Proximate Composition and Determination of Heavy Metal Concentration from Water and in Common Fish Species of Lake Tana

By: Hirut Geremew

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Advisors; Melese Abdisa (PhD)
Goraw Goshu (MSc)

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Approved by

	Signature	Date
Melesse Abdisa (PhD) (Advisor)	for	2/12/2014
Seyuom Leta (PhD) (Examiner)		10/11/2014
Tilahun Bekele (MSc) (Examiner)		2/12/2014
Dawd Gashu (PhD) (Chairman)		10/11/2014

DECLARATION

I, the undersigned, declare that this thesis is my original work and that all sources of materials used have been duely acknowledged.

Name......Hirut Geremew..................

Signature..

Date..........2/12/2014..........................

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LIST OF ACRONYMS AND ABBREVIATIONS

AAS	Atomic absorption spectrometer
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical chemists
ATSDR	Agency for Toxicology Substances and Disease Registry
EC	Electrical Conductivity
EU	European Union
EWI	Estimated Weekly Intake
FAO	Food and Agriculture Organization
FEPA	Federal Environmental Protection Agency
GFAAS	Graphite furnace Atomic Absorption Spectroscopy
LFDP	Lake Fisheries Development Project
MDL	Method detection limit
ND	Not detected
PH	Hydrogen Ion Concentration
PPMR	AAS reading of digest
PTWI	Provisional Tolerable Weekly Intake
PUFA	Poly Unsaturated Fatty Acid
SPSS	Statistical Package for Social Science
TDS	Total Dissolved Solid
UNEP	United State Environmental Protection Authority
USFDA	United State Food Drug Administration
UNEP	United Union Environment Program
WHO	World Health Organization

ABSTRACT

Three main fish species commonly utilized by commercial gillnet fishery from Lake Tana are Labeobarbus species, C.gariepinus and O.niloticus. As fish constitute an important part of human diet, it is imperative to examine the quality and safety aspects of fish that are of particular interest. Fish body composition is affected by exogenous and endogenous factors. A wide range of heavy metals are continuously introduced in to the aquatic environment mainly due to anthropogenic pollution and hence accumulated in fish tissue. As matter of afore-mentioned facts, this study was aimed to investigate the proximate composition of often used fish species, and determined heavy metal (Cr, Cd and Pd) concentration of the common fish species and habituated water of Lake Tana. Six sites were systematically selected from Lake Tana. The selections of the sampling sites were based on their proximity to expected anthropogenic emission sources. A total of 72 fish specimens were collected along with six samples (replicated three time per site) water of Lake Tana in February 2014. At the spot of sample collection, measurments for temperature, pH, electrical condactivity, and total dissolved solids. Both fish and water samples were transported to Food Science and Nutrition laboratory under cool and aseptic conditions using ice box. Samples were dried at 60 °C for 72 hrs, pulverized in a mortar, and stored in polyethylene bag until analysis for proximate composition and heavy metal concentration. Analyses were made based main effects: location, sex and species.Proximate composition was determined following the procedure of AOAC while heavy metals were wet digested and estimated using GFAAS. Data were analyzed using factorial treatment arrangement of ANOVA. There was significant variation between sex, location and species with regard to nutritional value. Generally, female fish had higher nutritional value as compared to their counter male. Proximate composition significantly varied between different sampling locations. O. niloticus had significantly highest levels of protein (18.82%) compared to both C. gariepinus (15.2%) and L.intermedius (15.44%) fish species. However, L.intermedius had significantly highest fat content (2.36%) and gross energy (83.1Kcal/100g) relative to both L.intermedius and C. gariepinus. Likewise there was significant variation on heavy metal accumulation between different sampling location and fish species. The trend of heavy metals accumulation were in the order of magnitude of Cr > Cd > Pd in all sampling location and fish species.The heavy metal concentration and physicochemical variables of Lake Tana were within the range of the recommended limit of WHO (2008). Anthropogenic pollution of the lake seems to pose no threat of heavy metal accumulation regarding the studied fish species. The heavy metal content of the studied fish species remained in line with food safety and quality regulation of WHO (1993) guidline for consumption. Hence, it was concluded that the consumption of L.intermedius, C. gariepinus, O. niloticus from lake Tana are good sources of protein and other nutrients and may not lead to health hazards induced by heavy metals.

Key words: LakeTana. *L.intermedius, C. gariepinus, O. niloticus* · Proximate composition · Heavy metals · Physico-chemical · Bioaccumulation. Food safety

1. INTRODUCTION

Fish, as human food, are considered source of protein, polyunsaturated fatty acids particularly omega-3 fatty acids, Calcium, Zinc and Iron. And it is considered one of the high nutrient sources for humans that contribute the lower the blood cholesterol and reduce the risk of stroke and heart diseases (Ali *et al.,* 2012; Krishna *et al.,* 2014). It is also sole accessible and affordable source of animal protein for poor households (Onyia *et al.,* 2010). Next to meat, fish is the only protein source that contains all the essential amino acids in right proportion and called complete protein. So consumption of fish provides important nutrients to a large number of people in the world and makes a very significant contribution to nutrition (Sandhya and Smita, 2013).

However the feeding habit, sex, species, seasonal variation and other factors greatly affect the nutrient composition of an individual fish species. Determination of some proximate profiles such as protein content, lipid, ash and other nutrients is often necessary to ensure that they are within the range of dietary requirement and commercial specifications (Stancheva1 *et al.,* 2013).

Since fish is a good source of human diet, furthermore it is the main route of exposure to heavy metals; therefore it is not surprising that polluted fish could be a dangerous dietary source of certain toxic heavy metals. A wide range of contaminants are continuously introduced into the aquatic environment mainly due to increased industrialization, technological development, growing human population, oil exploration and exploitation, agricultural and domestic wastes run-off (Akan *et al,* 2012). When released into the environment, deposited in aquatic organisms like fishes through the effects of bioconcentration, bioaccumulation and the food chain process and eventually threaten the health of humans that consume them (Enuneku *et al.,* 2013).

Among these contaminants, heavy metals constitute one of the most dangerous groups because of their persistent nature, toxicity, tendency to accumulate in organisms and undergo food chain amplification and more still, they are non-degradable (Enuneku *et al.,* 2013). Some of these metals are toxic to living organisms even at low concentrations, whereas others are biologically essential and become toxic at relatively high concentrations. When ingested in excess amounts heavy metals combine with body's biomolecules, like proteins and enzymes to form stable biotoxic compounds, thereby mutilating their structures and hindering them from the bioreactions of their functions (Javed and Usmani, 2011).

Like in other organisms, heavy metals are not destroyed by humans. Instead, they tend to accumulate in the body and can be stored in soft and hard tissues such as liver, muscles and bone and threaten the health of humans (Krishna *et al.,* 2014).There concentrations in aquatic ecosystems are usually monitored by measuring of concentrations in water, sediments and biota (Forero *et al.,* 2009).

Pollutants enter fish through a number of routes: via skin, gills, oral consumption of water, food and non-food particles (Nwani *et al.,* 2010). Fishes often accumulate large amounts of certain metals and assimilate them through ingestion of suspended particulates, food materials and sometimes by constant ion exchange process of dissolved metals across lipophilic membranes like the gills and adsorption of dissolved metals on tissue and membrane surfaces which becomes toxic at high levels and exert harmful effects on fish. Such metals are finally transferred to other animals including humans through the food chain (Asante *et al.,* 2014).

The amount of a metal bioaccumulated is influenced by various environmental and biological factors, leading to differences in metal bioaccumulation between different individuals, species, seasons, sites feeding habits, bio-concentration capacity of each species and the metal concentrations in the environment could be responsible for metals accumulation in various tissues in fishes (Adegbola *et al.,* 2012; Asante *et al.,*2014). The present study was carried out to investigate the nutritional value and bioaccumulation of heavy metals (Cr, Cd and Pb) in *C. gariepinus* and *O. niloticus* and *L.intermedius* from lake Tana. Also to investigated the heavy metal concentration of water and water quality mesurment (pH, Temperature, Total dissolved solids and Electrical conductivity) of lakeTana.

1.1. Statement of the problem

Published previous studies related to the present work in the study area are scarce. Despite the fact that Bahir Dar town is one of the fast growing urban and tourist centers in the country which is expected to flourish safe and quality food, the nutritional composition of fish species from lake Tana weren't comprehensively studied. Also effect of possible pollution on heavy metal concentration of biotic factors of Lake Tana remained unknown. No prior systematic study was conducted to understand the proximate composition of commonly used fish species by classifying them into such as species, size, gender, feeding season and physical activity (Ali *et al.,* 2012). On the other hand, chemical and biological composition of fish is subject to the general environment, genetic factors and their interaction (Dadebo *et al.,* 2013).

Depending on a city's level of waste management, such waste may be dumped in an uncontrolled manner, segregated for recycling purposes, or simply burnt. Poor waste management poses a great challenge to the well-being of city residents, particularly those living adjacent the dumpsites due to the potential of the waste to pollute water, food sources, land, air and vegetation. The poor disposal and handling of waste thus leads to environmental degradation, destruction of the ecosystem and poses great risks to public health (Raja *et al.,* 2009; Akan *et al.,* 2012). For instance, in Philippines *Labeobarbus* species in Lake Lanao has almost disappeared due to anthropogenic activities (Gebremedhin *et al*, 2013).

Apart from the scanty thorough study, lakes in Ethiopia may not be presumed free of these threats. As matter of these general facts, the biotic factors in lake Tana could pose worry because of the lake's location near the town of Bahir Dar and its surrounding which has become the focus of industry, recreational facilities in connection to urban tourism development. Consequently, the amount of waste being discharged directly into ground and surface water sources by activities in and around Bahir Dar is increasing (Mengistu, 2003) and hence anthropogenic activities of the Lake Tana and its adjoining wetlands are increasing with implication of profound negative impacts upon the biological, chemical and physical processes essential to maintaining the structure and functions of the Tana lake ecosystems (Minale and Kameswara, 2011). Sediments as well as organic and inorganic fertilizers from the agricultural fields that enter the lake by runoff may as well result in local eutrophication of the lake in question (Wondie ,2009).

Obviously, metal pollution could damage marine organisms at the cellular level and possibly affect the ecological balance. Fish can only metabolize heavy metals to a lesser extent because most of them are non biodegradable. Considering the various health risk and nutritional benefits associated with fish consumption, it has become important that examination of proximate composition and safety of fillets of fish could be examined in order to establish the safety level of table-sized fish species before consumption (Onyia *et al.,* 2010). Therefore, it was imperative that efforts be galvanized to undertake research hypothesizing that pollution of Lake Tana had no negative impact on safety and quality edible fish species in the lake.

1.2. Objective

General objective

The general objective of this study was to analyses the quality and safety of commonly edible fish species located in Lake Tana

Specific objectives

- To determine proximate composition of selected fishes fillet collected from lake Tana
- To determine pH, temperature,Total dissolved solids and Electrical condactivity of Lake Tana water
- To estimate accomulation of selected heavy metals in commonly edible fishes and their concentration in water from Lake Tana
- To examine compliance of of the concentration of each heavy metal in each selected fish species samples to the interest of consumers health

1.3. Significance of the study

Currently, Ethiopia has set no guideline values on the levels of heavy metals in fish resources. Therefore an effort to produce baseline data on the nutritional benefit associated with fish consumption in the studied lake could contribute to set in information. Also the findings of some proximate profiles could be necessary to ensure that they meet the dietary requirements and commercial specifications (Ondo-az *et al.,* 2013; Monalisa *et al.,* 2013). Further, the information is useful to help consumers choosing their preferred fish species for their nutritional security.

Moreover, if the pollution level of lake Tana in terms of accumulation of heavy metals in commonly consumed fish species: *C. gariepinus, O. niloticus* and *L. intermedius* is known, tourists aiming to recreate around Bahir Dar city will feel confidence and hence the study could contribute to promotion of urban tourism.

2. LITERATURE REVIEW

2.1. Fishery in Ethiopia

Fishery products are highly nutritious and an excellent means of obtaining dietary essentials, like protein, minerals and vitamins. The flesh of a fish in good condition is made up of five main chemical components namely protein, lipid, water, minerals and vitamins (Pawar and Sonawane, 2013). Generally it is appreciated as one of the healthiest and cheapest source of protein 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 populace because of its high palatability, low cholesterol and tender flesh (Onyia *et al.,* 2010).

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 50s, the rapid population growth, which resulted in a shortage of cultivable land and depletion of land resources, forced the people to look for other occupations and sources of food from water resources at a subsistence level Also, 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).

In 2008, the fish catch in Ethiopia was approximately 17,000 tons. The bulk of which, 74% originated from the six main lakes (Tana, Ziway, Langano, Awassa, Abaya and Chamo) and further 26% from other water bodies. 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 through proper management. The general view seems to be that the lakes in the south are heavily exploited. Only in 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). On the moment the Lake Tana fisheries contributes annually about 65 million Ethiopian Birr to the economy of the Amhara region. Approximately about 20% of the catch (14 million Ethiopian Birr's) is exported to Sudan (Gebremedhin *et al.*, 2013).

Ethiopians do not consume large quantities of fish, although there is no religious prohibition for the Christian and Moslem populations. 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 during those periods, though some strict followers will not eat any animal products. Tilapia is the dominant species caught and consumed in Ethiopia, 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 preferences and/or abundance: Nile perch (where available) is most expensive, followed by tilapia, catfish and barbus. Tilapia retains its value despite relative abundance, indicating strong consumer demand (Gordon *et al.,* 2007).

2.2. Selected fish species in Lake Tana for the study

Fishing in the Lake Tana fisheries is both artisanal and commercial. These days artisanal fishing (use of reed or papyrus boats) is being replaced by commercial fisheries using motorized boats. Fishing is mainly made in the north eastern flood plain of lake Tana where mass of Nile Tilapia fishes prefer and *Labeobarbus* fishes migrate for spawning. *Clarias gariepinus* is targeted by the commercial gillnet fishery when migrating between the flood plains (spawning areas) and the lake (Anteneh *et al.,* 2012).

Fish produced from Lake Tana varies by species, time and space (Yalew, 2010). The three main species groups targeted by commercial gillnet fishery are the *Labeobarbus* spp., African catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*) in lake Tana. *O. niloticus* is most abundant in the shallow littoral zone, while *C. gariepinus* and the larger piscivorous *Barbus* species are found mainly in the deeper open water area of the lake (Wudneh, 1998).

Although the large, older individuals proved to be vulnerable for increased mortality by the commercial gillnet fishery, it is known that, compared with *Labeobarbus* spp., *C. gariepinus* is only moderately susceptible to fishing pressure in Lake Tana. This is because *C. gariepinus* is found to be more resilient. However; *C. gariepinus* recently has become highly preferred fish by the commercial fishermen in Lake Tana area for dry fish export, especially to Sudan (Anteneh *et al.,* 2012).

O. *niloticus* is family *Cichlidae* and it is predominantly herbivore, feeding on macrophytes, algae and detritus (LFDP, 1998). The contemporary *Labeobarbus* species of Lake Tana (Ethiopia) form the only known remaining intact species flock of large cyprinid fishes, since the one in Lake Lanao in the Philippines has almost disappeared due to anthropogenic activities (Gebremedhin *et al.*, 2013).

The African big barb *Labeobarbus intermedius* is widely distributed in Northern Kenya and in most parts of Ethiopia. It is Benthopelagial, mainly littoral, Carnivorous (Mollusks, aquatic insects, fish). It is one of the commercially important fish species in Ethiopian fisheries (Dadebo *et al.,* 2013). African catfish (*Clarias gariepinus*) of the family *Claricdae,* Benthopelagial, Carnivorous Fish, (aquatic insects, mollusks) it is generally considered to be one of the most important tropical catfish species for aquaculture (Dsikowitzky *et al.*, 2012). It is the most dominant species during the rainy season upstream of the turbid Ribb River probably due to the availability of extended floodplain. When the water level starts to decrease (October December), *C. gariepinus* migrates back through the littoral zone towards the pelagiczone (Lake Tana) (Anteneh *et al.,* 2012).

2.3. Proximate compositions of fish

Proximate body composition is the analysis of water, fat, protein and ash contents of fish. Carbohydrates and non-protein compounds are presented in negligible amount and are usually ignored for routine analysis (Aberoumand, 2012). The measurement of some proximate profiles is often necessary to ensure that they meet the dietary requirements and commercial specifications (Onyia, 2010; Monalisa *et al.,* 2013; Ondo-az *et al.,* 2013). Fish is the cheapest source of animal protein and other essential nutrients required in human diet particularly of the low and middle income groups (Fawole *et al.,* 2007).

