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Accumulation of Certain Heavy Metals in Nile Tilapia (*oreochromis niloticus)* Fish Species Relative to Heavy Metal Concentrations in the Water of Lake Hawassa

By

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
APHP	American Public Health Association
BDL	Below Detection Limit
DO	Dissolved Oxygen
d.m.	Dry Mass
EU	European Union
EC	Electrical Conductivity
FAAS	Flame Atomic Absorption Spectroscopy
FAO	Food and Agriculture Organization
GFAAS	Graphite furnace Atomic Absorption Spectroscopy
HCL	Hallow Cathode Lamp
MDL	Method Detection Limit
ND	Not Detected
PTE	Potential Toxic Element
SPSS	Statistical Package for Social Science
TDS	Total Dissolved Solid
USEPA	United State Environmental Protection Authority
USFDA	United State Food Drug Administration
UNEP	United Union Environment Program
WHO	World Health Organization
pH	Hydrogen Ion Concentration
SS	Suspended solid
w.m.	Wet Mass
mg/L	milligram per Liter
µg/L	microgram per Liter
µg/g	microgram per gram
mS/cm	milli-Siemens per centimeter
^0C	Degree Celsius

ABSTRACT

The Tiliapia fish samples and water samples were collected from Lake Hawassa at three selected sampling sites namely Tikur Wuha, Dorie basana and Referral H. sites. The concentrations of certain heavy metals (copper (Cu), lead (Pb), nickel (Ni) and zinc (Zn)) in water and their accumulation in the edible tissue of Nile Tilapia fish (Oreochromis niloticus) were determined in samples collected from Lake Hawassa in the dry season of the area by flame and graphite furnace Atomic Absorption Spectroscopy. The results revealed that the dissolved heavy metal concentrations in water ranged from undetectable to 4.57 µg/L for copper (Cu) and also from undetectable to 0.25 µg/L for lead (Pb) but dissolved nickel (Ni) and zinc (Zn) concentrations were totally undetectable in water samples of Lake Hawassa water. The contents of investigated heavy metals in fish samples ranged between 1.80 - 4.8 µg/g for Copper (Cu), <1.95 – 2.23 µg/g for lead (Pb), <0.29 - 0.68 µg/g for nickel (Ni) and 21.4 – 40.70 µg/g for zinc(Zn). The highest accumulations of copper (Cu) and zinc (Zn) in edible muscle of Nile Tilapia were observed at Referral H. site. The highest accumulation of lead (Pb) and nickel (Ni) were observed at Tikur Wuha site and at Dorie Basana respectively Among the detected metals, zinc (Zn) showed a maximum accumulation in the edible muscle of Nile Tilapia fish from Lake Hawassa, the next was copper (Cu) and then lead (Pb) and the least was nickel (Ni). The concentrations of the metals (copper (Cu), lead (Pb), nickel (Ni) and zinc (Zn) in water of Lake Hawassa were far below the recommended limit by WHO, US EPA and FAO. The accumulations of copper (Cu) and zinc (Zn) in fish analysed in this study were below the recommended limit of FAO/WHO and EU, the levels of nickel (Ni) in the edible muscle of Nile Tilapia fish were also far below the maximum limit of USFDA. However the levels of lead (Pb) at Tikure Wuha exceeded the recommended limit of FAO/WHO and EU.

Key words; Bioaccumulation, Bio-indicator, Heavy metals, Nile Tilapia

1. INTRODUCTION

1.1 Background

Water pollution is a serious environmental problem in the world. It is the degradation of the quality of water that renders water unsuitable for its intended purpose. Anything which degrades the quality of water is termed as pollutant. Water pollutants can be broadly classified as major categories namely organic, inorganic, suspended solid and sediments, heavy metals, radioactive materials and heat (Botikin and Keller, 2008).

Many surface and ground water are being polluted with different pollutants. Some pollutants are directly discharged from industrial effluents and municipal sewage, and others come from polluted runoff in urban and agricultural areas. This situation has been exacerbated as a result of the rapid growth of population, increased urbanization and expansion of irrigation that more likely use different fertilizers, pesticides and herbicides, and other modern agricultural practices as well as lack of environmental regulations (FAO, 1992).

Of different pollutants, heavy metal pollution of aquatic environment has become a great concern in recent years because they are very harmful as a result of their non-biodegradable nature, long biological half-life and their potential to accumulate in different body parts of organism. They can also be concentrated along the food chain, producing their toxic effect at points after far removed from the source of pollution. Thus compared to other types of aquatic pollution, heavy metals pollution is less visible but its effects on the ecosystem and humans can be intensive and very extensive (Edem *et al.,* 2008).

The most common Potential Toxic Elements (PTE) listed by the United Stated Environmental Protection Agency (USEPA) are mercury (Hg), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn). Some of these PTEs are essential for the metabolic activities of living organisms. Potential toxic elements such as Cr, Cu, Ni and Zn are required by organisms at low level and become toxic at some higher levels. Non- essential elements including As, Cd and Pb are toxic and not required by organisms at any level (Poggio *et al.,* 2009). The toxicity of trace metals will vary greatly between organisms for the same trace metals, and between trace metals for the same organisms. Furthermore, trace metals will not necessarily follow the same rank order of toxicities between organisms, depending on differences between uptake rate, detoxification rates and excretion rate of the different organisms compared. However, the general order of toxicity of heavy metals is Hg> Ag> Cu> Cd >Zn> Ni> Pb> Cr> Sn and so on (Luoma and Rainbow, 2008). Heavy metals toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs (WHO, 1984).

Increased loading of heavy metals into a lake may have several ecological consequences. For instance elevated heavy metals concentrations may lead to toxic effects or bioaccumulation in aquatic environments. Bioaccumulation is the general term describing the net uptake of chemical from the environment by any or all possible routes (i.e, inhalation, ingestion and dermal from any source in the aquatic environment where chemicals are present. Bioconcentration is more specific term that used for describing accumulation from water alone (Tulonen et al., 2006). The accumulation of heavy metals in fish tissue depends on factors such as metal bioavailability, season of sampling, physical and chemical properties of water (Kargin, 1996).

Fish occupies the highest trophic level in aquatic systems. Besides that, it has high economical value, thus fish are suitable as water quality indicator organism. Fish is a good bio-indicator because it has a potential to accumulate heavy metals and other organic pollutants (Ahmed and Shubami-Othman, 2010). When Fish is exposed to high concentrations of heavy metals in water it may take up substantial quantities of these metals. Heavy metals can enter from contaminated water and can accumulate into the fish’s body by different routes. These metals concentrated at different contents in organs of fish body. Fish accumulates heavy metals in the tissue through absorption and humans can be exposed to heavy metals via food chain. This can cause acute and chronic effects in humans (Dogan and Yilmaz, 2007).

Bioaccumulation of metals reflects the amount of heavy metals ingested by the organism, the way in which the metals are distributed among the different tissues and extent to which the metal is retained in each tissue type (Sultana and Rao, 1998).

Lake Hawassa is one of the Rift Valley Lake of Ethiopia and it is used greatly for tourism attraction and supplies of fish for local communities. This lake serves to maintain the ecological system as habitat for various species of flora and fauna. Fish from Lake Hawassa are widely caught by local people for their daily diet or to be sold.

The study on heavy metal contamination in water and fish is vital to assess the current status of water pollution with heavy metal and threats to human health from heavy metal pollution of the Lake. The purpose of this study was to establish current concentrations of the metals (Cu, Pb, Ni and Zn) in Lake Hawassa's water and their accumulation in Tilapia fish.

1.2 Statement of Problems

Lake Hawassa is a fresh closed Lake playing an important role in the lives of many people in the region. It is the source of commercial fishery. It serves for recreation purpose and also is used for drinking water supply by the communities surrounding it. It is influenced by human activities such as agricultural practice, deforestation, industrialization and discharging of domestic sewages (Abayneh Ataro *et al.,* 2003). These may make the Lake Hawassa to receive various kinds of pollutants enriched with heavy metals through different mechanism. Tikur Wuha River is the only inflow river and it carries industrial effluent and agricultural runoff from the surroundings to the lake but there is no out flow river (Demeke Admasu, 1989; Abayneh Ataro *et al.,* 2003).

These different sources cause the accumulation of heavy metals in the Lake. The accumulation can be in water, suspended solid, sediment, fishes and aquatic plants. Some heavy metals may affect the growth of fish and/or are harmful to the consumer. The extent of accumulation in the fish is used as a bio-indicator for pollution of the lake water with the heavy metals. The loads of the pollutants in general and heavy metals in particular enter into the lake through different mechanism and their accumulations may vary with the seasons of the region (Kargin, 1996). Seasonal determination of the pollutants in general and heavy metals in particular in the lake is important for monitoring and protecting the lake and for examining threats to human health. Particularly, this study is considered only the dry season. This is due this season has higher temperature than the rainy season. The higher temperature can cause higher activities and ventilation rates in fish. The increasing temperature is made to increase the rate of metals accumulation in fish. The heavy metal concentrations in water during the dry season are also significantly higher than the rainy season (Obsohan and Eguavoen, 2008).

The study is conducted on the edible tissue (Fillet) of Nile Tilapia fish species which are the most common types of fish consumed by the local people and found widespread in the lake Hawassa. The determination of heavy metals in this fish relative to their concentration in water gives two closely related sets of information. The first is accumulation of heavy metals in fish which may be a threat to human health and the other is the pollution of water with heavy metals.

This study is undertaken to determine the current level of the concentrations of the heavy metals such as copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn) in water and their accumulations in the edible tissue of the Tilapia fish in dry season of Lake Hawassa. Other water parameters like temperature, dissolved oxygen, pH, and conductivity, total dissolved solid and suspended solid of the lake water were also determined in this study.

1.3 Objectives of the Study

1.3.1 General Objective

The general objective of the study is

- to investigate the accumulation of certain heavy metals (Cu, Pb, Ni and Zn) in edible muscle of Nile Tilapia fish (*oreochromis niloticus*) relative to heavy metal concentrations in water of Lake Hawassa in the dry season of the area.

1.3.2 Specific Objectives

The following are the specific objective of the study

- to determine some water quality of the Lake water in the dry season.
- to determine the concentrations of the metals (Cu, Pb, Ni and Zn) in water of Lake Hawassa in the dry season.
- to determine the accumulation of the metals (Cu, Pb, Ni and Zn) in the edible muscle of Nile Tilapia fish (*oreochromis niloticus)* of Lake Hawassa in the dry season.

- to provide the current information about the concentration of the metals (Cu, Pb, Ni and Zn) in water and amount of their accumulation in Nile Tilapia fish.
- to compare the current accumulation of the metals (Cu, Pb, Ni and Zn) in Tilapia Fish with the available maximum recommended guideline.

1.4 Research Questions

- Is the water quality of the Lake water favorable for the bioavailability of metals to the aquatic life?
- What are the concentrations of the heavy metals (Cu, Pb, Ni and Zn) in water and their accumulation in edible muscle of Nile Tilapia fish (*oreochromis niloticus*) of the Lake Hawassa water in the dry season?
- What types of relationship are there in the concentration of the heavy metals (Cu, Pb, Ni and Zn) in water and in the edible muscle Nile Tilapia fish (*oreochromis niloticus*) of the Lake Hawassa during the dry season of the area?
- Which metal is dominantly concentrated in water and accumulated in Nile Tilapia fish (*oreochromis niloticus*) during dry the season?

2. REVIEW OF LITERATURE

2.1 Environmental Pollution

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. According to Tokalioglu *et al.* (2003) and Moja, (2007), the impacts of pollution classified as primary and secondary. “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”. Pollution is now regarded as a global problem since pollutants can cross borders with the help of wind and water. Environmental pollution is insidious and its harmful effects only become apparent after periods of exposure. For this reason environmental monitoring is recognized as being vitally important in detecting the level and types of pollutants, and their source. Furthermore this monitoring helps to take measures to mitigate the effect in those seriously polluted areas.

Water pollution has an effect on oceans and inland water bodies. It refers to any chemical, biological or physical change in water quality that harms living organism or makes water unsuitable for desirable uses. Water pollutants include organic and inorganic chemicals, heavy metals, petrochemicals and microorganisms. Water pollution may also 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).

Soil contamination often occurs when chemicals are released by spillages from underground storage tank. Some common soil contaminants are chlorinated hydrocarbons (such as DDT), heavy metals such as lead (in paint and petrol), manganese (in industry and more recently in unleaded petrol), cadmium (in rechargeable batteries), chromium, zinc, arsenic and benzene. Pollutants in soils can runoff into rivers or lakes or leach into the groundwater, can ultimately cause accumulation in animals, plants and people (Mulligan *et al.*, 2001).

2.2 Heavy Metals in the Environment

Heavy metals 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. The heavy metal concentrations in the environment are due to natural sources (rock weathering, soil erosion, dissolution of water soluble salts) as well as anthropogenic source such as municipal wastewater, manufacturing industries, and agricultural activities (Güven and Akýncý, 2008).

Heavy metals are of much environmental concern currently. 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 different activities, and thus heavy metals have been emitted to and deposited in the environment. Metals can be retained for long period of time after entering the environmental medium such as soil (Tokalioglu, *et al.,* 2003; Moja, 2007).

