65

ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

CENTER FOR FOOD SCIENCE AND NUTRITION

Nutritional Value And Risk Assessment Of Toxic Heavy Metals Of Selected Fish Species From Freshwater Ecosystems In Ethiopia

Ph.D. Dissertation in Food Science and Nutrition

By:

Erkihun Massresha Asfaw

Submitted for the Fulfillment of Requirements for the Degree of Doctor of Philosophy in Food Science and Nutrition

September 2019

ADDIS ABABA UNIVERSITY

The Dissertation of **Erkihun Massresha** was reviewed and approved by the following:

A. SUPERVISORS

1) **Dr. Ashagrie Zewdu**

   Assistant Professor of Food Science and Nutrition

   Addis Ababa University

2) **Dr. Hintsa Mateos**

   Adjunct Professor of Molecular Nutrition

   Deakin University, Australia

3) **Dr. Paul Lewandowski**

   Associate Professor of Molecular Nutrition

   Deakin University, Australia

B. EXAMINERS

1. Prof. Gulelat Desse - External examiner
2. Mr. Kelebesa Urga - Internal examiner

C. CHAIR MAN

Dr. Paulos Getachew

Supervisors:

Dr Hintsa Mateos (Adjunct Professor)

Deakin University, Australia, School of Medicine

Department of Molecular Nutrition Unit

E-mail: hintsa.mateos@deakin.edu.au

Dr Ashagrie Zewdu (Asso. Professor)

Addis Ababa University, College of Natural Sciences

Center for Food Science and Nutrition

P.O.BOX 1176, Addis Ababa Ethiopia

E-mail: ashagrie.zewdu@aau.edu.et

Dr Paul Lewandowski (Asso. Professor)

Deakin University, Australia, School of Medicine

Department of Molecular Nutrition Unit

Email: Paul.Lewandowski@deakin.edu.au

ABBREVIATIONS AND ACRONYMS

AA	Amino acid
AAU	Addis Ababa University
ADI	Acceptable daily intake
ARA	Arachidonic acid, 20:4n-6
AAS	Atomic Absorption Spectrophotometry
ALA	Alpha linolenic acid,18:3n-3
ANOVA	Analysis of Variance
AOA	Antioxidant activity
AOAC	Association of Official Analytical Chemists
ASG	Acylated sterylglycosides
BHT	Butyl hydroxytoluene
CVD	Cardiovascular disease
CHD	Coronary heart disease
CSA	Central Statistical Agency of Ethiopia
DWB	Dry weight Base
DW	Dry Weight
DHA	Docosahexaenoic acid, 22:6n-3
EDI	intake of each heavy metal
EFA	Essential fatty acid
EIAR	Ethiopian Institute of Agricultural Research
EPA	Eicosapentaenoic acid, 20:5n-3
EU	European Union
FA	Fatty acid
FAME	Fatty acid methyl esters
FAO	Food and agriculture Organization
FRAP	Ferric reducing antioxidant capacity assay
GAE	Gallic acid equivalents
GC-FID	Gas chromatography - Flame Ionization Detector
GC-LC	Gas-liquid chromatography

GC-MS	Gas chromatography- mass spectrometry
HDL,HDL-C	High density lipoprotein, High density lipoprotein cholesterol
HI	Hazard index
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IS	Internal standard
IUPAC	International Union of Pure and Applied Chemistry
Kg	Kilogram
LA	Linoleic acid, 16:2n-6
LDL, LDL-C	Low density lipoprotein, Low density lipoprotein cholesterol
LC n-3 PUFA	Long chain omega-3 polyunsaturated fatty acids
MARD	Ministry of Agriculture and Rural Development
mg	Milligram
MUFA	Monounsaturated fatty acid
n-3	Omega-3
n-6	Omega-6
PUFA	Polyunsaturated fatty acid
RFD	Oral reference dose
Rpm	Revolution per minute
SD	Standard deviation
SE	Steryl fatty acid esters
SFA	Saturated fatty acids
SG	Steryl glycosides
SPSS	Statistical Package for Social Science
TC	Total cholesterol
TG	Triglycerides
THQ	Target hazardous quotient
WKU	Wolkite University
WW	Wet Weight
WHO	World Health Organization

ACKNOWLEDGMENT

First of all, I would like to praise the Almighty God for his wisdom and guidance throughout my life and this long journey which was really very tiresome.

I would like to take this opportunity to express my deepest gratitude for my supervisors Prof Paul Lewandowski, Dr Hintsa Mateos and Dr Ashagrie Zewdu for their guidance, encouragement and patience starting with the conception of the research idea till the final. I'm honored to have worked under your supervision.
I would like to express my deepest gratitude to my advisor Dr Ashagrie who is not only a good mentor but also a best friend throughout my stay in the Center. I really appreciate for his patience, dedication to follow my experiments in the lab, motivation to design my research and searching laboratory place and contact key persons despite your busy schedule. Really strives for my success and encourages me a lot to run over the success truck. I owe you Dr Ashagrie! I am indebted to Dr Hintsa Mateos and Prof Paul Lewandowski from Deakin University, for the unreserved support and follow up of my research. I am happy to have you both as an advisor. I am lucky to be design my research and reviewing my manuscripts despite your busy schedule. Dr Hintsa especially you had a significant contribution in my academic excellence, so thank you very much.

I acknowledge the financial support provided to me by Wolkite University (WKU) and Addis Ababa University (AAU), which has enabled me to perform my research without financial difficulty.

I would like to acknowledge Dr Kaleab Baye, Chair of the Center for Food Science and Nutrition for his support to complete my dissertation. Nothing was terrible whenever I share it with you. Thank you again! I would also like to thank Debebe, Yemane, and Woinishet for their support when I conduct experiments at the Center for Food Science and Nutrition. Tinsae should also deserve appreciation for facilitation of office requirements. My thanks also go to Admas Birahnu and Dr Zerihun Hailu for making my life smooth through facilitating things at Wolkite University during the study period.

Thanks are due to my friends Dr Ashenafi Geleta, Abinet Tekle, Dr Abebe Ayelign, Dr Tesfaye Deme, Dr Dawod Gashu and Dr Paulos Getachew for their friendship over the years. All of you deserves special appreciation for sharing your research and life experience. We had enjoyable and very memorable times. I am also grateful to my best friend Yemiserach Sheferaw for her support and encouragement throughout my life.

I would also like to thank my mother Abebech Belay and my father Gemechu Tegaye both are really my Hero and all my brothers and sister.

Most importantly of all, I express my deep sense of gratitude to my most affectionate and beloved wife Tsigereda Belege and my lovely children Fiyori, Keya, Amen and Adonay, for their love and encouragement. I honestly could not have completed this without their support.

ABSTRACT

One of the traditional sources of animal protein of the developing world is through livestock rearing. Unfortunately, the livestock production is under increasing pressure from the combined effects of human population growth, shortage of grazing land and expantion desertification. Therefore, it is important to look for fish, which is the cheap source of animal protein and an important source of high quality protein as it contains large amounts of essential amino acids. In addition, fish contains crude lipids, which supply the body with energy as well as omega-3 fatty acids- the healthy fats that play a role in regulating blood cholesterol and supporting proper brain functioning. Moreover, fish is rich in iron, zinc, magnesium, and copper. Despite this fact, biochemical composition and toxic metal accumulation of different fish species from various freshwater ecosystems had gained little emphasis in Ethiopia. Therefore, the present study was conducted to evaluate the nutritional quality, health benefits and toxic heavy metal risk assessment of selected fish species collected from four freshwater ecosystems in Ethiopia.

In this study, a total of 80 (40 females and 40 males) fish samples of two freshwater fish species, namely African catfish and Nile tilapia (*C. gariepinus* and *O. niloticus*) were collected from four freshwater bodies of Alwero River, Abay River, Lake Hawassa and Lake Ziway found in Ethiopia. The fish samples were transported to the Center for Food Science and Nutrition, Addis Ababa University for further analysis. Proximate analysis of the fish samples was done by Association of Official Analytical Chemists (AOAC) method. Minerals in fish samples were determined by Atomic Absorption Spectrophotometry (AAS). Heavy metals were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) with following a microwave digestion procedure.

The analysis of the proximate composition of both fish species (male and female) from all sampled sites for maximum and minimum dry matter content for male and female were respectively 7.96% and 11.76% ($p < 0.05$); 7.06% and 12.00% ($p<0.05$). Dry matter fish muscles content showed a variation among fish species and sites of fish collection in lakes and rivers. Even though sex has no significant ($p>0.05$) effect on dry matter content, female fish had higher (7.06 - 12.00% dry weight (DW) as compared to male fish (7.96 - 11.76%). The dry matter contents in the gill tissues of *O. niloticus* and *C. gariepinus* male fishes were in the range of 6.62 to 10.66% and for female fishes were in the range of 6.80 to 9.11% in DW and sex has no significant ($p>0.05$) effect on dry matter content of gill tissues from all sampled sites.

The results on the proximate composition of *O. niloticus* fish muscle from all sampled sites for the maximum and minimum protein, fat, ash and carbohydrate contents were 15.22% and 12.44% ($p < 0.05$); 2.76% and 2.15% ($p > 0.05$); 1.06% and 0.65% ($p > 0.05$); 5.47% and 1.55% ($p< 0.05$). For *C. gariepinus* maximum and minimum protein, fat, ash and carbohydrate contents were 20.23% and 10.75% ($p< 0.05$); 4.62% and 2.11% ($p < 0.05$); 1.55% and 1.09% ($p > 0.05$); 2.53% and 0.28% ($p< 0.05$), respectively.

The analysis of the proximate composition revealed that fat and ash content of the fish species ranged from 2.69 to 4.60 and 0.65 to 1.52 g/100g wet weight (WW), respectively. The lowest moisture was detected in *C. gariepinus* from Lake Ziway (71.11%) with high fat content (4.62 %). The protein content ranged from 12.44 - 15.22% and 10.75 - 20.23% for *O. niloticus* and *C. gariepinus* from all fresh water bodies, respectively. The difference in fat content was the major cause for the change in caloric values for *C. gariepinus* fish tissue ranged from a 61.99 -122.06 kcal/100g (260.36-512.65 kJ) followed by *O. niloticus* ranged from 69.75-82.16 kcal /100g (292.95-345.07 kJ) from all fresh water bodies. Sexes were not significant ($P > 0.05$) influence in terms of the four proximate compositions (protein, fat, ash and caloric values) measured in muscle tissues of *O. niloticus* and *C. gariepinus* collected from all sample sites.

Five essential mineral elements Fe, Ni, Cu, Mn, and Zn and seven toxic heavy metals (Hg, Sn, Pb, As, B, Cd and Cr) were analyzed for each of the fish species in muscle and gill tissues from four different freshwater bodies. Iron, zinc, copper, nickel and manganese contents were in the range of33.54 to 122.29 mg/kg, 20.21 to 47.43 mg/kg, 0.75 to 10.87 mg/kg, 0.16 to 1.16 mg/kg and 2.48 to 69.82 mg/kg DW, respectively. The result showed that iron and z inc were the most abundant minerals detected in gill and muscle tissues.

Toxic heavy metal concentration were assessed in gill and muscle tissues of *C. gariepinus* and *O. niloticus* collect from Alwero River, Abay River Lake Hawassa and Lake Ziway. In gill tissue, the highest accumulated heavy metals were Cd (0.58 mg/kg DW) and Pb (4.56 mg/kg DW) and were observed in *O. niloticus* species in all water bodies. The highest concentrations of Sn (222.89 mg/kg DW) and Hg (14.52 mg/kg DW) were observed in gills and muscle of *O. niloticus* from Lake Ziway and Alwero River, respectively, while the highest concentrations of Cr (4.95 mg/kg DW) and As (30.92 mg/kg DW) were observed in gills and muscle of *C. gariepinus* from Lake Ziway, respectively. A surprisingly too high As content was 30.92 mg/kg DW in muscle tissue of

C. gariepinus from Abay River and Hg content was 14.52 mg/kg DW in muscle tissue of *O. niloticus* from Lake Ziway, may be due environmental contaminants from municipal, agricultural and industrial sources may enter the food chain, accumulate in fish muscles. Those two fish species from Abay River and Lake Ziway are limiting its consumption for infant and pregnant women. However, the estimated daily intakes of all metals were below the acceptable daily intake (ADI) and oral reference dose (RfD) set by international guideline. The target hazardous quotient (THQ) and hazard index (HI) values were (<1) for all examined metals in both species in all sampled sites. There are no risk for consumptions of *O. niloticus* and *C. gariepinus* muscle meat for consumers. Therefore muscles of studied both fish species should be safe for utilization in human diet from those regions in Ethiopia.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER - 1 INTRODUCTION

1.1. Background

The world fish production is aroud 167.2 million tons, out of which 146.3 million tons used for human consumption and remaining is used for non-food purpose and discarded as waste material (FAO, 2016; Jag Pal et al., 2018). The demand for high quality fish and fishery products are growing significantly ever as they contain plentiful of beneficial healthy substances (FAO, 1986). Fish is one of the food items in diets of many countries. It is known to be a source of digestable protein rich in essential amino acids. It is also a source of lipids, which contains high amount of omega (n-3) fatty acids (FAs). It is also a rich source of minerals and vitamins, (fat-soluble) (Erkan and Bilen, 2009; Pawar and Sonawane, 2013).

Generally, it is acknowledged as one of the healthiest and cheapest sources of protein for low-income family and it has amino acid compositions that are higher in cysteine than most other sources of protein (Akan et al, 2012). Moreover, it is a high protein food consumed by a large percentage of the population because of its high palatability, low cholesterol and tender flesh (Onyia et al., 2010). However, one potential risk of detary fish intake is its content of heavy metals. Heavy metals are generally referred to as those metals which possess a specific density of more than 5 g/cm^3 and adversely affect the environment and living organisms. These metals are accumulated in the body and food chain, exbiting a chronic nature (Jaishankar et al., 2014). Fish is one of the existing organisms in aquatic ecosystems and consumption of fish from contaminated water bodies can be a danger for human health (Moiseenko et al., 2001; Castro et al., 2008). In future, it is important to detect the level of heavy metal concentration in fish organs in order to increase the awareness of the risk of fish consumption which may raise the toxic heavy metals concentration in edible part of fish tissues above the permissible level (Sivaperumal et al., 2007; Uysal et al., 2008). When it comes to estimate the potential risks to human health of heavy metals consumption from fish, several ways have been adopted such as calculating the carcinogenic effect and non-carcinogenic effect. Risk assessment is one of the fastest methods used to evaluate the impact of the hazards on human health and also needed to determine the level of treatment, which is meant to solve the environmental problem that occurs in everyday life (Zhang et al., 2012). These methods are typically based on the Target Hazard Quotients (THQ) assessed the non-carcinogenic health hazards for each individual metal through fish consumption, while the hazard index (HI) was estimated as the sum of the THQs (Peycheva et al., 2017).

The importance of fisheries to the Ethiopian economy until 50 years ago, was insignificant due to abundant land based resources and a sparse population density. But, from the 1940s and 1950s, the rapid population growth, which resulted in shortage of cultivable land and depletion of land resources, forced the people to look for other occupations and resources of food from water resources at a subsistence level. The rapidly growing demand for fish in the capital city by foreigners and modern town dwellers contributed to the start of commercial fishing as a new practice in Rift Valley Lakes (from the 1950s) and later in Lake Tana (late 1980s) (Yalew, 2012).

According to Assefa (2014), the fish catch in Ethiopia was approximately 15,000 tons of fishes, 74% of this catch originated from the six main lakes and the remaining 26% came from other bodies such as rivers, reservoirs, etc. As fish potential is estimated at 45,000-51,500 tons per year, according to several sources, less than 38% of this potential is currently exploited, demonstrating considerable room for expansion though proper management. The general view seems to be that the lakes in south of Ethiopia are heavily exploited. Lake Tana is off-take dramatically less than potential (15-20%). The traders in Addis Ababa substantiate this trend that fish size from the south is getting smaller and the catch is reduced from these over exploited water bodies (Yalew, 2012). In addition, to being recognized as a source of direct income of the Lake and River side communities in Ethiopia, fish is a very important food in the diet of individuals for its wealth of nutrients. Vila Nova et al (2005) pointed out that fish has a high nutritional value because it has high value protein, low level cholesterol and excellent quality of lipids which are characterized by its high content of long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) in particular eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). At the moment Lake Tana fisheries contribute annually about 65 million Ethiopian Birr to the economy of the Amhara region and approximately about 20% of the catch (Gebremedhine et al., 2013).

According to WHO (2012), fish and fish products provide the healthiest animal protein, however, Ethiopians consume around 10 kg per year (Dsikowitzky et al 2012), which is far below the recommended minimum consumption of fish, which is 12 kg/year or approximately 36 g of fish per day. Admittedly, the fish stands out for having in its composition high quality protein, retinol, vitamins D and E, iodine and selenium as well as n-3 FAs. Evidences from past studies have increasingly associated fish consumption to brain development, improving eye health, protection against CVD and some cancers. The fats and fatty acids of fish are highly beneficial and difficult to obtain from other food sources (FAO, 2013).

Ethiopians do not consume large quantities of fish, although there is no religious prohibition for the Christian and Muslim population Rather, this is a country with a strong tradition of livestock rearing and meat consumption. The Ethiopian Orthodox Church observes several fasting periods as well as fasting days every week when meat is not consumed. Most Christians consider fish acceptable food during those periodsthough some strict followers do not eat any animal products. Especially, Tilapia is the dominantspecies caught and consumed in Ethiopia during the fasting periods, although this does not hold for all groups and for all areas. In fish production areas, fish consumption patterns reflect the local availability of fish. Relative pricing gives some indication of preference and/or abundance. Nile perch is most expensive fish spicies followed by Tilapia, catfish and barbus. Tilapia retains its value despite relative abundance indicating strong consumer demand (Gordon et al., 2007).

Fish consumption was promoted in Ethiopian tradition due to its nutrients, consumers often neglect the nutritional importance of different fish species. Moreover, data on biochemical composition, risks and benefits associated with fish consumption of *O. niloticus* and *C. gariepinus* fish species from Alwero River, Abay River Lake Hawassa and Lake Ziway is limited.

This thesis comprises six chapters. The following is a brief summary of each chapter in this thesis. Chapter One presents the introduction, statement of the problem, justification and objectives of the study. Chapter Two contains a literature review of the current scientific knowledge on the proposed subject. Chapter Three evaluates the proximatecomposition (such as crude protein, crude fat, dry matter, carbohydrate and ash) of two fish species (*C. gariepinus* and *O. niloticus*) caught from Alwero River, Abay River, Hawassa and Zeway Lakes of Ethiopia. Chapter Four determines the essential mineral contents in the muscle and gill tissues of *C. gariepinus* and *O. niloticus* fish species collected from four different ecosystems in Ethiopia and identify the species and tissues with high mineral content, which can be recommended for use in altering mineral deficiencies in the country. Chapter Five determines the levels of seven heavy metals (B, Cr, Pb, Hg, Sn, As and Cd) in the edible and nonedible tissues of *C. gariepinus* and *O. niloticus* fish species caught in Alwero River, Abay Rivers, Hawassa and Ziway Lakes and assesses the health risks associated with their consumption Chapter Six presents summary of conclusion, limitation of the study and future research directions/ recommendations.

1.2. Statement of the Problem

In developing countries, fish is one of the preferred sources of animal proteins. It is easy to digest and contains essential nutrients for the maintenance of a healthy body (Fawoleet al., 2007). It is especially important for low and middle-income groups. Due to an ever-increasing awareness about healthy foods and acceptability, the demand for fish has increased tremendously because of its special nutritional qualities. Land degradation and expansion of industrialization in Ethiopia have raised the contamination of freshwater fish species. To satisfy the need of a continuously increasing population of thecountry, in addition to Lake source of fish species, it is wise to take a look at another freshwater bodies minor freshwater bodies like Alwero and Abay Rivers (Hussien, et al, 2010).

There are several works carried out on Ethiopian freshwater fish species which showed the initiation of interest in the studies ofbiochemical composition of the fishes. Frankly speaking, the present study is the first of its kind which was conducted on thefishes found in the Alwero and Abay Rivers. In light of the previous studies on theheavy metal pollution, the present study mainly focused in risk assessment of heavy metals such as Cd, Hg, Cr, B Pb, Sn, and Asin fish species of *O. niloticus* and *C. gariepinus* which are considered as sources of contaminated heavy metals in Ethiopian and therefore levels of contaminated heavy metals in these fishes should be detected since their health implications for these regions are still unknown.

The biochemical composition of fish muscles are greatly variable between and within the species depending upon different exogenous and endogenous factors (Kwetegyeka et al., 2008). Physiological status and age of fish also affect the nutritional quality of fish (Olssonet al., 2003). Nutritional content decreases in spawning period and is due to mobilization of fat related compounds to gametogenesis (Sharer, 1994; Bandarra*et* al., 1997; Iverson et al., 2002; Varlien et al., 2003). Patrick (2007) reported a monthly variation in fat content, which was mainly due to available diet and high temperature with season and geographical location. Studies on the nutritional quality of selected fish species from four different fresh water ecosystems in Ethiopia are scarce or nil, hence, discourage the traditional fish consumption habit which may be due to less motivation in its nutritional quality, consumers often neglect the nutritional importance of some fish species (Richard and Marcia, 2004).

The nutritional benefits of a discarded fish tissues (head, tail, skin, liver and gill) from fishes of Ethiopian freshwater bodies were not adequately reported and hence neglected and underutilized specially the gill tissues which contain high amount of accessible protein (Rustad, 2007). All these discarded raw materials that can be edible or inedible are left during the fish fillet preparations. The utilization of discarded fish fillet is very rare which might emanate from lack of knowledge of protein-base for products made from various parts of these fish species (such as fishmeal, fish sauce, fish silage and fertilizer). There is no information on the food/feed of gill tissues in protein and fat content of selected fish species from four different regions in Ethiopia.

Fishery sector contributes to food security by providing an accessible and cheap protein source for the poor that supplement other locally available food sources (Thorpe, 2005). These fish species are very crucial in solving problems arising with malnutrition because of their nutrient content (high protein, n-3 FAs, vitamin and mineral content). Furthermore, freshwater fish species play prominent role in ensuring food and nutrition security for a nation.

The measurement of proximate composition such as moisture, protein, lipid and ash content in fish is necessary to ensure that they meet the requirements of food regulations in terms of nutritional and commercial specifications (Osibona, 2011). The assessment of the proximate composition of the fish not only important in determining its nutritive value, but also for its better processing and preservation (Mridha et al., 2005).

Assessment of toxic heavy metals in fish from different freshwater bodies could be extremely important from the public health point of view. The presence of heavy metals in the aquatic environments leads to severe adverse effects on fish and has been a subject of concern for many decades. Since metals are non-biodegradable and cannot be metabolized into harmless forms they can easily be accumulated in fish. Consequently, human beings are potentially exposed to these contaminants through the food chain with the consumption of fish (Georgi et al., 2014). Considering the above facts, the present study was undertaken to investigate and evaluate the presence of toxic heavy metals in two fish species from four different ecosystems found in Ethiopia.

Therefore, the nutritional quality, health benefits and toxic heavy metal risk assessment of *O. niloticus* and *C. gariepinus* fish species in four freshwater ecosystems from Ethiopia were the general theme of this study. The present study therefore was undertaken in an attempt to achieve the following objectives:

1.3. Objectives of the study

1.3.1. General Objective

The aim of this study was to evaluate the nutritional quality, health benefits and toxic heavy metal risk assessment of *O. niloticus* and *C. gariepinus* fish species in four freshwater ecosystems from Ethiopia.

1.3.2. Specific Objectives

The specific objectives of this study were:

1. to analyze the proximate composition in selected tissues of freshwater fish species of *O. niloticus* and *C. gariepinus* from four freshwater ecosystems found in Ethiopia.

2. to assess essential element contents of *O. niloticus* and *C. gariepinus* freshwater fish spices from different lakes and river ecosystem found in Ethiopia

3. to determine the accumulation level and human health risk assessment of toxic heavy metals in edible tissue of *O. niloticus* and *C. gariepinus* spices from different freshwater bodies, Ethiopia.

CHAPTER -2 Literature review

2.1. Fish consumption and health

For the last two decades, many studies have reported the beneficial effects of fish consumption on the risk of coronary heart disease (CHD) mortality (He et al., 2004; Whelton et al., 2004). Intake of fish and fish oil were also reported to decrease the risk of other cardiovascular diseases (CVD) such as hypertension, stroke and cardiac arrhythmias (Jag Pal et al., 2018). Fish intake also increase insulin sensitivity (Ramel et al., 2008) and decrease the risk of non insulin-dependent (type II) diabetes (Kromann and Green, 1980; Nkondjock and Receveur, 2003). Furthermore, fish consumption hasbeen linked to decreased risk of many other diseases such as colorectal cancer (Gonzalez and Riboli, 2006), depression (Sontrop and Campbell, 2006) and rheumatoid arthritis (Calder, 2006).

The beneficial health effects of fish and fish products consumption have mainly been attributed to long chain omega- 3 polyunsaturated fatty acids (LC n-3 PUFA) since these fattyacids (FAs) are almost exclusively derived from seafood (He and Daviglus, 2005; Ortega, 2006). Less is known about the health effects of other constituents in fish such as fish protein, even though quantitatively protein is the major nutrient in fish. According to Jónsdóttir (2008), fish and fish products play an important role in the nutritional value because they are rich source of nutrients, other than PUFAs and concluded, thatweight loss is correlated with fish intake.

2.2. Health benefits of long chain n-3 polyunsaturated fatty acids

Fatty acids (FAs) are organic compounds consisting of a hydrocarbon chain and a carboxylic group. FA can be saturated, mono or polyunsaturated Omega-3 (n-3) and omega-6 (n-6) FAs are polyunsaturated fatty acids (PUFAs) which mean that they contain more than one double bond. They are called n-3 when the first double bond from the methyl end of the FA is placed at the third carbon atom. Moreover, in n-6 the first double bond is 6 carbons away from the non-acid end of the molecule. Fish contains long chain omega-3 polyunsaturated fatty acid (LC n-3 PUFA). The two main LC n-3 PUFAs are eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (C22:6n-3 DHA) (Hull et al., 2011).

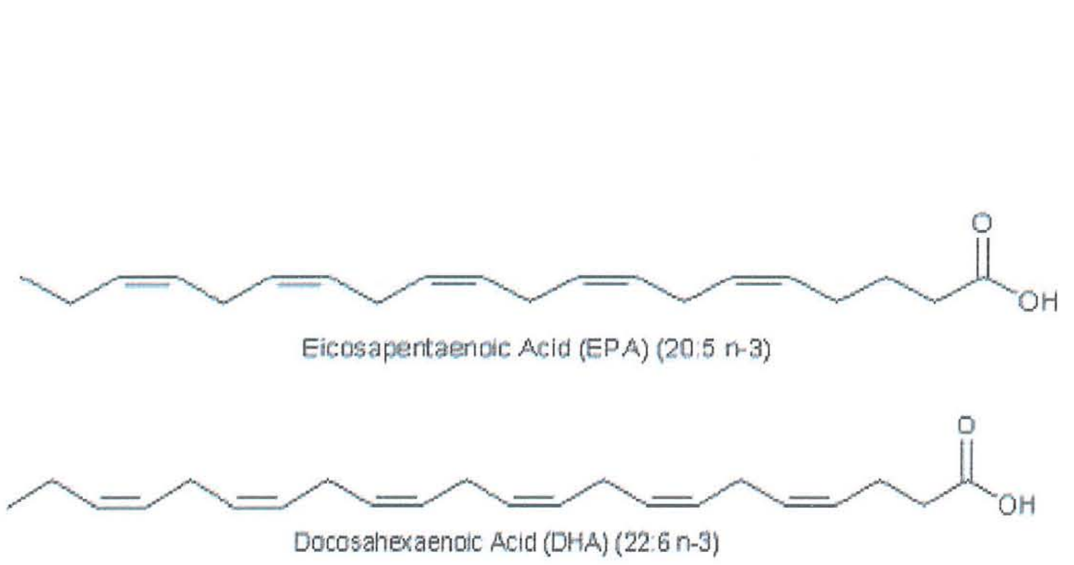

Figure 2.1- structure of DHA and EPA adapted from Huber et al. (2001)

EPA and DHA are the most important n-3 FAs in human nutrition. EPA and DHA are components of fish lipids. Fish such as mackerel, salmon, sardine, and tuna are excellent sources of EPA and DHA (Ackman, 2008). DHA is the predominant n-3 FA found in the brain and is linked to many aspects of neural function, including neurotransmission, ion channel regulation and gene expression (Sontrop and Campbell, 2006).

The human health benefits obtained by consuming fish is considered as the main reason for global increase of fish and shellfish production (Hoenselaar, 2012). Regarding the health benefits, several reliable studies have illustrated that n-3 consumption helps to prevent many diseases including mental disorders, asthma, high blood pressure, and some common cancers such as breast, colon, and prostate (Rose et al., 1999) and it can also protect against cardiovascular disease (CVD) (Karlsson et al (2017).

DHA improved visual acuity as well as other indexes of brain development in human infants (Innis et al., 2001; Innis, 2003; Lauritzen et al., 2004) Moreover meta-analysis indicated that by increasing every 20g/day fish intake the CVD risk decreased as much as 7% (He et al., 2004).

Recently there has been an increased interest in the remedial and public health communities concerning the role of highly unsaturated n-3 PUFAs in human health and well beings (Su et al., 2004). This dietary requirement is almost certainly due to the fact that fish is source of EPA and DHA, but humans, like other animals have limited conversion of these FA from the essential precursor's alpha-linolenic acid (ALA, 18:3n-3) (Riediger et al., 2009).

2.3. Health benefit of fish protein

The effects of fish protein on health have beeninvestigated considerably less than n-3 PUFA. Human studies on fish protein are few but several animal studies have shown that fish protein might have beneficial effect on human health, such as lowering blood pressure (Boukortt et al., 2004: Mateos et al., 2012), TC (Demonty et al., 2003; Wergedahl et al., 2004), TG (Demonty et al., 2003) and increasing insulin sensitivity (Lavigne et al., 2001; Tremblay et al., 2003). Lean fish consumption might therefore decrease the risk of developing the metabolic syndrome and thereby reducing the risk of CVD and type II diabetes.

Fish protein has high biological value, is easily digestible and is rich in essential amino acids (Costa, 2007). The effects that fish protein has on lipid metabolism could in part be attributed to its special amino acid (AA) composition. Fish protein has a low content of isoleucine, leucine, phenylalanine and tyrosine but a high content of arginine, alanine, methionine, cysteine and glycine, in comparison to casein (Yahia et al., 2005), whichmight affect clinical outcomes. Animal and human studies have also shown promising effects of fish protein on weight loss, where these effects might mainly lie in the high content of the amino acid taurine in fish (Zhang et al., 2004; Tsuboyama-Kasaoka et al., 2006). Ample fish consumption might therefore be an important component in the battle against obesity.
The bioactive effects of fish proteinon fish consuming population have not been investigated thoroughly in humans. On the other hand, the beneficial effects of LC n-3 PUFA are, well known and nowadays there are various supplements available containing FAs, in Brazilian Amazon, Scandinavian Japanese, Australia, New Zealand, the USA, China and other populations (Philibert et al., 2013). There is some evidence that the health effects of fish protein might have been underestimated and possibly, there is a basis for the development of functional food made of fish protein.

2.3.1. Cardiovascular diseases

Animal studies indicate that fish protein may influence cardiovascular risk factors by improving lipid metabolism and blood pressure. Fish protein might therefore have beneficial effects on cardiovascular risk independent of the intake of LC n-3 PUFA, even though human studies are limited. Long term consumption of fish appears to be associated with lower plasma LDL-C, therefore decreasing the risk of CVD (Fekete et al., 2009).

2.3.1.1 Blood pressure

Studies in rats have shown blood pressure loweringeffects of fish protein, compared to casein (Yahia et al., 2003a; Boukortt et al., 2004). These effects can be explained by the high arginine content of fish since nitric oxide, the metabolic product of arginine by the enzyme nitric oxide synthase, plays a crucial role as a vasorelaxant and lowers blood pressure (Yahia et al., 2003a). In addition, Yahia et al (2003a) found out that the fish protein dietmodified the composition of liver microsomal total lipids and liver phospholipids essentially by decreasig the proportion of arachidonic acid (ARA, 20:4,n-6), which is attributed principally to a diminution indelta-6 (Δ6) desaturation activity, the first step of arachidonate biosynthesis. This might havebeen caused by the low lysine to arginine ratio in fsh protein as compared to casein since a positive correlation has been reported between lysine/arginine ratio and liver microsomal Δ6 (n-6) desaturase activity in rats (Koba and Sugano, 1990). Despite the decreased proportion of ARA, which is the precursor of eicosanoids such as prostacyclin (PGI2), the production of the vasodilating PGI2 was increased. These conflicting results indicate that other factors influence eicosanoids biosynthesis in hypertensive rats independent of the ARA level. Yahia et al (200a) suggested that these factors might be vasoconstrictor hormones, including angiotensin II, vasopressin and epinephrine that have shown to stimulate PGI2 synthesis in vivo to constitute a homeostatic mechanism (Askari and Ferrerib, 2001).

2.3.1.2 Cholesterol

Fish protein has been found to reduce plasma total cholesterol levels in animal studies, compared to casein (Demonty et al., 2003; Yahia et al., 2003b; Wergedahl et al., 2004). Zhang and Beynen (1993) found that the cholesterol-affecting properties of a cod meal could be enhanced by the incorporation of higher proportions of fish protein in the diet, indicating a dose-dependent relation. Bergeron and Jacques (1989) demonstrated that fish protein diet caused fewer atherosclerotic lesions in rabbits, compared to casein diet, due to the cholesterol lowering effect.

Wergedahl et al (2004) found that Acyl-CoA cholesterol acyltransferase activity (ACAT) in rats significantly decreased by fish protein diet compared to casein diet. ACAT catalyzes the intracellular esterification of cholesterol and formation of cholesterol esters, thereby participating in accumulating cholesterol esters in macrophages and vascular tissues. By this mechanism, increased ACAT activity is thought to play a role in the progression of atherosclerosis (Carr et al., 1992) so this finding indicates that fish protein has a cardio protective role and is involved in the regulation of plasma cholesterol (Wergedahl et al., 2004). In humans, the effects of cod protein on cholesterol metabolism, compared to other animal proteins, appear to be different from those observed in rodents (Demonty et al., 2003). Lacaille et al (2000), found that fish protein did not affect total plasma cholesterol concentrations in men and a study by Gascon et al (1996) on premenopausal women showed the same results. Also in animal studies, isolated fish protein used leaving out nearly all possible effects of other nutrients, whereas lean fish as a whole is more often used in human studies, which can interfere with the results. Nevertheless, the fish food in young population resuted in a reduction in total cholesterol in the lean fish group that was of a similar degree as in the fatty fish group. The reduction was though only of borderline significance from the control group receiving no fish food when adjusted for weight loss (Gunnarsdo ttir et al., 2008), indicating that weight loss alone might be partly responsible for the cholesterol lowering effect. Unfortunately, the cholesterol lowering effect of the cod diet was mainly due to lowering of HDL-C rather than LDL-C, compared to the other diet groups, which is common in an energy-restricted diet containing $\leq$ 30 % from fat (Nordmann et al., 2006; Petersen et al., 2006).

2.3.1.3 Triglycerides

Fish protein might decrease plasma triglyceride (TG) levels in rodents (Hurley et al.,1995; Demonty et al., 2003; Shukla et al., 2006) and increase lipoprotein lipase (LPL) activity in adipose tissue (Demonty et al., 1998;Hodson et al., 2008) in comparison with casein. However, one should bear in mind that the protein-isolates tested in animal or human models are most likely not completely free of lipids. Thus, it cannot fully ruled out that some of the effects might originate from LC n-3 PUFA, which is possible in this case. Nevertheless, Demonty et al (2003) showed for the first time that cod protein lowers the rate of TGt secretion into the blood in rats, compared to casein, probably because of a reduction in hepatic triglyceride synthesis. The authors also suggested that the lowering effect of cod protein on TG secretion mightbe related in part to its AA composition, since cod, protein contains more arginine and has a lower lysine to arginine ratio than casein. However, Bergeron and Jacques (1989) found that serum TG were higher in rabbits feeding on fish diet compared to casein diet.
Human studies have shown that fish protein, compared with other animal proteins, might lower plasma VLDL TG in premenopausal women (Gascon et al., 1996) but did not affect total cholesterolemia and LDL-Apo protein B in postmenopausal women (Jacques et al.,1992). The

human trial of fish food consumption onadult people revealed interesting results on the effects of fish protein on serum TG concentration. The cod diet in that study lowered serum TG to a similar degree as the FO diet, a result that has not been seen previously in a human study (Gunnarsdottir et al., 2008).

Based on studies in rodents, it has also been proposed that cod protein may interact with dietary n-6 (Bergeron et al., 1991) and n-3 (Demonty et al., 1998) PUFA, mainly through specific alterations in hepatic lipid concentrations, to modulate plasma TG and cholesterol concentrations (Demonty et al., 2003). Therefore LC PUFA and fish protein might interact while improving cardiovascular health.

2.3.2. Obesity

According to WHO overweight and obesity are defined as "abnormal or excessive fat accumulation that may impair health" (WHO, 2006). A study by Yahia et al (2005) suggested positive effects of fish protein on weight loss in rats since rats fed fish protein diet did not gain as much body weight as rats fed casein protein diet despite similar food and energy intake. The authors reported that rats fed the fish protein diet had significantly lower total lipids in adipose tissue and therefore lower fat deposition, compared to rats fed the casein diet (Yahia et al., 2005). However, the reduced essential AAs (isoleucine, leucine, phenylalanine, tyrosine, valine and histidine) in fish protein, compared with casein, might have been responsible for this low growth (Yahia et al., 2003a).

Human study found that regular consumption of lean fish (3 x 150 g per week) increased weight loss in overweight and obese men during an eight-week energy restriction in comparison to an isocaloric control diet without seafood (6.5 ± 2.8 kg vs. 5.3 ± 3.0 kg) (Thorsdottir et al., 2007). However, a significant effect of lean fish consumption on weight loss not seen in women. Testing the weight loss was effects of lean fish eaten more frequently or in higher amounts than in fish consumers study is important, as lean fish might be an interesting possibility to increase weight loss options for overweight or obese individuals.

2.3.2.1. Weight loss

Particular amino acids (AAs) in fish protein might have beneficial effects on body weight, especially the amino acid taurine. Several studies have shown that taurine might promote weight loss. An early study by Zhang et al (2004) showed that taurine decreased body weight in obese, mainly due to inhibition of excess fat deposition in the body since taurine is highly concentrated in the gastrointestinal wall, influencing lipid metabolism.Tsuboyama-Kasaoka et al (2006) reported that dietary sulphur containing AA are important in the metabolism of fats to prevent obesity in mice fed high-fat diet by increasing both resting energy expenditure and gene expression involved in energy metabolism in white adipose tissue. The researchers concluded that obesity causes depletion of the blood taurine concentration, which then promotes further obesity, and that dietary taurine supplementation might interrupt this vicious circle and prevent obesity.

A human study by Zhang et al (2004) also found that seven-week supplementation with 3 g taurine (sulphur-containing AA) per day resulted in weight loss, possibly due to its beneficial effects on lipid metabolism.

2.4. Other effects of fish consumption on weight loss

2.4.1 Protein intake, fish consumption and satiety

Macronutrients have different effects on satiety independent of their caloric value. Studies demonstrated that dietary protein is the most satiating macronutrient compared with iso energetic ingestion of carbohydrate or fat in both human subjects and animal models (Bensaid, 2002; Leidy et al., 2007). An additional mechanism that might explain the positive influence of fish protein on body weight is therefore its possible effect on satiety. Previous studieshave indicated that fish protein might have stronger satiating effect when compared with beef and chicken protein (Borzoei et al., 2006).

However, in human study results indicated that lean fish consumption does not increase satiety compared to isocaloric diets of the same macronutrient composition including fatty fish,fish oil (FO) or control diet without seafood (Arnarson, 2007).

Furthermore, cod consumption even increased hunger scores measured on visual analogue scales (VAS) compared to the other diet groups. The researchers suggested that LC n-3 PUFA in the two other seafood groups had favorable effects on appetite by increasing satiety feelings (Arnarson, 2007; Parra et al., 2008). Another possible mechanism might be that the cod diet increased metabolic rate to a greater degree than the other diets (Arnarson, 2007) since fish AA has been shown to increase resting metabolic expenditurein mice (Tsuboyama-Kasaoka et al., 2006).

2.4.2 High-protein diets

Protein plays a key role in body weight regulation not only by increasing satiety but also by increasing thermogenesis and having favorable effect on body composition through maintenance or accretion of fat free mass (Paddon-Jones et al., 2008). Nutritional guidelines nowadays recommend that 10-20 % of the calorie content of the diet come from protein. However, in many popular diets, such as the Atkins Diet, 30-40 % of the calorie content comes from protein, at the expense of carbohydrates (Astrup, 2005). The high-protein content in the aforementioned diets may actually be the reason for their partial success in inducing weight loss (Astrup et al., 2004).

However, high-protein/low carbohydrate diets may suppress food intake, which causes the weight loss effects, due to producing ketosis. Ketosis results from the depletion of glycogen stores in the body, induced by a severe restriction of carbohydrates, causing acidosis that can have life threatening consequences (Herrin, 2003). High protein intake has also been associated with progression of renal diseases, increased calcium losses and possible harmful effects on bone mass, and the risk of adiposity in children and juvenile diabetes (Friedman, 2004; NNR, 2004). Tremblay et al (2007) reported that high-protein intake could have detrimental effects on glucose homeostasis by promoting insulin resistance and increasing gluconeogenesis. Emphasizing the quality rather than the quantity of protein has shown to modulate insulin resistance and revealed that protein derived from fish might have the most desirable effects on insulin sensitivity (Tremblay et al., 2007). Therefore further research is needed in the quantity of high protein intake with abnormal lipid profile in the human lipid profile.

2.5 Other major composition of fish tissues

2.5.1 Water

The main constituent of fish flesh is water, which usually accounts for about 80% of the weight of fresh white fish fillets (Huss, 1995; FAO, 2005, 2011). Whereas the average water content of the flesh of fatty fish is about 70%. Individual specimens of certain species may at times be found with water content anywhere between the extremes of 30% and 90 %. In the living fish, the water content usually increased and the protein content decreased as spawning time approaches. Thus is possible with cod for example to estimate the condition of the fish by measuring the water content of muscle. In cod, the water content of the muscle is slightly higher at the tail than at the head; this slight but consistent increase from head to tailis balanced by a slight reduction in protein content.

2.5.2 Protein

Proteins are the second most important fish constituent. These comprise structural protein, sarcoplasmic proteins and connectives tissue protein. Fish protein contains all the essential AAs and like milk, egg and mammalian meat proteins, have a very high biological value. In addition, fish proteins are excellent sources of lysine, methionine and cysteine. The amount of protein in fish muscle is usually between 15% and 20%, but values lower than 15% or high as 28% occasionally found in some species (FAO, 2005).

Amino acids

Fish is known to be a source of protein rich in essential AAs (lysine, methionine, cysteine, threonine, and tryptophan) (Zygmunt, Joanna and Maria, 2008), minerals, vitamins and LC n 3 PUFA that, amongst other benefits, have a hypo cholesterolemic effect (ant arteriosclerosis) (Fernandez and Venkatrammann, 1993; Ismail, 2005).

Amino acids (AAs) play a central role as the building blocks of proteins and as intermediates in metabolism and further help to maintain health and vitality. There are 20 AAs found in the human body, 18 of which are important in human nutrition. Eight AAs cannot be synthesized de novo by humans and other mammals and hence mustbe supplied in the diet; therefore, they are called essential AAs. The essential AAs are one is absent failure to obtain enough of even one of the essential AAs results in the degradation of the muscle proteins in the body (Zygmunt, Joanna and Maria, 2008).

Moreover, there is a group of amino acids which are not normally required in the diet but which must exogenously supplied to specific populations under special conditions, such as intensive growth, stress, or in some disease states. Such AAs havebeen classified as semi-essential. The remaining amino acids synthesized by the organism in sufficient amounts and henceare classified as nonessential amino acids. In addition, cystine and tyrosineare regarded by some authors (Boisen, Hvelplund and Weisbjerg, 2000) as semi-essential AAs as they are synthesized from methionine and phenylalanine, respectively. Therefore, the total AA requirements should include the sum of methionine + cystine (sulphur-containing AAs) and phenylalanine + tyrosine (aromatic AAs). Fulfilling the requirements as above, equivalent to the summed quantities alone may not be sufficient because methionine and phenylalanine cannot be synthesized from cystine and tyrosine, respectively (Boisen et al., 2000).

The nutritive quality of any food protein is determine by the following factors:

- The content of essential and nonessential AAs
- Should be similar to that found in the proteins of the body
- The energy supplied, which is essential for proteinsynthesis in the body
- The digestibility of the protein (Zygmunt, Joanna and Maria, 2008).

The quality of the proteins can be determined in relation to the composition of a standard protein, which is recognized as the most relevant for the assessment of the protein quality in the nutrition of all populations. The AA composition of a WHO standard protein hasbeen modified by the Joint Expert Committee of the FAO in 1991 (FAO/WHO, 1991), with relevance to the present knowledge. The evaluation of protein qualiy is carried out based on the amounts of limiting amino acids. These are the essential amino acids found in foodstuffs in the smallest quantities in comparison with a standard protein. The limiting AA content profoundly affects the net protein utilization, which is the ratio of the mass of AAs converted to proteins against that of AAs supplied. Therefore, foodstuffs that have different deficiencies in their essential AA profiles in comparison with a standard protein shouldbe mixed for consumption. For example, the proteins of cereal products are characterized by a low content of lysine and hence should be supplemented with proteins rich in this AA so as to optimize the utilization of the proteins supplied in the diet.