Water is required for the normal functioning of many biological molecules. It is present in two forms in the tissues, bound to the proteins and in the free form. These forms have well defined biological roles. There exists an inverse relationship between the water and lipid content of fish and the summation of both frequently spans a range of 78 to 88 %(Stancheva *et al.*,2013). The percentage of water is good indicator of its relative contents of energy, proteins and lipids. If lower the percentage of water, greater the lipids, protein contents and higher the energy density of the fish (Marichamy *et al.*, 2011; Naeem *et al.*, 2011). The high moisture content is a disadvantage in that it increases the fishes' susceptibility to microbial spoilage, oxidative degradation of polyunsaturated fatty acids and consequently decreases in the quality of the fishes for longer preservation time (Olagunju *et al.*, 2012).

The protein content is usually in the range of 15–20%. It is considered low if it is below 15% (Stancheva *et al.*, 2013).The fish species examined belonged to high-protein (15-20%) low-oil (<5%) category. They contained lower calorie content per unit of protein than do fatty fish, meats or poultry, and were an ideal source of animal protein for use in controlling diets.

Lipids are serves as source of energy during starvation and fasting. Fish can be grouped into four categories according to their fat content: lean fish (< 2 %), low fat (2 to 4 %), medium fat (4 to 8%), and high fat (> 8%) (Olagunju *et al.*, 2012). Fat content varies widely from species to species and from season to season; it can be as low as 0.5% in lean and starved fatty fish and can reach over 20% in some species (Emire and Gebremariam, 2009). The marine had a higher lipid content than the fresh water fishes hence their classification as high fat fishes. This indicates that the marine fishes are better sources of lipid in the body when consumed. The low concentrations of lipid in the muscles of the fresh water species could be due to poor storage mechanism and the use of fat reserves during spawning activities (Olagunju *et al.*, 2012).

The carbohydrate content in fish is generally very low and practically considered zero. The relatively low values of carbohydrate could be due to higher values of moisture and a relatively high value of protein content (Olagunju *et al.*, 2012).

2.4. Importance of fish

Fish have significant role in nutrition, income, employment and foreign exchange earning of the country. Fish and shellfish are the primary sources of animal protein and valuable in the diet because they provide a good quantity (usually 70% or more) or protein of high biological value, particularly sulphur containing amino acids. Next to meat, fish is the only protein source that contains all the essential amino acids in right proportion and called complete protein. Consumption of fish provides important nutrients to a large number of people in the world and makes a very significant contribution to nutrition (Sandhya and Smita, 2013).

Scientists report that societies with high fish intake, such as the Inuit and the Japanese have considerably lower rates of acute myocardial infarctions, other ischemic heart diseases and atherosclerosis .These medical benefits are thought to be due to richness in marine omega-3 poly unsaturated fatty acids (x-3 PUFA) (Saoud *et al.*,2008; Babalola *et al.*, 2011; Boran and Karaçam, 2010). Polyunsaturated fatty acids from fish have been reported to have preventive and/or curative effects for several diseases including arterial hypertension, cancers and inflammatory diseases. Generally fish fat contains a high proportion of polyunsaturated fatty acids, which may help to decrease the incidence of atherosclerosis, and heart related diseases (Akan *et al*, 2012).

2.5. Factors that affect proximate composition of fish

Chemical body composition of fish could illustrate its physiological condition and health (Saliu *et al.,* 2007). Both condition and quality of fish in lakes are affected by the environmental conditions (Ibrahim *et al.*, 2008) and other biotic and abiotic variables, such as hydrologic level, food availability and water temperature (Touhata *et al.,* 1998). Generally Fish body composition is affected by both exogenous and endogenous factors. Exogenous factors that affect fish body composition include the diet of the fish (composition, frequency) and the environment in which it is found (salinity, temperature). On the other hand, endogenous factors are genetic and linked to the life stage, age, size, sex and anatomical position in the fish (Alemu *et al.,* 2013). Several studies have shown significant changes in whole body composition or in the composition of specific organs or muscle tissues due to age, feeding frequency, migration, ration, season, sex, starvation and temperature (Fawole *et al.,* 2007; Naeem *et al.,* 2011; Ali *et al.*,2012).

It was also reported that changes in body components during starvation are dependent upon water, temperature, season, reproduction stage and age (Musa, 2009). Proximate composition varied fish's consumption or absorption capability and conversion potentials of essential nutrients from their diet or their local environment into such biochemical attributes needed by the organisms' body (Fawole *et al.*, 2007; Oniyo *et al.*, 2010).

2.5.1. Exogenous factors

Environmental factor

Physical factors such as climate (i.e. temperature, wind, precipitation, and Solar radiation) are also important determinants of water quality in lakes and all critically affect the lake's hydrologic and chemical characteristics, and indirectly affect the composition of the biological community (Najafpour *et al.*, 2008). The environmental conditions in water bodies are constantly changed by various natural and human induced factors. The features of the physico-geographical environment of the catchment area, as well as the morphometric parameters of the water body and its hydrological regime, accelerate or block the supply of organic matter to the lakes, which affects its trophic level, water pH and hardness, its electrolytic conductivity and colouring, light and oxygen availability, and consequently algae and plant species diversity (Chobot and Banaś, 2008).

Saeed (2013) reported that environmental factors as climate (seasonal variations and temperature) and drainage wastewater affect the physical and chemical characteristics of water as well as fish condition and quality. Water pollution is the most important factor affecting quality and quantity of fish production either in natural habitats or culture ponds. Also, water quality may be affected by the source of the water, rate of flow, nutrients and algae. Other factors like sewage and agricultural runoffs, various hazardous chemicals and natural contaminants (animal feces) reach the natural sources of water and also pollute the ground water by seeping (Hamill *et al.*, 2010).

Diet of the fish

The diet composition of fish may vary with in wide ranges on temporal and spatial conditions and environmental factors. The major factors that influence fish diet are fish size, maturity, condition, season (water level), bottom, depth, latitude, longitude and habitat types. Fluctuate, the quality and abundance of food items for fish vary significantly through time. A particular characteristic of fish is that individuals increase in size during their ontogeny and this increment in size is correlated with changes in food quality and quantity in aquatic systems and growth varies according to food availability in the environment (Dadebo *et al.,* 2013). The nature and quality of nutrients in most animals depend largely on their food type.

Moreover, the feeding habit of an individual fish species greatly affects the nutritional composition its flesh. Majority of fish usually consists of about 70-80% of water, 20-30% of protein and 2-12% of lipid. However, these values may vary considerably within and between species to species and as well with size, sexual condition, feeding and physical activity (Marichamy *et al.,* 2011).

There was variation in the consumption of the food objects by the different fish species. Despite similarity in the rank- order of some food objects in the species, the ingestion of the food objects varied significantly (Offem *et al.,* 2009). Laboratory studies on many fishes have shown that ration affects oxygen consumption and metabolic enzyme activities to some degree (Sullivan and Smith 1982). Yang *et al.,* (1993) obtaided that one species of deep-sea benthic fish, *S. alascanus,* held and fed in the laboratory, had a 68% higher respiratory rate compared to field-caught specimens, suggesting that food was indeed limiting in the environment. It was also evident that there was a significant effect of dietary protein on growth performance of the experimental fish. Weight gain and specific growth rate increased significantly with increasing dietary protein levels from 22.40% to 32.70% (Mohamed, 2005).

2.5.2. Endogenous factor

Species

The distributions or habitat selection of different fish species, or size classes within a species, in a lake depends on several factors including the physiological adaptation of the species to specific environments (Dadebo *et al.,* 2013), preference for specific feeding and spawning grounds, or refuges and shelter from predators (Philippart & Ruwet 1982).Different size classes of each species may also have different habitat preferences (Pet & Piet 1993), in most cases associated with the availability of suitable food and protection from predators for the young.

For instance, there are large differences in body form between cottids and flatfishes, both benthic groups, and their body forms and locomotory adaptations may lead to inherent differences in metabolism (Webb, 1990). Also An examination of white muscle composition of 18 species of benthic and benthopelagic fishes off of California found a significant increase in water content with depth of occurrence (Drazen, 2007). Other factors of variation between species and between individuals of the same species are closely related to the origin (fishing or farming), age, body weight, type of feeding, migratory behavior and reproductive status; it is widely known that reproductive activity causes stored energy expenditure in the form of lipids or proteins, depending on environmental conditions (Stancheval *et al.*, 2013).

Casallas *et al.* (2012) reported that protein content of catfish ranged from 15.71-16.2% which was lower than *Oreochromis* species (18.4-20.8%) protein content. The reverse result was reported by Ayeloja *et al.* (2013). Other authors Emire and Gebremedihn (2007) for *O. niloticus* in lake Ziway obtained that 79.87, 0.98, 18.52 0.37 for moisture, ash, protein and fat respectively. Moisture content for the three fish species in lake koka and lake Awassa by Dsikowitzky *et al.*, (2012) reported that *O. niloticus*, (83±2.5), *L. intermedius* (81±1.9) and *C. gariepinus*, (84±1.4). As well Ataro *et al.* (2003) revealed that moisture contents of tilapia and cat fishes collected from Lakes Awassa and Ziway obtained from different spots in the two lakes varied from 79.3%–82.5%.

Sex

Intrinsic factors like sex and size greatly influence various physiological processes, in a variety of animals (Naeem *et al.*, 2011). Such variations might be in all possibility due to difference in the biochemical construction of the tissues in respect to size (Shahana *et al.*, 2010).The variation among sex could be females were longer and heavier than males, which is very frequent in fish species (Pereira *et al.*, 2013). Several studies have shown significant changes in whole body composition or in the composition of specific organs or muscle tissues due to age, feeding frequency, migration, ration, season, starvation and temperature (Naeem *et al.*, 2011; Fawole *et al.*, 2007). Higher gross energy value in female fish compared to male may be attributed to relatively higher protein and fat in females than males (Ozogul *et al.*, 2010).

Muscles of female fish contain more organic materials and less water than male (Naeem *et al*, 2011). Ozogul *et al.,(*2010) obtained that moisture contents of male blue crabs and male swim crabs were significantly higher ($P<0.05$) than those found in female blue crabs and female swim crabs. Study conducted by Alemu *et al.* (2013) on effect of endogenous factors on proximate composition of Nile tilapia (*oreochromis niloticus*) fillet from lake Zeway revealed that, moisture content of male fish higher than female fish.

Finding of conducted on chemical composition and nutritional value of raw and fried allis shad fish, showed that female fish protein was higher than male fish (Pereira et al., 2013). The same result by Ozogul *et al.* (2010) on the effects of season and sex on the fatty acids and proximate compositions of the mantle of the cuttlefish *S. officinalis* showed that the level of protein contents of female of *S. officinalis* were significantly higher than those of male ($P<0.05$) for all seasons. In other study were investigated the protein contents of female blue crabs and female swim crabs were significantly higher ($P<0.05$) than those found in male blue crabs and male swim crabs (Ozogul *et al.,* 2010). Study conducted in Ethiopia Lake Zeway by Alemu *et al.,* (2013) showed the protein content of the female were slightly higher than the male fish.

Interaction effect of exogenous and endogenous factors

The body composition of fish is mainly influenced by both the endogenous and exogenous factors, which operate simultaneously (Alemu *et al.,* 2010). Several studies have shown significant changes in whole body composition or in the composition of specific organs or muscle tissues due to age, feeding frequency, migration, ration, season, sex, starvation and temperature. There was variation in the consumption of the food objects by the different fish species. The growth–ration relationship and energy allocation are affected by many factors, such as, feed type or composition, fish body size, and water temperature (Mohammad and Khalil, 2013).Mohammad and Khalil (2013) reported that growth performances were significantly affected ($P<0.05$) by water temperature and ration size. Mean weight gain and percentage of weight gain significantly increased with increasing water temperature from 24 to 28 °C and higher ($P<0.05$) at 3% ration size.

Other author Dadebo *et al.* (2013) obtained that average daily growth and specific growth rate were significantly ($P<0.05$) affected only by ration size at 3% level. Increment in size is correlated with changes in food quality and quantity in aquatic systems and growth varies according to food availability in the environment. Female fish were longer and heavier than males, which is very frequent in fish species (Pereira *et al.,* 2013). Chatzifotis *et al.* (2011) reported that the increase in crude protein in the diet from 40 up to 50% positively affected SGR (Specific growth rate) and FCR (Feed conversion ratio). He also showed that the increase in crude lipids in the diet from 12 up to 17% showed a tendency for improvement in SGR and FCR.

2.6. Environmental pollution

Environment is defined as the totality of circumstances surrounding an organism or group of organisms especially, the combination of external physical conditions that affect and influence the growth, development and survival of organisms. It consists of the flora, fauna and the abiotic and includes the aquatic, terrestrial and atmospheric habitats. The environment is considered in terms of the most tangible aspects like air, water and food, and the less tangible, though no less important, the communities we live in (Khan, 2011).

A pollutant is any substance in the environment, which causes objectionable effects, impairing the welfare of the environment, reducing the quality of life and may eventually cause death. Such a substance has to be present in the environment beyond a set or tolerance limit, which could be either a desirable or acceptable limit. Hence, environmental pollution is the presence of a pollutant in the environment; air, water and soil, which may be poisonous or toxic and will cause harm to living things in the polluted environment (Duruibe *et al.,* 2007).

Environmental pollution is caused due to the discharge of substances or energy into air, water, or land that may impart acute (short-term) or chronic (long-term) detriment to the quality of life. Pollutants may cause primary damage which has directly identifiable impacts on the environment or secondary damage in the biological food chain that are noticeable over long periods (Tokalioglu *et al.,* 2003 and Moja, 2007).

Over the last three decades there has been increasing global concern over the public health impacts attributed to environmental pollution, in particular, the global burden of disease. The

World Health Organization estimates that about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution.Most of these environment-related diseases are however not easily detected and may be acquired during childhood and manifested later in adulthood. Improper management of solid waste is one of the main causes of environmental pollution and degradation in many cities, especially in developing countries. Many of these cities lack solid waste regulations and proper disposal facilities, including for harmful waste. Such waste may be infectious, toxic or radioactive. Municipal waste dumping sites are designated places set aside for waste disposal (WHO, 2006).

Depending on a city's level of waste management, such waste may be dumped in an uncontrolled manner, segregated for recycling purposes, or simply burnt. Poor waste management poses a great challenge to the well-being of city residents, particularly those living adjacent the dumpsites due to the potential of the waste to pollute water, food sources, land, air and vegetation. The poor disposal and handling of waste thus leads to environmental degradation, destruction of the ecosystem and poses great risks to public health (Raja *et al.*, 2009; Akan *et al.*, 2012).

2.6.1. Heavy metals in the environment

The term "heavy metals" refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration. "Heavy metals" is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm3, or 5 times or more, greater than water (Anim *et al.*,2011). However, being a heavy metal has little to do with density but concerns chemical properties. Commonly the term heavy metal is used to refer to elements that are associated with pollution and toxicity problems. It include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag) chromium (Cr), copper (Cu) iron (Fe), and the platinum group elements (John and Sunday,2001;Duruibe *et al.*, 2007). Heavy metals like copper, iron and zinc are essential for fish metabolism while some others such as mercury, cadmium, arsenic and lead have no known role in biological systems. Heavy metals such as lead (Pb) and cadmium (Cd) are the most common toxicant that can be found in the marine Environment (Akan *et al.*, 2012).

The occurrence of metal contaminants especially the heavy metals in excess of natural loads has become a problem of increasing concern. The situation is as a result of the rapid population growth, increased urbanization and expansion of industrial activities, exploration and exploitation of natural resources, extension of irrigation and other modern agricultural practices as well as the lack of environmental regulations (Asante *et al.,* 2014). They are present in the environment in different forms such as in solid phase and in solution, as free ions, or absorbed to solid colloidal particles (Güven and Akýncý, 2008).

There have been instances where mass deaths resulted from heavy metal toxicity. These metals are dangerous as they tend to bioaccumulate in the food chain and they can be harmful to human and animals. The heavy metals risk pose to human and animals health is provoked by their long term persistence in the environment. Since the beginning of human kind we have used metals for environment. Metals can be retained for long period of time after entering the environmental medium such as soil (Tokalioglu *et al.,* 2003; Moja, 2007).

2.6.2. Source of heavy metal in the water body

A wide range of contaminants are continuously introduced into the aquatic environment mainly due to increased industrialization, technological development, growing human population, oil exploration and exploitation, agricultural and domestic wastes run-off (Irenosen *et al.,* 2013;Alex *et al.,*2013). They enter the aquatic environment naturally through weathering of the earth crust. The main natural source of heavy metals in water is weathering of minerals. Beside their natural occurrence, heavy metals may enter the ecological system through human activities, such as, mining, sewage sludge disposal, application of pesticides and inorganic fertilizers as well as atmospheric deposition (Asante *et al.,* 2014).

