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ECOLOGICAL AND SYSTEMATIC ZOOLOGY STREAM

Determination of Bioaccumulation and Food Chain Contamination of Heavy Metals and Organochlorine Pesticides in Tilapia (*Oreochromis niloticus*) and Abyssinian Ground Hornbill (*Bucorvus abyssinicus*), Lake Ziway.

In Partial Fulfillment of the Requirements for the Degree of
Master of Science in Biology (Ecological and Systematic Zoology)

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Abstract

The study had been carried out between July 2009 and June 2010 on the food chain components of Lake Ziway. Bioaccumilation and biotransference of heavy metals and organochlorine pesticides were measured and determined in tilapia (*Oreochromis niloticus*) and Abyssinian ground hornbill (*Bucorvus abyssinicus*) to evaluate ecological hazard levels. Heavy metals were determined using flame atomic absorption spectroscopy after digestion of the samples under reflux on kjeldhal hot plate apparatus using HNO_3, $HCLO_4$ and H_2O_2. Sample extraction for organochlorine pesticides analysis was carried out in a Soxhlet apparatus using mixtures of *n*-hexane and acetone (4:1) and clean up was carried out using SPE Cartridge (C-18 column). Gas chromatograph equipped with mass-spectrometer (GC-MS) was used for the detection and quantification of the organochlorine pesticides. Heavy metals; Cu, Zn, Cd and Pb were investigated with mean concentrations of 1.13 mg/kg, 27.13 mg/kg, 0.35 mg/kg and 0.28 mg/kg in tilapia and 8.91 mg/kg, 139.08 mg/kg, 0.225 mg/kg and 0.286 mg/kg in Abyssinian ground hornbill muscle tissue samples. The detected organochlorine pesticides were dieldrin, DDT and the metabolites of DDT (DDD and DDE). DDT and DDD are below the detection limit in tilapia and detected with mean concentration of 0.19ng/g and 0.14ng/g in Abyssinian ground hornbill samples. Dieldrin and DDE were detected in mean concentrations, 4ng/g and 0.45ng/g in tilapia and 11.8ng/g and 12.61ng/g in Abyssinian ground hornbill samples. Zn and Cu indicated biotransference up the food chain with 5 folds and 7.9 folds, respectively while Cd and Pb showed lower concentration in the predator bird. DDE and dieldrin were detected in the bird sample with 28 and 3 folds higher than the amount detected in the fish muscle tissue, respectively. From the detected heavy metals and organochlorine pesticides Zn, Cu, dieldrin, DDT and its metabolites indicated positive biotransference through the food chain. The concentrations of the detected heavy metals and organochlorine pesticides were compared with threshold limits set by international organizations and the levels are within the accepted limits.

Key words; Organochlorine, Bioaccumulation, Contaminant, Biotransference

1 Introduction

1.1 Background

Human beings started discharging chemicals into the environment since early times and thus increased as a consequence of rising population and technological development. But the effects of these chemicals on the natural environment have been recognized very lately. The chemicals introduced into the environment are generally categorized as inorganic and organic pollutants (Walker, 2004).

The inorganic pollutants include mainly heavy metal like Ag, Al, As, Cd, Co, Cr, Cu, Fe, Hg, Pb and Zn. Metals are redistributed naturally in the environment by geogenic, anthropogenic and biological cycles. Throughout much of the human history, they have been used to make utensils and machinery. Mining and smelting supplied the resources. More recently, additional benefits have been incorporated in industry, agriculture and medicine. These activities have increased environmental levels and exposure, not only to metal related occupational workers, but also to consumers of the various products (Klassen, 2001; Hodgson, 2004).

Certain metals are indispensible parts of biomolecules and one-third of the enzymes need them for the catalytic activity in the body of organisms (Celechovska *et al.*, 2008). The human body needs approximately 70 friendly trace metals, but there are 12 poisonous heavy metals. These heavy and toxic metals are trace metals that are at least 5 times denser than water. They are stable (can not be metabolized by the body), but bioaccumilate. Most heavy metals have no natural biological functions to the body (e.g. Cadmium, Lead and Mercury) and can be highly toxic and their toxic effects disrupt the existing biological balance. The route of contamination can be via inhalation, ingestion and skin adsorption. Once they enter the body, they accumulate in tissues faster than the body's detoxification pathways can dispose them and eventually a gradual build up of the chemicals will occur when released to the ecosystem through natural and anthropogenic routes (Beiglbock *et al*., 2002; Fernades *et al.*, 2008). No matter how many beneficial health supplements or procedures one takes, overload of heavy metals will be a detrimental for the healing function of the body (Ekipo *et al.*, 2008).

Heavy metals from natural and anthropogenic sources continuously enter the aquatic ecosystem where they pose serious threats because of their toxicity, long persistence, bioaccumulation and biomagnification in the food chain. The main reasons for increasing concentration of metals in the environment include the use of inorganic fertilizers and pesticides. The accumulation of such metals may block biochemical processes in the soil and facilitate the entry of toxic metals into food chains (He *et al*., 2005). However, the link between metal exposure and effects in aquatic system remained poorly known for the past several decades because biological responses differ among species, metals, physicochemical conditions and exposure routes. For example, marine crustaceans appear more sensitive to metals accumulated from food rather than from their aqueous environment (Hook and Fisher, 2001a, b).

Compared to essential micro and macro-nutrient ions, heavy metals have more tendencies to accumulate in the tissues of animals (Azmat *et al*., 2006). Among the heavy metals, zinc and copper have known essential functions in the living system. However, exposure of organisms to high concentration of these results in significant accumulation with toxic effects. Extreme dietary exposure of zinc can cause gastrointestinal disease, pancreatic damage and anemia (Al-Weher, 2008). Like zinc, copper is essential macronutrient but little is known about its trophic transfer (Croteau *et al*., 2004). Copper occurs in food in many forms and combinations which affect its bioavailability. It is known to be essential at low concentrations, but it is toxic at high levels (Iwegbu, 2008).

Because of the long term and widespread use of lead, it is one of the most ubiquitous of the toxic metals. Sources of lead in the environment include; fuel additives, lead pigments in paints, batteries, pipes and glazed ceramic food containers. Inorganic lead may be adsorbed through the skin, the respiratory system and gastrointestinal tract.

Ingested inorganic lead is absorbed more efficiently from gastrointestinal tract of children than of adults (Hodgson, 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 (Hodgson, 2004; Kocak *et al*., 2005).

Like lead, cadmium is among the heavy and toxic metals persisting in the tissues of organisms. This metal occurs in nature primarily in association with lead and zinc ores. Industrially, Cd is used as a pigment in paints and plastics, in electroplating and in making alloys and alkali storage batteries (e.g. Nickel - Cadmium batteries). Environmental exposure of cadmium is mainly from contamination of ground water, smelting and industrial uses as well as the use of sewage sludge as a food crop fertilizer. Grains, cereals and leafy vegetables usually constitute the main sources of Cd in food (Hodgson, 2004). It is bioaccumilative when wastes washed in the aquatic environment accumulate in the aquatic biomass, concentrate and pass up the food chain (Wang, 2002). Cd toxicity affects many target tissues and organs such as brain, heart, blood vessels, kidney and lungs. The toxicity may cause anemia, dry and scaly skin, fatigue, hair loss, heart disease, depressed immune response, hypertension, joint pain, kidney stone, liver disfunction, loss of sense of smell and lung cancer (Kocak *et al.*, 2005). Long term exposure through ingestion by Japanese population of contaminated water and food with Cd was associated with a crippling condition, Itai - itai disease (Kimbrough *et al*., 1999).

The organic pollutants include in general pesticides, and particularly the environmentally stable ones as basic concerns. Pesticide is a term used for a broad range of chemicals, synthetic or natural, that serve to control insects, fungi, bacteria, weeds, nematodes, rodents and other pests (Izu-Iyawu *et al*., 2007). The outburst of technological development and the continuously increasing population have resulted in enlarged production of basic agricultural products whose production and provision is associated with chemicals of organic origin applicable in cultivation (Kasiotis, 2009). At present agricultural pesticides are widely used to control pests, diseases, and weeds which may affect the quality and yield of crops. The frequent and broad use of these chemicals has been resulted in the contamination of the environment leading to the presence of their residues in food (Jos´e *et al*., 2004).

The 1940's and 1950's saw the introduction of the chlorinated hydrocarbon insecticides such as DDT (Dichloro Diphenyl Trichloroethane), Methoxychlor, Chloridane, Heptachlor, Aldrin. Dieldrin, Endrin, Toxaphone, Mirex and Lindane. Especially DDT and HCH are effective pest control chemicals widely used in agriculture and public health activities such as malaria eradication worldwide for the past several decades despite their persistence in the environment (Hodgson, 2004; Darko *et al*., 2008).

Persistent organic pollutants (POPs) are compounds that resist photochemical, biological and chemical degradation and remain in the environment for decades (Hodgson, 2004; Kasiotis, 2009). Under the Stockholm Convention, persistence is determined by evidences of half life of the chemicals in water for greater than 2 months and in soil or sediment for greater than six months (UNEP, 2001).

The basic characteristics of POPs that make them prone to environmental hazards includes high chemical and biological stability, low water solubility and high lipid solubility; which makes them bioaccumilative in the tissues of organisms, semi-volatility; a character that enables them move long distance in the atmosphere before deposition and that they can occur in areas never exposed for these chemicals. They volatilize in warm temperature and condense at cooler temperature, reaching their highest concentration in the cooler regions of the world. In addition to these properties, POPs are toxic to the environment and biota including human beings (Sankar *et al*., 2006; Kasiotis, 2009).

One of the dominant ecological stresses on the lake ecosystem is the presence of organic toxic pollutants. Of particular concern are those halogenated organic toxicants that bioaccumulate in the food web, such as polychlorinated biphenyl (PCBs), organochlorine pesticides (OCPs); DDT, Dieldrin, Chlordane, Chlorinated dioxins and Furans (Erkman and Kolenkaya, 2006; Magali *et al*., 2008). Organochlorines are among the agrochemicals that have been used extensively for a long period. Among the pesticides: Lindane, Aldrin, Endosulphan. Dieldrin and DDT have been used widely in agriculture as well as in mosquito, termite and tse - tse fly control programs (Kasozi *et al*., 2006).