According to Zygmunt et al (2008) fishproducts are particularly good sources of lysine, which is severely restricted in cereals, the most important staple food in the world. Reduced lysine in the diet may lead to mental and physical handicaps because it isan important precursor for the synthesis of glutamate, the most significant neurotransmitter in the mammalian central nervous system. Furthermore, the sulphur-containing essential AAs in fish products can supplement the corresponding deficiency in plant proteins. Thus, the proteins in a mixed diet can be utilized optimally for a healthy body constitution (Papes et al., 2001).

Therefore, a low consumption of fish and fish products in Ethiopia compared to that in other countries are due to inadequate promotion and a lack of sufficientinformation regarding their nutritional qualities.

2.5.3 Fat

The fat content of fish varies depending on the species as well as the season but, in general, fish have less fat than red meats. The fat content ranges from 0.2% to 25%. However, fats from fish contain LC n-3 PUFAs namely EPA and DHA, which are essential for proper growth of children and are not associated with the occurrence of CVD such as CHD (Elvevoll and James, 2002). In pregnant women, the presence of LC n-3 PUFA in their diets has been associated with proper brain development in unborn babies. In other studies, n3 FAs have also been associated with reduced risk of preterm delivery and low birth weight (Elvevoll and James, 2002). Fat also contributes to energy supplies and assists in the proper absorption of fat-soluble vitamins namely A, D, E and K (FAO, 2011). Taking all species into account, the fat content of fish can vary very much more widely than the water, protein or mineral content. Whilst the ratio of the highest to the lowest value of protein or water content encountered is not more than three to one, the ratio between highest and lowest fat values is more than three to one. There is usually considerable seasonal variation in the fat content of fatty fish. For example, a starved herring may have as little as 0.5% fat, whereas one that has been feeding heavily to replenish tissue may have a fat content of over 20%. Sardines, sprats and mackerel also exhibit this seasonal variation in fat content. As the fat content rises, so the water content falls, and vice versa; the sum of water and fat in a fatty fish is constant at about 80%. The fat is not always uniformly distributed throughout the flesh of a fatty fish. For example, in Pacific salmon there may be nearly twice as much fat in muscle from around the head as there is in the tail muscle. In white fish of the cod family, the fat content of the muscle is always low, usually below 1% and seasonal fluctuations in fat content are noticeable mainly in the liver where the bulk of the fat is stored. Fish lipids contrast greatly from mammalian lipids in that they include up to 40% of long chain FAs that are unsaturated and contain five or six double bonds. This difference has both health (anti-thrombotic activity of PUFAs) and technological (rapid development of rancidity) implications (Elvevoll and James, 2002).

2.6 Other nutritional components of fish

2.6.1 Carbohydrates

The amount of carbohydrate in white fish muscle is generally too small to be of any significance in the diet; hence, no values are given in the tables. In white fish, the amount is usually less than 1%, but in the dark muscle of some fatty species, it may occasionally be up to 2%. Some mollusks, however, contain up to 5% of the carbohydrate glycogen (FAO, 2011).

2.6.2 Minerals and vitamins

Humans require a suite of mineral elements in varying amounts for proper growth, health maintenance and general wellbeing (NRC, 1989; WHO, 1998). Fish based foods are an excellent source of most of the minerals which the body needs to develop properly and perform its functions. Calcium and phosphorus occur in fish fillets at about the same quantities as beef round (FAO, 2009). Although fish is very unlikely to be the only source of an essential mineral in the diet, fish does provide a well-balanced supply of minerals in a readily usable form. The table of mineral constituents of fish muscle gives values averaged from a large number of species and is intended to serve only as a rough guide. It would be impracticable in this short note, and of limited value, to give a detailed analysis for individual species. Composition tables for fish often include a value for total ash. Since ash consists largely of a number of different minerals, and the total rarely exceeds 1-2% of the edible portion, this figure has also omitted, except from the table of fish products (Watanae et al., 1997).

Vitamins can divided into two groups, those that are soluble in fat, such as vitamins A, D, E and K, and those that are soluble in water, such as vitamins B and C. All the vitamins necessary for good health in humans and domestic animals are present to some extent in fish, but the amounts vary widely from species to species, and throughout the year. The vitamin content of individual fish of the same species, and evenof different parts of the same fish, can also vary considerably. Often the parts of a fish thatare not normally eaten, such as the liver and the gut, contain much greater quantities of oil-soluble vitamins than the flesh. The livers of cod and halibut for example contain almost all of the vitamins A and D present in those species. In contrast, the same two vitamins in eels, for example, are present mainly in the flesh. Water soluble vitamins in fish, although present in the skin, the liver and gut, are more uniformly distributed, and the flesh usually contains more than half the total amount present inthe fish (FAO, 2009). In general, the vitamin content of white fish muscle is similar to that of lean meat and, with the exception of vitamin C, can usually make a significant contribution to the total vitamin intake of man and domestic animals.

However, it is difficult to generalize and to establish the mean mineral value because they depend on several factors such as species, sex, biological cycle, the portion of ash analyzed and ecological factors, such as season, place of development, nutrient availability, temperature and salinity of the water (Huss, 1995). Minerals classified based body function and used and it is important to note that not all metals are hazardous and toxic to fish and humans. They form part of a larger group of elements, some of which are essential to human health (Mertz, 1993). These can therefore classified as essential, non-essential or toxic.

2.6.3 Essential and Non-essential minerals

Minerals are inorganic compounds including macro-elements and microelements. The macro elements such as calcium, magnesium, sodium, potassium, and phosphorus are essential to human health (Joanna et al., 2009). Microelements such as zinc, iron, nickel, copper, and manganese, which occur in physiological concentrations play key roles in living processes and either an excess or deficit can disturb biochemical functions in humans (Mann and Trustwell, 2007; Joanna et al., 2009).

Some minerals are essential, non-essential or toxic to human health (Mertz, 1993). Essential elements which play a specific role in body metabolism include iron (Fe), copper (Cu), zinc (Zn), Nickel (Ni) and manganese (Mn). Non-essential elements are elements that have no known specific function in the body, but are also not considered toxic in any significant amount. Lastly toxic elements such as cobalt (Co), tin (Sn), chromium (Cr), cadmium (Cd), mercury (Hg), arsenic (As) and lead (Pb) are generally related to pollution and can have harmful effects on living organisms when exceeding certain concentrations fromthe natural occurring (Bosch et al., 2015). However, anthropogenic releases including industrial and domestic effluents, atmospheric sources and dumping of sewage sludge can give rise to higher concentrations of the metals relative to the normal backgroundvalues (Zarazua et al., 2006; Cicchella et al., 2008).

2.6.3.1 Essential elements

Iron

Iron (Fe) carries oxygen to the cells and is necessary for the production of energy, the synthesis of collagen, and the functioning of the immune system. Fe deficiency is common only among children and pre-menopausal women. Great care must taken not to take too much iron, as excess amounts are stored in the body's tissues and adversely affect the body's immune function, cell growth and heart health (Tzonou, 1998). Feabsorption can be blocked by calcium (Ca), magnesium (Mg), Mn, Zn, anti-acids and tetracycline, common antibiotic) (Rebouche et al., 1999). Deficiency of Fe results in anemia, whichis recognized by its symptom such as low blood iron level, small red blood cells and low blood hemoglobin values (Tortora, 1997). Fe toxicity usually results from a generic disorder calledhaemochromatosis. This disease causes over absorption and accumulation of Fe, which can result in severe liver and heart damage (Wardlaw & Insel, 1996).

Zinc

Zinc (Zn) is an essential element found in the tissue of animals and plants even at normal ambient concentrations. However, if plants and animals exposed to large concentrations of bioavailable Zn, significant bioaccumulations can result with possible toxic effects (Wardlaw & Insel, 1996). Zn is the most ubiquitous of all trace elements involved in human metabolism. More than one hundred specific enzymes require Zn for their catalytic function. If Zn is removed from the catalytic site, activity is lost; replacement of zinc restores activity. Zn participates in all major biochemical pathways and plays multiple roles in the perpetuation of genetic material, including transcription of DNA, translation of RNA, and ultimately cell division. When the supply of dietary Zn is insufficient to support these functions, biochemical abnormalities and clinical signs may develop. Studies in individuals with acrodermatitis enteropathica, a genetic disorder with Zn mal-absorption resulting in severe deficiency, have provided much insight into the functional outcomes of Zn deficiency. These include impairments of dermal, gastrointestinal, neurologic and immunologic systems (ASTDR, 1995).

Manganese

Manganese (Mn) is an essential element to both plants and animals. It is necessary for normal bone metabolism and important enzyme reactions. It also helps to maintain normal nerve, brain and thyroid function (Rebouche et al., 1999). While a deficiency of this mineral is uncommon, it is often lost in processed foods (Kimura & Itokawa, 1990: Craig, 1994). A deficiency of Mn may affect brain health, glucose tolerance, normal reproduction, and skeletal and cartilage formation. Aquatic food products are the best food sources of Mn and toxicity from Mn is uncommon (Keen et al., 1999). Exposure to high level of Mn can cause both mental and emotional disturbance, along with increased slowness and clumsiness of the body movements. The disease known as Mn is due to the accumulation of Mn in the brain form a permanent brain injury (ATSDR, 2001).

Nickel

No sign of nickel (Ni) deficiency has described for humans. Nonetheless, the presence of Ni enzymes in lower forms of life and the response of circumstantial evidence that Ni is essential for humans. Of course, the potential importance of Ni in human nutrition is not limited to deficiency. Like other mineral elements, nickel ingested in high amounts can have adverse effects. However, because of excellent homeostatic regulation, lifethreatening toxicity of Ni through oral intake is unlikely. Generally, greater than 250 µg/g of diet are required to produce signs of Ni toxicity (such as depressed growth and anemia) in animals; by weight extrapolation, this indicates that ingestion of over 250 mg of soluble Ni daily could producetoxic symptoms in humans. However, the generality excessive amounts of Ni intake cause a mildly toxic and long term exposure can causes decreased body weight, heart and liver damage and skin irritation (Rajeswari and Sailaja, 2014).

Copper

The essential role of copper (Cu) in maintaining normal health in both animals and humans has been recognized for many years. The average daily dietary requirement for Cu hasbeen reported by many scholars. Cu is required with iron for synthesis of hemoglobin.It works with many enzymes such as those involved in protein metabolism and hormone synthesis (Wardlaw and Insel, 1996; Tortora, 1997; ATSDR, 2001). Deficiency of Cu causes low white blood cell count and poor growth. Excess intake of Cu can cause vomiting nervous system disorder and Wilson's diseases (Wardlaw and Insel, 1996).

2.6.3.2 Non essentail elememts

Arsenic

Arsenic (As) is widely distributed in nature due to environmental sources (Goyer and Clarkson, 2001; WHO, 2011) and anthropogenic pollution which is largely due to smelting activities, glass manufacturing, manufacture and use of arsenic pesticides, herbicides, fungicides and wood preservatives (Castro-Gonzaleza & Mendez-Armenta, 2008). As has a complex chemistry and can present in several organic (trivalent and pentavalent arsenic) and inorganic (elemental, trivalent and pentavalent arsenic) forms which vary in their degree of toxicity. Inorganic As is seen as the most toxic form as it is stable and soluble and therefore absorbed by the digestive tract, abdominal cavity and muscles in the human body (WHO, 2011), whilst organic As does not accumulate in the human body due to rapid excretion (WHO, 2011). Inorganic As is often found in high levels in drinking water whereas organic As is primarily found in fish and meat (Castro-González & Méndez-Armenta, 2008). Fish and fish product can contain several times the amount of As than other foods and is therefore the main source of dietary intake in humans (Ysart et al., 2000; Llobet et al., 2003). Although high concentrations of As (up to 100 ppm) have been found in certain edible marine species (WHO, 2011; Burger et al., 2014), in most of these cases it is the total As concentrations that are measured instead of the toxic inorganic form (arsenite). Up to 90% of As in fish muscle is present in the non-toxic arsenobetain form (Goyer and Clarkson, 2001).

Cadmium

Cadmium (Cd) is a metal contaminant which is introduced into the environment through both natural processes (volcanic emissions and weathering of rocks) and anthropogenic activities such as the smelting of other metals, burning of fossil fuels, incineration of waste materials and the use of certain fertilizers (EFSA, 2009). In fish muscle most of the Cd present tends to bind to proteins (EFSA, 2009). Cd absorbed into the fish body thereforeis eliminated at a very slow rate, causing bioaccumulation in the body. Cd can enter fish by passive diffusion across the gills or by entering the marine food chain at the plankton and microorganism's level and thereby entering fish through the diet (Erasmus et al., 2004).

However, fish is still considered a major source of Cd (Castro-González & Méndez-Armenta, 2008), which has frequently been found to exceed maximum allowable limits ina number of commonly consumed fish species. Cd is highly toxic to humans and has a long biological half-life preventing the reduction of the accumulated body burden (Erasmus et al., 2004; EFSA, 2009). Effects on human health include hypertension and cardiovascular function, neurological disorders, carcinogenic effects and skeletal weakness and defects (Goyer and Clarkson, 2001). Cd exposure in humans is predominantly through food ingestion (Castro-González and Méndez-Armenta, 2008) where fish, meat contain 1 to 50 $\mu g \cdot kg^{-1}$ Cd (Goyer and Clarkson, 2001). The European Commission has set a provisional tolerance weekly intake (PTWI) of 7 μg/kg.bw (EC, 2006). The Food and Agriculture Organization of the United Nations presents species-specific maximum limits for Cd in fish from 0.05 mg·kg-1fresh weight in fishery products to 1.0 mg·kg-1 fresh weight in bivalves and cephalopods (FAO, 2003).

Lead

Lead (Pb) is one of the primary contaminant metal, which is present in the environment (Castro-González and Méndez-Armenta, 2008). Pb naturally occurs in rocks, soils and in the hydrosphere (Buljac et al., 2014). However, Pb is also the most widely used metal and industrial Pb contributes a considerable quantity to that found in the natural environment (Harlavan et al., 2010). Large amounts of Pb tetraethyl can completely be converted to aerosols through the combustion of gasoline, subsequently contributing to atmospheric Pb (Von Storch et al., 2003). The atmosphere in turn is the main source of Pb deposition in the marine environment, therefore acting as a Pb pathway from the terrestrial to the marine environment. Since it became evident that leaded petrol was the predominant source of atmospheric Pb (Reuer and Weiss, 2002), regulations were adopted on the allowable gasoline lead content (Von Storch et al., 2003). This reduction in anthropogenic Pb pollution was evident in a reduction in seawater Pb concentrations (Reuer and Weiss, 2002) form a direct link from terrestrial sources to effects in the marine environment. Once in the marine environment, Pb is easily absorbed into the fish's bloodstream and accumulated in the body tissues, bones, gills, kidneys, liver and scales (Nussey et al., 2000). It can thus enter the human body through the diet and can accumulate, especially when fish foodis consumed regularly. The toxicity of Pb is dependent on its chemical form (Erasmus, 2004) wherethe organolead compounds are more toxic than the inorganic Pb form (Munoz-Olivas and Camara, 2001). Pb is mostly found in its dissolved form in the ocean, of which a large proportion (50-70%) is organic compounds (Reuer & Weiss, 2002). As shown by a series of studies by Sánchez-Marín et al (2007), the bioavailability of Pb in the environment as organic compounds can significantly increase by the presence of dissolved organic matter (DOM). The more methyl or ethyl carbon groups linked to the Pb molecule, the higher its toxic effect (Munoz-Olivas & Camara, 2001). The marine environment is therefore a significant source of toxic Pb exposure in fish and humans due to consumption. In certain communities fish consumption is the main source of Pb exposure (Rubio et al., 2005) where excess exposure can result in neurological problems, haematological effects, renal failure, hypertension and cancer (Goyer and Clarkson, 2001; Munoz-Olivas and Camara, 2001). A PTWI of 50 µg/kg BW first set by the JECFA, which was replaced in 1993 by a new provisional tolerance weekly intake(PTWI) of 25 µg/kg BW for all age groups (JECFA, 2011).

Chromium

Chromium (Cr) exists in three main forms: metallic state, trivalent and hexavalent forms. While hexavalent Cr recognized as an industrial toxin linked to lung cancer, trivalent Cr is acknowledged as an essential nutrient. The latter is known to improve insulin sensitivity and, therefore, to influence carbohydrate, fat and protein metabolism. Diabetes and CHD are associated with low Cr concentration in human tissue (Santos et al., 2004).

Cobalt

The only known animal requirement for cobalt (Co) is as a constituent of vitamin B_{12}, which has 4% cobalt in its chemical structure. This means that a cobalt deficiency is really a vitamin B12 deficiency. Co deficiency symptoms include a loss of appetite, emaciation, weakness, anemia, and decreased production. Excessive amounts ofCo produce cardiomyopathy with a high mortality risk. No RDA (Relative Dietary Allowance) or Estimated Safe and Adequate Daily Dietary Intake has been set for Co (Santos et al., 2004).

Tin

Tin (Sn) occurs naturally in the Earth's crust, it has not been shown to be nutritionally essential for humans (EFSA, 2005). Sn compounds are found in various environmental media in both inorganic and organic forms (WHO, 2005). Sn maybe released to the atmosphere from both natural and anthropogenic sources and it is a component of many soils and released in dusts from windstorms, roads, and agricultural activities (WHO, 2005). In a survey of lakes and rivers, nearly 80% of samples were found to contain inorganic Sn at concentrations below 1 μg /liter; higher levels of up to 37 μg/liter were reported near pollution sources. Inorganic Sn concentrations ranging from 0.001 to 0.01 μg/liter havebeen reported for coastal waters, with levels of up to 8 μg/liter near pollution sources. Inorganic Sn concentrations in sediment ranged up to 8 mg/kg dry weight in coastal areas and up to 15.5 mg/kg in rivers and lakes (WHO, 2005). Sn has no known biochemical function. However, it could have a signs of chronic exposure to excessive intake of inorganic Sn include growth depression andattack the central nervous system (Ibrahim et al., 2006). For the general population, the diet is the main source of exposure to inorganic Sn. JECFA (2001) recently concluded that mean Sn intakes in seven countries ranged from <1 up to 15 mg/day per person, but maxi-mum daily intakes could reach 50–60 mg for certain individuals who routinely consume canned fruits, vegetables, and juices from unlacquered cans. Data presented in ATSDR (2003) indicate that organic Sn accounts for only a small fraction of total tin in most foods. On that basis, in this section, Sn figures are total Sn, but essentially represent inorganic Sn.

Mercury

Mercury (Hg) is a metal that is liquid at ambient temperature and pressure and canbe present in several different chemical forms and compounds in the environment. It is the metal that presents the most concern about seafood consumption and human health (Marcotrigiano and Storelli, 2003). Fish is considered the primary source of Hg in humans (Falcó et al., 2006). There are numerous reports of high levels of Hg in fish muscle, exceeding the allowable maximum limits. Due to anthropogenic input from various activities, freshwater, sediments and biota near cities, harbours and industrial areas tend to have higher Hg concentrations compared to rural locations (Costa et al., 2012).

A number of marine based studies have corroborated such claims where blackmouthed dogfish, carp spp. and catfish had overall higher Hg concentration when sampled from industrialized and developed sites compared to those areas considered rural or less developed (Horvat et al., 2003). Rivers also carry metal contaminants from inland industrial and agricultural sources towards the ocean, affecting marine fish in estuariesand near river mouths (Oosthuizen and Ehrlich, 2001).

In the current review the term 'mercury' (Hg) will refer to total Hg (tHg) which is the sum of the inorganic Hg (iHg), MeHg, EthHg and any other Hg forms present. Due to the significant role of diet in Hg accumulation (Mason et al., 2000), fish at higher trophic levels are more likely to be exposed to and accumulate higher levels of Hg than those at lower trophic levels (Das et al., 2000; Costa et al., 2012). This process of Hg accumulation in the food chain is referred to as bioaccumulation (Boening, 2000; Burger & Gochfeld, 2004). Additionally, Hg can be biomagnified within a single species with older/larger individuals having higher levels of concentrated Hg (Boening, 2000). Methylmercury has a longer half-life than inorganic Hg resulting in a strong correlation between the percentage of total Hg present as MeHg and the total Hg levels (Forsyth et al., 2004), therefore, the percentage of total Hg present as MeHg tends to approach 100% with increasing total Hg burden and fish size/age (Forsyth et al., 2004). The fish and seafood industry is still in need of time and cost effective, accurate way of determining levels of toxic MeHg for a true measurement of food safety for human consumption. The ratio of the total Hg burden present as MeHg can vary with more than 30% within one species and average ratios vary from approximately 50-100% (Forsyth et al., 2004). It is therefore clear that a fixed conversion factor will not provide accurate estimates of toxic Hg concentrations in health assessments. There are, however, significant correlations between the percentage of total Hg present as MeHg and the total Hg concentrations as well as between total Hg concentrations and fish size/age (Forsyth et al., 2004). Further research should therefore explore these relationships for the possibility of setting up a model for calculating toxic MeHg levels from total Hg measurements.

2.7 Possible harmful effects of fish intake

Despite numerous benefits, fish may be a pathway of exposure to heavy metal pollution. Concern has arisen over potential toxicity long-term persistence, bioaccumulation, and bio-magnification at various trophic levels present in some fish species. These contaminants are present in low levels in water bodies, but are concentrated in fish by bioaccumulation and bio magnification (Sidhu, 2003). Heavy metals have received considerable attention due to their toxicity, long-term persistence, bioaccumulation, and bio-magnification at various trophic levels (Ololade et al., 2008).

Toxic elemental contaminants are transferred into human metabolism through consumption of contaminated fish that leads to serious deterioration of human health status (Alinnor and Obiji, 2010) and highly toxic for consumers when exceeding the recommended safety concentrations (Basiony, 2014).

2.7.1 Toxic Heavy metal in fish tissues

Fish is one of the cheapest and available sources of animal protein and is an important dietary constituent. About 148 million tons of fish were supplied by the capture and aquaculture sector to the world population in 2010, of which about 128 million tons was utilized as food for people (FAO, 2012). The lack of sufficient protein is one of the most widespread nutritional deficiencies in many tropical countries (Ozkan, 2005). The content of minerals and all amino acids in fish tissues has an important role in human metabolism (Ozden, 2010). The levels of contaminants, especially toxic trace minerals in fish tissue are of particular interest because of potential risk to human (Canli and Atli, 2003).

Fish are often exposed to highly contaminated water, which leads to different changes ranging from biochemical modifications from single cells up to changes in the entire population (Bernet et al., 1999). Extents of bioaccumulation and biomarker responses in fish from contaminated sites provide information that can contribute to environmental monitoring programs planned for various aspects of environmental hazard assessment (Van der Oost et al., 2003).

Heavy metals are among biosphere pollutants of global concern due to their environmental persistence, ability to bio accumulate and magnify in the food chain and chronic toxicity to wildlife and humans (Papagiannis et al., 2004). In aquatic systems, fish are exposed to these environmental pollutants either from water via gills or/and from the diet. Henceforth, fish are the most suitable indicators for the load of aquatic pollution observed since they concentrate pollutants in different tissues (Fisk et al., 2001). Thus, bioaccumulation of pollutants canbe considered as an index of environmentalpollutants in the aquatic bodies. Studies carried out in Ethiopian Rift Valley Lakes have revealed the presence of heavy metals in water and sediment samples. The studies by Zinabu and Desta (2002) on the chemical composition of the effluent from Hawassa textile factory and its effects on aquatic biota with a focus on phytoplankton and fried fish clearly showed high concentration of heavy metals and indicated the effect of pollutants due to effluent from factories.

A recent study in Lake Hawassa revealed significant differences of heavy metals level among three fish species (Yohannes et al., 2013). Ataro et al (2003) investigated heavy metal concentration of some lake waters of Ethiopia. Since the past few years, anthropogenic pollution of lakes has become a serious concern because of the building of different factories, floriculture and hospitals in Hawassa. Studies indicate possible contamination of the lake water. Toxic heavy metals identified in the effluent of textile factory include As, Cr, Cu, Hg, and Pb that are poisonous for human and aquatic life (Zinabu and Desta, 2002;Yohannes, 2014). Fisheries must be sustainable for lake side and rive side communities as nutrition and creating income opportunities. These have bilateral benefit, one by keepingour ecosystem and health from pollution. Previously the industrial and domestic wastes disposal in to the lakes and rivers are highly affecting the ecosystem and the people living around the lake and riversides. However, no studies carried are out on gill and muscle tissues of selected fish species found in different (lakes and rivers) ecosystem from Ethiopia to address the level of toxic elements and indicators of heavy metal exposure for human health according to FAO/WHO (2011).

2.7.2 Methyl mercury

Among metals, methyl mercury (MeHg) is the compound of most concern (Costa, 2007). Larger, longer-living predators (e.g. tuna, swordfish and shark) have higher tissue concentrations of MeHg (as high as 1.0 mg/kg (Costa, 2007)) compared to smaller or shorter lived species (Mozaffarian and Rimm, 2006). MeHg in fish is bound to proteins in muscles so cooking and removal of skin does therefore not significantly reduce its concentration (Costa, 2007). MeHg is a highly reactive compound and promotes the formation of free radicals. It may also inactivate the antioxidant properties of glutathione and induce lipid peroxidation (Costa, 2007). Health effects of very high mercury exposure are paresthesias, ataxia and sensory abnormalities in adults, and delayed cognitive and neuromuscular development in fetuses. However, the health risks with chronic low exposure of MeHg, such as with fish consumption over time, are not as well documented (Mozaffarian and Rimm, 2006). It hasbeen suggested that MeHg may increase the risk of CVD by promoting atherosclerosis (Costa, 2007). The health risk and benefit of fish consumption was in challenging between MeHg and

LC n-3 PUFAs, due to fish is a primer sources for both compounds. The cardio protective effects of LC n-3 PUFAs greatly outweigh the possible harm by MeHg so the net effect of fish consumption is still beneficial. Fish oil capsules contain little to no mercury and herefore considered safe in moderate amounts (Foran et al., 2003). Among other metals, fish may also contain arsenic, cadmium and lead, which are not thought to be as harmful as MeHg (Costa, 2007).

2.8 Nutritional value of Ethiopian fish species a review

2.8.1. Overview of Ethiopian Fishery

Ethiopia is Africa's 10^{th} largest country with a surface area of approximately 1, 13 million square kilometer (Anon, 1994 cited by Wudneh, 1998). It is located in the northeastern part of the continent, or the horn of Africa, lying between 3-18^0 North and 33-48^0 East. It is bordered by Kenya in the South, Somalia in the East and Southeast in the East, Eritrea in the North and Sudan in the West and North-West. Ethiopia is the most populous landlocked country in the world, as well as the second-most populous nation on the African continent after Nigeria. Standing water bodies cover approximately 7 400 km and there are 7 185 km of rivers (Aragaw, 2010). Ethiopia is a federal republic with nine regional states and two charter cities. Ethiopia's fishery is inland and artisanal, with landings made at many dispersed sites where records are not kept very well. In 2010 total production was 18, 058 tons valued at approximately USD $600 000 (CSA 2012 cited by Aragaw, 2012).

According to a Central Statistics Agency (CSA) report in 2010, export quantity amounted to 849 tons, while import amounted to 421tons. The fisheries sector provides regular employment for thousands of poor people and seasonal or part-time employment for many more. The primary sector employs an estimated 13, 200 people of which 4 052 are fulltime fishers and the secondary sector is believed to employ an estimated 20,000 people (CSA, 2010). This work is closely linked to other activities such as farming, livestock rearing, and fuel wood collection. Ethiopia water bodies support a diverse aquatic life including more than 180 fish species, of which about 40 are endemic. Many artificial water bodies are also stocked with fish for fishery activities. Regulation of the sector is inefficient across a range of authorities specifically regarding management and data collection on fisheries in Ethiopia (Aragaw, 2011). Empirical modeling suggests that current total fish production potential is around 50 000 tons annually, although assessments in the mid-1990s, when landing were less than 10 000 tons, suggested that several lakes were already fully or over exploited. The empirical models generally do not take into account the effect of unsuitable management or fishing practices and have criticized for consequently grossly overstating the maximum sustainable yield (Aragaw, 1998).

2.8.2 Nutritional value of Ethiopian fish species

Fish is the main food source for the Neged weyto people or /minor ethnic group living around Lake Tana and other poor people living on the islands and surrounding of the Lake. According to Abebe (20001) fish is the only food source for these group of people who have long tradition in eating fish and hippopotamus meat by hunting and subsistence fishingactivity because of lack of access to farmland. They can't afford to use other food sources. Fish is a reserve of food against drought and hunger in areas around the lake and rivers it is the only and an important source of animal protein especially for the poor who cannot afford buying other animal protein sources. Animal protein is the most deficient or undersupplied nutrient in most rural communitie's diet (Aragaw 1998).

Fish is an important source of long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) and also a good source of protein. Ethiopian lakes harbor a rich fish biodiversity which vary in their nutrient composition. This review was examining data on the proximate and fatty acid content of six fish species from various Ethiopian Lakes. The fish species include the Nile tilapia (Oreochromis niloticus), African sharp tooth catfish (*Clarias gariepinus*), Nile perch (*Lates niloticus*), Barbs (*Barbus sp.*), Redbelly tilapia (*Tilapia zillii*) and Common carp (*Cyprinus carpio*). Species were selected based on their current commercial importance as well as their potential for nutrition. The content of protein, fat, moisture and ash ranged from 13.30 to 18.50%, 0.40 to 2.45%, 77.24 to 80.80% and 0.81 to 1.20%, respectively.

The fatty acid content of the fish ranged from 6.42 to 25.01 $mg.g^{-1}$ dry weight (DW) for saturated fatty acids (SFA), 2.02 to 24.62 $mg.g^{-1}$ DW for monounsaturated fatty acids (MUFA) and 7.88 to 24.12 $mg.g^{-1}$ DW for PUFA. Among the SFA, palmitic acid was the main fatty acid while oleic acid and docosahexaenoic acid (DHA) were the main MUFA and PUFA respectively. The highest content of eicosapentaenoic acid (4.74 $mg.g^{-1}$ DW) was found in *Barbs* from Lake Langeno while the highest docosapentaenoic acid (3.99 $mg.g^{-1}$ DW), DHA (11.53 $mg.g^{-1}$ DW) and total n-3 PUFA (20.61 $mg.g^{-1}$ DW) were found in Nile tilapia from Lake Haiq. The n-3/n-6 ratios ranged from 1.39 to 5.86 $mg.g^{-1}$ DW, with the highest ratio coming from Nile tilapia collected from Lake Haiq and the lowest from Redbelly tilapia from Lake Ziway.

Table 2.1 summarized nutritional value some fish species studied literature reviews in Ethiopia

Year, Author, dept. and University/research center	Title	Results	limitation
2014, Asmare *et al.* Biotechnology Research Institute, College of Medicine and Health Sciences and School of Food and Chemical Engineering, Bahir Dar University	Extraction and Analysis of Oil/Fat and Fatty Acids Content from Different Indigenous Fish form Lake Tana Source, Northwest Ethiopia	*Labeobarbus* spp. showed the highest oil/lipid content and the highest fatty acids.followed by *Clarius gariepinus* and *Oreochromis niloticus* showed the least in oil content. Sex has no significant effect	Different factors including fish species, sex, season and sites of collection were considered for the differences in oil/lipid content form samples (mainly containing body muscles)
2013, Alemu *et al.* Food Science and Nutrition program, Addis Ababa University	Effect of Endogenous Factors on Proximate Composition OfNile Tilapia (*Oreochromis niloticus* L.) Fillet (only one species observed From Lake Ziway Ethiopia	The moisture and fat contents increased with increase in age of fish from 4 to 5 years. Conversely, the contents of crude protein, ash and gross energy decreased with increase in age of fish from 4 to 5 years. Sex has no significant effect on proximate composition.	The effect of sex on body composition can't decide as insignificant because one species from one place can't confirmed for conclusion
2007, Mekonnen, Chemical engineering (food engineering) Addis Ababa University	Effect of Low Temperature Preservation on The Physicochemical and Microbiological Qualities of Selected Fish Species (only one fish species) From Lake Ziway Ethiopia	The influence days interval on tilapia fish fillets stored for up to 90 days on proximate composition and microbiological qualities of fresh	The natural antioxidant content of fish is not expressed nor is any way of adding manufacturing antioxidants
2014, Assefa ,Zeway Fisheries Resources Research Center	Review Fish Production, Consumptionand Management in Ethiopia from Tana, Ardibo and Lugo, Ziway, Langao Abijata, Shalla, Hawassa, Abaya, Chamo and Turkana	Domestic market for fish is small Fishery production is overexploited due to inappropriate fishing practice the potential of fish was underdeveloped and the management rule and regulation at federal level and regional level to control the overexploitation was very poor.	Rivers are not in this review, because rivers account around 40% of fish sources and management is ecologically balanced and environmentally friendly
2012, Zenebe *et al.* Sebeta National Fisheries and Aquatic Life Research Centre (NFALRC)	Effect of supplementary feeding of agro-industrial byproducts on the growth performance of Nile tilapia and in concrete ponds	All supplementary feeds nearly doubled the growth of *O. niloticus* in ponds. In addition to feed, ingredients, information on digestibility, palatability and levels of anti-nutritional substances should be determined under different agro-ecological conditions	Control cannot used for comparing those of farmed fish, because the main factors may easily be identified and give a general idea about feeding

Table 2.1 Continue

Year, Author, dept and University/research center	Title	Results	limitation
2012, Zenebe *et al.* Sebeta National Fisheries and Aquatic Life Research Centre (NFALRC)	Effect of supplementary feeding of agro-industrial byproducts on the growth performance of Nile tilapia and in concrete ponds	All supplementary feeds nearly doubled the growth of *O. niloticus* in ponds. In addition to feed, ingredients, information on digestibility and palatability should be determined under different agro-ecological conditions	Control cannot used for comparing those of farmed fish, because the main factors may easily be identified and give a general idea about feeding
2012, Jacobus *et al.* Leopold Amhara Region Agricultural Research Institute and Zoological Sciences Program Unit, Addis Ababa University	The composition of fish communities of nine Ethiopian lakes	Fish catchments management should be practiced to save the water bodies and their fish communities	Rivers are not in this review, because rivers account for only a some amount of biodiversity
2012, Kassahun, Department of Sustainable Agricultural Systems, BOKU-University	Proximate composition of selected potential feedstuffs for small-scale aquaculture in Ethiopia	Identified proximate composition of selected potential feedstuffs for small-scale aquaculture in Ethiopia **and** identified as dietary protein and energy source and feedstuffs with low *Crude Protein* and highest *Nitrogen -free Extracts* were identified as dietary energy source.	Fatty acid and mineral contents are also a affected by feed stuffs
2011, Asfaw Faculty of Life Sciences, AAU	Effect of Feed Quality On Growth Performance And Water Quality in Cage Culture System For Production of Nile Tilapia Ethiopia	The cage culture experiment did not produce significant results. Adverse effect to the water quality and plankton abundance	It was not possible to determine the digestibility and palatability of the tested diets and the presence of the essential amino acid in the diets chosen as an alternative of fish feed ingredients.

The nutritional composition of freshwater fish was found to differ between geographical localities (Zenebe et al., 1998). The changing biological and environmental conditions are a useful tool to the ecologist. Ecologists require information on meat composition to help create or maintain dam water atmosphere conducive for rearing a quality fish meat carcass. So far there has been research on other effects on fish, but not much research has been done on the nutritional value and heavy metal accumulation of the fish living in different ecosystems.

In conclusion, all the species collected from different Lakes of Ethiopia may be beneficial to human health. However, Nile tilapia from Lake Haiq and Barbs from Lake Langeno are the best for consumption due to higher levels of LC n-3 PUFA. The summary of reviews of Ethiopian fish related topics are shown in Table 2.1

CHAPTER -3

Evaluation of proximate composition in gill and muscle tissues of freshwater fish species from four freshwater ecosystems in Ethiopia

Abstract

Proximate composition of gill and muscle tissues of Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) fish species caught from four different freshwater bodies (Alwero River, Abay River, Lake Hawassa and Lake Ziway) in Ethiopia were examined. Proximate analysis of the fish samples was determined according to AOAC method. Protein content of fishes varied from 12.44 to 15.22 and 10.75 to 20.23 g/100g; fat content from 2.15 to 2.76 and 2.11 to 4.62% WW; ash content from 0.65 to 1.06 and 1.09 to 1.52 g/100g; and carbohydrate content from 1.55 to 5.47 and 0.28 to 2.53 in g/100g WW, respectively for *O. niloticus* and *C. gariepinus*. Protein content ranged from 12.44 to 15.22 % in muscle tissues of *O. niloticus* and 10.75 to 20.23 % for *C. gariepinus*. Crude fat content ranged from 2.15 to 2.76 % and 2.11 to 4.62 % in muscle tissues of *O. niloticus* and *C. gariepinus*, respectively. Protein content was ranged from11.42 to 17.36 % in gill tissues of *O. niloticus* and 10.58 to 20.05% for *C. gariepinus*. Crude fat content ranged fro m 2.91 to 3.47% and 2.69 to 4.60% in gill tissues of *O. niloticus* and *C. gariepinus*, respectively. In that order, the study points out that both gill and muscle tissues of the investigated freshwater fish species contain appreciable amounts of nutrients and the discarded fish tissue, such as gill contains significant amount of fat and protein, which is a valuable source of food for human consumption as well as a good substitute source to extract the fish lipid from muscle. Ash contents were significantly high ($P<0.05$) in the gills and low in the muscles. These fish species have high nutritional values and their consumption will contribute in the remedy of health and malnutrition problems among the population. Further research should be carried-out on freshwater fish species and their discharged waste body parts and essential amino acids should be included.

Keywords: Fish species. Freshwater. Lakes. Rivers. Muscle. Gill. Proximate composition

3.1. Introduction

Fish has been recognized as an excellent food source for humans for centuries and is preferred in the diet not only due to its excellent taste and high digestibility but also due to higher proportions of unsaturated fatty acids, essential amino acids and minerals for the fo rmation of functional and structural proteins (Abdullahi et al., 2001; Mehboob et al., 2003).

The fish muscles are mainly composed of proteins, fats and water in maximum percentage along with minerals and vitamins, all of which contribute to the overall meat composition (Sanatan, 2016). Moreover, fish has been the cheapest source of animal protein and a high protein food required in human diet particularly in the low and middle-income groups living in tropical countries suffering from lack of protein. This area is one of the most widespread nutritional deficiencies (Ozkan, 2005).

However, it is not fully known whether fish and shellfish have consistent nutrient composition due to exogenous and endogenous factors such as feeds, sex, temperature, pH, speci es and salinity (Inhamuns and Franco, 2008 Fabiola and Martha, 2012). Nutrient composition varies widely from species to species and within the same species (Fawole et al., 2007). These endogenous factors such as sex, organ location and species govern the majority of principles that determines the composition of fish (FAO, 2002; Noël et al., 2011). Proximate composition of body muscle of *O. niloticus* (male and female) analyzed shows that the moisture content was found to be higher in male, while protein, fat, ash and carbohydrate contents were higher in female. Moreover, different sexes were observed to have varying chemical composition (Alemu et al., 2013). African catfish grow significantly faster and larger than *O. niloticus* (Desta et al., 2007). Proximate composition of body muscles of *Puntius stigma* (male and female) analyzed shows that the moisture content was found to be higher in female, while protein, fat, ash, carbohydrate and minerals contents were higher in male. Besides, different sexes were observed to have varying proximate composition (Biro et al., 2009).

The crude fat content of the different fish species were significantly higher i n *C.gariepinus* than *Cyprinus carpio*, *Bagrus docmak*, *Labeobarbus intermedius* and *Labeobarbus nedgia* species (Tsegay et al., 2016). There are, therefore, a number of variables that can affect the overall proximate composition of fish tissues. However, there is little information on the effects of different species, tissues location and sex of the individual chemical composition of African catfish and *O. niloticus* tissues. To determine the overall biochemical composition of the two fish species, all the above-mentioned factors of variations need to be investigated. Therefore, in view of this fact, the present research was carried out to determine proximate composition of *Clarius gariepinus* and *Oreochromis niloticus* collected from four different freshwater ecosystems: Alwero River, Abay River, Lake Hawassa and Lake Ziway of Ethiopia.

The second objective was to investigate the gill wastes of these fish species, compare their nutritional values with muscle tissues. To the best of my knowledge, this is the first s tudy that explored the nutritional status of gill tissues of selected freshwater fish species collected from Lakes and Rivers in Ethiopia. These data will provide useful information for fish processing industries, nutrition and consumer groups.

3. 2. Materials and Method

3.2.1. Description of the study areas

The current study was conduceted in different freshwater bodies/areas found in four different regions in Ethiopia. One of the study area is Lake Hawassa which is located at 6°33'-7°33' N; 30°22'-38°29' E. The second is Lake Ziway which is found at 7°52'-8°8' N; 38°40'-38°56' E. The third is Alwero River that is located at 07^0 86′N:$34^0$50′E, and the forth is Abay River (Debere Maryam area) which is found at 11°36′93.6′′N; 037°24′53.85′′E, which are ecologically and economically important ecosystems in the Ethiopian (Fig. 3.1). They have an important nature value with a diverse avifauna. These freshwater bodies are also crucial for the subsistence of the local communities as fishing and they are highly productive. They have rich phytoplankton and zooplankton that support large populations of different fish species: *Oreochromis niloticus*, *Clarias gariepinus*, *Barbus intermedius*, *Barbus paludinosus*, *Lates niloticus*, *Bagrus docmak*, *Crucian carassius*, *Garra quadrimaculata* and *Aplocheilichthyes antinorii*; the first two of which are commercially and economically important (Hussien et al., 2010; Golubtsov et al., 2002, 2004).

3.2.2. Sampling

Fresh fish samples of Nile Tilapia (*Oreochromis niloticus*) and African Catfish (*Clarias gariepinus*) were collected with the help of local fishermen on the site area of Lake Ziway, Lake

Hawassa, Abay River and Alwero Rivers from February to October 2017. A total of eighty (80) samples, 20 samples from each freshwater body were collected (26-40 cm long).

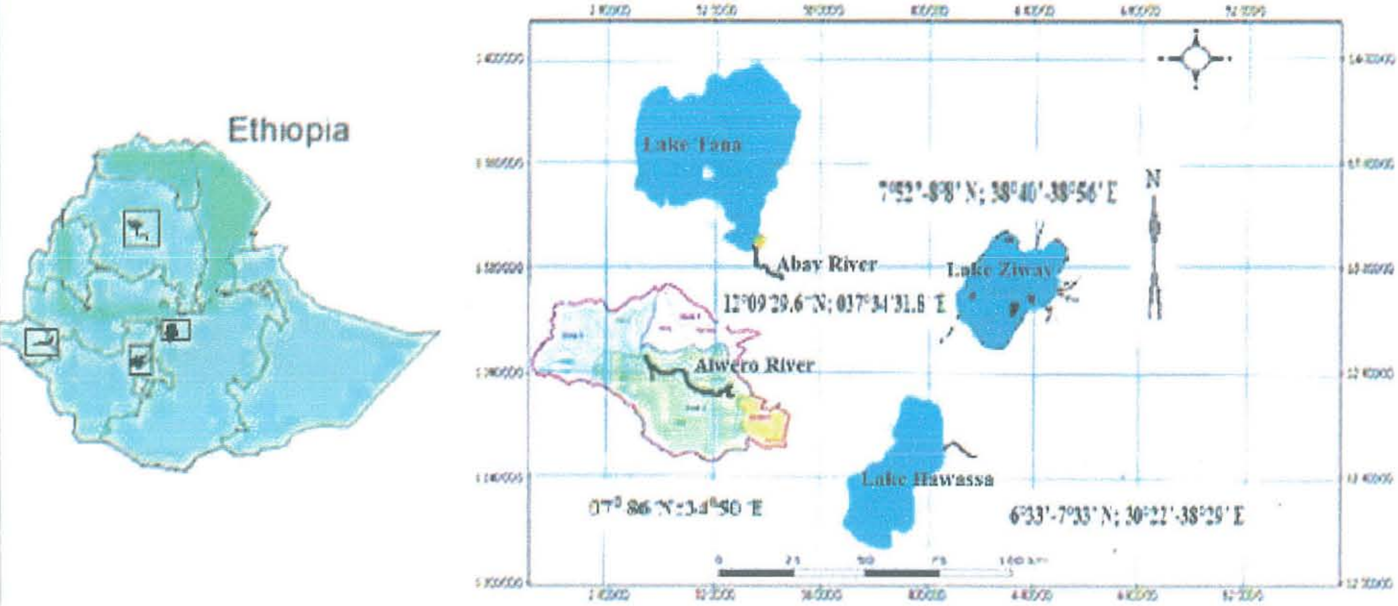

Figure 3.1 Geographical map of Ethiopia showing the location of study Site Rivers and Lakes. In the sketches of the four freshwater bodies.

The samples were collected from four freshwater bodies with different geogra phical location and ecosystems (lake and river). The samples were placed in icebox soon after collection to keep them fresh and then transported to Addis Ababa University, Center of Food Science and Nutrition Laboratory layered with flaked ice using icebox. Sex was identified by examining genital papilla located immediately behind the anus. In males, the genital papilla has only one opening (the urinary pore of the ureter) through which both milt and urine pass. In females the eggs exit through a separate oviduct and only urine passes through the urinary pore. Immediately after sex identification, length and weight of the fish were measured to the nearest 0.1 cm and 0.01g respectively.