Water pollution may occur in the form of thermal pollution and depletion of dissolved oxygen. It can come from single (point) sources or from larger and dispersed (non point) sources. Point sources discharge pollutants at specific location through drain pipes or sewer line into bodies of surface water. Non point sources such as runoff, are diffused and intermittent, and are influenced by factor such as land use, climate, hydrology, topography, native vegetation and geology. Common urban nonpoint sources include runoff from streets or fields; such runoff contains all sorts of pollutants, from heavy metals to chemicals and sediments. Rural source of non point pollution are generally associated with agriculture, mining, or forestry (Botkin and Keller, 2007). Roadways and automobiles now are considered to be one of the largest sources of heavy metals (Begum *et al.*, 2008).

The anthropogenic effect on faecal and chemical pollution at Bahir Dar Gulf of Lake Tana, Ethiopia was investigated in the period of October 2006 to February 2007 by Goshu *et al.* (2010). They revealed that the lake Tana environments are under growing stress from point and diffuse sources of pollution, and especially, the largest share of pollution comes from Bahir dar city since it has no centralized sewerage system and most of the sewerage lines have been directed to the lake. The wastewaters released from Felege hiwot hospital and from the municipality near St.George are major public health risk factors.

Heavy metals constitute one of the most dangerous groups because of their persistent nature, toxicity, tendency to accumulate in organisms and undergo food chain amplification and more still, they are non-degradable (Irenosen *et al.*, 2013).When released into the environment, they find their way into the aquatic systems and bioaccumulation and the food chain process and eventually threaten the health of humans that consume them. Heavy metals concentrations in aquatic ecosystems are usually monitored by measuring their concentrations in water, sediments and biota (Enuneku *et al.*, 2013).

2.6.3. Distribution of heavy metals in the water body

Water quality guidelines like WHO, EU and USEPA (UNEP, 2006) provide basic information about water quality parameters and ecological relevant toxicology threshold values to protect specific water uses. The quality of fresh water for fish should not allow accumulation of pollutants especially heavy metals in fish to such extent that they are potentially harmful (Alabaster and Lloyed, 1982).

Heavy metals in water can be partitioned into dissolved and suspended fraction. It is well known that most dissolved heavy metals are present as organic complexes in natural water (Prego and Cobelo-Garcia, 2003). A fraction of metals is bound to organic matters and particulate in water, which reduced the amount of metals for uptake by organism and the ability of metals to affect organism. It is known that bioavailability or toxicity of metals is directly corrected to concentration of free metals ions, which are not bounded to any matter, rather than to total concentrations (ATSDR, 2006).

In the aquatic environment, the trace elements are partitioned among the various environmental components (water, suspended solids, sediments and biota). The main processes governing the distribution of these elements in the marine environment are dilution, advection, dispersion, sedimentation and adsorption (Akan *et al.,* 2012). Metals after entering water, may precipitate, adsorb on solid surfaces, remain soluble, suspended in water or may be taken up by fauna and eventually accumulate in aquatic organisms that are consumed by human beings. All metals are virtually toxic if the exposure level is sufficiently high to exceed the tolerance limit. Specifically, some metals like (Cd, Pb, Hg) have been reported to be extremely dangerous to human health even at low level of concentration (Irenosen *et al.*, 2013).

When heavy metals enter water bodies, they change water quality, bind to sediments and accumulate in aquatic biota causing anemia, disturbance of physiological functions and mortalities of fish. Specifically, aquatic organisms experience histological and morphological changes in tissues; physiological changes like suppression of growth/development, poor swimming performance, changes in blood composition and circulation, behavior and reproduction, adversely affecting aquatic biodiversity. This adversely affects fish farming through decrease in revenue and profitability. Heavy metals also pose a serious threat to humans through ingestion of metal enriched aquatic organisms (John and Sunday, 2001).

2.6.4. Bioaccumulation heavy metals in fish

Fish may absorb dissolved elements and heavy metals from surrounding water and food, which may accumulate in various tissues in significant amounts (Jezierska and Witeska, 2006). Pollutants enter the fish through five main routes: via food or non food particles, gills, oral consumption of water and skin (Nwani *et al.,* 2010). Metal elimination routes are more than uptake routes, however metal accumulation is more rapid than metal elimination probably due to the presence of metal binding proteins in tissues (Kumar and Achyuthan, 2007).

Heavy metals are very toxic because, as ions or in compound forms, they are soluble in water and may be readily absorbed into aquatic organisms. Fish accumulate heavy metals directly because of the intimate contact they have with the aquatic medium and also because they have to extract oxygen from the medium by passing enormous volumes of water over their gills. They have been considered good indicators for heavy metal contamination in aquatic system because they are easy to obtain in large quantities as well as they occupy high trophic level in the aquatic food chain (Enuneku *et al.,* 2013). Meanwhile, fish are widely consumed in many parts of the world by humans, and polluted fish may endanger human health (Zheng *et al.*, 2007).

Pollutants enter fish through five main routes (food or non-food particles, gills, water and skin), absorbed into blood and then carried to either a storage point or to the liver for its transformation or storage. Pollutants that are transformed and not stored in the liver are excreted in bile or transported to other excretory organs such as gills or kidneys for elimination or stored in fat and enter the food chains and extent to many other problems to humans (Obasohan & Eguavoen, 2008; Jabeen and Chaudhry, 2009).

2.6.5. Factors for accumulation of heavy metals in the fish

In normal metabolism, fish may uptake heavy metals from water, food or sediment. However, the efficiency of metal uptake from contaminated water may differ in relation to ecological needs, metabolisms, and the contamination gradient of water, food and sediment as well as other environmental factors such as salinity, temperature and interacting agent (Ismail and Mat Saleh, 2012). Some of the factors that can influence the toxicity of metals include:

- The metal species in the water;
- The presence of other metals or pollutants;
- Abiotic factors such as temperature, pH, dissolved oxygen, hardness, salinity, etc.
- Biotic factors such as age, size, sex, stage in life history, adaptive capabilities; and
- Behavioural responses (Oldewage and Marx, 2006).

Generally, accumulation depends on metal concentration, time of exposure, way of metal uptake, environmental conditions (water temperature, pH, hardness, salinity), and intrinsic factors (fish age, feeding habits) (Jezierska and Witeska, 2006; Babatund, 2012). Similarly, it is also known that the metal accumulation in the tissues of fish is dependent upon the rate of uptake, storage and elimination (Ahmed and Bibi, 2010; Adegbola et al., 2012).

2.5.1. Environmental condition of the water

Fish are often used as subjects to investigate toxic substances present in water, as studies have indicated that fish are able to accumulate and retain heavy metals from their environment and that accumulation of metals in tissues of fish is dependent upon exposure concentration and duration as well as other factors such as salinity, temperature, dissolved oxygen concentration, water transparency, geographical locations, season and hardness of water (Cyrille *et al.,* 2012; Murtala *et al.,* 2012).The toxicity of heavy metal increase with high water temperature, oxygen concentration, basic pH and hardness of river water (Ogoyi *et al.,* 2011; Özparlak *et al.,* 2012). There are important interactions between heavy metals in the waters and physicochemical variables such as pH, total dissolver solid, temperature and electrical conductivity (Forero *et al.,* 2009).

pH of Water

pH value of water is important because many biological activities can occur only within a narrow range. Thus any variation beyond acceptable range could be fatal to a particular organism .The favorable range of pH is 6.5-9.0 at daybreak, are most suitable for fish production. The increase in pH in the rivers could be related to photosynthesis and growth of aquatic plants, where photosynthesis consumes CO_2 leading to arise in the pH values (Ghannam *et al.,* 2014).

pH is a measure of the concentration of hydrogen (H^+) ions dissolved in water. H^+ is the ion that causes acidity; however, it is also a cation. As a cation it is attracted to the negative charges of the soil and sediment particles. In acid conditions, there are enough H^+ ions in to occupy many of the negatively charged surfaces of clay and organic matter. Little room is left to bind metals, and as a result, more metals remain in the soluble phase (Cyrille *et al.,* 2012; Begum *et al.,* 2008; Koffi *et al.,* 2014; Shakweer and Abbas, 2005).

Low pH values might favor solubilization of metals in the water column, allowing a higher bioavailability for aquatic organisms. This is the case with Cr, which increases in toxicity with a reduction of pH. Low pH targets branchial tissue, causing an acute acid stress. This leads to an increased mucus secretion and inhibition of Na and Cl– exchange .On the other hand, chronic low pH stress is associated with reduced growth, reproductive failure, and increased accumulation of heavy metals (Forero *et al.,* 2009).

Water pH affects metal toxicity in several ways .Firstly, the speciation and the bioavailability of the metal may change in form between pH values of 4-8 .Secondly the uptake and toxicity of the metal can be affected by the changes in the sensitivity of the cell surface. It has been established that acid water as well as heavy metals affects the rhythmic valve movements in Anodonta cygna and may possibly change the amount of metal coming into contact with the soft part of the clam (Karthikeyan *et al.,* 2007).

Water acidification affects bioaccumulation of metals by the fish in an indirect way, by changing solubility of metal compounds or directly, due to damage of epithelia which become more permeable to metals, and on the other hand, competitive uptake of H+ ions may inhibit metal absorption. Various species of fish from the same water body may accumulate different amounts of metals. Interspecies differences in metal accumulation may be related to living and feeding habits (Jezierska and Witeska, 2006). The water pH, the nature and concentration of organic ligands, oxidation state and redox conditions within the environment could influence metal solubility (Koffi *et al.,* 2014).

Temperature

Temperature plays an important role in aquatic ecosystem health and affects the speed of chemical reactions, the metabolic rate of organisms, as well as how pollutants, parasites and other pathogens interact with aquatic residents. Temperature change depends mainly on the climatic conditions, sampling times and the number of sunshine hours (Ghannam *et al.,* 2014). Water temperature is one of the most influencing environmental factors affecting estuary dynamics and both the biological processes and water quality (Koffi *et al.,* 2014).The mechanism whereby heavy metal toxicity increases with higher temperature was attributed by to elevated respiratory activity(Shakweer and Abbas, 2005).

Water temperature may cause the differences in metal deposition in various organs. Higher temperatures promote accumulation of cadmium especially in the most burdened organs: kidneys and liver. Increased accumulation of metals by fish at higher temperatures probably results from higher metabolic rate, including higher rate of metal uptake and binding (Jezierska and Witeska, 2006). Higher temperatures can cause higher activity and ventilation rates in fish. This is due to increasing temperatures that lower the oxygen affinity of the blood and increases the rate of pollution accumulation. A higher metabolic rate may also induce more frequent feeding sessions, which in turn might result in increased metal concentrations, if these metals are taken up via the food chain (Obasohan & Eguavoen, 2008).

The distribution patterns of heavy metals in the water and fish organs increased in the hot seasons (spring and summer) which may be attributed to the release of heavy metals from sediments to the overlying water under the effect of both high temperature and fermentation process resulted from decomposition of organic matter (Yehia and Sebaee,2012). In addition, the values of heavy metals showed an obvious decrease in the water during cold season (winter and autumn) with a correspondent increase in the sediments due to precipitation of heavy metals from water column to the sediments under high pH values and the adsorption of heavy metals onto organic matter and their settlement downward (Yehia and Sebaee,2012). pH values (6.8-9) reported previously on lake Tana (Goshu *et al.,* 2010) and 5.7-7.7 reported by Getahun & Selassie (2013) in the same lake.

Electrical conductivity

Conductivity is a measure of the tendency of a liquid to conduct electricity reported that electrical conductivity is a useful parameter of water quality for indicating salinity. It is related to the amount of total dissolved solids (notably certain ionic salts) in the water body (Okorie and Oko, 2013). It is an indirect measure of the ions concentration. The highest values of electrical conductivity might indicate that drains are receiving large quantities of land run off and/or intensity industrial pollution.

Okorie and Oko (2013) reported that there was a positive correlation between the conductivity of the water and the temperature. The conductivity of the water is due to the presence of soluble salts of some metals in the water. This suggests that as conductivity of the water increases the temperature also increases.

Electrical conductivity of natural fresh waters varies greatly and may range from less than (20 μmhos/cm) in dilute waters to over several hundred or more in waters influenced by limestone or salt deposits. The highest values of electrical conductivity might indicate that drains are receiving large quantities of land run off and/or intensity industrial pollution and suggest potential irrigation problems in case of illegal and unofficial drainage use due to salinity hazards (EC should be <700 μmhos/cm) as adopted from (Ghannam *et al.*,2014).

Total dissolved solid

In water, total dissolved solids are composed mainly of carbonates, bicarbonates, chlorides, phosphates and nitrates of calcium, magnesium, sodium, potassium and manganese, organic matter, salt and other particles (Ghannam *et al.,* 2014).The density of water depends on the total dissolved solids that occur in natural water containing a complex mixture of cations and anions. TDS is an important indicator of the suitability of water for drinking, recreational, irrigation and industrial use. In water, total dissolved solids are composed mainly of carbonates, bicarbonates, chlorides, phosphates and nitrates of calcium, magnesium, sodium, potassium and manganese, organic matter, salt and other particles (Mahananda, 2010).

2.5.2. Biological conditions of the fish

Many researchers indicated that heavy metal bioaccumulation of fish is species-dependent. Feeding habits (as carnivores, herbivores, omnivores and limnivores) and habitats of species are strongly related to accumulation level (Akan *et al.,* 2012; Nwani *et al.,* 2010). In addition to species differences, variations of heavy metal concentrations in the different fish species can be also attributed to variety of reasons including; size (body weight and length), gender, age and growing rates of the of fish species as well as types of tissues analyzed, and physiological conditions (Raja *et al.,* 2009; Naeem *et al.,* 2011).

It has been reported that different organisms have different metabolic rates and different food requirements and amounts. Organisms with high food intake tend to accumulate more metals. Age of fish, lipid content in the tissue and mode of feeding are significant factors that affect the accumulation of heavy metals in fishes (Asante *et al.,* 2014; Ismail and Mat Saleh, 2012).

Heavy metals are accumulated in body tissue in different concentration. Gill tissue is an organ having a large surface and separates blood from water in fish and is very susceptible to changes in concentrations of the variables (heavy metals, temperature, pH etc.) in the environment. These variables affect the structural integrity of the gill and cause morphological changes. For this reason gills are good indicators of water pollution. Liver plays an important role in protecting inner homeostasis in vertebrates. Muscle tissue forms a major part of the body weight of fish when compared to other vertebrates and is also economically valuable (Javed and Usmani, 2011).

Furthermore, the physiological differences and the position of each tissue in the fish can also influence the accumulation of a particular metal. In other words, the amount of a metal accumulated is influenced by various environmental, biological and genetic factors, leading to the differences in metal accumulation between different individuals, species, age, tissues, seasons and sites (Ahmed and Bibi, 2010; Jabeen and Chaudhry,2009; Ismail and Mat Saleh, 2012; Cyrille *et al.*, 2012).

Yousafzai *et al.* (2010) obtained that *Labeo dyocheilus* accumulated 65.2% extra heavy metals burden as compared to *Wallago attu* they suggest that omnivorous fish may bioaccumulate more heavy metals than the carnivorous fish in natural habitats. There are 3 possible ways by which metals enter fish bodies: the body surface/epidermal tissue (skin), the gills and the digestive tract (Yousafzai *et al.*, 2010). Bioaccumulation factors and metal content in different species of fish appreciably vary with the bioavailability of metals in seawater, as well as to inter-specific differences of fish species (Nair and Joseph, 2010).

2.7. Food safety

Chemical hazards in food are potentially toxic substances that either occur naturally, such as aflatoxins and marine toxins, or are man-made. Man-made chemicals can be added to food intentionally, such as preservatives and colorants, they may be present as residues of pesticides and animal drugs, or they can unintentionally contaminate food through the environment or through the production process, for example, metals, cleaning agents and packaging materials used to keep food safe and fresh. Unintentional contamination may also occur through environmental pollution of the water, air and/or soil (WHO, 2009).

Sea foods have essential amino acids, fatty acids, protein, carbohydrates, vitamins and minerals. Among sea foods, fish are commonly consumed and, hence, are a connecting link for the transfer of toxic heavy metals in human beings. Metals transferred through aquatic food chains and webs to fish, humans and other animals are of more environmental concern to human health (Yigit and Altindag, 2006). Heavy metals have the tendency to accumulate in various organs of marine organisms, especially fish, which in turn may enter into the human metabolism through consumption causing serious health hazards (Raja *et al.,* 2009).

