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LEVELS AND EFFECTS OF ORGANOCHLORINE PESTICIDES AND HEAVY METALS IN AQUATIC ECOSYSTEM FROM THE RIFT VALLEY REGION, ETHIOPIA

(エチオピア・リフトバレーにおける有機塩素系農薬と重金属による汚染状況の解明と水圏生態系への影響評価)

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2014

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A Dissertation Submitted for the Degree of DOCTOR of PHILOSOPHY

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List of Abbreviations

$\delta^{13}C$	Stable Carbon Isotope ratio
$\delta^{15}N$	Stable Nitrogen Isotope ratio
AAS	Atomic Absorption Spectrometry
ADIs	Acceptable Daily Intakes
ANOVA	Analysis of Variance
As	Arsenic
ATSDR	Agency for Toxic Substances and Disease Registry
BMC	Benchmark Concentration
Cd	Cadmium
CHLs	Chlordanes
Co	Cobalt
Cr	Chromium
CSF	Cancer Slope Factor
Cu	Copper
DCM	Dichloromethane
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DDTs	Sum of DDT and metabolites
ECD	Electron Capture Detector
EDIs	Estimated Daily Intakes
EPA	Environmental Protection Agency
GC	Gas Chromatography
GIS	Geographic Information System
GPS	Global Positioning System
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HCHs	Sum of α–, β – γ – and δ– HCH isomers
Hg	Mercury
HNO_3	Nitric acid

HPTs	Heptachlors
HRs	Hazard ratios
IRS	Indoor Residual Spraying
IS	Internal Standard
LOD	Limit of Detection
Log	Logarithm (base 10)
LOQ	Limit of Quantification
lw	Lipid weight
N	Number of samples
nd	Not detected or below detection limit
Ni	Nickel
OCPs	Organochlorine Pesticides
OM	Organic matter
p	Level of Significance
PAN	Pesticide Action Network
Pb	Lead
PCA	Principal Component Analysis
POPs	Persistent Organic Pollutants
r	Correlation coefficient
RSD	Relative Standard Deviation
SIA	Stable Isotope Analysis
SRM	Standard Reference Material
TC*m*X	2,4,5,6-tetrachloro-*m*-xylene
UNEP	United Nations Environment Programme
USEPA	United States Environmental Protection Agency
V	Vanadium
WHO	World Health Organization
ww	Wet weight
Zn	Zinc

Preface

In this PhD thesis, levels and ecotoxicological risk assessment of organochlorine pesticides (OCPs) and heavy metals were investigated in fish and bird species as well as surface sediment samples from the Ethiopian Rift Valley region.

OCPs and heavy metals are ubiquitous and persistent contaminants with high bioaccumulation ability, and as a consequence high concentrations can be found in environmental and biota samples. In particular, species situated high on the food chain can accumulate very high concentrations of these environmental pollutants. OCPs and heavy metals are originated from natural (for heavy metals), and anthropogenic (for OCPs and heavy metals) sources. They have been associated with various toxic effects in humans and wildlife such as endocrine disruption, cancer, poisoning, serious illness and even death. Although the use of OCPs has been banned or restricted in developed nations, they are still being used for agricultural and public health purposes in developing countries like Ethiopia. Especially, one of the most controversial pesticide, DDT is being widely used in Ethiopia for agricultural and vector control. Furthermore, Ethiopia is burden with accumulated stockpiles of pesticides the so called "obsolete pesticides" since the first imported in the 1960s. The Ethiopian Rift Valley region which encompasses seven lakes is an important area for agricultural, commercial and industrial development of Ethiopia. At the same time it is one of the most environmentally vulnerable areas in Ethiopia. OCPs and heavy metals pollution in Ethiopia is anticipated mainly from anthropogenic sources.

In response to these concerns, the present study aimed to elucidate the bioaccumulation profiles and ecological risk assessment of OCPs and heavy metals from two Ethiopian Rift Valley Lakes – Lake Awassa and Lake Ziway. Twenty five surface sediment samples and three fish species; Tilapia (*Oreochromis niloticus*), Catfish (*Clarias gariepinus*) and Barbus (*Barbus intermedius*) from Lake Awassa, and five fish species; Tilapia, Zillii (*Tilapia zillii*), Carp (*Carassius* spp.), Catfish and Barbus and four bird species; Hamerkop (*Scopus umbretta*), African sacred ibis (*Threskiornis aethiopicus*), Marabou stork (*Leptoptilos crumeniferus*) and Pelican (*Pelecanus onocrotalus*) from/around Lake Ziway were collected for this research study.

The outline of this thesis is:

In chapter 1, a general introduction is presented on the background of the investigated pollutants (OCPs and heavy metals) and short description about Ethiopia and the study area.

Chapter 2 describes the distribution pattern, possible source identification and ecological risk assessment of OCPs and heavy metals from Lake Awassa. Chemosphere 91: 857–863 (2013) & Environmental Science and Pollution Research 20: 8663–8671 (2013).

Chapter 3 reports the ecotoxicological risk assessment of OCPs from Lake Ziway to evaluate the potential human health risk through fish consumption and to examine the risk posed by OCPs to the bird species under study. Ecotoxicology and Environmental Safety 106: 95–101 (2014) & Environmental Pollution 192: 121–128 (2014).

At the end, a general discussion and future perspectives are given based on all results presented in this thesis (Chapter 4).

SUMMARY: *DDTs, HCHs, heptachlors, and chlordane compounds were the most predominant and ubiquitous residues. In general, the contamination levels of OCPs on both lakes were dominated by DDTs, attributing to their current use in vector control, illegal usage for agriculture, contamination from past usage and spills from obsolete pesticides. In sediment samples, the levels of DDT metabolites (DDE and DDD) were exceeded the sediment quality guideline values, and thus identified as chemicals of potential ecological concern in Lake Awassa. There were significant differences in OCPs levels among the studied fish species in the present study. The carcinogenic hazard ratio exceeded the threshold value of one for most of the studied fish species. Cumulative daily consumption of fish showed a potential concern on human health problem–a lifetime cancer risk of greater than one in a million. High burden of DDTs was observed in all bird species under study. The level of DDE could pose deleterious effects on survival and/or reproduction in all bird species. According to the levels of heavy metals, they were found at low concentrations except to mercury (Hg), which exceeded the permissible limit (0.3 Hg µg/g ww) in B. intermedius fish species from Lake Awassa.*

Chapter 1:

General Introduction

Background

Persistent organic pollutants (POPs) are toxic chemicals, which are characterized by their high persistence in the environment, bioaccumulation and biomagnification ability along the food chain, and adverse effects on human health and the environment (UNEP, 2001). Due to their properties of high mobility (long-range transport) and resistance to degradation, POPs are now extensively distributed over large regions, including areas where they have never been used or produced (e.g. the Polar Regions). They are lipophilic and can become accumulated and biomagnified at the top of the food chain such as in fish, birds, mammals, and humans (www.chem.unep.ch/pops). Thus, POPs have been a major environmental issue, and drawing most scientific and public attentions. POPs include organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polycyclic aromatic hydrocarbons (PAHs), poly brominated flame-retardants (PBDEs), and their degradation products.