3.2.2.1. Sample Preparation

The fish samples were cut open and immediately a portion was used in moisture content determination. The remaining fish samples were dried on oven in 70 - 75 °C overnight (Ali and Mozhgan, 2015). The samples were removed from the oven, allowed to cool, and ground ed by a clean mortar and pestle. Then the grounded fine powder were stored in a dried plastic container with tight fitting lid until used for various analysis.

3.2.3. Proximate Composition Analysis

The proximate composition (moisture, crude protein, crude fat, ash and carbohydrates) of the two fish species were determined using (AOAC, 2000). All chemical analyses were carried out in duplicate. Results were expressed as g/100g dry and used a s wet weight by conversion based on fresh moisture content.

3.2.3.1 Moisture Content Determination

Principle: This was done based on the differences between the net weight and the weight after drying to a constant temperature at 105 ^{0}C. Moisture content was determined using AOAC (2000) method number 925.05. The sample dish was dried at 105^{0}C for one hour and placed in a desiccator for about 15-20 minutes to cool. This was weighed (M_1)-five gram (5g) of the sample was weighed into the moisture dish (M_2) and dried in an oven at 105^{0}C to a constant weight (M_3). The percent dry matter was determined using the following equation:

$$\textbf{Moisture content (\%)} = \left(\frac{M3-M1}{M2-M1}\right)X100 \ldots (1)$$

% Moisture = 100 - % dry matter

where :

$M_1 = Weight\ of\ dish$

$M_2 = Weight\ of\ fresh\ sample\ and\ dish$

$M_3 = Weight\ of\ Sample\ after\ drying$

3.2.3.2. Crude protein

Principle: - Nitrogen is used as an index of the protein and so termed as crude protein because it represents all of the nitrogen that is in the form of Non -Protein Nitrogen (NPN) such as nitrates, ammonia, urea and single amino acids, as well as the nitrogen present as true protein. The multiplication by a conversion factor of 6.25 is based on the assumption that true protein contains 16% nitrogen.

Procedure: - Crude protein contents of samples were determined according to the AOAC standard method 979.09 (AOAC, 2000). Briefly, around two gram (2 g) of dried sample was weighed and placed in Kjeldahl digestion tube in duplicate. To this, 6 ml of conc. H_2SO_4 was added. Then 3.5 mL hydrogen peroxide followed by 3 g of a mixture of $CuSO_4$ and K_2SO_4 (1:15 w/w) was added as a catalyst and digested at 370 ^{0}C until the solution turns to light green color. The digest was cooled and placed in an automatic distillation apparatus. During this step, water was first added to the solution followed by 35% sodium hydroxide solution to neutralize the system.

When distillation proceeds, the vapor was captured by 2% boric acid solution in another flask. This solution was finally titrated by 0.1M HCl to determine the total nitrogen content and phenolphthalein colorless was used as an indicator. Reagent blanks were also run side by side and the nitrogen content was subtracted from sample nitrogen. Percent nitrogen (%N) was calculated using the following equation:

$$\%N=\frac{(Vs-Vb)*N*14.007}{gram\ of\ sample}*100$$

Where; Vs-Volume of acid consumed by the sample, Vb-Volume acid consumed by the blank, N-Normality of HCl.

Crude protein, percent per weight = 6.25 * total nitrogen

3.2.3.3 Crude Fat

Principle: - This is the continuous extraction of fat content from a sample using a suitable solvent in a soxhlet extractor.

Procedure: - The amount of crude fat was determined by the Soxhlet extraction method according to AOAC, 920.39, 2000. Exactly 2.5 g of sample was weighed into an extraction thimble and covered with fat free cotton. Then 60 mL of diethyl ether (Sigma- Aldrich, USA) was added to pre weighed, cleaned and dried receiving flask and fitted into the apparatus. Water and heater was turned on to start extraction for 4 h. Then, the flask was dried in an oven at about 70 °C for 30 minutes to remove the solvent and cooled in a des iccator and weighed. The percent crude fat was determined by using the following formula:

$$\text{Crude Fat}\left(\frac{g}{100g}DM\right)=\frac{(\text{Weight of flask and fat extracted}-\text{Weight of empty flak})}{\text{Weight of dried sample used}}\text{x100}$$

3.2.3.4 Ash

Principle: - By ashing the sample, all the volatile organic constituents in the sample were burnt off leaving behind the non-volatile mineral elements.

Procedure:-The ash content was determined after the removal of organic matter by dry ashing according to (AOAC 923.03, 2000). Accurately weighed sample (M_1) (2 g) was placed into a pre-dried crucible (M_2) and charred in hot plate under the hood. The charred sample was placed in muffle furnace. The furnace was closed and ignited at 550 °C for 5 h until the sample became white/gray. The crucibles were cooled in a desiccator and weighed (M_3). The ash content was calculated using the following equation:

$$Ash\,(\%) = \frac{M_3 - M_1}{M_2 - M_1}\,x\,100\%$$

Where:

M_1 = *Weight of the dish*

M_2 = *Weight of fresh sample and dish*

M_3 = *Weight of ash and dish*

3.2.3.5 Determination of Carbohydrate (By Difference)

Available carbohydrates were determined by difference (i.e. subtracting the sum of pro tein, fat, ash, fiber and moisture content from 100) and the result was expressed as g/100g DM (FAO, 2003).

Total carbohydrates [%] =100- [%Moisture + % Protein+ % Fat + % Ash]

3.2.3.6 Estimation of the Gross energy value (calorific value)

The gross energy value of each samples were expressed as kcal/100g, was determined by multiplying the percentages of protein (PC) and fat (FC) contents with their respective standard factors of 4 and 9 kcal/100 g of fish samples (FAO, 2003; Jabeen and ch audhry, 2011) using the following equation:

Caloric value = (4PC + 9FC) kcal/100 g weight.

3.2.3.7 Determination of Dry Matter Content

The dry matter (DM) content of the representative gill and muscle samples were determined using moisture analyzer (HB43-S Halogen) continually measures the weight of the sample put in single pan and displays the reduction in moisture. Once drying has been completed, the moisture content of fish sample is displayed as the final result.

3.2.4. Statistical Analysis

Data were submitted to SPSS version 20.0-package software and analyzed using analysis of variance (ANOVA) to determine significant differences among the fish species. Tukey HSD test were performed for the comparison of means of samples. Differences were considered significant when $p < 0.05$.

Figure 3.2 Fish sampling area

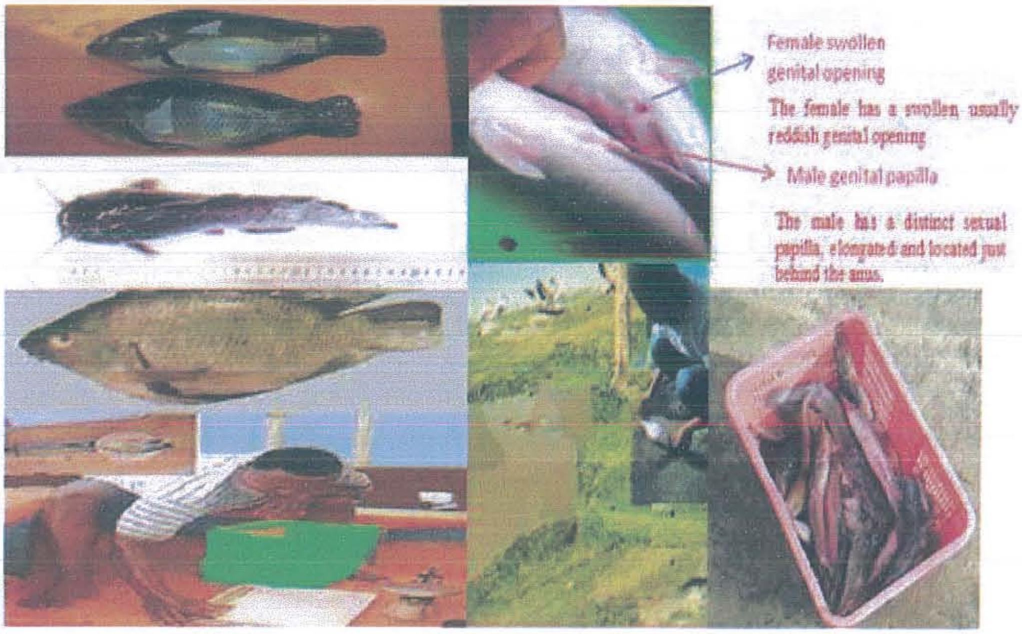

Figure 3.3 Fish sample collection, measurement and sex identification

Figure 3.4 Fish sample washing and desiccation

Figure 3.5 Fish tissues (muscle and gill) moisture determination

3.3. Results and discussion

Biometric data of *O. niloticus* and *C. gariepinus* species from four different water bodies

The biometric data measured using standard length of each fish was taken using metre rule, the length of fish was measured to the nearest 0.1 cm. The weights of the sampled fish were measured with weighing scale to the nearest 0.01gram (Ohaus Analytical weighing balance, China). For the adult fish, the total length and weight ranged from 26.0 - 31.50 cm, 276.0 - 667.81g and 33.0 - 40 cm, 252.0 - 693.92 g for *O. niloticus* and *C. gariepinus*, respectively.

Table 3.1 presents the biometric values of *O. niloticus* and *C. gariepinus* species studied. The total weights and lengths of *O. niloticus* from Alwero River, Abay River, Lake Hawassa and Lake Ziway fishes were 587.19±6.98 g, 328.89±14 g, 296.58±20.12 g, 299.45±18.38 g, 30.08±0.87 cm, 26.0±0.28 cm, 27.25±2.12 cm and 27.5±2.12 cm r espectively. For *C. gariepinus* the total weight and length of fishes were 605.42±25.76 g, 301.42±27.05 g, 262.28±14.54 g, 320.25±79.19 g, 36.44±1.55 cm, 35.92±1.98 cm, 35.75±1.41 cm and 35.5±2.12 cm from Alwero River, Abay River, Lake Hawassa and Lake Ziway respectively. The maximum and minimum lengths of *O. niloticus* were 26 cm and 31.50 cm, and for *C. gariepinus* were 33 cm and 40 cm, respectively ($p < 0.05$). The maximum and minimum weights for *O. niloticus* were 276 g to 667.81g and for *C. gariepinus* were 252 g and 693.92 g, respectively ($p < 0.05$). Based on the results, the *C. gariepinus* fish species had higher weight and length than the *O. niloticus* fish species and also River sources of fish species had higher weight and length than Lakes sources in both species ($p < 0.05$).

The range of values for weight and the length of the two fish species from different freshwater bodies with binomial and vernacular nomenclature are presented in Table 3.1. There were a high degree of inconsistency in biometric values of weight and length of the two species from the four different freshwater bodies, with least length and weight of 33.00 to 26.00 cm and 252.00 to 276.00 g respectively recorded for *C. gariepinus* and *O. niloticus* from all sampled sites, and falls in the range of 28.63 to 42.38 cm and 258.63 to 402.55 g reported for wild *C. gariepinus* and wild *H. bidorsalis* species from Nigeria, respectively (Onyia et al., 2013).

Table 3.1 Biometric data of two fish species from four different freshwater bodies

samples				Total Length (cm)		Total Body weight (g)	
Common Name	Scientific Name	Fish sources	N	Range	Mean± SD	Range	Mean± SD
Nile Tilapia	*O. niloticus*	Alwero River	12	29.0-31.50	30.08±0.87[c]	490.23-667.81	587.19±6.98[b]
		Abay River	10	26.0-28.0	26.83±0.72[e]	276.0-378.67	328.89±14.00[c]
		Lake Hawassa	10	26-29	27.25±2.12[d]	280-309.01	296.58±20.12[e]
		Lake Ziway	10	26-29	27.5±2.12[d]	286-312.89	299.45±18.38[d]
African Cat fish	*C. gariepinus*	Alwero River	8	35.0-38.0	36.44±1.55[a]	535.14-693.92	605.42±25.76[a]
		Abay River	10	33.0-40.0	35.92±1.98[a]	254.0-430.0	301.42±27.05[d]
		Lake Hawassa	10	35-36	35.75±1.41[ab]	252-272.56	262.28±14.54[f]
		Lake Ziway	10	34-37	35.5±2.12[ab]	264-376.50	320.25±79.19[c]
Total			80				

N= number of fishes sampled. Mean values ± SD, Means with the same superscript in the same column are not significantly different ($p \geq 0.05$).

On the other hand, the weight of fish samples showed direct proportional relationship with length and this result is similar to that reported and that length of the freshwater fish species alter depending upon the weight of the fish species by Gokhan et al (2010) and Oribhabor et al (2011).

Proximate Composition

Table 3.2 and 3.3 shows the proximate composition of *C. gariepinus* and *O. niloticus* tissues. Sex was found to have no significant ($P > 0.05$) influence in terms of the five proximate components (moisture, protein, fat ash and carbohydrate) measured in *C. gariepinus* and *O. niloticus* gill and muscle tissue, collected from Alwero River, Abay River, Lake Hawassa and Lake Ziway.The main component of fish tissues was moisture.

Moisture Content

The moisture content in muscle of *O. niloticus* from all four sampling sites ranged from 76.44 to 81.43% for male and from 77.37 to 80.62 % for female fish. For *C. gariepinus* the moisture content in muscle ranged from 71.15 to 83.32% for male and from 71.11 to 79.32 % for female. The highest moisture content (83.32 %) was recorded from Abay River male of *C. gariepinus* and the lowest (71.11 %) being from Lake Ziway female *C. gariepinus*. The present study found that the moisture contents of selected fish species from the same water bodies showed significant difference. For example, the moisture contents of fish samples collected from Alwero River were 78.86 % and 77.37% for male and female of *O. niloticus*, and 71.15% and 71.11% respectively for male and female of *C. gariepinus*, which showed significant differences among the fish species although they were collected from the same river.

As showed in Table 3.2 and 3.3, the moisture content of male fish is slightly higher than female fish recorded in all sample sites. However, moisture content is nearly the same for both sexes from similar freshwater bodies. In agreement to the present study, male fish is reported to have higher content of moisture than female fish (Islam and Joadder, 2005; Nargis, 2006; Bhavan et al., 2010; Cornelia, 2012). In addition, similar to our study male *O. niloticus* caught from Lake Zeway seen to have higher moisture content than female fish (Alemu et al., 2 013). Moreover, Alemu et al. (2013), reported moisture content of *O. niloticus was* 80.60 and 79.60% for male and female respectively.

The mean average of moisture contents was measured to be the highest in muscles 77.22% and lowest values were observed in gills 69.6% from all sampled sites. The moisture contents in muscle of the two fish species from all sample sites ranged from 71.11 to 82.32 % and the value is close to 75.0% to 80% reported in previous studies on muscle tissue of different fish species fr om Ethiopia (Mekonnen, 2007; Alemu et al., 2013; Tsegay et al., 2016). In line with the present result, Nwali et al (2015) and Osibona et al (2009) have also reported that moisture contents of *C. gariepinus was* (75.65%), which is similar to the results, ob tained in *C. gariepinus* caught from Lake Hawassa.

Another study by Osibona (2011) reported that a moisture content of C. gariepinus was *ranged from* 65.64 to 80.17 %. FAO (2010) and USDA (2010) set a limit moisture contents in fish muscle ranged from 78-90%, which are in good agreement with the present findings. As reported by Yeannes and Almandos (2003) and Wang et al (2005) moisture is a major component of the edible portion of fish tissues. This is also confirmed by the results of the present study. The finding shows that the moisture content is the main component of the tissues of *O. niloticus* and African catfish collected from various water bodies in Ethiopia. (Badolato et al., 1994).

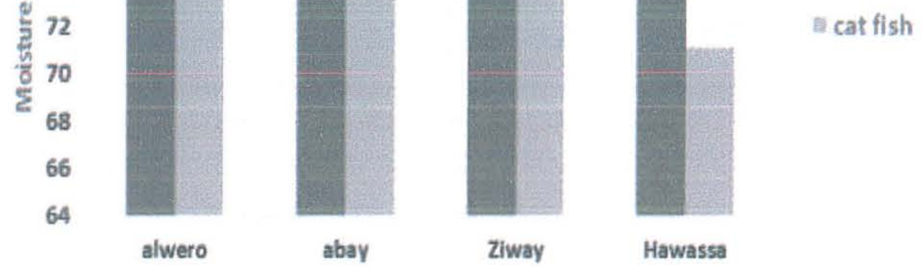

Figure 3.6 Muscle tissues moisture con tents (fresh body) of fishes collected from Alwero, Abay, Hawassa and Ziway freshwater bodies.

The moisture contents obtained from muscles of two different fish species showed a significant difference (P<0.05) and male of *O. niloticus* was showed the highest moisture content (81.43%) and female of *C. gariepinus* was the least moisture content (71.11%) (Figure 3.6).

Dry matter Content

The dry matter content shows the chemical potential of the fresh samples and reflects the true biological yield (Teye et al., 2011). Dry matter content of the two fish species in muscle and gill tissues ranged from 7.06 to 12.00 g/100g (dry base), and fal ls in the range of 14.35 to 7.96 g/100g dry base reported for *Govazym* and *Zeminkan of* fish species dry matter content, respectively (Ali and Mozhgan, 2015). There were significant differences (P < 0.05) in the dry matter content of in all sites of the two fish species.
Dry mater content of male of *O. niloticus* species were ranged from 8.03 to 11.76 g/100g and this value was significantly higher than that of the male *C. gariepinus which ranged from* 7.96 to 10.93g/100g. On the other hand, the dry matter cont ent of female of *O. niloticus* and *C. gariepinus* species were insignificantly (P > 0.05) different. The dry matter content in muscle and gill tissue was ranged from 7.75 to 12.00g/100g for female *O. niloticus* and from 7.06 to11.22g/100g for female of *C. gariepinus*. Table 3.2 and 3.3 shows the mean dry matter content of muscle tissue of *O. niloticus* and *C. gariepinus*. No significant (P>0.05) difference was observed between male and female fish species of all the muscle meat analyzed. But female fish had slightly higher dry matter content (7.06 to 12.00g/100g) compared to male fish species (7.96 to 11.76g/100g) observed in both fish species. Similar to the present study, the dry matter content of female *Puntius stigma* fish muscle is higher than male fish (Biro et al., 2009).

The gill tissues of fish species showed a significant variation in dry matter. The dry matter content in gill tissue of *O. niloticus* and *C. gariepinus* ranged from 6.62 to 10.66g/100g for male fish and for female in the range of 6.80 to 9.11g/100g, respectively, but sex has no significant (p>0.05) effect on dry matter content of gill tissues of *O. niloticus* and *C. gariepinus* species observed in all sampled sites (Alwero River, Abay River, Lake Hawassa and Lake Ziway). The dry matter content of the present study was lower than that reported for *Govazym* and *Zeminkan* species (14.35 to 7.96 %) Ali and Mozhgan (2015).

Table 3.2 Proximate composition (mean ± SD) of muscle tissue of two species of fish captured from four different freshwater bodies in Ethiopia

Parameter	Species	Alwero River		Abay River		Lake Hawassa		Lake Ziway		**FAO, 2010**
		M	F	M	F	M	F	M	F	
		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	
D. matter	CF	10.56 ± 0.47^{b}	9.81 ± 0.52^{b}	10.93 ± 057^{a}	11.22 ± 0.23^{a}	7.96 ± 0.52^{dc}	8.21 ± 0.42^{c}	8.21 ± 0.27^{c}	7.06 ± 0.32^{d}	
	NT	8.03 ± 0.43^{c}	7.75 ± 0.09^{d}	11.76 ± 0.74^{a}	12.00 ± 0.13^{a}	8.80 ± 0.31^{c}	8.76 ± 0.45^{c}	10.77 ± 0.21^{b}	10.68 ± 0.32^{b}	
Protein	CF	67.27 ± 0.23^{c}	66.79 ± 0.45^{c}	58.07 ± 0.89^{d}	58.66 ± 0.38^{d}	71.75 ± 0.18^{ab}	72.44 ± 0.44^{a}	70.82 ± 0.51^{b}	70.43 ± 0.15^{b}	15-28
	NT	67.09 ± 0.15^{b}	65.91 ± 0.02^{c}	58.66 ± 0.63^{c}	58.45 ± 0.82^{c}	60.34 ± 0.33^{d}	59.21 ± 0.04^{d}	70.01 ± 0.39^{a}	69.65 ± 0.02^{a}	
Fat	CF	13.92 ± 0.73^{c}	14.24 ± 0.59^{bc}	11.41 ± 0.11^{d}	11.42 ± 0.36^{d}	14.48 ± 0.93^{b}	15.05 ± 0.81^{a}	15.58 ± 0.23^{a}	16.16 ± 0.43^{a}	15-18
	NT	12.81 ± 0.27^{b}	13.36 ± 0.86^{a}	12.99 ± 0.31^{b}	12.95 ± 0.04^{b}	11.20 ± 0.08^{c}	10.12 ± 0.37^{d}	10.78 ± 0.40^{d}	11.03 ± 0.04^{c}	
Ash	CF	6.98 ± 0.63^{b}	7.14 ± 0.46^{a}	5.91 ± 0.79^{c}	7.41 ± 0.91^{a}	5.43 ± 0.63^{c}	5.11 ± 0.29^{c}	5.04 ± 0.38^{c}	5.16 ± 0.21^{c}	
	NT	4.15 ± 0.38^{ab}	4.49 ± 0.45^{a}	4.10 ± 0.08^{b}	4.03 ± 0.15^{b}	3.78 ± 0.41^{b}	4.97 ± 0.52^{a}	3.01 ± 0.22^{c}	3.97 ± 0.15^{b}	
CHO	CF	1.27 ± 0.33^{c}	2.02 ± 0.41^{c}	13.68 ± 0.33^{a}	11.29 ± 0.77^{b}	0.38 ± 0.34^{d}	0.01 ± 0.19^{d}	0.35 ± 0.60^{d}	1.19 ± 0.48^{c}	2-5
	NT	7.92 ± 0.23^{e}	8.49 ± 0.08^{e}	12.49 ± 0.23^{d}	12.57 ± 0.28^{d}	15.88 ± 0.56^{b}	16.94 ± 0.70^{a}	14.75 ± 0.32^{c}	4.67 ± 0.54^{f}	
Moisture	CF	79.37 ± 0.55^{b}	77.07 ± 0.43^{c}	83.32 ± 0.21^{a}	79.32 ± 0.19^{b}	74.76 ± 0.33^{d}	74.48 ± 0.83^{d}	71.15 ± 0.18^{e}	71.11 ± 0.12^{e}	78-90
	NT	81.43 ± 0.04^{a}	79.46 ± 0.23^{c}	76.44 ± 0.36^{f}	80.62 ± 0.41^{b}	79.40 ± 0.82^{c}	78.23 ± 0.84^{d}	78.86 ± 0.39^{d}	77.37 ± 0.50^{e}	

Mean±SD Value in the same rows with different superscript letters are significantly different ($p<0.05$) (Tukey Post Hoc Test) CF=Cat Fish (*C. gariepinus*), NT Nile Tilapia (*O. niloticus*) M=male and F= female: Values represent means and SD of duplicate determinations.

Table 3.3 Proximate composition (mean ± SD) of gill tissue of two species of fish captured from four different freshwater bodies in Ethiopia

Parameters	Species	Alwero River		Abay River		Lake Hawassa		Lake Ziway	
		M	F	M	F	M	F	M	F
		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
D. matter	CF	7.36 ± 0.33^{cb}	7.53 ± 0.37^{b}	7.98 ± 0.42^{b}	8.16 ± 0.46^{a}	6.37 ± 0.18^{ed}	7.72 ± 0.11^{b}	7.11±0.17d	6.97 ± 0.13^{d}
	NT	6.62 ± 0.05^{d}	6.80 ± 0.37^{d}	9.73 ± 0.17^{b}	9.06 ± 0.11^{b}	7.21 ± 0.04^{c}	7.17 ± 0.06^{c}	10.66±0.12a	9.11 ± 0.14^{b}
Protein	CF	35.02 ± 0.28^{e}	35.88 ± 0.21^{e}	42.59 ± 0.13^{c}	38.66 ± 0.12^{d}	56.60 ± 0.08^{ba}	57.44 ± 0.10^{ba}	58.35±0.45a	59.47 ± 0.48^{a}
	NT	38.39 ± 0.17^{d}	38.53 ± 0.25^{d}	45.47 ± 0.26^{c}	39.39 ± 0.24^{d}	54.25 ± 0.12^{b}	55.18 ± 0.14^{a}	56.08±0.49a	54.80 ± 0.52^{b}
Fat	CF	12.22 ± 0.39^{cb}	12.53 ± 0.34^{b}	10.43 ± 0.08^{d}	10.20 ± 0.02^{d}	12.21 ± 0.14^{cb}	12.76 ± 0.16^{b}	13.07 ± 0.19^{a}	13.95 ± 0.22^{a}
	NT	11.11 ± 0.43^{b}	12.26 ± 0.54^{a}	11.00 ± 0.59^{b}	10.86 ± 0.35^{cb}	9.74 ± 0.26^{d}	9.69 ± 0.29^{d}	9.50±0.84d	9.07 ± 0.90^{d}
Ash	CF	22.72 ± 0.25^{a}	22.81 ± 0.28^{a}	22.09 ± 0.42^{ba}	22.74 ± 0.44^{a}	15.88 ± 0.08^{c}	15.46 ± 0.08^{c}	15.26 ± 0.07^{c}	15.25 ± 0.05^{c}
	NT	17.84 ± 0.32^{a}	18.08 ± 0.18^{a}	12.27 ± 0.08^{d}	12.53 ± 0.06^{d}	14.05 ± 0.39^{c}	15.66 ± 0.39^{b}	14.44 ± 0.22^{c}	15.50 ± 0.28^{b}
CHO	CF	22.68 ± 0.07^{a}	21.25 ± 0.05^{a}	16.91 ± 0.32^{c}	20.24 ± 0.35^{b}	8.94 ± 0.09^{d}	6.62 ± 0.07^{e}	6.21 ± 0.34^{e}	4.36 ± 0.33^{f}
	NT	26.04 ± 0.13^{a}	24.33 ± 0.10^{b}	21.53 ± 0.02^{c}	21.53 ± 0.01^{c}	21.96 ± 1.02^{c}	12.30 ± 1.04^{d}	9.32 ± 0.14^{e}	11.52 ± 0.13^{d}
Moisture	CF	68.65 ± 0.27^{d}	68.15 ± 0.31^{d}	74.33 ± 0.39^{a}	73.54 ± 0.36^{b}	69.52 ± 0.17^{c}	68.35 ± 0.13^{d}	66.40 ± 0.57^{e}	65.54 ± 0.53^{f}
	NT	70.19 ± 0.39^{a}	70.46 ± 0.43^{a}	68.47 ± 0.73^{c}	70.39 ± 0.77^{a}	69.65 ± 0.27^{b}	69.15 ± 0.31^{b}	69.13 ± 0.63^{b}	68.21 ± 0.66^{c}

Mean±SD Value in the same rows with different superscript letters are significantly different ($p<0.05$) (Tukey Post Hoc Test) CF=Cat Fish (*C. gariepinus*), NT Nile Tilapia (*O. niloticus*): M= male and F= female Values represent means and SD of duplicate determinations.

The lower value of moisture content of the present study fish samples is very important for long shelf life, unfavorable for microbial growth and oxidative degradation of PUFAs (Omolara and Omotayo, 2008). On the other hand drying process has a positive influence on the protein, lipids and ash content of muscle and gill tissues of fishes (Olayemi et al., 2011).

Crude protein content

Muscle of the two fish species *(O. niloticus* and *C. gariepinus*) from all sample sites contain a lower amount of crude protein (58.45 to 70.01g/100g DW) compared to the values reported for *C. gariepinus* (91.99 g/100g DW) and *O. niloticus* (90.81 g/100g DW) from Nigeria (Adeniyi et al., 2011). The results of crude prote in content in the current study's samples were similar within the value of 68.4% for *C. gariepinus* and 71.46% for *O. niloticus* obtained from Nigeria and Sudan respectively (Olayemi et al., 2011; Egbal et al., 2017). Similarly, for *Govazym* and *Zeminkan* species crude protein of 77.44 and 72.49 (g/100g DW were respectively reported by Ali and Mozhgan (2015).

The protein content of *C. gariepinus* collected from Alwero River, Abay River, Lake Hawassa and Lake Ziway were 67.03, 58.36,72.10 &70.63 (g/100g DW) and 14.57, 10.81, 18.02 & 20.17 (g/100g WW) respectively. The highest and the lowest values of protein obtained from Lakes and Rivers respectively falls in the range of 15 to 28g/100g WW reported for fish muscles (FAO, 2010; USDA, 2010) except for *C. gariepinus* from Abay River. The protein content of *C. gariepinus* from Alwero River is similar with Lake Hashenge in the same fish species was15.44 g/100g WW recorded (Tsegay et al., 2016). Based on fish protein content stated by Stansby (1982) consider low-level protein content fish in muscle, when the fish contains less than 15% and high-level protein content, ranging from 15 to 20%. These results show the assertion made by Badolato et al. (1994) that protein is the second component with the highest contribution to the fish muscle. In the present study, African catfish fish species caught from Lake Hawassa and Lake Ziway contain protein content which ranged from 18.02 to 20.18% WW and Naile Tapia contains 15.09% WW and as a result both fish species can be classifi ed as rich protein species.

According to Cornelia (2012), the proximate composition of fish could not undergo any significant change between both sexes. Researchers have reported mixed and contradictory findings in this area. Some researchers have report ed that protein content of female fish were higher than male (Alemu et al., 2013). In contrast, other researchers have reported that male fish contain higher protein content than female (Bhavan et al., 2010; Cornelia, 2012). In the present study, the highe st values of protein content of *C. gariepinus* from Lake Hawassa was 70.01g/100g DW for male and 72.44g/100g DW for female. For *C. gariepinus* from Abay River the highest content of protein was 58.07g/100g DW for male and 58.66g/100g DW for female. This show ed that female fish collected from Lake Hawasa and Abay River contains slightly higher protein contents compared to the male fish, but sex has no significant effect on protein content. The result of the present study is similar with the work of Alemu et al (2011) who reported protein content of female fish as slightly higher compared to male fish. Regarding C. *gariepinus* caught from the two water bodies, the male fish contains slightly higher protein content (70.82 g/100g DW) than female fish (70.43 g/100g DW) caught from the Lake Ziway and the male from Alwero River contains protien 67.27 g/100g DW and the female fish contains 66.79 g/100g DW. However, these differences were not significant. Bhavan et al. (2010) and Cornelia (2012) have also reported that male fish contains higher protein content than female fish. Therefore, the crude protein content in muscles of *C. gariepinus* species from all sampled sites followed a decreasing order: Lake Hawassa > Lake Ziway > Alwero River > Abay River.

The protein content of muscle tissues of the two species in all sampled sites ranged from 58.07 to 72.44 g/100g DW, while in gill tissues were recorded from 35.02 to 59.47 g/100g DW. Similar results for muscles were recorded for *C. gariepinus* (68.4 g/100g DW) and for *O. niloticus* (71.46g/100g DW) obtained from Nigeria and Sudan respectively (Olayemi et al., 2011; Egbal et al., 2017). A significant higher protein content obtained in the fish sample of the current study indicated that protein nitrogen was not lost during th e drying period of sampled preparation. This is in accordance with the findings of Tao and Linchun (2008).

Both fish species in the present study from Lakes Ziway and Hawassa have an adequate source of protein, which makes *C. gariepinus* from these lakes an important source of dietary protein similar to the result reported from Lake Hashenge (Tsegay et al., 2016). The result of this study showed that all the analyzed fish samples were good sources of protein. The protein content was different from one source to another source in similar fish species observed. This may be associated with stage of sexual growth and feeding conditions (El-Serafy et al., 2005) and it depends on the difference in fish absorption capability and conversion potentials of essentia l nutrients from their diets (Adewoye and Omotosho, 1997).

The crude protein results of fish muscle in the present study agreed with those reported for *Liza falcipins* and *O. niloticus* freshwater fish species from Cameroon in the range of (62.80 to 52.47 g/100g DW) (Tenyang et al; 2016). According to Ogbonnya and Ibrahim (2009), protein content in muscles of *C. gariepinus* from Nigeria recorded as 67.21 g/100g DW. The result of the present study was slightly higher than the reported in the above fish specie s but comparable with Şahin et al (2011) reported for *Salmo trutta labrax* Pallas and *Salvelinus fontinalis* Mitchill fish species in the rage of 73.82 to 69.51 g/100 g DW from Turkey.

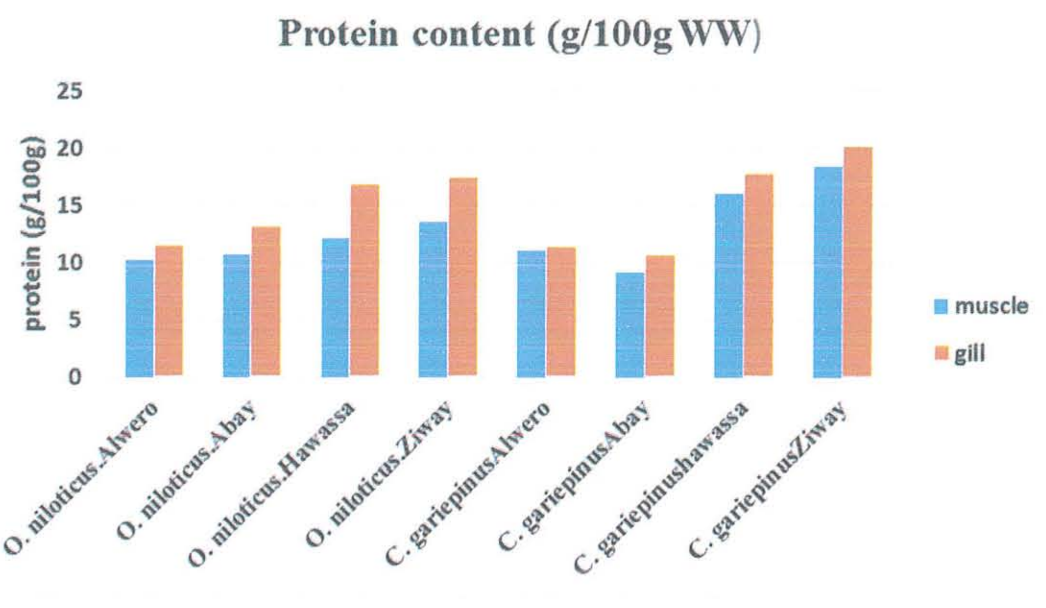

Figure 3.7 Protein content in gill and muscle tissues of the two fish specie

The present study confirmed the difference of protein content of sample fish species in terms of certain essential nutrients which are available in the local environment (Hernández and Elena, 2012; Onyia et al., 2013). Therefore, protein content in fish muscles showed a significant difference based on the sampled sites. This may be due to the difference in water temperature, stage of life and salinity (Fabiola and Martha, 2012) and the diet of the fish (Zenebe, 2010). Thus, the different mean percentage of protein for muscle and gill tissues of the two fish spe cies are presented in Figure 3.

Crude fat content

The crude fat content of both fish species from all four sites ranged from 10.12 to 16.16 g/100g DW in muscle tissues and 9.07 to 13.95 g/100g DW in gill tissues. This is comparable to the same fish species of African catfish from Nigeria that contained around 12.50g/100g DW (Olayemi et al., 2011).

The fat content of fish from different freshwater bodies of *O. niloticus* and *C. gariepinus* species were determined and significant variation was observed as shown in Table 3.2 and 3.3. Analysis of results showed that *C. gariepinus* contain the highest fat as compared to *O. niloticus* fish species ($P<0.05$ each sites). The fat content of *C. gariepinus* from all sampled sites ranged from 11.41 to 16.16g/100g DW whereas for *O. niloticus* ranged from 10.12 to 13.36 g/100g DW. This result was in agreement with that of Olayemi et al (2011) who reported 12.50% for *C. gariepinus*, Oladipo and Bankole (2013) 11.02% and 10.13% for *C. gariepinus* and *O. niloticus* respectively, and Obaroh et al (2015) who reported values of 16.87% for *C. gariepinus* in DW. Male and female fat content of *O. niloticus* in gill tissues from Alwero, Abay, Hawassa and Ziway freshwater bodies were 11.11% and 12.26%, 11.0% and 10.86%, 9.74% and 9.69%, 9.50% and 9.07% respectively. The fat content in gill tissues of *C. gariepinus* for male and female from the above fresh water bodies were 12.22 and 12.53%, 10.43 and 10.20%, 12.21 and 12.76%, 13.07 and 13.95% DW respectively.

Gill tissues are discarded parts of fish. Fish's gills were analyzed and the fat content was compared and was similar with muscles of both fish species observed in the present study. The mean average value of fat was higher in the muscle (15.87% and 11.69 %) than in the gill (13.51% and 13.09% DW) in *C. gariepinus* and *O.niloticus* respectively. The differences were significant ($P < 0.05$) among the fish species and types of tissues. Similar results were reported in the muscle and gill of *Schilbe mystus* fish species from Nigeria with 15.25% and 12.39% DW recorded (Babangida, 2009). Therefore, the non-edible part of fish body parts (gill) contain apprecia ble amount of fat and this tissues have a potential valorization of sub products f rom fish's muscles processing.

The positive and negative correlation between fat and moisture cont ent in Lakes sources fish as compared to Rivers may be attributed to the ecological needs, metabolism and degree of pollution in water bodies, as well as salinity and temperature of water (Barwick and Maher, 2003). The variation fish length in fat and moisture content may be affected if the fish in state of development of gonad and spawning, the fish expends great deal of energy with fat as its principal sources (Caponio et al., 2004). Analysis result of crude fat content showed a difference among the fish species with *C. gariepinus* having the highest value 4.45% and 4.62% in male and female respectively whereas for *O. niloticus* had the lowest (2.76%) in both sexes.

The differences in fat content between the two species may be due to the feeding style of the species. *C. gariepinus* which mainly depends on small fish, aquatic insects and mollusks and have high fat accumulation in their tissues as compared to Herbivorous fish *O. niloticus* species mostly feed algae plankton (FAO, 2002; Noël et al., 2011; Dsikowitzky et al., 2012).

Sex has no significant ($p>0.05$) effect on fat content, but female fish had higher fat as compared to male. Similar to present study the fat content of female *O. niloticus* was found to have higher fat content compared to male fish (Alemu et al., 2013). The lower moisture content in female fish species can be attributed to muscles of female fish contain more fat and less water than male (Amer et al., 1991).

Ash content

The muscle tissues of the two fish species showed significant difference in their ash content with a range of minimum ash content for male *O.niloticus* (3.01 g/100g) from Lake Ziway and maximum for female *C. gariepinus* (7.41 g/100g) from Abay River. In gill tissues minimum ash content was found in male *O. niloticus* (12.27 g/100g) from Abay River and maximum found in female *C. gariepinus* (22.81 g/100g) from Alwero River. A significant ($p<0.05$) variation in ash content was found between male and female fish. In agreement to the present study, Alemu et al (2013) reported higher ash content in female *O. niloticus* fish than male fish.

The higher ash content in female fish as compared to male fish may be due to increased minerals contents to correct ionic balance during starvation. Between the two fish species significant differences ($p< 0.05$) were found and the ash content of *O.niloticus* (4.06%) is the lowest ash content as compared to *C. gariepinus* species (6.02%). Fish sample collected from river sources were higher in ash content as compared to lake sources of similar fish species. In present study, the higher ash content were found in gill tissues as compared to muscle tissues. This result may be associated with higher amount of skeleton in the tissues and species (Antony, 2013). The ash content in our study was comparable with those reported by Oladipo and Bankole (2013) for *C. gariepinus* (3.85% DW) and by Babangida (2009) for muscle (6.71%) and gill (20.23%) of *Schilbe mystus* species. Emmanuel et al (2011) also reported similar result in *C. gariepinus* (12.79 to 17.64%). On the other hand, the ash content obtained from the present stu dy were lower than those reported by Oladipo and Bankole (2013) for *O. niloticus* (20.02%) and *C.gariepinus* (17.69%).

Carbohydrate content

The content of carbohydrate in the present study was low across all samples of fish species. Carbohydrate values ranged between 1.27 to 13.68 % for *C. gariepinus* and from 7.92 to 25.71% for *O. niloticus*. The results showed difference among the fish species. The highest carbohydrate content was visible in *O. niloticus* (25.71%) from Lake Hawassa and lowest was observed in the muscle of *C. gariepinus* (1.27%) from Alwero River. The content of carbohydrate in gill tissue sampled fish species ranged from 16.91 to 28.16% DW from both rivers.

The results showed variation among the fish tissues. The gill tissues contained significantly higher ($P<0.05$) content of carbohydrate than the muscle tissues. Carbohydrate content also varied significantly between male and female. The value falls within the range of 7.92 to 12.49% DW for male and from 8.49 to 12.57% for female in muscles of *O. niloticus*. For *C. gariepinus* carbohydrate ranged from 1.27 to 13.68% for male and from 2.02 to 11.29% for female found at both Rivers. For Lake source of fish, species the gills have the highest carbohydrate content which ranged from 6.62 to 21.96 %. Least carbohydrate recorded in muscle tissues of the two species ranged from 0.01 to 16.94% DW. Therefore based on fish tissues significantly ($P < 0.05$) differ in carbohydrate contents observed.

The higher carbohydrate in gill tissues than muscles at all four site may be due to relatively higher protein and fat in females than males (Amer et al., 1991). The highest value of carbohydrate in the present study were higher compared to those reported by Olayemi et al (2011) for C . *gariepinus* (1.80) in Nigeria. Tsegay et al (2016) investigated carbohydrate contents in five freshwater fish species and found 2.07% for *C. gariepinus* and 3.76% for *L. intermidius* from lake Hashenge, Ethiopia. A species of *C. gariepinus* recorded 2.07% of carbohydrate in mu scle tissues (Tsegay et al., 2016) in good agreement with the present finding in similar speci es from Abay and Hawassa sites.

Gross energy content

The amount of carbohydrate in fish muscle is generally too small to be of any significance in the diet (Murray and Burt, 1969). The gross energy contents of gill and muscle tissues were found to be in the range of 291.93 to 395.30 Kcal/100g (Table 3.4). The gross energy values reported in this study were relatively high compared to those demonstrated by Alemu et al (2013) for *O. niloticus* (60.2 Kcal/100 g). The average values of gross energy for *O. niloticus*, from four different sampled sites between 316.22 - 339.35 kcal per100g and for *C. gariepinus* which ranged from 291.93 to 395.30 kcal/100g. The caloric value, of *O. niloticus* from four different sampled sites ranged from 316.22 to 339.35 kcal /100g DW, except getting to the species *O. niloticus* from Abay River was 302.83 kcal and similar species from Lake Ziway which presented the maximum 343.44 kcal/100g DW. For *C. gariepinus* species a gross energy values was in the range of 299.60 to 387.27 kcal /100g DW, except for *C. gariepinus* from Abay River which was 291.93 kcal and the same species from Lake Ziway, which presented the maximum of 395.30 kcal/100g. The lat ter being the species with the highest fat content studied. Similar to present study, the caloric value of *Zeminkan* and *Govazym* fish species were 333.79 kcal/100g and 352.68 kcal/100g (Ali and Mozhgan, 2015).

According to Jabeen and Chaudhry (2016) the gross energy values of *C. carpio*, *L. rohita* and *C. mrigala*, obtained from River Indus, Pakistan, ranged from 454 to 481.6 kcal/100g. These values were higher than the present study. Gross energy value of *C. gariepinus* was higher than *O. niloticus* in all sampled sites, except from Abay River. The fish species significantly ($p< 0.05$) affected caloric value. In the present study, the higher gross energy found in male and female *C. gariepinus* 394.36 and 395.32kcal/100g DW from Alwero River are in agreement with that of Onyia et al (2010) who reported similar results from fish species caught in Benue River.

The whole body proximate composition

The result analysis of the whole body of proximate composition of two sampled fish species showed variation among the fish species. There is a significant difference ($P<0.05$) in moisture, crude fat, crude protein and carbohydrate contents between the fish species. The protein content of the *O. niloticus,* which was obtained from the four freshwater bodies were lower than 15-28% recommended by FAO (2012), while that of *C. gariepinus*, only from the two Lake Hawassa and Lake Ziway fall within this range (Table 3.5). Proteins are the building blocks of AAs and certain of them are important in human diet for the maintenance of good living. The fat contents of the two fish species ranged from 2.01 to 4.20 g/100 g WW, this result was lower than 15 -18% recommended by FAO (2012). The values of the fat contents obtained in the two fish species from all sample sites were higher than 0.70±0.02 g/100g WW reported by Alemu et al (2013) but lower than 5.74±0.27 g/100g WW reported by Tsegay et al (2016).

