkbox"/>	Dark, discolored water (water color 20 PCU or higher) <input type="checkbox"/>	Visibility extremely reduced due to high color <input type="checkbox"/>	
	No evident disturbance	Minimum disturbance	Moderate disturbance	High disturbance	
Vegetation Quality <input type="checkbox"/>	Diverse, expected native vegetation (emergent or submersed), less than 5% nuisance taxa	Mostly expected native plants, but moderate growths (6%-20% of lake segment) of nuisance macrophytes, or more than 50% of lake covered with plants	Large masses (21%-40%) of nuisance macrophytes (e.g., Hydrilla, hyacinth, cattail, etc.) or algal mats	Lake choked (>40%) with nuisance macrophytes (Nile cabbage, hyacinth, etc.) or algal mats, or few plants present at all (e.g., plants removed)	
	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1	
Storm water Inputs <input type="checkbox"/>	Storm water enters system via sheet flow over non-cultivated and/or natural vegetation	Some direct storm water inputs (ditches, pipes, cultivated vegetation < 10%) but good BMPs in place	Moderate direct inputs of storm water (ditches, pipes, cultivated vegetation 11%-50%) but few BMPs in place	Much direct input of storm water (ditches, pipes, cultivated vegetation > 51%) and no or ineffective BMPs in place	

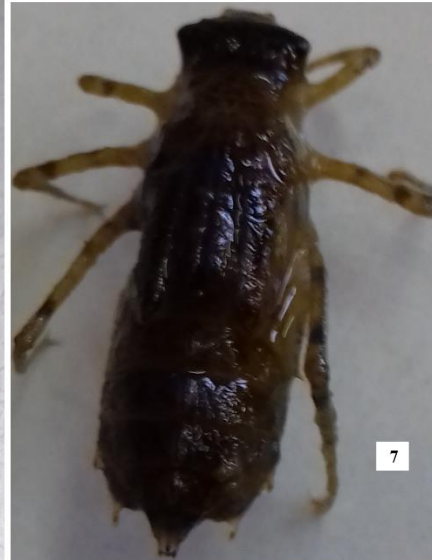
	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
Bottom Substrate Quality <input type="checkbox"/>	Diverse mixture of sand, detritus, with small amounts of CPOM/mud/muck. SAV may be present					Mixture of sand or clay and detritus with higher % CPOM/mud/muck content. SAV may be present					Moderate layer of CPOM/ mud/muck, or hard packed sand only, or moderate algal growth (mats or Chara) on bottom					Thick deposits of CPOM, or fine detritus and anaerobic muck/mud/silt, or algal growth or nuisance plants cover bottom				
	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
Lakeside Adverse Human Alternations <input type="checkbox"/>	Very few man-made structures, roads, or other disturbance adjacent to lake (<10%)					Moderate disturbance visible (structures, roads or other), 10%-49% lakeside affected					Many structures, roads or other human disturbance visible (50%-70%) lakeside affected)					Highly developed or disturbed (>70% of lakeside affected)				
	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
Upland Buffer Zone <input type="checkbox"/>	Expected native vegetation between uplands and littoral zone, greater than 90% of shore with >18 m buffer					89%-51% of shoreline with >18m buffer or >75% with 10m to 18m buffer					50%-30% of shoreline with >18m buffer or 50%-74% with 10m to 18m buffer					< 29% of shoreline with >18m buffer				
	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
Adverse Watershed Land Use <input type="checkbox"/>	Score the potential effects from adverse human land uses, based on a continuum of amount and type, with least to most adverse as follows: Native vegetation, Silviculture, Pasture or Citrus, Low Density Residential, Row Crops, Commercial, High Density Residential, Urban, Industrial																			
	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
TOTAL SCORE	<input style="width: 80px; height: 20px; border: 2px solid black;" type="text"/> COMMENTS:																			
ANALYSIS DATE:	ANALYST:										SIGNATURE:									