Sources of contamination of the aquatic environment includes, direct spraying, drift, atmospheric transport, agricultural and residential run off, individual misuse and improper disposal (Jones *et al*., 2009). In addition to these, obsolete pesticides are also sources of contamination. FAO described obsolete pesticides as those that can no longer be used and therefore requires disposal. These include banned, expired, unlabeled, and/or unidentified pesticides in all forms. Such pesticides are major public health and environmental concerns as many of these pesticides are toxic, stored in open or inappropriate stores and leaking or corroded containers, thus posing serious hazards on organisms and the environment.

Environmental occurrence of persistent OCPs are a global rather than a regional problem, because chlorinated pesticides used in tropical regions will be carried by long range atmospheric transport and ultimately end up in polar and environmentally pristine regions and certainly have impact on the ecosystem (Bordajandi *et al*., 2003; Kasozi *et al*., 2006). In 2001, the Stockholm Convention on persistent organic pollutants has acknowledged persistent organic pollutants as a global problem (UNEP, 2001).

In the Stockholm Convention, 12 persistent organic pollutants were detected as the “Dirty Dozens”. Among these chemicals, eight of them are organochlorine pesticides; Aldrin, Chloridane, DDT, Dieldrin, Endrin, Heptachlor, Mirex and Toxaphene (UNEP, 2001; Bordajandi *et al*., 2003). These pesticides have been used extensively worldwide in the early 1950's. However, due to their persistence in the environment, most of them are no longer allowed to be in use in many developed countries. Despite the restriction and ban in developed countries they are still produced and used in developing countries to a large extent. Since these countries still allow their use in agriculture and public health programs, these pesticides have led to contamination of the environment and the food chain (Sankar *et al*., 2006).

Due to their resistance to degradation, persistent organic pollutants are not metabolized and, therefore, when an organism containing a pollutant is consumed, the pollutant is simply passed on the predator and accumulates in the fatty tissues since they are lipophilic (Yelena *et al*., 2005; Bentzen *et al*., 2008). By consuming many prey species, an organism may build up very high concentration of the pollutant in its tissues. This process may continue up the food chain leaving the top predators with very high and sometimes lethal concentrations of the pollutants. Their accumulation in the food chain as such or as their metabolites makes OCPs ubiquitous and causing concern on the animals at the top of food chains (Tursov *et al*., 2002; Barwick and Maher, 2003; Croteau *et al*., 2005).

However, contamination exposure may occur directly through ingestion, inhalation, or dermal exposure, significant contamination can also occur through food chain transport. Species not normally in direct contact with contaminated media may become exposed through ingestion of contaminated prey, promoting accumulation or magnification of contaminants into higher trophic levels. Studies on earth worm in soils contaminated with organochlorines and heavy metals can accumulate quantities of contaminants known to be deleterious to sensitive species (Bracher and Bider, 1982). Oysters also can concentrate DDT from 0.001 ppm in sea water to 700 ppm in their bodies. In general, organisms can bioaccumulate to this extent through feeding relations with different components of the food web, even though the concentration of the pollutant is less in the aquatic environment (Winter, 1992; Croteau *et al*., 2004).

The potential exposure of predatory species may be enhanced by the altered behavior of contaminant - exposed prey. Affected prey may be easier to catch, leading predators to concentrate their foraging efforts on contaminated sites and thus increasing their direct exposure and the transfer of contaminants through trophic levels. As contaminants move through food chains, they may be translocated from the sources and migratory individuals may transport them to considerable distances, resulting in potential exposure and effects in organisms that otherwise would not be in contact with the contaminated sites (Braestrup *et al*., 1974).

Different researches have shown that persistent chemicals have the ability to bioaccumulate in organisms directly from the water, and bioaccumilated and biomagnified up in the food chain, causing organisms higher in the food chain becoming contaminated with higher concentrations of the chemicals than their prey. So organochlorine pesticides and heavy metals are said to be bioaccumilative since, organisms retain in their bodies at a concentration higher than in their food and water that originally contain them. When predators higher in the food chain consume prey contaminated with such chemicals, this will lead to a very high concentration of contaminants in their bodies. Subsequently they pose a risk of causing adverse effect to human health and the environment (Morrison *et al*., 1996; Hargrave *et al*., 2000, Lee *et al*., 2000).

Bioconcentration is the intake of chemical contaminants through an organisms' epithelial tissues or gills, and subsequent concentrations of that chemical contaminants in the organisms' tissues to a level that exceeds ambient environmental concentrations. The process by which chemical contaminants are concentrated at levels that increases with each step in a food chain is bioaccumulation and, biomagnification is the accumulation of chemical contaminants in the tissues of organisms that exceed chemical equilibrium from dietary absorption of chemicals (Gobias *et al*., 1999).

Bioconcentration, bioaccumulation and biomagnification of chemical contaminants, are dynamic processes that involve many interconnected variables. For example, the potential of a chemical to bioconcentrate, bioaccumilate or biomagnify in organisms and food webs is dependent upon the properties of the chemicals (e.g. hydrophobicity, lipophilocity and resistance to degradation), Environmental factors (such as salinity, temperature, concentration of other organic chemicals and redox potential), biotic factors (e.g. the organisms mode of feeding, trophic position and lipid concentration), and metabolism and bioavailability (such as current chemical input, transport mechanism and degree of contamination) play a big role (Gobias *et al*., 1999; Lee *et al*., 2000; Shin and Lam, 2001).

The transfer of trace metals and persistent organic pollutants from a food source to consumers takes through a process of biotransference. Biomagnification in this case occurs when increases in concentration of trace metals and organic pollutants are observed at least in two trophic levels in a food chain. Elevated pollutant concentrations in higher trophic group organisms could pose a threat to the organisms themselves or to human consumers. Low concentration of the pollutants in water column and increased level in sediments indicates trophic transfer as the main route of accumulation (Barwick and Maher, 2003; Lerman and Hull, 2007). So diet, through the food chain, plays a significant role in the bioaccumulation ant biomagnifications of the pollutants (Bordajandi *et al*., 2003).

Among the Organochlorine pesticides, Dichloro Diphenyl Trichloroethane (DDT), has been the most widely used one in agriculture and health sectors for several decades and it is considered as a priority pesticide. It is one of the most controversial pesticides of all the time and among the 12 persistent organic pollutants that are restricted and targeted for ultimate elimination under the international treaty on POPs (UNEP, 2001; Ssebugere *et al*., 2009).

Although DDT was synthesized in 1874, its insecticidinal property was not noticed until 1939, when Dr. Paul Muller, a Swiss chemist, discovered its effectiveness as an insecticide. During World War II, the United States used large quantities of DDT to control vector born diseases, such as typhus and malaria to which U.S troops were exposed. After the war, DDT use became widespread in agriculture, public health and households (Hodgson, 2004).

Like other OCPs, DDT is characterized by high persistence, low polarity, low aqueous solubility and high lipophilocity, and as a result it has a potential to bioaccumilate in fatty tissues. Its persistence initially considered as a desirable attribute, latter becomes the basic concern for public health. The publication by Rachel Carson, 'The Silent Spring' in 1962 stimulated this concern and eventually led to the ban of DDT and other chlorinated pesticides in different countries. Though complete ban on DDT was imposed in many developed countries, it was banned either only for agricultural use in countries like India, but still allowed for malaria control by the WHO program (Rajendran *et al.*, 2005). From an African perspective, this might be understandable, since malaria still is a tremendous problem killing one child every 30 seconds in sub Saharan Africa (Hileman, 2006).

The toxic property of organochlorine pesticides result in adverse effect on the immune and respiratory systems and critical organs, hormones systems disfunction, damage of the reproductory system, sex linked disorders, developmental disorders, carcinogenic effects and shortened lactation periods in nursing mammals. Chronic exposure to these chemicals can also result in death in organisms. Consequently exposure to large quantities can have adverse effect on the entire population (Bowman *et al.*, 2006; Grandjean and Landrigan, 2006).

DDT has been implicated in a broad range of adverse human health and environmental effects including reproductive failure, endocrine disruption and egg shell thinning, and hence poor reproductive success resulting from bioacccumilative property (Beard, 2005; Turusov *et al.*, 2002). DDT and some of its metabolites are potentially carcinogenic and are known to be environmentally estrogens; they mimic hormones binding to and activateing the estrogen and androgen receptors, thereby often producing estrogen - like effects (Robinson *et al*., 1985).

Like DDT, dieldrin is among the POPs considered as persistent in the convention. It is a highly effective insecticide for soil dwelling pests and for the protection of wooden structures against termites and wood borers. Although the use is severely banned in the 1970's, it is still used in termite control in some countries (Hallegue *et al*., 2003). Animal studies demonstrated that dieldrin can cause a wide variety of health problems such as; neurological defects, immune system disfunction and multiplying the risk of carcinogenic processes (Hoffman, 1996). During the past several decades, increase of testicular anomalies including testicular cancer (Adami, 1994) and a defect in human semen have received considerable attention and studies revealed the potential toxic effect of dieldrin on the male and female genetalia (Auger *et al*., 1995; Hallegue *et al*., 2003). The pesticide passes through the blood/testis barrier and affects both the production and maturation of sperm in the epididymis. The study observed in adult rat assured that exposure to dieldrin impairs both gametogenesis and steroidogenesis (Hallegue *et al*., 2003). In addition to this study, towards the end of 1960's, the substantial decline in the population of a number of avian species due to a decrease in the reproduction success was shown to be a big concern and the effect of the contaminant accumulation and toxicity has been studied (Blues *et al*., 1979).

African countries are using pesticides before 50 years for combating agricultural pests and controlling disease vectors, especially malaria. The pesticide use may still be small representing only 2 – 4 % of the total amount used globally. But, the continent has accumulated an estimate of 50,000 tons of obsolete pesticide stocks over a period of 4 decades. According to the FAO report, some African countries including Ethiopia have more than 1000 tons of obsolete pesticide stocks being sold in open market (FAO, 1992; Hodgson, 2004).