Table 3.4 Mean ± SD gross energy content in kcal /100 g of *O. niloticus* and *C. gariepinus* tissues from four freshwater bodies (DW)

			Gross energy content in kcal /100 g		Mean
Fish source	Sex	Body Parts	*O. niloticus*	*C. gariepinus*	Difference (MD)
Alwero River	Male	Muscle	383.65 ± 0.81^{a}	394.36 ± 0.26^{b}	10.71**
		Gill	253.55 ± 0.36^{f}	250.06 ± 0.29^{f}	3.49*
		Average	318.60±1.16	322.21±1.82	
	Female	Muscle	383.88 ± 0.23^{a}	395.32 ± 0.31^{b}	11.44**
		Gill	264.46 ± 0.14^{e}	256.29 ± 0.38^{f}	8.17**
		Average	324.17±1.43	325.81±1.86	
Abay River	Male	Muscle	351.55 ± 0.34^{b}	334.97 ± 0.32^{c}	16.58**
		Gill	280.88 ± 0.22^{e}	264.23 ± 0.19^{f}	16.65**
		Average	316.22±1.08	299.60±0.82	
	Female	Muscle	350.35 ± 0.62^{b}	337.42 ± 0.26^{c}	12.93**
		Gill	255.3 ± 0.36^{f}	246.44 ± 0.51f	8.86**
		Average	302.83±0.99	291.93±2.01	
Lake Hawassa	Male	Muscle	342.16 ± 0.27^{b}	417.32 ± 0.63^{a}	75.16**
		Gill	304.66 ± 0.53^{d}	336.29 ± 0.71^{c}	31.63**
		Average	323.41±0.39	376.81±0.7	
	Female	Muscle	327.92 ± 0.92^{c}	425.21 ± 0.33^{a}	97.29**
		Gill	307.93 ± 0.25^{d}	344.6 ± 0.30^{c}	36.67**
		Average	317.93±0.33	384.91±1.24	
Lake Ziway	Male	Muscle	377.06 ± 0.37^{a}	423.5 ± 0.24^{a}	46.44**
		Gill	309.82 ± 0.17^{d}	351.03 ± 0.24^{c}	41.21**
		Average	343.44±0.26	387.27±1.49	
	Female	Muscle	377.87 ± 0.66^{a}	427.16 ± 0.18^{a}	49.29**
		Gill	300.83 ± 0.91^{d}	363.43 ± 0.22^{c}	62.60**
		Average	339.35±0.29	395.30±0.98	

At each level of parameter, values with different superscripts in the same column are significantly different ($p < 0.05$). **= Statistically Significant at $P \leq 0.05$ when compared between the two sites; * = Not Statistically Significant at $P \leq 0.05$.

The highest values of the carbohydrate contents (5. 47% WW) obtained in present study from *O. niloticus* species higher than 3.76±0.22 percentage WW reported by Tsegay et al (2016). For *C. gariepinus* from the four sample sources, the higher value (2.44%) was lower than 2.07±0.08 percentage reported in similar species by Tsegay et al (2016).

According to FAO (2012), the composition of a particular species often appears to vary from one fish species to another, and from male to female, but the basic causes of changes in composition are usually variation in the amount and quantity of food that the fish eats and the amount of movement it takes. Proteins are the building blocks of amino acids and certain types of them are important in human diet for the maintenance of good living.
The fat contents of the two fish species ranged from 2.01 to 4.20 g/100g WW, and this result was lower than 15-18% recommended by FAO (2012). The values of the fat contents obtained in the two fish species from all sample sites were higher than 0.70±0.02 g/100g WW reported by Alemu et al (2013) and lower than 5.74±0.27 g/100g WW reported by Tsegay et al (2016). The highest values of carbohydrate content (5.47% WW) obtained in *O. niloticus* species from all sample sites were however higher than 3.76±0.22% WW reported by Tsegay et al (2016).

The proximate composition of the present study vary from one fish species to another and in male and female similar fish species. This may be due to water, temperature difference, stage of life, environmental salinity and (Fabiola & Martha, 2012) the diet of the fish (Zenebe, 2010).

In wet weight (WW) the gross energy contents of *O. niloticus* muscle ranged from 72.78 to 82.16 kcal/100g and for *C. gariepinus* were ranged from 61.99 to 122.06 kcal/100g and these are in agreement with that of Porto et al (2016) which reported caloric value for seven fish species (ranged from 87 to 136 kcal /100g) from Itapecuru River in Brazil. Tsegay et al (2016) reported for the species *C. gariepinus*, in lake Hashenge, Ethiopia, gross energy value of around 133.42 kcal /100g and it is higher than the present study of *C. gariepinus* (121.52 kcal/100g in WW). Higher gross energy value found in female as compared to male fish may be attributed to relatively higher protein and fat in females than males (Amer et al., 1991; Alemu et al., 2013).

The values of crude fat were higher in *C. gariepinus* than *O. niloticus* species collected from all freshwater bodies. This may be due to the diet composition of the fish (Zenebe, 2010). The amount of protein and fat in this study for both whole and fillets of the *C. gariepinus* was found to be higher than previously reported studies for *O. niloticus* species (Alemu et al., 2013), whereas very closer than previously reported study for *O. niloticus* and *B. docmak* (Mekonnen, 2007:Tsegay et al., 2016).

Tsegay et al (2016) reported that *C. gariepinus* contained 5.74% crude fat from Lake Hashenge, Ethiopia. Beyza and Akif (2009) reported that *C. gariepinus* contained 5.02% crude fat and according to Rosa et al (2007), the mean crude fat content of *C. gariepinus* was 5.70%, which was higher than the present study. Samy et al (2012) have reported that feed composition has a major influence on the proximate composition of salmonids. Based on the fat content, both fish species categorized as lean fish as fat contents of these fishes were lower than 5% by weight content (Bennion and Scheule, 2003).

Table 3.5 Mean average of the whole body in g/100g WW of the proximate composition of fishes analyzed.

Parameters	Species	Alwero River		Abay River		Lake Hawassa		Lake Ziway	
		Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Moisture (%)	NT	75.39^{a}	7.16	73.99^{c}	6.44	74.11^{bc}	6.66	73.40^{cd}	35.68
	CF	73.31^{b}	6.95	77.63^{a}	5.23	71.78^{c}	4.02	68.56^{d}	3.65
Protein (%)	NT	10.29^{c}	4.95	10.74^{c}	2.85	12.18^{b}	0.89	13.63^{a}	2.55
	CF	11.14^{c}	5.58	9.17^{d}	3.14	16.1^{b}	2.67	18.51^{a}	2.07
Fat (%)	NT	2.43^{a}	0.62	2.55^{a}	0.73	2.17^{a}	0.34	2.20^{a}	0.58
	CF	2.88^{b}	0.61	2.01^{b}	0.39	3.41^{a}	0.81	4.20^{a}	0.84
Ash (%)	NT	3.09^{a}	9.65	2.33^{c}	5.90	2.74^{b}	7.41	2.73^{b}	8.12
	CF	4.37^{a}	11.11	3.54^{b}	11.14	3.10^{b}	7.36	3.33^{b}	7.18
CHO (%)	NT	4.55^{b}	12.01	5.12^{a}	8.71	5.30^{a}	3.79	3.35^{c}	3.79
	CF	3.65^{a}	14.37	3.58^{a}	4.31	2.19^{b}	5.36	2.10^{b}	3.19

Mean ±SD Value in the same rows with different superscript letters are significantly different ($p<0.05$) (Tukey Post Hoc Test) CF=Cat Fish (*C. gariepinus*), NT Nile Tilapia (*O. niloticus*): Values represent means and SD of duplicate determinations.

Conclusion

This study showed a variation in proximate composition of the fish species. The result obtained indicated that muscle and gill tissues of the investigated freshwater fish species were found to be good in their protein, fat, gross energy and ash composition. The fat content tends to increase with decrease in moisture content. The crude protein and fat content of C. gariepinus from Lakes Zeway and Hawassa are superior. While the two sampled sites, Alwero and Abay fish species are good source of ash and moisture contents. The discarded gills contain appreciable amount of fat and protein contents, may have a potential solution to fill the gap between demand and supply of fish sauces, fish meal, and fish silage and, also specifically a viable alternative to substitute fish oil sources extract from fish muscles in supply of fish oil to aquaculture sectors.

Fish discarded gill tissue can also be used for food product development of various value added products such as protein and fat. These fish protein and fat can be used as a functional ingredient in bakery substitutes, soups and infant formulas. The protein present in the fish gill can be utilized in animal feed in the form of fishmeal and sauce or can be used in the production of fertilizers.

CHAPTER-4

Concentration of essential metals in selected tissues of two fish species from different freshwater ecosystem found in Ethiopia

Abstract

Fish are rich sources of essential micronutrients and can contribute in combating malnutrition caused by micronutrient deficiency. The objective of this study was to determine the essential mineral contents in gill and muscle tissues of *O. niloticus* and *C. gariepinus* freshwater fish species collected from four freshwater bodies (Lake Ziway, Lake Hawassa, Alwareo and Abay Rivers) in Ethiopia. A total of eighty (80) fish samples, 20 samples from each freshwater body were collected from February to October 2017. Atomic Absorption Spectrometry (AAS) was used for mineral analysis. Five mineral elements namely, copper (Cu), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn) were analyzed in muscle and gill tissues of both fish species from each sample sites. Iron and Zinc were the most abundant minerals detected in gill and muscle tissues of both species. The values of Zn obtained was significantly higher ($p<0.05$) in muscle tissue of *O. niloticus* (27.07 mg/kg DW) and *C. gariepinus* (30.76 mg/kg DW) from Lake Ziway. Nickel showed the highest level (1.86 mg/kg) in *C. gariepinus* collected from Ziway Lake while the lowest level was in *O. niloticus* (0.74 mg/kg) from Lake Hawassa. Concentration of Cu in muscle of selected fish species are in the range of 0.74 to 7.92 mg/kg DW, the highest found in muscle tissues of C. gariepinus and the lowest 0.74 mg/kg in O. niloticus muscle. There was significant ($p<0.05$) difference in the content of Cu between fish species in all sample sites. Concentrations of Mn in the fish samples of the four freshwaters varied from 3.20-16.9 mg/kg in muscle and 2.48-69.82 mg/kg in gill tissues on DW ($P<0.05$ at Alwero river). The highest concentrations of metals in the muscles observed in the order of accumulation are Fe > Zn > Mn > Cu > Ni and Fe > Zn > Mn > Ni> Cu for *C. gariepinus* and *O. niloticus* species. The levels of essential elements in muscle tissues of *O. niloticus* and *C. gariepinus* from all sample sites did not exceed the permissible limits set for minerals by ATSDR, FAO and WHO.

Keywords: Lake. River. Muscle. Gill. Mineral content. Freshwater. Fish species

4.1. Introduction

The freshwater fish species are nutritionally precious sources of various mine rals, which the body needs to develop properly and perform its functions. Cu, Zn, Mn, Ni and Fe are essential elements, only required in trace amount and carefully regulated by physiological mechanisms in most organisms (Grusak and Cakmak, 2005). The main roles of these elements can be described as functional and structural because of their important roles in the biological system (Skonberg and Perkins, 2002; USDA, 2003; Fallah et al., 2011). These metals play a catalyzing role in the enzymatic systems by binding their ions to substrates, especially during oxidation-reduction reactions. Structurally, they stand out for their role as integrators of the body's organic compounds (Mendil et al., 2010). The content of these metals in fish body is important with r espect to both nature management and human consumption of fish (Papagiannis et al., 2004).

Minerals are chemical constituents used by the body in many ways; they are yield no energy, but have important roles to play in many activities in the body (Malhot ra, 1998). Every form of living matter requires these inorganic elements or minerals for their normal life processes (Ozcan, 2004). When these elements were not adequately provided to the body, the individual may suffer from mineral deficiency diseases for example goiter, stunted growth anaemia, osteoporosis and genetic disorders (Bhandari and Banjara, 2014; Fumio et al., 2012; Hsieh et al., 2011).

Micronutrient deficiencies are widespread in populations of developing countries and the deficiency in one of the micronutrients has important implications for the pathogenesis of diseases, for morbidity and severity (Hernell and Lönnerdal, 1996; Kawarazuka and Bene, 2011). The WHO reported that about 2 billion of the world's population is suffering from mineral and vitamin deficiencies and the majority of these are in the third world countries (FAO/WHO, 2001). According to CSA and ICF International (2016) among pre-school children, 38% are stunted, 10% are wasted, 29% are underweight and 2% are overweight in Ethiopia. Fishery is an important sector for fight against poverty and nutritional deficiencies (Ozkan, 2005; Tsegay et al., 2016). Fish is commonly situated at the top of the food chain and they can accumulate large amounts of some essential minerals (Al-Yousuf et al., 2000; Jarić et al., 2011). These fish are used as sources of essential nutrients required for fulfilling the gap of lack of mineral and vitamin deficiencies infants and adults diet (Abdullahi et al., 2001).

The minerals composition of *C. gariepinus* and *O. niloticus* tissue have been extensively investigated in various parts of the world (Tariku et al., 2015; Sayad et al., 2016; Tenyang et al., 2016; Tsegay et al., 2016; Abelti, 2017). According to Tsegay et al (2016) the mineral content of different species widely vary from the same water bodies *C. gariepinus* has high content of Fe and Zn than the other four freshwater fish species from Lake Hashenge and this species has a great importance due to the beneficial effect on human health. In view of the importance of various minerals, composition of many freshwater fish species has been partially described. In general the distribution of metals in fish body varies between fish species, depending on sex, age development, environmental factors, temperature and other physiological factors (Lidwin-Kaźmierkiwicz et al., 2009: Yilmaz et al., 2010: Fallah et al., 2011). Findings from temperate lake studies show that the variability of mineral composition greatly affected both within and between species (Effiong and Fakunle, 2013).

There are, therefore, a number of variables that can affect the overall mineral content of fish organ. Nonetheless, there is little information on the effects of environmental factors and fish organ location on the individual elements of *C. gariepinus* and *O. niloticus*. Hence, this study aimed at determining the mineral composition in tissues of selected fish species, both aforementioned factors of gaps need to be investigated. Therefore, in view of this fact, the present researc h was carried out to determine the concentration of essential elements in muscle and gill tissues of selected economic importance freshwater fish species from different ecosystem: Alwero River, Abay River, Lake Hawassa and Lake Ziway found in Ethiopia.

The focus of this particular research was to - (i) identify the species and tissues (gill and muscle) with high minerals content which can be recommended for use in fighting mineral deficiencies in the country (ii) compare with international guideline and other world literatures and (iii) estimating the daily intake of those fish species by the local community from Alwero River, Abay River, Lake Hawassa and Lake Ziway fish collected.

4.2. Material and methods

4.2.1. Sample Preparation

The ash obtained after dry ashing at 525 ^{0}C until the colour of the ash became white/gray and the ash was treated with 7 ml of 6N HCl to wet it completely. 15 ml of 3N HCl was added and the dish was heated on the hot plate until the solution just boils. Th en, it has been cooled and filtered. 10 ml of 3N HCl was add to the dish and heated until the solution just boils. Finally, cooled and filtered into 50ml volumetric flask. Blank digestion was also carried out.

4.2.2. Mineral Determination by Atomic Absor ption Spectrophotometery (AAS)

Procedure: The digested sample was analyzed for mineral contents by AAS (AA-7000 Shimadzu, Japan). Different cathode lamps were used for each mineral. The equipment was run for standard solutions of each mineral before and during determination to check that it was working properly. The concentration of Fe, Zn, Mn, Cu and Ni were determined using the method described by AOAC (2005). The concentrations of minerals recorded in terms of "ppm" were used as milligrams of the minerals per kilogram of dry matter (mg/kg DM) of the minerals by using the formula below:

$$\mathbf{Conc}\left(\frac{\mathbf{mg}}{\mathbf{kg}}\mathbf{DM}\right) = \frac{\text{Conc(ppm)} * \text{Dilution Volume} * \text{Dilution factor}}{\text{Weight of sample (g)} * \text{Dry matter content}}$$

4.3 Statistical Analyses

Data analyses were performed using IBM Statistical 23 software. Significance of differences in the average contents of manganese, zinc, copper, nickel and iron in the muscle and gills of two selected fish species were statistically analyzed using Tukey's test. In this case, a significance of differences in the concentration of metals (between all sampling sites within a single tissue) was calculated by Univariate analysis of variance (ANOVA) and there was significant difference at $P \leq 0.05$.

4.4. Result and Discussion

The two fish species of *C. gariepinus* and *O. niloticus* were investigated in the present study are the most preferred market fish for consumers of rural and urban areas of Ethiopia (Hussien et al., 2010). The nutritional quality of these selected fish species assessed based on their essential mineral profile in muscle and gill tissues as compared to those of the standard set for minerals by FAO (1983), FAO/WHO (2001) and ATSDR (2005).

Concentration of the selected essential elements in the fish samples

Among the studied minerals, Fe and Zn are the most abundant elements in both tissues followed by other metals in all sampled sites. Iron is an essential element for most life on globe, including human and needed for a number of highly multipart processes that constantly take plac e on a molecular level and that is crucial to human life, transportation of oxygen around human body (Gupta, 2014). The concentration of Fe was the highest in the studied tissues of fish sampled. In the fishes analyzed Fe content the maximum in both the ti ssues and the highest value was 122.29 mg/kg DW, observed in gill of *O. niloticus* from Abay River were significantly ($P<0.05$) higher than the rest of three freshwater sampled fish tissues considered in the present study.

Content of Fe was 95.87 mg/kg DW recorded in gill of *C. gariepinus* from Lake Hawassa. The concentration of Fe in *C. gariepinus* ranged between 41.08-89.04 mg/kg DW in muscle tissue while it ranges between 49.77 and 55.35 mg/kg DW from all sample sites of *O. niloticus*. In Lake Hawassa and Abay River, the highest Fe concentration was detected in *C. gariepinus* and *O. niloticus*, respectively. Varying concentration of Fe was observed in all the fish samples but *O. niloticus* from Lake Hawassa has the lowest mean concentration (Table 4.1). In both fish species from all four sampled sites, Fe, Zn and Mn were detected high in the muscle tissues (Figure 4.1 and 4.2).

The rank of mean elements concentration for *C. gariepinus* fish species collected from Alwero River was Fe > Zn > Cu > Mn > Ni in muscle tissue and > Zn > Mn > Cu > Ni in gill tissues respectively. For fish caught from Abay River the concentration of essential minerals were ranked as Fe > Zn > Mn > Cu > Ni and in the gill was Fe > Zn > Mn > Ni > Cu respectively. For fish collected from Lake Hawassa the rank of mean element contents in the muscle was Fe>Zn>Mn>Cu>Ni and whereass in gill tissues were ranked as Fe>Zn>Mn>Ni>Cu. For fish caught from Lake Ziway the ranking in the muscle was Fe>Zn>Mn>Ni>Cu and in gill was Fe>Zn>Mn>Ni>Cu respectively.

The rank of mean elements concentration for *O. niloticus* fish species collected from Alwero River was Fe > Zn > Cu > Mn > Ni in muscle tissues and Mn > Fe > Zn > Cu > Ni in gill tissues respectively. For fish caught from Abay River, the ranking in the muscle was Fe > Zn > Mn > Cu > Ni and in gill the rank was Fe > Zn > Mn > Cu> Ni respectively. For fish collected from Lake Hawassa the rank of mean elements contents in the muscle was Fe > Zn > Mn > Cu > Ni and in gill tissue the rank was Fe > Zn > Mn > Cu > Ni. For fish caught from Lake Ziway the rank in the muscle was Fe > Zn > Mn > Ni > Cu and in gill the rank was Fe > Zn > Mn > Cu > Ni respectively.

The mean Fe concentration range (51.66 to 89.04mg.kg^{-1}DW) in muscle part fishes of this study was higher than values reported for *O. niloticus* (18.7 to 53.9 mg/kg DW) but lower in gill tissues (120 to 196 mg/kg DW) from Lakes Hawassa and Ziway (Kebede and Wondimu, 2004). My result was also higher than other fish species from Lake Hashenge which ranged from 16.04 to 25.89 mg/kg DW (Tsegay et al., 2016) and also similar with Tariku et al (2015) which reported Fe content of *O. niloticus* and *Bagrus docmac* as 105.94 ± 1.35 mg/kg and 115.61 ± 1.68 mg/kg DW collected from Lake Abaya, respectively.

Fe content in gill tissues of the studied fish samples were higher than muscle tissues. Similar to present study, the gill tissues of *Channa punctatus* and *Labeo rohita* species, higher Fe content observed than muscle tissues (Javed and Usmani, 2011). The Fe content of different fish species from different sampled sites were different and the highest Fe was found from Lake Hawassa while the least from Lake Ziway.

The gill shows high concentration of iron than muscle. Consequently, it can be selected as bio amplifier tissue to assess the level of iron in hydrobiology of the lakes. All the mean concentrations of Fe are below the ATSDR, (2004; 2005) limit of 100mg/kg DW for consumable fish muscle, but Fe can be bioaccumulate in human body over years of continues consumption of fish.

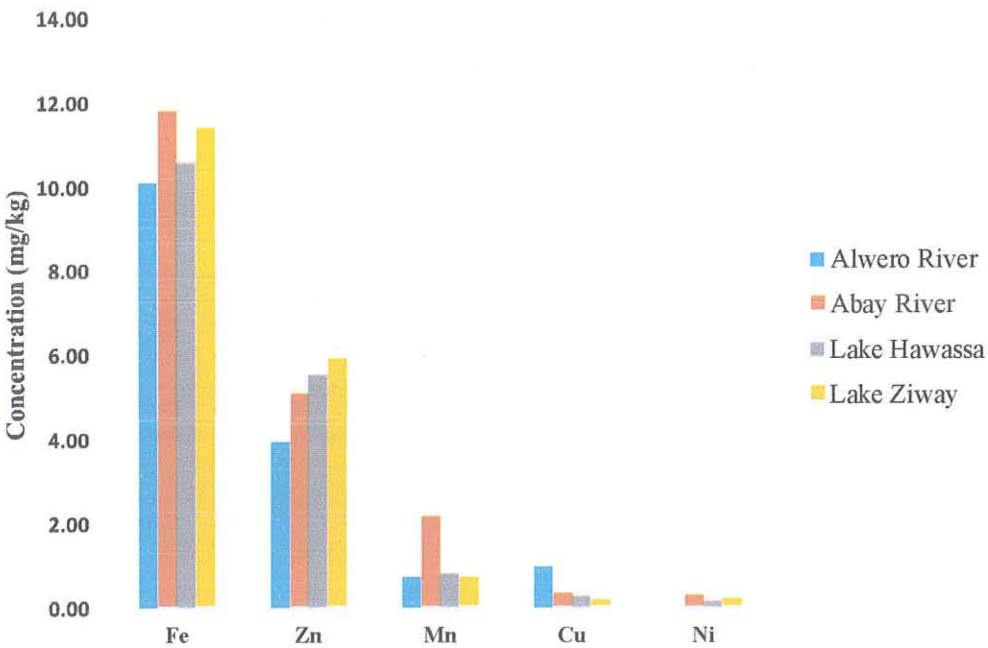

Figure 4.1 Essential minerals concentrations in the muscles of *O. niloticus* species.

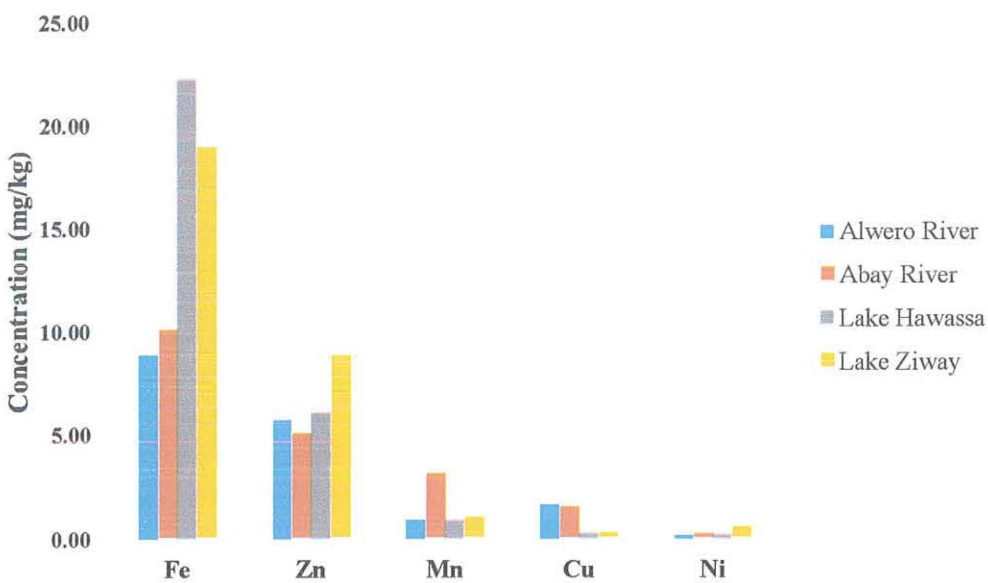

Figure 4.2 Essential minerals concentrations in the muscles of *C. gariepinus* species.

Whereas, the recommended nutrient intake of Fe for female adults between the ages 19 -55 years is 24 mg/day. The muscle tissues of *O. niloticus* and *C. gariepinus* from all sampled sites can provide 37% of the daily requirement for women if 100 g of fish is consumed.

Zinc (Zn) metal was the second highest detected mineral in the present study at all sampled sites. Zinc is involved in most metabolic pathways in humans and Zn deficiency can lead to loss of appetite, growth-retardation, skin change and immunological abnormalities (Malakootian et al., 2011; Özden, 2013). Zn distribution in tissue of *O. niloticus* and *C. gariepinus* from all sampled sites ranged from 20.21–30.76 mg/kg in muscle and 21.47–47.13 mg/kg DW in gill tissues. The highest concentration of Zn in this study was recorded in gill tissue of *O. niloticus* (47.13 mg/kg DW) from Abay river site and the lowest recorde d in muscle tissue of *O. niloticus* (20.21 mg/kg DW) from Alwero river. Fish takes up Zn directly from water, especially via mucous and a gill, as this study confirms, the higher accumulation of Zn in gill tissues as compared with muscles tissues. Therefore, the values of Zn obtained in present study was significantly higher ($p<0.05$) in gill tissues of selected fish species.

The content of Zn in muscle tissue of *C. gariepinus* was higher (30.76 mg/kg) from Lake Ziway, and is significantly higher ($p < 0.05$) than the other water bodies of similar fish sampled. The muscle concentration of Zn in *O. niloticus* was found to be (27.07 mg/kg DW) from Lake Ziway. From the above points, Zn content of the two fish species from similar water body have a significant difference ($P<0.05$). The higher Zn content found in *C. gariepinus* than *O. niloticus* happened in the present study may be due to *C. gariepinus* feeds mainly on fish, aquatic insects and mollusks. Therefore, feeding habits and trophic level of fish species are one factor for differences in mineral content btween *C. gariepinus* and *O. niloticus* (Desta et al., 2006).
The highest Zn concentration in present study was lower than values obtained by Akpanyung et al (2014) reported in *C. nigrodigitatus* species from Ibaka and Ifiayong Rivers, Nigeria. Ermias et al (2015) reported 21.11 mg/kg DW from Lake Hawassa and Tariku et al (2015) reported 14.02 mg/kg in muscle of *O. niloticus*. John et al (2010) found highest Zn content in muscle (10.80 mg/kg) in *C. gariepinus* from Nigeria: Tenyang et al (2016) obtained 1.13mg/kg in Tilapia fish species in Lake Maga, Cameroon. However, the result of Zn content of *O. niloticus* from Lake Hawassa (25.99 mg/kg DW) agreed with the reported content of *O. niloticus* which ranged from 27 to 30.92 mg/kg DW (Ataro et al., 2003).

The FAO (1983) set a limit of daily human intake for Zn of 30 mg/kg, and therefore, Lake Zeway provides 100% daily intake of Zn metal which can satisfy the consumption of the two fish species.

According to Tenyang et al (2016) Zn recommended dietary allowance for adult is 8 to 11 mg per day, if consumed the highest concentration of Zn in both muscles of fish species from the present study can provide 38 % of the requirement in 100 g of fish, making the m a good source of Zn. This species can be beneficial to populations who suffer from malnutrition.

Manganese (Mn) in human nutrition acts as co -enzyme and recognized as essential element for human (Özden et al., 2010). Concentrations of Mn in the fish tissues from all sampled sites varied from 3.20 to 16.9 mg/kg in muscle and also for gill tissue ranged from 2.48 to 69.82 mg/kg DW. The highest Mn content was found in muscle tissues of *C. gariepinus* from Abay River (16.9 mg/kg DW) followed by *O. niloticus* from Abay River (10.11 mg/kg DW). Whilst, *O. niloticus* from Lake Ziway sample had the least Mn content represented 3.20 mg/kg DW followed by *C. gariepinus* from Lake Ziway sample (3.48mg/kg DW).

The Manganese content of the selected fish species varies significantly ($p<0.05$) in both tissues for each sampled sites. Similar to present study, the Mn content of gill tissue of *C. gariepinus* and *Barbus intermedius* fishes are higher than muscles (Asefa and Beranu, 2015).

In the present study, the muscle mean r ange content of Mn (3.20 -10.11 mg/kg DW) was higher than *O. niloticus* (3.20 - 10.11 mg/kg DW) (Kebede and Wondimu, 2004), *C. gariepinus* (1.76 - 2.49 mg/kg DW) (Ataro et al., 2003), 0.10 mg/kg in Tilapia (Abraha et al., 2012) as well as in five fish species (0.16 - 0.52 mg/kg DW) from Lake Hashenge (Tsegay et al., 2016). Values were documented for others fish species of *P. maclareni* and *C. maclareni* in Cameroon (88.0 mg/kg) and (66.8 mg/kg DW), commercial fish species in Littoral region of Cameroon (10.2-46.6 mg/kg DW) (Nkwelle, 2012; Tenyang et al., 2014). While lower than the present study Mn content reported in range muscle of *O. niloticus* (4 - 5 mg/kg) by Tariku et al (2015) and Asefa, (2015) from Abaya Lake and Tendaho Reservoir, respectively. According to Pirestani et al (2009), the recommended dietary allowance for Mn is 3.8 mg per day, but 100g of *C. gariepinus* from Abay can contribute to 45% of the daily requirement. The Mn concentration levels in the two fish species from all sampled sites excced the WHO (1984) limit of Mn (2.50 mg/kg) for fish and fish products, but in WW the content of Mn in present study was within the range of RDA and hence fish tissue can be considered a good source of this mineral.

As Fe and Zn, Cu is also part of many enzymes but occur in very low levels in food. It is also involved in stimulating the body defense system and required for antibody development (Burke and Miller, 2006). The recommended daily requirement of Cu in human nutrition ranges from 1.5-2.5 mg/100g (Widman and Medeiros, 2000).

Concentration of Cu in muscle of selected fish species from this work ranged from 0.74 to 8.28 mg/kg DW. This result was much lower than the recommended daily intake, assuming a single serving of 100g fish per day, but this is not a concern because Cu deficiencies are rare. The highest Cu concentration was 8.28 mg/kg DW in muscle of *C. gariepinus* from Alwero River, while the least 0.74 mg/kg in *O. niloticus* from Lake Ziway. There is significant ($p<0.05$) difference in the concentration of Cu between the two fish species and sampled sites observed in this study.

Copper contents were found to be significantly ($P\leq0.05$) higher in the gill tissue of *O. niloticus* which ranged from (0.66 to10.87 mg/kg DW) than *C. gariepinus* which ranged (0.66 to 8.28 mg/kg DW) at all sampled sites. Cu revealed its maximum accumulation in the gill tissue of *O. niloticus* fish species than muscles tissue, and the present study was comparable to *O. niloticus* (Kebede and Wondimu, 2004; Javed and Usmani, 2011).

The Cu content in muscles of the study fish species (0.74 -8.28 mg/kg DW) was in agreement with that reported (7.10 mg/kg DW) in some common fish species from Cameroon (Tenyang et al., 2016). On the other hand, the Cu content of our sample was lower than the three freshwater fish species (3.0 -11.28 mg/kg DW) in Lake Abaya (Tariku et al., 2015), in muscle for similar fish species from Aligarh, India ranged from 9.0 - 15 mg/kg DW (Javed and Usmani, 2011), but higher than five freshwater fish species (0.7-3.49 mg/kg DW) from Lake Hashenge, Ethiopia (Tsegay et al., 2016). The permissible limits for Cu set by FAO/ WHO (1989b) is 30 ppm, which exceeds the maximum concentration obtained in the present study. According to Widman and Medeiros (2000) the recommended daily requirement of Cu in human nutrition is ranged from 1.5 -2.5 mg.

The Cu content of the two fish species in the present study ranged from (0.27 -1.72 mg/kg WW) which is much lower than the recommended daily intake, assuming a single serving of 100g of fish per day. Cu content of all fish sampled in the present study did not exceed the maximum level (30 mg/kg) FAO/WHO (1984). There were significant differences between fish species at all sample sites. The Cu content obtained in this work is lower compared to those obtained by Ermias et al. (2015) (13.83 mg/kg DW) in muscles of *O. niloticus* from Hawassa Lake. Similar result obtained to the present reported by Ataro et al (2003), Gebrekidan et al (2012), Tsegay et al (2015) and Tariku et al (2015). The Cu content in muscle tissues which ranged (0.74 - 8.28 mg/kg) in the different fish in the present study was far below the recommended daily intake, when assuming around 100g of *C. gariepinus* from Alwero river contribute 11% for daily requirement.

Nickel (Ni) is one of trace elements required in mg/day quantity (Mann and Trustwell, 2007). Ni element is classified in probably as an essential element. Very little information is known about this element. Ni plays some roles in body functions including enzyme functions in very small amounts (Poonkothai and Vijayavathi, 2012). It may be beneficial to activate some enzyme systems, but its toxicity at higher levels is more evident. Ni toxicity is not very common in humans because Ni absorption is very low (Divrikli et al., 2006).

In the present study, lower content of Ni was de tected. *C. gariepinus* contained the highest value (1.86 mg/kg DW) and *O. niloticus* had the lowest (0.74 mg/kg DW). The fish species have different Ni concentrations from different sampled sites. The Ni content in muscle tissues of *O. niloticus* and *C. gariepinus* ranged from 0.74±0.01 - 1.86±0.01 mg/kg DW. In the muscle tissue, Ni showed the highest level (1.86 mg/kg) in *C. gariepinus* caught from Ziway Lake while the lowest level was in *O. niloticus* (0.74 mg/kg) from Lake Hawassa. All the mean concentrations of Ni are below the FAO (1983) limit of 5.0 mg/kg WW for consumable fish, but Ni can be_bioaccumulate in human body over years of continues consumption of fish. Therefore, people should be worried of its bioaccumulation over time as higher concentrations of Ni can elevate incidences of lung and nasal cancer (Duda-Chodak and Blaszczyk, 2008).

The Ni content obtained in the present study was lower as compared to those of Asefa's (2015) reported 2.17 mg/kg found in *C. gariepinus* from Tendaho Reservoir, Tariku et al. (2015) 8.83 mg/kg found in *O. niloticus* from lake Abaya and Kebede and Wondimu, (2004) ranged from 7.8-15.9 mg/kg DW and 34.8-42.4 mg/kg DW respectively found in muscle and gills tissues for *O. niloticus* from Lakes Hawassa and Ziway. However, higher as compared to those obtained by

Abraha et al (2012) and Gebrekidan et al (2012) for *O. niloticus* 0.04 and 0.041 mg/kg from Lake Hashenge, respectively.
The permissible limit set by FAO (1984) for Ni element, the maximum is 5.0 mg/kg WW, this data was higher than the result in the present study, but Ni toxicity in humans is not very common occurrence because the absorption of Ni is very low (Özden, 2008). The mean concentration of Ni found in muscle tissue of all evaluated fish species (0.028 to 0.53 WW) was below the critical limits set by (FAO, 1983 and USFDA, 1993), which may not cause severe health effects on fish consumers. As of WHO (1993), the concentration of Ni recommended for dietary allows around 0.1-0.3 mg/day, if around 500g of muscle of the two fish species from those regions can provide 100% of the requirement in 100g of fish muscles. The total dietary intake of Ni of 0.3mg per day reported by WHO in 1993 (Al bader, 2008). This value is 10 times lower than the maximum value obtained for Ni in the present study, which was (0.028 mg/100g WW).

The present study showed that, regardless of the type of fish tissues, the levels of all minerals were higher in gill tissues as compared to muscles at all sampled sites. Unfortunately, gill tissues discarded parts of fishes; however, the muscle tissue more concerned because WHO/FAO mostly recommended dietary allowance in fish tissues mostly based on muscle tissues. Our study showed that mineral content in muscle tissue of *O. niloticus* was lower than *C. gariepinus* obtained at all sampled sites.

The statistical analysis of the minerals data showed significant differences ($p<0.05$) between these two species in each sites. The muscle tissue of *C. gariepinus* from all sites the maximum minerals content for each element was as follows: Fe (89.04 mg/kg); Ni (1.86 mg/kg); Zn (30.76 mg/kg); Cu (8.28 mg/kg) and Mn (16.9 mg/kg DW). This result was agreed with Asefa and Beranu (2015) reported in investigated three fish species, the highest levels of major metals obtained in *C. gariepinus* as compared to other two fish species of *O niloticus* and *B. intermedius* from Tendaho Reservoir. This may be due to carnivores feeding mainly on fish, aquatic insects and mollusks. This species obtained at the top trophic level and could gain more minerals from water, air and the Mollusca (includes snails, slugs and mussels) (Desta et al., 2007). In the present study, *C. gariepinus* species contained higher minerals accumulation in their muscles as compared to *O. niloticus*.

Table 4.1 Mineral concentrations (mean ± SD, mg/kg DW) *O. niloticus* and *C. gariepinus* fish species muscle and gill tissues in four different ecosystem of water bodies

Source of Water bodies	Fish species	Tissue	Cu	Fe	Zn	Ni	Mn
Alwero River	*O. niloticus*	Muscle	5.05 ± 0.01^{d}	51.66 ± 0.00^{j}	20.21 ± 0.01^{l}	0.08 ± 0.00^{h}	3.83 ± 0.01^{g}
		Gill	10.87 ± 0.01^{a}	58.38 ± 0.00^{h}	41.11 ± 0.00^{c}	1.87 ± 0.00^{b}	69.82 ± 0.00^{a}
		average	7.96 ± 4.13^{c}	55.02 ± 4.76^{i}	30.67 ± 14.77^{f}	0.98 ± 1.27^{f}	36.82 ± 46.67^{b}
	C. gariepinus	Muscle	7.92 ± 0.01^{c}	41.08 ± 0.01^{k}	26.48 ± 0.01^{i}	0.99 ± 0.01^{f}	4.45 ± 0.01^{g}
		Gill	2.04 ± 0.01^{fg}	80.69 ± 0.01^{f}	$43.74\pm0.01b^{c}$	1.55 ± 0.00^{c}	28.94 ± 0.00^{c}
		average	4.98 ± 4.16^{d}	60.89 ± 28.01^{h}	35.11 ± 12.2^{d}	1.27 ± 0.4^{de}	16.70 ± 17.32^{d}
Abay River	*O. niloticus*	Muscle	1.57 ± 0.01^{g}	55.35 ± 0.01^{i}	23.78 ± 0.01^{k}	1.33 ± 0.01^{d}	10.11 ± 0.01^{e}
		Gill	4.39 ± 0.01^{e}	122.29 ± 0.01^{a}	47.13 ± 0.02^{a}	1.99 ± 0.00^{a}	37.85 ± 0.01^{b}
		average	2.98 ± 1.99^{f}	88.82 ± 87.8^{de}	35.46 ± 16.52^{d}	1.66 ± 0.47^{bc}	23.98 ± 19.62^{c}
	C. gariepinus	Muscle	8.28 ± 0.01^{b}	54.30 ± 0.00^{i}	27.07 ± 0.01^{i}	1.07 ± 0.01^{e}	16.90 ± 0.01^{d}
		Gill	0.75 ± 0.03^{j}	33.54 ± 0.06^{l}	21.47 ± 0.11^{l}	0.92 ± 0.04^{f}	8.41 ± 0.03^{f}
		average	4.52 ± 5.33^{e}	43.92 ± 14.68^{k}	24.27 ± 3.96^{k}	1.00 ± 0.11^{ef}	12.66 ± 6.0^{df}
Lake Hawassa	*O. niloticus*	Muscle	1.31 ± 0.03^{h}	49.77 ± 0.04^{jk}	25.99 ± 0.01^{j}	0.74 ± 0.01^{g}	3.85 ± 0.01^{g}
		Gill	1.32 ± 0.01^{h}	60.37 ± 0.01^{gh}	44.49 ± 0.00^{b}	0.66 ± 0.00^{gh}	2.48 ± 0.01^{j}
		average	1.32 ± 0.01^{h}	55.07 ± 7.50^{i}	35.24 ± 13.08^{d}	0.70 ± 0.06^{g}	3.17 ± 0.97^{h}
	C. gariepinus	Muscle	1.07 ± 0.01^{i}	89.04 ± 0.01^{d}	24.39 ± 0.01^{k}	$0.83\pm0.01f$	3.55 ± 0.01^{h}
		Gill	0.66 ± 0.01^{j}	95.87 ± 0.01^{b}	25.14 ± 0.01^{jk}	1.15 ± 0.01^{e}	3.71 ± 0.01^{h}
		average	0.87 ± 0.29^{j}	92.46 ± 4.83^{c}	24.77 ± 0.53^{k}	0.99 ± 0.23^{ef}	3.63 ± 0.11^{g}
Lake Ziway	*O. niloticus*	Muscle	0.74 ± 0.01^{j}	52.33 ± 0.01^{j}	27.07 ± 0.00^{i}	0.82 ± 0.00^{f}	3.20 ± 0.01^{hi}
		Gill	1.54 ± 0.01^{g}	62.02 ± 0.01^{h}	37.67 ± 0.01^{cd}	0.65 ± 0.00^{gh}	3.00 ± 0.01^{hi}
		average	1.14 ± 0.57^{i}	57.18 ± 6.85^{h}	32.37 ± 7.5^{e}	0.74 ± 0.12^{g}	3.10 ± 0.14^{h}
	C. gariepinus	Muscle	0.89 ± 0.01^{j}	66.23 ± 0.02^{gh}	30.76 ± 0.01^{f}	1.86 ± 0.01^{b}	3.48 ± 0.01^{h}
		Gill	1.30 ± 0.04^{h}	90.46 ± 0.01^{d}	28.39 ± 0.01^{h}	1.55 ± 0.01^{c}	4.47 ± 0.01^{g}
		average	1.10 ± 0.29^{i}	78.35 ± 17.13^{g}	29.58 ± 1.68^{g}	1.71 ± 0.22^{bc}	3.98 ± 0.70^{gh}

Values Means, at each column with the same superscript are not significantly different at $p<0.05$

Daily Consumption

Concentrations of essential elements in fishes and comparison with international diet ary standards and guidelines (Wet weight muscle)

The concentrations of metals in fish were reported on dry basis (DW) as well as wet weight (WW) in the literature reported by Cohen et al (2001). Based on WW rather than DW, minerals documents in fish muscle and comparison made with international organization literature reports.
To evaluate the essential mineral content of selected freshwater fish species found in fish samples from Alwero River, Abay River, Lakes Hawassa and Ziway and identify the species and tissues with high mineral content which can be recommended for use in combating miner al deficiencies in the country, a comparison is made with reference values for fish muscles (Table 4.2). The validity of such comparison may appear questionable. However, in the absence of national standards and guidelines, a comparison of this kind may pr ovide preliminary idea about minerals profile of Ethiopian freshwater fish species.

European Union (EU, 2006) has summarized dietary standards and guidelines. The guideline sets limits for Cu, Mn, Ni and Zn as 0.5, 0.52, 0.5 and 50 mg/kg WW, respectively. The maximum permissible limits proposed by WHO/FAO (1989a) for Cu 30mg/kg, for Mn 9 mg/kg, for Ni 80 mg/kg, for Fe 100mg/kg and for Zn 40 mg/kg WW.

Iron contents in the two sp ecies in four sampled sites in the range of 8.93 - 22.26 mg/kg WW, and significantly higher ($p \leq 0.05$) in in muscle of *C. gariepinus* from Lake Hawassa. The highest concentration was 22.26 mg/kg recorded in muscle of *C. gariepinus* from Lake Hawassa, while the lowest value of 8.93 mg/kg was recorded in *C. gariepinus* from Alwero River. The highest concentration of Fe found in the present study were far below the maximum permissible (MPL) 100 mg/kg of Fe established by (ATSDR, 2004; 2005).

Manganese was detected in the entire samples studied. The lowest concentration was (0.70 mg/kg WW) detected in muscle of *O. niloticus* from Lake Ziway, while the highest concentration was (3.13 mg/kg WW) detected in muscle of *C. gariepinus* from Abay River. The concentrations of

Mn in fish sampled from Alwero River, Lakes Hawassa and Ziway sites are below the MPL of Mn 1.00 mg/kg established by ATSDR, (2004; 2005).

Copper is an essential part of several enzymes and it is necessary for the synthesis of hemoglobin (Stern, 2010). The present study showed the highest Cu content was (1.72 m g/kg WW) in the muscle of *C. gariepinus* from Alwero River, while the lowest level was (0.23 mg/kg WW) detected in the muscle of *C. gariepinus* from Lake Ziway. However, the highest value of Cu in present works was below the MPL established by ATSDR and FAO guideline 30 mg/kg. The present study concentrations of Cu in all sampled sites of the two species found below the recommended guideline of FAO and ATSDR (FAO, 1983: ATSDR, 2005).

Zinc toxicity is rare but concentrated in water up to 40 mg/kg, may induce toxicity, characterized by symptoms of irritability, muscular stiffness and pain (Iweala et al., 2014). In the present study , the highest concentration of Zn was (8.79 mg/kg WW) found in the muscle of *C. gariepinus* from Lake Ziway, while the lowest was (3.96 mg/kg WW) in the muscle of *O. niloticus* from Alwero River. Therefore the concentration of Zn varied among fish species observed in the present work, this may occur due to the chemical form of Zn in the diets and other components such as Calcium, Phosphorus and Phytic acid may affect the fish tissues (FAO, 2009). According to FAO (1983), guideline the maximum concentration of Zn is 30 mg/kg. The concentrations of Zn in present study in all the sampled sites were below the FAO (1983) food guideline.