Infants and children are potentially more vulnerable to the effects of ingesting chemical hazards, owing to still developing organ systems and higher exposure. Children consume more food per unit of body weight than adults: in the case of infants, twice the amount. Moreover, developing organs and tissues are more susceptible to the toxic effects of certain chemicals. For example, excessive exposure to lead or methylmercury during gestation or early childhood may cause serious damage to the developing brain with consequent loss of intellectual potential, while an adult experiencing the same exposure will suffer no great effect on his/her intellectual capacity. Symptoms related to prolonged low-level exposure may not be apparent until later in life and, when they do occur, may be chronic and irreversible. Serious illness due to long-term exposure to various toxic chemicals may include damage to the immune and nervous systems, impairment of reproductive function and development, congenital anomalies in the offspring, cancer, and organ-specific damage (WHO, 2009).

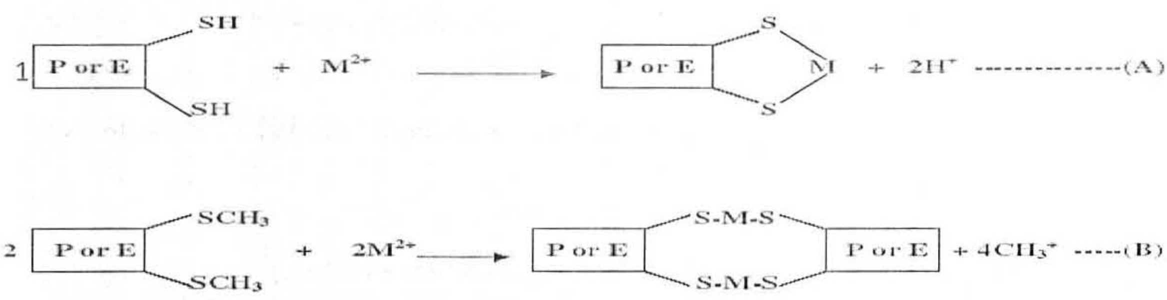
Deadly diseases like edema of eyelids, tumor, congestion of nasal mucous membranes and pharynx, stuffiness of the head and malfunctions in genetic makeup, gastrointestinal cavity, muscular, reproductive and neurological systems caused by some of these heavy metals have been documented (John and Sunday,2011).

2.8. Biochemistry of heavy metal toxicity

Essential metals are used either an electron donor system or function as ligands in complex enzymatic compounds. This trace metals are only used in trace amount by the organism and usually they are found in small concentration in the environment. The amount of heavy metals in the organism does not exceed the level which allows the enzyme system to function without interference. The excess amount of heavy metal in the organism can be regulated by homeostasis. But, if the heavy metal concentration at the source of supply such as water and food is too high, the homeostasis mechanism ceases to function and the essential heavy metals act in either an acutely or chronically toxic manner (Javed and Usmani, 2011).

On absorption, the pollutant is carried in blood stream to either a storage point or to the liver for transportation or storage. Pollutants transformed in the liver may be stored there or excreted in bile or transported to other excretory organs such as gills, skin or kidneys for elimination or stored in fat which is an extra hepatic tissue (Nwani *et al.,* 2010). Pollution of aquatic system by heavy metals inhibits primary production, nitrogen fixation, mineralization of carbon, nitrogen, phosphorous; litter decomposition, and enzyme synthesis and activities in the sediments and surface waters (Irenosen *et al.,* 2013).

The poisoning effects of heavy metals are due to their interference with the normal body biochemistry in the normal metabolic processes. When ingested, in the acid medium of the stomach, they are converted to their stable oxidation states (Zn2+, Pb2+, Cd2+, As2+, As3+, Hg2+ and Ag+) and combine with the body's biomolecules such as proteins and enzymes to form strong and stable chemical bonds. The equations below show their reactions during bond formation with the sulphydryl groups (-SH) of cysteine and sulphur atoms of methionine (-SCH3) (Ogwuegbu and Ijioma, 2003).



Where:

(A) = Intramolecular bonding; (B) = Intermolecular bonding; P = Protein; E = Enzyme; M = Metal

Fig.1: Biochemical reaction between heavy metals and protein

The hydrogen atoms or the metal groups in the above case are replaced by the poisoning metal and the enzyme is thus inhibited from functioning, whereas the protein–metal compound acts as a substrate and reacts with a metabolic enzyme.

If the body cannot utilize the product formed from the heavy metal – protein substrate, there will be a permanent blockage of the enzyme, which then cannot initiate any other bio-reaction of its function. Therefore, the metal remains embedded in the tissue, and will result in bio-dysfunctions of various gravities bioactivities. When ingested, they combine with the body's biomolecules, like proteins and enzymes to form stable biotoxic compounds, thereby mutilating their structures and hindering them from the bioreactions of their functions (Duruibe *et al.,* 2007).

Furthermore, a metal ion in the body's metallo-enzyme can be conveniently replaced by another metal ion of similar size. Thus Cd2+ can replace Zn2+ in some dehydrogenating enzymes, leading to cadmium toxicity. In the process of inhibition, the structure of a protein molecule can be mutilated to a bio-inactive form, and in the case of an enzyme can be completely destroyed (Duruibe *et al.,* 2007). For instance, lead has been observed to interfere with the calcium-dependent release of neuro-transmitters. Also, lead, cadmium and vitamin D have been shown to have a complex relationship affecting mineralization of bone, and there exists a more direct influence involving impairment of 1-25-dihydroxy vitamin D synthesis in the kidney (Igwegbe *et al.,* 2014).

2.9. Risk assessment

Studies have shown that muscle is not an active tissue in accumulating heavy metals. This may reflect the low levels of metallothionein, low molecular weight binding proteins, in the muscle. Metals that enter the body via food are carried by the blood bound to proteins, where they move first move in to the liver and gradually into the muscle tissues (Yohannes *et al.,* 2013).

Provisional Tolerable Weekly Intake is an endpoint used for food contaminants such as heavy metals with cumulative properties. Its value represents permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods. Provisional Tolerable Weekly Intakes (PTWI) is set for substances, such as heavy metals, that are contaminants in food and are known to accumulate in animals and humans. Like in other organisms, heavy metals are not destroyed by humans. Instead, they tend to accumulate in the body and can be stored in soft and hard tissues such as liver, muscles and bone and threaten the health of humans (Elnabris, 2012).

The human health risk assessment, for certain metals, was estimated by comparing the metal intake from the consumption rate of seafood with the Provisional Tolerable Weekly Intake (PTWI) according to the calculation made by (El-Sadaaw *et al.,* 2013) using the average consumption of Kg fish/year for Kg body weight. The amount of heavy metals taken weekly by a person can be calculated by following this equation:

$$\text{Amount of metal taken weakly by a person} = \text{Concentration of metal in muscle } (\mu g/g) \times \text{Average consumption } (g)$$

2.10. Selected heavy metals for study

2.10.1. Chromium

Cr is essential element and an important enzyme cofactor, which may become toxic when accumulating in liver and spleen. Zhang *et al.* (2007) reported that compared to Chinese food standards, muscle Cr from bottom fish exceed more than 50% of the Chinese food standards. Cr is an essential trace element in humans and some animals but in excess, it could have undesirable lethal effect on fish and wildlife (Akoto *et al.,* 2014; Anim *et al.,* 2011).

Chromium concentration in natural waters is usually low. Elevated concentration can result from industrial and mining processes. It is used in industry for electroplating, steelmaking alloys, in chrome plating, rubber manufacturing, leather tanning and for fertilisers (Oldewage and Marx, 2006). Cr is particularly dangerous as it can accumulate in many organisms, sometimes as much as 4000 times above the level of the surrounding environment as was noted in aquatic algae (Oldewage and Marx, 2006). Due to Cr is particularly dangerous as it can accumulate in many organisms, sometimes as much as 4 000 times above the level of the surrounding environment as was noted in aquatic algae (Oldewage and Marx, 2006).

2.10.2. Cadmium

Cadmium is widely known to be a highly toxic non–essential heavy metal and it does not have a role in biological process in living organisms. Thus, even at its low concentration, cadmium could be harmful to living organisms (Makimilua, 2013). It is a natural element in the earth crust and usually found as a mineral with other elements. All soils and rocks, including coal and mineral fertilizer, have some cadmium in them. In industry and consumer products, it is used for batteries (Ni-Cd batteries of mobile phones), pigments, metal coatings and plastics. Cadmium is also a constituent in many other things such as alloys. It enters air from mining, industry, and burning coal and household wastes. Its particles can travel long distance in air before falling to ground or water (Elnabris *et al.,* 2012; Mudga, 2010).

Cadmium could originate from water, sediments and food and may accumulate in the human body as may induce kidney dysfunction, skeletal damage, reproductive deficiency, carcinogenic, teratogenic, Genotoxic, damage to the central nervous system and produce psychological disorder (Akoto *et al.,* 2014; Cyrille *et al.,* 2012).

Cadmium have high affinity for thiol groups, make proteins and peptides susceptible to structural modifications in sub-cellular compartments and tissues as in skeletal muscle. Some authors have already observed that cadmium alters calcium homeostasis (Cyrille *et al.,* 2012). Also, cadmium upon getting to the environment may interact with the calcium metabolism of animals and in fish may cause hypocalcaemia, probably by inhibiting calcium uptake from the water. However, high calcium concentrations in the water protect fish from cadmium uptake by competing at uptake sites (Adegbola *et al.,* 2012).

2.10.3. Lead

Lead is a microelement naturally present in trace amounts in all biological materials in soil, water, plants and animal (Korai et al., 2008). Pb is considered as a toxic even at low concentration but non-essential metal implying that it has no known function in the biochemical processes (Korai et al., 2008; Akoto et al., 2014). Lead enters the aquatic environment through soil erosion and leaching gasoline combustion, municipal and industrial wastes and runoff .Higher levels of Pb often occur in water bodies near highways and large cities due to high gasoline combustion. Also, dust which holds a huge amount of lead from the combustion of petrol in automobile cars led to increase Pb content (Saeed and Shaker, 2008). The concentration of lead in natural water increases mainly through anthropogenic activities (Krishna *et al.,* 2014).

The main source of lead contamination are smelting works, application of waste water treatment sludge's to soil, transportation, rain, snow, hail and other, approximately 98% of lead in the atmosphere originates from the human activates (Korai *et al.*,2008). Lead (Pb) is used in storage batteries, cable coverings, plumbing, ammunition, manufacture of tetraethyl Pb, sound absorbers, radiation shields around X-ray equipment and nuclear reactors, paints, while the oxide is used in producing fine "crystal glass" and "flint glass" with a high refractive index for achromatic lenses, solder and insecticides(Mudga,2010).

Pb is known to induce renal tumours, reduce cognitive development, increase blood pressure in adults, gastrointestinal disorders and some liver impairment (Akoto *et al.*, 2014).Pregnant women exposed to lead were found to have high rates of still births and miscarriages, mental retardation among children and Hyper tension has also been reported. Lead poising is accompanied by symptoms of intestinal cramps, peripheral nerve paralysis anemia, and fatiage (Krishna *et al.*, 2014).Lead is cancer-causing agent and adversely effects reproduction, liver and thyroid function and disease resistance. Fishes exposed to high levels of lead exhibit a wide-range of effects including muscular and neurological degeneration and destruction, growth inhibition, mortality, reproductive problems, and paralysis (Ahmed and Bibi, 2010).

3. MATERIALS AND METHODS

3.1 Study area

The study samples were collected from lake Tana. Lake Tana is the largest highland lakes in Ethiopia with an area of about 3200 Km^2 and is situated in the northwestern part of the country at an altitude of about 1800 m. It is shallow lake with an average minimum depth of 8 m and maximum depth of 14 m. It embeds 37 islands and has a catchment area of 16,500 km^2. It is turbid and well-mixed (Gebremedhin *et al.,* 2013). Seven large permanent rivers feed the lake as well as about 40 small seasonal rivers. The main tributaries to the lake are Gilgel Abbay (Little Blue Nile), Megech River, Gumara River and the Ribb River. The Blue Nile is the only out flowing river (Wondmagegne, 2012).

At least 15,000 people are believed to live on the 37 islands in the lake Tana. Over 200,000 beneficiaries of the lake Tana live in Bahir Dar city, which is the largest city on the lakeshore. The majority of the populations around the lake are dependent on rainfed agriculture. Cultivation practices are primitive and crop production and livestock rearing are closely integrated (McCartney, 2010).

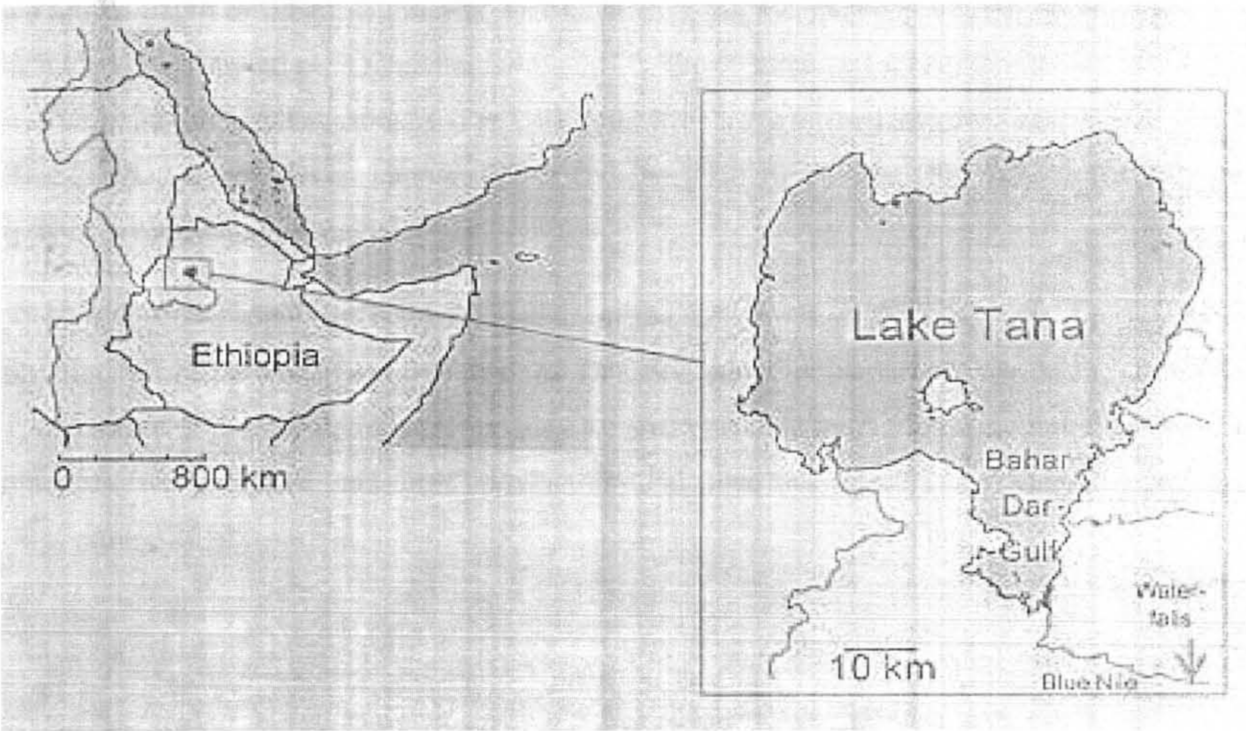


Fig.2: The study site: Lake Tana and Bahir Dar town

The potential for wild fish production of Lake Tana is estimated to be 13,000 tonnes per annum. However, the current production is only about 1,000 tonnes per year. For the people living on the islands and around the lakeshore, boat transportation is vital and is the only means of transportation to connect them to the city of Bahir Dar. For many centuries, the Negede or Weito people have built boats known as'*tanquas*' from the papyrus reeds and are widely used. Neverthless, in recent times, some modern fishing boats have been introduced. Ferries also make regular services between the islands (McCartney, 2010). Three main fish species targeted by commercial gillnet fisheries are the large *Labeobarbus* species, African catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*) (Gebremedhin *et al.,* 2013).

3.2. Selection of sampling stations

Diagnostic survey was undertaken in order to systematically select six sampling stations. Following the six sites were seleceted based on their proximity to expected anthropogenic emission sources. Six sampling sites were fixed based on the hypothesis that five sites within the Gulf are antropogenically more influenced so as to represent negatively impacted parts of the lake while the sixth site was made to correspond to less affected water body and hence used as control. Brief description of the selected sites is as follows.

The location of sampling site one (Micheal church) was where fish production and marketing enterprise takes place with anticipated release of quite substantial amount of fish offal and contaminants from transportation boats. The second sampling site (Prison) was included in connection to the onshore jail which could pose leachate from solid and liquid waste released from the premises. The location of site 3 (Hospital) was near by Felege Hiwot referral hospital chosen for possible release of hospital effluent. Site four (Blue Nile outlet) was made to be at the outlet of Blue Nile river. This site was considered control against systematically selected polluted loctions. Fivth sampling site (Alima building) was near the onshore Alima building seleceted for possible contamination of the lake by sewage released from the hotel and people who come for onshore recreation. The final site (Kuriftu risort), is located near Hidar 11 recreational centre where not only one of the city's sewerage lines empty their watses but also quite a lot of people take on shore cloth washing, bathing and swimming which were presumed as possible sources of contamination.