Metals are introduced into the environment by a wide range of natural and anthropogenic sources and with anthropogenic sources being either domestic or industrials. They occur naturally at levels that are considered not to have toxic effects to living organisms. The natural levels of metals are normally increased through various anthropogenic processes. Currently, anthropogenic inputs of metals are higher than the natural input and this may pose a great threat to aquatic life in particular, and to whole ecosystems in general (Weiner, 2008).

In natural aquatic ecosystems, heavy metals occur in low concentration. In recent times, however, the occurrence of metal contaminants in excess of natural loads has become a problem of increasing concern. Heavy metals contamination of the aquatic environment may lead to deleterious effects from localized inputs which may be acutely or chronically toxic to aquatic life within the affected area (Calamari & Naevel, 1994).

The term heavy metal is a general collection term applying to the group of metals and metalloids with an atomic density greater than 6g/cm^3 (Alloway and Ayres, 1993). Commonly the term is used to refer to elements that are associated with pollution and toxicity problems. The table below shows the major metals in natural water and their chemical species according to Ward, (1995).

Table 2.1 Major chemical species found in natural water

Metals	chemical symbol	chemical species
Lithium	Li	Li^+
Aluminum	Al	$Al(H_2O)_6^{3+} \longrightarrow [Al(OH)_4]^-$
Chromium	Cr	$Cr(OH)_2(H_2O)_4^+$, CrO_4^{2-}
Manganese	Mn	Mn^{2+}, $MnSO_4$, $MnCl^+$
Iron	Fe	$[Fe\ (OH)_2]^+$, $[Fe\ (OH)_4]^-$
Cobalt	Co	Co^{2+}
Nickel	Ni	Ni^{2+}
Copper	Cu	Cu^{2+}, $Cu(OH)^+$, $Cu^{2+}SO_4^{2+}$, $Cu^{2+}CO_3^{2-}$
Zinc	Zn	$Zn(OH)^+$, $Zn(OH)_2$, $ZnCl^+$, $ZnCl_2$, $ZnCO_3$, Zn^{2+}
Selenium	Se	Se (IV), Se((VI)
Molybdenum	Mo	MoO_4^{2-}
Cadmium	Cd	$CdCl^+$, $CdCl_2$, $CdCl_3^-$, Cd^{2+}
Cesium	Cs	Cs^+
Mercury	Hg	$HgCl_2$, $HgCl_3^-$, $HgCl_4^{2-}$, HgOHCl, $Hg(OH)_2$
Lead	Pb	Pb^{2+}, $PbCO_3$, $PbCl^+$, $PbCl_2$, $PbCl_3^-$,$Pb(OH)^+$, $Pb(OH)_2$, $Pb(OH)_3^-$, $Pb_3(OH)_4^{2+}$, $Pb(OH)_4^{4+}$

Some of these elements are required by most living organisms in small amounts and they are also referred to as micronutrients. All metals, however, can be toxic to aquatic organism where present at high levels, causing direct effects such as histological damage or a reduction in survival, growth and reproduction of the species it influences (Heath, 1987).

Generally, the pattern of the flow of anthropogenic sources of heavy metals in the environment is illustrated in the figure below as proposed by Chalermsupanimit (2006)

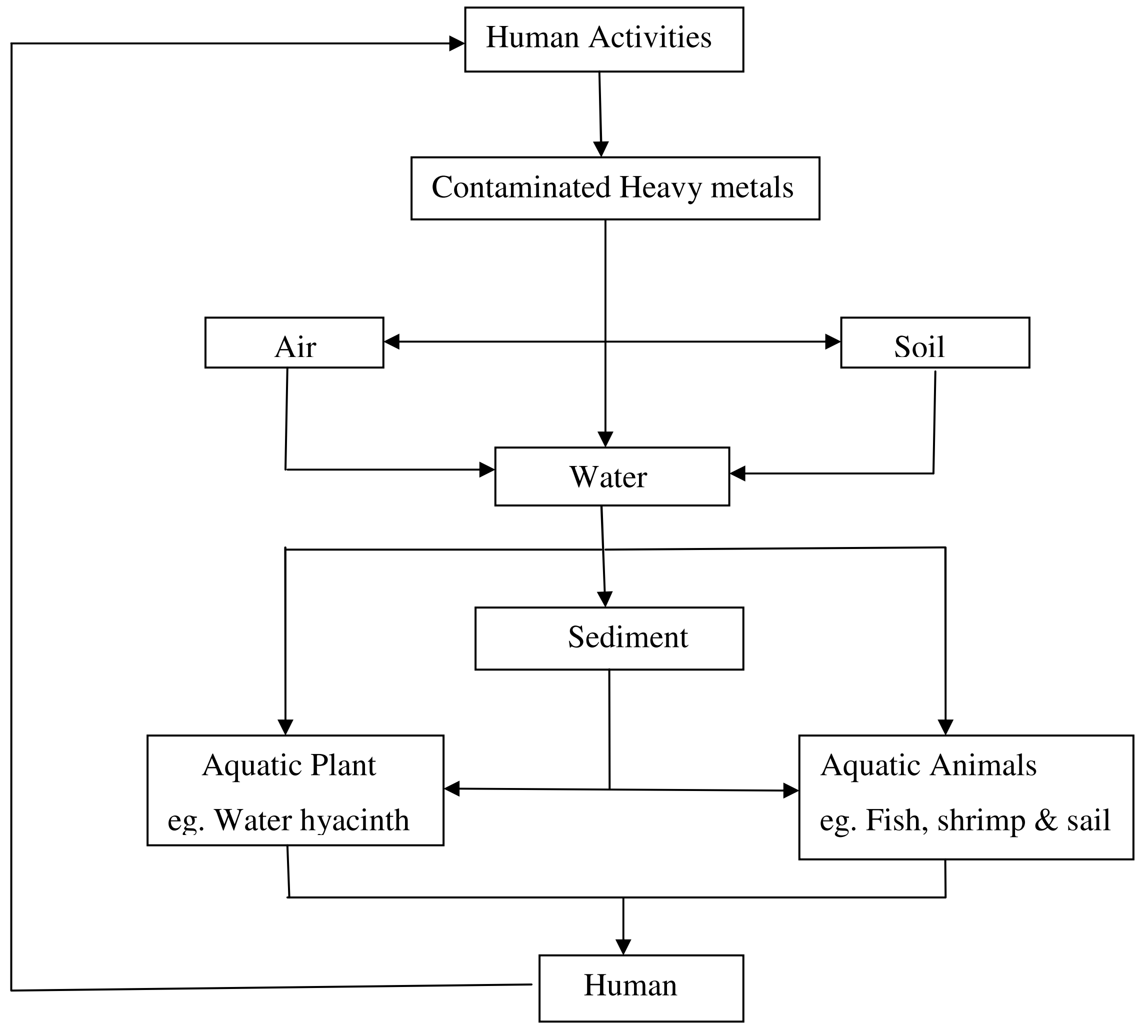


Figure 2.1 Anthropogenic sources of heavy metals flow in environment

2.3 Distribution of Heavy Metals in the Aquatic Environment

Once in the aquatic environment, heavy metals are partitioned among various aquatic environmental compartments (water, suspended solids, sediments and biota). The metals in the aquatic environment may occur in dissolved particulate and complex form. The majority of metal contaminants partition onto particulate matter such as clay minerals, Fe and Mn oxides/hydroxides, carbonates, organic substances (e.g., humic acids) and biological materials (e.g., algae and bacteria) (Calmano *et al.,* 1993).

The main process governing distribution and partition are dilution, advection, dispersion, sedimentation, and adsorption/desorption. Thus speciation under the various soluble forms is regulated by the instability constants of the various complexes and by physicochemical properties of water (pH, dissolved ions, redox and temperature). However, several mechanisms indicate that heavy metals in water may be removed due to (1) adsorption onto particulate; (2) chemical transformation into insoluble form; and (3) precipitation and sedimentation (Balasubramania *et al*., 1997)

Adsorption could be the first step in the ultimate removal of metal from water. In the course of distribution, permanent or temporary storage of metal takes place in the sediments of both freshwater and marine environments (Aksu *et al*,, 1998). Microbial activity and redox processes may change the properties of sediments and affect the composition of interstitial water (Vale *et al*., 1998). Reworking to the sediments by organisms will also bring heavy metals from sediments to the surface water. The extent of the sediment resuspension and dispersal depends on water movements in the immediate dredge/disposal area. Strong currents, such as those during spring tides, tend to disseminate resuspended material, whereas neap tides tend to localize impacts (Vale *et al*., 1998).

The transformation of heavy metals in aquatic environments occurs as biochemical mediated reduction, methylation, demethylation, and oxidation of single metal species. Redox reactions may also facilitate some transformations. The biochemical processes are carried out by microorganisms and algae. Heavy metals are taken up by both fauna and flora of the aquatic environment. This uptake could provoke an increase in the concentration of metals in an organism; if the excretion phase is slow, this can lead to the bioaccumulation phenomenon. Some heavy metals have been shown to undergo biomagnifications through the food chain (Suter, 1993).

2.4 Accumulation of Heavy Metals in Fish

Fish are used as bio-indicator of aquatic ecosystems for estimation of heavy metal pollution and potential risk for human consumption (Agarwal *et al.,* 2007). Bioaccumulation of metals in fish takes place directly, from the water by gills and indirectly from food (Barron, 1990).

The metals such as copper, zinc, iron, and cobalt are essential and have important biochemical functions in the organism as opposed to non-essential metals like lead, cadmium, mercury, and arsenic. Essential metals are used either an electron donor system or function as ligands in complex enzymatic compounds. The essential 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 (Bryan and Hummerstone, 1973). 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.

The function of uptake and excretion in fish is determined the accumulation of metal in fish. The gills are likely sites of metal uptake from water due to their large surface area and the close proximity of the internal constituent of the body and external environment (Wepener, 1997). Within the body, the degree of accumulation in different tissues is dependent on the binding of the metal to specific ligands. Dallinger *et al*. (1987) stated that as far as fish is concerned, there are three possible ways by which metals may enter the body (i) the body surface, (ii) the gill, (iii) the alimentary tract. But little is known about the uptake of heavy metals through the skin. It can be assumed that the body surface of fish is more or less impervious to harmful substances in the surrounding water.

Heavy metals have an effect on different aquatic organisms but its effect is often complex and difficult to interpret. Dissolved oxygen, pH, salinity, temperature and hardness of water have been shown to be factors that influence the physiology of an organism and the rate of uptake of heavy metals. “The main factors concerned in determining the seasonal variation of heavy metal levels in aquatic biota are the extent of pollutant delivery into the aquatic environment, the weight change occurring in the organisms and the direct effects of salinity, temperature and other water qualities which vary seasonally” (Chaudhari, *et al*. 1996).

2.5 Bioavailability of Metals

The toxicity of trace pollutants to aquatic organisms is related to the bioavailable fraction of contaminants available for assimilation. Bioavailability is defined as the fraction of the total amount of a chemical substance that can be taken up by living organisms within a certain time span (Wang *et al*., 2002). Bioavailability and bioaccumulation of contaminants in an aquatic environment is mainly dependent on the partitioning behaviour or binding strength of the contaminant to sediment. Dissolved or weakly adsorbed contaminants are more bioavailable to aquatic biota compared to more structurally complex mineral-bound contaminants which may only become bioavailable upon ingestion with food. For example, metals in the aquatic phase are the most bioavailable compared to particulate, complexed or chelated forms. Cadmium is less bioavailable in seawater than freshwater due to complexation with chloride ions and organic binding competition with calcium and magnesium, whereas copper bioavailability increases with increasing salinity (Forstner *et al*., 1989).

Factors affecting metal bioavailability and bioaccessability include metal speciation and biotransformation, availability of complexing ligands (e.g., organic carbon, chloride, carbonate, sulfide, manganese and ferrous oxides), competition by other cations for membrane adsorption sites (e.g., calcium, magnesium), pH, redox, particle sorption, sediment and soil physicochemical properties and hydrology. Weathering or aging of metals over time also can reduce their bioavailability. Hydrogen ion activity (pH) is probably the most important factor governing metal speciation, solubility from mineral surfaces, transport, and eventual bioavailability of metals in aqueous solutions. pH affects both solubility of metal hydroxide minerals and adsorption-desorption processes. Most metal hydroxide minerals have very low solubility under pH conditions in natural water. Because hydroxide ion activity is directly related to pH, the solubility of metal hydroxide minerals increases with decreasing pH, and more dissolved metals become potentially available for incorporation in biological processes as pH decreases. Ionic metal species also are commonly the most toxic form to aquatic organisms (Salomons, 1995).

Temperature exerts an important effect on metal speciation, because most chemical reaction rates are highly sensitive to temperature changes (Elder, 1989). An increase of 10 ^{0}C can double biochemical reaction rates, which are often the driving force in earth surface conditions for reactions that are kinetically slow, and enhance the tendency of a system to reach equilibrium. Temperature may also affect quantities of metal uptake by an organism, because biological process rates typically double with every 10^{0}C temperature increment. Because increased temperature may affect both influx and efflux rates of metals, net bioaccumulation may or may not increase (Luoma, 1983).