However, chemical pesticide use in Ethiopia was historically low, recent developments in increased food production and expansion in floriculture industry has resulted in higher consumption of chemical pesticides. Recently, Ethiopia has been considered as having the largest accumulations of obsolete pesticides in the whole of Africa. It was estimated that there were 402 stores at 250 sites containing 1,500 tons of obsolete pesticides (Asferachew Abate and Tadesse Amera, 2008). This estimate does not include the massive but unquantifiable amounts of pesticides soaked in soils, in water bodies, contaminated building materials, pallets, shipping containers and other miscellaneous items.

The Ethiopian Obsolete Pesticides Disposal Project, a project that mainly aimed at removing obsolete pesticides has been operational in Ethiopia for the last few years. However, it is obvious that as the obsolete pesticides are removed, new pesticides are imported and are possibly contributing to further accumulation. While pesticides have increased agricultural production and improved public health, evidences in the last few decades have shown that they could also be detrimental to human health and the ecosystem (Asferachew Abate and Tadesse Amera, 2008).

In environmental studies, certain organisms provide valuable information about chemical states of their environment not through their absence or presence but ability to concentrate environmental toxins within their tissues. Since fish spend their entire lives in the aquatic environment and are often found at higher feeding levels of the aquatic food chain, they incorporate chemicals from the environment into their body tissues through feeding relationships. The result from fish tissue analysis is widely used as an important indicator of contamination of aquatic environments and their consecutive food chains (Tenora *et al*., 2000; Sures, 2001; Ucman *et al*., 2001; Hodgson, 2004).

However, environmental fate of persistent residues persistent chemicals is an issue that at present receiving considerable attention due to international residue limit requirements in food, drinking water supplies as well as export products (Westborn *et al*., 2008), studies on the effect of the residues on different species in Ethiopia are nonexistent. The main aim of the present study is aimed to assess the size and effect of accumulated chemicals in the food chain by considering fish and fish eater birds of one of the Ethiopian lakes suspected to be vulnerable for pollution.

The fish selected for this study, Nile Tilapia (*Oreochromis niloticus)* is distributed in the tropical and sub-tropical Africa and Middle East. It primarily occurs in fresh water habitats, but, due to its tolerance for low oxygen level and salinity, can occur in estuarine (Njiru *et al*., 2004). *Oreochromis niloticus* is selected in this study to determine bioaccumulation and biomagnifcation of heavy and toxic metals and organochlorine pesticides due to:

- Its feeding ecology; it is an omnivorous species able to utilize food sources of various trophic levels with preference to phytoplankton, zooplanktons, bacteria and organic materials (detritus) at different degree of decomposition (Setlikova and Adamek, 2004). Thus, the feeding ecology contributes for the possibility of contamination and can serve as an indicator of aquatic environment level of contamination as well an ideal transport route for the contamination of terrestrial consumers.
- Its abundance in the lake; *Oreochromis niloticus* accounts more than 90 % of fish production in Lake Ziway (Huib and Herco, 2006) and it currently forms the third commercially important species next to *Lates niloticus* and *Obola argentea* (Njiru *et al.,* 2004; Setlikova and Adamek, 2004). This enhances the possibility of contamination of organisms higher in the food chain.

- Provide chief sources of food and livelihood for the people living in the vicinity of the lake. Many communities living around the rivers and lakes depend on fishery as a source of livelihood (Josephine *et al*., 2006). So the degree of vulnerability of the community is expected to be high to the contaminants that the present findings will initiate further studies by extending the food chain levels and by giving emphasis to human consumer as part of the ecological component.

The other component of the food chain under study is a fish eater bird, Abyssinian ground hornbill (*Bucorvus abyssinicus*) is a large bird found in Sub-Saharan Africa distributed along the dry savannah, steppe and woodland habitats. It has a varied diet including small animals. This fish eating bird is selected for the study due to its predatory habit on tilapia. The short tongue of the hornbill disables it from manipulating the prey. It sends the prey to the throat and then ingests the whole part (Kimberly *et al*., 2002). This provides it with ingestion of all parts of the fish and magnifies well the accumulation.

Using the above given components of the food chain, the study dealt with heavy and toxic metals (Zn, Cu, Pb, and Cd) and Organochlorine pesticides (Dieldrin, DDT and its metabolites) detected in the study area.

2. Objectives

2.1 General Objective:

- Analyze the effect of heavy and toxic metals and persistent organochlorine pesticides on the food chain of Lake Ziway.

2.2 Specific Objectives:

- to measure bioaccumulation of heavy and toxic metals and organochlorine pesticides (from the muscle tissues of Tilapia (*Oreochromis niloticus*) and Abyssinian ground hornbill (*Bucorvus abyssinicus*) samples taken from Lake Ziway;

- to identify the heavy metals and Organochlorine pesticides that can biotransfer to Abyssinian ground hornbill (*Bucorvus abyssinicus*) samples taken from Lake Ziway;

- assess the concentration of heavy metals and organochlorine pesticides from the tissues of Tilapia and Abyssinian ground hornbill samples taken from Lake Ziway by comparing with the threshold levels if it poses threat to the resident organisms and human consumer.

3. Materials and Methods

3.1 Description of the Study Area

Lake Ziway is one of the freshwater Rift Valley lakes of Ethiopia. It is located about 160 km South of Addis Ababa. The catchments of the lake are indicated in figure 1.

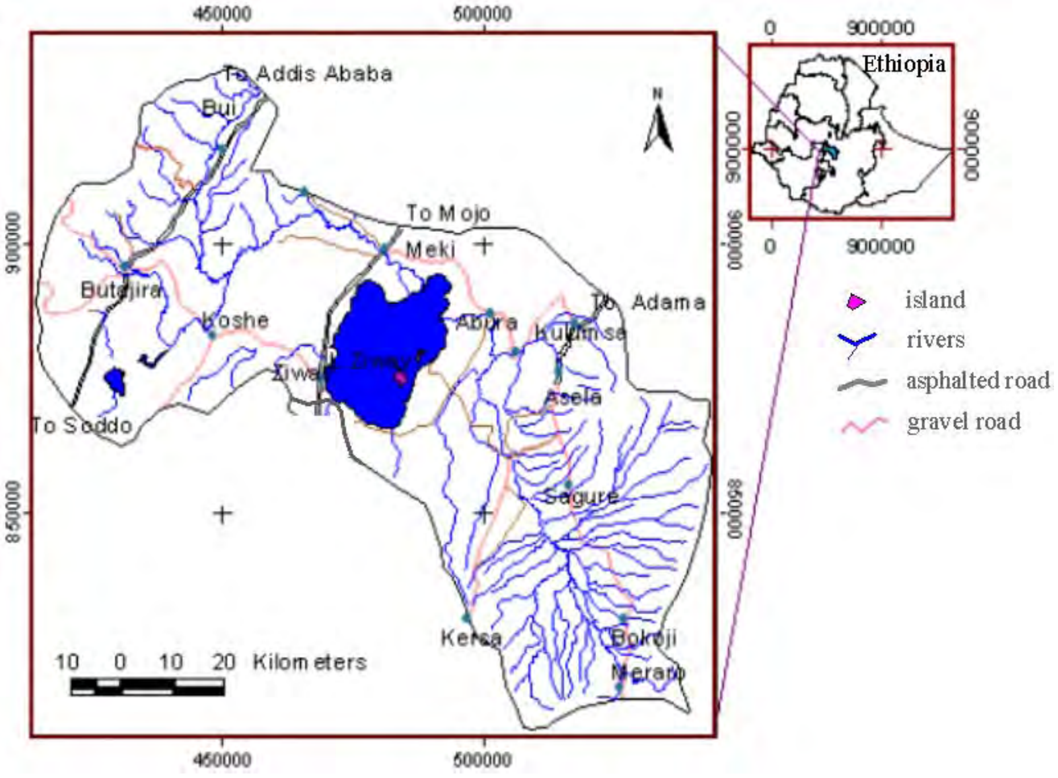


Figure 1. Catchment area of Lake Ziway

The lake is 25 km long and 20 km wide, with a surface area of 434 km^2. It has a maximum depth of 4 m and an elevation of 1,846 m (Awoke Kebede and Taddese Wondimu, 2004). The lake has five islands and fed primarily by two rivers, Meki and Katar, but does not always have an outflow, although in some years it drains into Lake Abijatta (Blundell, 2001).

Lake Ziway is known for its population of birds and hippopotamus. In addition, it supports a fishing industry mainly Nile tilapia (*Oreochromis niloticus*), which represents 90 % of the landings in the Lake as in many other Ethiopian lakes. Based on an average area of 434 km^2, Lake Ziway could produce 2250 tons of fish on annual basis (Huib and Herco, 2006).

Recently, large - scale foreign and national horticulture and floriculture enterprises have been settling down in the area due to suitable climatic and socio - economic conditions. These enterprises claim part of the available water resources in the area in addition to smallholder agriculture, domestic water use and fishery. This has resulted in lower lake water levels and increased the salinity and alkalinity level. In addition, since the floriculture and horticulture need more fertilizers, insecticides and herbicides, nutrient enrichment has been observed in the lake (Huib and Herco, 2006).

Due to the geohydrological conditions in the area, groundwater may contain trace elements that are hazardous to horticulture and floriculture. Another potential risk of irrigation with groundwater is the elevated concentration of bicarbonate, which may cause problems with respect to the soil structure and permeability. It is noted that hazardous concentrations of Arsenic and Boron could be locally present in the groundwater (Haile Gashaw, 1999**;** Berhanu Erco *et al.*, 2006).

There is also evidence for spraying of insecticides like DDT, Hexachlorohexanes (HCH) and Dieldrin against the vectors of malaria in order to control the epidemy. The use of persistent chemicals for such purposes is suspected to increase the accumulation in the lake (Yihenew Alemu, 2007).