Appendix 2. Some of the identified macroinvertebrate families of Lake Hawassa



Hirudinae: 1. Glossiphoniidae and 2. Piscicolidae

Gastropoda: 3. Physidae, 4. Planorbidae and 5. Pleuroceridae



Odonata families: 6. Aeshnidae, 7. Libellulidae, and 8. Coenagrionidae



Hemiptera: 9. Nepidae, 10. Naucoridae, 11. Notonectidae and 12. Belostomatidae

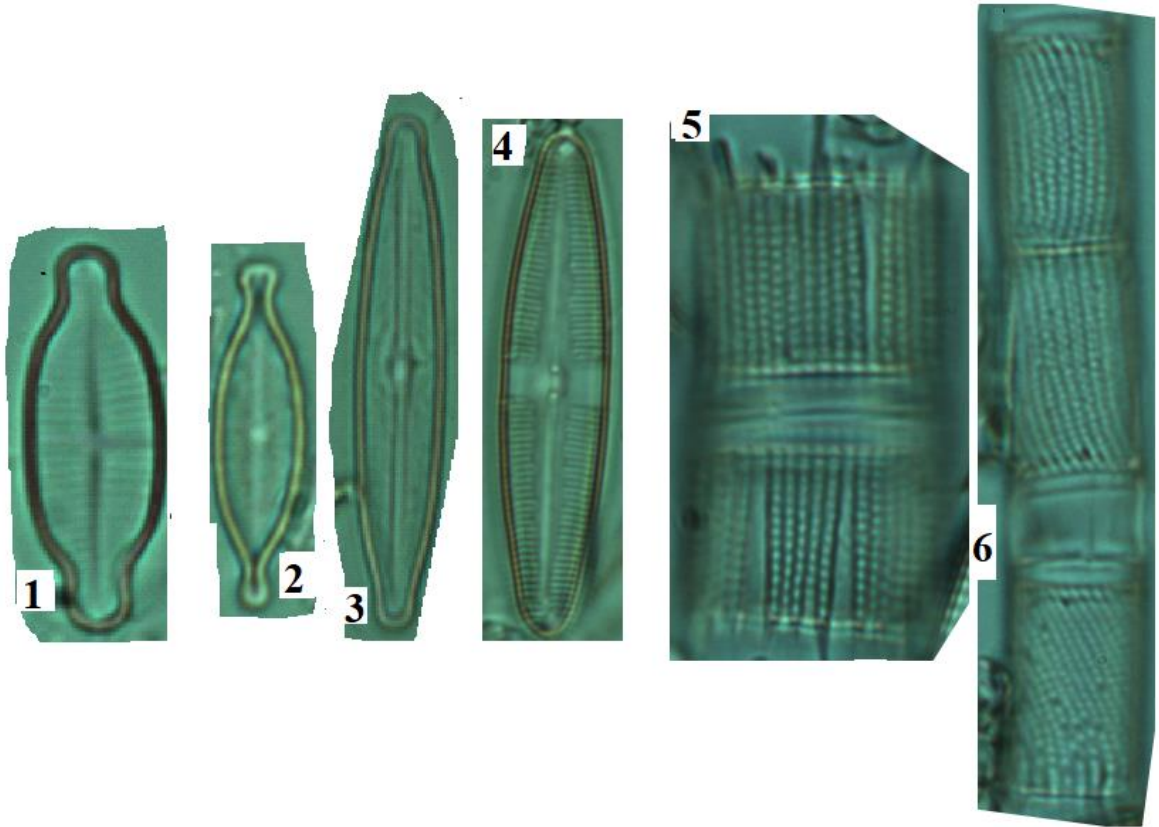


Coleoptera: 13. Noteridae, 14. Dytiscidae and 15. Hydrophilidae

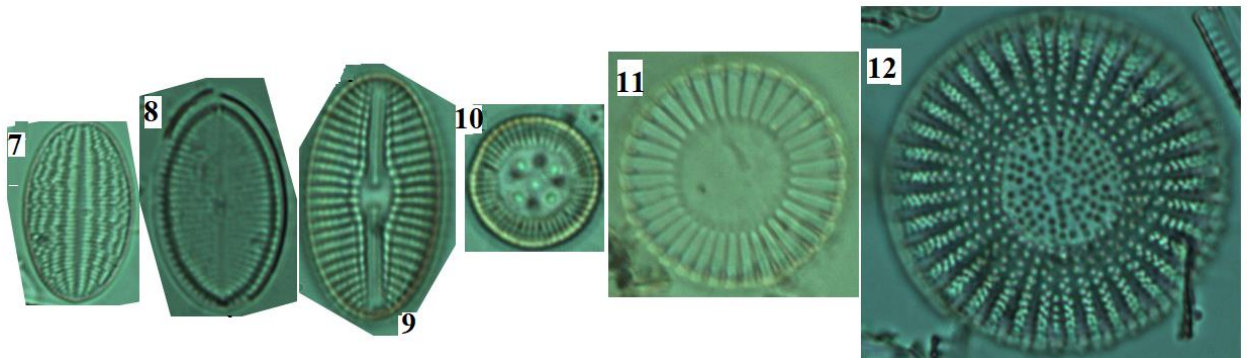


Diptera: 16 and 17. Chironomidae

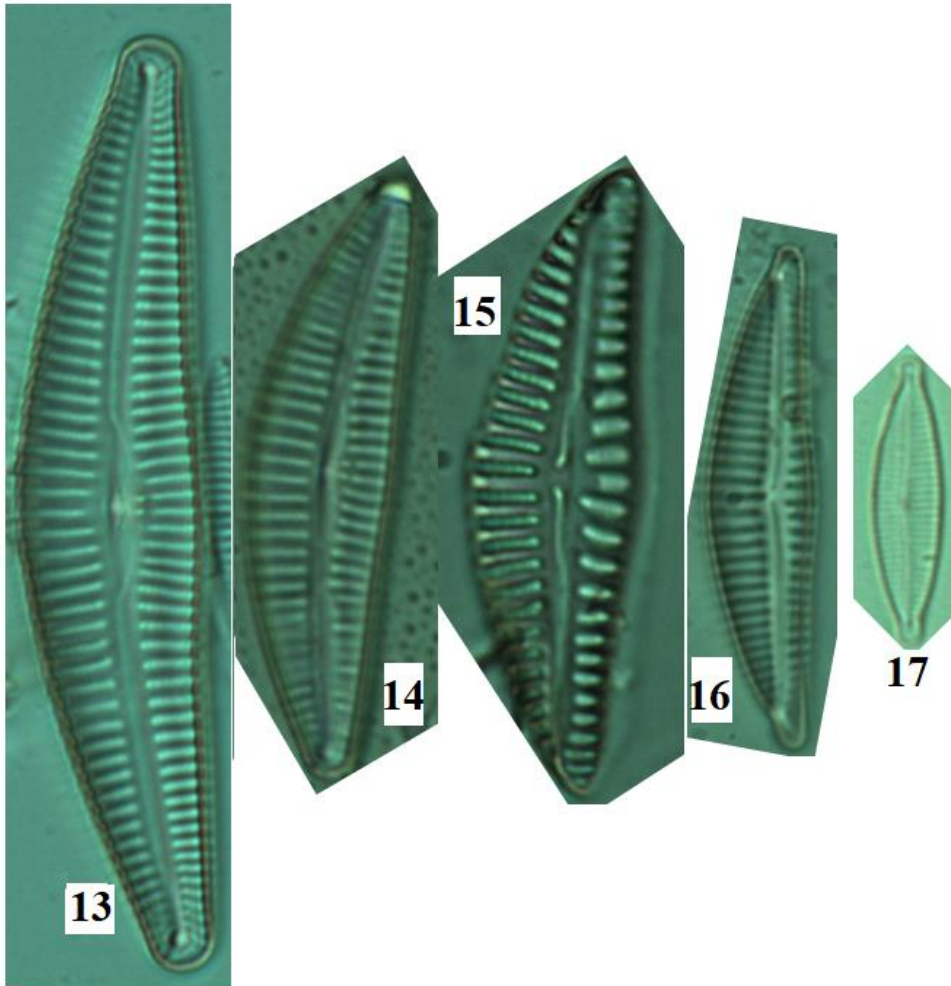
Appendix 3. Some of the identified diatom species of Lake Hawassa