In recent organic carbon-rich sediments, trapped interstitial fluids can commonly form a strongly reducing (anoxic) environment. Low redox potential in this environment can promote sulfate reduction and sulfide mineral deposition. During diagenesis, much of the non-silicate-bound fraction of potentially toxic metals such as arsenic, cadmium, copper, mercury, lead, and zinc, can be co-precipitated with pyrite, form insoluble sulfides, and become unavailable to biota. Seasonal variation in flow rates or storms that induce an influx of oxygenated water can result in rapid reaction of this anoxic sediment and thereby release significant proportions of these metals. Pyritization and/or de-pyritization of trace metals probably can be an important process in controlling bioavailability of many trace metals, especially in the aquatic environment (Morse, 1994).

2.6 Heavy Metals in the Study

2.6.1 Copper

Copper is one of the world's most widely used metals. The most common copper-bearing ores are sulfides, arsenates, chlorides, and carbonates (Weiner, 2008). It reaches aquatic systems through anthropogenic sources such as industry, mining, plating operations, usage of copper salts to control aquatic vegetation or influxes of copper containing fertilizers (Nussery, 1998). Copper is an essential trace element to plants, animals and even humans, and although the concentration of copper is usually low in nature, it happens in adequate quantities for growth in all aquatic environment. “It is required for bone formation, maintenance of myelin within the nervous system, synthesis of haemoglobin, component of key metalloenzymes, plus it forms an important part of cytochrome oxidase, and assorted other enzymes involved in the redox reactions in the cells of animals. It is also essential for cellular metabolism, where its concentration is well regulated, but becomes toxic at elevated levels.” (Pelgorm *et al.,* 1995).

Although copper is important, it is toxic when concentrations exceed that of natural concentrations (< 0.05 µmol/L) (Stouthart *et al.,* 1996). The toxicity of copper in aquatic organisms is largely attributable to Cu^{2+} that forms complexes with other ions (Nussey, 1998). A reduction in water dissolved oxygen, hardness, temperature, pH, and chelating agents can increase the toxicity of copper (Nussey, 1998). Organic and inorganic substance can easily complex the cupric form of copper, which is the most common speciation of this metal, and it is then absorbed on to particulate matter. Therefore, the free ion is rarely found except in pure acidic soft water. The chemical speciation of copper strongly depends on the pH of water (Stouthart *et al,* 1996). Copper, in water, particulate at high pH (alkaline) and is thus not toxic, while at low pH (acidic) it is mobile, soluble and toxic (Nussey, 1998).

Acute poisoning result from ingestion of excessive amount of copper salt and can lead to “nausea, vomiting, stomachache and diarrhea and may produce death. Copper fume, dusts and mist from industries exposure affect to upper respiratory tract. At low concentration, copper can result in anemia, gastrointestinal disturbances, bone development abnormalities, and death. Copper toxic effects in fish include; change biochemistry, anatomy, physiology and behavior. It damages the gill and head area of fish, could probably cause mucous to accumulate on the gill area” (Lewis and Lewis, 1971).

2.6.2 Lead

The major sources of lead in the environment are automobile exhaust, industrial wastewater, wastewater sludge and pesticides (Balba *et al.,* 1991). The global mean lead concentration in lakes and rivers is estimated to be between 1.0 to 10.0 µg/L (Weiner, 2008). Lead enters the aquatic environment through erosion and leaching from soil, leads dust fallout, combustion of gasoline, municipal and industrial waste discharges, runoff of fallout deposit from streets and other surfaces as well as precipitation (DWAF, 1996).

Lead is toxic and a major hazard to human and animals. Lead has two quite distinct toxic effects on human beings, physiological and neurological. “The relatively immediate effects of acute lead poisoning are ill defined symptoms, which include nausea, vomiting, abdominal pains, anorexia, constipation, insomnia, anemia, irritability, mood disturbances and coordination loss. In more severe situations neurological effects such as restlessness, hyperactivity, confusion and impairment of memory can result as well as coma and death” (Ansari, *et al*. 2004).

The main targets of lead toxicity are “the hematopoitic and nervous systems. Several of the enzymes involved in the synthesis of heme are sensitive to inhibition by lead. Besides this, the nervous system is another important target for lead toxicity, especially in infants and young children where the nervous system is still developing. Even at low levels of exposure, children may show hyperactivity, decreased attention span, mental deficiencies and impaired vision. Lead damages the arterioles and capillaries resulting in cerebral edema and neuronal degradation. Another system affected by lead is the reproductive system. The exposure can cause reproductive toxicity, miscarriages and degenerated offspring” (Kocak *et al*., 2005).

Several effects of lead toxicity have been reported on the exposure of fish to lead. These include (1) behavioral deficits in fish within a day of exposure to sub lethal concentration (2) a deficit in metabolism and survival (3) decreasing in growth rate and development (4) a deficit in behavior and learning (5) increased mucus formation in fish and (6) the level at 50 µg/g in the diet are associated with reproductive effects in some carnivorous fish (Eisler, 1997).

2.6.3 Nickel

Nickel is found in many ores as sulfides, arsenides, antimonide, silicates and oxides. Its average crustal concentration is about 75 mg/kg and in aquatic ecosystem, dissolved nickel concentrations are generally between 0.005 and 0.01 mg/L (Galvin, 1996). Chemical and physical degradation of rocks and soils, atmospheric deposition of nickel-containing particulates, and discharges of industrial and municipal wastes are released nickel into ambient waters (USEPA, 1986; WHO, 1991). The main anthropogenic sources of nickel in water are primary nickel production, metallurgical processes, combustion and incineration of fossil fuels, and chemical and catalyst production (USEPA, 1986). The atmosphere is a major conduit for nickel as particulate matter. The atmospheric loading of nickel comes from both natural sources and anthropogenic activity. Nickel particulates eventually precipitate from the atmosphere to soils and waters. Soil-nickel enters waters with surface runoff or by percolation of dissolved nickel into groundwater (Weiner, 2008)

.

Nickel is one of the most mobile heavy metals in aquatic environment. It ions tend to be soluble at pH value below 6.5, and above 6.5 they mostly form insoluble nickel hydroxides (Dallas and day, 1993). Nickel pollution of aquatic system is controlled largely by co-precipitation and sorption with hydroxides of iron and manganese. In surface water, sediments generally contain more nickel than the overlying water (Weiner, 2008)

Nickel is an essential micronutrient required to produce red blood cells but it is known to be toxic at high intakes. The toxicity of Ni to aquatic life has been shown to vary significantly with organism species, pH and water hardness (Birge and Black, 1980). According to Nebeker *et al.* (1985), nickel has been show moderately toxic to fish and aquatic invertebrate when compared to other metals. "The long term exposure can cause decrease in body weight, heart and liver damage and skin irritation. Nickel accumulates in fish tissues and causes alterations in gill structure, including hypertrophy of respiratory and mucus cells, separation of the epithelial layer from the pillar cell system, cauterization and sloughing, and necrosis of the epithelium" (Nath and Kumar, 1989).

2.6.4 Zinc

Zinc is a very common environmental contaminate and usually outranks all other metals and it is commonly found in association with lead and cadmium (Finkelman, 2005). Major sources of zinc to aquatic environment include the discharge of domestic wastewater, manufacturing processes involving metals and fallout atmosphere.

Zinc is an essential element for human, animal, and certain types of plant. "The cell of living organisms contain zinc as one of the main component of various enzymes, such as carbonate anhydrase, carboxy peptidase, superoxide dismutase, lactase, dehydrogenase, phosphatase and glutamate dehydrogenase. It is also necessary for a healthy immune system, cell division and synthesis of protein and collagen which is great for wound healing and healthy skin. However, a higher amount of it can cause anemia, pancreas damage and lower levels of high density of lipoprotein cholesterol" (Finkelman, 2005).

Even though zinc is an essential trace element and help in homeostatically control in fish, but at high concentration of zinc can be toxic to fish (Counture and Rajotte, 2003). Acute toxic zinc concentrations result in gill damage, which interferes with respiration, leading to hypoxia. Chronically toxic are generally extensive deterioration of liver, kidneys, heart, and muscle. Chronic sub-lethal zinc concentration can also delay or inhibit the growth sexual maturity and reproduction of the fish, and can also induce pathological and morphological abnormality in adult fish (Somasundaram *et al*., 1984).

Zinc toxicity is modified by water chemical factors including dissolved oxygen concentration, hardness, pH and temperature of the water (Nussey, 1998) and can also be changed through other heavy metals compounds and alkaline earths metals. High temperature tend to increase zinc toxicity, while increase in water hardness, alkalinity and organic chelators can reduce its acute lethality and low dissolved oxygen content in water increases the toxicity of zinc (Chapman, 1978).

2.7 Relevant Studies in Ethiopia and Other Africa Countries

Abayneh Ataro *et al*. (2003) studied trace metals in fish from Lake Hawassa and Ziway. The results indicated the range of concentration 1.03-2.78 µg/g in Lake Hawassa and 1.03-1.98 µg/g in lake Ziway for Cu and 23.04 -30.92 µg/g in Lake Hawassa and 26.29-30.92 µg/g in Lake Ziway for Zn. The accumulations of Pb and Ni were below 1.66 and 0.99 in Tilapia fish of both lakes. Selamawit Geta (2010) found 1.35 of Cu, 0.35 of Pb and 27.13 of Zn in the muscle of Tilapia fish from Lake Ziway.

The concentrations of Pb in Lake Victoria ranged from 0.04 to 0.94 mg/L and its accumulation in the muscle of fish were ranged from 3.6 to 20.3 µg/g as studied by Tole and Jenipher (2003) and Kisamo (2003) found the concentrations of Cu, Pb and Zn in water of Lake Victoria were < 0.01 mg/L, 0.35- 0.36-0.63 mg/L and 0.01-5.62 mg/L and their accumulatons in the range of 2.3 -6.6 µg/g, 0.01-28.0 µg/g and 17-179 µg/g, respectively. Sanarathne and Pathirathe (2007) estimated the accumulation of heavy metals in fish inhabiting Bolgada Lake in Sri Lanka. The results showed that the accumulation ranged from 0.8 - 9.3µg/g wet mass for Cu, 0.1 - 3.0µg/g wet mass for Pb and 5.9 - 11.5µg/g wet mass for Zn.

Saeed and Shaker (2008) assessed the heavy metals pollution in water and sediments and their effect on Nile Tilapia (Oreochromis Niloticus) in the northern delta Lakes in Egypt. The results indicated that the concentrations of heavy metals in water were maximum at the Lake Manzala (1.36-0.68 mg/L for Cu and 0.32-0.66 mg/L for Zn) while minimum at Lake Edku (0.002- 0.054 mg/L) but the highest Pb concentration was at Borollus Lake (0.11-0.31 mg/L) and the lowest was at Lake Edku (ND – 0.084 mg/L). The accumulations of Cu, Pb and Zn were highest in the fish of Lake Manzala (44.84 µg/g d.w for Cu, 10.1µg/g d.w. for Pb and 212.44 µg/g d.w for Zn) but the lowest accumulations were recorded at Lake Borollus for Cu (1.77µg/g d.w.) and for Zn (9.88 µg/g d.w.), and at Lake Edku for Pb (0.59 µg/g d.w.)

3. MATERIALS AND METHODS

3.1. Description of Study Area

Lake Hawassa is a medium sized lake in the Ethiopian rift valley, with a total drainage basin of 1250 km (Zinabu Gebremariam, 1988; Zerihun Desta *et al.,* 2006). The Lake has an area of 88 km^2, the mean depth is 11m, and maximum depth is 22m (Elias Dedebo, 2002). The Hawassa region has a dry, sub-humid climate with one rainy and one dry season. The mean annual rainfall is around 1154 mm, distributed throughout the eight rainy months; March to October and November to February is the dry season (Zerihun Desta *et al.,* 2006).

The Lake supports six fish species: Nile tilapia *Oreochromis niloticus* (L.), African catfish *Clarias gariepinus* (B.), African big barb *Barbus intermedius* (R.), small barb *Barbus paludinosus* (P.), the cyprinid *Garra quadrimaculata* (R.), and the cyprinodont *Aplocheilichthyes antinorii* (V.). The local artisan fishery depends mainly on Nile Tilapia and to a lesser extent on Cat fish and Big Barb (Bjørkli, 2004; Zerihun Desta *et al.,* 2006). The river Tikur Wuha is the only inflow, but there is no surface outflow from the lake. There are four factories operating within the catchments of Lake Hawassa and except the flour factory that has no liquid discharge, the Hawassa textile, Ceramics, and Sisal factories discharge their effluent directly to Tikur Wuha River and eventually to the Lake (Bjørkli, 2004; Zerihun Desta *et al.,* 2006). Additionally, wastewaters from urban areas, agricultural fields and the referral hospital are released or washed up by runoff and eventually reach the lake.

The Lake is important for the tourism industry and fishing activities and generates income to the regional government as well as local community. The monitoring of this lake through periodical assessment of the status of pollution in general and the accumulation and distribution of heavy metals in particular is very crucial.