Pesticide, now classified as POPs, is a term used for a broad range of chemicals, synthetic or natural that serve for preventing, destroying, repelling or mitigating any pest and disease. Although there are benefits to the use of pesticides, there are also drawbacks such as potential toxicity to humans and other animals (Walker et al., 2001). According to the Stockholm Convention on POPs, 8 of the 12 most dangerous and persistent organic chemicals the so called "**dirty dozen**" are chlorinated pesticides such as aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), and toxaphene (UNEP, 2001). The reports on widespread environmental contaminations and toxic effects of chlorinated pesticides lead to restrictions on the use of these compounds in developed countries. However, still ongoing use of these pesticides for agriculture and public health purposes in the developing countries especially Africa continent make them a big concern in the aquatic and terrestrial ecosystems.

OCPs are relatively large group of chemicals which have been widely used throughout the globe. Five major groups belong to this class are: DDTs: *o*,*p*′–DDT, *p*,*p*′–DDT, *o*,*p*′–DDE, *p*,*p*′–DDE, *o*,*p*′–DDD and *p*,*p*′–DDD, hexachlorocyclohexanes (HCHs: α–, β–, γ– and δ–HCH), cyclodienes (aldrin, dieldrin, endrin, chlordane, heptachlor), toxaphene, and mirex (Hoffman et al., 2000). Among the OCPs, DDT is one of the most controversial pesticides of

all the time that has been widely used in agriculture for the control of pests and in health sectors for malaria control.

In this doctoral thesis, DDT and its metabolites (i.e. *p,p′*–DDE, *p,p′*–DDD, *p,p′*–DDT, and *o,p′*–DDT), HCHs (α–, β–, γ–HCH), chlordanes (CHLs), heptachlors (HPTs) and HCB were of the most concern (Fig. 1).

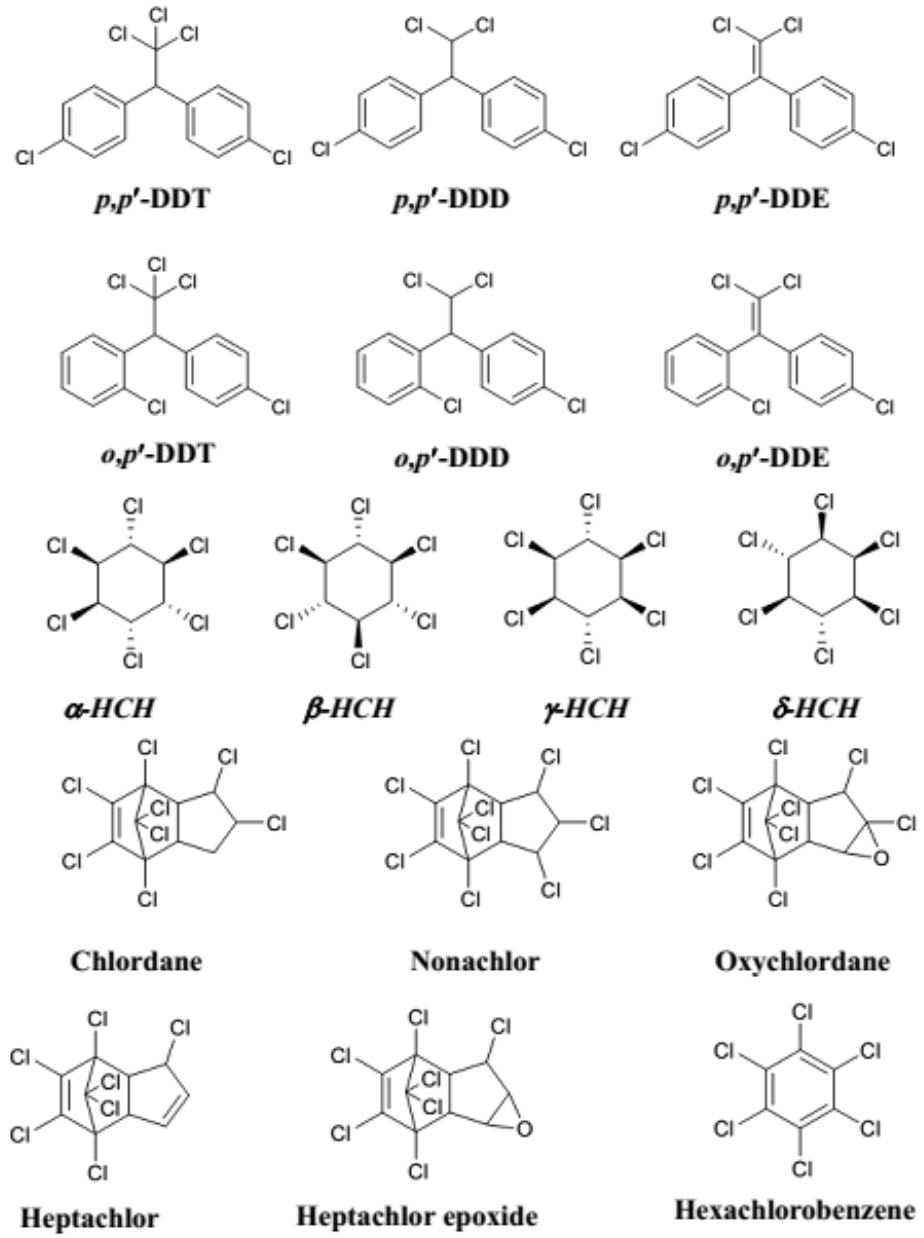

Fig. 1. Chemical structure of the main OCPs analyzed in this thesis

DDT was first synthesized by a German chemist named Othmar Zeidler in 1874 and its insecticidal properties of DDT were discovered in 1939 by Swiss chemist, Paul Muller. Technical DDT product generally contains about 75% *p,p'*-DDT, 15% *o,p*'–DDT, 5% *p,p*'–DDE, < 5% others (Yang et at., 2005). In natural environment, DDT degrade into DDE under aerobic conditions via oxidative dehydrochlorination, and DDD under anaerobic condition via reductive dechlorination (Fig. 2) (Hitch and Day, 1992). In the human body, DDT can break down into DDD less toxic to human health and DDE, a marker of chronic exposure. DDT, DDE, and DDD are readily accumulates in human fatty tissue, and some of the accumulated chemicals leave the body very slowly. Levels in fatty tissues may either remain relatively the same over time or even increase with continued exposure (ATSDR, 2002).