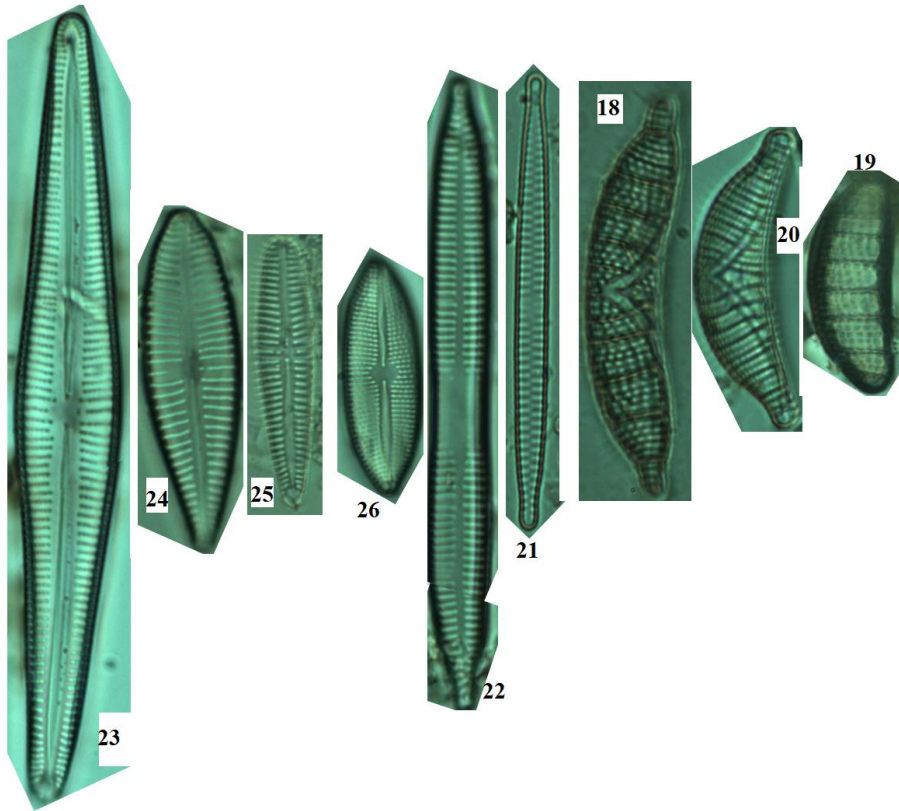
1. *Achnanthidium exiguum*, 2. *Achnanthes impexa* , 3. *Achnanthidium minutissimum*, 4. *Caloneis bacillum*, 5. *Aulacoseira granulata*, and 6. *A. ambigua*



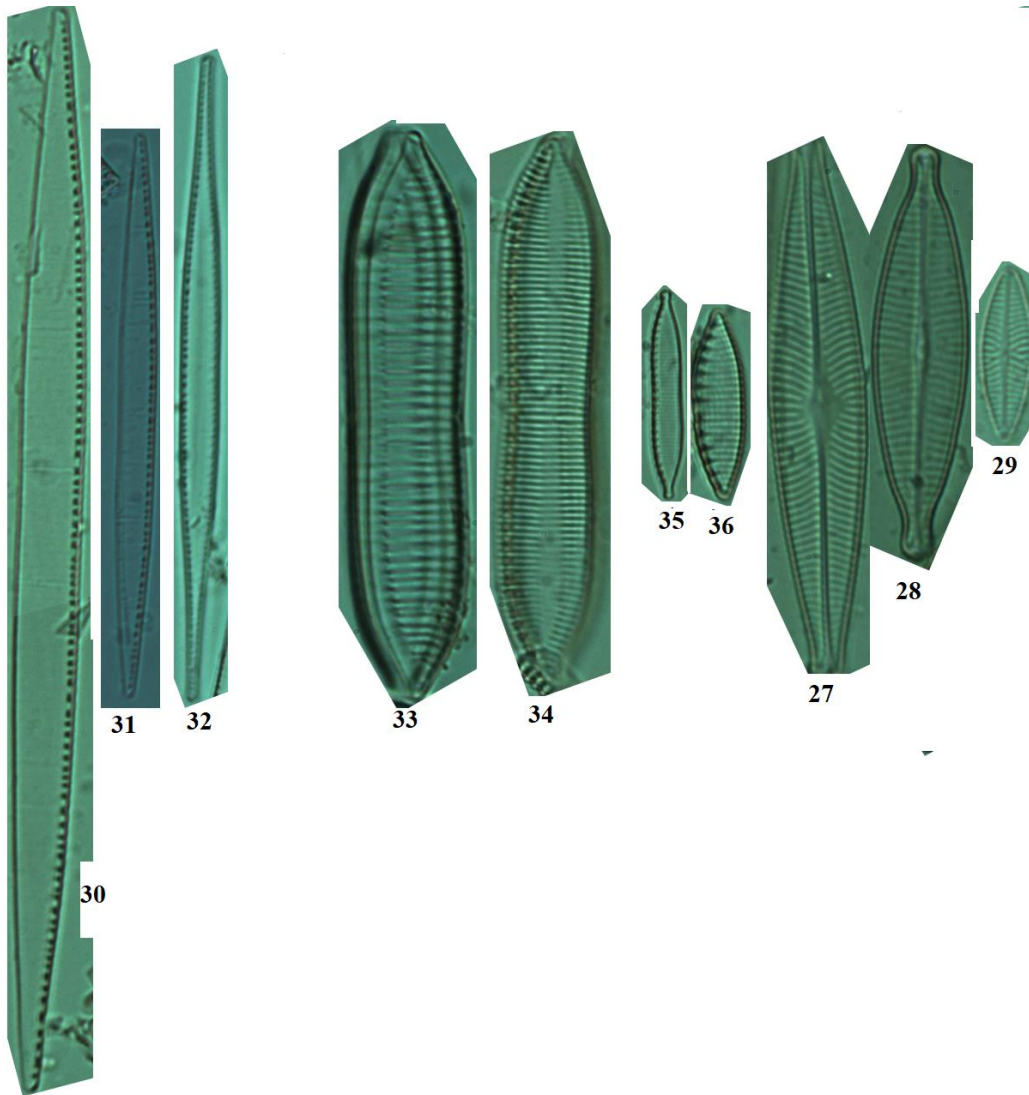
7. *Cocconeis placentula*, 8. *Cocconeis placentula* var. *lineata*, 9. *Diploneis smithii*, 10. *Pantocsekiella ocellata*, 11. *Cyclotella meneghiniana*, and 12. *Cyclostephanos dubius*.