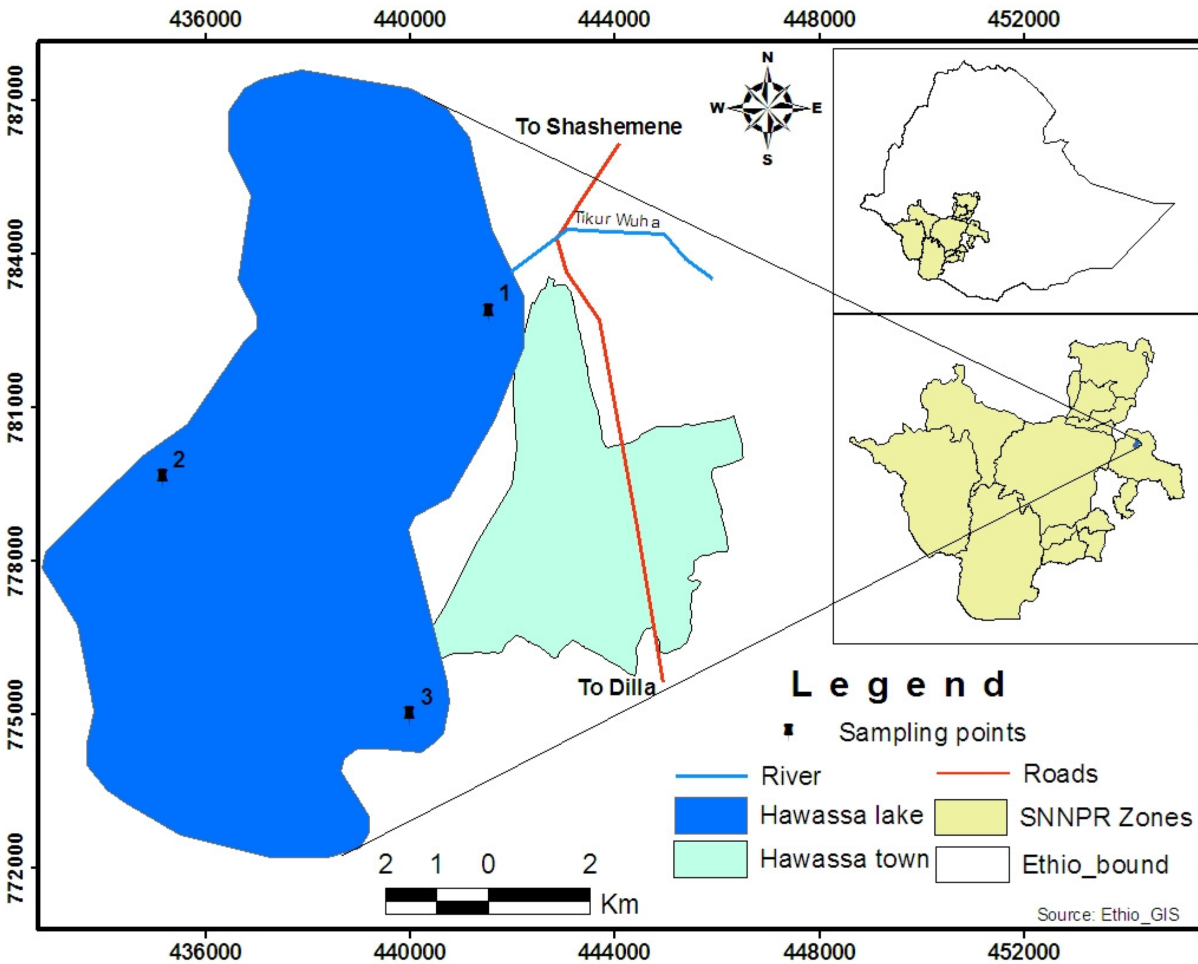


Figure 3.1 Map of Lake Hawassa with sampling sites

3.2. Selection of Sampling Stations

An observational trip in the study area was undertaken using a motor boat in order to select three sampling stations. The sampling sites were used during the sampling period. The sampling of water and fish were done on December 1 to 4/2010 and January 24 to 27/2011. Three sampling sites in the lake were selected by considering the relative sources of pollution. Each selected sampling site has been received different types of pollutants from different sources of pollution. Due to the domination of these three sources of the pollution in lake, the following three sites are selected.

Sampling Site-1 (Figure 3-1) is near the entry of the Tikur Wuha River. This is an area where the river inputs are high to the lake. The factories like Hawassa Textile, Ceramic and Sisal release their effluent into the river and then the river finally discharges their input into Lake Hawassa. The site is also close to the highway which may be receiving particulate pollutants related to motor vehicles. The sample site is named as Tikur Wuha.

Sampling Site-2 (Figure.3-1) is located in the opposite side of Amora Gedel which is located near the Lake Hawassa and commonly used for recreational purpose. The site name is after the local village that is found near to Lake Hawassa is Dorie Basana and it does not have point source of pollution but there may be non-point source of pollution from the agricultural land and soil of the area. The site is called by its local village name Dorie Basana.

Sampling Site-3 (Figure.3-1) is located on the side of a hospital known as the referral Hospital. This is an area where the lake is directly receiving the effluent of the Hospital as well as urban runoff. This sampling site is named as Referral H.

3.3 Collection of Samples

Water samples and fish samples were collected from the three selected sites of the Lake Hawassa in December, 2010 and January, 2011. Three replicate surface water samples were collected from three different points of the same station using 1L polyethylene sampling bottle during the sampling period. Bottled samples were taken to laboratory using an ice box at 4 ^{0}C. Then, the three replicated samples for each site were composited for all analytical procedures conduct in the laboratory.

Three Tilapia fish species samples were caught and collected at each sampling site with the help of fishermen. After collection, the samples were immediately dissected in the field and only the edible tissue (fillet) was transferred to plastic bags and keeps in an ice box at 4^{0}C and then transported to the laboratory.

3.4 Instrumentations and Apparatus

All digestion works were carried out by using Heating Mantle (98-II-B Magnetic stirring electric sleeve). The fish samples were oven dried and grounded in mortar and pestle. The 250 mL round bottle flask was used for digestion purpose. PG-990 Atomic Absorption Spectrometer, equipped with the deuterium lamp method and the self reversal method background correction system were used for analysis of heavy metals in water and fish samples. The GF-990 graphite furnace power supply and ASC-990 programmable automatic sample loader were used for GFAAS and ASA-900 automatic sample was used for Flame Atomic Absorption Spectrophotometer (FAAS). The PG data station with AAwin software provided control of the spectrometer, furnace and autosampler. The operating parameters for the elements were set as recommended by the manufacture. For flame measurement, a 10 cm long slot burner head, a lamp for each metals and acetylene flame were used. Argon gas was used as the inert gas for graphite furnace measurements. Pyrolytic coated graphite tubes with a platform were used for metals determinations by GFAAS.

3.5 Reagents and Standard Solution

All the chemicals used were of analytical reagent grade. Deionized water was used for all dilutions throughout the study. Nitric acid, HNO_3 (69%), and hydrogen peroxide, H_2O_2 (30%), were used for digestion. Working standards were prepared by diluting concentrated stock solution of 1000 mg/L for Cu, Ni and Zn and 1000 µg/L for Cu, Pb and Ni in deionised water. The matrix modifier $NH_3H_2PO_4$ and $Mg(NO_3)_2$ were used for Graphite Atomic Absorption Spectrophometery (GFAAS).

3.6 Determination of Physicochemical Parameters

The following physicochemical variables of water were determined in situ at the three sampling sites using the instruments indicated in parenthesis; temperature and dissolved oxygen (CO 411, ELMETRON), pH (Wagtech, pH meter), conductivity and total dissolved solid (Wagtech, EC/TDS). Suspended solid (SS) of the water was determined with Hack Spectrophotometer in the laboratory.

3.7 Preparation of samples

The water samples were filtered through Whitman 541 filter paper immediately after the samples have been transported to the laboratory. The filtered samples were acidified with HNO_3 and were kept at 4 ^{0}C prior to analysis.

In order to obtain a representative sample, composites were prepared by taking the edible tissues (fillet) of the three fish samples at each sampling site. The fish samples were oven dried at 105^{0}C until they reached a constant weight (Jackson, 1992). Each dried sample was then ground into a fine powder using porcelain mortar and pestle, and thereafter all powdered tissues were kept in desiccators prior to further chemical analysis.

3.8 Digestion of Fish Samples

The powdered fish samples were thoroughly homogenised before subjecting them to digestion and were digested using concentrated nitric acid and hydrogen peroxide (1:1) v/v according to FAO methods (Daziel and Baker, 1983). 1g of dried and powdered fish samples was weighted and transferred into 250 mL round bottled flask and the mixture of 10 mL of concentrated HNO_3 (65%) and 10 mL of H_2O_2 (30%) was added . 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 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 deionized water.

3.9 Analysis of Heavy Metals

Concentration of Cu, Pb, Ni and Zn were determined in water and fish samples. The analyses of metals in water and fish samples were carried out by both; furnace atomic absorption spectrometry and flame atomic absorption spectrometry. The PG-990 Atomic Absorption Spectrophotometer equipped with a graphite furnace and ASC- 990 autosampler for GFAAS and ASC-900 autosampler for flame was used for determinations. The operating conditions for Cu, Pb, Ni and Zn analysis by FAAS and/or GFAAS were indicated in Table 3.1 & 3.2. Calibration of the instrument was carried out with range of standard solution. After calibration, the samples were aspirated into the AAS instrument according to standard method (APHP, 1998). The samples were analysed in duplicates, and the blank determinations in duplicates were also run in the same manner during the analysis.

The water samples and fish samples were analysed by GFAAS for Pb and by FAAS for Zn determinations. The fish samples for Cu and water samples for Ni were analysed by FAAS. The water samples for Cu and fish samples for Ni were analysed by GFAAS. Modifiers [Mg $(NO_3)_2$ and $NH_4H_2PO_4$] were used for GFAAS to eliminate matrix interferences.

Table 3.1 Instrumental conditions for the flame analysis on the PG-990 AAS

Element	Cu	Ni	Zn
Wavelength(nm)	324.7	232.0	213.9
Slit width (nm)	0.4	0.2	0.4
Lamp	HCL	HCL	HCL
Lamp current (mA)	5.0	5.0	5.0
Detection limit (mg/L)	0.004	0.008	0.003
Gas	Acetylene	Acetylene	Acetylene

Table 3.2 Instrumental conditions for the furnace analysis on the PG-990 AAS

Element	Cu	Pb	Ni
Wavelength (nm)	324.7	283.3	232.0
Slit width (nm)	0.4	0.4	0.2
Lamp	HCL	HCL	HCL
Lamp current(mA)	3.0	3.0	4.0
Detection limit (pg/mL)	3.43	3.88	8.57
Inert gas	Argon	Argon	Argon

3.10 Recovery Test

The digestion method and AAS analysis were validated by measuring the recovery of copper, lead, nickel and zinc 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 50 µL for Cu, 1.5 mL for Pb, 1mL for Ni and 50 µL for Zn 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) the formula

$$\text{Recovery} = \frac{\text{Conc. Spiked Sample} - \text{Conc.Unspiked Sample}}{\text{Conc.Analyte Added (Spiked)}} X\ 100\% \qquad \text{Equation 3.1}$$

The recovery percentages of spiked fish sample were obtained as shown in Figure 3.2 and the results for metals under investigation (Cu, Ni, Pb and Zn) varied between 78.28% and 93.85%. The obtained 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.

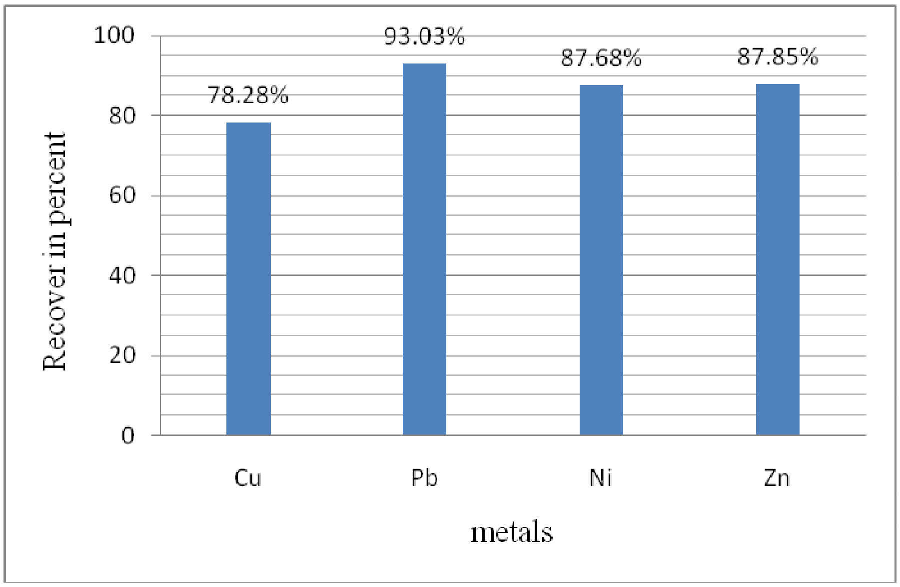


Figure 3.2 Recovery Percentage of the heavy metals in spiked fish samples

3.11 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 PG-990 Atomic Absorption Spectrophotometer was made with standard solutions. Five working calibration standards were prepared by serial dilution of concentrated stock solution of 1000 mg/L for copper and zinc and nickel and 1000µg/L for Cu, Pb and Ni. 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 concentrations of standard solutions that were used to calibrate the FAAS and GFAAS were given in Table 3.3. The absorbance of each standard solution for FAAS and GFAAS was provided in the Appendix 2. The correlation coefficient of each metal was determined from the calibration curve which was provided in Appendix 3

Table 3.3 Standard solution for metals analyzed

Metals	Standard solution for FAAS (mg/L)	Standard solution for GFAAS (µg/L)
Cu	0.25, 0.5, 1.0, 1.5, 2.0	1, 10, 20, 30, 40
Pb	-------------------------	1, 25, 50, 75, 100
Ni	0.25, 0.5, 1.0, 1.5, 2.0	10, 20, 30, 40, 50
Zn	0.25, 0.5, 1.0, 1.5, 2.0	--------------------

The correlation coefficient (R^2) which was obtained from the calibration curve (Appendix 3) of each metal was greater than 0.99 and it was given in Table 3.4

Table 3.4 Correlation coefficient (R^2) value for each metal

Metals	R^2	Remark
Cu	0.998	GFAAS
	0.999	FAAS
Pb	0.998	--------
Ni	0.999	GFAAS
	0.999	FAAS
Zn	0.995	---------

3.12 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).