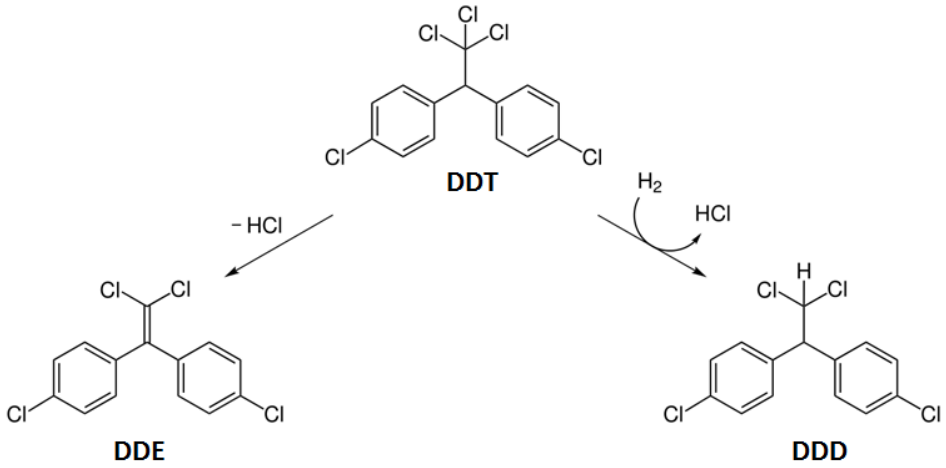

Fig. 2. Degradation of DDT to form DDE and DDD

In addition to insects, DDTs are toxic to humans and wildlife. They are well known to cause reproductive failure and eggshell thinning in bird species (UNEP, 2003; Stokstad, 2007), and have been shown to have an endocrine disrupting activity and probable carcinogenic activity (e.g. breast cancer) in human (Snedeker, 2001; Eskenazi et al., 2009). Concern over the use of DDT began in the 1960s, when the famous book "S*ilent Spring*" by Rachel Carlson described a series of harmful effects on the environment and wildlife resulting from pesticide use (Carlson, 1962). The US Environmental Protection Agency (EPA) has classified DDT, DDE, and DDD as class B2 "probable" carcinogens. According to the

Stockholm Convention, the global production and use of DDT is now limited for controlling disease vectors such as malaria mosquitoes (www.pops.int). Until locally appropriate and cost-effective alternatives are available for a sustainable transition from DDT, WHO recommends using it for indoor residual spraying (IRS) (WHO, 2007).

IRS is the application of insecticide to the inside of dwellings, on walls and other surfaces that serve as a resting place for malaria-infected mosquitoes. IRS kills mosquitoes when they come in contact with treated surfaces, and preventing disease transmission. In African countries, an intensive campaign to eliminate malaria begun in 1950s and DDT is applied on a broader scale to defeat malaria through IRS in accordance with WHO recommendations and guidelines (WHO, 2007). From an African perspective this might be understandable, since malaria still is a tremendous problem causing nearly a million deaths, with children under 5 years of age and pregnant women most severely affected (WHO, 2012). In global use, organochlorines, carbamates and organophosphates were used for residual spraying. DDT, the only organochlorine reported was used in higher quantities than any other insecticide class and was exclusively applied in IRS. Of the global use of DDT, 82% was in India alone; and the remainder was used in Africa with Ethiopia contributing the highest 11.3%, followed by Mozambique 2.2%, Namibia 1.3%, and South Africa 1.2% of the global use (van den Berg et al., 2012).

Heavy metals are natural elements in the environment. However anthropogenic releases, including industrial and domestic effluents, urban storm, water runoff, landfill leachate, atmospheric sources, and dumping of sewage sludge can give rise to higher concentrations of the metals relative to the normal background values (Zarazua et al., 2006; Cicchella et al., 2008). Heavy metals are considered as critical contaminants of aquatic ecosystems due to their ability being deposited in suspended particulate matter and sediments that can be a long-term source of contamination and high bioaccumulation potential in food chains (Erdogrul and Erbilir, 2007; Luo et al., 2010). The exposure to some heavy metals has been associated with a great many adverse health effects. Some essential metals can damage human health at relatively high exposure levels, and nonessential metals are toxic at even very low concentrations (Debelius et al., 2011).

Situated in the Horn of Africa, Ethiopia is the second populous country in Africa with a population of greater than 90 million, and one of the fastest growing non-oil dependent country. Ethiopia's economy is dependent on agriculture and over 85% of the population live in rural areas relies on agricultural production for its livelihood. The agricultural sector plays a central role in the economic and social life of the nation, and is a dominant source of foreign currency earnings contributes about 46% of the gross domestic product (GDP), 90% of foreign export earnings, and 80% of employment (Demese et al., 2010). Thus, agricultural development in Ethiopia is important and requires environmental consciousness in order for the sector to sustainably develop. However, population growth, land degradation, shrinking farm size, pests and diseases result in food insecurity. Thus, the government of Ethiopia considered a viable option to overcome these problems by agricultural intensification associated with increased use of external inputs such as fertilizers and pesticides (Malin, 2004; PAN, 2006).

In Ethiopia, DDT has been sprayed outdoors (for agricultural use) as well as indoors for malaria control by reducing the density and longevity of vector mosquitoes using IRS (PMI, 2013). DDT spraying is commonly conducted during the rainy months from June to October. About 400 metric tons of active-ingredient DDT per year is used for IRS in many parts of the country including the Rift Valley, a malaria epidemic prone region (van den Berg et al., 2009). In addition to this, Ethiopia has one of the largest stockpiles of obsolete pesticides in Africa since first imported in the 1960s. They can no longer be used for their intended purpose, and are accumulated in old and improper storage facilities that allow concentrated levels of toxins to leak into the environment and threatening the terrestrial and aquatic ecosystems. These obsolete pesticides are mostly OCPs such as aldrin, chlordane, DDT, dieldrin, heptachlor and lindane. OCPs pollution in Ethiopia is expected mainly from three sources namely IRS, obsolete pesticides and agricultural uses.