3.2 Sample Collection

Fifteen Tilapia (*Oreochromis niloticus*) and three Abyssinian ground hornbill (*Bucorvus abyssinicus*) specimens were collected. Fish samples were collected from different parts of the lake by the local fishermen and bird samples were collected using nets. The fish specimens were washed with the lake water prior to dissection. Then, were filleted separately using stainless steel and the muscle selected only for organochlorine analyses were wrapped with aluminum foil. The fillets, taken for metal analysis were wrapped with polyethylene sheets. The specimens of the bird were dissected separately and muscles from different parts were taken and wrapped in the same manner as that of the fish sample. Finally, the samples were kept in an ice box during transportation to the analytical laboratory of the Department of Chemistry, Addis Ababa University and kept under -20^{o}C until used.

3.3 Chemicals and Apparatus

3.3.1 Chemicals

To carry out the experiment; HNO_3 (69-72 %), $HCLO_4$ (70 %), H_2O_2 (30 %), acetone, *n*-hexane, sodium sulphate anhydrous, methanol and standards of organochlorine pesticides (Dieldrin, DDT, DDE and DDD) and Heavy metal (Cu, Zn, Cd and Pb) were used.

3.3.2 Apparatus

For sample preparation and analysis of heavy metals and organochlorine pesticides; blender, mortar and pestle, digital analytical balance, oven, round bottom flasks, water bath, vials, volumetric flasks, Soxhlet extractor, Kjeldahl apparatus hot plate, SPE cartridge (C-18 column), rotary evaporator, atomic absorption spectroscopy (AAS) and Gas chromatograph-mass spectrometer (GC-MS) were used.

3.4 Sample Preparation

Sample preparation was necessary for most samples and remained one of the major time consuming steps in most analysis. It can be as simple as dissolution of a desired weight of sample in a solvent prior to analysis and complicated utilizing appropriate instrumental techniques.

3.4.1 Sample Preparation for Heavy Metal Analysis

3.4.1.1 Homogenization of Tissue Sample

First, the different specimens of the tissues of tilapia and Abyssinian ground hornbill were homogenized separately using blender until mixed well. Then, the tissue was allowed to dry in an oven at 150°C till it reached constant weight. Using mortar and pestle, the dried samples were ground into free flowing powder.

3.4.1.2 Sample Digestion

The digestion process used perchloric acid (70 %), nitric acid (69-72 %) and hydrogen peroxide (30 %) and was done with some modifications of the method used by Al-Weher (2008). Using these reagents and 1 g of dried and powdered muscle tissues of *Oreochromis niloticus* and *Bucorvus abyssinicus* samples, different methods were tested. Optimizing of the digestion procedures involved some changes of parameters such as reagent volume, digestion temperature and digestion time. Accordingly, procedures were tested for digestion of the samples as indicated in Table 2.

Applying the optimized procedure, 1 g of dried and powdered tissue was weighted and transferred into a 100 ml round bottom flask and 9 ml of freshly prepared (69-72 %) nitric acid- (70 %) perchloric acid (2:1) was added. After 10 minutes, 1 ml of 30% H_2O_2 was added to the tilapia sample and 2 ml to the Abyssinian ground hornbill muscle tissue sample. Then the samples were digested in a reflux on kjeldahl digestion apparatus by setting the temperature first at 150^{o}C for 10 minutes and then increased to 300^{o}C. Sample digestion took a total of 3.30 hrs for tilapia and 4 hrs for Abyssinian ground hornbill samples. After digestion was over, the samples were allowed to cool for 30 min, filtered and transferred into 25 ml volumetric flasks and made up to volume with deionized water. Triplicate digestions were carried out for each sample and the digested and diluted samples were kept in a refrigerator until the level of the heavy metals in the sample solutions were determined by flame atomic absorption spectroscopy (FAAS). The blank solutions were prepared by digesting the mixture of reagents following the same digestion procedure and diluted to 25 ml with deionized water.

3.4.2 Sample Preparation for Organochlorine Analysis

The analytical procedures used for the determination of organochlorine pesticides in the environmental samples typically involves a number of equally relevant steps for sampling, sample preparation, separation and detection of target compounds, identification, quantification and data handling.

3.4.2.1 Homogenization and Preparation for Extraction

Tissue samples were first mixed well using blender, and then 10 g of homogenized tissue was taken and ground with 30 g of Na_2SO_4 anhydrous using mortar and pestle in order to reduce the water content and help to open up the tissue structures which enable good penetration of solvent into sample matrix. Then the homogenized sample was transferred into extraction thimble.

3.4.2.2 Sample Extraction

Following Erkman and Kolenkaya (2006), the sample contained in the thimble was taken into a soxhlet extractor. A 200 ml of solvent *n*-hexane and acetone (4:1) was used in a 500 ml round bottom flask and kept in a water bath at 45^{o}C for 10 hrs. After that, the extract was filtered, cooled and concentrated to 2 ml using rotary evaporator at 30^{o}C.

3.4.2.3 Sample Clean Up

Clean up was carried out according to the procedure described in Darko *et al*., (2008). The extract was cleaned in a pre-conditioned SPE Cartridge (C-18 column). The conditioning steps were, first the column was washed with 5 ml of methanol, then rinsed with 5 ml of acetone and finally with 5 ml of deionized water. After conditioning of the column, the extract was allowed to pass through at a flow rate of 1ml/min. After that, the stationary phase was washed with 1 ml of 30 % methanol followed by 1 ml of deionized water and left to dry for 15 min. The pesticide residue adsorbed in the cartridge was rinsed five times with 0.5 ml of *n*-hexane. Finally, the solvent was evaporated from the solution until it dried and the residue was dissolved with 1 ml of *n*-hexane and kept in a refrigerator until GC-MS analysis.

3.5 Instrumentation

3.5.1 Atomic Absorption Analysis Conditions

For the analysis of the heavy metals (Cu, Zn, Cd and Pb), Buck Scientific Model 210VGP (East Norwalk, USA) Atomic Absorption Spectrophotometer was used. The instrument was used with the operating conditions indicated in Table 1. Calibration of the instrument was carried out with ranges of standard solutions prepared from 1000gm/L stock solutions for Cu, Zn, Cd and Pb. After calibration, the sample solutions were aspirated into the AAS instrument and direct readings of the metal concentrations were recorded. Three replicate determinations were carried out on each sample. The same analytical procedure was employed in the determination of the heavy metals in each blank solution.

Table 1 Instrumental operating condition for the determination of heavy metals in *Oreochromis niloticus* and *Bucorvus abyssinicus* muscle tissue samples

Heavy metal	**Wavelength (nm)**	**Detection limit (mg/L)**	**Slit width (nm)**	**Lamp current (mA)**
Cu	324.8	0.04	0.7	2.0
Zn	213.9	0.01	0.7	2.0
Pb	283.2	0.12	0.7	3.0
Cd	228.9	0.014	0.7	2.0

3.5.2 Chromatographic Separation Conditions

Organochlorine pesticides were analyzed using an Agliant 6890 Gas chromatograph (GC) equipped with an Agliant 5975 inter mass selective network system (MS). The GC-MS analysis was carried out by adjusting the oven and detector temperature at 120^{o}C and 280^{o}C. The injector temperature was 230^{o}C for 7 min, 240^{o}C for 2 min and reached a final temperature of 285^{o}C. The sample volume used was 2 µL with split less inlet mode and 33 min solvent delay time. The instrument was calibrated using standard solutions of dieldrin, DDT, DDD and DDE. After calibration of the instrument, 2 µL of samples were injected and the concentrations were indicated with peak areas of the chromatograms. Triplicate determinations were carried out for both tilapia and Abyssinian ground hornbill samples. The same procedure was employed for the blank and spiked sample analysis.

3.6 Determination of Method Validity

In the analysis of heavy metals and organochlorine pesticides on tilapia and Abyssinian ground hornbill samples, reference materials, blanks and spikes were included. Blank analysis was carried out in order to check interference from instruments. Individual reference standards of heavy metals (Cu, Zn, Pb and Cd) and organochlorine pesticides (dieldrin, DDT, DDD and DDE) were used to identify and quantify the levels of residues. Arithmetic means and standard deviations were calculated from positive quantifiable samples only.

3.6.1 Method Detection Limit

For the determination of method detection limits, blank solutions were prepared. For metal analysis, reagents were mixed and digested with the optimum procedure, cooled, filtered and diluted to 25 ml using deionized water. After digestion of triplicate blank solutions, triplicate readings were obtained. For organochlorine analysis, 30 gm of sodium sulphate anhydrous was extracted in a Soxhlet apparatus, cleaned and determined using GC-MS as the original samples. Then the method detection limit of each heavy metal and organochlorine pesticide was calculated as three times the standard deviation of the concentrations detected in the blank samples.

3.6.2 Recovery Analysis

3.6.2.1 Recovery Analysis for Heavy Metals

Spiking experiments in which known volume and concentration of standard solutions were employed on the samples in order to determine recovery. From the stock solution (1000 mg/L), intermediate standard solutions (10 mg/L) were prepared for heavy metals (Cu, Zn, Cd and Pb) by diluting with deionized water. 6.5 ml of Cu, 30 ml of Zn, 6.5 ml of Cd and 6.5 ml of Pb from 10 mg/L solutions were added to 1 g of muscle tissue of tilapia. The same amount of solutions were added to 1 gm of Abyssinian ground hornbill samples except increased volume of Zn (35 ml) used. Then triplicate samples were digested with the optimized procedures and diluted to 25 ml with deionized water. The spiked samples were analyzed with the same procedure as used for original samples and triplicate readings were recorded and mean concentration and relative standard deviations were calculated.

3.6.2.2 Recovery Analysis for Organochlorine Pesticides

Spiking of known concentration of organochlorine pesticides were carried out for recovery determination. First 100 mg/L solution was prepared and further diluted to 1mg/L using *n*-hexane. Then 1 ml of DDT, DDE and DDD and 0.5 ml of dieldrin standard solutions were taken and spiked on 10 g of tilapia and Abyssinian ground hornbill samples. After spiking; extraction, concentration, clean up and GC-MS analysis followed the same procedure used for the original samples and the results of recoveries were calculated from triplicate concentration.