The major source of Ni for humans is food and uptake from natural sources, trace amounts of Ni act as activator of some enzyme systems (Nas-NRC, 1975). The upper tolerable intake level of Ni for children (1-3 years old) and males/females (19-70 years old) are 7 mg/day and 40 mg/day, respectively (National Academy of Medicine, 2001). In addition, the WHO recommends 0.1 -0.3 mg of this element for daily intake (WHO, 1996). The Ni concentration detected in the present study were ranged from 0.02 to 0.53 mg/kg WW. The highest concentration of Ni was (0.53 mg/kg WW) detected in the muscle of *C. gariepinus* from Lake Ziway, while the lowest concentration was (0.02 mg/kg WW) in the muscle of *O. niloticus* from Alwero River. The maximum permissible limit of Ni is 1.0 mg/kg WW established by ATSDR and FAO/WHO. Therefore in the present study Ni was found lower than the maximum permissible limits set by (FAO/WHO, 2009; ATSDR, 2004; 2005).

Table 4.2 shows a comparison between the concentrations of five minerals in the pres ent study with concentrations in maximum permissible limits set by different international organization. The present study result varied between all sampled sites; this may be due to differences natu ral environment of collected fish sampled. This result approve by Bahnasawy et al (2009) reported mineral content of fish in tissues differ within metal concentrations and chemical, physical characteristics of water bodies from which fish were sampled.

The concentrations of minerals in two fish species from all sampled sites were acceptable by the international guideline limits. But, the highest Cu metal detected in the present work was lower as compared to those proposed by US EPA (1983), FAO (1983), WHO/FAO. (1989b), CCC (1994) and MAFF (2000) for fish muscles in WW, while higher than maximum permissible limits set by FSANZ (2002), EU (2006) and FAO/WHO (2011) (Table 4.2).

Table 4.2 Mineral concentrations the two fish species in muscle tissue (mg/kg WW)

Fish name and sources	Cu	Fe	Zn	Ni	Mn
O. niloticus fromAlwero River	0.99	10.13	3.96	0.02	0.75
O. niloticus from Abay River	0.33	11.78	5.06	0.28	2.15
O. niloticus from Lake Hawassa	0.28	10.59	5.53	0.16	0.82
O. niloticus from Lake Ziway	0.16	11.38	5.88	0.18	0.70
C. gariepinus from Alwero River	1.72	8.93	5.76	0.22	0.97
C. gariepinus from Abay River	1.53	10.06	5.01	0.20	3.13
C. gariepinus from Lake Hawassa	0.27	22.26	6.10	0.21	0.89
C. gariepinus from Lake Ziway	0.23	18.92	8.79	0.53	0.99

In general the concentration of those essential minerals in muscles of selected fish species from the present study were far below the maximum permissible limits set by different international guidelines, the analyzed fish species may be considered as more benefited for human consumption (Table 4.2). The maximum concentration of Fe detected in the present work was lower than that proposed by WHO/FAO. (1989b) but higher than FAO/WHO (2011) for Fe in fish muscles. For Zn the maximum concentration was lower than those proposed by USEPA (1983), FAO (1983), WHO/FAO (1989b), CCC (1994), MAFF (2000) and FAO/WHO (2011) for the permissible limits of Zn in fish muscles, whereas lower than FSANZ (2002) proposed.

Daily consumption safety

According to FAO (2011), the average quantity of fish consumed per a person (assuming a 70-kg person) per day in Ethiopia is 27.49 g/day. Multiplying this value by the average concentration of each metal (Cu, Zn, Mn, Ni and Fe) analyzed in fish sampled, the average daily intake (consumption) of metals from fish sampled can be estimated.

The daily consumption of Cu, Fe, Zn, Ni and Mn in the fishes of this study were ranged from 0.006 to 0.047, 0.25 to 0.61, 0.11 to 0.24, 0.0006 to 0.0145 and 0.019 to 0.086 mg per day, respectively (Table 4.3). The average daily intake of minerals through fish consumption can be ordered as follows: Fe>Zn> Mn>Cu>Ni.

Table 4.3.The estimated daily intake (mg/day) of minerals by humans in Alwero River, Abay River Lake Hawassa and Lake Ziway from fish muscles

Fish name and sources	Cu	Fe	Zn	Ni	Mn
O. niloticus Alwero River	0.027	0.28	0.11	0.0006	0.021
O. niloticus Abay River	0.009	0.32	0.14	0.0077	0.059
O. niloticus Lake Hawassa	0.008	0.29	0.15	0.0044	0.022
O. niloticus Lake Ziway	0.004	0.31	0.16	0.0049	0.019
C. gariepinus Alwero River	0.047	0.25	0.16	0.0060	0.027
C. gariepinus Abay River	0.042	0.28	0.14	0.0055	0.086
C. gariepinus Lake Hawassa	0.007	0.61	0.17	0.0058	0.024
C. gariepinus Lake Ziway	0.006	0.52	0.24	0.0145	0.027

The estimated maximum total dietary intakes of the five minerals in these fishes from present study were 0.047, 0.61, 0.24, 0.0145 and 0.086 mg per day for Cu, Fe, Zn, Ni and Mn, respectively (Table 4.3). For Cu, the maximum value of consumption from fish per day obtained from the present study was 0.047 mg which was far below the maximum acceptable daily intake value proposed by WHO in 1996, which limited it by 0.25 mg/day for adult (WHO 1996). Based on the results from this work, with assumption that 100 g of fish muscle is consumed, *C. gariepinus* from Alwero River can provide over 50% of recommended daily intake of Cu.

Maximum daily intake of Zn, in the present study was 0.24 mg per day; it was far below as comparing with international standards, 15 mg/day for adult men and 12mg/day for adult women IPCS (1991) and 20 mg/day for adults JECFA (1982). It can be concluded that there was no health risk by consumption of around 500g of Zn from the present study. Zn is usually taken to stimulate the immune system and human growth. It is a component of many metallo -enzymes, important for gene expression (Wood, 2000).

For Fe, the maximum value of consumption from fish per day obtained from the present study was 0.61 mg which was far below the maximum acceptable daily intake value proposed by (EFSA, 2009) and (FAO/WHO, 2001) which sets limited to 10 mg/day for adult men and 15 mg/day for adult female (EFSA, 2009). For male and female 9-50 years old intakes are ranged from 8 to18 mg per day respectively (FAO/WHO, 2001). Therefore, from the present study it be concluded that, the highest Fe content in *C. gariepinus* muscle tissue was (2.44g/day DW) and by consumption of this species, around 500g can contribute at 81 % of the daily requirement.

The recommended daily requirement of Cu, Fe, Zn, Ni and Mn in human nutrition were set to 2-3 mg, 9-17 mg, 5-20 mg, 0.15-7 mg and 2-9 mg per day respectively (WHO, 1981; JECFA1982; WHO, 1985; NAM, 2001; EFSA, 2009). The concentration of Cu, Fe, Zn, Ni and Mn in muscles of selected fish species from all sampled sites in this study were lower as compared to the international recommended daily intake, as assuming a single serving of 100g of fish per day. In the present study fish species the highest content of minerals could be provide 13% for Cu, 8% for Fe, 5% for Zn, 10% for Ni and 4% for Mn requirement for men and women 70 kg body weight if 100 g of fish is consumed. For Ni and Mn, the maximum daily intake values obtained from the present study were 0.0145 and 0.086 mg per day, respectively. These values were far below the maximum TDI values set by EU and WHO, set as 0.5 and 4 mg per day, respectively (WHO, 1985; EU, 2006). Therefore, the daily intake of Fe, Zn, Cu, Mn and Ni for regular consumption (more than 500g per day) of the two fish species from different water bodies in the present study may meet the daily requirement proposed by different international organization and also may not has any hazardous effect on human health.

RDA (Recommended Daily Allowed)

To ensure the complete daily nutritional needs of a person, a balanced diet is important and this has to bring the right amount of nutrients and energy for health and wellbeing. It is important to set nutritional standards that refer to the nutrient amount to be taken by a human being to meet daily nutritional needs (FAO/WHO, 2001)

The nutritional standard that we took into consideration in this study is the RDA (Recommended Daily Allowed) which corresponds to the average daily intake level adequate to meet the needs of nearly all the healthy persons in a particular life stage and gender (Table. 4.4). Nutritional standards are intended to protect the entire population from the risk of nutritional deficiencies, to provide the basis for assessing the nutritional adequacy of the average diet of a population, for food education and food labelling. In this study we take into account the daily intake of mineral salts of an average human being (male/female, 30-55 years) in good health and average weight (70kg).

The average concentration for each mineral element has been calculate d in 150g of fresh fish, which is the average weight of a fish fillet for a normal meal. The table also shows the recommended daily values (RDA) for each mineral element expressed by mg/diet. Clearly through the values shown on table 4.5 it was then possible to talk about the nutritional value of *O. niloticus* and *C. gariepinus* and also to see in percentage how much these values meet the recommended daily nutritional needs (RDA).

Table 4.4. Recommended Daily Allowance (RDA) of some essential elements

Element	Unit of measure	Men 30 – 55 years	Women 30 – 55 years	Reference
Fe	mg/diet	8 – 10	18 - 20	(SCF 1993)
Zn	mg/diet	15	12	(Van Dokkum 1995)
Mn	mg/diet	1-10	1-10	(WHO 1991)
Cu	mg/diet	1 – 2.3	0.9 – 1.8	(Van Dokkum 1995)

Taking into account the data in table 4.5 it has been calculated the daily intake percentage of Fe, Zn, Cu and Mn in a portion of fish fillet (150g). Table. 4.6 and 4.7 shows the values of essential minerals concentrations in 150g of edible portion with the related value of RDA required to meet the needs of a middle age man/woman (35-55 of age). Through this set of data it was possible to identify how much in percentage these fish products, considered here, meet the daily needs required to maintain a good health.

Table 4.5 Daily intake % (mg/150g WW) of some minerals in fish muscle

Fish name and sources	Cu	Fe	Zn	Ni	Mn
O. niloticus Alwero River	0.15	1.52	0.59	0.003	0.11
O. niloticus Abay River	0.05	1.77	0.76	0.04	0.32
O. niloticus Lake Hawassa	0.04	1.59	0.83	0.02	0.12
O. niloticus Lake Ziway	0.02	1.71	0.88	0.03	0.11
C. gariepinus Alwero River	0.26	1.34	0.86	0.03	0.15
C. gariepinus Abay River	0.23	1.51	0.75	0.03	0.47
C. gariepinus Lake Hawassa	0.04	3.34	0.92	0.03	0.13
C. gariepinus Lake Ziway	0.03	2.84	1.32	0.08	0.15

Table 4.6. Daily percentage intake (for men and women 30-55 yrs) of Fe, Cu, Zn and Mn in *O. niloticus*

Minerals	**Daily intake % of *O. niloticus* contribution**							
	Alwero River		Abay River		Lake Hawassa		Lake Ziway	
	Men	Women	Men	Women	Men	Women	Men	Women
Fe	15.2	7.6	17.7	8.9	15.9	8	17.1	8.6
Zn	3.9	4.9	5.1	6.3	5.5	6.9	5.9	7.3
Mn	1.1	1.1	3.2	3.2	1.2	1.2	1.1	1.1
Cu	6.5	8.3	2.2	2.8	1.7	2.2	0.9	1.1

Table 4.7. Daily percentage intake (for men and women 30-55 yrs) of Fe, Cu, Zn and Mn in *C. gariepinus*

Minerals	**Daily intake % of *C. gariepinus***							
	Alwero River		Abay River		Lake Hawassa		Lake Ziway	
	Men	Women	Men	Women	Men	Women	Men	Women
Fe	13.4	6.7	15.1	7.6	33.4	16.7	28.4	14.2
Zn	5.7	7.2	5	6.3	6.1	7.7	8.8	11
Mn	1.5	1.5	4.7	4.7	1.3	1.3	1.5	1.5
Cu	11.3	14.4	10	12.8	1.7	2.2	1.3	1.7

The table 4.6 and 4.7 confirms what we have said previously, iron and copper are the elements that give a greater contribution to meet the daily nutritional needs (respectively for Fe: 33.4% and Cu: 14.4% in *C. gariepinus* from L. Hawassa, and Alwero River ; while for Cu and Fe

8.3 and 17.7% in *O. niloticus* from Lake Ziway and Alwero River). Following in descending order was recorded Zn (respectively 11% in *C. gariepinus from Lake Ziway.* and 7.3 % in *O. niloticus* from Lake Ziway), and finally Mn (4.7 % in *C. gariepinus and 3.2 % in O. niloticus* from Abay River.

Considering the values of Fe the percentages obtained seem to be too high compared to what has been obtained in other sources, stating that an average serving of fish provides an average daily intake of about 9.2 % or a little bit more (Tsegay et al. 2016). These results suggest the possible geographical and environment effect the results increasing to a great extent the recorded values (Kebede and Wondimu, 2004; Dsikowitzky et al., 2012; Ermias et al., 2015).

Conclusion

Significant variation was observed among the fish species and tissues in their mineral content in all sample sites. The fish species included in this study are rich in essential minerals and can play a great role in combating micronutrient malnutrition. Fe, Zn and Cu are the most abundant elements in both tissues followed by other metals in all sampled sites. The rank of mean elements concentration for both species were Fe > Zn > Cu > Mn > Ni. Among fish species, C. gariepinus from L. Ziway and L. Hawassa showed high Zn and Fe content respectively. Hence,150 gram of fish muscles are consumed, the species of C. gariepinuscanprovideover15%ofthe daily recommended of Cu, Fe, Zn and Mn ,if fish consumed from Abay River, Lake Hawassa, Lake Ziway and Alwero River ,respectively.

CHAPTER -5

Determination of Selected Heavy Metals and Human Health Risk Assessment in Fishes from different freshwater ecosystem found in Ethiopia

Abstract

The objective of this study was to assess the bioaccumulation of heavy metals (Cr, As, B, Hg, Cd, Pb, Sn and Co) in the muscle and gill of *O. niloticus* and *C. gariepinus* fish species collected from four different regions of freshwater bodies in Ethiopia in order to determine the value daily intake of heavy metals by consumption of fish and human health risk assessment. In the present study, the highest concentrations of Co (1.16 mg/kg), Cd (0.58 mg/kg) and Pb (1.91mg/kg DW) were observed in the gill of *O. niloticus*. The highest concentrations of Sn (222.89 mg/kg) and Hg (14.52 mg/kg DW) were observed in gills and muscle of *O. niloticus*, respectively, while the highest concentrations of Cr (4.95 mg/kg) and As (30.92 mg/kg DW) were observed in gills and muscle of *C. gariepinus*, respectively. Freshwater *O. niloticus* from Lake Ziway different from the other three freshwater bodies based on Sn metal concentrations in gills, while *C. gariepinus* from Abay River different from the other three sampled sites based on A s metal concentrations in muscles. This indicates that the metal levels detected in tissues seem to reflect the pollution level of sediment and planktons rather than the usual pollution state of the water. The most correlations between element accumulation and fish sources and tissues from Lake Ziway were recorded in the species of *O. niloticus*. Although the maximum levels of Hg were above the permissible tolerable limits set by FAO (1993) (i.e >0.05 mg/kg WW), the estimated daily intakes of all metals were below the ADI and RfD set by international guideline. The THQ and HI values were (<1) for all examined metals in both species in all sampled sites. There are no risk for consumptions of *O. niloticus* and *C. gariepinus* fillets in reasonable amounts for consumers. Therefor muscles of studied both fish species is safe for utilization in human diet from those regions in Ethiopia.

Keywords: Heavy Metals. Fish. Lake Hawassa. Lake Ziway. River Alwero. River Abay

5.1. Introduction

Environmental pollution is a universal problem. Heavy metals are one of the most vital pollutants since industries have a continuous raised emission of pollutants and therefore, contamination of aquatic ecosystems, has been receiving increased worldwide attention over the last few decades. (Dawoud et al., 2009). The biological accumulation of toxic heavy metals in existing organisms are transfered from one trophic level to another in food chain. Fish is one of the existing organism in aquatic ecosystem and consumption of fish from contaminated water bodies can be danger for human health (Moiseenko et al., 2001; Castro et al., 2008). Hereafter, it is important to detect the level of heavy metal concentration in fish organs in order to guarantee that it does n ot expose any hazard to the human and maintain contents under permissible level (Sivaperumal et al., 2007; Uysal et al., 2008). Various species of fish are mostly used as bio -indicators of heavy metals contamination (Svobodova et al., 2004). Fishes are the most important organisms in the aquatic food chain, which are sensitive to heavy metals contamination and destroyed the aquatic life due to the impact of human activities that discharge their waste such as heavy metals into the aquatic ecosystems (Rashed, 2001). As result of this, fish species are not only indicates the pollution status of aquatic ecosystem but has a significant impact on the food web due to fish is one of the main sources of long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) and protein-enriched food all over the world (Mansour and Sidky, 2002; Chi et al., 2007; Racine and Deckelbaum, 2007). Heavy metals are natural elements in the environment, and exceeding in certain amount from the natural occurring by human activities, such as many industrial and agricultural activities (Bosch et al., 2015). The same fish species from different regions vary with the fish size, weight and nutritional values but generally, there are differences in disposal of untreated municipal, agricultural and industrial wastes in to lakes and rivers freshwater bodies and obtain in fish species muscles, which agree with the food safety and quality control standards (Marushka, 2018). It is important to note that not all metals are hazardous and toxic to humans and now, fish meat marketing are widely distributed in the Ethiopian lake and river side communities. With changing environmental conditions under increasing anthropogenic influences, the nature of the Ethiopian freshwater bodies are also changing. Environmental contaminants from municipal, agricultural and industrial sources may enter the food chain, accumulate in organisms and affect their survival. Hence, the abundance and quality of commercially important fish species, an important ecosystem service of the freshwater bodies, may be at risk (Hussien et al., 2010 Asmare et al., 2016; Meko et al., 2017). Furthermore, consumption of contaminated fish is one of the main exposure routes to toxic metals for humans (Castro-González and Méndez-Armenta, 2008).

Alwero River, Abay River at mouth, Lakes Hawassa and Ziway are Ethiopian freshwater bodies, which are affected by agricultural fertilizers, pesticides, and anthropogenic pollution, hence risks the life of fish species as well as human being (Hussien et al., 2010 ; Margareta 2015; Kiflom and Tarekegn, 2015). This information is desirable because Rivers and Lakes, source of fish species are important bio indicators of toxic heavy metal contamination. Several researchers have evaluated the fish's quality of muscle analysis, because higher and relatively stable concentrations would be obtained in fish samples. These, fishes have been proposed as bio indicators to monitor a variety of contaminants in the freshwater ecosystem (Kebede and Wondimu, 2004; Dsikowitzky et al., 2012; Ermias et al., 2015).

Heavy metals, which incidentally spread because of human day to day activities, are harmful even in small quantity. These heavy metals are non-biodegradable and may result to world environmental problems. Even though heavy metals such as (Ni, Sn and Cr) are vital components for many genetic activities in the human body, high concentration of them may result in health difficulties to human. Similarly, Cd, Hg, As, and Pb have no nutritional importance to human even at small level, they are toxic metals may be not lethal to fish species but the concentration of such metals in their tissues creates hazards when used as food for human consumption (Saidu et al., 2018).

In recent years risk assessment of heavy metals for the lake and river side communities have been of great importance, because of high protein content and minerals are essential for human diet, but the contaminated fish represent serious food safety concern across the globe (Tuzen, 2009). Sometimes the contaminants exceed the legal limits set by EU (2006), FAO/WHO (2010), regulations for food; they did not always represent risk to human health (Copat et al., 2012; Alipour et al., 2014).

Usually, health benefits and fish consumption are informed through different international organizations but rarely is there insight into potential health risks (Antonijevic et al., 2007; Pieniak et al., 2010). Consequently, knowledge of toxic heavy concentrations in fish tissues are important with respect to human health (Ebrahimpour and Mushrifah, 2010). There are a number of toxic heavy metals that can affect the overall nutritional quality of tissues of different fish species.

However, there is little information on the effect of various species, tissues and ecosystems on the individual toxic heavy metals of Nile Tilapia (*O. niloticus*) and African Cat fish (*C.gariepinus*) fish tissues. To determine the overall toxic heavy metal accumulation of the two fish species tissues, all the aforementioned factors of variations need to be investigated. Therefore, in view of this fact, this study: (1) investigates eight heavy metal (Ni, Cr, Pb, Hg, Sn, B, As and Cd) concentrations in edible and non-edible parts of *O. niloticus* and *C. gariepinus* from Alwero River, Abay River, Lakes Hawassa and Ziway, (2) find out whether the fish species from the sources have elevated concentrations of these heavy metals in their tissues that could causes them dangerous for human consumption, (3) examine if there were differences in tissue accumulation in two fish species (4) estimate the daily intake, comparing it with ADI and calculating the HQ to assess the human health risk from consumption of these selected fish species and comparing the results generated in the present study with the permissible tolerable daily intake (PTDI) of these heavy metals proposed by European Union EU FAO and WHO.

5.2. Material and Methods

5.2.1. Reagents

All reagents and chemicals used in the study were of analytical grade. De -ionized water was used to prepare all aqueous solutions. All plastic and glassware used were rinsed and soaked in 10% (v/v) HNO_3 overnight. They were rinsed with de-ionized water and dried prior using. All acids (Nitric, HNO_3; Sulfuric, H_2SO_4 and Hydrochloric, HCl) and oxidants (Hydrogen peroxide, H_2O_2) were of highest quality from Merck, Germany.

5.2.2. Microwave Digestion of Fish Samples

The microwave digestion technique was widely used as a new alternative for conventional acid digestion (Ozparlak et al., 2012; Zeitoun and Mehana, 2014). Then the dried fish samples are crushed and powdered using porcelain mortar and pestle. According to Bashir et al (2013), the dried powder tissues samples of 0.5 g is weighed into Teflon carousels from each fish tissues containing 10 ml of 65% nitric acid and added 2 ml of 30% H_2O_2. Then, 4 ml of concentrated nitric acid, 2 ml of concentrated sulphuric acid and 1 ml of concentrated hydrochloric acid were slowly added. Then digested at high pressure in a microwave oven (Microwave Accelerated Reaction System, with a regulated pressure and temperature). After digestion, the samples are transferred to 50 ml conical tubes and diluted with 3% nitric acid to the 50 ml mark, further samples are diluted to a ratio of 1:10, 3 % nitric acid for final analysis, and blanks were performed to check for any possible contamination. The homogenized samples were digested in a microwave oven digestive system using nitric acid (65 % HNO_3) and hydrogen peroxide (30 % H_2O_2) in Teflon vessels.

5.2.3. Blank Preparation

At each step of the digestion processes of the samples acid blanks (laboratory blank) were done using an identical procedure to ensure that the samples and chemicals used were not contaminated. They contain the same digestion reagents as the real samples with the same acid ratios but without fish sample. After digestion, acid blanks were treated as samples and diluted with the same factor. They were analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES) before real samples and their values were subtracted to check the equipment to read only the exact values of heavy metals in real samples. Each set of digested samples had its own acid blank and was corrected by using the blank sample.

5.2.4. ICP-OES analysis

All measurements were performed with an ICP-OES (AMETEK). Horticoop Ethiopia (Horticulture) PLC Service carries out the ICP-OES spectrometer analysis. The homogenized samples digested in microwave oven were used for determination of Hg, Sn, Cd, B, Co, Pb, As and Cr in the samples. The concentrations were expressed as mg/kg DW.

5.2.5. Health risk assessment

Under regular consumption habits, it is important to assess the daily intake of heavy metals from fish. The general formulas that are used to calculate the estimated daily intake of each heavy metal (EDI) in this work are equations: (1)

$$EDI = C_{fish} \times [dc_{fish} / bw] \quad (1)$$

Where Cfish is the mean concentration of heavy metals in fish muscle (mg/kg WW), dCfish and bW are daily per capita consumption of fish (g/day) recorded by the FAO (2011), and average body weight of 70 kg for an adult person.

5.2.5.1 Target hazard quotient (THQ)

The target hazardous quotient (THQ), target carcinogenic risks (CR) represents a complex parameter, which is introduced by the US Environmental Protection Agency (EPA 1989; 2015)

THQ was calculated (USEPA, 2015) by the following equation (2)

$$\mathbf{THQ} = \frac{\mathbf{EF\ x\ ED\ x\ FIRx\ C}}{\mathbf{RfD\ xWAB\ x\ ATn}}\mathbf{x10^{-3}} \qquad \mathbf{(2)}$$

Where: EF = exposure frequency (360 days year-1); ED = exposure duration (65 years for adults), equivalent to the average lifetime (WHO, 2018); FIR = fish ingestion rate (kg person $^{-1}$ day^{-1}), (0.027kg person^{-1} day^{-1} for adults 70kg); C = metal concentration in fish (mg kg^{-1}); RfD = oral reference dose (mg kg $^{-1}$ day^{-1}); WAB=average body weight (kg), (70 kg for adults); ATn = average exposure time for non-carcinogens (365 days year-1×ED), for carcinogenic: 70 years x 365 days. Years^{-1}

Hazard index (HI)

In this study, cumulative health risk was evaluated by summing of the individual metal THQ value and expressed as total THQ (TTHQ) (called hazard index, HI). A total HI was working by summing all the calculated THQ values of heavy metals as described by equation: (3).

$$\mathbf{HI}= \sum_{i=1}^{n} THQ \qquad \mathbf{(3)}$$

When HQ and HI are less than one, there is no obvious risk. If it becomes more than one, the consumption of fish might impose health hazard to the consumer, especially to susceptible people like children and pregnant women (Cheung et al., 2008).

5.2.5.2. Cancer risk (CR)

The cancer risk over a lifetime exposure obtained using cancer slope factor (CSF), is provided by USEPA (USEPA, 2000).

Carcinogenic risk can be evaluated by the following linear equation: (4)

$$\mathbf{Cancer\ risk = (CDI\text{-}RfD)*SF} \qquad \mathbf{(4)}$$

CDI is chronic daily intake dose of carcinogens (mg/kg/day). The CDI values described by equation: (5).

$$\mathbf{CDI}_{fish} = \frac{\text{Cfishx FIR x ED x EF}}{\text{BW x AT}} 10^{-3} \qquad \textbf{(5)}$$

Where cancer risk is a unitless probability of an individual developing cancer, CDI is chronic daily intake dose of carcinogens (mg/kg/day), RfD =oral reference dose (mg kg^{-1} day^{-1}); and SF is the carcinogenicity slope factor (mg/kg/day). The slope factor (SF) converts estimated daily intake averaged over a lifetime of exposure directly to incremental risk of an individual developing cancer (U.S. EPA, 1989). Slope factors of As for ingestion, was 1.5, mg/kg/day (Ferreira-Baptista and De Miguel, 2005; Lim et al., 2008) and Pb is (8.5 × 10 -3 mg/kg/day)-1 (U.S. EPA, 2005).

The cancer risk caused by a variety of carcinogens is the sum of carcinogenic r isk of individual carcinogens in the possible exposure pathways, which is the total cancer risk (CR). According to the U.S. EPA (2015), the value of cancer risk in the range of 10^{-6} to 10^{-4} is an acceptable or tolerable risk, a risk of less than 10^{-6} can be ignored, and a risk exceeding 10^{-4} is considered to unacceptable (Yaya et al., 2017).

5.3. Statistical analysis

Data analyses were performed using the statistical package SPSS (version 23). Univariate Analysis of Variance, followed by Tukey's test to assess whether the means of metal concentrations were varied significantly among fish species and between sites less than 0.05 were considered statistically significant ($p < 0.05$).

5.4. Result and Discussion

Toxic Heavy Metal Analysis

This study was undertaken to investigate heavy metal concentrations in muscles and gills of two commercially important fish species from four different freshwater bodies found in Ethiopia, and to detect whether their levels are potentially harmful for human health if include d in the diet. *C. gariepinus* and *O. niloticus* were selected because they are the most commonly consumed fish species in Ethiopia. The levels of heavy metals were determined in the muscles in each species because of their importance for human consumption. It is well known that it is very difficult to compare the metal concentrations even between the same tissues in different species because of the differences in many factors such as, the freshwater environments concerning the type and the level of water pollution, feeding habits (whether omnivorous or carnivorous), and level of fish presence in water benthic fish. Kamaruzzaman et al (2010) indicated that there was a relationship between metal concentration and several intrinsic factors of fish such as organis m size, genetic composition and age of fish. Considering all these factors, it was very difficult to compare metal concentrations between the same and different fish species from different freshwater sources in this study. Consequently, it is normal to have some fluctuations in some heavy metal concentrations; different heavy metals present different distribution patterns in the different body parts of fish such as muscle and gill (Table 5.1).

Table 5. 1 shows that the havey metals like Hg, Sn and As are maximum in concentration but boron (B) is below the limits of detection < 0.019 mg/kg. Since the maximum levels of these heavy metals found in muscle and gill tissues of fishes are far below the thresholds, the analyzed fish species may be considered as safe for human consumption relative to the analyzed metals. This pollution of heavy metals may be come through contributory rivers and many chemicals from the large farmland as well as horticulture, domestic and chemical industry found near the towns' drainage lines, which is connected with the freshwater bodies and it, would be directly or indirectly accumulated in the body of the fish.

Accumulation of toxic metals by fish species from four sites

Cobalt (Co) is beneficial for humans because it is a part of vitamin B12 (Ikem and Egiebor, 2005). However, exposure to high levels of Co can result in lung and heart defects and dermatitis. Co is used to treat anemia in pregnant women because it stimulates the production of red blood cells. An excesses intake of Co may cause overproduction of red blood cells (Kalagbor et al., 2014).

Co concentration in fish tissues ranged from 0.07 mg/kg in *C. gariepinus* to 1.16 mg/kg in *O. niloticus* as shown in Table 5.1. The maximum concentration of Co found in gills of *O. niloticus* from Abay River was 1.16 mg/kg DW; this value was slightly higher than the maximum permissible level (EC, 2013).

According to Ataro et al (2003) the Co concentration as in the range of <0.71 mg/kg in muscle for the same fish species from Lake Hawassa and Ziway. This result was higher than the maximum value obtained in the present study.

Table 5.1 Heavy metal concentrations (mean ± SD, mg/kg DW) selected fish muscle and gill tissues in four different ecosystem of water bodies.

Source of Water bodies	*O. niloticus*							
	Tissue	Co	Cr	Cd	Hg	Sn	Pb	As
Alwero River	Muscle	0.08 ± 0.00^{e}	1.55 ± 0.01^{m}	0.08 ± 0.00^{d}	0.81 ± 0.00^{f}	51.50 ± 0.00^{e}	0.49 ± 0.00^{h}	ND
	Gill	0.65 ± 0.01^{c}	3.32 ± 0.01^{gh}	0.41 ± 0.01^{a}	1.46 ± 0.01^{d}	126.74 ± 0.01^{b}	3.73 ± 0.00^{b}	3.35 ± 0.00^{j}
	Average	0.37 ± 0.04^{d}	2.44 ± 1.25^{ij}	0.25 ± 0.24^{b}	1.13 ± 0.46^{de}	89.12 ± 53.2^{c}	2.11 ± 2.29^{e}	1.68±2.37k
Abay River	Muscle	0.41 ± 0.01^{d}	2.57 ± 0.01^{i}	0.33 ± 0.00^{b}	1.57 ± 0.01^{d}	6.55 ± 0.01^{g}	1.91 ± 0.01^{ef}	17.65 ± 0.01^{b}
	Gill	1.16 ± 0.01^{a}	3.40 ± 0.02^{g}	0.58 ± 0.01^{a}	2.40 ± 0.02^{c}	12.18 ± 0.01^{f}	4.56 ± 0.02^{a}	11.18 ± 0.01^{g}
	Average	0.79 ± 0.53^{h}	2.99 ± 0.59^{h}	0.46 ± 0.18^{a}	1.99 ± 0.59^{cd}	9.37 ± 3.39^{g}	3.24 ± 1.87^{c}	14.42 ± 4.57^{d}
Lake Hawassa	Muscle	ND	1.48 ± 0.01^{mn}	0.16 ± 0.01^{c}	0.74 ± 0.02^{f}	0.90 ± 0.01^{l}	ND	ND
	Gill	ND	1.49 ± 0.00^{m}	0.08 ± 0.01^{d}	0.66 ± 0.01^{f}	0.33 ± 0.00^{lm}	ND	0.50 ± 0.00^{m}
	Average	-	1.49 ± 0.01^{m}	0.12 ± 0.06^{c}	0.70 ± 0.06^{df}	0.62 ± 0.41^{l}	-	0.25 ± 0.035^{mn}
Lake Ziway	Muscle	ND	2.13 ± 0.01^{j}	0.08 ± 0.01^{d}	14.52 ± 0.01^{a}	1.48 ± 0.01^{k}	ND	ND
	Gill	ND	1.46 ± 0.01^{n}	0.08 ± 0.01^{d}	0.65 ± 0.01^{f}	222.89 ± 0.01^{a}	ND	3.32 ± 0.01^{j}
average	Average	-	1.80 ± 0.47^{l}	0.08 ± 0.00^{d}	7.59 ± 9.81^{b}	112.19 ± 156.6^{b}	-	1.66 ± 0.00^{k}
Fish species	*C. gariepinus*							
	Tissue	Co	Cr	Cd	Hg	Sn	Pb	As
Alwero River	Muscle	0.08 ± 0.01^{e}	1.73 ± 0.01^{l}	0.16 ± 0.01^{c}	1.32 ± 0.01^{c}	5.03 ± 0.01^{i}	0.08 ± 0.00^{i}	0.16 ± 0.01^{n}
	Gill	0.49 ± 0.00^{cd}	3.11 ± 0.00^{ef}	0.41 ± 0.00^{a}	1.72 ± 0.00^{d}	7.77 ± 0.00^{h}	3.02 ± 0.00^{d}	12.83 ± 0.01^{f}
	average	0.29 ± 0.29^{d}	2.42 ± 0.98^{j}	0.29 ± 0.18^{b}	1.52 ± 0.28^{de}	6.40 ± 1.94^{hi}	1.55 ± 2.08^{f}	6.50 ± 8.96^{i}
Abay River	Muscle	0.16 ± 0.01^{de}	2.13 ± 0.01^{i}	0.25 ± 0.01^{bc}	1.56 ± 0.01^{d}	4.59 ± 0.01^{j}	0.82 ± 0.01^{g}	30.92 ± 0.01^{a}
	Gill	0.08 ± 0.01^{e}	1.19 ± 0.03^{no}	0.17 ± 0.03^{c}	1.25 ± 0.03^{de}	4.66 ± 0.03^{j}	0.42 ± 0.03^{h}	0.58 ± 0.03^{m}
	average	0.12 ± 0.06^{de}	1.66 ± 0.66^{m}	0.21 ± 0.06^{bc}	1.41 ± 0.22^{de}	4.63 ± 0.05^{j}	0.62 ± 0.28^{g}	15.75 ± 21.45^{c}
Lake Hawassa	Muscle	ND	2.73 ± 0.01^{gi}	0.08 ± 0.01^{d}	0.99 ± 0.01^{f}	0.74 ± 0.01^{l}	ND	13.48 ± 0.01^{e}
	Gill	0.08 ± 0.01^{e}	4.95 ± 0.01^{a}	0.16 ± 0.01^{c}	0.82 ± 0.01^{f}	35.12 ± 0.01^{d}	ND	0.99 ± 0.01^{lm}
	Average	0.04 ± 0.06^{e}	3.84 ± 1.57^{b}	0.12 ± 0.6^{c}	0.91 ± 0.12^{f}	17.93 ± 24.32^{df}	-	7.24 ± 8.83^{h}
Lake Ziway	Muscle	ND	4.70 ± 0.01^{d}	0.08 ± 0.01^{d}	0.57 ± 0.01^{g}	0.57 ± 0.01^{l}	ND	ND
	Gill	ND	4.72 ± 0.01^{c}	0.16 ± 0.01^{c}	0.41 ± 0.01^{hi}	2.68 ± 0.01^{gk}	ND	ND
	Average	-	4.71 ± 0.01^{cd}	0.12 ± 0.06^{c}	0.49 ± 0.11^{h}	1.63 ± 1.49^{k}	-	-

Values followed by different superscript in the same columns are significantly different at $\alpha<0.05$* Comparison is made with the mean value of muscle and gill tissue

In the same Lakes, the concentration of Co in muscle of *O. niloticus* was recorded as 2.47-3.59 mg/kg (Kebede and Wondimu, 2004). The Co concentration in present work was lower as compared to Tariku et al (2015) who reported the highest concentration of Co (11.11 mg/kg DW) in muscles of *O. niloticus* from Abay Lake, but similar with a result obtained by Gebrekidan et al(2012) in muscle of *O. niloticus* recorded as 1.61 mg/kg in DW from Lake Hashenge. By comparison, the concentration of Co in the present study was lower than those reported in *C. gariepinus* (Javed and Usmani, 2011) and in Tilapia (Kebede and Wondimu, 2004).

In the present study, the cobalt levels were in agreement with the values in the above literature. The detected concentrations of Co in *C. gariepinus* muscle from Abay River (0.16 mg/kg DW) is similar with *O. niloticus* from Lake Hashenge (0.16 mg/kg DW) (Abraha et al., 2012), but is lower than in samples from Lake Abaya (11.11 mg/kg DW) (Tariku et al., 2015), and from Lakes Ziway and Hawassa (0.71mg/kg DW) (Alrto et al., 2003). Co concentrations in *O. niloticus* muscle samples from Abay River (0.4 mg/kg DW) is lower than in samples from the Lakes Hawassa and Ziway (2.47-3.59 mg/kg DW) (Kebede and Wondimu, 2004), and Lake Hashenge samples (1.61 mg/kg DW) (Gebrekidan et al., 2012).

The Co content in different species of the fish shows wide fluctuations, minimum Co content is observed in the species *C. gariepinus* and *O. niloticus* from Rivers as compared to Lakes. The results show that Co is low in both Hawassa and Ziway Lakes, but there is significance difference between Alwero and Abay River at ($P<0.05$). The concentration of Co in muscles of Tilapia from Abay River is slightly higher than Co concentration from Alwero River. This may be due to the fact that agricultural activities around the Abay River are more dominant than that of Alwero River.

According to EC (2013), the maximum permissible level of Co in fish muscles was 0.05 mg/kg WW. Co concentrations in this study fishes ranged from 0.008 to 0.41 mg/kg DW. For comparison based on WW the present study ranged from (0.09 to 0.002 mg/kg) (Table 5.2); therefore, consumption of 100g muscle of *O. niloticus* and *C. gariepinus* fish species from Alwero River, Abay River, Hawassa and Ziway Lakes do not pose any potential health risks to human health. In the gill tissues of *O. niloticus* from Abay River (1.16 mg/kg DW), the Co values are slightly higher than the prescribed limits by EC (2013) in WW.

Table 5.2 Heavy metal concentrations (mg/kg WW) selected fish muscles from four different freshwater ecosystems.

Sampled of fish species and location	Pb	Hg	Co	Cr	Cd	Sn	As
O. niloticus from Alwero River	0.10^{b}	0.16^{e}	0.02^{b}	0.30^{f}	0.02^{c}	10.10^{a}	ND
C. gariepinus from Alwero River	0.41^{a}	0.33^{b}	0.09^{a}	0.55^{c}	0.07^{a}	1.39^{b}	3.76^{b}
O. niloticus from Abay River	ND	0.16^{e}	ND	0.31^{f}	0.03^{c}	0.19^{f}	ND
C. gariepinus from Abay River	ND	3.16^{a}	ND	0.46^{d}	0.02^{c}	0.32^{e}	ND
O. niloticus from L. Hawassa	0.02^{c}	0.29^{cd}	0.02^{b}	0.38^{e}	0.03^{c}	1.09^{c}	0.03^{d}
C. gariepinus from L. Hawassa	0.15^{b}	0.29^{cd}	0.03^{b}	0.39^{e}	0.05^{b}	0.85^{d}	5.73^{a}
O. niloticus from L. Ziway	ND	0.25^{c}	ND	0.68^{b}	0.02^{c}	0.19^{f}	3.37^{c}
C. gariepinus from L. Ziway	ND	0.16^{e}	ND	1.34^{a}	0.02^{c}	0.16^{f}	ND

The concentrations of chromium (Cr) in gill and muscle tissues are different with a range of 1.48 - 4.7 mg/kg in muscle and 1.19-4.95 mg/kg in gill tissues of the two fish species. Cr was detected in all the fish sampled sites. The concentration of Cr in gil l tissues is slightly higher than muscle tissues but no significant difference was seen between the two tissues of the fish species sampled in all sites.

The highest muscle concentration of Cr was (4.70 mg/kg DW) in *C. gariepinus* from Lake Ziway and the lowest was (1.148 mg/kg DW) in muscles of O. *niloticus* from Lake Hawassa. The gill tissues of *C. gariepinus* from Lakes Hawassa and Ziway have the highest Cr values (4.95 and 4.72 mg/kg) respectively, while the lowest value was (1.19 mg/kg) found in *C. gariepinus* from Abay river. The results show that there was no significant difference in the concentration of Cr between Alwero and Abay sites, but there is significance difference between Ziway and Hawassa at ($P<0.05$) for both fish species.

The concentration of Cr in muscle of *O. niloticus* from Lake Ziway was higher than that of Lake Hawassa for *C. gariepinus* muscles. In the present study, gill tissues high accumulation of Cr as compared to muscles, this result agrees with that of Negi & Maurya (1015) study which investigated in different freshwater fish species from Bhagwanpur pond India. Similar to the present study, Tariku et al (2015) reported 6.50 mg/kg; Rosseland et al (2007) reported 3mg/kg in DW but lower Cr concentration than that reported by Negi and Maurya (2015) in Cameroon (13.01

to 17.63 mg/kg). The present results were lower than that reported by Asefa and Beranu (2015) from Tendaho Reservoir (0.76 mg/kg DW); Dsikowitzky et al (2012) from Lakes Hawassa and Koka (0.32 and 0.01 mg/kg), respectively; Gebrekidan et al (2012) from Lake Hashenge (0.37 mg/kg DW.

The proposed limits of Cr set by FAO/WHO (1989b) for edible fish muscles are about 50 mg/kg DW. Therefore, FAO/WHO (1989b) proposed limits of Cr concentration far above as compared with the present study.

Cadmium (Cd) has a long half-life in humans and it is a carcinogenic element (Jaishankar et al., 2014). Cd can be accumulated in the kidneys and cause kidney failure and has an adverse effect on fertility (NIFES, 2016). In the present study, the concentration of Cd, was significantly lower ($p<0.05$) in the muscle of *C. gariepinus* from both lakes as compared to *O. niloticus* from Lake Ziway and Alwero river.

The concentration of Cd was 0.08 mg/kg in muscle of O. *niloticus* from Lake Ziway and Alwero River, followed by 0.16, 0.25 and 0.33 mg/kg in *C. gariepinus* from Alwero River, in *C. gariepinus* and *O. niloticus* from Abay, respectively. Whereas, the concentration of Cd in gills was significantly higher ($p<0.05$) in *O. niloticus* from Lake Hawassa (0.08 mg/ kg) as compared to *O. niloticus* from Abay River (0.58 mg/ kg).

The results obtained in this study were lower than the values reported by Gebrekidan et al (2012) for *O. niloticus* from Lake Hashenge and Kiflom and Tarekegn (2015) for muscle tissues of *C. gariepinus* and *O. niloticus* (1.45 mg/kg and 1.85 mg/kg DW, respectively) from Lake Hawassa. The present study is comparable with the literature reported by Asefa and Beranu (2015) which found 0.23-0.31 mg/kg; Kiflom and Tarekegn (2015) found 0.4-0.46 mg/kg; Tariku et al (2015) found 0.37 mg/kg and Ataro et al (2003) recorded <0.24 mg/kg dry DW in similar fish species from Ethiopian Lakes in the level of Cr in muscle tissue.

When the two tissues are compared in all fish sampled, the musc le had the lowest levels of Cd as compared to the gill tissues. This is because of the inactive nature of this tissue, which is responsible for its ability to accumulate heavy metals largely (Eneji et al., 2011). The proposed limits of Cd set by FAO/WHO (1989b) for edible fish muscle is about 0.5 mg/kg. Therefore, the

FAO/WHO (1989b) proposed limits show higher concentrations of Cd as compared with the present study.

Lead (Pb) is known to cause hematological, behavioral and reproductive effects at levels a bove the tolerable limit (FAO/WHO, 2011). The concentration of Pb was 0.08 mg/kg in the muscle of *C. gariepinus,* and *O. niloticus* from Alwero River followed by 1.91 mg/kg DW in muscle of *O. niloticus* from Abay. Whereas, concentration of Pb in gill tissues were significantly ($p<0.05$) different between *C. gariepinus* (0.42 mg/ kg) and *O. niloticus* (4.56 mg/kg) from Abay River respectively. The Pb content of *C. gariepinus* in this study was similar to those obtained by Ataro et al (2003) in *O. niloticus* and *C. gariepinus* from Lakes Ziway and Hawassa, which were recorded around 1.66 mg/kg DW. In addition, Kebede and Wondimu (2004) reported in muscle of *O. niloticus* (1.89 mg/kg) and in gill tissue (20.37 mg/kg) from Lakes Hawassa and Ziway respectively.

This idea is supported by Barwick and Maher (2003) who suggested that the ability of fish to accumulate heavy metals depends on ecological needs, metabolism and degree of pollution in sediment, water and food as well as salinity and temperature of water.

By comparison, the muscle concentration of Pb in the present study was similar with those reported by Ataro et al (2003), Kebede and Wondimu (2004), Dsikowitzky et al (2012), Asefa and Beranu (2015) and Kiflom and Tarekegn (2015) in *O. niloticus* species, but it different from those reported by Cohen et al (2001), Kiflom and Tarekegn (2015) and Negi and Maurya (2015) (Table 5.3).