The Ethiopian Rift Valley region, part of the Great Rift Valley, extends for more than 1000 km from the Afar depression at Red Sea-Gulf of Aden junction to the Turkana depression. The region encompasses seven lakes namely Lake Ziway, Abijata, Langano, Shalla, Awassa, Abaya and Chamo of great importance in agricultural, tourism and industrial development for the country. The lakes are used for irrigation, soda abstraction, fish farming and

recreation. However, rivers that flow into some of these lakes are heavily loaded with contaminants of natural and anthropogenic origin such as discharges from factories and domestic sources. The region is also a densely populated area confined with various agricultural activities where there is still an increasing trend of pesticide usage (Jansen et al., 2007). Lake Awassa located at 6°33'–7°33' N; 30°22'–38°29' E, and Lake Ziway at 7°52'–8°8' N; 38°40'–38°56' E are an ecologically and economically important ecosystems in the Ethiopian Rift Valley region. They have an important nature value, with a diverse avifauna and a population of hippopotamus. The lakes are also crucial for the subsistence of the local communities as fishing ground, and also has an important touristic potential. However, both lakes are threatened by the deteriorating water quality due to agricultural runoff and insufficiently treated sewage, water extraction, erosion problems, and overfishing.

Objectives of the thesis

There is still a scarcity of data on the contamination status and ecological impacts of POPs residue in general and OCPs in particular in biota and environmental samples from Ethiopia. The level of exposure due to the long time use of these pesticides in the country and the impact of long time presence of such huge quantities of obsolete pesticides has not been assessed at all. Therefore, it is mandatory to access the levels of environmental pollutants for the well faring of environmental aspect, human being and wildlife health.

The main objectives of this doctoral thesis are:

i) To investigate the bioaccumulation levels of heavy metals and OCPs in sediments, fish and bird species from two Ethiopian Rift Valley Lakes – Lake Awassa and Lake Ziway.
ii) To assess the ecotoxicological risk of these environmental pollutants.

This study is expected to deliver up to date information on the levels of the pollutants in the aquatic ecosystem and is significant in establishing baseline data regarding the pollution status. The outcome of this study will therefore, help different government offices and policy makers like ministry of health and water, and environmental agencies to generate guidelines for sustaining the social, ecological and economic benefits of human and wildlife health.

Chapter 2:

Ecological Risk Assessment of OCPs and Heavy Metals from Lake Awassa, Ethiopia

2.1.

Organochlorine pesticides and heavy metals in fish from Lake Awassa, Ethiopia: Insights from stable isotope analysis

Abstract

The levels and bioaccumulation of organochlorine pesticides (OCPs) and heavy metals were studied in muscle and liver of three fish species, with two trophic levels, from Lake Awassa, Ethiopia. DDTs were the predominant organic pollutant in all species with a maximum level of 73.28 ng/g wet weight (ww). *p,p′*–DDE was the predominate congener and showed a significant ($p < 0.001$) relationship with $\delta^{15}N$, which indicates that DDTs could be biomagnified in the food web of the lake. Generally, high levels of heavy metals (Cd, Co, Cr, Cu, Ni, Pb, Zn and Hg) were found in liver samples as compared to muscles. The levels of Cd, Co, Cu, Ni, and Pb in liver samples showed negative correlation with $\delta^{15}N$. They were found markedly higher in the lower trophic level fish species ($p < 0.05$) that indicates biodilution whereas; Zn level showed positive correlation with $\delta^{15}N$.

Keywords: Bioaccumulation, OCPs, Heavy metal, Fish, Lake Awassa

Introduction

Organochlorine pesticides (OCPs) and heavy metals are among biosphere pollutants of global concern due to their environmental persistence, ability to bioaccumulate and magnify in the food chain and chronic toxicity to wildlife and humans (Jones and de Voogt, 1999; Papagiannis et al., 2004). In aquatic systems, fish are exposed to these environmental pollutants either from water via gills or/and from the diet. Henceforth, fish are the most suitable indicators for the burden of aquatic pollution monitoring since they concentrate pollutants in their tissues and enabling the assessment of transfer of pollutants through the trophic web (Fisk et al., 2001; Boon et al., 2002). Thus, bioaccumulation of pollutants can be considered as an index of environmental pollutants in the aquatic bodies. It is therefore useful to link a pollution load to the trophic position of fish species. Stable isotope analysis (SIA) has been widely employed, using stable nitrogen isotope ratio ($\delta^{15}N$) to characterize an organism's trophic position while stable carbon isotope ratio ($\delta^{13}C$) signatures have been used to determine the source and flow of carbon in a food web (Cabana and Rasmussen, 1994; Hecky and Hesslein, 1995).

The Ethiopian Rift Valley region that encompasses seven principal lakes namely Lake Ziway, Abijata, Langano, Shalla, Awassa, Abaya and Chamo is a densely populated area confined with agro industry enterprises and various agricultural farms especially floriculture and horticulture industry (Jansen et al., 2007). Lake Awassa, the smallest of the Rift Valley lakes (90 km^2 in area), lies to the west of Awassa town and about 275 km south of Addis Ababa, capital of Ethiopia. The lake is an endorheic basin and eutrophic lake with agricultural and industrial activities in its catchment. Four public factories operate within the catchment of lake discharge their wastes directly to River Tikur Wuha and eventually to the lake (Desta, 2003). These activities as well as population growth have substantially increased the burden of contamination. Recent studies on fish fillets have revealed high levels of mercury (Hg) in *Barbus* fish species from the lake (Desta et al., 2006, 2008). Wastes from urban areas, agricultural fields and the regional hospital in Awassa drain to the lake (Desta, 2003), but the levels of pollutants especially pesticides reaching the lake have never been studied. As to the best of our knowledge, this is the first study on the bioaccumulation of organochlorine pollutants in individual fishes and species in Lake

Awassa, Ethiopia.

The objective of this study are, therefore; (i) to investigate the levels of OCPs and heavy metals in three fish species and as well as to study their bioaccumulation profiles, which reflect the state of pollution, from the insights of stable isotope analysis, and (ii) to estimate an indication of public health risk levels due to the pollutants associated with fish consumption.