4. Result

Procedures were tested for sample digestion and the results of digested samples after cooling and filtration are indicated under Table 2.

Table 2 Procedures tested during optimization of methods for digestion of *Oreochromis niloticus* and *Bucorvus abyssinicus* muscle tissue samples

No	Sample size	Reagent added			Intial Temp.	Final Temp.	Digestion Time	Color of the digested sample
		HNO_3 (67-72 %)	$HCLO_4$ (70 %)	H_2O_2 (30 %)				
1	1 g	8 ml	2 ml	1.5 ml	150^oC	300^oC	3.00 hr	Clear and yellow
2	1 g	8 ml	3 ml	1.5 ml	150^oC	300^oC	3.00 hr	Clear and pale yellow
3	1 g	7 ml	3 ml	1.5 ml	150^oC	300^oC	3.00 hr	Clear and slightly pale yellow
4	1 g	6 ml	3 ml	1.5 ml	150^oC	300^oC	3.00 hr	Clear & colorless
5	1 g	5 ml	2 ml	1.5 ml	150^oC	300^oC	3.00 hr	Clear and yellow
6	1 g	6 ml	3 ml	1 ml	150^oC	300^oC	3.00 hr	Clear and almost colorless
7	1 g	6 ml	3 ml	1 ml	150^oC	300^oC	3.5 hr	Clear and colorless
8	1 g	5 ml	2 ml	1 ml	150^oC	350^oC	3 hr	Clear and pale yellow
9	1 g	6 ml	3 ml	1 ml	150^oC	300^oC	4.00 hr	Clear and colorless
10	1 g	6 ml	3 ml	1 ml	150^oC	270^oC	4.00 hr	Clear and slightly yellow
11	1 g	5 ml	2 ml	1 ml	150^oC	300^oC	4.00 hr	Clear and pale yellow
12	1 g	5 ml	2 ml	1.5 ml	150^oC	300^oC	4.00 hr	Clear and slightly pale yellow

13	1 g	6 ml	3 ml	2 ml	150^{o}C	300^{o}C	2.00 hr	Clear and nearly yellow
14	1 g	6 ml	3 ml	2 ml	150^{o}C	300^{o}C	3.00 hr	Clear and colorless
15	1 g	6 ml	3 ml	2 ml	150^{o}C	3.50^{o}C	3.00 hr	Clear and colorless
16	1 g	6 ml	3 ml	2 ml	150^{o}C	300^{o}C	4.00 hr	Clear and Colorless (for the Bird sample)

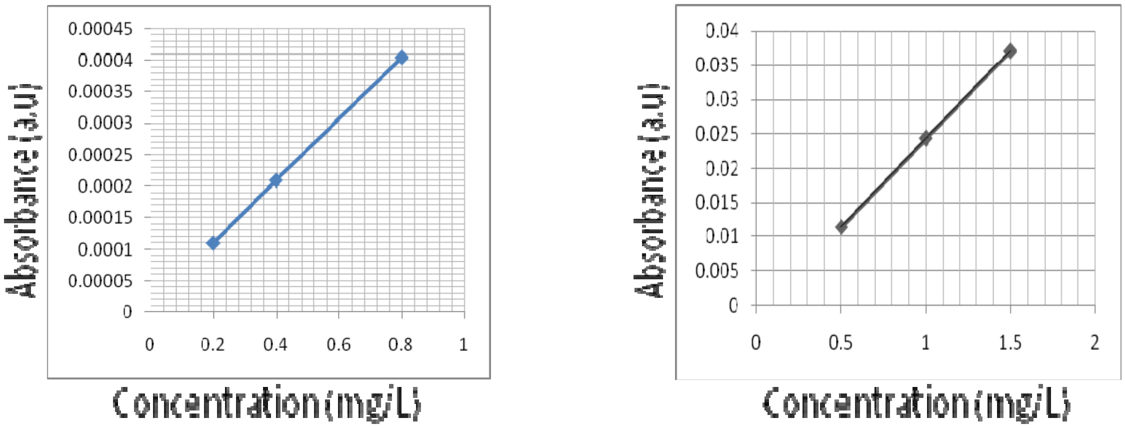
From the tested procedures, the digestion procedure that underwent complete digestion, produced clear and colorless solution, consumed minimal reagent volumes and shorter digestion time was selected. Accordingly, the procedure used 6 ml of HNO_3 (69-72 %), 3 ml of $HCLO_4$ (70 %) and 1 ml of H_2O_2 (30 %) with initial temperature of 150^{o}C and final temperature of 300^{o}C for 3.5 hrs was selected as optimum procedure for the digestion of tilapia samples and with a change only in the volume of H_2O_2 (2 ml) and additional 30 min (a total of 4 hrs) for the Abyssinian ground hornbill samples digestion.

The ranges of concentrations of standard solutions used for heavy metals Cu, Zn, Cd and Pb and the corresponding linearity of the calibration graphs are indicated under Table 3.

Table 3 Concentration ranges of standard solutions and linearity of calibration for the determination of heavy metals

Heavy metal	**Range of standards (mg/L)**	**Linearity**
Zn	0.2, 0.4, 0.6, 0.8	0.9665
Cu	0.5, 1, 1.5	0.9996
Pb	1.2. 2.4, 4.8	0.9999
Cd	0.25, 0.5, 1	0.9999

Calibration graphs of the heavy metals under investigation (Cu, Zn, Cd and Pb) were constructed from the concentration of standard solutions and their respective absorbance as indicated in Figure 2 (a-d).



A = 0.0005375C + 1.3712E^{-5}

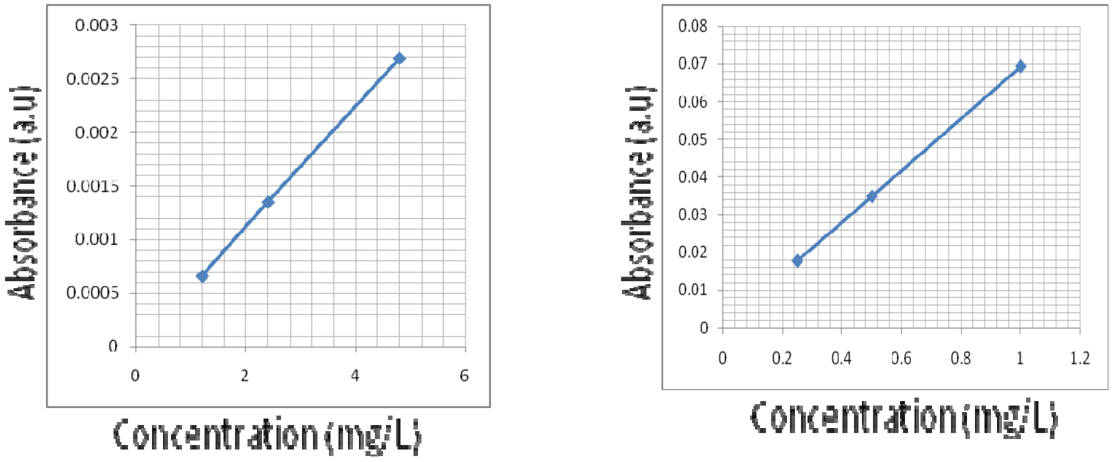
$R^2 = 0.9665$

2a. Zn standard solutions

A = 0.02564C - 8.48^{-5}

$R^2 = 0.9996$

2b. Cu standard solutions



A = 0.0005642C + 4.0208E^{-6}

$R^2 = 0.9999$

2c. Pb standard solutions

A = 0.0688C + 1.65287E^{-4}

$R^2 = 0.9999$

2d. Cd standard solutions

Figure 2**. Calibration graphs of the analyzed heavy metal standard solutions**

Analysis of residues of heavy metals (Cu, Zn, Cd and Pb) in Lake Ziway on tilapia and Abyssinian ground hornbill showed detection of all of the metals in the muscle tissues with mean concentrations of 1.13 mg/kg, 27.13 mg/kg, 0.248 mg/kg and 0.35 mg/kg in tilapia and 8.91 mg/kg, 139.1 mg/kg, 0.225 mg/kg and 0.286 mg/kg in Abyssinian ground hornbill samples as summarized in Table 4. Each measured results are average of three samples analyzed three times each (n =9) at 95% confidence limit.

Table 4. Mean and standard deviation of detected heavy metals (mg/kg) in *Oreochromis niloticus* and *Bucorvus abyssinicus* muscle tissue samples

Samples	Heavy metals			
	Cu	Zn	Pb	Cd
Oreochromis niloticus	1.13 ± 0.02	27.13 ± 0.11	0.248 ± 0.0115	0.35 ± 0.016
Bucorvus abyssinicus	8.91 ± 0.04	139.1 ± 0.61	0. 225 ± 0.014	0.286 ± 0.017

As indicated in Table 4, the analyzed heavy metal concentration decreased in the sequence of Zn>Cu> Cd>Pb in the samples of tilapia and Abyssinian ground hornbill.

The concentrations of heavy metals (Cu, Zn, Cd and Pb) in the digested and diluted blank samples were detected using AAS and limits of detection for the developed procedure were calculated as three times the standard deviation of the blank solutions obtained from three readings for each replicate. The values are summarized in Table 5.

Table 5.Method detection limits for heavy metals

Heavy metal	Cu	Zn	Pb	Cd
Method detection (mg/L)	0.04	0.01	0.12	0.014

The efficiency of the optimized procedure was evaluated by analyzing the digests of spiked muscle tissues of both *Oreochromis niloticus* and *Bucorvus abyssinicus* samples. The percentage recoveries of the detected heavy metals ranged from 92.4 % to 102.1 % for tilapia and 91.09 % to 106.4 % for Abyssinian ground hornbill samples with relative standard deviations (% RSD) less than 10 % with 95 % confidence limit as indicated in Table 6.