3.3. Collection of samples

Samples of water and fish were collected in February 2014. Both fish and water samples were taken at all sampling sites with the help of fisherymen. They were requested to capture fishes of different size at negotiable price. Fishing sites were bound within the radius of the fixed smapling locations with the aid of the investigator. Four average-sized specimens per fish species (two fishes per sex and six fish per site) were seleceted and bought from the local fishermen. The sections of the bought fishes were made to represent eqaual proportion of the both sexes per sepecies. Sex identification was made by the skilled technical assistant to the principal investigator. The identification of sex was by examining genital papilla located immediately behind the anus of the fish.

After collection of the appropriate samples, length and weight of the fish were determined (Table 1). The samples were immediately washed by de-ionized water, dissected and only edible tissue (fillet) was transferred to plastic bags and keept in an ice box at about 4^0C for transportation to Chemical and Food Engineering Department of Bahir Dar University laboratory. Samples were kept in deep freezer until transported to Center Food Science and Nutrition laboratory of Addis Ababa University.

Table 1 Some basic descriptions of the studied fish species in Lake Tana

Species	Local name	Habitat	Feeding mode	Food source	Mean weight (g)	Mean length (cm)
O. niloticus	Kereso	Benthopelagial	Herbivorous	Mainly algae	243.5±45.2	23.71±1.2
C. gariepinus	Ambaza	Benthopelagial	Carnivorous	Fish, aquatic insects, mollusks	280.3±31.2	34.14±1
L. intermedius	Niche assa	Benthopelagial	Carnivorous	Mollusks, aquatic insects, fish	252.1±50.1	28.95±2.3

Source: Dsikowitzky *et al.* (2012);

Further, a total of six water samples were collected from six sampling locations. Samples at each site were replicated three times for composite analysis using 1L polyethylene sampling bottle. Samples of surface water were collected at depth of about 50 cm from the water surface to prevent the contamination heavy metals from surface air. Bottled samples were taken to laboratory along with the fillet samples in an ice box at temperature of about 4 ^{0}C. The three replicated water samples at each site were composited for all analytical procedures conducted in the laboratory.

3.4. Chemical analysis

3.4.1. Preparation of the instrument and apparatus

All glassware were soaked overnight in 10 % (v/v) nitric acid, rinsed with distilled water for about three times and dried before using. The fish samples were oven dried and grounded in mortar and pestle. Heavy metal digestion was carried out by using kjelddhal apparatus. Two hundered fity milliliter capacities round bottom flask was used to collecet digeted samples. For the purpose of protein analysis kjelddhal apparatus, for fat soxhlet, for ashing furnace and for moisture dtermination oven were used. Analyst 600 graphite Atomic Absorption Spectrometer, were used for analysis of heavy metals in water and fish samples.

3.4.2. Reagents and standard solution

All the chemicals used were of analytical reagent grade. De-ionized water was used for all dilutions throughout the study. HNO_3 (69%), and hydrogen peroxide, H_2O_2 (30%), were used for digestion of fish sample. Working standards were prepared by diluting concentrated stock solution of 1000 mg/L for Cd, Pb and Cr dilution carried out in stage used de-ionized water. Matrix modifiers: 0.05mg of $NH_4H_2PO_4$ + 0.003mg of Mg $(NO_3)_2$, for lead (Pb), 0.05mg of $NH_4H_2PO_4$+0.003mg of Mg $(NO_3)_2$ for cadmium (Cd) and 0.015mg Mg $(NO_3)_2$ for chromium (Cr) were used for Graphite Atomic Absorption Spectrophometery (GFAAS). Matrix modifiers are important to react with the analyte to stabilize the element.

3.4.3. Determination of physicochemical parameters

Physicochemical variables of sample water were determined. These were electrical conductivity, pH, temperature, and total dissolved solids. The pH and temperature of sample water was measured with probe coupled pH/Temp/Meter (Model CE 370 pH meter 01186, EU). Electrical conductivity (EC) and total dissolved solids (TDS) were measured with Cond//TDS meter (Model CE 470 Cond Meter 01189). All the physio-chemical measurements were taken by dipping the probe about 3-5 cm below the water surface (APHA, 1995).

3.4.4. Preparation of samples

Three replicated water samples for each site were composited for all analytical procedures conducted in the laboratory. From the composited sample 100 ml water were taken and filtered using 0.45μm filters. The filtered samples were stored in pre-cleaned polyethylene bottles (cleaning with 10 % HNO_3, 48 h). Concentrated 0.5 ml HNO_3 was added to each sample, to prevent precipitation of metals, reduce adsorption of the analytes onto the walls of containers and to avoid microbial activity, and then water samples were stored at 4°C until the analyses.

In order to obtain a representative fish sample, composites were prepared by taking the edible tissues (fillet) of the fish samples at each sampling site for each sex and species. The fish samples were thawed at room temperature and samples were oven dried at 60 ^{0}C for 72 hrs. Each dried sample was then ground into a fine powder using porcelain mortar and pestle. All the powdered tissues were kept in polyethylene bag for proximate and heavy metal analysis.

3.4.5. Digestion of fish samples

The powdered fish samples were thoroughly homogenized before subjecting them to digestion. Powdered samples were digested using concentrated nitric acid and hydrogen peroxide (1:1) v/v according to FAO methods CF Daziel and Baker (1983). One gram of dried and powdered fish samples was weighted and transferred into 250 mL round bottled flask. Mixture of 10 mL of concentrated HNO_3 (65%) and 10 mL of H_2O_2 (30%) was added to the sample flask. The flask was covered with a watch glass and left aside until the initial vigorous reactions occur. Then, the samples were heated on a Heating Mantle (kjeldhal apparatus) to 130 ^{0}C until dissolution inside a fume hood to reduce the volume to 3-4 mL. After that, the samples were allowed to cool, were filtered and diluted to 50 mL in volumetric flask with de-ionized water. The actual concentration of each metal was calculated according to (Olaifa *et al.,* 2004) using the following formula:

Actual concentration of metal in Sample = PPMR X Dilution factor

Where PPMR = AAS reading of Digest

$$\text{Dilution factor} = \frac{\text{Volume of digest used}}{\text{Weight of Sample digested}} \qquad \text{Equation....... (1)}$$

3.4.6. Analysis of heavy metals

Concentration of Cr, Pb and Cd were determined in water and fish samples. The analyses of heavy metals in water and fish samples were carried out by graphite furnace atomic absorption spectrometry .The Analyst 600 Graphite Atomic Absorption Spectrophotometer equipped with a graphite furnace autosampler was used for determinations. The operating conditions for Cr, Pb and Cd analysis by GFAAS were indicated in (Table 2). Calibration of the instrument was carried out with range of standard solution. After calibration, the samples were aspirated into the AAS instrument. The samples were analysed in duplicates, and the blank determinations in duplicates were also run in the same manner during the analysis.

Table 2 Sample assay conditions by graphite atomic absorption spectrometry

Parameter	Cd	Cr	Pb
Sample	Water + Fish	Water + Fish	Water + Fish
Method	GFAAS*	GFAAS	GFAAS
Wave length	228.8	357.9	283.3
Tube/site	Pyro/platform	Pyro/platform	Pyro/platform
Pretreatment temp (^{0}C)	700	1500	850
Atomization temp (^{0}C)	1400	2300	1500
Low slit (nm)	0.7	0.7	0.7
Matrix modifier	0.05mg of $NH_4H_2PO_4$ +0.003mg of Mg $(NO_3)_2$	0.015mg Mg $(NO_3)_2$	0.05mg of $NH_4H_2PO_4$ + 0.003mg of Mg $(NO_3)_2$

*GFAAS = graphite furnace atomic absorption spectrometry

3.4.7. Validation of analytical methodology (Recovery test)

The digestion method and AAS analysis were validated by measuring the recovery of cadmium, lead and chromium spiked to fish samples. The known volume and concentration of standard solutions were employed on the samples in order to determine recovery. The volume of 1.5ml for Cd, Pb and Cr was added to 1g of powdered fish sample. The spiked samples were then digested in the same way as fish sample. The final volume of the digestion was diluted to 50 mL and run on AAS and metal contents determined from the calibration curve.

The amount of spiked metals recovered after the digestion of spiked samples was used to calculate percentage recovery using Burns *et al.* (2002) formula.

$$\text{Recovery} = \frac{\text{conc. Spiked sample - unspiked sample c}}{\text{Conc. Analyte added (spiked)}} \times 100\% \qquad \text{Equation .... (2)}$$

The recovery percentages of spiked fish sample were obtained as shown in Figure 2. Rresults for metals under investigation (Cd, Pb and Cr) varied between 94.6% and 100%. The obtained

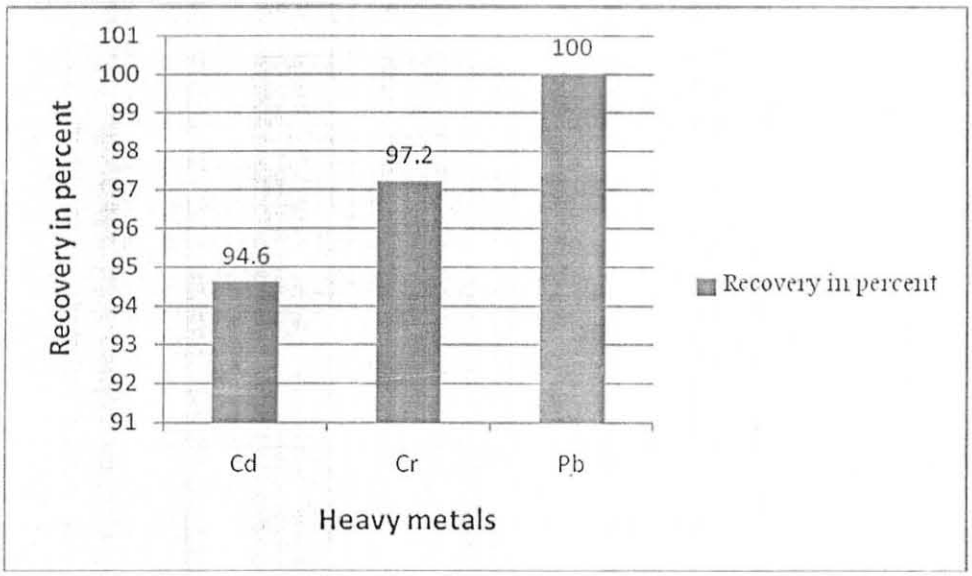


Fig. 3: Recovery percentage of the heavy metals in spiked fish samples

results are in acceptable range which is mostly no less than 70% and no greater than 125% (Machado and Griffith, 2005) and which revealed that the digestion method and the AAS analysis were reliable.

3.4.8. Calibration procedure

Calibration curves for each heavy metal were set to ensure the accuracy of the atomic absorption spectrophotometer and to confirm that the results of determination were true and reliable. The calibration of the analyst 600 Atomic Absorption Spectrophotometer was made with standard solutions. Calibration standards were prepared by serial dilution of concentrated stock solution of 1000 mg/L for Cd, Cr and Pb. These solutions and blank were aspirated into AAS. A calibration curve of Absorbance Vs concentration was established for each metal and used for determination of metal concentration in the samples of fish and water. The calibration curve of each heavy metal was given in appendix 1.

3.4.9. Method detection limit

Method detection limit (MDL) is defined as the minimum concentration of analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero, and is determined from analysis of a sample in a given matrix containing the analyte (USEPA, 1997).

Method detection limit for fish samples was established using the blank reagent (mixture HNO_3& $H2O_2$) which was used to digest the fish sample. Seven replicate fish blanks were digested in the same condition as fish sample. The method detection limits were calculated according to Childress *et al.*, (1999) formula:

$$MDL= (S) \times (t) \qquad \text{Equation .... (3)}$$

Where MDL= Method Detection Limit

S= standard deviation of the seven replicate analysis

t= student's value for 99% confidence level and standard deviation estimated with n – 1 degrees of freedom

The concentrations of the metals in fish samples below MDLs were rejected and only above MDLs were reported in this study.

3.5. Proximate composition analysis

3.5.1. Moisture content

Moisture content was determined by Oven drying method following the procedure of AOAC (1998). Empty dishes were dried using air drying oven for 1 hour at 105^0C, transferred to the desiccators (with granular silica gel), cooled for 30 minutes, and were weighed. Replicates of the minced samples were mixed thoroughly and five gram of composite fresh fillet was transferred to the dried and weighed dishes. The dishes and their contents were placed in the drying oven and dried for 3 hrs at 105^0C in an oven until constant weights were obtained, and then the dishes and their contents were cooled in desiccators to room temperature and reweighed. The moisture content was determined by measuring the weight of a sample before and after the water was removed by evaporation:

$$\text{Moisture content} = \frac{\text{Weight of wet sample- Weight of dried sample}}{\text{Weight of wet sampl}} \text{X } 100$$

3.5.2. Crude protein

Crude protein in the sample fish fillets were quantified method following the procedure of AOAC (1998) by Kjeldahl methods. 0.5 g of powdered fish fillet was weighed into Kjeldahl digestion flask and then digested by heating at 370 0C for four hours in the presence of 6 mL Sulfuric acid, 3.5 mL H_2O_2, 3 g of catalyst Copper Sulfate (CuSO4) and Potassium sulfate (K_2SO_4). After digestion was completed, formed clear solution was cooled for 30 minutes and neutralized by addition of 25 mL NaOH (40 %) and diluted using 25mL distilled water. Twenty five mL of distilled water, 25 mL of Boric acid and 3 drops of Methyl blue was added to receiving flask 250 mL capacity connected to the distiller by tube. The distillation process was terminated when the volume of receiving flask reached between 200 to 250 mL. Note: all reagents were added to the blank except the sample. The nitrogen content was estimated by titration of the borate anion formed with 0.1N HCl. The amount of Nitrogen was calculated using the formula:

$$\% N = \frac{N\ HCl \times (\text{Volume of HCl titrates sample} - \text{Volume of HCl titrates blank}) \times 14g \times 100}{\text{Gram of sample mole}}$$

Crude protein = 6.25 x N

3.5.3. Crude fat

Crude fat was determined method following the procedure of AOAC (1998) by semi continuous solvent extraction methods (Soxhlet method). Accordingly, for all sample categories, 2 g of dried and ground sample was placed in a porous cellulose extraction thimble and thimble was covered with fat free cotton. The thimble was placed in an extraction chamber which is suspended above a flask containing the solvent (50 mL of diethyl ether) and below a condenser. The flask which had dried in drying oven at 1050C containing boiling chips was placed inside the extraction chamber and heated at 550C and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. At the end of the extraction process, which typically lasts for 3 hours, the flask containing the solvent and lipid was removed, the solvent was evaporated in drying oven at 70 0C and the mass of lipid remaining was quantified gravimetrically and calculated from the difference in weight of the extraction flask before and after extraction as percentage. The crude fat in the initial sample was calculated as:

$$\text{Fat content} = \frac{\text{Weight of fat} \times 100}{\text{Weight of sample}}$$

3.5.4. Ash content determination

To determine the ash content AOAC method (AOAC, 2000) was used. Briefly, duplicates of 2.50 g of homogenized samples were placed in pre-washed, dried, weighed and marked crucibles, to be ashed at 550 0c in Muffle Furnace for eight hours. Then, samples were cooled in desiccator and weigh again. The ash content was calculated as follows:

$$\% \text{ ash (wet basis)} = \frac{\text{wt after ashing} - \text{tare wt of crucible}}{\text{Original sample wt}} \times 100$$

Eventually protein and fat content in wet base was recalculated from dry base using the formula:

$$\% \text{ Proximate in wet} = \frac{\% \text{ Proximate in dry (100-Moisture content)}}{100}$$

3.5.5. Gross energy value

Gross energy values (kcal/g) was calculated by overall addition of the protein content multiplied by 4 and the total lipids content multiplied by 9 and using Atwater's conversion factors. The result was expressed as kcal per 100 gram.

Gross energy value= 4 x protein content + 9 x fat content

3.7. Estimation of heavy metal intake dose

Heavy metal intake dose estimation was based on the assumption by FAO (2011). According to FAO (2011) report, Ethiopians are traditionally meat eaters. The eating habits have been shifting in favor of fish in communities and areas where there is a regular and sufficient fish supply. In the community suppiled with sufficient fish quantity, annual fish consumption can exceed 10 kg per person. Therefore average value of 0.19 kg per week can be used for the calculation of the estimated weekly fish intake (EWI). Further, the dose of heavy metal intake can be drived as a product of EWI and accumulation of the heavey metal in the fish. On such logical frame work, the possible heavey metal intake of consumers of fish from Lake Tana was caluculated. To express the intake on fresh (wet) basis, dry weight EWI values were divided by an average moisture factor of 4.98. Finally, possible heavey metal intake was reported on fresh fillet basis. The reported figures were compared with WHO (1993) safety margins.