Materials and methods

Study area and sample collection

Lake Awassa (surface area: 90 km^2; mean depth: 11 m) is a fresh closed lake, without an out flow situated in the Ethiopian rift valley (Fig. 1). The littoral area is covered with emergent and sub-mergent macrophytes and inhabited by diverse species of benthic and bird fauna (Kibret and Harrison, 1989; Tilahun et al., 1996). The lake is highly productive. It has a rich phytoplankton and zooplankton that support large populations of six fish species: *Oreochromis niloticus*, *Clarias gariepinus*, *Barbus intermedius*, *Barbus paludinosus*, *Garra quadrimaculata* and *Aplocheilichthyes antinorii*; the first three of which are commercially and economically important (Golubtsov et al., 2002).

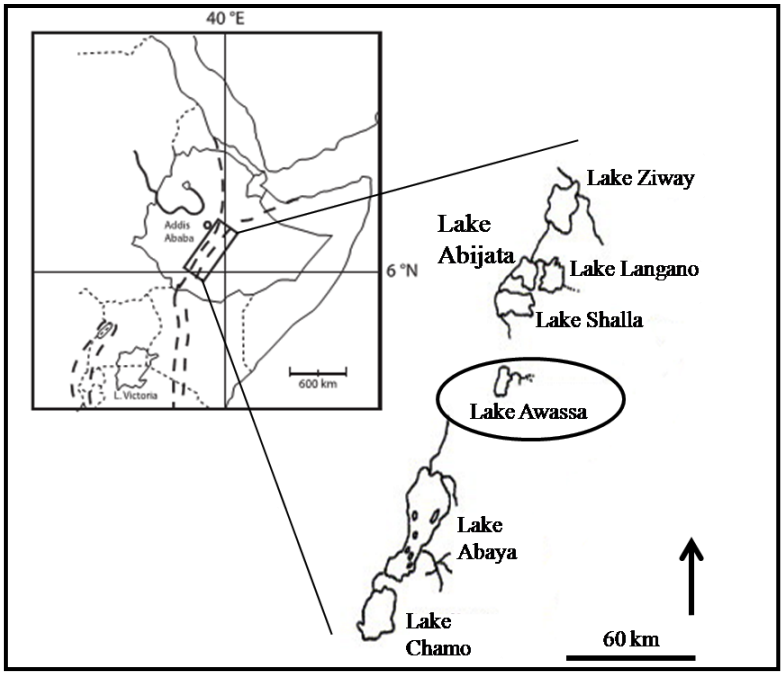

Fig. 1. Geographical map of Ethiopia showing the location of Lake Awassa in the Ethiopian Rift Valley

Sampling

A total of 49 representative fish samples from three fish species, *O. niloticus* (n = 20), *C. gariepinus* (n = 18) and *B. intermedius* (n = 11) were bought from local fishermen at shore in January 2011. Information about the samples by species is given in Table 1. The freshly collected adult fish individuals were thawed and dissected carefully to obtain liver and muscle. The separated tissues were frozen in ice box until keep at –20 ^{o}C in deep freezer unit. The frozen samples were transported to Japan for analysis. Muscle samples for SIA and OCPs determinations; while muscle and liver tissues for heavy metals analysis were taken from each specimen.

Table 1. Biometric data and lipid content (median and range); stable isotope ratio values and concentration of DDTs (ng/g wet weight) in muscle of three fish species from Lake Awassa, Ethiopia

Species (common name)	N		Standard length (cm)	Weight (g)	Lipid (%)	$\delta^{15}N$ (‰) Mean ± SD (Range)	$\delta^{13}C$ (‰) Mean ± SD (Range)	Σ-DDT Mean ± SD (Range)
O. niloticus	20	Median	22	311	0.49	8.45 ± 0.4 [b]	-21.1 ± 0.3 [a]	1.80 ± 1.25
(Tilapia)		Range	(19 - 26)	(200 - 436)	(0.03 - 1.23)	(7.96 - 9.58)	(-21.46 - 20.14)	(0.63 - 5.19)
C. gariepinus	18	Median	36	426	0.32	9.49 ± 1.4 [a]	-20.9 ± 1.2 [a]	9.35 ± 7.64
(Catfish)		Range	(26 - 44)	(152 - 731)	(0.07 - 2.45)	(7.45 - 11.81)	(-22.41 - 19.43)	(2.26 - 30.84)
B. intermedius	11	Median	27	309	0.68	10.39 ± 1.5 [a]	-20.4 ± 0.7 [a]	21.34 ± 23.17
(Barbus)		Range	(21 - 32)	(150 - 548)	(0.26 - 1.71)	(8.46 - 12.26)	(-21.59 - 19.44)	(6.82 - 73.28)

N = number of fishes sampled.

Mean values ± standard deviation (range values).

Values with different letters (a, b) within a column are significantly different at $p < 0.05$ level (Tukey test is applied).

Materials

A standard mixture (DDTs, HCHs, Chlordanes, Drins, Heptachlors and hexachlorobenzene) at 10 μg/mL was purchased from Dr. Ehrenstorfer GmbH, Germany. Florisil (60-100 mesh) from Kanto Chemical Corp. (Tokyo, Japan) was activated at 130 ºC in oven for 12 h. The organic solvents used (diethyl ether, acetone and *n*-hexane) were pesticide grade and anhydrous sodium sulfate for pesticide residue and PCB analysis were obtained from Kanto Chemical Corp., Tokyo, Japan.

For metal analysis; nitric acid, atomic absorption spectrometry grade and hydrogen peroxide were purchased from Kanto Chemical Corp. All glass vessels were soaked in 1:1 nitric acid for 12 h then rinsed with de-ionized water several times. For Hg analysis, the sample containers, quartz boats, were furnacing at 800 ºC for 5 h.

Stable isotope analysis

Small sub-samples of muscle tissues were dried at 60 ºC and ground to a fine powder with a mortar and pestle. A mixture of chloroform:methanol (2:1 v/v) was used to remove lipids from the samples and dried the residue. Stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) were measured using an isotope ratio mass spectrometer equipped with an elemental analyzer (Fisons NA1500-Finnigan MAT 252). $\delta^{15}N$ and $\delta^{13}C$ were expressed as the deviation from standards according to the following equation:

$$\delta X\ (‰) = [(R_{sample} / R_{standard}) - 1] \times 1000$$

Where X is ^{13}C or ^{15}N and the corresponding ratio R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. PDB and atmospheric nitrogen were used as a standard for carbon and nitrogen, respectively (Minagawa and Wada, 1984; Minagawa et al., 2005). Replicate measurements of internal laboratory standards indicate replicate error within ±0.2‰ for both measurements.