Table 6. Percentage recovery (Mean and RSD) of detected heavy metals in the muscle tissues of *Oreochromis niloticus* and *Bucorvus abyssinicus*

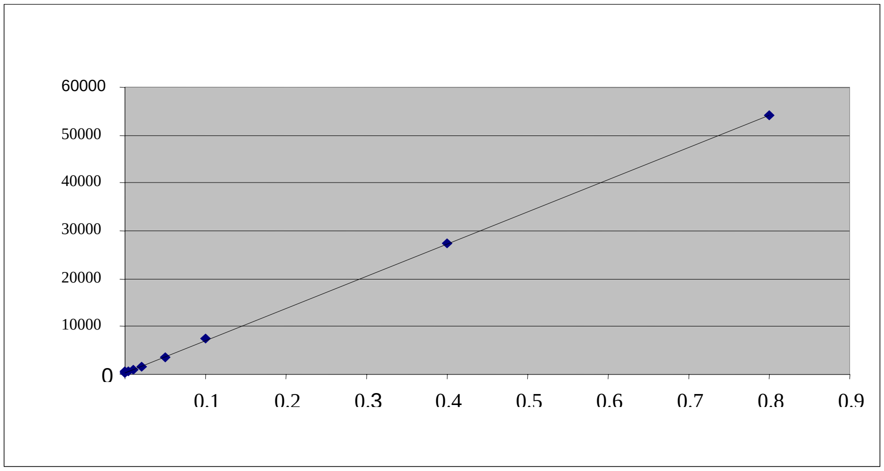
Heavy metal	Sample	Spiked concentration (mg/kg)	Recovery (%) ± %RSD
Cu	*Oreochromis niloticus*	0.26	99.6 ± 1.82
	Bucorvus abyssinicus	0.26	106.4 ± 4.22
Zn	*Oreochromis niloticus*	1.2	102.1 ± 0.41
	Bucorvus abyssinicus	1.4	104 ± 0.37
Pb	*Oreochromis niloticus*	0.26	92.4. ± 4.4
	Bucorvus abyssinicus	0.26	91.09 ±5.3
Cd	*Oreochromis niloticus*	0.26	97.3 ± 4.02
	Bucorvus abyssinicus	0.26	94.06 ± 5.74

The ranges of standard solutions and the resulting linearity of the calibration graphs were indicated in Table 7.

Table 7. Concentration ranges and linearity of the studied organochlorine pesticides

Organochlorine pesticides	**Range (µg/g)**	**Linearity**
Dieldrin	0.0005 - 0.8	0.9999
DDT	0.0005 - 0.8	0.9999
DDD	0.0005 - 0.8	0.9999
DDE	0.0005 - 0.8	0.9997

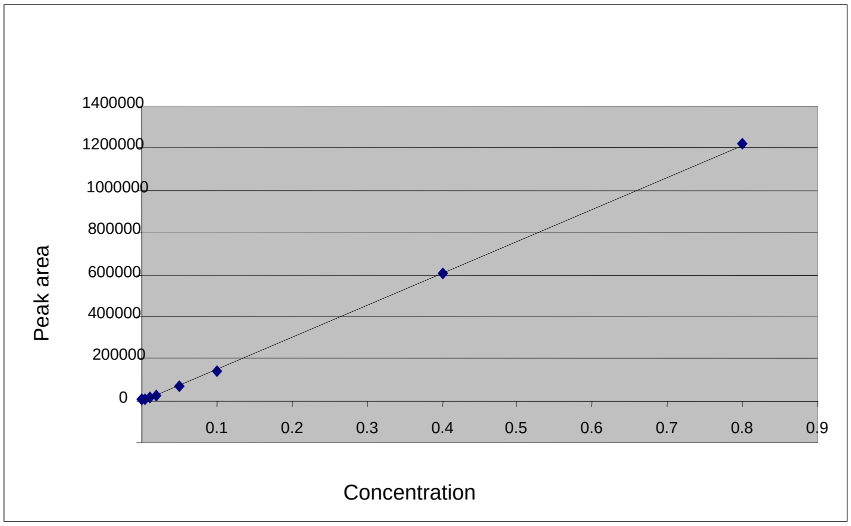
The calibration graphs are constructed using concentrations of the standard solutions and their respective peak areas as indicated in Figure 3(a-d).



$$Y = 67390X - 366.97$$

$$R^2 = 0.9999$$

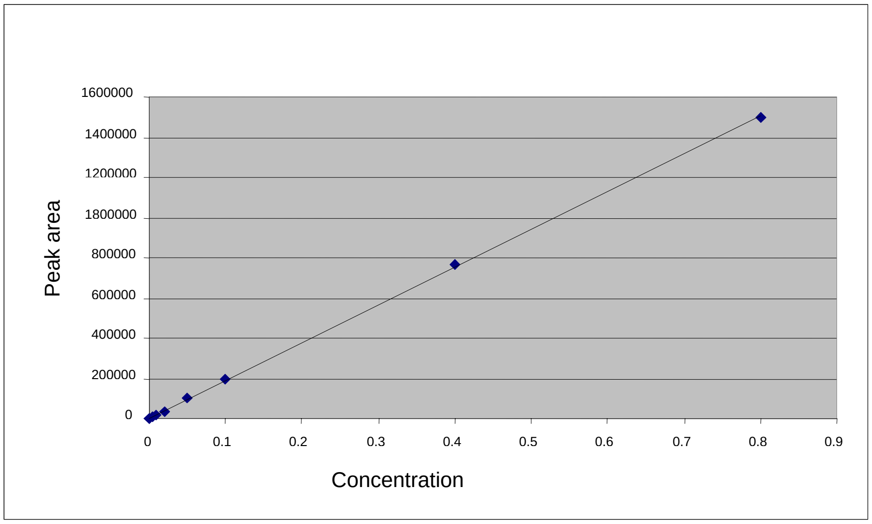
3a. Dieldrin standard solutions



$Y = 2E^{+6}X - 2337.5$

$R^2 = 0.9999$

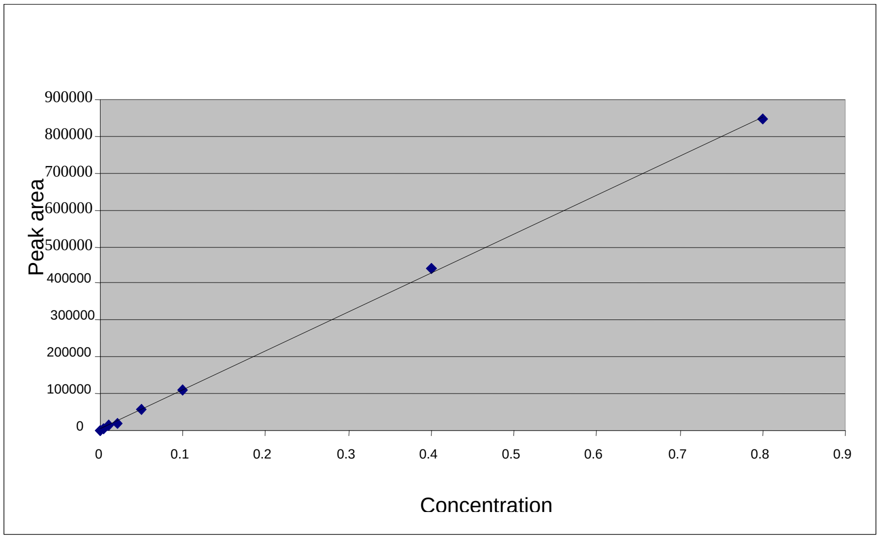
3b. DDT standard solutions



$Y= 2E^{+6}X-1230.4$

$R^2= 0.9999$

3c. DDD standard solution



$Y= 1E^{+6}X+2973.5$

$R^2=0.9997$

3d. DDE standard solutions

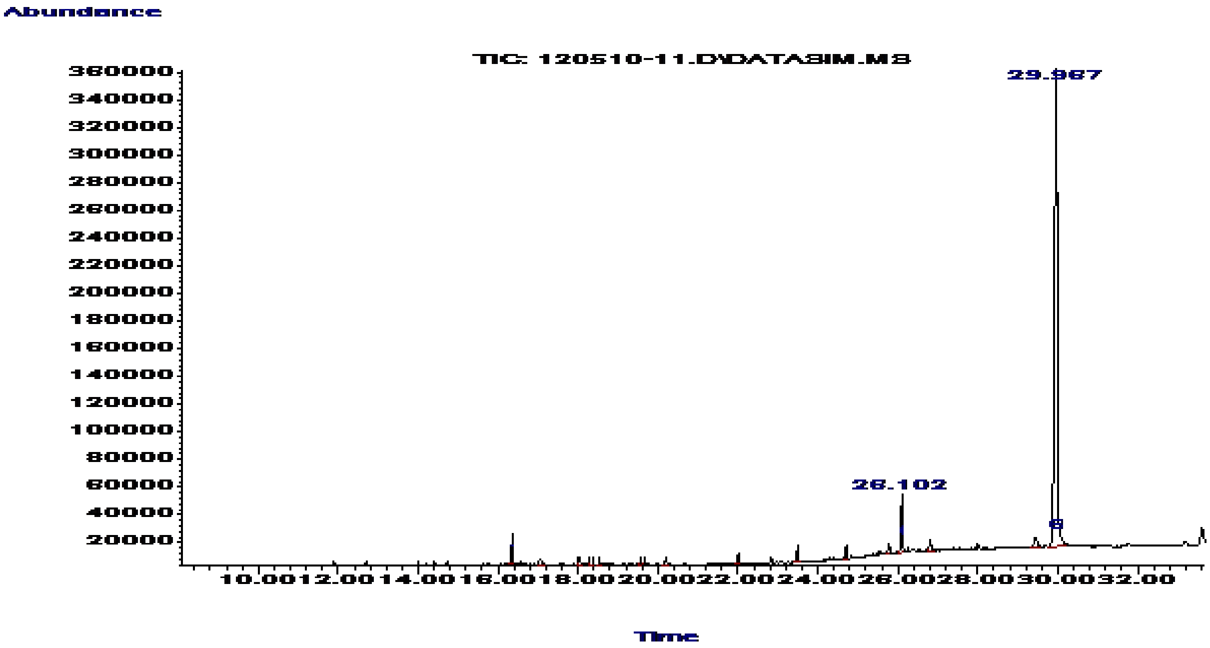
Figure 3. Calibration graphs for the detected organochlorine pesticides

Dieldrin, DDT and its metabolites were detected from the analyzed samples of tilapia and Abyssinian ground hornbill. The concentrations of the detected organochlorine pesticides are summarized in Table 8.