3.6. Statistical analysis

Statistical analyses of data were carried out using SPSS version 21. While treatments were factorially arranged, one way ANOVA in case of water sample, two way ANOVA in case of heavy metals and three way ANOVA in case of proximate analysis was used to test statistical significance of main effects. Significantly different mean values of the responses were separated at $p < 0.05$. The mean separation technique followed Duncan multiple range test.

4. RESULTS AND DISCUSSION

4.1. Proximate composition and energy values in relation to fish species

The mean and standard error of proximate composition of the three fish species was presented in (Table 3). It was shown that fish speceies significantly affected the proximate composition of the fish fillets (Table 3). Mean protein content of *O. niloticus* (18.82%) was significantly higher than *L.intermedius* (15.44%) followed by *C. gariepinus* (15.2%). The moisture content of the *O. niloticus* (78.99 %) was significantly lower compared to both *C. gariepinus* (80.45) and *L. intermedius* (80.37). The mean value of effct of species on fat was significantly highest for *L. intermedius* (2.36%) while it was lowest mean value (0.58%) for *O. niloticus*.The mean ash content of both *O.niloticus* and *L. intermedius* was significantly higher compared to that of *C. gariepinus*. Neverthless, *L. intermedius* gave the highest mean gross energy content relative to *O. niloticus* which was followed by *C. gariepinus*.

Table 3: Mean ± SE proximate composition in percent and gross energy (GE) content in kcal /100 g of *O. niloticus, C. gariepinus and L.intermedis* fillet in wet basis (n=3)

Fish species	N	Parameter				
		Protein	Moisture	Fat	Ash	Gross energy
O. niloticus	24	18.82±0.01[a]	78.99±0.03 [b]	0.58±0.02[c]	1.44±0.01 [a]	79.78±0.07[b]
C. gariepinus	24	15.2±0.01[c]	80.45±0.03 [a]	1.76±0.02 [b]	1.3±0.01 [b]	76.71±0.07[c]
L. intermedius	24	15.44±0.01[b]	80.37±0.03 [a]	2.36±0.02[a]	1.44±0.01 [a]	83.1±0.07[a]

N= Sample size of fish. Values with different superscripts with in the column of parameters were significantly different ($p<0.05$)

The present study is consistent with findings of Casallas *et al.* (2012) who reported protein values of *Oreochromis sp.* (18.4%-20.8%) were significantly higher than that of *C.garipiness* (15.71%-16.2%). On the other hand, different authors (Olagunju *et al.*, 2012; Ayeloja *et al.*, 2013) reported that *C. garipiness* significantly higher protein value compared to *O.niloticus*. Quantitatively, protein is the second major component in muscle tissues of fish. Its content in fish is considered low if it is below 15% (Stancheva *et al.*, 2013). In this study all the mean protein values were above 15% and indicated that the studied fish species were rich source of protein to consumers.

Casallas *et al.,* (2012) obtained that *Oreochromis sp.* (72.3-76.9) had lower moisture content than *C. gariepinus* (76.8-77.91) which is in agreement with present finding. The reverse was reported by other researchers (Fawole *et al;* Olagunju *et al.*, 2012).The percentage of water is a good indicator of its relative content of energy, protein and lipid.The high moisture content is a disadvantage in that it increases the fishes' susceptibility to microbial spoilage, oxidative degradation of polyunsaturated fatty acids and consequently decreases in the quality of the fishes for longer preservation time (Olagunju *et al.,* 2012).

Reports of both Ayeloja *et al.,* (2013) and Casallas *et al.,* (2012) were in agreement with present finding. They revealed that the fat content was significantly higher in *C. garipinus* than in *O. niloticus*. However the opposite were obtained by Olagunju *et al.* (2012). The present result showed that those species had low content of lipid in their muscles. This may be expected in fresh water fishes despite marine fishes have higher lipid content for the reason that of diversified feed source in their environment than the fresh water fishes (Olagunju *et al.,* 2012). According to Olagunju *et al.* (2012) O. *niloticus and C. gariepinus* could be classified as a lean, whereas *L. intermedius* was low fat species. Therefore, *L. intermedius* could be more useful for production of fish oil relative to both fish species.

Ash value of present study is consistent with the result of Olagunju *et al.* (2012) who reported that the ash content of *Oreochromis species* was signiftlycanly higher than *C. garipiness*. Casallas *et al.* (2012) and Ayeloja *et al.* (2013) revealed that ash content of *C. gariepinus* higher than that of *O. niloticus* which is not parallel with the present finding.

Higher gross energy value in *L. intermedius* as compared to *O. niloticus* and *C. gariepinus* may be attributed to relatively higher fat in L. *intermedius* than the other two fish species. No data is available in literature for proximate composition of *L. intermedius* and *C. gariepinus* in Ethiopia's lakes for comparison. In general, however, different researchers reported that there was significant difference in proximate composition between different species (Fawole *et al.*, 2007; Mazumder *et al.*,2008; Oniya *et al.*, 2010; Karemoh and Amakiri, 2013; Ondoazi *et al.*, 2013).

The difference of proximate composition in the obtained values of the three fish species may be due to fish's consumption or absorption capability and conversion potentials of essential nutrients from their diet or their local environment into such biochemical attributes needed by the organisms body are different between fish species (Fawole *et al.*, 2007).

4.2. Proximate composition and energy values in relation to sex

The mean proximate composition in male and female of three fish species *O. niloticus, C. gariepinus* and *L. intermedius* were investigated and compared (Table 4). Sex of the studied fish speceies affected the proximate composition and gross energy content. Except for mean moisture content, all other proximate values and gross energy content of female fish were significantly higher ($p<0.05$) compared to that of male fish. In the case of mean values of moisture, however, male fish gave significantly higher mean values (80.58%) compared to that of female (79.29%) at $p<0.05$.

Table 4: Mean ± SE proximate composition in percent and gross energy content in kcal /100 g of three fish species fillet in relation to sex in wet basis (n=3)

Sex	N	Parameters				
		Moisture	Protein	Fat	Ash	Gross Eergy
Male	36	80.58±0.02[a]	15.99±0.01[b]	1.290. 01[b]	1.32±0.01[b]	75.17±0.06[b]
Female	36	79.29±0.02[b]	16.98±0.01[a]	1.84±0.01[a]	1.47±0.01[a]	84.55±0.06[a]

N= Sample size of fish. Values with different superscripts with in the column of parameters were significantly different (p<0.05)

The mean moisture content of present study is similar with Stancheva *et al.* (2013) who reported that the major component of the fish fillet was moisture (78 to 88 %). Similar to the present finding, Ozogul *et al.* (2010) obtained that moisture contents of male blue crabs and male swim crabs were significantly higher ($p<0.05$) than those found in female blue crabs and female swim crabs. In contrast study conducted by Alemu *et al.* (2013) on proximate composition of Nile tilapia fillet from lake Zeway revealed that moisture content of male fish were non significantly higher than that of female fish.

Significantly higher protein values of migrating allis shad female fish (20.1%) relative to the male (19.0 %) fish and for resident allis shad female fish (17.9%) compared to male (17.3%) was repodted by Pereira *et al.* (2013) which is inline with present study. Similar result by Ozogul *et al.* (2010) on the effects of season and sex on the fatty acids and proximate compositions of the mantle of the cuttlefish *S. officinalis* showed that the level of protein contents of female of *S. officinalis* were significantly higher than those of male ($p<0.05$) across all seasons. In other study the protein contents of female blue crabs and female swim crabs were significantly higher ($p<0.05$) than those found in male blue crabs and male swim crabs (Ozogul *et al.* 2010). On the other hand study conducted in Ethiopia Lake Zeway by Alemu *et al.* (2013) showed the protein content of the female were slightly but not significantly higher than that of the male fish.

The fat content of present study is corresponding with the findings of Ozogul *et al.* (2010) reported that *S.officinalis* female fish (0.94%) were significantly higher than male (0.84%). On the other hand different aouthors obtained that there was non significant different in fat content between different sex fishes (Alemu *et al.*, 2013; Pereira *et al.*, 2013).

Present investigation is similar with the report of Aydin (2014) who stated ash content of female (1.86%) jinga shrimps were significantly higher relative to male (1.10%) jinga shrimps. However, as differed from the present findings, Alemu et al., (2013) and Pereira et al., (2013) found that the ash content was not significantly different between female and male fish.

Higher gross energy value in female fish compared to male may be attributed to relatively higher protein and fat in females than males (Ozogul *et al.,* 2010).The difference in proximate composition between sex may be because of intrinsic factors like sex and size greatly influence various physiological processes (Naeem *et a*l., 2011). Such variations might also be due to difference in the biochemical reactions of the tissues in respect to size (Shahana *et al.,* 2010). In this study it was clear that females were longer and heavier than males as reported byPereira *et al.* (2013).

4.1.3 Proximate composition and energy values in relation to location

The effect of location on protein contenet of fish fillets were generally ststistically significant at $p<0.05$ (Table 5). Fish fillets colleceted from Kuriftu risort contained significantly lower mean protein value (15.78%) compared fillets colleceted from other locations followed by mean protein value (16.27%) of Prison and value (16.59%) of Blue Nile outlet (Table 5). Mean protein content of fillets colleceted from Hospital (16.69%) was significantly higher than values of filletes from Blue Nile outlet but not from mean values of fillets from Micheal, which inturn is statistically non significant from mean fillets' value (16.83%) of Alima building.

Table 5: Mean ± SE proximate composition percent and gross energy content (GE) in kcal /100 g of the three fish species fillet in different location in wet basis (n=3)

Location	N	Parameters				
		Protein	Moisture	Fat	Ash	Gross Energy
Micheal	12	16.76±0.02^{bc}	79.71±0.04^{e}	1.88±0.01^{b}	1.42±0.01^{c}	84.04±0.1^{b}
Prison	12	16.27±0.02^{e}	80.19±0.04^{b}	1.1±0.01^{f}	1.23±0.01^{e}	73.51±0.1^{f}
Hospital	12	16.69±0.02^{c}	79.51±0.04^{f}	1.26±0.01^{e}	1.36±0.01^{d}	78.20±0.1^{d}
Blue Nile outlet	12	16.59±0.02^{d}	80.04±0.04^{c}	2.31±0.01^{a}	1.43±0.01^{c}	87.17±0.1^{a}
Alima building	12	16.83±0.02^{ab}	79.79±0.04^{de}	1.57±0.01^{c}	1.45±0.01^{bc}	81.48±0.1^{c}
Kuriftu risort	12	15.78±0.02^{f}	80.37±0.04^{a}	1.29±0.01^{d}	1.47±0.01^{ab}	74.78±0.1^{e}

N= sample size of fish. Values with different superscripts with in the column of parameters were significantly different (p<0.05)

Moisture content were significantly (p<0.05) affected by location (Table 5). Fish fillet collected from Kuriftu risort gave significantly higher moisture mean value (80.37%) compared to fillets collected from other location followed by mean value (80.19%) of Prison and mean value (80.04%) of Blue Nile outlet. Mean moisture content of fillet collected from Hospital (79.51%) were statsicaly lower compared to other locations followed by mean value (79.71%) of Micheal and mean value (79.79%) of Alima building but fish fillet gathered from Micheal and Alima building were not significantly (p>0.05) different.

Sample location significantly affected the fat content of fish fillet at p<0.05 (Table 5). Significantly higher mean value (2.31%) of fat conten of fish fillet were collected at Blue Nile outlet and the lower mean value (1.1%) of fat content of fish fillet were collected at Prison. All locations were significantly different in their mean value of fish filletes of fat content.

The effect of location on ash contenet of the fillets were ststistically significant at p<0.05 (Table 5). Fish fillet collected from Kuriftu resort were significantly higher mean value (1.47%) than ash conten compared to other locations but not significantly different from Alima building mean value (1.45%). Also Alima building were not significantly differ from Blue Nile outlet and Micheal mean value of ash content.The lower mean value (1.23%) of ash content were obtained at Prison.

Gross energy were significantly ($p<0.05$) affected by location (Table 5). The highest mean value (87.17) of gross energy was recorded for Blue Nile outlet while the lowest mean value (73.51) was at Prison. The GE value of all other locations varied between the highest value of Blue Nile outlet and lowest value of Prison.

Present study is inline with the finding of Ali *et al.* (2001) on *Channa punctata* collected from two different locations. The authors reported significant effect of location on proximate composition. The same result was reported in Egypt that different location had significantly different values (Tawwab, 2004). Study conducted in Egypt on three cichlid species proved that environmental factors as climate (seasonal variations and temperature) and drainage wastewater affect the physical and chemical characteristics of water as well as fish condition and quality (Saeed, 2013).

Variation on chemical body composition of fish may be attributed to environmental conditions (Ibrahim *et al.*, 2008) and other biotic and abiotic variables, such as hydrologic level, food availability and water temperature (Touhata *et al.*, 1998). The environmental conditions in water bodies are constantly changed by various natural and human induced factors. Variation in proximate composition may result from the features of the physico-geographical environment of the catchment area, as well as the morphmetric parameters of the water body and its hydrological regime, accelerate or block the supply of organic matter to the lakes, which affects its trophic level, water pH and hardness, its electrolytic conductivity and colouring, light and oxygen availability, and consequently algae and plant species diversity (Chobot and Banaś, 2008). Hence variation on proximate composition of fish fillet in the present findings collected from different location may be due to availability of diet and water quality.

4.1.4. Interaction effect of species, sex and location

Exept there was no significant interaction effect among sex and species on the ash content, different leveles of main effects combined gave significant interaction for moisture, protein, fat, ash and GE ($p< 0.05$) appendix 2. For instance, two way and three way interaction of location, sex, and species gave significant effect of on mean protein values of fish fillets. Therefore, it implies optimum level of mean proximate value response of the fillets requires curvilinear combination of the different levels of the main effects.

The body composition of fish is mainly influenced by both the endogenous and exogenous factors, which operate simultaneously (Alemu *et al.,* 2010). The significant interaction in proximate composition in relation to location, sex and species may be due to the interaction effect of endogenous and exogenous factors.

Ahmad *et al.* (2002) obtained that feed intake and feed conversion ratio (FCR) were significantly affected by protein level, fish size and their interaction. Also they reported that fish growth was significantly affected by protein level and fish size. Therefore the variation reported in present study for proximate composition in different sex with different species may be due to the interaction effect fish's consumption or absorption capability and conversion potentials of essential nutrients (Fawole *et al.,* 2007; Oniyo *et al.,* 2010) with relation to fish size that signify female fish are longer and heavier than male fish (Naeem *et a*l., 2011).

Chatzifotis *et al.* (2011) reported that the increase in crude protein in the diet from 40 up to 50% positively affected SGR (Specific growth rate) and FCR (Feed conversion ratio). He also showed that the increase in crude lipids in the diet from 12 up to 17% showed a tendency for improvement in SGR and FCR. There was variation in the consumption of the food objects by the different fish species. Despite similarity in the rank- order of some food objects in the species, the ingestion of the food objects varied significantly (Offem *et al.,* 2009). Fish proximate composition variation from different site for the three fish species might be the interaction effect of the quantity of feed and water quality in the specific location (Dadebo *et al.,* 2013) and associated with the feeding on natural feed preferably phytoplankton and zooplankton feed and metabolic efficiency of nutrient from their diet for those fish species (Effiong and Mohammed, 2008).

4.2. Heavy Metal Analysis

4.2.1. Physico-chemical characteristics and heavy metal concentration of the water

Physico-chemical and heavy metal concentrations were significantly ($p<0.05$) affected by location (Table 6). Temperature obtained from Kuriftu risort significantly higher temperature mean value (25.7°C) compared to temperature obtained from other locations followed by mean value (25.3°C) of Alima building and mean value (24.95 °C) of Hospital however there was no significant different between Kuriftu risort with Alima building. Again Alima building was not significantly different from Hospital. Significanly lower mean value (20.65 °C) of temperature was obtained at Blue Nile outlet compaired to other locations.

Blue Nile outlet is recorded significantly ($p<0.05$) higher pH mean value (8.85) compaired to other locations followed by Alima building mean value (8.55) and mean value (8.53) of Kuriftu risort. However, Kuriftu resort, Alima building, Prison and Micheal were not significantly ($p>0.05$) varied in their pH mean values. Significantly lower pH mean value (8.21) was indicated at Hospital which was not significantly different with Micheal mean pH value (8.36).

Only Blue Nile outlet were significantly lower mean total dissolved solids value compaired to other locations. All other locations were not significantly varied in their total dissolved solid mean values.Which is the same for electrical conductivity.