The concentration of Pb in gill tissues in present study was higher as compared with the muscle tissues in all sampled sites; gill tissues may be more active to fix metals as compared to muscle tissues. This idea agrees with Nussey (2000) who specified that the concentrations of metals in gills reflect their concentration in water. High concentration of Pb in the gill tissues agree with various authors worked on similar fish species (Kebede and Wondimu 2004; Negi and Maurya, 2015). According to FAO (1983), acceptable residue limits of Pb in fish species is 0.5 mg/kg WW. In the present study, Pb concentration in all fish sampled was far below the premisble limit set by FAO but *O. niloticus* from Abay and Alwero Rivers whereas *C. gariepinus* from Abay River exceeds the acceptable residue limits of Pb in fish muscles.

Mercury (Hg) is one of the most toxic heavy metals in the environment and naturally occurring element, which can be present in foodstuffs by natural causes (Castro -González & Méndez-Armenta, 2008). Fish ingest contaminated mercuric food, which passes through the gastrointestinal tract and gets distributed, accumulated or detoxified by the liver (Mieiro et al., 2011).

In the present study, the concentration of Hg in muscles ranged from 0.57 mg/kg in *C. gariepinus* to 14.52 mg/ kg in *O. niloticus* from Lake Ziway. Whereas, concentration of Hg in gills was significantly higher ($p<0.05$) in *C. gariepinus* (0.41 mg/ kg) from Lake Ziway as compared to *O. niloticus* (2.40 mg/ kg DW) from Abay River. The highest Hg concentration was 14.52 mg/kg DW detected in *O.* niloticus from Lake Ziway as compared to the other fish samples. This result may be attributed to feeding habit and habitat of *O. niloticus* as it differ when compared with *C. gariepinus*. This result agreed with the finding of Dsikowitzky et al (2012) that *O. niloticus* fish species from Lake Hawassa contained Hg in muscle tissues than the muscle tissues of *C. gariepinus*.

In the present study, the concentration of Hg in muscle tissues of *O. niloticus* from Alwero River was (0.81 mg/kg), in *O. niloticus* from Hawassa was 0.74 mg/kg, in *C. gariepinus* from Hawassa was (0.99 mg/kg) and in *C. gariepinus* from Lake Ziway was 0.57 mg/kg DW. The results found in this study were similar with the work of Dsikowitzky et al (2012) who obtained in a range of 0.3 to 1.25 mg/kg and 0.2 to 1.6 mg/kg DW) in muscles of *C. gariepinus* from Lake Hawassa and Lake Koka, respectively. Similar to the present study, Margareta (2015) reported 0.05 mg/kg WW of Hg concentration in *C. gariepinus* from Lake Tana, which is higher than the 0.006 mg/kg WW recorded in *O. niloticus* from the same area. Based on fish tissues, the higher concentration of Hg was found in the muscles as compared to the gills obtained in different freshwater fish species reported by Dsikowitzky et al (2012) from Ethiopian Rift Valley and Maury-Brachet et al (2006) from Amazonian Basin. In the present result, the higher concentration of Hg was found in the herbivorous (*O. niloticus*) fish muscles (14.52 mg/kg) from the Lake Ziway, while the lowest level was in predatory fish (*C. gariepinus*) caught in the Lake Ziway. Therefore, the concentrations of Hg were, in most cases, higher in the muscle than in the gills but not in all sampled sites observed. The difference of this result may be due to its differences in fish species with types of metal accumulation in different organs, which are species specific and reflected the respective diets. This ideas are supported by Canli and Atli (2003), who reported that levels of heavy metals in fi sh fluctuate in the same species from different aquatic environments and also Mason et al. (2000) observed that inorganic Hg is mostly concentrated in detoxifying organs such as the liver, whereas methyl mercury is relatively evenly distributed in freshwater fish tissue. With our data set, the principal distribution of total Hg between liver and muscles cannot be assessed. The distribution of inorganic and methyl mercury in the two investigated fish species should be investigated in the future because the two species are important for human nutrition. The concentration of Hg in the muscles of all fish samples were beyond the WHO permissible limits of 0.5 mg/kg (WHO, 1990) except C. gariepinus from Abay River.

Arsenic (As) is classified as organic and inorganic. Organic As is not toxic as the inorganic form (Castro-Gonzaleza and Mendez-Armenta, 2008). According to Neff (2004) most of total As (usually 50 to more than 95%) in fish is in the nontoxic organic forms such as arsenobetaine. But inorganic As is very toxic and that poses the greatest threat to human safety (Fabris et al., 2006). Therefore the guideline values only concern inorganic form of As, and the value of 10% of total As can be used as an estimate of inorganic As (Cheung et al., 2008). Inorganic As is considered carcinogenic and is related mainly to lung, kidney, bladder, and skin disorders (ATSDR, 2003). USEPA guidelines established maximum inorganic As in fish muscle 1.5 mg/kg WW or 6 mg/kg DW (Mishra et al., 2007).

According to Cheung et al (2008) the concentration of As estimated 10% of the total As level in fish. For this work the average inorganic As concentration in muscle and gill tissues of the two fish species collected from four different water bodies ranged from 0.016 to 3.12 mg/kg and 0.05 to 1.28 mg/kg DW, respectively. Highest mean concentration of As in muscle tissue of *C. gariepinus* was 3.12 mg/kg recorded at Abay River whereas the minimum concentration of 0.016 mg/kg was recorded in the same fish species at Alwero River. The highest concentration of As in gill tissue of *C. gariepinus* was 1.28 mg/kg recorded at Alwero River, whereas the minimum concentration of 0.05 mg/kg was recorded in *O. niloticus* fish species at Lake Hawassa. Asrsenic was not detected in muscle tissues of *O. niloticus* fish species collected at Alwero, Lake Hawassa and Lake Ziway and was not detected in gill and muscle tissues of *C. gariepinus* from Lake Ziway. The maximum values of As indicate that in the muscle tissues of carnivorous fish species caught in the Abay River near to Lake Tana was (3.09 mg/kg). Arsenic (As) concentrations found in this fish species from Abay River was much higher than Lake Hawassa (1.35 mg/kg) of the same species. This result showed that muscles of *C. gariepinus* accumulate As is higher than *O. niloticus* species observed in the present study. According to Vieira et al (2011), the results of Asarsenic concentration is low in most fish, being always the highest concentration found in muscle than gill tissues. The present study similarly observed the concentration of As higher in muscle tissues than gill tissues, but fish contains higher amounts of As concentration as compared to the oth er edible foods staff (ATSDR, 2007). Therefore, due to the guideline values only concern inorganic As, in this study, the levels of inorganic As in all samples were lower than the guidelines established by the USEPA (Mishra et al., 2007). So, it is difficu lt to judge the potential health risk related to As concentration in fish. There is no available data on the As levels in fish to compare with Ethiopian river source fish species. However, various reports are available from other countries. The highest concentration of As (30.92 mg/kg DW) in this work was in *C. gariepinus* from Abay River and it was higher than that of 6.44 mg/kg DW in muscle tissue of kutum (*Rutilus frisii kutum*) from the southern Caspian Sea (Hosseini et al. (2015), 0.2 mg/kg DW in muscl e of *O. niloticus* from Lake Hawassa (Dsikowitzky et al, 2012) and 0.01 mg/kg in muscle of *S. glanis* from Bovan Reservoir in Serbia (Milošković and Simić, 2015), but lower than that of 66.75 mg/kg As content recorded in muscle of *L. poecilopterus* from Malaysia.

Tin (Sn) is relatively less toxic than the six heavy metals investigated in the present study. The concentration of Sn is ranged from 0.74 to 51.5 mg/kg in muscle tissues from all sampled sites. The highest concentration of Sn is 51.5 mg/kg in muscl e of *O. niloticus* from Alwero River and the lowest is found in *C. gariepinus* from Lake Hawassa (0.74 mg/kg) DW. The gill tissues are ranged from 0.33 to 222.89 mg/kg. The highest concentration of Sn obtained was (222.89 mg/kg) in *O. niloticus* from Lake Ziway and 126.74 mg/kg in *C. gariepinus* from Alwero River, whereas the lowest concentration was recorded in *O. niloticus* from Lake Hawassa. In the present work, the highest Sn concentration obtained, but previously works did not report any obvious content and effect with high concentration of Sn metal in any fish species from Ethiopia. Sn mean concentrations of the present work was the lowest as compared to Ashraf et al (2012) who reported 153.45 mg/kg of Sn in *T. trichopterus* fish species from Malaysia. Only limited data are available on the toxicological effects of inorganic Sn present in food. Sn has no known biochemical function. However, it could have signs of chronic exposure to excessive intake of inorganic Sn which can cause headaches and stomach upset leading to diarrhea (Ibrahim et al., 2006). Based on the result of this study, the concentration of Sn in muscle tiss ues are below the permissible limits set by SADOH (2004), which is 50 mg/kg WW; therefore, it is safe for humans to consume these fish species from all the sampled sites of the study.

Comparison of heavy metal concentration from the current study with oth er studies

The comparisons of the present study with the previous studies of the same freshwater bodies as well as other freshwater bodies compiled in Table 5.3.

The comparison was made between the concentrations of metals in the present study with concentrations in other studies conducted on the same and different fish species in various countries. The variations of heavy metal concentrations in fish from different areas of the world may be due to differences in metal concentrations and chemical, physic al characteristics of freshwater from which fishes were sampled (Bahnasawy et al., 2009). The levels of Cobalt (Co) content in analyzed fish were the highest concentrations of Co (0.41 mg/kg) and (1.16 mg/kg) were observed in muscles and gills of *O. niloticus* from Abay River, respectively. Alrto et al (2003) has reported that the Co levels were in the range of <0.71 mg/kg in muscles for the same fish species from Lake Hawassa and Ziway.

The maximum mean accumulation of Co found by Alrto et al (2003) in *O. niloticus* species collected from Hawassa Lake was 0.07 mg/kg and from Lake Abaya 11.11mg/kg of Co evaluated by Tariku et al (2015). According to Kebede and Wondimu (2004) the mean accumulations of Co in *O. niloticus* fish species from Lake Hawassa was 2.47 mg/kg although the same author has found the mean levels of heavy metal accumulation of Co in *O. niloticus* from Lake Ziway (3.59 mg/kg). The maximum mean accumulation of Co found by Gebrekidan et al (2012) and Abraha et al (2012) in *O.niloticus* from Lake Hashenge were 1.61 mg/kg and 0.16 mg/kg respectively. By comparison, the present results were different from those reported by Tariku et al (2015), Gebrekidan et al (2012) and Kebede and Wondimu (2004) but similar to those of Abraha et al (2012) and Alrto et al (2003).

In the present study, gill tissues accumulate high concentration of Chromium (Cr) than muscles. This result agrees with the work of Negi and Maurya (1015) which reported on different freshwater fish species from Bhagwanpur pond India and Cameroon, and found that Cr concentrations were generally higher in the gills than in the muscles. The concentration of Cr was higher than literature reported from Cameroon (13.01 to 17.63 mg/kg) (Negi and Maurya, 2015), but lower than reported from Tendaho Reservoir (0.7 6mg/kg) (Asefa and Beranu, 2015), in Lake Hawassa and Koka (0.32 and 0.01 mg/kg) (Dsikowitzky et al., 2012) and in Lake Hashenge (0.37 mg/kg) (Gebrekidan et al., 2012).

The maximum mean accumulation of Cr in the present study was 4.7 mg/kg in *C. gariepinus* from Lake Ziway. By comparison, the levels of Cr in our study was higher than those reported in the Lake Hawassa and Ziway with the same fish species (Alrto et al., 2003). The results of the present were lower than those reported by Negi and Maurya (2015) in Labeo rohita (12.01 mg/kg) from Bhagwanpur pond India but higher than those reported by Asefa and Beranu (2015), Abraha et al (2012) and Gebrekidan et al (2012) and in similar range to those of Tariku et al (2015) 6.50 mg/kg and Rosseland et al (2007) 3 mg/kg DW are comparable with the present study.

The cadmium (Cd) results obtained in this study were lower than values reported by Gebrekidan et al (2012) for *O.niloticus* from Lake Hashenge. Kiflom and Tarekegn (2015), reported 1.45 mg/kg and 1.85 mg/kg of Cd concentration in muscle tissues of *C. gariepinus* and *O. niloticus* from Lake Hawassa respectively. The present study level of Cd in the muscle tissue was comparable with literature reported by Asefa and Beranu (2015) 0.23-0.31 mg/kg, Kiflom and Tarekegn (2015) 0.4-0.46 mg/kg, Tariku et al (2015) 0.37 mg/kg and Ataro et al (2003) recorded <0.24 mg/kg in similar fish species from Ethiopian Lakes.

The lead (Pb) content of *C. gariepinus* in this study was similar to those obtained by Ataro et al (2003) in *O. niloticus* and *C. gariepinus* from Lake Ziway and Hawassa (1.66 mg/kg DW). Kebede and Wondimu, (2004) also reported in muscle ranging from 1.89 mg/kg to 20.37 mg/kg in gill tissues of *O. niloticus* from Hawassa and Ziway Lakes. High concentration of Pb in the gill is in agreement with various authors working on similar species to the present study. Higher Pb concentrations in the gills compared with the muscles were found in *O. niloticus* from two Lakes (Kebede and Wondimu 2004) in *Labeo rohita* species (Negi and Maurya, 2015).

The concentration of Mercury (Hg) in muscle tissues of *O. niloticus* from Alwero River (0.81 mg/kg), *O. niloticus* from Hawassa (0.74 mg/kg), *C. gariepinus* from Hawassa (0.99 mg/kg) and *C.gariepinus* from Lake Ziway (0.57 mg/kg) DW found in this study was similar with the 0.3 to 1.25 mg/kg and 0.2 to 1.6 mg/kg DW of Hg concentration obtained by Dsikowitzky et al (2012) in muscles of *C. gariepinus* from Lakes Hawassa and Koka, respectively. According to Margareta (2015), higher Hg accumulation was found in *C. gariepinus* (0.05 mg/kg) than in *O. niloticus* (0.006 mg/kg) WW from Lake Tana, and this result contrasts with a previous study by (Desta et al., 2006,2007) and Dsikowitzky et al (2012). A higher concentration of Hg was also determined in the muscles compared with the gills for different freshwater fish species from Ethiopian Rift Valley Region (Dsikowitzky et al., 2012) and from Amazonian Basin (Maury-Brachet et al., 2006). Therefore, the higher concentration of Hg in the herbivorous fish muscles was 14.52 mg/kg from the Lake Ziway in the present study.

The maximum mean accumulation of inorganic Arsenic (As) in the present study was 3.092 mg/kg in *C. gariepinus* from Abay River. By comparison, the levels of As in the present study was higher than reported 0.04mg/kg and 0.07mg/kg in *C. gariepinus* species and 0.13 mg/kg and 0.05mg/kg in *O. niloticus* from Lake Hawassa and Koka respectively (Dsikowitzky et al., 2012). The present result is also higher than the 0.01 mg/kg in muscle of Cat fish (*S. glanis*) from Serbia Bovan reservoir (Milošković and Simić, 2015) but lower than the reported content of As (6.44 mg/kg DW) in muscle tissue of kutum (*Rutilus frisii kutum*) from the southern Caspian Sea (Hosseini et al., 2015). It is clear that fish contains higher amounts of As than the other foods.
Therefore, due to the guideline values only concern inorganic form of As, in this study, the levels of As in all samples based on WW were lower than the guidelines established by FAO, (2004) and WHO (2004).
The mean average Sn concentrations of the present work were the lowest as compared to Ashraf et al (2012) who reported 153.45 mg/kg of Sn in the *T. trichopterus* fish species from Malaysia. Also based on the result of this study, the maximum concentration of Sn in muscle tissues was below the permissible limits set by FAO/WHO (1995), which is 50 mg/kg DW. Therefore, the consumption of the fish species collected from various regi ons in Ethiopia are safe for human.

Table 5.3 Comparison of level of metals in Nile Tilapia (*O. niloticus*) and African cat fish *(C. gariepinus)* muscle of present study with other reported literature values and fishes dietary standards guidelines (mg/Kg DW)

Fish species	Cr	Cd	Hg	Pb	As	Fish source	Reference
O. niloticus	1.55±0.01	0.08±0.02	0.81±0.01	0.49±0.01	ND	Alwero River	Present study
	2.57±0.01	0.33±0.01	1.57±0.01	1.91±0.01	17.65±0.01	Abay River	Present study
	1.48±0.01	0.16±0.01	0.74±0.02	ND	ND	Lake Hawassa	Present study
	2.13±0.01	0.08±0.01	14.52±0.01	ND	ND	Lake Ziway	Present study
		<0.24		<1.66		Hawassa and Ziway	Ataro et al., 2003
	0.53	0.23		0.52		Tendaho Reservoir	Asefa and Beranu, 2015
	6.50	0.37		ND		Lake Abaya	Tariku et al., 2015
		1.85	0.08	0.55		Lake Hawassa	Kiflom &Tarekegn, 2015
		0.40	0.09	1.86		Lake Ziway	Kiflom & Tarekegn, 2015
	0.4	0.17		0.12		Lake Hashenge	Abraha et al., 2012
	0.54	0.003-0.2	0.05-0.24	0.02-0.3	0.26	Hawassa	Dsikowitzky et al., 2012
	0.19	0.008	0.06-0.07	0.03-0.054	0.056	Koka	Dsikowitzky et al., 2012
			0.024-0.14			Ziway	Tadiso et al.2011
		1.04		1.89		Hawassa and Ziway	Kebede &Wondimu, 2004
	0.37	0.58		1.24		Lake Hashenge	Gebrekidan et al., 2012
C. gariepinus	1.73±0.01	0.16±0.01	1.32±0.01	0.08±0.01	0.16±0.01	Alwero River	Present study
	2.13±0.01	0.25±0.01	1.56±0.01	0.82±0.01	30.92±0.01	Abay River	Present study
	2.73±0.01	0.08±0.01	0.99±0.01	ND	13.48±0.01	Lake Hawassa	Present study
	4.70±0.01	0.08±0.01	0.57±0.01	ND	ND	Lake Ziway	Present study
		<0.24		<1.66		Ziway and Hawassa	Ataro et al., 2003
	0.76	0.31		0.73		Tendaho Reservoir	Asefa and Beranu, 2015
	0.36	0.004	0.31	0.34		Hawassa	Dsikowitzky et al., 2012
	1.45	0.01	0.38	0.25		Koka	Dsikowitzky et al., 2012
		1.45	0.07	3.58		Lake Hawassa	Kiflom &Tarekegn, 2015
		0.46	0.09	3.58		Lake Ziway	Kiflom & Tarekegn, 2015
	0.35	ND	0.20	0.015		Lake Ziway	Yohannes, 2014

As=totalarseni

Generally, the muscles of *O. niloticus* and *C. gariepinus* species in the present study gave a comparable level comparing with other results. The concentrations of toxic metals (Hg, Sn and total As) in muscle tissues of *O. niloticus* from Lake Ziway and Alwero River and in muscle of *C. gariepinus* from Abay River respectively showed higher levels than others reported in the literature in Table 5.3.

The whole body mean average concentration of Cr, Cd, Sn and Hg in fish muscle of selected fish species from Lake Ziway was relatively higher than the rest of sampled site fish species. Therefore, this result shows Lake Ziway has relatively higher concentration of the heavy metals, and this might be due to many reasons, some of which are: the chemical spray of pesticides in vegetable farmlands near the Lake and flowering industry effluents discharging through the river found near Ziway town (Yohannes, 2014). The presence of high level of As metal in *C. gariepinus* from Abay River might be due the use of As pesticides, herbicides and fungicides in the farmlands around Abay River (Järup, 2003).

The presence of high level of Sn metal in gill tissues of *O. niloticus* from Lake Ziway is important to our knowledge, since no other studies about Tin metal in fish species from Ethiopian water bodies were carried out, so there is a l ack of comparable data for explanation.

Dietary intake of heavy metals

Concentrations of heavy metals in selected fish species and comparison with international dietary standards and guidelines

Currently, Ethiopia has set no guideline values on the levels of heavy metals in fish resources. With the view to making preliminary evaluation of the safety to the consumer of fishes from Alwero River, Abay River, lakes Hawassa and Ziway comparison was made with international standards guidelines values. Table 5.4 shows the maximum acceptable concentrations in fish muscles of the seven metals described in the present study set by many international organizations as WHO (1989b), EC (2013) and FAO/WHO (2011).

The range of Co was between 0.02 and 0.09 mg/kg WW. The maximum average concentration of Co in the present study was 0.009 mg/g in *O. niloticus* (Table 5.4). This value exceeded the maximum acceptable limit of Co in fish muscles proposed by EU (2013), which set maximum limit of Co in fish muscles by 0.005 mg/kg WW.

The range of Cr was 0.31 to 1.34 mg/kg WW found from both fish species in the present study. The maximum acceptable limit of FAO/WHO, which limited the maximum concentration of Cr in fish, muscles by 0.5 mg/kg. The maximum content of Cr in the present fish muscles was lower than the limits prescribed by FAO/WHO (1995).

For Pb the range was from ND to 0.4 mg/kg WW. The maximum acceptable limit of FAO/WHO and EC, which limited the maximum concentration of Pb in fish muscles by 0.3 mg/kg. FAO, in 1983, proposed the maximum acceptable concentration of Pb in fish muscles by 0.4 mg/kg (FAO, 1983). The maximum level of Pb was higher than the permissible tolerable limits set by FAO/WHO (2011) and EC (2006), but comparable with the maximum limits identified by WHO (1983).

The range of Cd was 0.02 and 0.07 mg/kg WW. The maximum content of Cd in the fish muscles of *O. niloticus* from Abay River was 0.07 mg/kg WW. This value is nearly 4 times lower than the maximum acceptable limit of Cd in fish set by EC (2006) which detected it by 0.3 mg/kg and lower than the limits prescribed by FAO/WHO (2011) and FAO (1983), which are set maximum limit of Cd in fish muscles by 0.1 mg/kg and 0.05 mg/kg WW respectively. For Hg the range are from 0.16 mg/kg to 3.15 mg/kg WW.

The maximum content of Hg in the fish muscles of *O. niloticus* from Lake Ziway was 3.15 mg/kg WW. This value is nearly 4 to 6 times higher than the maximum acceptable limit of Hg in fish set by FAO/WHO (2011) to be 0.5 to 1 mg/kg WW. The maximum content of Hg in the present fish muscles was higher than the limits prescribed by FAO/WHO (2011).

The range of Sn was 0.19 to 10.10 mg/kg WW. The maximum content of Sn in the present fish muscles of *O. niloticus* from Alwero River was 10.10 mg/kg WW. This value is nearly 5 times lower than the maximum acceptable limit of Sn in fish set by FAO/WHO, EU and SADOH, which detected it by 50 mg/kg WW. The maximum content of Sn in the present fish muscles was lower than the limits prescribed by FAO/WHO (1995), DOH (2004) and EU (2013).

Table 5.4 Comparison of maximum acceptable levels of heavy metals in muscles of two selected fish species (mg/kg WW) with international standards guidelines

Organization guidelines	Pb	Hg	Cr	Cd	Sn	TAs	In As
EC, 2013					50		
WHO, 1989			0.5				
FAO/WHO, 2011	0.3			0.1			
WHO/FAO, 1995					50	0.5	
FAO, 2004a						1.5	
WHO, 1985. 2004a						2.0	
SADOH, 2004		1.0			50	3.0	
FAO, 1983	0.4	0.5		0.05			
EC, 2006	0.3	0.5	0.5	0.03			
FSANZ, 2000						0.01	0.05
Sampled fish species and location	**Pb**	**Hg**	**Cr**	**Cd**	**Sn**	**As**	**In As**
O. niloticus from Alwero	0.10	0.16	0.30	0.02	10.10	0.00	0.000
O. niloticus from Abay	0.41	0.33	0.55	0.07	1.39	3.76	0.376
O. niloticus from Hawassa	0.00	0.16	0.31	0.03	0.19	0.00	0.000
O. niloticus from Ziway	0.00	3.16	0.46	0.02	0.32	0.00	0.000
C. gariepinus from Alwero	0.02	0.29	0.38	0.03	1.09	0.03	0.003
C. gariepinus from Abay	0.15	0.29	0.39	0.05	0.85	5.73	0.573
C. gariepinus from Hawassa	0.00	0.25	0.68	0.02	0.19	3.37	0.337
C. gariepinus from Ziway	0.00	0.16	1.34	0.02	0.16	0.00	0.000

[a] FAO, 1983, 2004 and WHO, 1985. 2004 (Ahmad and Al-Mahaqeri, 2015)

Total arsenic (TAs) content ranges from 0 to 5.73 mg/kg WW, but 10% inorganic Arsenic (IAs) from the TAs was 0.03 to 0.57 mg/kg. The maximum content of IAs and TAs in the present fish muscles of *C. gariepinus* from Abay River was 03.15 mg/kg and 5.73 mg/kg WW respectively. The TAs value is nearly 5 times higher than the maximum acceptable limit in fish set by FAO (2004) as 1.5 mg/kg WW, but IAs value was lower than the limits prescribed by FAO/WHO (1995), FAO (1983, 2004), WHO (1985. 2004) and SADOH (2004) which seting at 0.5, 1.5, 2 and 3 mg/kg WW respectively. These results were higher for TAs but lower than the maximum recommended limit in fish set by FAO/WHO and SADOH. Therefore all results of toxic heavy metals concentrations in selected fish species studied showed that Co, Cr, Pb, Hg and TAs were slightly higher than the maximum recommended limit in fish muscle set by FAO, HWO, SADOH and EU and human consumption of high quantities of these metals may lead to various health defects depending on the metal and the quantities and frequency of consuming in lake and river side communities.

Recommendations should be taken into consideration and periodical monitoring of Cr, Pb, As and Hg level in Lake Ziway, Alwero and Abay Rivers are needful, especially for *O. niloticus* and *C. gariepinus* fish species, which showed the highest concentrations of toxic heavy metals.

Health risk evaluation for daily consumption safety

Though Ethiopians are traditionally meat eaters, eating habits have been shifting in favor of fish in areas and communities where there is a regular and sufficient supply. In those communities, annual fish consumption can exceed 10 kg person^{-1} (FAO, 2011). To calculate the daily intake of fish in the present study, the figure was used. Researcher from Ethiopia (Dsikowitzky et al., 2012) was also used 10 kg person^{-1} fish consumption in similar study from from Awassa and Koka Rift Valley Lakes.

Therfore, this value (0.0274 kg/day or 27.40 g day^{-1}) was used for the calculation of the estimated daily intake (EDI), and target hazard quotient (THQ) of heavy metals through fish from Alwero River, Abay River Lakes Hawassa and Ziway using equation 1, 2 and 3. Daily intake values of heavy metals (Cr, Cd, Hg, Pb, Sn and As) for a 70 kg person (PTDI70) via the consumption of two fish species from four regions in Ethiopia are shown in Table 5.4, and the data of PTDI suggested by the FAO/WHO (2010), JECFA (2003) and FSANZ (2003) are also listed. Carcinogenic risk (CR) and chronic daily intake (CDI) dose of carcinogens (mg/kg/day) was calculated using equation 4 and 5.

Even though the concentrations of seven analyzed metals in the tested fish samples did not exceed the legal limits set by various agencies, the toxic potency of these analyzed metals depends on exposure doses. Thus, combining the determined concentration of contaminant and the estimated daily fish consumption limit is considered as a great tool for evaluating the balance between benefits and risks (carcinogenic and non-carcinogenic), observed in this study.

As a first step of estimation of human health risk, daily intake of every metal through ingestion of the fish was estimated using equation one (1) and the daily consumption of Co, Cr, Cd, Hg, Sn, Pb and As in all fish species in this study ranged from $7.83x10^{-6}$ to $35.2x10^{-6}$, $1.17x10^{-4}$ to $5.24x10^{-4}$, $7.83x10^{-6}$ to $27.4x10^{-6}$, $6.26x10^{-5}$ to $124x10^{-5}$, $6.26x10^{-5}$ to $395x10^{-5}$, $0.78x10^{-5}$ to $16x10^{-5}$ and $1.17x10^{-5}$ to $2.24x10^{-3}$ mg/day/person, respectively.

The highest average value of EDI as observed for TAs ($2.24x10^{-3}$ mg/person/day) consumed from muscles of *C. gariepinus* from Abay River and for Hg $1.24x10^{-3}$ mg/person/day) consumed muscles of *O. niloticus* collected from Lake Ziway. The lowest average value was for Co, ($7.83x10^{-6}$mg/person/day) consumed muscles of *O. niloticus* from Alwero River. The EDI results were compared with the respective recommended daily dietary intake of individual metal suggested by various agencies including Joint FAO/WHO Expert Committee on Foo d Additive (JECFA) for Hg (JECFA, 2003), Food and Agriculture Organization and World Health Organization for Cd and Pb (FAO/WHO, 2010), National Research Council for Co (NRC, 1989), Food Standards Australia New Zealand for Sn and As (FSANZ, 2003). Table 5. 5 showed that the EDI values for all the metals were far below the respective recommended levels. From the human health point of view, the EDI of each heavy metals was lower than the respective accepted daily intake (ADI) and RfD guidelines set by the JECF A (2003) and FSANZ (2003), US EPA (2009), FAO/WHO (2010) and US EPA (2010).

Table 5.4 represents the concentration of heavy metals in fish muscle as wet basis and the contents were below the corresponding allowable levels, except for Pb, Hg, Cr and As. Th e rate excess for Pb was 36.67% in *C. gariepinus* from Abay River, for Hg was 100% in Tilapia fish from Lake Ziway, for Cr was 80% in *C. gariepinus* from Lake Ziway and for IAs was 14.6% in *C. gariepinus* from Abay River.

According to our result, even though the levels Hg in *O. niloticus* species from Lake Ziway in the examined muscles of this fish were above the permissible tolerable limits set by FAO (1993) (i.e >0.05 mg/kg) there is no risk for human health from consuming this fish from Lake Ziway, due to the EDI of this examined metal was lower than that of respective reference dose and ADI prescribed by various agencies. Therefore, consumption of the examined fishes should be considered as safe for human health (Table 5. 5).

In addition, levels of Co, Cr, Pb, Hg and TAs in the examined muscles of fish species from Lake Ziway and Abay rivers were above the permissible tolerable limits set by EU and WHO/FAO. There are no concern for human health from consuming these fishes. However, fish contamination levels should be monitored on a regular basis to detect changes that could become a risk to human safety and provide solutions to reduce and control the pollution inputs to Lake Ziway and Abay River, because of the increasing amounts of the mentioned metals. The calculation of potential health risks (non-carcinogenic and carcinogenic effects) associated with the consumption of fish contaminated with (Hg, Pb, As, Co, Sn, Cr and Cd) for an individual adult from different region in Ethiopia are presented in Table 5.6.

Target hazard quotient (THQ) values of the selected heavy metals due to consumption of the examined two fish species by average Ethiopian adults shown in Table 5.5. The IAs from TAs, only 10% (Giri and Singh, 2013). The present study was used 10% out of the TAs for calculation of IAs. In the non-carcinogenic health risk, all the seven heavy metals (Hg, Pb, As, Co, Sn, Cr and Cd) concentrations in the fish tissues have the lowest potential of health risk for fish consumption. The value of THQ obtained in the present study for consumption of *O. niloticus* and *C. gariepinus* were <1.00. The higher THQ value obtained for Hg was $2.71E^{-01}$ in muscle of *O.* niloticus from Lake Ziway and for TAs $2.62E^{-01}$in *C. gariepinus* from Abay River while the lowest was for Co ($2.72E^{-07}$). The THQ values for individual metals and the THQ or HI values for combined metals were lower than one. This implies that no health risks for consumers due to the intake of either individual metals or combined metals from these four regions in Ethiopia (Table 5.6).

This, can suggest that these levels of human exposure to the analyzed metals should not cause any deleterious effect during an entire lifetime. Similar to the present study, Copat et al (2013) reported that the THQ values for six metals in fish and shellfish from the eastern Mediterranean Sea were all below one. That means that there is no risk for developing chronic systemic effects due to the intake of the above-analyzed metals.

In order to reveal such carcinogenic health effects, attempt was made to calculate cancer risk (CR) on concentrations of heavy metals such as Pb and As only, due to absence of the value of SF for the remaining of five heavy metals.

Table 5.5. Estimated daily intakes (EDI) and hazard quotient of metals in muscles of two fish species from Alwero River, Abay River, Lake Hawassa and Lake Ziway, Ethiopia

Area	Metals	Co	Cr	Cd	Hg	Sn	Pb	Tot-As	In-As
	ADI(mg kg-1bw/w)1	0.0217[a]	0.233[b]	0.07[c]	0.0033[d]	0.029e	0.25[c]	0.009[e]	3 E^{-03}[f]
	PTWI (mg kg-1bw/w)1	0.1519[a]	1.631[b]	0.49[c]	0.0231[d]	0.203e	1.75[c]	0.063[e]	0.021[f]
Alwero	EDI (mg kg-1bw/d)2 (*O. niloticus*)	$7.83E^{-06}$	$1.17E^{-04}$	$7.83E^{-06}$	$6.26E^{-05}$	$3.9E^{-04}$	$3.91E^{-05}$	ND	ND
	EDI(mgkg-1bw/d)2 (*C. gariepinus*)	$7.83E^{-06}$	$1.49E^{-04}$	$11.7E^{-06}$	$11.4E^{-05}$	$42.7E^{-05}$	$0.78E^{-05}$	$1.17E^{-05}$	$1.17E^{-06}$
Abay	EDI (mg kg-1bw/d)2 (*O. niloticus*)	$35.2E^{-06}$	$2.15E^{-04}$	$27.4E^{-06}$	$12.9E^{-05}$	$54.4E^{-05}$	$16E^{-05}$	$147E^{-05}$	$147E^{-06}$
	EDI(mgkg-1bw/d)2 (*C. gariepinus*)	$11.7E^{-06}$	$1.53E^{-04}$	$19.6E^{-06}$	$11.4E^{-05}$	$33.3E^{-05}$	$5.87E^{-05}$	$224E^{-05}$	$224E^{-06}$
Hawassa	EDI (mg kg-1bw/d)2 (*O. niloticus*)	ND	$1.21E^{-04}$	$11.7E^{-06}$	$6.26E^{-05}$	$7.44E^{-05}$	ND	ND	ND
	EDI(mg kg-1bw/d)2 (*C. gariepinus*)	ND	$2.66E^{-04}$	$7.83E^{-06}$	$9.79E^{-05}$	$7.44E^{-05}$	ND	$132E^{-05}$	$132E^{-06}$
Ziway	EDI (mg kg-1bw/d)2 (*O. niloticus*)	ND	$1.80E^{-04}$	$7.83E^{-06}$	$124E^{-05}$	$12.5E^{-05}$	ND	ND	ND
	EDI(mgkg-1bw/d)2 (*C. gariepinus*)	ND	$5.24E^{-04}$	$7.83E^{-06}$	$6.26E^{-05}$	$6.26E^{-05}$	ND	ND	ND

[1]ADI: Acceptable daily intake, calculated from provisional tolerance weekly intake; PTWI (ADI= PTWI/7); [2]EDI: Estimated Daily Intake; TAs= Total arsenic; IAs = Inorganic arsenic; [a]Nordic Council of Ministers (2015); [b]Cheung et al., 2008; [c]FAO /WHO, 2010; [d]JECFA (2003).; [e]FSANZ 2003; [f]ANZFA 1999.

Table 5.6: Estimated THQ and HI (mg kg^{-1}bw/d) of metals due to consumption of two fish species from four freshwater in Ethiopia.

Area	Metals	Co	Cr	Cd	Hg	Sn	Pb	In-As	HI
	RfD (mg kg-1 day-1)1	0.1g	0.003g	0.001h	$1.6E^{-04}$h	54.98j	0.004h	$3E^{-04}$i	
Alwero	THQ (mg kg-1bw/d) (*O. niloticus*)	$2.74E^{-06}$	$1.37E^{-03}$	$2.74E^{-04}$	$1.37E^{-02}$	$2.5E^{-06}$	$3.42E^{-04}$	ND	$1.57E^{-02}$
	THQ (mg kg-1bw/d) (*C. gariepinus*)	$2.74E^{-06}$	$1.74E^{-03}$	$4.11E^{-04}$	$2.48E^{-02}$	$2.7E^{-07}$	$6.85E^{-05}$	$1.4E^{-04}$	$2.72E^{-02}$
Abay	THQ (mg kg-1bw/d) (*O. niloticus*)	$1.23E^{-05}$	$2.51E^{-03}$	$9.59E^{-04}$	$2.83E^{-02}$	$3.5E^{-07}$	$1.40E^{-03}$	$1.7E^{-02}$	$5.03E^{-02}$
	THQ (mg kg-1bw/d) (*C. gariepinus*)	$4.11E^{-06}$	$1.78E^{-03}$	$6.85E^{-04}$	$2.48E^{-02}$	$2.1E^{-07}$	$5.14E^{-04}$	$2.6E^{-02}$	$5.40E^{-02}$
Hawassa	THQ (mg kg-1bw/d) (*O. niloticus*)	ND	$1.42E^{-03}$	$4.11E^{-04}$	$1.37E^{-02}$	$4.7E^{-08}$	ND	ND	$1.55E^{-02}$
	THQ (mg kg-1bw/d) (*C. gariepinus*)	ND	$3.11E^{-03}$	$2.74E^{-04}$	$2.14E^{-02}$	$4.7E^{-08}$	ND	$1.5E^{-02}$	$4.02E^{-02}$
Ziway	THQ (mg kg-1bw/d) (*O. niloticus*)	ND	$2.10E^{-03}$	$2.7E^{-04}$	$2.7E^{-01}$	$8E^{-08}$	ND	ND	$2.7E^{-01}$
	THQ (mg kg-1bw/d) (*C. gariepinus*)	ND	$6.12E^{-03}$	$2.74E^{-04}$	1.37E-02	$3.9E^{-08}$	ND	ND	$2.01E^{-02}$

TAs= Total arsenic, [1]RfD: Reference doses for metals;Hosseini et al., 2015; [g] Asefa (2015) Reference doses for Cr & Co set by FAO ; [h]USEPA (2009) Reference doses (RfD) for Cd, Hg & Pb set by U.S. EPA; [i]USEPA (2010) Reference doses (RfD) for As ; [j]USEPA (2000) Reference doses (RfD) for Sn set by WHO/FAO

Table 5.7 Estimated cancer risk for lifetime fish consumption of two metals from Alwero River, Abay River, Lake Hawassa and Lake Ziway, Ethiopia

Metals	Fish species	Alwero area		Abay area		Hawassa area		Ziway area	
		CDI	CR	CDI	CR	CDI	CR	CDI	CR
Pb	*O. niloticus*	$1.59E^{-05}$	$1.35E^{-07}$	$6.50E^{-05}$	$5.53E^{-07}$	ND	ND	ND	ND
	C. gariepinus	$3.17E^{-06}$	$2.69E^{-08}$	$2.38E^{-05}$	$2.02E^{-07}$	ND	ND	ND	ND
In-As	*O. niloticus*	ND	ND	$5.96E^{-05}$	$8.94E^{-05}$	ND	ND	ND	ND
	C. gariepinus	$4.76E^{-07}$	$7.14E^{-07}$	$9.08E^{-05}$	$1.36E^{-04}$	$5.3E^{-05}$	$8.01E^{-05}$	ND	ND

CDI= chronic daily intake dose (CDI, mg/kg/day; CR=cancer risk (unitless)

Inorganic Pb and As metals are classified as carcinogenic elements to humans (Vieira et al., 2011); which tends to accumulate mainly in the liver and kidney of humans. This phenomenon is attributed to the precipitation of protein by Pb, through the interaction of Pb ions with the sulfhydryl (-SH) groups of proteins (ATSDR, 2007). According to Azmi et al (2009) As is frequently associated with, kidney, lung, skin, and bladder cancer in human bodies. The value of slope factors (SF) for As is $(1.5 \text{ mg/kg/day})^{-1}$ and for Pb is $(8.5 \times 10^{-3} \text{ mg/kg/day})^{-1}$ (U.S.EPA, 2005).

Due to absence of the value of SF for Cr, Hg, Cd, Sn and Co heavy metals in present work, such comparison was not possible for these heavy metals. However, the risk of cancer due to exposure to TAs was a concern since the values of CR value was $1.36 E^{-03}$ in muscle of *C. gariepinus* from Abay River, which was higher than the acceptable range of 10^{-6} to 10^{-4}. The acceptable lifetime CR considered by USEPA is 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10000), but the toxic IAs used as 10% and applied in present study was $1.36E^{-04}$ which was, in the acceptable range (Table 5.7).

Conclusion

The two fish species of *C. gariepinus* and *O. niloticus* from four different freshwater bodies were sampled and carried out to provide information on heavy metal concentrations in muscle and gill tissues.The heavy metal distribution between the different fish tissues showed statistically significant differences.

The accumulation of Hg and As are more preferential in the muscle of the two fish species than in the gill tissues. Comparison of the heavy metals with the acceptable limits set by international guideline showed that the levels of Cd and Sn were below the acceptable limits. However, Pb, Cr, As, and Hg level was higher than the permissible limit in wet weight basis.

The consumption of both fish species from the four freshwater bodies in Ethiopia is not free of risks but that the complex THQ and HI parameters used in health risk assessment of heavy metals provides a better image than using only a simple parameter like the conte nt of the metals in the muscles.

CHAPTER-6

General Conclusion, Limitation and Recommendations

7.1. Conclusion

The present study showed that both the muscle and gill tissues of *Oreochromis niloticus* and *Clarias gariepinus* have an appreciable amount of gross energy value, crude protein, crude fat, ash content, and essential minerals such as Fe, Zn, Cu and Mn. As compared to *O. niloticus*, with the *C. gariepinus* fish species in all sample site areas were found to be rich in all nutrients. There was a great variability with respect to the crude protein and fat contents between fish species, which may be important for the selection of fat and protein-rich fishes for aquaculture breeding purpose.

The superiority of *C. gariepinus* muscle tissues from Lakes Ziway and Hawassa in crude protein and fat content, respectively. While the two sampled sites, Alwero River and Abay River fish species are good source of ash and moisture contents. The discarded gills contain appreciable amount of fat and protein contents, may be have a potential solution to fill the gap between demand and supply of fish sauces, fish meal, and fish silage and, also specifically a viable alternative to substitute fish oil sources extract from fish muscles in supply of fish oil to aquaculture sectors.
The fish species included in this study was rich in essential minerals and can play a great role in combating micronutrient malnutrition. Fe, Zn and Cu are the most abundant elements in both tissues followed by other metals in all sampled sites.

Fe, Cu & Zn minerals that give a greater contribution to meet the daily nutritional needs (respectively in 150g WW muscles of *C. gariepinus* from L. Hawassa, Abay river and L. Ziway). The superiority of *C. gariepinus* in nutritional value. In contrast higher accumulation of toxic heavy metals in gill and muscle tissues observed in *C. gariepinus* than *O. niloticus*. The consumption of both fish species from four freshwater bodies, in Ethiopia is no risk for consumers.

7.2 Limitations of the study

This study was the first in its kind; it came up with strong findings in relation to the effect of different ecosystem freshwater bodies on the proximate composition, essential minerals and heavy metal contamination on *O. niloticus* and *C. gariepinus* fish species collected from Alwero River, Abay River, Lake Hawassa and Lake Ziway, Ethiopia. Therefore, it may be used as scientific baseline information for subsequent studies and for different organizations engaged in freshwater fish oil processing, food fortification and related programs in the coun try.

The studysummarizes the proximate composition, essential minerals content, the level of toxic heave metal concentration in selected fish species. However, the study used different human risk assessement for toxic heavy metal contamination fish species. Whereas this study didn't evaluate the total lipid profile of selected fish species and the correlation human plasma phospholipid status and freshwater consumption in Ethiopia.

Fish and human blood samples were collected during the study and All samples were prepared in Food Science and Nutrition laboratory of AAU for ana lysis of fatty acids and human plasma phospholipids using a gas chromatography (GC) instrument and thus, the result may be affected by GC machine failed technically.

7.3. Recommendations and future perspectives

Consumption fishes are associated with several health benefits. However there is a limited awareness, the fish demand is only seasonal and inadequate fish intake habit with Ethiopia's people. Based on these findings, the following recommendations were forwarded:

- The research centers require information on nutritional quality of these fish tissues and include other discarded body parts (liver and head) on total lipid and fatty acids profile of *C. gariepinus* and *O. niloticus* need be study.
- Fish discarded gill tissue can also be used for food product development of various value added products such as protein and fat. These fish species high protein and fat contents can be used as a functional ingredient in bakery substitutes, soups and infant formulas. The protein present in the fish gill can be utilized in animal feed in the form of fishmeal and sauce or can be used in the production of fertilizers.

- Currently, Ethiopia has set no guideline values for dietary recommendations for levels of plasma phospholipid LC n-3 PUFA concentrations in men and women, therefore study on nutritional benefit associated with fish consumption very important.
- The quality controlling authority should set guideline values on the leve ls of heavy metals in fish resources.
- High accumulation of As and Hg in the muscle of C. gariepinus and O. niloticus caught from Lake Ziway and Abay River are alarming and still, this needs to be further examined in future studies
- The Sher Ethiopia floriculture industry is affecting the ecology (fishes) of Lake Ziway. The effluent treatment plants have to be in place before the waste water is discharged to the water body to minimize the contamination of fish tissues.