Analysis of organochlorine pesticides

Fish fillet of 10 g was homogenized with anhydrous sodium sulfate and placed into acetone/hexane pre-washed extraction thimble. The sample was extracted in a Soxtherm apparatus (S306AK Automatic Extractor, Gerhardt, Germany) for 6 h with 150 mL mixture of hexane:acetone (3:1 v/v). The extract was concentrated to approximately 2 mL using

rotary vacuum evaporator, which then diluted to 10 mL with hexane. An aliquot of 20% of the extract was taken for gravimetric lipid determination and the rest was subjected for clean-up process after solvent evaporation. It was performed on a glass column packed with 6 g of activated florisil topped with anhydrous sodium sulfate. Elution was carried out with 80 mL of hexane containing 25% diethyl ether. The effluent was concentrated to about 2 mL and then to near dryness under gentle nitrogen flow. The extract was redissolved in 100 μL n-decane and transferred to GC-vials for analysis.

Analysis of OCPs was carried out with a gas-chromatography equipped with ^{63}Ni electron capture detector (GC-ECD: Shimadzu GC-2014, Kyoto, Japan). An ENV-8MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used for separation. 1 μL of each sample was injected in splitless mode. The GC oven temperature was programmed from 100 °C (1 min hold); ramp at 12 °C/min to 180 °C; 4 °C/min to 240 °C, and finally at 10 °C/min to 270 °C (5 min hold). The temperatures of injector and detector were 250 °C and 320 °C, respectively. Helium was used as the carrier gas with a flow rate of 1.0 mL/min and nitrogen as the make-up gas at a flow rate of 45 mL/min.

Analysis of heavy metals

Approximately 1.5 g of individual samples were dried in an oven at 40 ^{o}C and digested in a closed microwave extraction system, Speed Wave MWS-2 microwave digestion system (Berghof, Germany). Briefly, the dried samples were placed in prewashed digestion vessels followed by acid digestion using 6 mL of nitric acid (65%) and 1 mL of hydrogen peroxide (30%). The digestion vessels were capped and placed into a 10-position turntable conditions followed by a ramped temperature programme: ramp to 160 ^{o}C (5 min hold); and increase to 190 ^{o}C (15 min hold). After cooling, samples were transferred into plastic tubes with 0.1 mL of lanthanum chloride and diluted to a final volume of 10 mL with Milli-Q water. A reagent blank was prepared using the same procedure. A Hitachi polarized Zeeman atomic absorption spectrophotometer (AAS) (Model Z-2010, Hitachi High-Technologies, Tokyo, Japan) equipped with a graphite furnace was used for quantification.

For the analysis of total mercury (Hg), an auto MA-3000 mercury analyzer (Nippon Instruments Corporation, Tokyo, Japan) was used for quantification based on direct analysis

system. Certified fish reference standard materials; DORM-3 (Fish protein, the National Research Council, Canada) and DOLT-4 (Dogfish liver, the National Research Council, Canada) were used for calibration and analytical performance studies. Hg recoveries were between 90% and 105% for the certified standard materials. The method detection limit was determined as 0.2 ng/g.

Quality assurance and quality control

The OCPs were identified by comparing their retention time with reference to the corresponding standard. The concentrations of the target analytes were quantified from the peak area of the sample to that of the standard peak area. The correlation coefficients (r^2) for the calibration curves were all greater than 0.995. For each set of 10 samples, a procedural blank and spiked blank were run to check for interference and cross-contamination. The mean recovery of OCPs for the spiked blanks was 90 ± 11%. Spiking experiments using fortified samples, *O. niloticus* at 5 ng/g of the composite standards showed recovery ranged from 70% to 110% for all OCPs. To further test the precision and accuracy of the analytical method, the standard reference material SRM 1947 (Lake Michigan Fish Tissue) was analyzed using the same procedures. Accepted recoveries ranged from 75% to 115% with RSD less than 12% were obtained. Limits of detection based on 3:1 signal to noise ratio (S/N) were between 0.05 and 0.1 ng/g for all OCPs.

For heavy metals, replicate blanks and the reference materials DORM-3 and DOLT-4 were used for method validation and quality control. Replicate analysis of these reference materials showed good accuracy, with recovery rates ranged from 80% to 115%.

Statistical analysis

All the statistical analyses were performed using JMP 9 (SAS Institute) in order to evaluate the significant differences of data among the studied species. The slope of the regression between the log-transformed concentrations of *p,p′*-DDE and DDD, and $\delta^{15}N$ was used as index of bioaccumulation of Σ-DDT among the three fish species. Linear regression analysis was employed to analyze relations between heavy metals concentration in liver and $\delta^{15}N$. All the statistical analyses were performed at the significant level of 0.05 ($p < 0.05$).

Results and discussion

Stable isotopes (δ^{13}C and δ^{15}N) analyses

Values of δ^{13}C and δ^{15}N for fishes analyzed ranged from −22.41‰ to −19.43‰ and from 7.45‰ to 12.26‰, respectively (Table 1). No significant difference of δ^{13}C and significant difference of δ^{15}N amongst fish species were observed ($p < 0.05$). The mean δ^{15}N values of *C. gariepinus* (9.49‰) and *B. intermedius* (10.39‰) were significantly higher than that of *O. niloticus* (8.45‰) ($p < 0.05$). Relative trophic positions of individual fish species based on δ^{13}C and δ^{15}N signatures (Fig. 2) indicating a higher trophic level of the two species, *C. gariepinus* and *B. intermedius*. The δ^{15}N values of fishes from Lake Awassa indicated that the carnivorous species, *C. gariepinus* and *B. intermedius* fed at nearly the same trophic level.