There was no significant ($p>0.05$) variation in total Chromium concentration between different sampling locations. Cadmium concentration were not detacted in Prison, Hospital and Blue Nile outlet.Cadmium were significantly ($p<0.05$) higher mean concentration (0.0195μg/L) in Alima building followed by mean concentration value (0.0072μg/L) of Micheal and mean concentration value (0.0002μg/L) of Kuriftu risort. Significantly ($p<0.05$) higher lead mean concentration value (0.039 μg/L) were obtained in Prison compired to other locations followed by Hospital mean concentration value (0.0261μg/L) and mean concentration value (0.0219 μg/L) of Alima building. There was no significant ($p>0.05$) different between mean concentration value of Hospital and Alima building. Micheal was significantly ($p<0.05$) lower lead mean concentration value compaired to other locations however there was no significant ($p>0.05$) different in lead mean concentration values between Micheal, Blue Nile outlet and Kuriftu risort.

The temperature of present finding of lake Tana water values are inline with the range of average water temperature values (19 -26 °C) reported previously nine Ethiopian lake by Vijverberg *et al.*(2012) but slightly below the range of 21.7 to 26.7°C reported on lake Tana by wudneh, (1998) and Wood and Talling, (1988). Okorie and Oko (2013) reported that there was a positive correlation between the conductivity of the water and the temperature. The conductivity of the water is due to the presence of soluble salts of some metals in the water (Ezzat et al., 2012). This suggests that as conductivity of the water increases the temperature also increases.

Table 6: Descriptive values of physio-chemical and heavy metal concentrations of water from lake Tana (n=2)

:ation / neters	Micheal Mean ± SD	Prison Mean ± SD	Hospital Mean ± SD	Blue Nile outlet Mean ± SD	Alima building Mean ± SD	Kuriftu risort Mean ± SD	WHO*
ɔerature	23.1±0.52^{e}	24.8±0.52^{d}	24.95±0.52^{cd}	20.65±0.52^{f}	25.3±0.52^{bc}	25.7±0.52^{ab}	-
	8.36±0.13^{bc}	8.49±0.13^{b}	8.21±0.13^{c}	8.85±0.13^{a}	8.55±0.13^{b}	8.53±0.13^{b}	6.5-9.5
(ppt)	0.06±0.002^{a}	0.07±0.002^{a}	0.065±0.002^{a}	0.045±0.002^{b}	0.07±0.002^{a}	0.06±0.002^{a}	500mg/L
3/m)	0.135±0.005^{a}	0.135±0.005^{a}	0.135±0.005^{a}	0.115±0.005^{b}	0.14±0.005^{a}	0.135±0.005^{a}	250µS/cm
g L^{-1})	0.0148±0.0006^{a}	0.0133±0.0006^{a}	0.011±0.0006^{a}	0.0118±0.0006^{a}	0.0154±0.0006^{a}	0.0136±0.0006^{a}	50 µg/L
ıg L^{-1})	0.0072±0.0035^{b}	ND	ND	ND	0.0195±0.0035^{a}	0.0002±0.0035^{c}	3 µg/L
.g L^{-1})	0.0009±0.004^{c}	0.039±0.004^{a}	0.0261±0.004^{b}	0.0012±0.004^{c}	0.0219±0.004^{b}	0.0068±0.004^{c}	10 µg/L

Considering the mean concentrations between the six sampling sites, Values with different superscripts within the row were significantly different ($p<0.05$).

ND: Not detected

** Guideline values for drinking water quality from the world health organization (WHO, 2008)

It was observed that the sample location with the highest temperature in Kuriftu risort also had the highest electrical conductivity. The highest values of electrical conductivity might indicate the lake Tana were received land run off at kuriftu risort (Goshu et al., *et al.*, 2010).

Present study is within the range of pH values 6.8-9 reported previously on lake Tana by Goshu *et al.* (2010) but slightly higher the range of 5.7-7.7 reported in lake Tana by Getahun & Selassie (2013). Other authors Enuneku *et al.,* (2013) and Saeed and Mohammed, (2012) also the same with present study they revealed that there were significant variation between different sampling site in pH values. Favorable range of pH is 6.5-9.0 most suitable for fish production (Saeed and Mohammed, 2012). pH values of the present study for all collected water samples from different location in lake Tana were within the permissible limits of WHO (2008). PH of present study indicates that lake Tana is suitable for fish production. The pH of Hospital water was low and this may be due to the discharge of wastewaters released from Felege hiwot hospital (Goshu *et al.,* 2010).

Total dissolved solids from lake Tana in Bahir Dar gulf by Goshu *et al.,* (2010) were reported between 0.02-0.42 which is in the range of current study. The same finding also reported for the same lake by Getahun & Selassie (2013). Other researchers Enuneku *et al.,* (2013) and Saeed and

Mohammed, (2012) revealed that there were significant variation between different sampling site in total dissolved solids values. TDS is an important indicator of the suitability of water for drinking, recreational, irrigation and industrial use. In water, total dissolved solids are composed mainly of carbonates, bicarbonates, chlorides, phosphates and nitrates of calcium, magnesium, sodium, potassium and manganese, organic matter, salt and other particles (Mahananda, 2010). Higher concentrations of TDS in the impacted location water were thus likely originating from sewage, urban runoff and wastewater (Goshu *et al.,* 2010). TDS values of the lake were below the WHO (2008) limit for drinking water (Table 6).

Present finding is in the range of previous work done by Getahun & Selassie, (2013) and Goshu *et al.* (2010) that were carried out in the same lake. Other authors Enuneku *et al.* (2013) and Saeed and Mohammed, (2012) also the same with present finding they revealed that there were significant variation between different sampling site in electrical conductivity values. Conductivity is a measure of the tendency of a liquid to conduct electricity.Thus the observed conductivity of the water is due to the presence of soluble salts of some metals in the water.The highest values of electrical conductivity might indicate that impacted site are receiving large quantities of land run off and/or industrial pollution (Ezzat *et al.*, 2012).The electrical conductivity of the lake Tana water samples indicating that the lake is not heavily loaded with ions. Lake Tana was not go beyond the WHO (2008) Limit of drinking water showed in (Table 6).

All obtained metal concentrations are below the values from previous studies of water from lake Awassa and lake Ziway (Ataro *et al.,* 2003; Nigussie *et al.,* 2010; Dsikowitzky *et al.,* 2012) and other study in lake Gudbahri, eastern Tigray of northern Ethiopia by Desta and Weldemariam (2013). Study condacted by Dsikowitzky *et al.,* (2012) reported that there was no significat different between different sample locations in heavy metals concentration of the lake which were more or less the same with present finding. The heavy metal concentrations in the lake water were relatively low, even though the systems receive a constant input of wastewaters (Goshu *et al.,* 2010). This may be due to degree or type of pollution. As a result temperature and pH conditions were not favorable for the solubility of the metals; it was not expected to find high amounts of dissolved metals in water samples in the lake Tana. In addition TDS and EC were not expected to elevate the metal concentration in the lake because they are still not go above the

WHO limit. Furthermore, the concentrations heavy metal is very low showing that there was no measurable effect of the waste water discharges on the potentially impacted sites. The concentrations of Cd, Cr and Pb in water from Lake Tana were below background values for WHO (2008) (Table 6).

4.2.2. Comparison of Heavy metals in lake water and fish muscle

In present study Chromium and cadmium concentrations were lower in the water samples compared to fish muscle (Table 6). Pb was highest concentration relative to chromium and cadmium concentration in the water sample. However Chromium and cadmium were higher concentrations and lead was not detected in the examined fish species fillets (Table 7).

Present finding reported that heavy metals concentrations were lower in the water whereas higher in the fish fillet which is the same with other researchers (Ataro *et al.,* 2003; Nigussie *et al.,* 2010; Dsikowitzky *et al.,* 2012; Desta and Weldemariam, 2013). Particularly Cr is dangerous as it can accumulate in many organisms, sometimes as much as 4 000 times above the level of the surrounding environment as was noted in aquatic algae (Oldewage and Marx, 2006). Fish accumulate toxic chemicals such as heavy metals directly from water and diet, and contaminant residues may ultimately reach concentrations hundreds or thousands of times above those measured in the water, sediment and food (Babatunde, 2012).

Fish attracted a lot of attention as bioindicators for monitoring aquatic pollution, due to their relatively large body size, long life cycle, position in the aquatic food chain and their use for human consumption (Enuneku *et al.,* 2013). So the reason for fishes accumulates large amounts of certain metals than water were they accumulate large amounts of certain metals and assimilate them through ingestion of suspended particulates, food materials and sometimes by constant ion exchange process of dissolved metals across lipophilic membranes like the gills and adsorption of dissolved metals on tissue and membrane surfaces which becomes toxic at high levels and exert harmful effects on fish. Such metals are finally transferred to other animals including humans through the food chain (Asante *et al.,* 2014).

4.2.3. Heavy metal concentrations in fish fillet in relation to location

Heavy metals accumulation were significantly affected by location at ($p<0.05$) indicated in table 7. Lead was not detected in all sampling locations. Chromium mean concentration values were significantly ($p<0.05$) varied in all sampling locations. At Blue Nile outlet chromium concentration was not detected. Significantly higher chromium mean concentration value (41.17 µg/L) was recorded in Micheal. Kuriftu resort was significanyly ($p<0.05$) lower mean concentration value (14.34µg/L) compaired to other locations chromium mean concentration values.

Significantly ($p<0.05$) higher cadmium mean concentration value (1.62 µg/L) was attained in Prison compired to other locations followed by cadmium mean concentration value (1.42 µg/L) of Micheal and mean concentration value (0.71 µg/L) of Hospital. Although cadmium mean concentration value of Micheal and Prison were not significantly different. The same for Hospital, Alima building and Kuriftu risort were not significantly ($p>0.05$) varied in their cadmium mean concentration values.Cadmium was not detected at Blue Nile outlet.

Present investigation are concides with Kebede and Wondimu ,(2004) study condacted in lakes Awassa and Ziway revealed that trace elements data showed significant difference between the two lakes but Dsikowitzky *et al.,* 2012 reported that there was no significant variation between different sampling staions which was contrasted with present finding. Ogoyi *et al.* (2011) reported that water temperature, pH value, dissolved oxygen concentration and water transparency are factors influence heavy metal concentrations in the different fish species. It is also documented that the geographical locations could lead to different metal concentrations even in the same fish species (Özparlak *et al.,* 2012).

Table 7: Heavy metal concentrations in different location of the three fish species from Lake Tana

Location	N	Parameters		
		chromium	cadmium	lead
Micheal	12	41.17 ± 0.08^{a}	1.42 ± 0.08^{a}	ND
Prison	12	33.23 ± 0.08^{c}	1.62 ± 0.08^{a}	ND
Hospital	12	39.55 ± 0.08^{b}	0.71 ± 0.08^{b}	ND
Blue Nile outlet	12	ND	ND	ND
Alima building	12	30.68 ± 0.08^{d}	0.5 ± 0.08^{b}	ND
Kuriftu risort	12	14.34 ± 0.08^{e}	$.072\pm0.08^{b}$	ND

N=12 sample size of fish.Considering the mean concentrations between different location, Values with different superscripts within the column were significantly different ($p<0.05$). Concentrations are given in µg kg−1 wet basis ND: Not detected

As indicated in (Table 6) the physico- chemical parameters of the water were significantly different between the sampling sites. The different concentration the heavy metal in fish muscle between the sampling sites might be due to that physico-chemical property of the lake. Additional factor could be due to degree of pollution and form metal in the water (Ogoyi *et al.*, 2011; Özparlak *et al.*, 2012).

4.2.4. Metal accumulation in relation to fish species

Heavy metals accumulation were significantly ($p<0.05$) affected by species (Table 8). Chromium concentration was the highest concentration in all fish species. Metals accumulation were as such order Cr> Cd >Pd in all fish species. Lead was not detected in all fish species fillets. Chromium concentrations was significantly ($p<0.05$) different among different fish species. *C.gariepinus* was significantly higher chromium accomulation value (42.56 μg/kg) relative to both *O. niloticus* (7.43μg/kg) and *L.intermedius* (29.5μg/kg) chromium mean accumulations in fish fillets.

Cadmium accumulation was significantly ($p<0.05$) affected by different fish specie. Significantly ($p<0.05$) higher cadmium mean accumulation value of fillets (1.14μg/kg) was recorded in *O.niloticus* relatively to cadmium mean accumulation value of (0.27μg/kg) *L.intermedius* fillets.*O.niloticus* and *L.intermedius* were no significant ($p>0.05$) varied in cadmium mean accumulation values of fillets.

Table 8: Heavy metal concentrations in the three commercially important fish species from Lake Tana, guideline values from WHO (1985)/FEPA (2003)/FAO (1983) in fish Food in mg/L

Fish species	N	Parameters		
		Chromium	Cadmium	Lead
O. niloticus	24	7.43±0.06 ^{c}	1.14±0.05 ^{a}	ND
C. gariepinus	24	42.56±0.06 ^{a}	1.08±0.05 ^{a}	ND
L. intermedius	24	29.5±0.06 ^{b}	0.27±0.05 ^{b}	ND
Limit in fish food*		0.15-1	2	2

N=sample size of fish.Considering the mean concentrations between the three fish species, Values with different superscripts within the column were significantly different ($p<0.05$).Concentrations are given in μg kg−1 wet basist
ND: Not detected

*Limit set by WHO (1985), FEPA (2003), and FAO (1983).

Accomulation of Cd and Cr were very low in *O. niloticus*, C. *gariepinus* and *L. intermedius* of Lake Tana than fishes from Lake Awassa and Ziway. Present study revealed that the presence of Species-specific heavy metals accumulation which is more or less consides with investigation reported by Dsikowitzky *et al*.(2012) higher Hg levels in *L. intermedius* than in *C. gariepinus* were detected from lake Awassa. The reverse were revealed by Ataro *et al*.(2003) from Lake Awassa and Lake Ziway which was absence of significant difference in concentrations of the analyzed trace metals between *O.niloticus* and *C.gariepinus*.

Higher Cr levels in *C. gariepinus* than in *L. intermedius* were detected, although both species inhabit the deeper parts of the lake and both are carinivorous.The distributions or habitat selection of different fish species, or size classes within a species, in a lake depends on several factors including the physiological adaptation of the species to specific environments (Dadebo *et al.*, 2013). Different size classes of each species may also have different habitat preferences (Pet & Piet 1993), in most cases associated with the availability of suitable food and protection from predators for the young.

Hence, feeding habits and size of the species may affect the Cr accumulation of *C.gariepinus*. As it showed in (Table1) *C.gariepinus* were longer and heavier relative to *L. intermedius*.Therefore the differences may be due to *C. gariepinus* inhabit the deeper parts of the lake as a result species dependents on sediment and burrows in mud. Zhang *et al.,* (2007) reported that muscle Cr from bottom fish exceed more than 50% of the Chinese food standards. Some metals were not fully dissolved within the water column but precipitated into sediments, causing a higher risk of contact and further absorption by benthic organisms and the bottom-dwelling fauna preyed upon by this species (Forero *et al.,* 2009).

Other than habitat and size the rate of bioaccumulation of heavy metals in organisms depends on the ability of organisms to digest the metals (Eneji *et al.,* 2011). *O. niloticus* is mainly feeding on algae and zooplankton (Dsikowitzky *et al.,* 2012) and is therefore on a lower trophic level than the other two fish species. A lower Cr level in this species compared with *C. gariepinus* is in accordance with the concept of Cr biomagnification in aquatic food. The accumulations of Cr, Cd and Pb in the edible muscle of the three fish species collected from Lake Tana were far below the FAO (1983), FEPA (2003) and WHO (1985) Limit (Table 8). Hence the consumption of this fish seems to pose no threat to human health regarding to these metals.

4.2.5. Interaction effect of location and species in metal accumulation

The statistical analysis revealed that there was significant ($p<0.05$) interaction effect among the different location and the three fish species (appendix 3). Bioaccumulation factors and metal concentration in different species of fish appreciably vary with the bioavailability of metals in seawater, as well as to inter-specific differences of fish species (Nair and Joseph, 2010). So the reason for the interaction effect between location and species might be the interaction effect of bioavailability of heavy metal in the water (Yehia and Sebaee, 2012) and feeding habits (as carnivores, herbivores, omnivores and limnivores) and habitats of species (Akan *et al.,* 2012; Nwani *et al.,* 2010).

4.2.6. Risk assessment

This study was undertaken to investigate heavy metal concentrations in edible parts (muscles) of three commercially important fish species in lake Tana, and to examine whether their levels are potentially harmful for human health if included in the diet. Provisional Tolerable Weekly Intakes (PTWI) is set for substances, such as heavy metals, that are contaminants in food and are known to accumulate in animals and humans. Its value represents permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods.