List of references

Abelti AL (2017) Minerals Content and Fatty Acids Profile of Nile Tilapia (*Oreochromis niloticus*) Fillet from Lake Zeway: Effect of Endogenous Factors. *J Nutr Food Sci* 7: 574.

Abdel-Baki, A., Dkhil, M., & Al-Quraishy, S. (2011). Bioaccumulation of Some Heavy Metals in Tilapia Fish Relevant to Their Concentration in Water and Sediment of WadiHanifah, Saudi Arabia. *African Journal of Biotechnology*.10 (13):2541-2547.

Abdullahi S. A., Abolude D. S. & Ega R. A. (2001). Nutrient quality of four oven dried fresh water catfish in Northern Nigeria. *J. Trop. Biosci*. pp.: 70.

Ackman, R.G. (2008). Fatty acids in fish and shellfish." In Fatty acid in Food and their health implication, *by Ching K uang Chow*, 155-185. Boca Raton.

Ackman RG (2007) Fatty Acids in Fish and Shellfish 8. Fatty acids in foods and their health implications. 19:155.

Adarme V, T.; Lim, D.; Timmins, M.; Vernen, F.; Li, Y.; Schenk, P. (2012). Microalga bio factories: A promising approach towards sustainable omega-3 fatty acid production. *Microb. Cell Fact.J*. Page-11.

Adeniyi O.R, Ademosun A.A and Alabi O.M. (2011). Proximate composition and economic values of four common sources of animal protein in South -western Nigeria. *J. Zootecnia Trop.*, 29(2): 231-234.

Adeniyi, S.A., Orikwe C.L, Ehiagbonare J.E and Joshia S.J. (2012). Nutritional Composition of three different fishes (*C. gariepinus, M. electricus and Tilapia guineenisis*). *Pak. J. Nutr.*, 8: 793-797.

Adewoye SO, Omotosho JS. (1997). Nutrient Composition of some freshwater Fishes in Nigeria. *J. Bio Sci. Res. Common*, 11(4):333-336.

Adewumi AA, Adewole HA, Olaleye VF. (2014). Proximate and elemental composition of the fillets of some fish Species in Osinmo Reservoir, Nigeria, Agric. Biol. *J North Am*. 5(3):109-117.

Ahmad, A.K, Sarah, A. Al-Mahaqeri. (2015). Human Health Risk Assessment of Heavy Metals in Fish Species Collected from Catchments of Former Tin Mining: *International Journal of Research Studies in Science, Engineering and Technology Volum* e 2, Issue 4, PP 9 21.

Akan J.C, Mohmoud S, Yikala B.S, Ogugbuaja V.O. (2012). Bioaccumulation of some heavy metalsin fish samples from river Benue in Vinikilang, Adamawa State, Nigeria. *American Journal of Analytical Chemistry*. 2012; 3: 727-736.

Akpanyung E.O., Ekanemesang U.M., Akpakpan E.I., Anadoze N.O. (2014). Levels of heavy metals in fish obtained from two fishing sites in Akwa Ibom State, Nigeria. *Afr. J. Environ Sci. Technol.* 8, (7), 416.

Akpinar, M.A., Görgün, S., Akpinar, A.E. (2009). A comparative analysis of the fatty acid profiles in the liver and muscles of male and female Salmo trutta macrostigma. *Journal of Food Chem.* 112: 6-8.

Alas A, Ozcan MM, Harmankaya M (2014). Mineral contents of head, caudal, central fleshy part, and spinal columns of some fishes. *J. Environ. Monit. Assess*. 186:889-894.

Al-Busaidi, M., Yesudhason, P, Al-Mughairi, S., Al-Rahbi, W. A, Al-Harthy, K. & Al-Mazrooei, N. (2011). Chemosphere toxic metals in commercial marine fish in Oman with reference to national and International Standards. *J. Chemosphere*. 85(1):67-73.

Alemu, L. A.; Melese, A. Y.; Gulelat, D. H. (2013). Effect of endogenous factors on proximate composition of Nile tilapia (*O. niloticus* L.) fillet from Lake Zeway. *American Journal of Research Communication*, 1(11): 405-410.

Aliasgharpour M and Rahnamaye F. M. (2013). Trace Elements in Human Nutrition: A Review. *International journal of medical investigation* 2 (3):115-128.

Ali, A. and Mozhgan, K. R. (2015). Influences of drying methods processing on nutritional properties of three fish species Govazym stranded tail, Hamoor and Zeminkan. *International Food Research Journal.* 22(6): 2309-2312.

Ali, H. M. and Abdel-Satar, M.A. (2005). Studies of some heavy metals in water, sediment, fish and fish diet in some fish farms in El-Fayoum Province, Egypt. Egyptian *Journal of Aquatic Research.* 31(2): p. 261-273.

Alinnor, I. J., & Obiji, I. A. (2010). Assessment of trace metal composition in fish samples from Nworie River. *Pakistan Journal of Nutrition*, 9 (1), 81 – 85.

Alipour, H., Pourkhabbaz, A. and Hassanpour, M., (2014). Estimation of potential health risks for some metallic elements by consumption of fish. *Journal of Water Quality, Exposure and Health,* 7(2), 179-185.

Al-Yousuf M.H., El-Shahawi M.S., Al-Ghais S.M. (2000). Trace metals in liver, skin and muscle of *Lethrinus lentjan* fish species in relation to body length and sex. *J.Sci. Total Environ.* 256, 87.

Amer, H.A., Sedik, M.F., Khalafalla, F.A. and Awad, H.A. (1991). Results of chemical analysis of prawn muscle as influenced by sex variations. *Die Nahrung*. 35:133-138. 256, 87.

Anim AK; Ahialey EK; Duodu GO; Ackah M; Bentil NO (2011); Res. *J. Environ. Earth Sci*., 3(1), 56.

Antonijevic B., Matthys C, Sioen I., Bilaum. Van Camp J., Willems J.L., De Henauw S. (2007). Simulated impact of a fish based shift in the population n-3 fatty acids intake on exposure to dioxins and dioxin-like compounds. *Food Chem. Toxicol*. 45, (11), 2286.

Antony M.M. (2013). Comparative evaluation of proximate composition of Etroplus suratensis from ecologically different areas of ashtamudi Lake, Kerala. *International Journal of Science and Research (IJSR) IS SN (Onli ne)*: 2319-7064.

ANZFA. (2002). Australia New Zealand Food Authority, Contaminants in foods—metals, full assessment report, Proposal P157 , ANZFA, *Publishing Services, Canberra. ISBN* 0 642 345910.

ANZFA (1999). Australia New Zealand Food Authority, Contaminants in foods metals, full assessment report, Proposal P157, ANZFA, *Publishing Services, and Canberra. ISBN* and Canberra.

AOAC (Association of Official Analytical Chemists) (2007) Methods 968.09, 969.33, 972.10, 996.06. Official methods of analysis, 18th edn. 2005; Current through revision 2, (On-line). AOAC International, Gaithersburg, MD.

AOAC. (2000). Association of Official Analytical Chemists Official Methods of Analysis. *17th Edition. W. Hortuntzed (Ed.)*, Washington, USA. 21- 447.

Appeal LJ, Miller ER, Selder AJ, Whelton PK. (1993). Does supplementation of diet with fish oil reduces blood pressure. A meta-analysis of controlled clinical trial. *Archives of Internal Medicine,* 153:1429–38.

Aragaw, C., (1998). Development and Management Approaches for inlan d Fisheries with Special references to Lake Tana. Portsmouth, England, *A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master* of Fisheries. University of Portsmouth, England.

Aragaw, C. (2010). Pond fish farming in practice: Challenges and opportunities in Amhara Region. Sciences Association (EFASA), Editors: Seyoum Mengistu and Brook Lemma, *Addis Ababa University Press, Addis Ababa, Ethiopia*. pp. 61 -68.

Arnarson A (2007). The effect of energy-restricted diets, varying in fish protein and fish oil, on appetite and the level of adiposity signals and appetite-regulatory hormones in young, overweight adults in Europe. *Master thesis*, Reykjavik, Iceland.

Arts, M.T., R.G. Ackman and B.J. Holub, (2001). Essential fatty acids" in aquatic ecosystems: A crucial link between diet and human health and evolution. Can. *J. Fish. Aquat. Sci*., 58(1): 122-137.

Asefa W, Beranu T (2015). Levels of Some Trace Metals in Fishes Tissues, Water and Sedi ment at Tendaho Water Reservoir, Afar Region, Ethiopia. *J Environ Anal Toxicol* 5: 313.

Asfaw Alemayehu Kebede. (2011). effect of feed quality on growth performance and water quality in cage culture system for production of Nile Tilapia (*Oreochromis niloticus*, (*LINNAEUS, 1758*)) in Lake Hora-Arsedi, Ethiopia. *Master thesis Addis Ababa university school of graduate studies* faculty of life sciences Ethiopia.

Askari B and Ferrerib NR (2001). Regulation of prostacyclin synthesis by angiotensin II and TNF-alpha in vascular smooth muscle. *J. Prostaglandins*, 63: 175-187.

Assefa Mitike Janko. (2014). Fish production, consumption and management in Ethiopia. *Research Journal of Agriculture and Environmental Management*. Vol. 3(9), pp. 460-66.

Ashraf, M. A., Maah, M. J. and Yusoff, I. (2012). Bioaccumulation of Heavy Metals in Fish Species Collected from Former Tin Mining Catchment. *J. Environ. Res*., 6(1):209-218.

Asmare` Amuamuta, Zewdie Mekonnen and Agmassie Agazie (2014). Extraction and Analysis of Oil/Fat and Fatty Acids Content from Different Indigenous Fish of Lake Tana Source, Northwest Ethiopia. *World Journal of Fish and Marine Sciences* 6 (5): 417-423.

Astrup A (2005). Editorial: The satiating power of protein a key to obesity prevention? *American Journal of Clinical Nutrition*, 82: 1-2.

Astrup A, Meinert Larsen T and Harper A (2004). Atkins and other low -carbohydrate diets: hoax or an effective tool for weight loss? *Lancet American Journal of Clinical Nutrition*, 364: 897-899.

Ataro, A., Wondimu, T and Chandravanshi, B. S. (2003). Trace metals in selected fish species from lakes Awassa and Ziway, Ethiopia. SINET: *Ethiopian Journal of Science*, 26, 103-14.

ATSDR (1995). (Agency for Toxic Substances and Disease Registry). Toxicological Profile for Zinc CAS# 7440-66, Atlanta, GA: U.S.

ATSDR (2001) (Agency of Toxic Substances and Disease Registry). Toxicological Profile for Manganese CAS# 127-18-4, Atlanta, GA: U.S.

ATSDR (2003) Toxicological profile for tin and compounds (update). Draft for public comment. Atlanta, GA, US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

ATSDR. (2007) (Agency for Toxic Substances and Disease Registry). Toxicological Profile for Arsenic (Update). U.S. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA.

Authman, M.M.N., Abbas, H.H., A bb as, W.T. (2012). Assessment of metal status in drainage canal water and their bioaccumulation in *Oreochromis niloticus* fish in relation to human health. *Environmental Monitoring and Assessment*, doi: 10.1007/s10661-012-2599-8.

Aytegeb Anteneh Chekol. (2013). Management and Livelihood Opportunity of Lake Tana Fishery, *Ethiopia. Master thesis in International Fisheries* Management. Norwegian College of Fisheries Science Arctic University Norway.

Azmi .Jr M, Junidah R, Siti M.A, Safiah M, Fatimah S, Norimah A, Poh B, Kandiah M, Zalilah M, Wan A.M.W. (2009). Body mass index (BMI) of adults: findings of the Malaysian Adult Nutrition Survey (MANS), *Malaysian journal of nutrition*, 15 - 97-119.

Babalola A. F, S. R. Adeyemi, O. A. Olushola, M. O. Mutiat, O. S. Olajuyigbe, and R. G. Akande, (2011). Proximate and mineral composition in the flesh of five commercial fish species in Nigeria. *Intern. J. of Food Safety*. 13: 208-213.

Babangida A. (2009). Seasonal variations in the proximate and mineral contents of three fresh water fishes in Mairuwa reservoir, Faskari, Katsina state, department of biological sciences; *master thesis at Ahmadu Bello University*, Zaria, pp 52-56.

Badawi N.ES, Barakat A.A, El Sherbini S.A, and Fawzy H.M. (2013). Prevalence of overweight and obesity in primary school children in Port Said city, *Egyptian Pediatric Association Gazette*, vol.61, no.1, pp.31–36, 2013.

rosenbergii Collected from Natural Culture Environments. *International Journal of Biology*. 2:2311-18.

Biro, J., Hancz, C., Szabo, A. and Molnar, T. (2009). Effect of sex on the fillet quality of Nile tilapia fed varying lipid sources. *Ital.J.Anim. Sci.8:25-227.*

Boening, D. W. (2000). Ecological effects, transport, and fate of mercury: A general review. *J. Chemosphere,* 40(12), 1335–1351.

Borzoei S, Neovius M, Barkeling B, Teixeira-Pinto A and Rossner S (2006). A comparison of effects of fish and beef protein on satiety in normal weight men. *European Journal of Clinical Nutrition*, 60: 897-902.

Boukortt FO, Girard A, Prost JL, Ait-Yahia D, B ouchenak M and Belleville J (2004). Fish protein improves the total antioxidant status of streptozotocin induced diabetes in spontaneously hypertensive rat. *J.Medical Science Monitor*, 10: 397-404.

Bosch A. C, O'Neill B, Sigge G.O, Kerwath S.E and Hoffman L.C. (2015). Heavy metals in marine fish meat and consumer health: a review. *Journal of the Science of Food and Agriculture* : 01-02.

Buckley, Jonathan D., Alison M. Hill, Catherine M Milte, Alison M Coates, and P R Howe.(2007). Dose-dependent effects of docosahexaenoic acid-rich fish oil on erythrocyte docosahexaenoic acid and blood lipid levels." *British Journal of Nutrition*, 99(5):1083-8.

Buljac M., Bogner, D., Brali, M., Periš, N., Buzuk, M., Vrinic, S., & Vladislavic, N. (2014). Cadmium and lead distribution in marine soil sediments, terrestrial soil, terrestrial rock, and atmospheric particulate matter around Split, Croatia. *J. Analytical Letters*, 47(11), 1952–1964.

Burger, J., & Gochfeld, M. (2004). Mercury in canned tu na: White versus light and temporal variation. *Journal of Environmental Research*, 96(3), 239–49.

Burger, J., Gochfeld, M., Jeitner, C., Pittfield, T., & Donio, M. (2014). Heavy metals in fish from the Aleutians: Interspecific and locational differences. *Journal of Environmental Research*, 131, 119–30.

Burke J.M. and Miller, J. E. (2006). Control of Haemonchus contortus in goats with a sustained - release multi-trace element/vitamin ruminal bolus containing copper. *J. vet. Parasitol.* 141: 132-137.

Calder, P. (2006). *n*-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am. J. Clin. Nutr.83*, 1505–1519.

Carr TP, Parks JS and Rudel LL (1992). Hepatic ACAT activity in African green monkeys is highly correlated to plasma LDL cholesteryl ester enrichment and coronary artery atherosclerosis. *Journal of Arteriosclerosis and Thrombosis*, 12: 1274-1283

Campose H, Khoo C and Sacks FM. (2005). Diurnal and acute pattern of postprandial Apo lipoprotein B-48 in VLDL, IDL and LDL from normal lipidemic human. *Journal of Atherosclerosis*; 181: 345–51.

Canli, M., & Atli, G. (2003). The Relationships between Heavy Metal (Cd, Cr, Cu, Fe, Pb, Zn) Levels and the Size of Six Mediterranean Fish Species. *Journal of Environmental Pollution.* 121: 129-136.

Caponio, F., Lestingi, A., Summo, C., Bilancia, M.T. and Laudadio, V. (2004). Chemical characteristics and lipid fraction quality of sardines (*Sardina pilchardus W.*): influence of sex and length. *J. Appl. Ichthyology*. 20:530-535.

Castro-González, M. I., & Méndez-Armenta, M. (2008). Heavy metals: Implications associated to fish consumption. *Journal of Environmental Toxicology and Pharmacology*, 26(3), 263–71.

CCC, (1994). Commission of the Chinese Criterion. Chinese national criterion collection GB 2736-94: Healthy standard for fresh water fish. *Chinese national criterion press* 32: Beijing, 24/1/.

Central Statistical Agency and ICF International (2016). Ethiopia Demographic and Health Survey 2011. Addis Ababa, Ethiopia and Calverton, Maryland, USA: *Central Statistical Agency and ICF International.*

Cheung K, Leung H, M. Wong M. (2008). Metal concentrations of common freshwater and marine fish from the Pearl River Delta, South China, *Archives-Journal of Environmental Contamination and Toxicology*, 54; 705-715.

Cicchella D, de Vivo B, Lima A, Albanese S, McGill RAR, Parrish RR. (2008). Heavy metal pollution and Pb isotopes in urban soils of Napoli, Italy. *Journal of Geochemistry* 8: 103-112.

Cohen, T., Shane, S.Q. H. and Richard, F.A. (2001). Trace metals in fish and invertebrates of three California Coastal Wetlands. *Journal of Marine Poll. Bull.*, v. 42, p.224-230.

Copat, C., Arena, G., Fiore, M., Ledda, C., Fallico, R., Sciacca, S. and Ferrante, M., (2013). Heavy metals concentrations in fish and shellfish from Eastern Mediterranean Sea: consumption advisories. *Journal of Food and Chemical Toxicology*, 53, 33–37.

Copat, C., Bella, F., Castaing, M., Fallico, R., Sciacca, S., & Ferrante, M. (2012). Heavy Metals Concentrations in Fish from Sicily (Mediterranean Sea) and Evaluation of Possible Health Risks To Consumers. *Bull Environ Contam Toxicol. J.* 88 (1): 78-83.

Cornelia A.B. (2012). Investigation of the chemical composition and nutritional value of smoothhound shark (Mustelus mustelus) meat. Thesis is presented in partial fulfillment of the requirements for the *degree Master of Science in Food Science* at the University of Stellenbosch. 2012.

Costa LG (2007). Contaminants in fish: risk-benefit considerations. Ahvaz *journal of hygiene rada itoksikologiju,* 58: 367-374.

Costa JV, Silva ARV, Moura IH, Carvalho RBN, Bernardes LE, Almeida PC. (2012). an analysis of risk factors for arterial hypertension in adolescente students. *Rev. Journal Latino-Am. Enferm.* 20 (2):289-95.

Costa, M. F., Landing, W. M., Kehrig, H. A., Barletta, M., Holmes, C. D., Barrocas, P. R. G., Malm, O. (2012). Mercury in tropical and subtropical coastal environments. *Journal of Environmental Research*, 119, 88–100.

Craig, W.J. (1994). Iron status of vegetarians. *Am. J. Clin Nutr.* 59, 1233S-1237S.

Das, K., Lepoint, G., Loizeau, V., Debacker, V., Dauby, P., & Bouquegneau, J. M. (2000). Tuna and dolphin associations in the North-east Atlantic: Evidence of different ecological niches from stable isotope and heavy metal measurements. *Journal of Marine Pollution Bulletin,* 40(2), 102–109.

Dawoud AS, Salem AH, Samah AS (2009) Clinico Pathological Studies On the Impact of Heavy Metal Pollution on Catfish in River Nileain Damieta and Dakhlaia Governorayters. *Egypt J Comp Path Clinic Path* 23: 37-61

Gebrekidan A, Desta MB, Gebremedh YW. (2012). Bioaccumulation of Heavy Metals in Fishes of Hashenge Lake, Tigray, Northern Highlands of Ethiopia. *American Journal of Chemistry.* 2: 326-334.

Demonty I, Deshaies Y, Lamarche B and Jacques H (2003). Cod protein lowers the hepatic triglyceride secretion rate in rats. *Journal of Nutrition*, 133: 1398-1402.

Demonty I, Deshaies Y and Jacques H (1998). Dietary proteins modulate the effects of fish oil on triglyceridemia in the rat. *Journal of Lipids*, 33: 913-921.

Desta, Z., Borgstrøm, R., Røsseland, B. O, & Dadebo, E. (2007). Lower than expected mercury concentration in piscivorous Clarias gariepinus (Burchell). *Science Journal of Total Environment*, 376, 134 –142.

Desta, Z., Borgstrøm, R., Røsseland, B. O., & Gebremariam, Z. (2006). Major difference in mercury concentrations of the African big barb, Labeobarbus intermedius (R.) due to shiftsin trophic position. *Journal of Ecology of Freshwater Fish*, 15, 532 –543.

Dsikowitzky L, Mengesha M, Dadebo E, de Carvalho C.E.V and Sindern S. (2012). Assessment of heavy metals in water samples and tissues of edible fish species from Awassa and Koka Rift Valley Lakes, Ethiopia. *Journal of Springer. Environ Monit Assess*, vol, 12, pp 2777-8.

Duda-Chodak A, Blaszczyk U. (2008). The impact of nickel on human health review. *J. Elementol.* 13(4): 685-696

Ebrahimpour M., Mushrifah I. (2010). Seasonal variation of cadmium, copper, and lead concentrations in fish from a freshwater lake. *Journal of Biol. Trace Elem. Res.* 138, (1-3), 201.

EU, (2013). The European Parliament and of the Council as regards maximum levels for arsenic, cadmium, lead, nitrites, volatile mustard oil and harmful botanical impurities. *Official Journal of the European Union*, L 328/86.

EC (2006). The Commission of the European Communities. Commission regulation (EC) No 1881/2006. *Official Journal of the European Union*, L 364, 5-24.

EDHS (2011). Ethiopia Demographic and health Survey. (2011). September 2006. Central Statistical Agency Addis Ababa, Ethiopia, ORC Macro Calverton, Maryland, USA.

Effiong BN, Fakunle JO (2013). Proximate composition and fatty acid profile in some commercially important fish species from lake Kainji, Nigeria. *Int J Biol Pharm Allied Sci.* 2:849-56.

EFSA (2009). European Food Safety Authority Scientific Opinion. Cadmium in food: Scientific opinion of the panel on contaminants in the food chain adopted on 30 January 2009. *The EFSA Journal*, 980, 1–139.

EFSA (2005). Nutrition and Allergies on a request from th e Commission related to the Tolerable Upper Intake Level of Tin. *The EFSA Journal*, 254, 1-25.

Egbal O. A, Abd-Alrhman M. A, Sheham J. E and Heba H. A. (2017). Proximate and mineral composition of some commercially important fishes in Jebl Awlia Reservoir, Sudan. *International Journal of Fisheries and Aquaculture Research* Vol.3, No.1, pp.51-57.

El-Serafy, S.S, Ibrahim S.H. and Mahmoud, S.A. (2005). Biochemical and histo -pathological studies on the muscles of the Nile Tilapia (*Oreochromis niloticus*) in Egypt. Egypt *J. Aqua. Bio. & Fish.* 9: 81– 96.

Elvevol, El and James, D. (2002). Fish oils in fetal and neonatal development. FAO/ WHO Joint expert consultation on diet, nutrition and the prevention of chronic diseases. Geneva, 28 januuary-1 February.

Emmanuel BE, Oshionebo C and NF Aladetohun (2011). Comparative Analysis of the Proximate Compositions of Tarpon Atlantic sand Clarias gariepinus from Culture Systems in South - Western Nigeria. *Africa Journal of food, agriculture and development*. Volume 11 No. 6

Eneji, I. S., Sha, Ato, R., & Annune, P. A. (2011). Bioaccumulation of heavy metals in fish (Tilapia zilli and Clarias gariepinus) organs from River Benue, North Central Nigeria. *Pakistan Journal of Analytical and Environmental Chemistry*, 12, 25 –31.

EPHI, (2015). The Ethiopian Public Health Institute. Ethiopia Steps Report on Risk Factors for Non-Communicable Diseases (NCDs) and Prevalence of Selected NCDs. EPHI & MOH, Addis Ababa, Ethiopia.

Erasmus, C. P., Rossouw, G., & Baird, D. (2004). The concentration of ten heavy metals in the tissues of shark species Squalus megalops and Mustelus (Chondrichthyes) occurring along the Southeastern coast of South Africa. University of Port Elizabeth.

Erkan N, Bilen G. (2009) Effect of essential oils treatment on the frozen storage stability of chub mackerel fillet. *Journal fur Verbraucherschutz und Lebensmittelsicherheit*. 5(1):101-110.

Ermias Haile, Sisay Tadesse, N.Surendra Babu and Milkyas Endale. (2015). Analysis of Selected Metals in Edible Fish and Bottom Sediment fro m Lake Hawassa, Ethiopia. J. *Elixir Appl. Chem.* 82; 32610-32616.

EU (2006). European Union. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants food stuff. Food stuffs; http: //ec. europa.eu/food/food/chemicalsafety /contaminants/ Mk contaminants final web_en.pdf.

Fabiola Hernández, Martha Elena. (2012). Nutritional Richness and Importance of the Consumption of Tilapia in the Papaloapan Region Redvet. *Rev. Electrón.vet*. 13:6-12.

Falcó, G., Llobet, J. M., Bocio, A., & Domingo, J. L. (2006). Daily intake of arsenic, cadmium, mercury, and lead by consumption of edible marine species. *Journal of Agricultural and Food Chemistry*, 54(16), 6106–12.

Fallah A.A., Saei-Dehkordi S.S., Nematollahi A., Jafari, T. (2011). Comparative study of heavy metal and trace element accumulation in edible tissues of farmed and wild rainbow trout (Oncorhynchus mykiss) using ICP-OES technique. *Journal of Microchem*. 98, 275.

FAO (1983). "Compilation of Legal Limits for Hazardous Substances in Fish and Fishery Products," FAO Fisheries Circular No. 464, 1983, pp. 5-100.

FAO/WHO (1984) List of Maximum Levels Recommended for Contaminants by the Joint FAO/WHO Codex Alimentarius Commission. 2nd Edition, FAO/WHO, Rome, 1-8.

FAO, (1986). Food and Nutrition paper manuals of food quality control food analysis: quality, adulteration and tests of identity. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO/WHO. (1989). National Research Council Recommended Dietary 626 Allowances (10th ed). National Academy Press. Washington, DC. USA.

FAO/WHO. (1991). Protein quality evaluation. Report of the Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper 51, Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO/WHO (2001) Human Vitamin and Mineral Requirements, Report of a Joint FAO/WHO Expert Consultation, Bangkok, Thailand. Food and Nutrition Division, FAO, Rome.

FAO (2002). Chemical composition Quality and quality changes in fresh fish. Available fromhttp://www.fao.org/docrep/v7180e/V7180E05.htm.

FAO, (2003). Heavy Metals Regulations Legal Notice No 66/2003. Food and Agriculture Organization Rome.

FAO, (2005). United Nation Food and agricultural organization, Nutritional Elements of fish. FAO, Rome, 2005.

FAO, (2009) Food and Agriculture Organization of the United Nations, the state of food and agriculture, livestock in the balance, Available from: http://www.fao.org/catalog/inter-e.htm. Rome, 2009. Accessed Jun 21, 2013

FAO/WHO, (2010). Joint FAO/WHO food standards programmed codex committee on contaminants in foods Fourth Session. CF/4 INF/1 April 2010, pp. 1 –86.

FAO, (2011). fisheries and aquaculture topics. Quality and safety of fish and fish products. In: FAO fisheries and aquaculture department. Rome, Italy.

FAO (Food and Agricultural Organization). (2012).The state of world fisheries and aquaculture. Retrieved from http://www.fao.org/docrep/016/i2727e/i2727e.pdf.

FAO (2016). The State of World Fisheries and Aquaculture. Food and Agriculture O rganization Rome, 2016, 200.

Fawole OO, Ogundiran MA, Ayandiran TA, and Olagunji OF. (2007). Proximate and mineral composition in some selected fresh water fishes in Nigeria. *Internet Journal of Food Safety* 9 52-55.

Fekete, K, T Marosvölgyi, V Jakobik, and T Decs. (2009). methods of assessment of n-3 long-chain polyunsaturated fatty acid status in humans: a systematic review." *American Journal of Clinical Nutrition*, 89(6):2070S-2084S.

Fernandez, G., & Venkatrammann, J. (1993). Role of omega-3 fatty acids in health and disease. *Journal of Nutrition Research*, 1 (Suppl. 13), 19–45.

Ferrari P, Jenab M, Norat T, et al. (2007). Lifetime and baseline alcohol intake and risk of colon and rectal cancers in the European prospective investigation into cancer and nutrition (EPIC). *Int J Cancer*; 121:2065–72.

Ferreira-Baptista, L.; De Miguel, E. (2005). Geochemistry and risk assessment of street dust in Luanda, Angola: A tropical urban environment. Atmos. *Journal of Environ*. 39, 4501–4512.

Field, C.J., Johnson, I.R. and Schley, P.D. (2002). Nutrients and their role in host resistance to infection. *Journal of Leukocyte Biology*, 71, 16-32.

Forsyth, D. S., Casey, V., Dabeka, R. W., & McKenzie, A. (2004). Methyl mercury levels in predatory fish species marketed in Canada. *Food Additives and Contaminants.J*, 21(9), 849–56.

Fumio K, Yasuo K, Terue K, Yoshimori K, Hideki K, Baatar P, Judger O, Ulziburen . C (2012). Influence of essential trace minerals and micronutrient insufficiencies on harmful metal overload in a Mongolian patient with multiple sclerosis. *J. Curr. Aging Sci*. 5:115-125.

Gascon A, Jacques H, Moorjani S, Deshaies Y, Brun LD and Julien P (1996). Plasma lipoprotein profile and lipolytic activities in response to the substitution of lean white fish for other animal protein sources in premenopausal women. *American Journal of Clinical Nutrition*, 63: 315-321.

Gebremedhin, S., Budusa, M.Mingist, M. and Silverberg, J. (2013). Determining factors for fisher's income: the case of Lake Tana, Ethiopia. *International Journal of Current Research.* Vol. 5, Issue, 5, pp.1182-1186.

Gebreyes YF, Goshu DY, Geletew TK, Argefa TG, Zemedu TG, Lemu KA, et al. (2018) Prevalence of high blood pressure, hyperglycemia, dyslipidemia, metabolic syndrome and their determinants in Ethiopia: Evidences from the National NCDs STEPS Survey. Journal.pone 13 (5):1-18.

Georgi I. Zhelyazkov, Dian M. Georgiev, Lilko K. Dospatliev, Yordan S. Staykov. (2014). Determination of Heavy Metals in Roach (Rutilus rutilus) and Bleak (Alburnus alburnus) in Zhrebchevo Dam Lake. *Journal of Ecologia Balkanica*, Vol. 5, pp. 15-20.

Giri. S, and Singh A.K. (2013). Assessment of human health risk for heavy metals in fish and shrimp collected from Subarnarekha river India, *International journal of environmental health research,* 1-21

Gokhan G., Mustafa C and Halit F. (2010). Length- weight relationships of marine fishes off Yumurtalık coast (İskenderun Bay), Turkey," *Turkey Journal of Zoology*, 34 101 -104.

Gonzalez CA and Riboli E (2006). Diet and cancer prevention: where we are, where we are going. *Journal of Nutrition and Cancer*, 56: 225-231.

Gordon, A., Sewmehon, D., Melaku, T. (2007). Marketing systems for fish from Lake Tana, Ethiopia: opportunities for improved marketing and livelihoods. Improving. Productivity and Market Success of Ethiopian Farmers Project, *Working Paper No. 2 ILRI, Nairobi, Kenya* 49 pp.

Goyer, R. A., & Clarkson, T. W. (2001). Toxic Effects of Metals. *In Toxic Agents. J*, pp. 811-867.

Grusak M.A. and Cakmak I. (2005). Methods to improve the crop delive ry of minerals to human and livestock. In: Broadley M.R., White P.J. (Eds.), *Plant Nutritional Genomics, Blackwel,* 265-286.

Gunnarsdottir I, Tomasson H, Kiely M, Martinez JA, Bandarra NM, Morais MG and Thorsdottir I (2008). Inclusion of fish or fish oil to weight-loss-diets for young adults; effects on blood lipids. *International Journal of Obesity*, 32: 1105-1112.

Gupta, C. P. (2014) Role of Iron (Fe) in Body. *IOSR Journal of Applied Chemistry* (IOSR-JAC), 7, 38-46.

Harlavan, Y., Almogi-Labin, A., & Herut, B. (2010). Tracing natural and anthropogenic Pb in sediments along the Mediterranean coast of Israel using Pb isotopes. *Journal of Environmental Science & Technology,* 44(17), 6576–6582.

He KA, Song Y, Daviglus ML, Liu K, Van Horn L, Dyer AR and Greenland P (2004). Accumulated evidence on fish consumption and coronary heart disease mortality: a meta - analysis of cohort studies. *Circulation. J,* 109: 2705-2711.

Hernández F, Elena, M (2012) Nutritional Richness and Importance of the Consumption of Tilapia in the Papaloapan Region (Riqueza nutricionale importancia delconsumo de la mojarra tilapia enlaregión del Papaloapan). *Redvet Rev Electrón vet*. 13:1-12.

Herrin M (2003). Nutrition councelling in the treatment of eating disorder s. Brunner-Rout ledge, New York.

Hodson, L, C M Skeaff, and B A Fielding. (2008). Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake." *Progress in Lipid Research. J,* 47(5):348-80.

Hoenselaar, R. (2012). Saturated fat and cardiovascular disease: The discrepancy between the scientific literature and dietary advice. *Journal of Nutrition*: 28(2):118-23.

Hosseini S. S. P., Hosseini S. E., Soltani M., Kamali A., Valinassab T. (2015) Proximate composition and protein electrophoresis pattern of muscle from black mouth croaker (Atrobucca nibe) in the Oman Sea. *Journal of Food Hygiene* 3(1):11-24.

Horvat, M., Nolde, N., Fajon, V., Jereb, V., Logar, M., Lojen, S., Drobne, D. (2003). Total mercury, methyl mercury and selenium in mercury polluted areas in the province Guizhou, China. *The journal of Science of the Total Environment*, 304(1-3), 231–56.

Hull, M. A. (2011). Omega-3 polyunsaturated fatty acids. *Best Practice & Research in Clinical Gastroenterology.J,* 25(4-5), 547-554.

Huss H (1995). Quality and Quality changes in fresh fish. FAO Fisheries Technical paper No. 348, Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy.

Hussien, A., Gashaw, T., Abebe, C., (2010). The Riverine Fishery Resource in Gambella Regional State. Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia, pp 78.

Ibrahim, D.; Froberg, B.; Wolf, A.; Rusyniak, D.E. (2006). Heavy metal poisoning: Clinical presentation and pathophysiology. *Clin. Lab. Med.J.* 26, 67–97.

Ikem A. and Egiebor N. (2005). Assessment of trace elements in canned fishes (mackerel, tuna, salmon, sardines and herrings) marketed in Georgia and Alabama (United States of America). *Journal of Food Composition and Analysis*, 8: 771-787.

Ilich JZ, Kerstetter JE (2000) Nutrition in bone health revisited: a story beyond calcium. *J Am Coll Nutr* 19 (6):715–737.

Inhamuns AJ, Franco MR (2008) EPA and DHA quantification in two species of freshwater fish from Central Amazonia. *J. Food Chem*. 107:587-91.

Innis, S.M., Gilley, J., & Werker, J. (2001). Are human milk long-chain polyunsaturated fatty acids related to visual and neural development in breast-fed term infants? *The Journal of Pediatrics*, 139, 532-538.

Innis SM. (2003). Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. *Journal of Paediatrics*; 143:S1–8.

Ismail, H. M. (2005). The role of omega-3 fatty acids in cardiac protection: an overview. J. *Frontiers in Bioscience,* 10, 1079–1088.

Iverson SJ, Kathryn JF, Shelley LCL (2002). Fat content and Fatty acid composition of forage fish and invertebrates in prince William Sound, Alaska: Factors contributing to among and within species variability. *Journal of Marine Ecology Progress Series* 241 161-181.

Iweala, E.E.J.; Olugbuyiro, J.A.O.; Durodola, B.M.; Fubara-Manuel, D.R.; Okoli, A.O. (2014) Metal contamination of foods and drinks consumed in Ota, Nigeria.Res. *J. Environ. Toxicol.* 8, 92–97.

Jabeen F and Chaudhry A.S. (2011). Chemical compositions and fatty acid profiles of three freshwater fish species. *Journal of Food Chemistry* 125: 991–996.

Jacques H, Noreau L and Moorjani S (1992). Effects on plasma lipoproteins and endogenous sex hormones of substituting lean white fish for other animal-protein sources in diets of postmenopausal women. *American Journal of Clinical Nutrition*, 55: 896-901.

Jacquot, R. (1961). Organic constituents of fish and other aquatic animal foods. In Fish as Food, Vol. 1, ed. G. *Borgstrom. Academic Press*, New York, USA.

Jag Pal, BN Shukla, Ashish Kumar Maurya, Hari Om Verma, Gayatri Pandey and Amitha. (2018). A review on role of fish in human nutrition with special emphasis to essential fatty acid. *International Journal of Fisheries and Aquatic Studi es.* 6(2): 427-430.

Jaishankar, M. Mathew, B. B., Shah, M. S., Gowda, K. R. S (2014). Bio sorption of Few Heavy Metal Ions Using Agricultural Wastes. *Journal of Environment Pollution and Human Health*; 2(1):1–6.

Jarić I., Višnjić-Jeftić Ž, Cvijanović G., Gačić Z., Jovanovi, L., Skorić S., Lenhardt, M. (2011). Determination of differential heavy metal and trace element accumulation in liver, gills, intestine and muscle of starlet (Acipenser ruthenus) from the Danube River in Serbia by ICP-OES. *Microchem. Journal.* 98, 77.

Javed.M, Usmani.N (2011). Accumulation of heavy metals in fishes: A human health concern. *International journal of environmental sciences*. Volume 2, No 2.

JECFA. (2011). Safety evaluation of certain food additives and contaminants. WHO Food Additive Series, 64, 1–551.

JECFA, (2003). Summary and conclusions of the sixty first meeting of the joint FAO/WHO Expert Committee on Food Additives (JECFA), Vol. 2003. Rome: FAO/WHO.

JECFA (2001) Safety evaluation of certain food additives and contaminants. Prepared by the 55th meeting of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, (WHO Food Additive Series 46). (http: // www .inchem .org/documents /jecfa/jecmono/ v46je01. htm).

JECFA, (1982). Joint FAO/WHO Expert Committee on Food Add itives. Evaluation of certain food additives and contaminants. Twenty-sixth report of the Joint FAO/ WHO expert Committee on Food Additives. Technical Report Series 683, Geneva, Switzerland, 1982. http://whqlibdoc.who.int/trs/WHO_TRS_683.pdf (a ccessed 04.02.16).

Joanna L, Elzbieta T, Marek J. L. (2009). Essential mineral components in the muscles of six freshwater fish from the Mazurian Great Lakes (northeastern Poland). *Arch. J Pol. Fish.* (2009) 17: 171-178.

Jónsdóttir M.P. (2008). Health effects of lean fish consumption in overweight and obese young adults following an energy-restricted diet for eight weeks. Master thesis, in human nutrition Department of Food Science and Human Nutrition, Faculty of Science University of Iceland. P 59

Justi KC, Hayashi C, Visentainer JV, De Souza NE, Matsushita M (2003) The influence of feed supply time on the fatty acid profile of Nile tilapia (*Oreochromis niloticus*) fed on a diet enriched with n-3 fatty acids. *J.Food Chem*. 80:489-93.

Kalagbor IA, Barisere V, Barivule G, Barile S and Bassey C. (2014). Investigation of the Presence of Some Heavy Metals in four Edible Vegetables, Bitter leaf (Vernonia amygdalina), Scent leaf (Ocimum gratissimum), water leaf (Talinum triangulare) and Fluted Pumpkin (Telfairia occidentalis) from a Cottage Farm in Port Harcourt Res. *J. of Environ and Earth Sc.* 6 (1): 18 – 24.

Kamaruzzaman, Y.B., Ong, C.M. and Rina, Z. S. (2010). Concentration of Zn, Cu and Pb in some selected marine fishes of the pahang coastal waters, malaysiay. *American journal of applied sciences*. 7(3): p. 309- 314.

Karlsson T, Rosendahl-Riise H, Dierkes J, Drevon C. A, Tell G. S & Nygård O. (2017). Associations between fish intake and the metabolic syndrome and its components among middle-aged men and women: the Hordaland Health Study. *Journal of Food & Nutrition Research,* 61:1, 1347479,

Kassahun A, Waidbacher H and Zollitsch W. (2012). Proximate composition of selected potential feedstuffs for small-scale aquaculture in Ethiopia. *Livestock Research for Rural Development* .24 (6).

Kawarazuka N. and Bene, C. (2011). The potential role of small fish species in improving micronutrient deficiencies in developing countries: Bulding evidence. *Journal of Public Health nutrition*.14, 1927-1938.

Kebede A and Wondimu T. (2004). Distribution of trace elements in muscle and organs of tilapia, Oreochromis niloticus, from lakes Awassa and Ziway, Ethiopia. *Bull. Chem. Soc. Ethiop*. 18(2), 119-130.

Keen, C.L.; Ensunsa, J.L.; Watson, M.H. (1999). Nutritional Aspects of Manganese from Experimental Studies. *Journal of Neurotoxic ology*, 20, 213-23.

Kimura, M.; Itokawa, Y. (1990). Cooking Losses of Minerals in Foods and Its Nutritional Significance. *J. Nutr. Sci. Vitaminol* (Tokyo). 36, S25-S33.

Koba K and Sugano M (1990). Dietary protein influences polyunsaturated fatty acid patterns of rat tissue lipids. *Journal of Nutritional Science and Vitaminology*, 36: 173.

Kobayashi, M., Sasaki, S., Kawabata, T., Hasegawa, K., Akabane, M. & Tsugane, S. (2001) Single measurement of serum phospholipid fatty acid as a biomarker of specific fatty acid intake in middle-aged Japanese men. *Eur. J. Clin. Nutr.* 55: 643–650.

Kwetegyeka J, Mpango G, Grahl-Nielsen O (2008) Variation in fatty acid composition in muscle and heart tissues among species and populations of tropical fish in Lakes Victoria and Kyoga. *Journal of Lipids*. 43:1017-1029.

Lauritzen, L., Jorgensen, M.H., Mikkelsen, T.B., Skovgaard, M., Straarup, E.M., Olsen, S.F., Hoy, C.E., & Michaelsen, K.F. (2004). Maternal fish oil supplementation in lactation: Effect on visual acuity and n-3 fatty acid content of infant erythrocytes. *J.Lipids*, 39, 195-206.

Lavigne C, Tremblay F, Asselin G, Jacques H and Marette A (2001). Prevention of skeletal muscle insulin resistance by dietary cod protein in high fat-fed rats. *American Journal of Physiology Endocrinology and Metabolism,* 281: 62-71.

Leidy HJ, Carnell NS, Mattes RD and Campbell WW (2007). Higher protein intake preserves lean mass and satiety with weight loss in pre-obese and obese women. *J. Obesity (Silver Spring)*, 15: 421-429.

Li Z, McNamara JR, Fruchart JC, Luc G, Bard JM, Ordovas JM, Wilson PW, Schaefer EJ. (1996). Effects of gender and menopausal status on plasma lipoprotein subspecies and particle sizes. J Lipid Res.37 (9):1886-96.

Lidwin-Kaźmierkiwicz M., Pokorska K., Prota-Sowicki M., Rajkowska M. Wechterowicz Z. (2009). Content of selected essentials and toxic metals in meat of freshwater fish from West Pomeranian, Poland. *Pol. J. Food Nutr. Sci*. 59, (3), 219.

Lim, H.S.; Lee, J.S.; Chon, H.T.; Sager, M. (2008). Heavy metal contamination and health risk assessment in thevicinity of the abandoned Songcheon Au-Ag mine in Korea. *J. Geochem. Explor.* 96, 223–230.

Llobet, J. M., Falcó, G., Casas, C., Teixidó, a, & Domingo, J. L. (2003). Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. *Journal of agricultural and food chemistry,* 51(3), 838–42.

Lunn, J, and H E Theobald. (2006). the health effects of dietary unsaturated fatty acids. *British J. Nutrition Foundation* 31(3):178-224.

MAFF. (2000). Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1997. In: Aquatic Environment Monitoring. Report No. 52. *Center for Environment, Fisheries and Aquaculture Science*, Lowestoft, UK.

Mann, J, and Truswell A, S. (2007). Essentails of human nutrition. New yourk: Oxford.pp34.

Marcotrigiano, G. O., & Storelli, M. M. (2003). Heavy metal, polychlorinated biphenyl and organochlorine pesticide residues in marine organisms: Risk evaluation for consumers. *J.Veterinary Research Communications*, 27(1 Supplement), 183–195.

Marushka L. (2018). Fish consumption and nutritional health among first nations in Canada. PhD dissertation, Department of Biology Faculty of Science, University of Ottawa Canada.

Mason, R. P., Laporte, J., & Andres, S. (2000). Factors Controlling the Bioaccumulation of Mercury, Methylmercury, Arsenic, Selenium, and Cadmium by Freshwater Invertebrates and Fish. *Archives of Environmental Contamination and Toxicology journal*, 38(3), 283-297.

Mateos, H.T., Lewandowski, P.A., and Su, X.Q. (2012). Effects of dietary fish oil replacement with flaxseed oil on tissue fatty acid composition and expression of desaturase and elongate genes. *Journal of the Science of Food and Agriculture*, 92,418–426.

Maury-Brachet, R., Durrieu, G., Dominique, Y., & Boudou, A. (2006). Mercury distribution in fish organs and food regimes: Significant relationships from twelve species collected in French Guiana (Amazonian basin). *Journal of the Science of the Total Environment*, 368, 262–27.