The method detection limit of each element depends on the sample matrix as well as the instrument, the type of automizer and use of chemical modifier. For water samples with a sample matrix (i.e., low concentration of dissolved solids and particulates) the method detection limit will be close to instrument detection limit. In digests containing higher concentrations of dissolved solids, interference effects may lead to an increase in the method detection limit. The MDL protects against incorrectly reporting the presence of a compound at low concentrations in case when noise and actual analyte may be indistinguishable. The MDL concentration does not imply accuracy or precision of the quantitative measurement (Childress *et al*., 1999)

Method detection limit for fish samples was established using the blank reagent (mixture HNO_3& H_2O_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 for fish blanks (mixture of HNO_2 and H_2O_2 that used to digest the fish samples) were calculated according to Childress *et al*., (1999) formula:

MDL= (S) x (t) Equation 3.2

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 calculated values of MDL of the metals Cu, Pb, Ni and Zn are given in the Table 3.5

Table 3.5 Method detection limit of fish samples analysis

Metals	Method Detection Limit (µg/g)
Cu	1.27
Ni	0.29
Pb	1.95
Zn	1.55

The large MDLs of metals compare to the instrumental detection limit in this study might be due to the sample matrix effect of the digested fish samples, the instrument conditions, adjustment of the autosampler and the chemical modifier (for GFAAS). The concentrations of the metals in fish samples below MDLs were rejected and only above MDLs were reported in this study.

3.13 Statistical Analysis

Statistical analysis of data was carried out using SPSS 16.0 statistical package program. One-way ANOVA (Analysis of Variance) was performed for statistically significant difference in the mean value of heavy metal concentrations and physicochemical parameters between the three sampling sites. Difference in mean values were accepted as being statistically significant if $P < 0.05$. The concentrations of all metals in fish sample are expressed in µg/g and the water concentrations are expressed in µg/L.

4. RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Physicochemical Variables of Water

The results of the physicochemical parameters are presented in Table 4.1. The minimum mean value of temperature was recorded at Tikure Wuha site (21.38 ^{0}C) while the relatively maximum value was observed at Referral H. site (22.53 ^{0}C). The maximum mean value of dissolved oxygen (DO) was measured at Dorie Basana site (8.43 mg/L) and minimum of 5.20 mg/L recorded at Referral H. site. The pH values of the lake water were slightly alkaline, with the lowest mean reading was recorded at Tikur Wuha site (8.64) while the relatively maximum value recorded at the Dorie Basana site (8.75).

The higher mean value of electrical conductivity was recorded at Tikur Wuha site (835.83 µS/cm) while the lower mean value (820.50 µS/cm) was recorded at Dorie Basana site. The minimum mean value of total dissolved solid (TDS) was recorded in Dorie Basana (491.83 mg/L) and maximum of 501.17 mg/L recorded at Tikur Wuha. The maximum mean value of suspended solid (SS) was observed at Tikur Wuha site (17.67 mg/L) and minimum of 9.67 mg/L recorded at the Dorie Basana site.

Table 4.1 The mean (± SD) value of some physicochemical properties of Lake Hawassa water at the three sampling sites

Physicochemical Parameters	Sampling Sites			P - value
	Tikur Wuha	Dorie Basana	Referral H.	
Temperature (^{0}C)	21.38 ± 0.38	22.18 ± 0.62	22.53 ± 0.33	P=0.169
DO (mg/L)	6.80 ± 0.70	8.43 ± 0.68	5.20 ± 0.08	P=0.00
pH	8.64 ± 0.02	8.75 ± 0.02	8.73 ± 0.03	P=0.10
EC (µS/cm)	835.83 ± 6.03	820.50 ± 1.32	832.33 ± 0.57	P=0.00
TDS(mg/L)	501.17 ± 3.51	491.83 ± 0.76	499.00 ± 0.50	P=0.00
SS(mg/L)	17.67 ± 0.58	9.67 ± 0.58	14.67 ± 0.28	P=0.00

4.1.2 Heavy Metals Concentration in Water

The dissolved metal concentrations in Lake Hawassa water were measured at the three sampling sites (TikurWuha, Dorie Basana and Referral H.) during the dry season of the area. The concentrations of dissolved zinc and nickel in Lake Hawassa were found to be below the instrumental detection limit (FAAS) in all sampling sites. But the copper concentrations ranged from undetected at Referral H. site to 0.005 mg/L at Dorie Basana and the lead concentrations also ranged from undetected at Dorie Basana and Referral H. sites to 0.003 mg/L at TikurWuha site. The concentrations of the metals in the three sampling sites of Lake Hawassa are presented in Table 4.2.

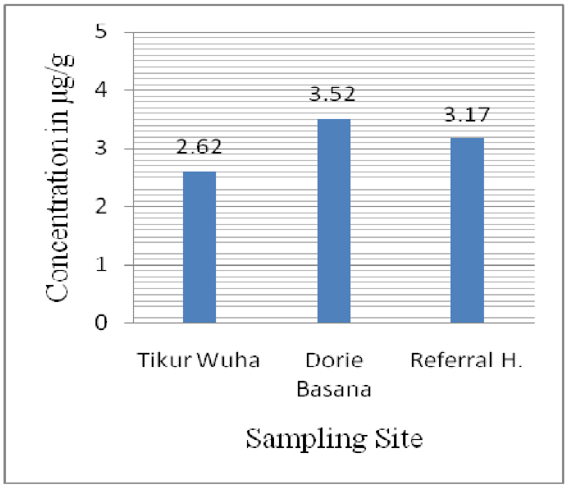
Table 4.2 Concentration of dissolved metals in Lake Hawassa water at the three sampling sites (in mg/L)

Metals	Sampling Sites		
	Tikur Wuha	Dorie Basana	Referral H.
Cu	ND – 0.003	ND – 0.005	ND
Pb	ND- 0.003	ND	ND
Ni	ND	ND	ND
Zn	ND	ND	ND

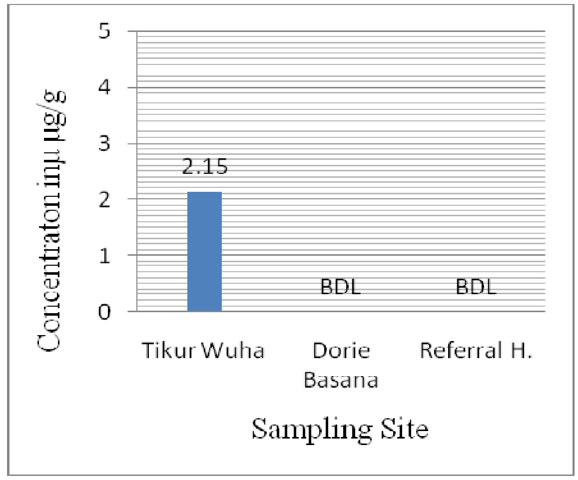
ND= Not detected

4.1.3 Accumulation of Metals in Tilapia fish

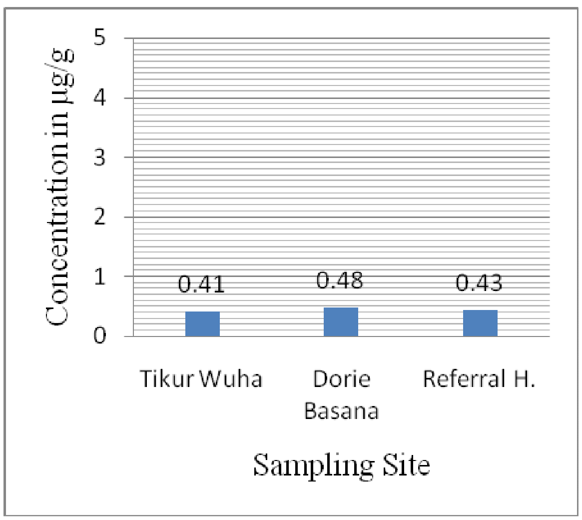
The mean concentration of Cu (3.52 µg/g) recorded in the fish sample collected at Dorie Basana was the highest while the lowest mean concentration (2.62 µg/g) was recorded in sample obtained at Tikur Wuha site. The highest mean concentration of Zn was 29.93 µg/g at Referral H. site and the lowest mean concentration of 25.38 µg/g was measured at Tikur Wuha site. The concentrations of Pb ranged from below the detection limit at Dorie Basana and Referral H. sites to the mean concentration 2.15 µg/g at Tikur wuha sites. Ni concentrations in Tilapia fish samples ranged from below the detection limit at Referral H. to maximum mean concentration of 0.48 µg/g at the Dorie Basana site. The mean concentrations of the Cu, Ni and Zn at each site were also illustrated in Figure 4.1 (a-c)



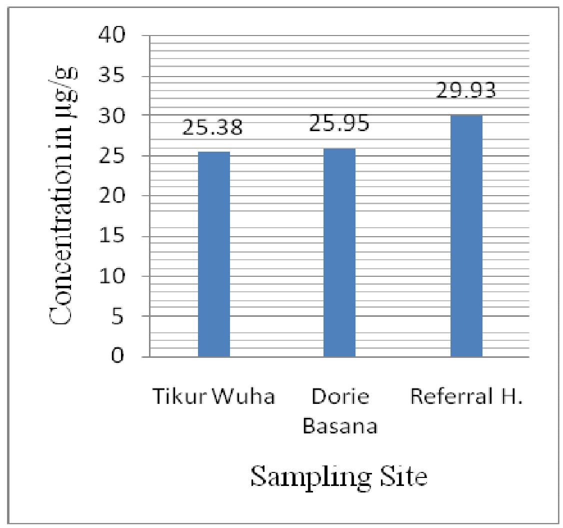
4.1a: Cu accumulation



4.1b: Pb accumulation



4.1c: Ni accumulation



4.1d: Zn accumulation

Figure 4.1 (a-d) Accumulation of Cu, Pb, Ni, Zn in edible muscle of Tilapia fish of Lake Hawassa

4.2 Discussions

4.2.1 Physicochemical Properties

The physical, chemical and biological contents of water determine the quality of water. 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).

Surface water temperature is one of the important factors affecting aquatic environments for two reasons. First, water temperature affects nearly all other water parameters and second aquatic organisms are adapted to certain temperature range. It exerts an important effect on metal speciation because most chemical reaction rates are highly sensitive to temperature change (Prosi, 1989). Due to increased temperature may affect both uptake and elimination rates of metals, so net bioaccumulation may or may not increase. The mean temperature of this study ranged between 21.38 ^{0}C to 22.53 ^{0}C. The three sampling sites were not significantly varied (P= 0.169) in temperature between the three sampling sites. That means the temperature was the same throughout the sampling sites.

Dissolved oxygen (DO) of water has a great significance to aquatic organisms and is considered to be the factor which reflects physical and biological process taking place in a water body. It is crucial in the production and support of life. Water body receives oxygen gas mainly from two sources (1) from atmosphere and, (2) during the process of photosynthetic of chlorophyll bearing plant. The concentration of DO in natural water body depends on surface agitation due to temperature, respiration rate of living organism and decomposition rate of organic matters (Mathur *et al*., 2008).

In this study, mean DO concentration ranged between 8.43 mg/L to 5.20 mg/L. The highest concentration was measured at Dorie Basana site while the lowest concentration was observed at Referral H. site. The low level of dissolved oxygen in the Referral H. site could have been due to the high organic load from the effluent of the Referral Hospital as well as the slightly elevation of water temperature. The highest mean value of DO (8.43 mg/L) recorded in the Dorie Basana site may be due to the relatively free from organic load. The DO values were significantly different (P=0.00) among the three sampling sites. According to the range of DO given by USEPA (Weiner 2008), the value of DO at Dorie Basana was in the range of good quality of water (above 8.0) but the DO value at Tikur Wuha was within the range slightly polluted (6.5-8.0 mg/L) and at Referral H. site moderately polluted (4.5-6.5 mg/L).

pH has an impact on solubility and bioavailability of metals in the natural water. The lower the pH indicates the higher the solubility of heavy metals, and thus increase in metal bioavailability (Waite *et al.*, 1984). In this study, the Lake Hawassa water was found to be slightly alkaline where pH varied from 8.64 to 8.75.