Like in other organisms, heavy metals are not destroyed by humans. Instead, they tend to accumulate in the body and can be stored in soft and hard tissues such as liver, muscles and bone and threaten the health of humans (Elnabris, 2012). Table 9 shows PTWI of heavy metals given by WHO (1993).

Table 9: Comparison of concentrations of heavy metals in muscle tissue of three fish species from LakeTana in µl kg−1 wet weight with WHO (1993) recommendation

Heavy metals	Fish species	Mean conc. fish muscle	Estimated human intake	Tolerable human intake
Cd	*O.niloticus*	1.143±0.05	0.21	420
	C. gariepinus	1.083±0.05	0.09	420
	L. intermedius	0.272±0.05	0.02	420
Pb	*O. niloticus*	ND	-	1500
	C. gariepinus	ND	-	1500
	L.intermedius	ND	-	1500

Estimated weekly intake of metals was calculated as described in "Human exposure estimates". Provisional tolerable weekly intake (PTWI) in microgrammes per kilogramme human body weight was given by WHO in 1993 (Cd, Pb) and calculated for an adult of 60 kg

The estimated weekly intake of heavy metals considering the amount of consumed fish and the measured metal levels in the fish muscle were calculated ("Human exposure estimates"). Table 9 shows that these weekly intake estimates were considerably far below than the tolerable human intake values provided by WHO (1993). This result implies that Cd and Pb pose no public health hazard through consumption of the fish species considered in the present study. No tolerable human intake values for Cr from food were provided by WHO, may be because Cr is not very toxic when introduced by the oral route (Adegbola *et al.*, 2012).

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From the present study it can be concluded that, there is variation among sex, location and species with regard to proximate composition and gross energy content. Female fish contained significantly higher protein, fat, ash and gross energy as compared to male. While male moisture content was significantly higher than female fishes. Different sampling locations and fish species significantly affected proximate composition. From a nutritional point of view, the three fish species demonstrated acceptable quality; in particular, *O.niloticus* had the highest levels of protein and ash content compared to the other species. However, consumers who need to reduce the risk of cardiovascular disease and diabetes should be cautious about the *L.intermedius* due to its high fat content. Statistical analysis also revealed that there was significant interaction effect among location, sex and species. Consumers may receive some health benefits consuming those three fish species due to its nutritional value.

This study as well reported that the heavy metal concentration and physicochemical variables such as temperature, pH, TDS and EC of sampling location of Lake Tana was below or within the range of the recommended limit of WHO (2008). Cr and Cd accumulations in muscle of the three fish species showed that there was variation throughout the lake and their accumulations are lower than safety threshold values provided by the FAO(1983), FEPA (2003) and WHO (1985). Hence the anthropogenic pollution of this lake seems to pose no threat of heavy metal accumulation regarding to these fish species. Also, the present work has elucidated the eating quality of lake Tana fishes and safety for the consumers'consumption.

5.2 Recommendations

Studied parameters varied based on main effects. Therefore is important that factors considered in the present study should be taken into account when selection of the fish species in Lake Tana is desired. Although all results of heavy metal concentrations studied showed that regular consumption of the three fish species may not cause any obvious harm effect on human health, the following recommendations should be taken into consideration:

- ✓ The responsible body should stop the disposal of waste matter and resolve lack of sewerage system. If not the elevation of metal concentrations in the affected lake would only be a matter of time.
- ✓ Further research concerning the whole lake area and other metals (Hg, As, Cu, Zn, Fe...) could be important.
- ✓ Further research on the interactiob between the water column and the sedment in the bottom of the lake and fish and other biotic components of the ecosystem

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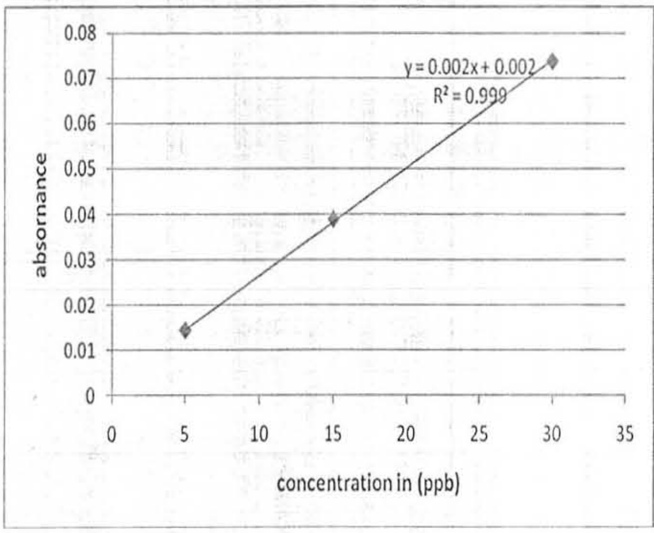
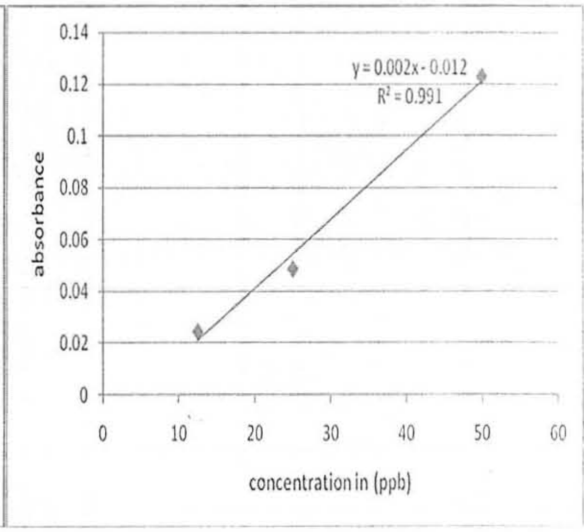
APPENDICES

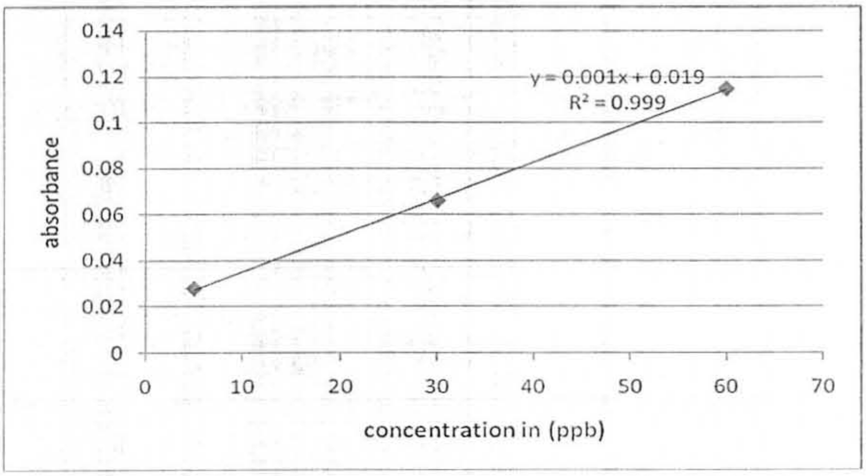
Appendix 1 Calibration curve of Cd, Cr and Pb



Calibration curve of Cd for water and fish analysis by GFAAS



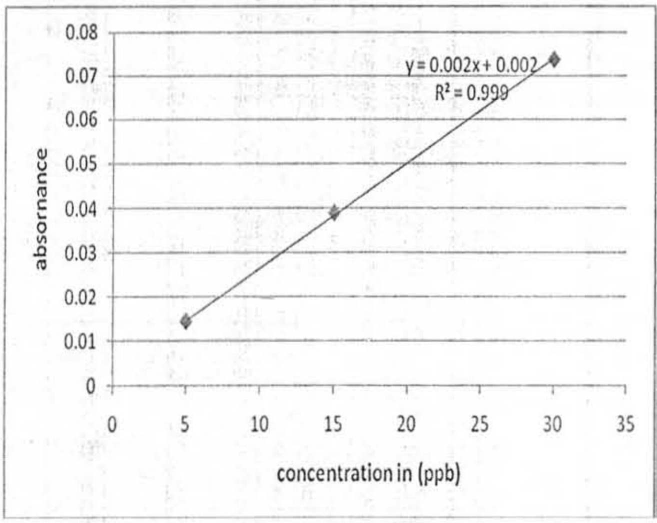
Calibration curve of Cr for water and fish samples samples analysis by GFAAS



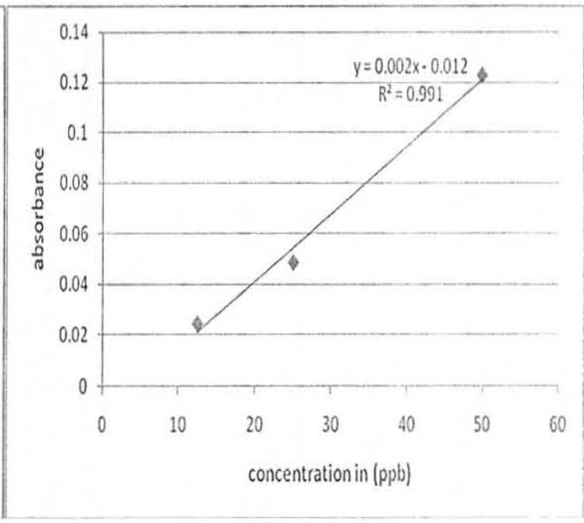
Calibration curve of Pb for water and fish samples analysis by GFAAS

APPENDICES

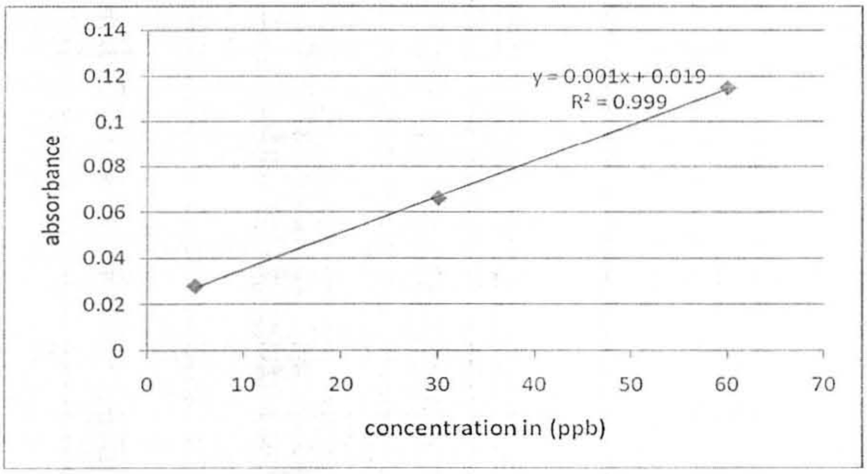
Appendix 1 Calibration curve of Cd, Cr and Pb



Calibration curve of Cd for water and fish analysis by GFAAS



Calibration curve of Cr for water and fish samples samples analysis by GFAAS



Calibration curve of Pb for water and fish samples analysis by GFAAS

Appendix 2 The ANOVA table of proximate analysis

Tests of Between-Subjects Effects

Dependent Variable: protein

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	444.342[a]	35	12.695	953.353	.000
Intercept	29372.648	1	29372.648	2205705.720	.000
LOCATION	14.396	5	2.879	216.217	.000
SEX	26.324	1	26.324	1976.792	.000
SPECIES	295.088	2	147.544	11079.660	.000
LOCATION * SEX	3.537	5	.707	53.122	.000
LOCATION * SPECIES	95.378	10	9.538	716.231	.000
SEX * SPECIES	1.314	2	.657	49.354	.000
LOCATION * SEX * SPECIES	8.303	10	.830	62.353	.000
Error	.959	72	.013		
Total	29817.949	108			
Corrected Total	445.301	107			

a. R Squared = .998 (Adjusted R Squared = .997)

Tests of Between-Subjects Effects

Dependent Variable: moisture

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	171.229[a]	35	4.892	154.382	.000
Intercept	690147.425	1	690147.425	21778609.087	.000
LOCATION	9.340	5	1.868	58.946	.000
SEX	45.270	1	45.270	1428.560	.000
SPECIES	48.395	2	24.198	763.594	.000
LOCATION * SEX	3.316	5	.663	20.928	.000
LOCATION * SPECIES	56.500	10	5.650	178.295	.000
SEX * SPECIES	4.402	2	2.201	69.457	.000
LOCATION * SEX * SPECIES	4.005	10	.401	12.639	.000
Error	2.282	72	.032		
Total	690320.935	108			
Corrected Total	173.510	107			

a. R Squared = .987 (Adjusted R Squared = .980)

Tests of Between-Subjects Effects

Dependent Variable: fat

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	121.716[a]	35	3.478	3210.088	.000
Intercept	267.058	1	267.058	246514.777	.000
LOCATION	18.636	5	3.727	3440.527	.000
SEX	8.107	1	8.107	7483.488	.000
SPECIES	59.632	2	29.816	27522.387	.000
LOCATION * SEX	.611	5	.122	112.889	.000
LOCATION * SPECIES	27.968	10	2.797	2581.703	.000
SEX * SPECIES	2.937	2	1.468	1355.314	.000
LOCATION * SEX * SPECIES	3.824	10	.382	353.007	.000
Error	.078	72	.001		
Total	388.852	108			
Corrected Total	121.794	107			

a. R Squared = .999 (Adjusted R Squared = .999)

Tests of Between-Subjects Effects

Dependent Variable: ash

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.111[a]	35	.089	23.728	.000
Intercept	210.844	1	210.844	56284.681	.000
LOCATION	.720	5	.144	38.463	.000
SEX	.599	1	.599	160.025	.000
SPECIES	.496	2	.248	66.203	.000
LOCATION * SEX	.153	5	.031	8.187	.000
LOCATION * SPECIES	.985	10	.099	26.295	.000
SEX * SPECIES	.009	2	.004	1.146	.324
LOCATION * SEX * SPECIES	.148	10	.015	3.956	.000
Error	.270	72	.004		
Total	214.224	108			
Corrected Total	3.381	107			

a. R Squared = .920 (Adjusted R Squared = .881)

Tests of Between-Subjects Effects

Dependent Variable: total energy

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8533.837[a]	35	243.824	1136.463	.000
Intercept	688894.725	1	688894.725	3210937.393	.000
LOCATION	2565.244	5	513.049	2391.320	.000
SEX	2373.516	1	2373.516	11062.953	.000
SPECIES	733.777	2	366.889	1710.067	.000
LOCATION * SEX	50.832	5	10.166	47.386	.000
LOCATION * SPECIES	2317.258	10	231.726	1080.074	.000
SEX * SPECIES	57.679	2	28.840	134.422	.000
LOCATION * SEX * SPECIES	435.530	10	43.553	203.001	.000
Error	15.447	72	.215		
Total	697444.010	108			
Corrected Total	8549.284	107			

a. R Squared = .998 (Adjusted R Squared = .997)

Appendix 3 The ANOVA table of Physicochemical Parameters and Heavy Metals

Tests Of Between-Subjects Effects

Dependent Variable: Chromium

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	28249.868[a]	17	1661.757	36173.207	.000	1.000
Intercept	25281.000	1	25281.000	550318.055	.000	1.000
location	7792.147	5	1558.429	33923.966	.000	1.000
species	7567.584	2	3783.792	82365.772	.000	1.000
location * species	12890.137	10	1289.014	28059.314	.000	1.000
Error	.827	18	.046			
Total	53531.695	36				
Corrected Total	28250.695	35				

a. R Squared = 1.000 (Adjusted R Squared = 1.000)

Tests of Between-Subjects Effects

Dependent Variable: cadmium

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	31.550[a]	17	1.856	44.705	.000	.977
Intercept	24.950	1	24.950	601.004	.000	.971
location	10.801	5	2.160	52.036	.000	.935
species	5.683	2	2.841	68.442	.000	.884
location * species	15.066	10	1.507	36.292	.000	.953
Error	.747	18	.042			
Total	57.247	36				
Corrected Total	32.297	35				

a. R Squared = .977 (Adjusted R Squared = .955)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chromium	Between Groups	.000	5	.000	1.700	.268
	Within Groups	.000	6	.000		
	Total	.000	11			
Lead	Between Groups	.002	5	.000	28.573	.000
	Within Groups	.000	6	.000		
	Total	.002	11			
Temprature	Between Groups	36.227	5	7.245	189.009	.000
	Within Groups	.230	6	.038		
	Total	36.457	11			
Total disolved solid	Between Groups	.001	5	.000	10.400	.006
	Within Groups	.000	6	.000		
	Total	.001	11			
Electrical condactivity	Between Groups	.001	5	.000	3.720	.070
	Within Groups	.000	6	.000		
	Total	.001	11			
PH	Between Groups	.451	5	.090	9.777	.008
	Within Groups	.055	6	.009		
	Total	.507	11			