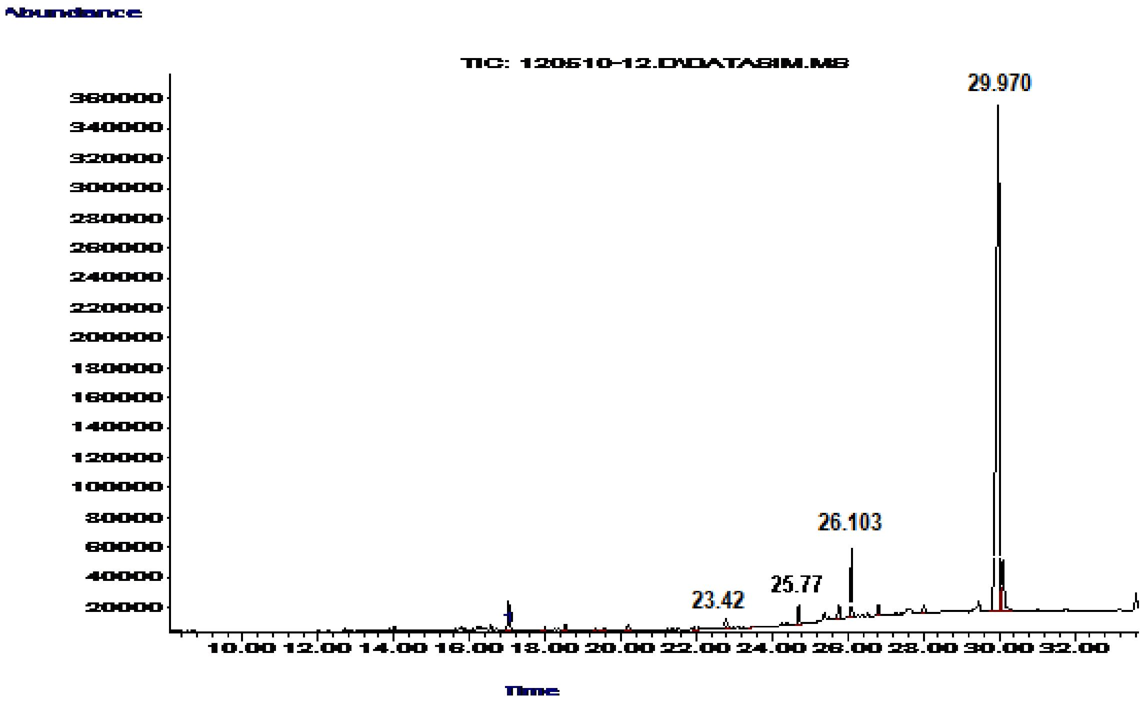
Table 8. Mean and standard deviations (ng/g wet weight) of the detected organochlorine pesticides in *Oreochromis niloticus* and *Bucorvus abyssinicus* samples (ND = not detected)

Samples	Organochlorine pesticides			
	Dieldrine	DDT	DDD	DDE
Oreochromis niloticus	4 ± 0.51	ND	ND	0.45 ± 0.05
Bucorvus abyssinicus	11.8 ± 0.87	0.19 ± 0.012	0.14 ± 0.0097	12.6 ± 0.94

From the detected organochlorine pesticides, Dieldrine was detected in a relatively higher concentration in all of the samples of both *Oreochromis niloticus* and *Bucorvus abyssinicus*. DDT and one of its metabolites, DDD, were not detected at all in fish samples but occurred in minute concentration in the analyzed fish eater bird tissue while DDE, the other metabolite of DDT was low in fish but detected with higher concentration in the bird’s tissue. Chromatogram results of the analyzed samples are indicated in figure 4a and 4b.



4a. *Oreochromis niloticus* sample



4b. *Bucorvus abyssinicus* sample

Figure 4. GC-MS Chromatograms of *Oreochromis niloticus* and *Bucorvus abyssinicus* samples

Samples were considered positive when their residue levels were≥ the limit of detection (LOD). The limits of detection for the detected pesticides are indicated in Table 9.

Table 9. Method Detection limits of detected Organochlorine pesticides

Organochlorine pesticides	Dieldrin	DDT	DDE	DDD
Method detection (mg/L)	0.0034	0.00025	0.0029	0.0023

Recoveries with majority above 70 % were obtained from spiked representative samples in triplicate from 0.5 mg/L for dieldrin and 1 mg/L for DDT, DDE and DDD standards. The majority of the relative standard deviations are below 10 % as indicated in Table 10.

Table 10. Percentage recovery (Mean and %RSD in ng/g wet weight) of organochlorine pesticides

Organochlorine pesticides	Dieldrin	DDT	DDD	DDE
Recovery (%) ± %RSD	136.5 ± 12.5	71.48 ± 7.73	67.5 ± 6.90	84.82 ± 7.04

5. Discussion

Optimum method was selected for sample digestion from the tested procedures with preconditions producing clear and colorless solutions with minimum reagent volume, less time and digestion temperature. The method fulfilling such conditions considered to be optimum (Griepink and Tolg, 1989). The efficiency of methods used for sample preparation were evaluated with spiked recoveries and the detected heavy metals indicated a recovery above 90 % and majority of organochlorine pesticides showed above 70 % with relative standard deviations below 10 % that the method used were efficient.

Of the metals that were measured in the samples of Lake Ziway, Zn showed the highest concentration in both species with mean concentrations of 27.57 mg/kg in the fish muscle tissue and 139.08 mg/kg in the predator bird sample. The amount detected in the Abyssinian ground hornbill was about five folds higher than the amount detected in *Oreochromis niloticus.* Following Zn, Cu was detected in higher concentration in the bird tissue with mean concentration of 8.91 mg/kg which is about 7.9 folds higher than the detected amount in the tilapia muscle tissue. In contrast to Zn and Cu, the concentrations of Cd and Pb in the predator bird samples were lower than the concentrations detected in the fish sample. Different studies investigated the lower concentration of Cd in organisms higher in the food chain. A study conducted in Lake Macquarie of Australia, indicated higher concentration of cadmium in prey species than in the predator (Barwick and Maher, 2003). In food chain biotransference, some metals show less trophic enrichment up the food chain while biomagnifying in lower trophic groups, particularly in invertebrates. This trend can be attributed to the capability of detoxification of vertebrate systems (Croteau *et al*., 2005).

However, heavy metal uptake and accumulation occurs mainly from water, sediments and food (Canli *et al*., 1998), the efficiency of assimilation in different organisms might be affected by many factors such as; ecological needs, feeding habits, habitat, metabolism, biology and physiology of the organisms (Arellano *et al*., 1999). Additional environmental factors such as salinity, temperature, season and interacting agents also have impact on this pattern (Heath, 1987).

From the metals detected, the concentration of Zn is high. This is alarming for fish and fish consuming organisms. However, it is one of the essential elements for both animals and humans with deficiency marked by retarded growth, loss of taste and hypogonadism leading to decreased fertility, it becomes toxic above the threshold limits. Its toxicity is rare but at concentration above the limit may induce stiffness and pain, loss of appetite and nausea (Al-Wheher, 2008).

Unlike Zn concentration, the amount of Cu detected in both species were far below the threshold limits. Most of the minerals of copper are relatively insoluble in water that it is mostly available in surface and ground water due to use of copper containing pesticides for agricultural purposes. Like Zn, it is an essential element in human metabolism but can cause anemia, liver damage and bone and connective tissue disorder at excessive levels. The toxicity of copper depends upon the hardness of water bodies and that it is more toxic in soft waters (Taha, 2004).

Two of the metals analyzed in this study, Cd and Pb, have no biological functions and that they are bioaccumilative and highly toxic even at low exposure. They were detected in tilapia with higher concentration than in bird samples. It is known that Pb toxicity damages the brain, kidney, liver and the reproductive system when it exceeds the tolerable limits in humans (Ekipo *et al*., 2008).

In the analyzed samples of *Oreochromis niloticus* and *Bucorvus abyssinicus*; dieldrin, DDT and its metabolites were detected. Dieldrin was detected in the muscle tissue of both of the species analyzed. DDT and one of its metabolite, DDD, were not detected in fish at all but detected in 66 % of Abyssinian ground hornbill samples though the concentration is low.

In this investigation, the concentration of DDE was high and this observed trend of the occurrence of the metabolite of DDT in high concentration could be attributed to the decomposition of the parent compound used in the past. The relative concentration of the parent compound and its metabolites are very useful in providing information on sources and history of input to the environment and possible degradation pathways involved. DDT/DDE provides a useful index to know whether the input at a given site occurred recently or in the past. Generally, a low value (≤ 0.33) indicates past input of DDT, which has at the time of analysis been largely converted into DDE (Strandberg and Hites, 2001). In the present finding, the concentration of DDT ranged from non-detectible level to 0.19ng/g and its metabolite DDE, ranged from 0.45ng/g to 12.8ng/g that it can be concluded that past input of DDT was detected in the analysis.

Urbanization, expansion of agricultural activities and health control mechanisms depend on industrial products which later result in contamination of the aquatic environment and biota. Aquatic environments are the ultimate global sinks for contaminants which originate from industrial discharges, domestic sewage and more frequently as in the case of pesticide pollution, can be attributed to precipitation and agricultural off. Once they have contaminated, lakes flush out their contents slowly. This makes them more sensitive to pollution and affects the organisms either through direct contamination or food chain transfer (Rajendran *et al*., 2005; Darko *et al*., 2008). The residues of the organochlorine pesticides in Lake Ziway biota have likely to be originated via runoff, atmospheric deposition and leaching due to high malaria epidemy in the area and agricultural practices including large horticulture and floriculture industries in the vicinity of the lake.