Mehboob S, Samreen TT, Hassan M, Nadeem S, Rafique RM (2003) Proximate composition of muscle, liver and gonad of wild and farmed Labeo rohita (Rohu). *Pak J Zool.* 35:307-310.

Meko T, Kebede A, Hussein A, Tamiru Y. (2017). Review on Opportunities and Constraints of Fishery in Ethiopia. *International Journal Poultry Fish Science*. 1(1):1-8.

Mekonnen Melaku. (2007). Effect of low temperature preservation on the physicochemical and microbiological qualities of selected fish species of lake Ziway. *Master Thesis in Food Engineering of Addis Ababa University* Ethiopia.

Memon N.N. (2011). Analytical characterization of lipids in river Indus and farm fish species from Sindh, Pakistan. Ph.D. *Thesis National Center of Excellence in Analytical Chemistry, University Of Sindh, Jamshoro*, PAKISTAN.

Mendil D., Demirci Z., Tuzen M., Soylak M. (2010). Seasonal investigation of trace element contents in commercially valuable fish species from the Black sea, Turkey. *Journal of Food and Chemical Toxicology*, 48, 865.

Mertz W. (1993). Essential trace metals: New definitions based on new paradigms. *J.Nutr Rev* 51:287–295.

Milošković A., Simić V. (2015). Arsenic and Other Trace Elements in Five Edible Fish Species in Relation to Fish Size and Weight. Potential Health Risk for Human Consumption, Serbia, *Pol. J. Environ. Stud.* Vol. 24, No. 1, 199-206.

Moiseenko, T. I., & Kudryavtseva, L. P. (2001). Trace Metal Accumulation and Fish Pathologies in Areas Affected by Mining and Metallurgical Enterprises in the Kola Region, Russia. *J.Environmental Pollution*. 114: 285-297.

Mozaffarian, D., and E.B. Rimm (2006) Fish intake, contaminants and human health evaluating the risk and benefits. *J. Am. Med. Assoc.*, 295: 1885-1899.

Mridha MA, Lipi SY, Narejo NT, Uddin MS, Kabir MS, Karim M (2005). Determination of Biochemical Composition of Cirrhinus Reba (Hamilton-1822) from Jessore, Bangladesh. *Journal of Science & Technology University Peshwar* 29(1) 1-5.

Munoz-Olivas, R., & Camara, C. (2001). Speciation related to human health. In L. Ebdon, L. Pitts, R. Cornelis, H. Crews, O. F. X. Donard, & Quevauviller (Eds.), Trace Element Speciation for Environment. *Journal of Food and Health* (pp. 331–352). Cambridge, UK: The Royal Society of Chemistry.

Murray, J. and Burt, J.R. (1969). The composition of fish. Torry Advisory. Note 38, Torry Research Station, Aberdeen. Accessed on 31 August 2016. http: // www. fao.org/ wairdocs/ tan/x5916e/x5916e00.htm

Nargis A (2006) Seasonal variation in the chemical composition of body flesh of koi fish Anabas testudineus (Bloch) (Anabantidae: Perciformes). *Bangladesh J Sci Ind Res*. 41:219-26.

Nas-NRC (1975). National Academy of Sciences-National Research Council. "Division of Medical Sciences, Medical and Environmental Effects of Pollutants Nickel," *National Academic Press,* Washington DC.

Neff, J.M. (2004). Bioaccumulation in marine organisms. Journal Elsevier Ltd, 469P.

Negi R.K and Maurya Aarti. (2015). Heavy metal concentrations in tissues of Major carp and exotic carp from Bhagwanpur fish pond, India. *Journal of fisheries and aquatic science*. Volume 10(6):543-552.

Nkondjock A and Receveur O. (2003). Fish-seafood consumption, obesity, and risk of type 2 diabetes: an ecological study. *Journal of Diabetes & Metabolism*, 29: 635-642.

NNR, (2004) Nordic Nutrition Recommendations. Integrating nutrition and physical activity. Nordic Council of Ministers, 4th edition, Copenhagen, Denmark.

Noël L, Chafey C, Testu C, Pinte J, Velge P, Guerin T. (2011). Contamination levels of lead, cadmium and mercury in imported and domestic obsters and large crab species consumed in France: differences between white and brown meat. *J. Food. Comp. Anal.*, 24: 368–375.

Nordmann AJ, Nordmann A, Briel M, Keller U, Yancy WS Jr, Brehm BJ and Bucher HC (2006). Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Archives of Internal Medicine Journal*, 166: 285 293.

NRC (1989). National Research Council (US). Food and Nutrition Board: Recommended Dietary Allowances, 10th ed. Washington, DC: National Academy Press.

Nussey, G., Van Vuren, J. H. J., & Du Preez, H. H. (2000). Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, Labeo umbratus (Cyprinidae), from Witbank Dam, Mpumalanga. *J.Water SA*, 26(2), 269–284.

Nwali B.U, Egesimba P.C, Okechukwu U, Ogbanshi M.E. (2015). Assessment of the nutritional value of wild and farmed Clarias gariepinus. *In t. J Curr Microbiol App Sci.* 4(1):179-182.

Obaroh, I. O., Haruna, M. A. and Ojibo, A. (2015). Comparative study on proximate and mineral element composition of Clarias gariepinus from the cultured and wild sources. *European Journal of Basic and Applied Sciences;* Vol. 2 No. 2,

Ogbonnya C and Ibrahim M.S. (2009). Effects of drying methods on proximate composition of cat fish (Clarias gariepinus). *World Journal of Agricultural Sciences*. 5(1):114-116.

Oladipo I. C. and Bankole S. O. (2013). Nutritional and microbial quality of fresh and dried C. gariepinus and Oreochromis niloticus. *Int. J. Appl. Microbiol. Biotechnol. Res* Vol. 3(4), pp. 3-6.

Olayemi F. F, Adedayo M.R, Bamishaiye E.I and Awagu E. F. (2011). Proximate composition of catfish (Clarias gariepinus) smoked in Nigerian stored products research institute (NSPRI): Developed kiln. *International Journal of Fisheries and Aquaculture* Vol. 3(5), pp. 96-98.

Ololade, I. A., Lajide, L., Amoo, I. A., & Oladoja, N. A. (2008). Investigation of heavy metals contamination of edible marine sea food. *African Journal of Pure and Applied Chemistry*, 2, 121 – 131.

Olsson GB, Olsen RL, Carlehog M, Ofstad R (2003). Seasonal variations in chemical and sensory characteristics of farmed and wild Atlantic halibut (Hippoglossus hippoglossus). *J.Aquaculture* 217 191–205.

Onyia, L. U, Michael, K.S., Manu, J.M. and Sabo, M. (2 013). Comparison of Nutrient Values of Wild and Cultured Heterobranchus bidorsalis and Clarias gariepinus. *Nigerian Journal of Fisheries and Aquaculture* 1(1): 7 – 12.

Onyia, L.U, Milam, C.; Manu, J.M and Allison, D.S. (2010). Proximate and mineral composit ion in some freshwater fishes in upper river Benue, Yola, Nigeria. *Continental J. Food Science and Technology* 4: 1 – 6.

Oosthuizen, J & Ehrlich, R. (2001). The impact of pollution from a mercury processing plant in KwaZulu-Natal, South Africa, on the health of fish eating communities in the area: An environmental health risk assessment. *International Journal of Environmental Health Research,* 11(1), 41– 50.

Oribhabor B.J. Ogbeibu, A.E. and Udo, M.T. (2011). The Length -Weight Relationships of Brackish Water/Marine Fish Species Assemblage in a Niger Delta Mangrove Creek, Nigeria," *Current Research Journal of Biological Sciences*, 3(6): 616 -621.

Ortega RM (2006). Importance of functional foods in the Mediterranean diet. *J.Public Health Nutrition,* 9: 1136-1140.

Osibona A.O. (2011). Comparative study of proximate composition, amino and fatty acids of some economically important fish species in Lagos, Nigeria. *African Journal of Food Science Vol.* 5(10), pp.581-588.

Osibona AO, Kusemiju K, Akande GR. (2009). Proximate composition and fatty acids profile of African catfish (*C. gariepinus*). *J.Acta Satech*; 3(1):85-89.

Osman, H., Suriah, A.R., Law, E.C. (2001). Fatty acid composition and cholesterol content of selected marine fish in Malaysian waters. *J.Food Chemistry* 73, 55–60.

Özcan, M. (2004). Mineral contents of some plants used as condiments in Turkey. *Journal of Food chemistry*, 84(3), 437-440.

Özden O. (2008). Study of seasonal heavy metal and macronutrient mineral profile of mussels using inductively coupled plasma mass spectrometry methods. *Fresenius Environmental. Journal Bulletin.* 17(9a): 1300-1306.

Özden Ö., Ulusoy S, Erkan N. (2010). Study on the behavior of the trace metal and macro minerals in Mytilus galloprovincialis as a bio indicator species: the case of Marmara Sea, Turkey. *J. Verbr. Lebensm*. 5: 407-412.

Ozkan O. (2005). Changes in amino acid and fatty acid composition during shelf-life of marinated fish. *J Sci Food Agric*. 85:2015-2020.

Ozparlak, H., Arslan, G., & Arslan, E. (2012). Determination of Some Metal Levels in Muscle Tissue of Nine Fish Species from Besysehir Lake, Turkey. *Turkish Journal of Fisheries and Aquatic Sciences.* 770: 761-770.

Paddon-Jones D, Westman E, Mattes RD, Wolfe RR, Astrup A and Westerterp -Plantenga M. (2008). Protein, weight management, and satiety. *American Journal of Clinical Nutrition*, 87: 1558-1561.

Papagiannis I., Kagalou I., Leonardos J., Pe -Tridis D., Kalfakakou V.(2004). Copper and zinc in four freshwater fish species from Lake Pamvotis (Greece). *J. Environ. Int.* 30, 357.

Papes, F., Surpili, J. S., Langone, F., Trigo, J. R., & Arruda, P. (2001). The essential amino acid lysine acts as precursor of glutamate in the mammalian central nervous system. *JFEBS Letters,* 488, 34–38.

Parra D, Ramel A, Bandarra N, Kiely M, Martinez JA and Thorsdottir I (2008). A diet rich in long chain omega-3 fatty acids modulates satiety in overweight and obese volunteers during weight loss. *Journal of Appetite*, 51, 676-680.

Patrick S (2007). Seasonal variation in highly unsaturated fatty acid composition of muscle tissue of two fishes endemic to the eastern Mediterranean. *Journal of Ecology of food and nutrition* 46 77-89.

Pawar S.M., Sonawane S. R. (2013). Fish muscle protein highest source of energy. *International Journal of Biodiversity Conservation* 5(7):433-435.

Petersen M, Taylor MA, Saris WH, Verdich C, Toubro S, Macdonald I, Rossner S. et al. (2006). Randomized, multi-center trial of two hypoenergetic diets in obese subjects: high - versus low-fat content. *International Journal of Obesity (London)*, 30: 552-560.

Peycheva K, Makedonski L and Stancheva M, (2017). Trace Elements Concentrations in Black Sea Mussel (*Mytilus galloprovincialis*) and Rapa Whelks (*Rapana venosa*) from Bulgarian Black Sea Coast and Evaluation of Possible. *Chemistry Research Journal,* 2(6):236-250.

Pieniak, Z., Verbeke, W., & Scholderer, J. (2010). Health-related Beliefs and Consumer Knowledge as Determinants of Fish Consumption. *Journal of Human Nutrition and Dietetics.* 23(5): 480-8.

Pirestani S, Sahari A, Barzegar M, Seyfabadi SJ (2009). Chemical compositions and minerals of some commercially important fish species from the South Caspian Sea. *Int. Food Res. J.* 16:39-44.

Philibert A, Fillion M, DeGuire J.R, Weiler H.A, Passos. C.J.S, Lemire M and Mergler D. (2013). Plasma Phospholipid Omega-3 Fatty Acids and Freshwater Fish Consumption in the Brazilian Amazon. *Journal of Food and Nutrition Sciences*, 4, 137-149.

Poonkothai M. and Vijayavathi S. (2012). Nickel as an essential element and a toxicant. *International Journal of Environmental Sciences*, 1(4), 285-288.

Porto H.L.R, de Castro. A.C.L, Filho V.E.M and Baptista G.R. (2016). Evaluation of the Chemical Composition of Fish Species Captured in the lower Stretch of Itapecuru River, Maranhão, Brazil. *Int'l Journal of Advances in Agricultural & Environmental Engg. (IJAAEE) Vol*. 3, Issue 1, ISSN 2349-1523 EISSN 2349-1531.

Racine, R.A. and R.J. Deckelbaum, 2007. Sources of very long chain unsaturated omega-3 fatty acids: Eicosapentaenoic acid and docosahexaenoic acid. *Curr. Opin. Clin. Nut. Metab. Care,* 10: 123-128.

Rajeswari T.R and Sailaja N. (2014). National Seminar on Impact of Toxic Metals, Minerals and Solvents leading to Environmental Pollution. *Journal of Chemical and Pharmaceutical Sciences. JCHPS Special Issue* (3) 175-181.

Ramel A, Martinez A, Kiely M, Morais G, Bandarra NM and Thorsdottir I (2008). Beneficial effects of long-chain n-3 fatty acids included in an energy -restricted diet on insulin resistance in overweight and obese European young adults. *Journal of Diabetologia*, 51: 1261-1268.

Rashed M.N. (2001). "Monitory of Environmental Heavy Metals in Fish from Nasser Lake," *Environment Internationa Journall*, Vol. 27, No.1, pp. 27-33.

Rebouche, C.J.; Carnitine, I.; Shils, M.E.; Olson, J.A.; Shike, M.; Ross, A.C. (1999). Modern Nutrition in Health and Disease. 9th ed. Philadelphia: Lippincott, Williams and Wilkins; pp. 505-512.

Reuer, M. K., & Weiss, D. J. (2002). Anthropogenic lead dynamics in the terrestrial and marine environment. Philosophical Transaction of the Royal Society of London A, 360(1801), 2889–2904.

Richard, Y. and Marcia, H. G. (2004) Cross-national meat and fish consumption: exploring the effects of modernization and ecological context. *J. Ecol. Econ.* 48, 293-302.

Riediger, N.D., R.A. Othman, M. Suh and M.H. Moghdasian (2009). A systemic review of the roles of n-3 fatty acids in health and disease. *J. Am. Diet. Assoc.*, 109: 668-679.

Rosa R, Bandarra NM, Nunes ML. (2007). Nutritional quality of African catfish *Clarias gariepinus* (Burchell1822): A positive criterion for the future development of the European production of Siluroidei. *Int. J Food Sci and Tech*. 42:342-351.

Rose, D. P., & Connolly, J. M. (1999). Omega-3 fatty acids as cancer chemo preventive agents. *Journal of Pharmacology & Therapeutics*, 83(3), 217-244.

Rosseland B.O., Rognerud S., Collen P., Grimalt J.O., Vives I., Massabuau J.-C., Lackner R., Hofer Ret al. (2007). Brown trout in Lochnagar: Population and contamination by metals and organic micro pollutants. Pale environ. Res. 12. *Journal of Springer*, 503 pp 3986-7.

Rustad, T. (2007). Physical and chemical properties of protein sea food by products. In F. shahidi (Ed.), maximizing the value of marine by product (pp.3-21). *USA: Wood head publishing.*

SADOH (2004). South African Department of Health. Regulation relating to maximum levels for metals in foodstuffs. *Foodstuffs, Cosmetics and Disinfectants Act, 1972* (Act 54 of 1972), No. R. 500(April).

Saidu, S. Garba, A. Isah, M. Mukhtar, C. Muhammad, Zubairu A. Y & Waziri F .T. (2018). Assessment of well water quality in Yabo local government area, Sokoto state, Nigeria, *International Journal of Pure & Applied Science Published by Oxford Research and Publications Vol.* 14 No.1.

Samy Y, Mohamed M, Mohamed E, Walid N. (2012). Flesh quality differentiation of wild and cultured Nile tilapia (*Oreochromis niloticus*) populations. *Afri. J Biotech*. 11(17):4086-4089.

Sánchez-Marín, P., Lorenzo, J. I., Blust, R., & Beiras, R. (2007). Humic acids increase dissolved lead bioavailability for marine invertebrates. *Journal of Environmental Science & Technology*, 41(16), 5679–5684.

Santos, E.E.; Lauria, D.C.; Porto da silveria, C.L. (2004). Assesment of Daily Intake of Trace Elements Due to Consumption of Food Stuffs by Adult Inhabitations of Rio De Janeiro City, Sci. *Journal of Total Environ*. 329, 69-79.

SCF (Scientific Committee on Food) (1993). Reports of the Scientific Committee on Food (31st series). Commission of the European Community, Luxembourg, pp. 177-189.

Schulze MB, Fung TT, Manson JE, Willett WC and Hu FB (2006). Dietary patterns and changes in body weight in women. *Journal of Obesity (Silver Spring)*, 14: 1444-1453.

Sharer KD (1994). Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Journal of Aquaculture* 119 63–88.

Shukla A, Bettzieche A, Hirche F, Brandsch C, Stangl GI and Eder K (2006). Dietary fish protein alters blood lipid concentrations and hepatic genes involved in cholesterol homeostasis in the rat model. *The British Journal of Nutrition*, 96: 674-682.

Sidhu, K. S. (2003). Health benefits and potential risks related to consumption of fish or fish oil. *Journal of Regulatory Toxicology and Pharmacology*, 38(3), 336–344.

Sivaperumal, P., Sankar, T. V, & Nair, P. G.V. (2007). Food Chemistry Heavy Metal Concentrations in Fish, Shellfish and Fish Products from Internal Markets of India Vis -A-Vis International Standards. *Journal of Food Chemistry*. 102: 612-620.

Skonberg D.I and Perkins B.L. (2002). Nutrient composition of green crab (Carcinus maenus) leg meat and claw meat. *Journal of Food Chemistry*, 72, 401–404.

Sontrop J and Campbell MK (2006). Omega-3 polyunsaturated fatty acids and depression: A review of the evidence and a methodological critique. *J. Preventive Medicine*, 42: 4-13.

Stansby, M.E. (1982). Properties of fish oils and their application to handling of fish and to nutritional and industrial use. *In. Martin RE, Fl ick GJ, Hebard CE, Ward DR (Eds) Chemistry and biochemistry of marine food products. Avi Publishing Wesport, Connecticut*. 75-92.

Stern, B.R. (2010). Essentiality and Toxicity in Copper Health Risk Assessment: Overview, Update and Regulatory Considerations. *Journal of Toxicology & Environmental Health-Part A,* 73, 114-127.

Su, X.Q., Antonas, K.N., & Li, D. (2004). Comparison of n-3 polyunsaturated fatty acid contents of wild and cultured Australian abalone. *International Journal of Food Sciences and Nutrition,* 55, 149-154.

Svobodova Z, Celechovska O, Kolara. J, Randak T and Zlabek V. (2004). "Assessment of Metal Contamination in the Upper Reaches of the Ticha Orlice River," *Czech Journal of Animal Science,* Vol. 49, No. 4, pp. 458-641.

Tao, W. and Linchun, M. (2008). Influences of hot air drying and microwave drying on nutritional and odorous properties of Grass Carp (Ctenopharyngodon idellus) fillets. *Journal of Food Chemistry* 110 (3): 647-653.

Tariku B, Amare A, Tesfaye H, Tsegu L, Alle M. (2015). Determin ation of the Levels of Major-Essential, Minor-Essential and Toxic Metals in Tilapia (Oreochromis niloticus), Nile Perch (*Lates niloticus*) and Bagrus (*Bagrus docmac*) of Lake Abaya, Ethiopia. *International Journal of Modern Chemistry and Applied Science*. 2(1), 50-56.

Tenyang N, Ponka R, Tiencheu B, Djikeng F.T and Womeni H.M, (2016). Proximate Composition, Fatty Acid and Mineral Contents of Four Freshwater Fish from Maga Lake (Far North Region of Cameroon). *American Journal of Food Science and Technology, vol.* 4, no. 3: 64 -69.

Teye, E., Asare, A. P., Amoah, R. S., & Tetteh, J. P. (2011). Determination of the dry matter content of cassava (*Manihot esculenta, Crantz*) tubers using specific gravity method, ARPN Journal of Agriculture and Biological Science. 6(11), 23–28.

Theodros G, Atkure D, Habtamu T, Geremew G, Alemayehu B, Abebe B. et al. (2017). Magnitude and predictors of excessive alcohol use in Ethiopia: Findings from the 2015 national non-communicable diseases STEPS survey. *Ethiop.J. Health Dev.* 31(Special Issue):312-319.

Thomas, Lothar. (1998).Clinical Laboratory Diagnostics, use and assembly of Clinical Laboratory Results.

Thorpe, A., (2005). Mainstreaming fisheries into national development and poverty reduction strategies: current situation and opportunities. *FAO Fisheries Circular No*. 997, FAO, Rome.

Thorsdottir I, Tomasson H, Gunnarsdottir I, Gisladottir E, Kiely M, Parra MD, Bandarra NM, Schaafsma G and Martinez JA. (2007). Randomized trial of weight -loss-diets for young adults varying in fish and fish oil content. *International Journal of Obesity (London),* 31: 1560-1566.

Tietz, N W. (2001). Text Book of Clinical Chemistry. Philadelphia: WB Saunders.

Tortora, G.J. (1997). Introduction to Human Body; the Essential of Anatomy and Physiology, *4th ed., John Wiley and Sons*: New York, pp. 472-474.

Tremblay F, Lavigne C, Jacques H and Marette A (2007). Role of dietary proteins and amino acids in the pathogenesis of insulin resistance. *Annual Review of Nutrition.J,* 27: 293-310.

Tremblay F, Lavigne C, Jacques H and Marette A (2003). Dietary cod protein restores insulin - induced activation of phosphatidylinositol 3- kinase/Akt and GLUT4 translocation to the T-tubules in skeletal muscle of high-fat-fed obese rats. *Journal of Diabetes*, 52: 29-37.

Tsegay Teame, P Natarajan, Zelealem Tesfay. (2016). Proximate and mineral composition of some commercially important fish species of tekeze reservoir and lake Hashenge, Ethiopia. *International Journal of Fisheries and Aquatic Studies*. 4(1): 160-164.

Tsuboyama-Kasaoka N, Shozawa C, Sano K, Kamei Y, Kasaoka S, Hosokawa Y and Ezaki O (2006). Taurine (2-aminoethanesulfonic acid) deficiency creates a vicious circle promoting obesity. *Journal of Endocrinology*. 147: 3276-3284.

Tuzen M. (2009). Toxic and essential trace elemental contents in fish species from the Black Sea, Turkey. J. *Food Chem. Toxicol*. 47, (8), 1790.

Tzonou, A. (1998). Dietary Iron and Coronary Heart Disease Risk: A Study from Greece. *Am. J. Epidemiol*. 147: 161-166.

USDA, (2010). United State Department of Agriculture, Agricultural Research Service, and National Nutrition Data base for standard reference. Rel ease, 23. Nutrition Laboratory.

USDA (2003). National Nutrient Database for Standard Reference, Release 16. Nutrient Data Laboratory.

USEPA (1983). Methods for Chemical Analysis of Water and Wastewater, EPA Report 600/4-79-020, Office of Water, Cincinnati, Ohio.

USEPA (1989). United States Environmental Protection Agency. Risk Assessment Guidance for Superfund, Volume I: Human Health Evaluation Manual; Office of Emergency and Remedial Response: Washington, DC, USA.

USEPA (2005). United States Environmental Protection Agency. Guidelines for carcinogen risk assessment. Risk Assessment Forum. EPA/630/P-03/001F, March, 2005.

USEPA, (2009). United States Environmental Protection Agency, Risk-based concentration table. Philadelphia: United States Environmental Protection Agency, Washington, D C.

USEPA (2015). United States Environmental Protection Agency). Regional Screening Level (RSL) Summary Table, November.

Uysal, K., Emre, Y., & Köse, E. (2008). The determination of heavy metal accumulation ratios in muscle, skin and gills of some migratory fish species by inductively coupled plasma-optical emission spectrometry (ICP-OES) in Beymelek Lagoon (Antalya/Turkey). *Micro chemical Journal*. 90 (1): 67-70.

Van Dokkum W. (1995).The intake of selected minerals and trace elements in European countries. Nutr Res Rev 8: 271-302.

Vieira C, Morais S, Ramos S, Delerue-Mato C, Oliveira M. (2011). Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: intra and interspecific variability and human health risks for consumption. *Journal of Food and Chemical Toxicology,* (49) 923-932.

Von Storch, H., Costa-Cabral, M., Hagner, C., Feser, F., Pacyna, J., Pacyna, E., & Kolb, S. (2003). Four decades of gasoline lead emissions and control policies in Europe: A retrospective assessment. *The Science of the Total Environment Journal,* 311(1-3), 151–76.

Wang, Y. Liu, Yong-Jian; Tian, Li-Xia; Xiao, Wei Ping. (2005). Effects of dietary carbohydrate level on growth and body composition of *juvenile tilapia, Oreochromis niloticus, O. aureus*. *Aquaculture Res., Oxford, vol*. 36, p. 1408-1413.

Wardlaw, G.M.; Insel, P.M. (1996). Persp ective in Nutrition, 3rd ed., Mosby Year Book: *Boston, pp.* 524-562.

Welch A.A, Bingham S.A, Ive J, Friesen M.D, Wareham N.J, Riboli E and K. T. Khaw K.T. (2006). Dietary Fish Intake and Plasma Phospholipid n-3 Polyunsaturated Fatty Acid

Concentrations in Men and Women in the European Prospective Investigation into Cancer-Norfolk United Kingdom Cohort, *The American Journal of Clinical Nutrition* , Vol. 84, No. 6, pp. 1330-1339.

Wergedahl H, Liaset B, Gudbrandsen OA, Lied E, Espe M, Muna Z, Mørk S and Berge RK. (2004). Fish protein hydrolyses reduces plasma total cholesterol, increases the proportion of HDL cholesterol, and lowers acyl-CoA: cholesterol acyltransferase activity in liver of Zucker rats. *Journal of Nutrition*, 134: 1320-1327.

Whelton SP, He J, Whelton PK and Muntner P. (2004). Meta-analysis of observational studies on fish intake and coronary heart disease. *American Journal of Cardiology,* 93: 1119-1123.

WHO: (2014). Overweight and obesity, Obesity and overweight http: // www .who.int/ media Centre /factsheets/fs311/en/.

WHO. (2011). Arsenic in Drinking-water. World Health Organaization Guidelines for Drinking-Water Quality.

FAO, (2010). Nutritional Elements of Food and Agricultural Organization Fish. Rome.

WHO, (2008). Global database on body mass index an interactive surveillance tool for monitoring nutrition transition. BMI classification. *Site downloaded from: http: // www.who. Int / bmi/Index. Jsp? Intro Page=intro3.html (Accessed June 2017).*

WHO, (2006). Obesity and overweight. Fact sheet No.311. *Site downloaded from: http://www.who.int/mediacentre/factsheets/fs311/en/index.html (Accessed June 2017).*

WHO (2005). Tin and inorganic tin compounds. World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

WHO/FAO (2002). Diet, Nutrition and the Prevention of Chronic Diseases WHO Technical Report No 916. Report of a joint WHO/FAO expert consultation. Geneva: World Health Organization.

WHO/FAO, (1998); Food and Agriculture Organization of the United Nations (FAO). Vitamin and mineral requirements in human nutrition, 2nd ed., WHO and FAO, Bangkok, Thailand, 1998, pp 1- 340.

WHO (1991). Manganese. Environmental Health Criteria 17, World Health Organization, Geneva.

WHO/FAO, (1989b). Evaluation of food contaminants. 33rd Report of the Joint FAO/WHO Expert Committee on Food Additives. Technical Report Series 776, Geneva, Switzerland.

WHO (1989a). "Environmental Health Criteria 108: Nickel. International Programme on Chemical Safety," World Health Organization, 1989. http: \\ www. Inchem .org / doc uments/ehc

WHO, (1985). World HealthOrganization, guidelines for drinking water quality. Recommendation WHO, Geneva.

Wood, R. J. (2000). Assessment of marginal Zinc status in humans. *Journal of Nutrition*, 130, 1350 -1354.

Wudneh, T., (1998). Biology and Management of Fish Stocks in Bahir Dar Gulf, Lake Tana, Ethiopia. Ph.D. Dissertation, Wageningen, the Netherlands, *Agricultural University institute of Animal Science*. 143p.

Yahia DA, Madani S, Prost E, Prost J, Bouchenak M and Belleville J (2005). Fish protein improves blood pressure but alters HDL2 and HDL3 composition and tissue lipoprotein lipase activities in spontaneously hypertensive rats. *European Journal of Nutrition*, 44: 10-17.

Yahia DA, Madani S, Prost E, Prost J, Bouchenak M and Belleville J (2003b). Tissue antioxidant status differs in spontaneously hypertensive rats fed fish protein or casein. *The Journal of Nutrition*, 133: 479-482.

Yahia DA, Madani S, Savelli JL, Prost J, Bouchenak M and Belleville J. (2003a). Dietary fish protein lowers blood pressure and alters tissue polyunsaturated fatty acid composition in spontaneously hypertensive rats. *Journal of Nutrition*, 19: 342-346.

Yalew A. (2012). Fish production, processing and utilization in the Lake Tana fisheries. Project report, *Polish development cooperation programme*. Pp 20-23.

Yeannes, Maria Isabel.; Almandos, Maria Elsa. (2003). Estimation of fish proximate composition starting from water content. *J. Food Compos. Anal., San Diego*, vol. 16, p. 81-92.

Yilmaz, A. B., Sangün, M. K., Yağlıoğlu, D., & Turan, C. (2010). Metals (major, essential to non-essential) composition of the different tissues of three demersal fish species from İskenderun Bay, Turkey. *Food Chemistry*, 123, 410.

Yohannes B.Y (2014) levels and effects of organ chlorine pesticides and heavy metals in aquatic ecosystem from the rift valley region, Ethiopia. *A Dissertation degree of doctor of philosophy. Hokkaido University* Sapporo, Japan.

Yohannes, Y.B., Ikenaka, Y., Nakayama, S.M.M., Saengtienchai, A., Watanabe, K., Ishizuka, M., (2013). Organochlorine pesticides and heavy metals in fish from Lake Awassa, Ethiopia: Insights from stable isotope analysis. *Chemosphere* 91, 857–863.

Ysart, G., Miller, P., Croasdale, M., Crews, H., Robb, P., Baxter, M., Harrison, N. (2000). 1997 UK Total Diet Study dietary exposures to aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc. *Journal of Food Additives and Contaminants,* 17(9), 775–86.

Zarazua G, Avila-perez P, Tejeda S, Barcelo-Quintal I and Martineth T. (2006). Analysis of total and dissolved heavy metals in surface water of a Mexican polluted river by total reflection X-ray florescence spectroscopy, Spectrochimia Acta part B: *Atomic Spectroscopy,* 61, 1180-1184.

Zeitoun, M. M., & Mehana, E. E. (2014). Impact of Water Pollution with Heavy Metals on Fish Health : Overview and Updates. *Global Veterinaria Jor.* 12(2): 219-231.

Zelalem A.A, Molla G, Kidist N.T, Meseret T and Dagmawi A. (2015). Risk Factors of Overweight and Obesity among High School Students in Bahir Dar City, North West Ethiopia: School Based Cross-Sectional Study. *Hindawi Publishing Corporation Advances in Preventive Medicine Volume 2015,* pp 2-9.

Zenebe T, Ahlgren G, Gustafsson IB, Boberg M (1998a) Fatty acid and lipid content of Oreochromis niloticus L. in Ethiopian lakes dietary effects of phytoplankton. *J. Ecol Freshw Fish.* 7:146-58.

Zenebe T, Ahlgren G, Boberg M (1998b) Fatty acid content of some freshwater fish of commercial importance from tropical lakes in the Ethiopian Rift Valley. *J Fish Biol.* 53:987-1005.

Zenebe T. (1988). Studies on some aspects of the biology of *Oreochromis* niloticus L. (*Pisces: Cichlidae*) in Lake Zeway, Ethiopia. M.Sc thesis. Addis Ababa University. 78.

Zenebe Tadesse. (2010). Diet composition impacts the fatty acid contents of Nile Tilapia, Oreochromis niloticus L., in Ethiopian highland lakes, Verh. *Internat. Verein.J. Limnol.* 30(9):1363-1368.

Zenebe T, Abeba W G, Mulugeta J, Fekadu T and Fasil D. (2012).Effect of supplementary feeding of agro indistryial by products on the growth performance of Nile tilapia (*O. Niloticus L.*) in concert pond. *Ethiop. J. Biol.sci.* 11 (1): 29-41.

Zhang, H.; Wang, Z.F.; Zhang, Y.L.; Hu, Z.J. (2012). The effect s of the Qinghai Tibet railway on heavy metals enrichment in soils. *Jour. Sci. Total Environ*. 439C, 240–248.

Zhang M, Bi LF, Fang JH, Su XL, Da GL, Kuwamori T and Kagamimori S (2004). Beneficial effects of taurine on serum lipids in overweight or obese no n-diabetic subjects. *Journal of Amino Acids*, 26: 267-271.

Zhang X and Beynen AC (1993). Influence of dietary fish proteins on plasma and liver cholesterol concentrations in rats. *The British Journal of Nutrition*, 69: 767-777.

Zinabu, G.M. and Desta, Z. (2002). The Chemical Composition of the Effluent from Hawassa Textile Factory and Its Effects on Aquatic Biota. SINET: *Ethiopian Journal of Science*, 25, 263-274.

Zmijewski T, Kujawa R, Jankowska B, Kwiatkowska A, Mamcarz A. (2006). Slaughter yield, proximate and fatty acid composition and sensory properties of rapfen (*Aspius aspius L.*) with tissue of bream (*Abramis brama L.*) and pike (*Exox lucius L.*) *J Food Comp Anal.* 19:176-181.

Zygmunt, U., Joanna, S, Maria, A., (2008). Protein quality and amino acid profiles of fish products available in Poland, Sea Fisheries *Institute in Gdynia, Testing Laboratory, ul, 1Kolla, taja Str., Gdynia* PL 81-332, Poland.

ANNEXES

Annex A-mineral elements concentration (mg/kg dry basis and wet basis) of selected fish species from four fresh water bodies

Element	Tissue	Fe		Cu		Zn		Ni		Mn	
		DW	WW	DW	WW	DW	WW	DW	WW	DW	WW
Alwero Tilapia	muscle	51.66	10.13	5.05	0.99	20.21	3.96	0.08	0.02	3.83	0.75
	gill	58.38	11.45	10.87	2.13	41.11	8.06	1.87	0.37	69.82	13.69
	average	55.02	10.79	7.96	1.56	30.66	6.01	0.98	0.19	36.83	7.22
Abay Tilapia	muscle	55.35	11.78	1.57	0.33	23.78	5.06	1.33	0.28	10.11	2.15
	gill	122.29	26.02	4.39	0.93	47.13	10.03	1.99	0.42	37.85	8.05
	average	88.82	18.90	2.98	0.63	35.46	7.54	1.66	0.35	23.98	5.10
Hawassa Tialpia	muscle	49.77	10.59	1.31	0.28	25.99	5.53	0.74	0.16	3.85	0.82
	gill	60.37	12.84	1.32	0.28	44.49	9.47	0.66	0.14	2.48	0.53
	average	55.07	11.72	1.32	0.28	35.24	7.50	0.70	0.15	3.17	0.67
Ziway Tilapia	muscle	52.33	11.38	0.74	0.16	27.07	5.88	0.82	0.18	3.20	0.70
	gill	62.02	13.48	1.54	0.33	37.67	8.19	0.65	0.14	3.00	0.65
	average	57.18	12.43	1.14	0.25	32.37	7.04	0.74	0.16	3.10	0.67
Alwero Cat fish	muscle	41.08	8.93	7.92	1.72	26.48	5.76	0.99	0.22	4.45	0.97
	gill	80.69	17.54	2.04	0.44	43.74	9.51	1.55	0.34	28.94	6.29
	average	60.89	13.24	4.98	1.08	35.11	7.63	1.27	0.28	16.70	3.63
Abay Cat fish	muscle	54.30	10.06	8.28	1.53	27.07	5.01	1.07	0.20	16.90	3.13
	gill	33.54	6.21	0.75	0.14	21.47	3.98	0.92	0.17	8.41	1.56
	average	43.92	8.13	4.52	0.84	24.27	4.49	1.00	0.18	12.66	2.34
Hawassa Cat fish	muscle	89.04	22.26	1.07	0.27	24.39	6.10	0.83	0.21	3.55	0.89
	gill	95.87	23.97	0.66	0.17	25.14	6.29	1.15	0.29	3.71	0.93
	average	92.46	23.11	0.87	0.22	24.77	6.19	0.99	0.25	3.63	0.91
Ziway cat fish	muscle	66.23	18.92	0.89	0.25	30.76	8.79	1.86	0.53	3.48	0.99
	gill	90.46	25.85	1.30	0.37	28.39	8.11	1.55	0.44	4.47	1.28
	average	78.35	22.38	1.10	0.31	29.58	8.45	1.71	0.49	3.98	1.14

WW= wet weight, DW= dry weight

Annex B- Proximate composition muscle tissue (g/100g dry basis and wet basis) of selected fish species from four fresh water bodies

Fish species		Dry basis				Wet basis			
O. niloticus.	Parameters	Alwero River		Abay River		Alwero River		Abay River	
		male	female	male	female	male	female	male	female
	Moisture%	8.03	7.75	11.76	12	81.43	79.46	76.44	80.62
	Protein%	67.09	65.91	58.66	58.45	13.15	12.92	12.48	12.44
	Fat %	12.81	13.36	12.99	12.95	2.51	2.62	2.76	2.76
	Ash%	4.15	4.49	4.1	4.03	0.81	0.88	0.87	0.86
	CHO	7.92	8.49	12.49	12.57	1.55	1.66	2.66	2.67
C. gariepinus	Moisture%	10.56	9.81	10.93	11.22	79.37	77.07	83.32	79.32
	Protein%	67.27	66.79	58.07	58.66	14.62	14.52	10.75	10.86
	Fat %	13.92	14.24	11.41	11.42	3.03	3.10	2.11	2.11
	Ash%	6.98	7.14	5.91	7.41	1.52	1.55	1.09	1.37
	CHO	1.27	2.02	13.68	11.29	0.28	0.44	2.53	2.09
		Dry basis				Wet basis			
O. niloticus.	Parameters	L. Hawassa		L. Ziway		L. Hawassa		L. Ziway	
		male	female	male	female	male	female	male	female
	Moisture%	8.8	8.76	10.77	10.68	79.4	78.23	78.86	77.37
	Protein%	60.34	59.21	70.01	69.65	12.84	12.60	15.22	15.14
	Fat %	11.2	10.12	10.78	11.03	2.38	2.15	2.34	2.40
	Ash%	3.78	4.97	3.01	3.97	0.80	1.06	0.65	0.86
	CHO	24.68	25.7	16.2	15.35	5.25	5.47	3.52	3.34
C. gariepinus	Moisture%	7.96	8.21	8.21	7.06	74.76	74.48	71.15	71.11
	Protein%	71.75	72.44	70.82	70.43	17.94	18.11	20.23	20.12
	Fat %	14.48	15.05	15.58	16.16	3.62	3.76	4.45	4.62
	Ash%	5.43	5.11	5.04	5.16	1.36	1.28	1.44	1.47
	CHO	8.34	7.4	8.54	8.25	2.09	1.85	2.44	2.36

Annex C- Heavy metal concentration (mg/kg dry basis and wet basis) of selected fish species from four fresh water bodies

Source	Fish species	Tissue	Co		Cr		Cd		Hg		Sn		Pb		As	
			d.w	w.w	d.w	w.w	d.w	w.w	d.w	w.w	d.w	w.w	d.w	w.w	d.w	w.w
Alwero	*O. niloticus*	Muscle	0.08	0.02	1.55	0.30	0.08	0.02	0.81	0.16	51.5	10.10	0.49	0.10	ND	ND
		Gill	0.65	0.13	3.32	0.65	0.41	0.08	1.46	0.29	126.74	24.85	3.73	0.73	3.35	0.66
		Average	0.37	0.07	2.435	0.48	0.25	0.05	1.135	0.22	89.12	17.47	2.11	0.41	1.68	0.33
	C. gariepinus	Muscle	0.08	0.02	1.73	0.38	0.16	0.03	1.32	0.29	5.03	1.09	0.08	0.02	0.16	0.03
		Gill	0.49	0.11	3.11	0.68	0.41	0.09	1.72	0.37	7.77	1.69	3.02	0.66	12.83	2.79
		Average	0.29	0.06	2.42	0.53	0.29	0.06	1.52	0.33	6.4	1.39	1.55	0.34	6.50	1.41
Abay	*O. niloticus*	Muscle	0.41	0.09	2.57	0.55	0.33	0.07	1.57	0.33	6.55	1.39	1.91	0.41	17.65	3.76
		Gill	1.16	0.25	3.4	0.72	0.58	0.12	2.4	0.51	12.18	2.59	4.56	0.97	11.18	2.38
		Average	0.79	0.17	2.99	0.64	0.46	0.10	1.99	0.42	9.37	1.99	3.24	0.69	14.42	3.07
	C. gariepinus	Muscle	0.16	0.03	2.13	0.39	0.25	0.05	1.56	0.29	4.59	0.85	0.82	0.15	30.92	5.73
		Gill	0.08	0.01	1.19	0.22	0.17	0.03	1.25	0.23	4.66	0.86	0.42	0.08	0.58	0.11
		Average	0.12	0.02	1.66	0.31	0.21	0.04	1.405	0.26	4.625	0.86	0.62	0.11	15.75	2.92
Hawassa	*O. niloticus*	Muscle	ND	ND	1.48	0.31	0.16	0.03	0.74	0.16	0.9	0.19	ND	ND	ND	ND
		Gill	ND	ND	1.49	0.32	0.08	0.02	0.66	0.14	0.33	0.07	ND	ND	0.5	0.11
		Average	ND	ND	1.49	0.32	0.12	0.03	0.7	0.15	0.615	0.13	ND	ND	0.25	0.05
	C. gariepinus	Muscle	ND	ND	2.73	0.68	0.08	0.02	0.99	0.25	0.74	0.19	ND	ND	13.48	3.37
		Gill	0.08	0.02	4.95	1.24	0.16	0.04	0.82	0.21	35.12	8.78	ND	ND	0.99	0.25
		Average	0.04	0.01	3.84	0.96	0.12	0.03	0.905	0.23	17.93	4.48	ND	ND	7.235	1.81
Ziway	*O. niloticus*	Muscle	ND	ND	2.13	0.46	0.08	0.02	14.52	3.16	1.48	0.32	ND	ND	ND	ND
		Gill	ND	ND	1.46	0.32	0.08	0.02	0.65	0.14	222.89	48.45	ND	ND	3.32	0.72
		Average	ND	ND	1.795	0.39	0.08	0.02	7.585	1.65	112.19	24.39	ND	ND	1.66	0.36
	C. gariepinus	Muscle	ND	ND	4.7	1.34	0.08	0.02	0.57	0.16	0.57	0.16	ND	ND	ND	ND
		Gill	ND	ND	4.72	1.35	0.16	0.05	0.41	0.12	2.68	0.77	ND	ND	ND	ND
		Average	ND	ND	4.71	1.35	0.12	0.03	0.49	0.14	1.63	0.46	ND	ND	ND	ND

w.w = wet weight, d.w= dry weight, ND= No detected

Annex D- Proximate composition gill tissues (g/100g wet and dry basis) of selected fish species from four fresh water bodies

Sources	Nile Tilapia	Protein%		Fat %		Ash%		Carbohydrate%		Moisture%	
	Sex	w.w	d.w	w.w	d.w	w.w	d.w	w.w	d.w	w.w	d.w
Alwero River	male	11.46	38.39	3.32	11.11	5.32	7.84	7.77	6.04	70.19	6.62
	female	11.37	38.53	3.62	2.26	5.33	8.08	7.18	4.33	70.46	6.80
Abay River	male	14.34	45.47	3.47	11.00	3.87	12.27	6.79	21.53	68.47	9.73
	female	11.65	39.39	3.21	10.86	3.71	12.53	8.33	28.16	70.39	9.06
Lake Hawassa	male	16.49	54.25	2.96	9.74	4.27	14.05	6.67	21.96	69.65	7.21
	female	17.03	55.18	2.99	9.69	4.83	15.66	3.8	19.47	69.15	7.17
Lake Ziway	male	17.31	56.08	2.93	9.50	4.46	14.44	2.88	19.98	69.13	10.66
	female	17.4	54.80	2.88	9.07	4.92	15.50	3.66	20.63	68.21	9.11
	African cat fish										
Alwero River	male	10.98	35.02	3.83	2.22	7.12	22.72	7.11	2.68	68.65	7.36
	female	11.43	35.88	3.99	2.53	7.26	22.81	6.77	1.25	68.15	7.53
Abay River	male	10.92	42.59	2.67	10.43	5.66	22.09	4.34	16.91	74.33	7.98
	female	10.23	38.66	2.7	10.02	6.02	22.74	5.35	20.24	73.54	8.16
Lake Hawassa	male	17.26	56.60	3.72	12.21	4.84	15.88	2.73	15.31	69.52	6.37
	female	18.18	57.44	4.04	12.76	4.89	15.46	2.09	14.34	68.35	7.72
Lake Ziway	male	19.58	58.35	4.39	13.07	5.12	15.26	2.08	13.32	66.40	7.11
	female	20.51	59.47	4.81	13.95	5.26	15.25	1.5	11.33	65.54	6.97

w.w= wet weight, d.w= dry weight