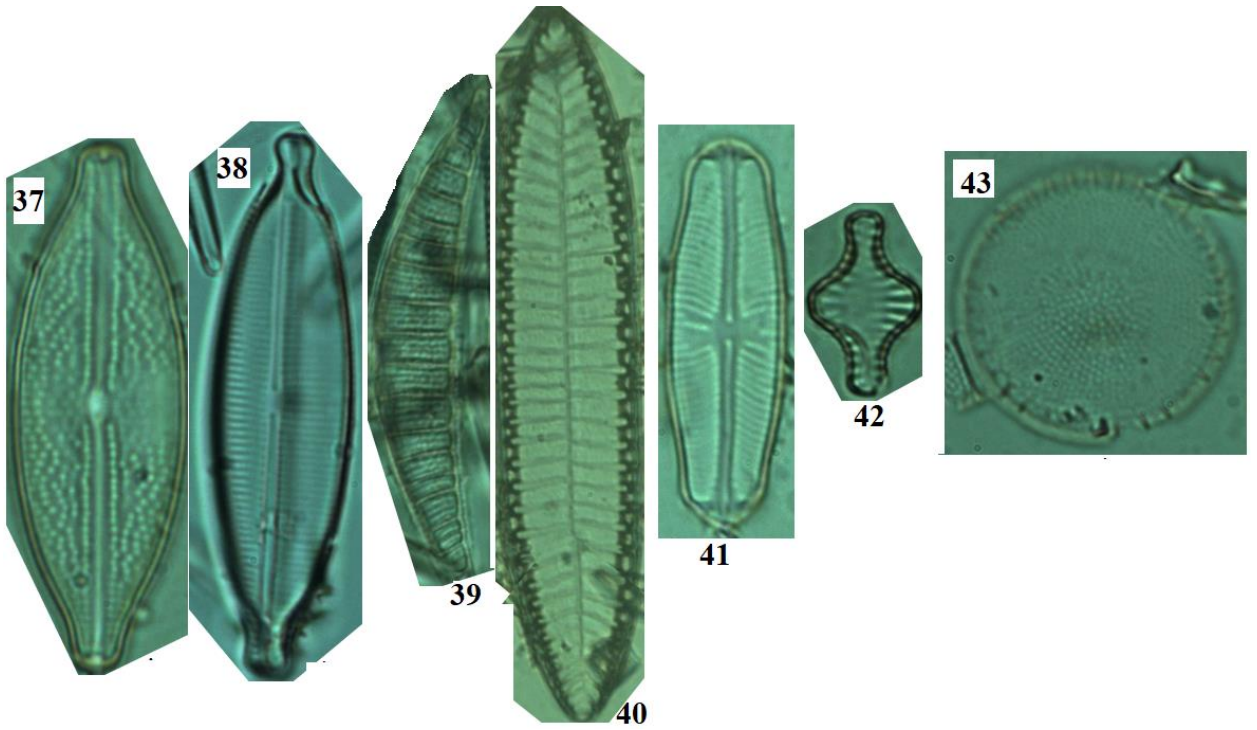
13. *Cymbella cistula*, 14. *C. turgidula*, 15. *Encyonema muelleri*, 16. *E. silesiacum*, and 17. *Encyonema microcephala*