The alkalinity of natural water is controlled by the concentration of hydroxide and represented by a pH greater than 7. This is usually an indication of the amount of carbonates, and bicarbonates that shift the equilibrium producing [OH^-]. This is happening due to the amount Carbon dioxide in water will be converted into H_2CO_3 which acidify the water to a pH of about 6. If any alkaline earth metals (sodium, calcium and magnisum, etc) are present, the carbonate and bicarbonate formed from solublisation of CO_2 will interact with alkaline earth metals increasing the alkalinity shift the pH up over 7. Other contributors to an alkaline pH include boron, phosphorous, nitrogen containing compounds and potassium (Bellingham, 2008). Hence this pH values do not support the bioavailability of most dissolved heavy metals in Lake Hawassa. The pH of this study was significantly varied (P= 0.10) among the three sampling point of the Lake Hawassa

The pH values of Lake Hawassa water were within range of the WHO limit and US EPA limit for drinking water and for freshwater aquatic life (Table 4.3).

Table 4.3 Guideline values for some selected parameters

Physicochemical properties	Fisheries and aquatic life		Drinking water		
	EU	USEPA	WHO	EU	USEPA
PH	6 – 9	6.5 – 9.0	6.5 - 9.5	6.5 – 8.5	6.5 – 8.5
EC (µS/cm)	–	–	250	250	–
TDS (mg/L)	–	–	500	–	500
SS (mg/L)	–	–	–	–	–

Electrical conductivity (EC) is the ability of a material to carry electrical current. In water, it is generally used as a measure of the mineral or other ionic concentration. Michaud (1991) defined EC as the total amount of dissolved ions in the water and is used to estimate the amount of total dissolved salts in water. Only ionizable materials contribute to conductivity. The conductivity levels of Lake Hawassa ranged from 820.50 µS/cm to 835.83 µs/cm. The EC values of the Lake Hawassa were greater than the WHO Limit of drinking water (Table 4.3).

Total dissolved solid (TDS) and suspended solid (SS) are indicators for general water quality as they directly affect the aesthetic value of water by increasing turbidity. Solid suspended matter in water bodies has high surface area than the bulk sediment as a result increases the availability of toxic metals (Ramesser and Ramjeawon, 2002). 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. Total Dissolved solid (TDS) includes those materials dissolved in the water, such as, bicarbonate, sulphate, phosphate, nitrate, calcium, magnesium, sodium, organic ions, and other ions. These ions are important in sustaining aquatic life. However, high concentrations can result in damage to organism’s cell (Mitchell and Stapp, 1992).

The range of TDS in Lake Hawassa in this study ranged from 491.83 mg/L to 501.17 mg/L. The values showed that there was significant difference (P=0.00) between the sampling sites of Lake Hawassa and the TDS values of the lake were below the WHO limit for drinking water

Suspended solid (SS) can come from silt, decaying plant and animals, industrial wastes, sewage, etc. They have particular relevance for aquatic organisms that are dependent on solar radiation and those whose life forms are sensitive to deposition. High concentrations have several negative effects, such as decreasing the amount of light that can penetrate the water, thereby slowing photosynthetic processes which in turn can lower the production of dissolved oxygen; high absorption of heat from sunlight, thus increasing the temperature which can result to lower oxygen level; low visibility which will affect the fish’ ability to hunt for food; clog fish’ gills;

prevent development of egg and larva. It can also be an indicator of higher concentration of bacteria, nutrients and pollutants in the water (Tarazona and Munoz, 1995).

The suspended solid in this study ranged between 9.67 mg/L to 17.67 mg/L. The highest mean value was recorded at Tikur Wuha site while the lowest mean was observed at Dorie Basana site. They were significantly different (P = 0.00) among sampling sites.

4.2.2 Heavy Metal Concentration in Water

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 (Cambell, 1995; ATSDR, 2006)

In the present study, the concentrations of heavy metal in water samples from almost all the sampling sites were found to be below the instrumental detection limit. However, in some samples Cu concentrations of 0.005 mg/L and 0.003 mg/L were recorded at Dorie Basana and at Tikur Wuha sites respectively, and 0.003 mg/L of lead was recorded at Tikur Wuha site. The possible explanation for the higher concentration of copper at Dorie Basana site could be the contribution of the non-point sources of pollutions, especially from agricultural fields that might be used copper containing fertilizer and pesticides and for the concentration of lead at Tikur Wuha site might be the closeness of the site to the highway.

The undetectable concentrations of copper, lead, nickel and zinc in water samples from the sampling sites might be the result of adsorption and accumulation of metals by suspendered solid as well as for nickel and zinc due to detection limit of the FAAS. Chapman (1992) stated more than 50% of the total metals present (and up to 99.9%) in water are usually adsorbed onto suspended particles. The solubility of heavy metals is predominately controlled by the water pH (Osmond *et al.,* 1995), water temperature (Iwashita and Shimamura, 2003; Papafilippaki *et al.,*

2008) and the redox environment of the lake water (Osmond *et al.,* 1995; Iwashita and Shimamura, 2003; Papafilippaki *et al*., 2008). The behavior of metals in surface water is a function of the substrate sediment composition, the suspended solid composition and the water chemistry (Osmond *et al.,* 1995).

The water chemistry of the system controls the rate of adsorption and desorbtion of metals to and from sediment. Adsorption removes the metal from the water and stores the metal in the sediment and suspended solid. Desorption returns the metal to the water column, where recirculation and bioassimilation may take place. Metals may be desorbed from the sediment and suspended solid if the water experiences increases in salinity, decreases in redox potential, or decreases in pH. A lower pH increases the competition between metals and hydrogen ions for binding sites. A decrease in pH may also dissolved metals-carbonate complexes, releasing metals ions into the water column (Osmond *et al.,* 1995). A decreased redox potential, as it is often seen under oxygen deficient conditions, will change the composition of metal complexes and release the metals into the overlying water (Osmond *et al.,* 1995). During the sampling period of this study, the dissolved oxygen was found to be in the range 4.97 mg/L to 8.51 mg/L in the lake. Also, in all the sites pH of the water measured to be alkaline. These oxygen and pH conditions are not favorable for the solubility of the metals; it was not expected to find high amounts of dissolved metals in water samples.

When the dissolved metal concentrations in the lake water in the three sampling sites were compared with international standards, the obtained results obviously showed that the concentration of the heavy metals (Cu, Pb, Ni and Zn) in water did not exceed WHO (1993), USEPA (2006) and FAO (1985) (Table 4.4). In the Lake Hawassa, the concentration of suspended solid (SS) were ranged from 9.67 mg/L to 17.67 mg/L. These concentrations of SS were enough to make the metals unavailable for an organism. This might be due to most metals being absorbed into suspended particulate matter. Several mechanisms indicated that heavy metals in water are removed due to (1) adsorption onto particulate; (2) chemical transformation in to insoluble form; and (3) precipitation and sedimentation (Balasubramania *et al*., 1997)

Table 4.4 Recommended Limit of the metals (Cu, Pb, Ni and Zn) in water (in mg/L) by various organizations

Guideline	Cu	Pb	Ni	Zn
USEPA (2006)	1.5	0.05	0.1	0.5
WHO (1993)	2	0.05	0.02	5
FAO (1985)	0.2	–	0.2	2

4.2.3 Accumulation of Heavy Metal in Fish

Fish are important aquatic organism that are used as bio-indicators of aquatic ecosystems for estimation of heavy metal pollution and risk potential for human consumption (Argawa *et al.,* 2007). Bioaccumulation of metals in fishes takes place directly, from the water by gills and indirectly from food (Barron, 1990). Bioaccumulation of metal by an organism is the consequences of the interactions between physiological factor (growth, weight loss, absorption and accumulation), chemical factors (metal concentration, speciation and bioavailability) and environmental factors (temperature, pH, water hardness, conductivity, salinity and food concentration (Casas and Bacher, 2006).

Copper and zinc are essential elements and are regulated by physiological mechanisms in most organisms. However, they show toxic effects when organisms are exposed to levels higher than normally required (Biney *et al*., 1994). In this study, the mean accumulations of Cu and Zn in the edible muscle of Nile Tilapia collected from Lake Hawassa ranged from 2.62- 3.52 µg/g and 25.38 – 29.93 µg/g dry weight, respectively. The levels of Cu and Zn in the muscle were not significantly different (P = 0.612 for Cu and P = 0.683 for Zn) among the three sampling sites of Lake Hawassa. This shows that there is no variation of the accumulations of Cu and Zn in the edible muscle of Nile Tilapia fish throughout the lake.

Nickel is also an essential micronutrient required to red blood cells. It is known to be toxic at high intakes. The long term exposure can cause decrease in body weight, heart and liver damage and skin irritation. The toxicity of Ni to aquatic life has been shown to vary significantly with organism species, pH and water hardness (Birge and Black, 1980). In this study, the mean accumulation of Ni in muscle of Nile Tilapia fish was ranged from below detection limit to 0.48 µg/g. The accumulation of Ni in muscle of Tilapia fish was no significant difference (P = 0.834) among the three sampling sites.

Lead is non essential elements which is accumulated in fish tissues and is harmful to human health. The accumulation of Pb in the edible muscle of Tilapia fish collected from Lake Hawassa ranged from below detection limit at Gore Basana and Referal H. sites to mean accumulation 2.15 µg/g at Tikur Wuha site. The high accumulation of Pb at Tikur Wuha site might be the closeness of the site to the high way and receive pollutants that related to vehicle emission. The accumulation of Pb was significantly different (P=0.00) among three sampling point of the Lake. This could show that the accumulation of Pb in fish muscle was differed from one place of the lake to other. This might be connected with the exposure of the sites to different source of pollution.

The comparison between the present study with international standards are compiled in the Table 4.5. The FAO/ WHO (1989) limit for Cu and Zn in fish are 30 µg/g and 40 µg/g, respectively and the EU (2001) limit for Cu in fish is 10 µg/g but Zn level does not have limited by EU. The accumulations of Cu and Zn in the edible muscle of Tilapia collected from all sampling sites of Lake Hawassa were lower than the FAO/WHO and EU Limit. Hence the consumption of this fish seems to pose no threat to human health regarding to these metals.

The hazardous of Ni metals is notified by the USFDA (1993) but not covered by the EU and FAO/WHO. According to USFAD (1993), the maximum recommended limit for Ni is 70-80 µg/g, and the samples analyzed in this study showed that the mean accumulation of Nickel in Nile Tilapia Fish muscle up to 0.48 µg/g. Thus the accumulations of Ni in all samples were far below the maximum limit of USFDA.

The FAO/WHO and EU limit of Pb in the fish muscle are 0.5 µg/g and 0.1µg/g, respectively. The levels of Pb in the edible muscle of Tilapia fish from Gore Basana and Referral H. sites were substantially lower than the maximum limit recommended by FAO/WHO and EU. But the Pb levels in muscle of Tilapia fish from Tikur Wuha site exceeded the FAO/WHO and EU limit.

Table 4.5 Comparison of heavy metals accumulation in Tilapia fish of Lake Hawassa with international standard (in µg/g)

Metals	Sampling Sites			
	Cu	Pb	Ni	Zn
Tikur wuha	2.62	2.15	0.41	25.38
Dorie Basana	3.52	<1.95	0.48	25.95
Referral H.	3.17	<1.95	0.43	29.93
FAO/WHO	30	0.5	–	40
EU	10	0.1	–	–
USFDA	–	–	70 - 80	–

4.2.4 Comparison of Current Result with Reported Literature

4.2.4.1 Water

The comparison of the current study of heavy metals in water with reported literature was compiled in Table 4.6. Authman and Abbas (2007) found the concentration of Cu and Zn in Lake Qarun of Egypt in the range 1.25- 2.59 mg/L and 0.0096 - 0.18 mg/L, respectively. This lake receives mainly agricultural and sewage drainage water from Fayounce province and neighboring cultivated land (Authman and Abbas, 2007). The concentrations of Cu and Zn in Qarun were below the WHO (1993) but Cu concentration was higher than FAO (1985). The concentrations of Cu and Zn in Lake Qarun were significantly higher than the finding of the present study

Kisamo (2003) reported Cu conconcentration was < 0.01 mg/L and Zn concentration was in the range 0.01- 5.62 mg/L in Lake Victoria of Tanzania. The Lake Victoria in the Tanzania side received the pollutants that are come from mining area, industrial and agricultural activities (Kisamo,2003). The concentration of Cu in Lake Victoria was lower than the USEPA (2006), WHO (1993) and FAO (1985) but the concentration of Zn was higher than the USEPA (2006), WHO (1993) and FAO (1985) (Table 4.4). The concentration of Zn in Lake Victoria was higher than the finding of the present study of Lake Hawassa

Senarathne and Pathirathe (2007) reported that the Borgada Lake water in Sir Lanka contains Cu in the range of 13.2 - 135.5 mg/L and Zn in the range 58.2 - 227.6 mg/L. The main sources of pollutants in Lake Borgada were urban and industrial wastes from multiple sources(Senarathne and Pathirathe, 2007). The concentrations of the two metals in this lake were higher than the USEPA (2006), WHO (1993) and FAO (1985) (Table 4.4). The concentrations of Cu and Zn in the Borgada Lake were too much higher than the present study.