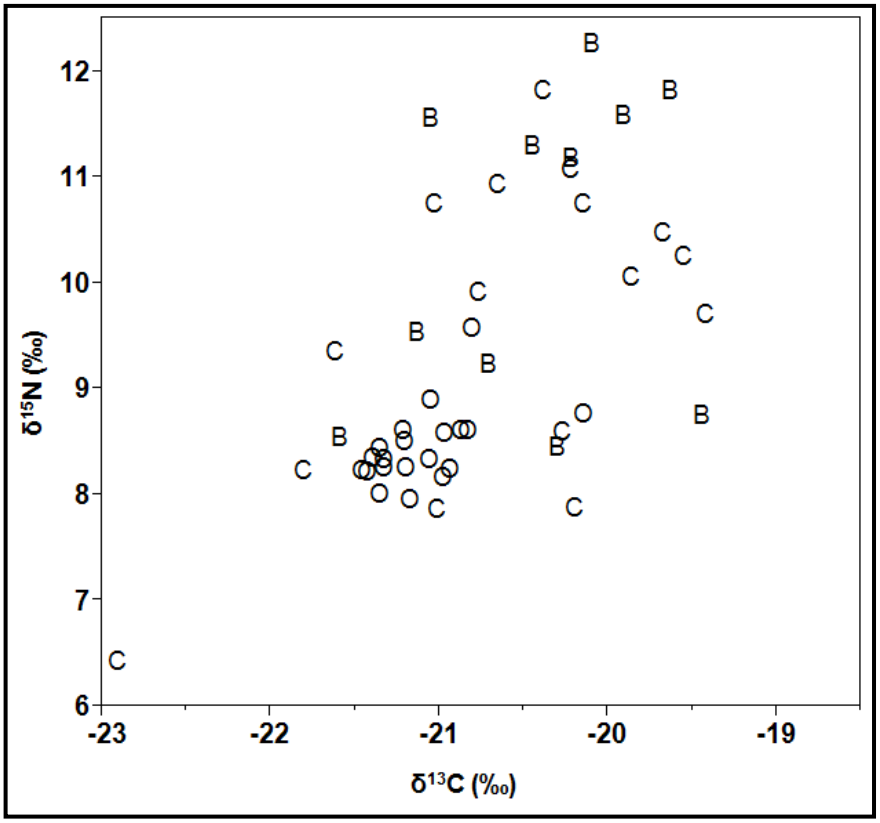

Fig. 2. Relationship between stable isotope ratios in all the fish species (O, *Oreochromis niloticus*; C, *Clarias gariepinus*; B, *Barbus intermedius*)

Concentration of OCPs

Among the analyzed organochlorine residues, DDT and its metabolites were the most abundant pollutants than other OCPs. The concentrations of other OCP components were generally low, under detection limits and were detected in a lesser frequency. The possible reasons for the presence of high level of DDTs may be attributed to the run-off and atmospheric deposition from DDT which is used for agricultural and malaria control activities in the area (Biscoe et al., 2005). This dominance of DDTs among the analyzed OCPs in fish species has also been documented in other studies (Erdogrul et al., 2005; Covaci et al., 2006).

Significantly different DDTs levels were found among the fish species. Mean concentrations of Σ-DDT were in the range of 1.80–21.34 ng/g (mean 10.83 ng/g ww) and presented in Table 1. The total DDTs concentrations were present in the order of: *B. intermedius* > *C. gariepinus* > *O. niloticus*. This result might be attributed to their different habitats, feeding habits and position in the trophic level. The *O. niloticus* is an herbivorous feeding mode, mainly feeds on planktons and lives in pelagic areas; whereas *C. gariepinus* and *B. intermedius*, carnivorous fish species, are at higher trophic levels and prefer different habitats than *O. niloticus*. DDTs levels were higher in *B. intermedius* and *C. gariepinus* which are benthic and bentho-pelagic species, respectively as sediment plays role in the remobilization of contaminants in aquatic systems. A similar finding, high levels of organochlorine pesticide residues in benthic species, was also observed in the Ouémé River catchment in the Republic of Benin (Pazou et al., 2006).

Technical DDT generally contains 75% *p,p′*-DDT, 15% *o,p′*-DDT, 5% *p,p′*-DDE, and <5% others (Yang et al., 2005). The relative percentage of DDTs is shown in Fig. 3. The *p,p′*–DDE was the predominant DDT congener (41% on average) detected followed by *p,p′*–DDD, which is accounted for 18% on average. Additionally, *o,p′*–DDT was detected at much higher percentage (*o,p′*–DDT:*p,p′*–DDT = 0.80 ± 0.36) as compared to the technical DDT composition (*o,p′*–/*p,p′*–DDT ≅ 0.2). Similar result (*o,p′*–/*p,p′*-DDT = 0.81 ± 0.55) was found in fish from lakes of the Tibetan plateau (Yang et al., 2010). According to a study by Qiu et al., (2005), Dicofol type DDT pollution is characterized by high ratio of

o,p′–DDT/*p,p′*–DDT (~7). In the present study, *o,p′*–/*p,p′*–DDT ratios were still higher than the technical DDT mixture. Thus, the lake might be moderately be impacted by the usage of dicofol. Recently due to the expansion of horticulture and floriculture farms in the Ethiopian Rift Valley region, the pesticide dicofol is used by small farm holders and large flower farms (Tadesse and Asferachew, 2008; Emana et al., 2010).