The basic properties of persistent chemicals; lipophilocity, low water solubility and persistence for chemical and biological degradation make them bioaccumilative and hence simply pass up the food chain from prey to predator and biomagnify in the organisms higher in the food chain (Bracher and Bider, 1982; Barwick and Maher, 2003; Croteau *et al*., 2005). This trend is also observed in the present study with exception of Cd and Pb.

To accurately predict biomagnification in nature, physiological biodynamics, food web structures and trophic positions have to be considered. These factors seem to provide an initial set point at lower trophic levels that determine the concentration from which contaminant transfer up the food chain. Traditional approaches ascribing trophic position only as the sole predictor of contaminants fail in predicting some heavy metal enrichment along food webs (Croteau *et al*., 2004).

From the detected heavy metals of this study, the essential ones, Zn and Cu indicated higher concentrations in *Bucorvus abyssinicus* than in *Oreochromis niloticus.* This can be attributed to bioaccumulation and biomagnification of the metals while the concentration of Cd and Pb was low in contrast. In the study conducted to measure biotransference and biomagnification of heavy metals in a food chain, organisms lower in the food chain indicated higher concentration of Cd than the higher ones. The concentration was highest in authotrophs and herbivores than carnivores and omnivores. In such trend, the concentration was the least in fish. The study indicated the ability of vertebrate systems to detoxify and excrete the chemical from the body (Barwick and Maher, 2003). The low concentration of heavy and toxic metals in *Bucorvus abyssinicus* can be attributed to physiological advancement in order to equilibrate intake and loss rate.

Study dealing on the toxic effects of heavy metals indicated the impact of methallothioneins, metal binding proteins with high affinity for heavy metal cations and leading to reduction of the bioavailability (Lavery *et al*., 2009). Organisms can detoxify by compartmentalization in granules and membrane bounded vesicles or binding to specific soluble proteins such as methallothioneins. These biochemical characteristics make them unique in playing a primary role in regulating the concentration of free metal ions in cells (Viarengo *et al*., 1993). The attachment of the metals to methallothionein might be the reason for the detection in lower amount in the muscle tissue of the predator bird. On the other hand, high accumulation of Cd is associated with low concentration of Zn and vice versa (Gupta and Mathur, 1983). So, high concentration of Zn might have affected the availability of Cd in the muscle tissue of *Bucorvus abyssinicus*.

The findings of this study indicated higher concentrations of all the detected organochlorine pesticides in *Bucorvus abyssinicus* than in *Oreochromis niloticus* samples. From the detected organochlorine pesticides, DDT and DDD were below the detection limits in the fish but were detected in the bird muscle tissue in minimal concentration. Dieldrin was detected with higher concentration in the bird tissue than the fish. Low concentration in the biota can be attributed to different factors like bioavailability of the chemical, feeding ecology, food chain length and age of the individual.

In a study conducted in Uganda, DDT and its metabolites were not detected in *Oreochromis niloticus* while these were detected in other fish species analyzed in the same study area. One of the conclusions for this event was based on the feeding ecology and age of the specimen collected. As the collected specimens were young, their diet mainly consists of planktons and as a result the contaminants were not detected. The matured ones can shift their diet to zooplanktons, aquatic insects and invertebrates, smaller fishes and terrestrial invertebrates (Ssebugere *et al.*, 2009). So, the diet shift and elongated food chain might result in higher accumulation of the contaminants.

Trophic transfer of contaminants have been limited by the difficulty of discriminating food webs and accurately ascribing trophic positions of organisms in addition to other interfering factors. But, recent approaches in biomagnification study are using stable isotopes of carbon and nitrogen to understand food web structure and in turn advance the understanding of the accumulation of persistent pollutants through aquatic food web. Stable nitrogen isotopes ratios (^{13}N: ^{14}N) provides a continuous measure of trophic positioning of the organisms within a food web because of the predictable fractionization

(3-4%) of the lighter isotope to the heavier one for a given element from predator to prey (Bargogli *et al.*, 1998: Barwick and Maher, 2003). However, in the present study, this method was not applied due to unavailability of instruments used to provide stable isotope ratios.

From the analysis of organochlorine pesticides in Lake Ziway's fish and fish eater bird samples, dieldrin was detected at 3 folds in *Bucorvus abyssinicus* sample and DDE about 28 folds high. DDT and DDD were not detected in the fish samples but only occurred in low concentration in the fish eater bird. From this finding, it can be concluded that food chain transfer is one of the routes of contamination leaving organisms higher in the food chain with the risks even if low and undetectable concentrations occur in the environmental media and organisms low in the food chain.

Persistent pollutants have a particular significance in ecotoxicology since they are highly persistent and all have the potential to be toxic to living organisms above the tolerable levels (Storelli *et al*., 2005). So the organic community has been facing with the challenges of defining and adopting standards for the levels of contaminants in the environment. Since persistent pollutants pose potential health hazards, maximum residue limit has been recommended in the environment, biota and human consumption by various agencies. Fish can be considered as one of the most significant indicator in fresh water systems for the estimation of metal pollution levels (Rashed, 2001). The commercial and edible species have been widely investigated in order to check for those hazardous chemicals to human health (Begum *et al*., 2005).

The concentration of the detected heavy metals in the present study in fish tissue was compared with international standards for human consumption; FAO (Food and Agriculture Organization, 1983), WHO (World Health Organization, 1971), EC (European Community, 2001) and EPA (Environmental Protection Agency, 2002). The FAO and WHO limit for Cu and Zn is 30 mg/kg and for Cd and Pb, the acceptable values are 0.05 mg/kg and 0.01. The EPA limit for Cd and Pb is 0.03 mg/kg and 0.05 mg/kg. The EC threshold limit for Cd is 0.05 mg/kg.

The obtained results of the present study showed that the concentration of Zn and Cu are within the acceptable limits of FAO and WHO. Though Cd and Pb are above the threshold limits accepted by WHO, their concentration is within the limits of FAO, EC, EPA and UNEP. Both metals have no biological function in organisms rather they are potentially toxic even at trace concentrations (Mwashot, 2003). The effects of acute toxicity of cadmium are high blood pressure, kidney damage, and distraction of red blood cells (Gupta and Mathur, 1983). The concentration of Zn is also below the acceptable level only with three magnitudes low and so the level in fish tissue is alarming.

In this study, dieldrin and DDT with its metabolites were detected. All of them are known to be carcinogenic and can cause different disorders in humans when bioaccumulated above their limits. Dieldrin is highly persistent and is highly toxic to fish and aquatic invertebrates but it is moderately toxic to bird species within the threshold limits. It is neurotoxic and also affects the reproductory system (Sapozhnikova *et al*., 2004). DDT and its metabolites, DDE and DDD, are highly persistent in the environment with half life up to 15 years and are toxic to aquatic species through bioaccumilation. However, DDE was detected in higher concentration than DDT, the concentration was not above the threshold limits for the protection of predatory birds and inhibition for human consumption. Therefore, the detected concentration in fish tissue may not cause risk for avifauna and humans at present but continuous study is needed to monitor the level.

Different organizations have set levels of organochlorine pesticides for human consumption; FAO recommended a level of 300ng/g as maximum acceptable limit for DDTs while the Canadian limit is 500ng/g (Mwevura *et al*., 2002). The FDA (2001) limits are 5000ng/g for DDTs and 300ng/g for dieldrin, but the academy of Science and National Academy of Engineering (NAS/NAE, 1972) recommended a limit of 1000ng/g for total DDTs and100ng/g for dieldrin. From the comparison of these limits, the detected concentrations of organochlorines are within the acceptable limits of different organization and the concentration in Lake Ziway tilapia is not alarming for human consumption.

6. Conclusion and Recommendations

The study determined heavy metals and organochlorine pesticide concentrations in muscle tissues of *Oreochromis niloticus* and *Bucorvus abyssinicus* of Lake Ziway and evaluated bioaccumulation, food chain contamination and ecological hazard level of the toxicants.

In the heavy metal analysis, copper, zinc, lead and cadmium were detected. From these heavy metals, the two essential ones, Cu and Zn indicated higher concentrations in the predator bird sample than in tilapia but cadmium and lead concentrations were lower in the bird sample than fish. Dieldrin, DDT and its metabolites; DDD and DDE, were the detected organochlorine pesticides in the analyzed species of Lake Ziway. Like Zn and Cu concentrations, organochlorine pesticides were higher in the fish eater bird sample than in fish muscle tissue. The higher concentration of the contaminants in the predator can be attributed to different factors. The finding indicated higher concentration of DDE, one of the metabolites of DDT than the concentration of the parent compound. This higher DDE/DDT value showed minimized use of DDT in recent times in the area.

The residue levels of the detected contaminants were compared with different acceptable limits set by various international organizations. Except the threshold levels set by WHO for lead and cadmium, all the heavy metals as well organochlorine pesticides are within the acceptable limits that the levels are not alarming for fish, predator bird and for human health.

Although the contaminants have not reach levels posing threat to the aquatic biota, predatory birds and humans, measures should be taken. The following suggestions are recommended in order to monitor and protect the ecosystem:

- Measurements of residues of heavy metals and organochlorine pesticides should be regularly carried out.
- Establishment of reference laboratory with analytical instruments is needed.
- Environmental concerned organizations which are governmental or non-governmental such as Ministry of Agriculture, Health and Environmental Protection and others should take due attention on the contamination of the environment and biota with such chemicals.
- Education of the rural farmers about the use of safe pesticides and fertilizers should be encouraged.
- Environmental pollution information centers should be established to guide people and sensitize them about the toxicity of persistent chemicals.
- Replacement of chemical use by biological methods for increased crop productivity and pest and vector control should be initiated.
- Measurement of the residue levels from different organisms by using discrete and longer food chains should be carried out.
- Continuous and extensive studies on residue levels, effects and food chain transfer through out the country should be carried out to know the burden of persistent chemicals in different areas.

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