Saeed and Ibrahim (2008) reported the concentrations of Cu were in the range 0.002 - 0.054 mg/L in Lake Edku, 0.020 - 0.050 mg/L in Lake Borollus and 1.36 - 0.68 mg/L in Lake Manzala, and the concentrations of Zn ranged 0.004 - 0.05 mg/L in Lake Edku, 0.027 - 0.077mg/L in Lake borollus and 0.23 - 0.66 mg/L of Northern Delta Lakes of Egypt. Lake Edku receive pollutants from sewage and agricultural runoff, Lake Borollu mainly received agricultural drainage water and Lake manzala receives sewage, agricultural and industrial wastewater (Saeed and Ibrahim, 2008). The concentrations of Cu and Zn in these three lake water were below the USEPA (2006), WHO (1993) and FAO (1985) (Table 4.4). The concentrations of Cu and Zn in the present study were too much lower than the concentrations that were found in the three above lakes.

Öztürk *et al* (2009) found 0.01 mg/L of Cu in Avsar Dam Lake St1 & St2 of Turkey. The lake received pollutants related to human and agricultural activities (Öztürk *et al,* 2009). The concentration of cu in this lake was lower than the USEPA (2006), WHO (1993) and FAO (1985) (Table 4.4). The concentration of Cu of this lake was higher than the finding of the present study.

Tole and Jenipher (2003) in Kenya and Kisamo(2003) in Tanzania found the concentrations of Pb in Lake Victoria was in the range of 0.04-0.094 mg/L and 0.35 – 0.63 mg/L respectively. The lake received pollutants from urban center, industrial activities and road as well as the geological contributions of the lake (Tole and Jenipher, 2003). The concentration of Pb in Victoria lake was slightly higher than the recommended limit of USEPA (2006), WHO (1993) and FAO (1985) (Table 4.4). The Pb concentration in this lake was higher than the finding of the present study.

Senarathne and Pathiratne reported 6.5-7.59 mg/L range of Pb in Lake Bolgada in Sirlanka. Saeed and Ibrahim (2008) found the Pb concentration ranged from ND- 0.084 mg/L in Lake Edku,0.11-0.31 mg/L in Lake Borollus and 0.012- 0.22mg/L in Lake Manzala of Northern Delta Lakes of Egypt. Öztürk *et al* (2009) reported the average concentrations of Pb were 0.01 mg/L and 0.005 mg/L in Avsar Dam Lake St1 and St2, respectively.

The Pb concentration in Lake Bogada, Edku, Borollus and Manzala was higher than the USEPA (2006), WHO (1993) and FAO (1985) (Table 4.4) but the Pb concentration in Avsar Dam Lake was lower than USEPA (2006), WHO (1993) and FAO (1985) (Table 4.4). In addition to this, the concentration of Pb from all lakes mentioned in the above was higher than the finding of present study.

Öztürk *et al* (2009) also found the concentrations of Ni in Avsar Dam Lake St1 and St2 were 0.004 mg/L and 0.006 mg/L, respectively. The concentration of Ni in this lake was lower than the WHO (1993) and FAO (1985) (Table 4.4) It is clear that the concentration of Ni in Avsar Dam Lake were slightly higher than the finding of the present study.

The possible explanation of the variation in the concentrations of metals between the above all mentioned lakes and Lake Hawassa could be due to the different sources of pollution, their exposure time for pollutants, the water chemistry of the lake and the geological nature of the lake.

Table 4.6 Comparison of heavy metals in Lake Hawassa water (mg/L) with reported Literature

Location	Cu	Pb	Ni	Zn	Reference
L. Hawassa,	ND - 0.005	ND – 0.003	ND	ND	Present study
L. Victoria, Keny.	–	0.04- 0.094*	–	–	Tole& Shitsama (2003)
L.Victoria, Tanza.	<0.01	0.35 - 0.63	–	0.01- 5.62	Kisamo (2003)
L. Qarun, Egypt	1.25 - 2.59	–	–	0.096- 0.18	Authman & Abbas (2007)
L. Borgada, (Sir Lanka)	13.2-135.5	6.5 - 7.59	–	58.2- 227.6	Senarathne &Pathirathe (2007)
L. Edku, Egypt	0.002 -0.054	ND - 0.084	–	0.004- 0.5	Saeed & Shaker (2008)
L. Borollus, »	0.02-0.05	0.11-0.31	–	0.026-0.077	“
L. Manzala, »	1.36-0.68	0.012- 0.22	–	0.32- 0.66	“
Avsar Dam lake st1, Turkey	0.01	0.01	0.004	–	Öztürk *et al* (2009)
Avsar Dam, » lake st2	0.01	0.005	0.006	–	“

ND= Not detected, * = given in µg/L

4.2.4.2 Fish

The comparisons of the present study with the previous studies of the same lake as well as other lakes were compiled in Table 4.7. The maximum mean accumulation of copper and zinc found by Abayneh Ataro *et al*. (2003) in Tilapia species collected from the same lake of this study were 1.85 µg/g of copper and 27.00 µg/g of zinc and from Lake Ziway 1.98 µg/g of Cu and 30.92 µg/g of Zn. Selamawit Geta (2010) found the mean accumulations of Cu and Zn in Nile Tilapia fish from Lake Ziway were 1.13 µg/g and 27.13 µg/g, respectively. In 2003, the sources of pollution in Lake Hawassa were through Tikur Wuha and agricultural activities but the Referral Hospital was under construction and it did not contribute pollutants to the Lake. The accumulations of Cu and Zn in Tilapia fish from Lake Hawassa and Ziway were lower than the

FAO/WHO and EU recommended limit. Their accumulations were also lower than the maximum result obtained in present study (Cu=4.80 µg/g and Zn =40.7 µg/g).

Saeed and Shaker (2008) found 2.80, 1.77 and 48.84 µg/g of Cu and 27.6, 9.88, and 212.44 µg/g of Zn in *oreochromis niloticus* fish species from Lake Edku, Borollus and Manzala of Northern Delta Lakes of Egypt, respectively. The accumulations of Cu and Zn in fish from Lake Edku and Borollus were lower than the FAO/WHO and EU recommended limit but in lake Manzala their accumulations were too much higher than the FAO/WHO and EU recommended limit. The accumulation of these metals showed that the fish from Lake Manzala has higher values but Borollus has lower values of Cu and Zn than present study. And these metals in fish from Lake Edku were within the range of the present study.

Kisamo (2003) found the accumulation of Cu (2.3 – 6.6 µg/g) and Zn (17 - 179 µg/g) in fish from Lake Victoria in Tanzania. Cu accumulation in fish from this lake was lower than the FAO/WHO and EU recommended limit but Zn accumulation was higher than FAO/WHO limit. The maximum values of both metals in fish from Lake Victoria were higher than the finding of the study.

Yilmaz (2009) reported Cu (3.91 µg/g) and Zn (84.72 µg/g) in fish from Lake Köyceğiz of Turkey. Cu accumulation was lower than the FAO/WHO and EU recommended limit but Zn accumulation was higher than the FAO/WHO limit. The values were within the range of present study in Cu accumulation but higher in Zn level in fish muscle. Sanarathne and Pathiratne (2007) reported the levels of copper and zinc were in the range 0.8-9.3µg/g and 5.9-11.5µg/g in fish from Borgada Lake of Sir Lanka. Cu and Zn accumulations were lower than the FAO/WHO and EU recommended limit. The maximum value of Cu (9.3 µg/g) was almost two times higher than the present study but the range level of Zn was lower than this study.

Abayneh Ataro *et al* (2003) found that the accumulation of Pb and Ni in Nile Tilapia fish were below 1.66 and 0.99 respectively from the Lake Hawassa and Ziway. Selamawit Geta (2010) found 0.35 µg/g of Pb in the muscle of Nile Tilapia fish from Lake Ziway. Pb accumulation in fish from Lake Ziway was lower than FAO/WHO limit but higher than the EU limit. The mean values of these metals in the present study ranged from below detection limit to 2.15 µg/g for Pb and from below detection limit to 0.48 µg/g for Ni. These results showed that the accumulations of Pb in fish samples at Tikur Wuha sampling site of the present study were slightly increased but the accumulations of Ni in this study were lower than the previous study.

Tole and Shitsama (2003) in Kenya and Kisamo (2003) in Tazania reported the levels of Pb in muscle of fish were 3.6 - 20.3 µg/g and 0.01 – 28 µg/g from Lake Victoria of respectively. Pb accumulation in fish from Lake Victoria was higher than the FAO/WHO and EU limit. Saeed and Shaker (2008) determined 0.59, 0.016 and 10.1µg/g of Pb in muscle of *oreochromis niloticus* fish species from Lake Edku, Borollus and Manzala, respectively. Pb accumulation in fish from Lake Edku and Manzala was higher than FAO/WHO and EU limit but from Lake Borollus lower than FAO/WHO and higher than EU limit. The values of Pb in Lake Victoria of both countries and Lake Manzala were significantly higher than the present study, but the values in Lake Edku and Borollus were lower than the value that was found in some samples of this study.

Öztürk *et al.* (2009) reported the mean concentration of Ni in muscle of fish was 1.27 in Avsar dam Lake of Turkey. Ni accumulation was lower than USFDA recommended limit. This value was greater than the finding of the present study.

Senarathne and Pathirthe (2007) found the range of Pb was 0.1 to 3.0 µg/g w. m. in muscle of fish from Lake Borgada of Sir Lanka and Yilmaz (2009) recorded accumulations of Pb in fish muscle were found 1.12 µg/g from Lake Köyceğiz in Turkey. Öztürk *et al.* (2009) also founded 2.14 µg/g of Pb in fish muscle from Lake Avar Dam of Turkey. Pb accumulation in fish from Lake Borgada, Köyceğiz and Avar Dam were higher than the FAO/WHO and EU limit The accumulations of Pb in fish muscle from Lake Borgada, Köyceğiz and Avar Dam were comparable with the finding of the present study

Table 4.7 Comparison of heavy metal accumulations (µg/g, dry mass) in fish muscle from Lake Hawassa with Literature report

Location	Cu	Pb	Ni	Zn	Reference
L.Hawassa, Eth.	2.62 – 3.52	<1.95- 2.15	<0.29- 0.48	25.38- 29.93	Present study
L.Hawssa, Eth.	1.39 – 1.85	<1.66	<0.99	24.42- 27.00	Abayneh Ataro, *et al.*, (2003)
L.Ziway, Eth.	1.03 – 1.98	<1.66	<0.99	26.29- 30.92	"
L.Ziway, Eth.	1.35	0.35	–	27.13	Selamawit Geta (2010)
L.Victoria, Ken.	–	3.6 - 20.3	–	–	Tole& Shitsama (2003)
L.Victoria, Tanz	2.3 – 6.6	0.01 – 28.0	–	17 – 179	Kisamo (2003)
L. Edku, Egypt	2.80	0.59	–	27.6	Saeed& Shaker (2008)
L. Borollus »	1.77	0.016	–	9.88	"
L. Manzala »	44.84	10.1	–	212.44	"
L.Borgada (Sir Lanka)	0.8-9.3*	0.1- 3.0*	–	5.9 - 11.5*	Senarathne & Pathira the (2007)
Avsar DamLake (Turkey)	3.85*	2.14*	1.27*	–	Öztürk *et al.* (2009)
L. Köyceğiz (Turkey)	3..91	1.12	–	84.72	Yilmaz, 2009

*= given in w. m.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study revealed that the physicochemical variables such as temperature, DO, pH, TDS and SS of Lake Hawassa were below or within the range of the recommended limit of EU, WHO, USEPA. Only temperature of the lake water was not significantly different (P = 0.169) among the three sampling sites and it showed uniform throughout sampling points but the P-values of DO, pH, EC, TDS and SS were showed that these parameters were varied from one sampling site to another.

The pH of this study showed that the lake water was slightly alkaline which might have an effect on the availability of dissolved metals.

The concentrations of dissolved Ni and Zn in the lake water were undetectable. However, Cu at Dorie Basana site and Tikur Wuha and Pb at Tikur Wuha were detected in some samples of water.

In fish samples, the concentrations of Cu, Ni and Zn were detected in all sampling sites but Pb was detected only at Tikur Wuha sampling site. In the detected samples: concentration of Zn > Cu > Pb> Ni.

The P-values for Cu, Ni and Zn accumulations in muscle Tilapia fish showed that there no variation throughout the lake and their accumulations are lower than the FAO/WHO (for Cu and Zn), EU (for Cu) and USFDA (for Ni) limit. Hence the consumption of this fish seem to pose no threat to human health regarding to these metals.

The high accumulation of Pb in edible muscle of Nile Tilapia fish was observed only at Tikur Wuha site which exceeded the recommended limit of FAO/WHO and EU. The high concentration of Pb at Tikur wuha site might be caused by the closeness of the site to the highway.

5.2 Recommendations

Parameters such as temperature, DO, PH and others should be monitored regularly to ensure the sustainable use of the Lake Hawassa's water for different activities in general and to maintain the aquatic life and water environment in particular.

Even though heavy metal concentrations in water were very low or negligible, they levels in muscle of Tilapia fish were relatively high. The potential sources of these heavy metals to the fish in the aquatic system should be identified and quantified, hence further research is recommended.