18. *Epithemia adnata*, 19. *E. frickei*, 20. *E. sorex*, 21. *Fragilaria capucina*, 22. *Ulnaria contracta*, 23. *Gomphonema gracile*, 24. *G. parvulum*, 25. *G. olivaceum* and 26. *Mastogloia elliptica*.



27. *Navicula radiosa*, 28. *N. capitatoradiata*, 29. *N. tenella*, 30. *Nitzschia palea* var. *debilis*, 31. *N. palea*, 32. *N. gracilis*, 33. *Tryblionella umbilicata*, 34. *T. apiculata*, 35. *Nitzschia desertorum*, 36. *N. frustulum*



37. *Anomoeneis sphaerophora*, 38. *Craticula ambigua*, 39. *Rhopalodia gibberula*, 40. *Surirella linearis*, 41. *Sellaphora pupula*, 42. *Staurosira construens*, 43. *Thalassiosira faurii*

Appendix 4. List of reference taxa used to calculate the reference macroinvertebrate metrics (abundant/accumulated in reference sites)

Taxa	Reference sites		Non-reference sites	
	% Contribution	% Accumulation	% Contribution	% Accumulation
Cordulegastridae	0.03	100	0	0
Gomphidae	0.03	100	0	0
Notonectidae	4.72	69.67	2.40	30.33
Belostomatidae	7.28	57.36	6.32	42.63
Naucoridae	15.60	99.70	0.06	0.30
Gelastocoridae	2.48	99.68	0.00	0.32
Nepidae	0.45	87.69	0.074	12.31
Veliidae	0.28	92.11	0.03	7.89
Mesoveliidae	0.38	87.27	0.06	12.73
Cicadellidae	0.49	100	0	0
Curculiodae	0.01	100	0	0
Hydrophilidae	1.33	61.99	0.95	38.01
Dyticidae	0.65	72.57	0.29	27.43
Noteridae	0.72	54.49	0.70	45.51
Scirtidae	0.01	100	0	0
Baetidae	9.31	60.35	7.16	39.65
Caenidae	1.18	76.41]	0.43	23.59
Polymitarcyidae	3.93	94.66	0.26	5.34
Pyralidae	0.11	51.85]	0.12	48.15
Corydalidae	0.09	73.33	0.04	26.67
Sericostomatidae	0.38	75	0.15	25
Leptoceridae	0.31	97.5	0.01	2.5
Labiduridae	0.06	88.89	0.01	11.11

Appendix 5. List of reference taxa used to calculate the reference diatom metrics
(abundant/ accumulated in reference sites)

List of Diatom spp.	Reference Sites		Non-reference Sites	
	% Contribution	% Accumulation	% contribution	% Accumulation
AGVA	0.03	100	0	0
AMIT	15.23	80.88	3.69	19.12
CBAC	0.33	62.5	0.21	37.5
CLEP	0.07	100	0	0
CPLA	0.27	61.54	0.17	38.46
CPLI	0.53	72.73	0.21	27.27
CRCU	0.03	100	0	0
CTGL	3.13	69.63	1.40	30.37
ECAE	0.93	82.35	0.21	17.65
ELSE	5.67	72.34	2.22	27.66
ENCM	8.57	70.60	3.66	29.34
ENMU	2.23	66.34	1.16	33.66
FTEN	1.37	57.75	1.03	42.25
GANT	0.07	66.67	0.03	33.33
MELL	0.17	100	0	0
NCPR	0.23	63.63	0.14	36.36
NPAD	0.07	66.67	0.03	33.33
NPHY	0.47	77.78	0.14	22.22
NTNL	7.93	65.03	4.38	34.97
NZCL	0.17	100	0	0
UCON	0.5	57.69	0.38	42.31
UUAC	7.57	67.96	3.66	32.04