Even though the accumulations of most heavy metals in muscle of Tilapia fish were below the standard set by FAO/WHO and EU, the load of heavy metals input to the lake from the probable sources should be quantified and a proper measure should be taken in order to keep the fish safe for consumption and to maintain the aquatic ecosystem.

Even though the Pb accumulation in Tilapia fish at Tikur Wuha site was higher than the standard set by FAO/WHO and EU, the Tilapia fish from this site should be consumed until extended and extensive study will be conducted on Tilapia fish from the Lake to recheck the Pb accumulation and to determine whether the fish is consumed or not.

It is important to continuously monitor heavy metals concentration in water and their accumulation in fish of the lake to know the current pollution status of the lake water.

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APPENDICES

Appendix 1 Graphite Furnace Temperature Program

Element	Step	Temp ^0C	Ramp Time (sec.)	Hold Time (sec.)	Internal gas Flow (mL/min)	Gas
Cu	1	70	5	10		High
	2	110	10	10		High
	3	600	10	15		High
	4	2100	0	3	Yes	off
	5	2200	1	2		High
Pb	1	70	10	10		High
	2	110	10	10		High
	3	450	10	15		High
	4	1800	0	3	Yes	off
	5	1900	1	2		High
Ni	1	80	5	10		High
	2	110	10	15		High
	3	800	15	10		High
	4	2000	0	3	Yes	off
	5	2100	1	2		High

Appendix 2 Standard concentrations and their absorption

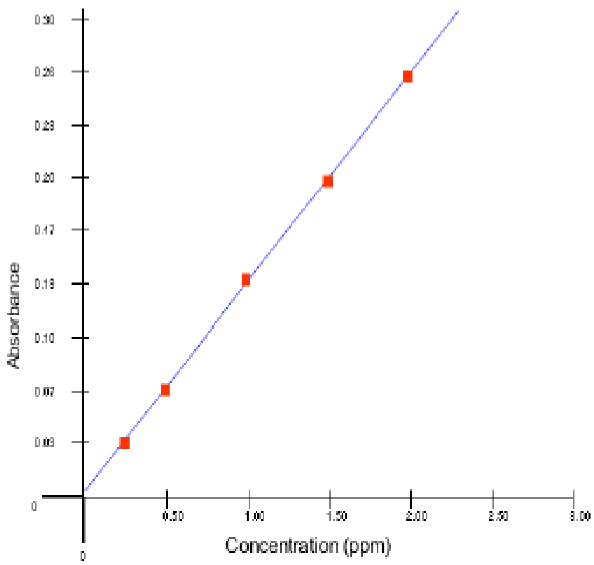
Standard concentration (in mg/L) and its Absorbance of metals analysed by flame AAS

Cu		Ni		Zn	
concentration	Absorbance	concentration	Absorbance	Concentration	Absorbance
0.25	0.034	0.25	0.02	0.25	0.093
0.5	0.067	0.5	0.06	0.5	0.160
1	0.134	1	0.13	1	0.25
1.5	0.195	1.5	0.19	1.5	0.33
2	0.260	2	0.26	2	0.40

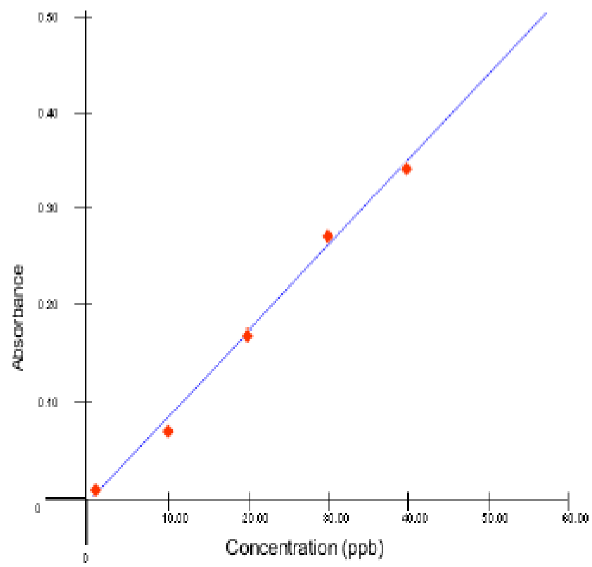
Standard concentration (in µg/L) and its Absorbance of metals analysed by graphite AAS

Cu		Pb		Ni	
concentration	Absorbance	concentration	Absorbance	Concentration	Absorbance
1	0.01	2	0.006	10	0.018
10	0.07	25	0.109	20	0.048
20	0.17	50	0.199	30	0.070
30	0.27	75	0.290	40	0.097
40	0.34	100	0.398	50	0.118

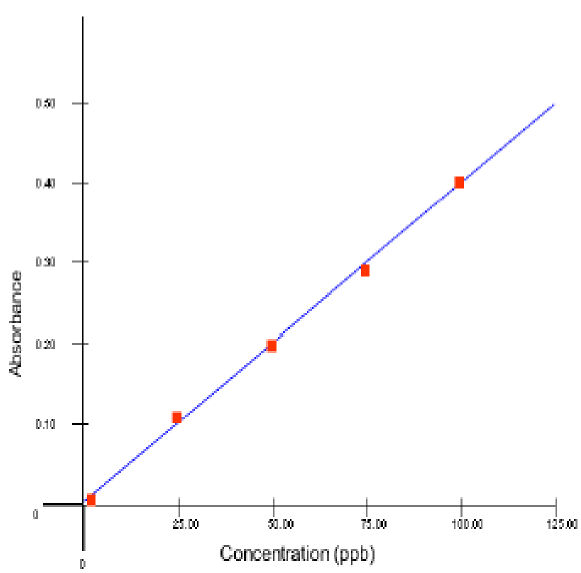
Appendix 3 Calibration Curve of Cu, Pb, Ni and Zn



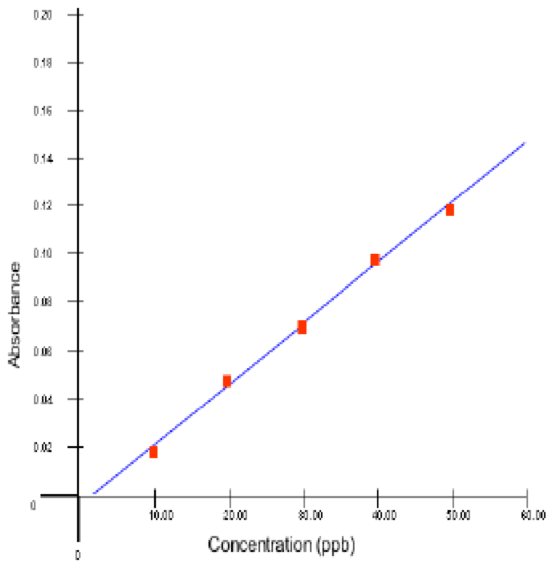
Calibration curve of Cu for fish sample analysis by FAAS



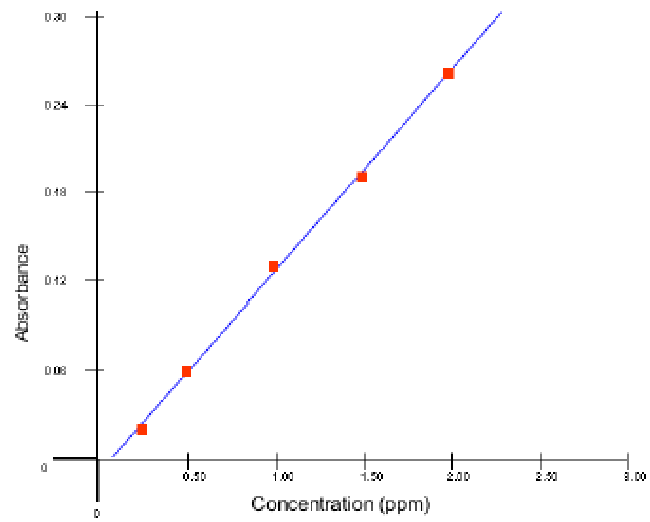
Calibration curve of Cu for water sample analysis by GFAAS



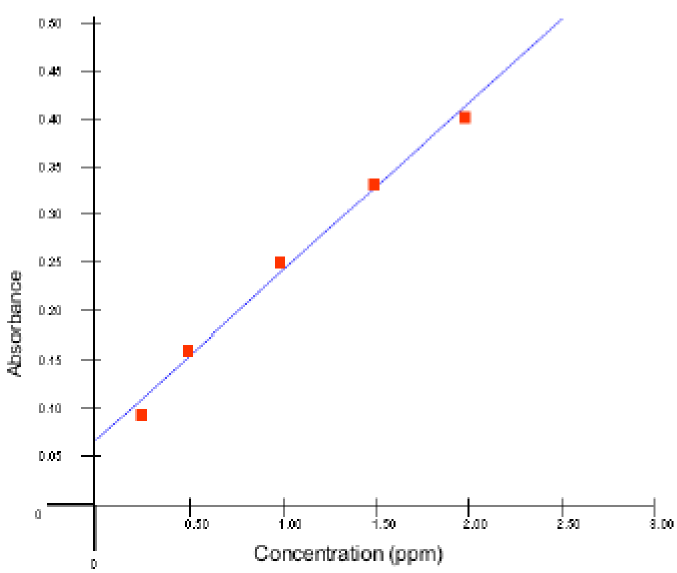
Calibration curve of Pb for water and fish samples analysis by GFAAS



Calibration Curve of Ni for fish samples analysis by GFAAS



Calibration Curve of Ni for water samples analysis by FAAS



Calibration curve of Zn for fish and water samples analysis by FAAS

Appendix 4 Physicochemical parameters and concentration of heavy metals in water and heavy metals accumulation in Tilapia fish

Some physicochemical parameters at the three sampling sites of Lake Hawassa

Physicochemical Parameter	Sampling Sites								
	Tikur Wuha			Dorie Basana			Referral H.		
Temperature (^{0}C)	20.95	21.70	21.50	22.73	21.50	22.300	22.85	22.55	22.20
DO (mg/L)	6.76	6.89	6.76	8.45	8.48	8.35	5.28	5.22	5.12
pH	8.64	8.63	8.66	8.73	8.77	8.75	8.75	8.72	8.76
EC (µS/cm)	829.50	836.50	841.50	821.00	819.00	821.50	832.00	832.00	833.00
TDS(mg/L)	497.50	501.50	504.50	492.00	491.00	492.50	498.50	499.00	499.50
SS(mg/L)	18	17	18	10	10	9	15	14.5	14.5

The concentrations of dissolved metals (Cu, Pb, Ni & Zn in Lake Hawassa at three sampling sites (in mg/L)

Metals	Sampling Sites								
	Tikur Wuha			Dorie Basana			Referral H.		
Cu	0.003	ND	ND	0.005	ND	ND	ND	ND	ND
Pb	0.003	ND	ND	ND	ND	ND	ND	ND	ND
Ni	ND	ND	ND	ND	ND	ND	ND	ND	ND
Zn	ND	ND	ND	ND	ND	ND	ND	DN	ND

The accumulation of the metals (Cu, Pb, Ni & Zn) in the edible muscle of Nile Tilapia Fish of Lake Hawassa at the three sampling sites (in µg/g)

Metals	Sampling Sites								
	Tikur Wuha			Dorie Basana			Referral H.		
Cu	2.40	2.10	3.35	3.80	2.55	4.20	4.80	1.80	2.90
Pb	2.06	2.23	ND	ND	ND	ND	ND	ND	ND
Ni	0.33	0.36	0.55	0.38	0.39	0.68	0.53	0.32	0.21
Zn	21.40	24.65	30.09	25.55	21.00	31.30	40.70	22.75	26.36

Appendix 5 The ANOVA values of Physicochemical parameters

		Sum of Squares	df	Mean Square	F	Sig.
Tem	Between Groups	1.046	2	.523	2.586	.169
	Within Groups	1.011	5	.202		
	Total	2.057	7			
DO	Between Groups	15.553	2	7.776	1.263E3	.000
	Within Groups	.031	5	.006		
	Total	15.583	7			
pH	Between Groups	.014	2	.007	13.337	.010
	Within Groups	.003	5	.001		
	Total	.017	7			
EC	Between Groups	447.552	2	223.776	67.133	.000
	Within Groups	16.667	5	3.333		
	Total	464.219	7			
TDS	Between Groups	163.302	2	81.651	66.204	.000
	Within Groups	6.167	5	1.233		
	Total	169.469	7			
SS	Between Groups	80.167	2	40.083	150.313	.000
	Within Groups	1.333	5	.267		
	Total	81.500	7			

Appendix 6 The ANOVA values of the heavy metals

		Sum of Squares	df	Mean Square	F	Sig.
Cu	Between Groups	1.235	2	.618	.534	.612
	Within Groups	6.940	6	1.157		
	Total	8.175	8			
Pb	Between Groups	6.902	2	3.451	1.194E3	.000
	Within Groups	.014	5	.003		
	Total	6.916	7			
Ni	Between Groups	.008	2	.004	.188	.834
	Within Groups	.109	5	.022		
	Total	.117	7			
Zn	Between Groups	36.925	2	18.462	.407	.683
	Within Groups	272.204	6	45.367		
	Total	309.129	8			