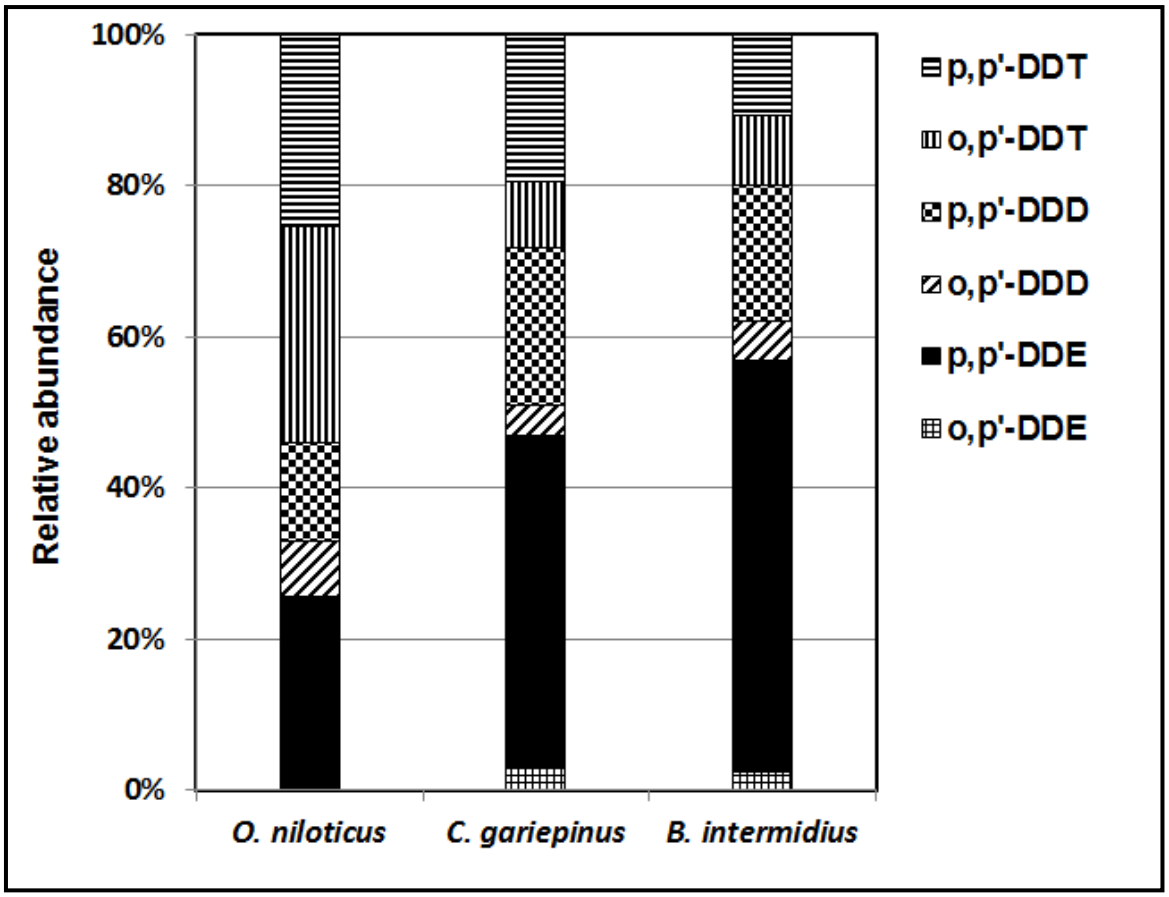

Fig. 3. Relative abundance of individual DDT components (to Σ-DDT) in three fish species from Lake Awassa

Heavy metal concentrations

The concentration of heavy metals expressed as μg/g wet weight in liver and muscle samples is shown in Table 2. The results confirm the differences of heavy metal accumulation in the tissues. It is apparent that all samples are contaminated with different levels of heavy metals, and metal concentrations in livers of examined species were generally higher than those in muscles. Both the essential elements, Cu and Zn, had the highest concentration of all elements with a maximum concentration of 582.4 and 160.23 μg/g wet weight, respectively in *O. niloticus* and *C. gariepinus* livers. The high levels in liver were expected in view of its storage and detoxification functions. Studies have shown that muscle is not an active tissue in accumulating heavy metals. This may reflect the low levels of metallothionein, low molecular weight binding proteins, in the muscle (Karadede and Ünlü, 2000; Mansour and Sidky, 2002).

However, in this study relatively high concentration of Hg with a maximum concentration of 0.59 μg/g wet weight was observed in the muscle of *B. intermedius* species (Table 2). This fish species was found to primarily exist in the littoral habitat, with mollusks being their predominant food item (Desta et al., 2006). Mercury concentrations in the *B. intermedius* ranged from 0.02 to 0.59 μg/g, and were positively related with body weight ($R^2 = 0.560$, $p < 0.01$) (data not shown). Metals that enter the body via food are carried by the blood bound to proteins, where they first move into the liver and gradually into the muscle tissues (Edwards et al., 2001). Hg appears to be very mobile in the fish organism, whereas other metals remain in the liver or other organs like gill and kidney.

Table 2. Mean and range of heavy metal concentrations (µg/g wet weight) in liver and muscle tissues of the examined fish species

Species	Tissue	Cd	Co	Cr	Cu	Ni	Pb	Zn	Hg
O. niloticus	Liver	**0.18** [a]	**1.02** [a]	0.25 [a]	**219.68** [a]	**0.48** [a]	**0.08** [a]	13.51 [b]	0.05 [b]
		(0.04 - 0.65)	(0.64 - 1.97)	(0.09 - 0.85)	(52.9 - 582.4)	(0.18 - 1.71)	(0.03 - 0.48)	(5.83 - 20.20)	(0.013 - 0.154)
C. gariepinus	Liver	0.05 [b]	0.08 [b]	0.42 [a]	47.08 [b]	0.07 [b]	0.04 [a]	**62.33** [a]	0.04 [b]
		(0.01 - 0.28)	(0.04 - 0.20)	(0.10 - 1.18)	(7.58 - 136.4)	(ND - 0.21)	(0.01 - 0.13)	(12.96 - 160.23)	(0.013 - 0.059)
B. intermedius	Liver	0.03 [b]	0.06 [b]	**0.62** [a]	12.92 [b]	0.15 [b]	0.04 [a]	29.34 [b]	0.09 [a]
		(0.01 - 0.09)	(0.03 - 0.13)	(0.17 - 3.15)	(4.03 - 22.78)	(0.01 - 0.70)	(0.02 - 0.10)	(18.31 - 39.0)	(0.015 - 0.18)
O. niloticus	Muscle	ND	0.006 [a]	0.07 [a]	0.54 [a]	0.01 [a]	0.004 [a]	3.68 [b]	0.02 [b]
			(0.003 - 0.02)	(0.03 - 0.12)	(0.44 - 0.72)	(0.004 - 0.04)	(ND - 0.07)	(2.81 - 5.29)	(0.01 - 0.04)
C. gariepinus	Muscle	ND	0.005 [a]	0.07 [a]	0.58 [a]	0.004 [b]	0.003 [a]	3.67 [b]	0.04 [b]
			(ND - 0.02)	(0.03 - 0.20)	(0.47- 0.75)	(0.001 - 0.008)	(ND - 0.02)	(2.35 - 5.50)	(0.01 - 0.09)
B. intermedius	Muscle	ND	0.001 [b]	0.04 [b]	0.65 [a]	0.002 [c]	0.003 [a]	5.30 [a]	**0.26** [a]
			(ND - 0.002)	(0.02 - 0.11)	(0.52 - 0.87)	(0.001 - 0.003)	(ND - 0.007)	(3.76 - 7.56)	(0.02 - 0.59)

ND indicates not detected or results were lower than the limit of detection.

Values with different letters (a, b,c) within a column are significantly different at $p < 0.05$ level (Tukey test is applied).

Highest values are indicated in bold.

Relationships between stable isotope and concentration of pollutants

Stable isotopes of nitrogen ($\delta^{15}N$) have been employed widely to determine the trophic positions of organisms and used to evaluate the biomagnification potential of contaminants through an aquatic food web (Hoekstra et al., 2003; Campbell et al., 2005). Hence, relations between $\delta^{15}N$ and log-transformed concentration of DDTs and heavy metals were examined to investigate the trophic level dependent accumulation of those pollutants among the studied fish species.

The two degradation metabolites, *p,p′*–DDE and *p,p′*–DDD, were detected in all species and used to study DDT bioaccumulations. Concentrations of the metabolites showed a significant increase ($p < 0.001$) with increasing $\delta^{15}N$ values on wet weight bases, Fig. 4.

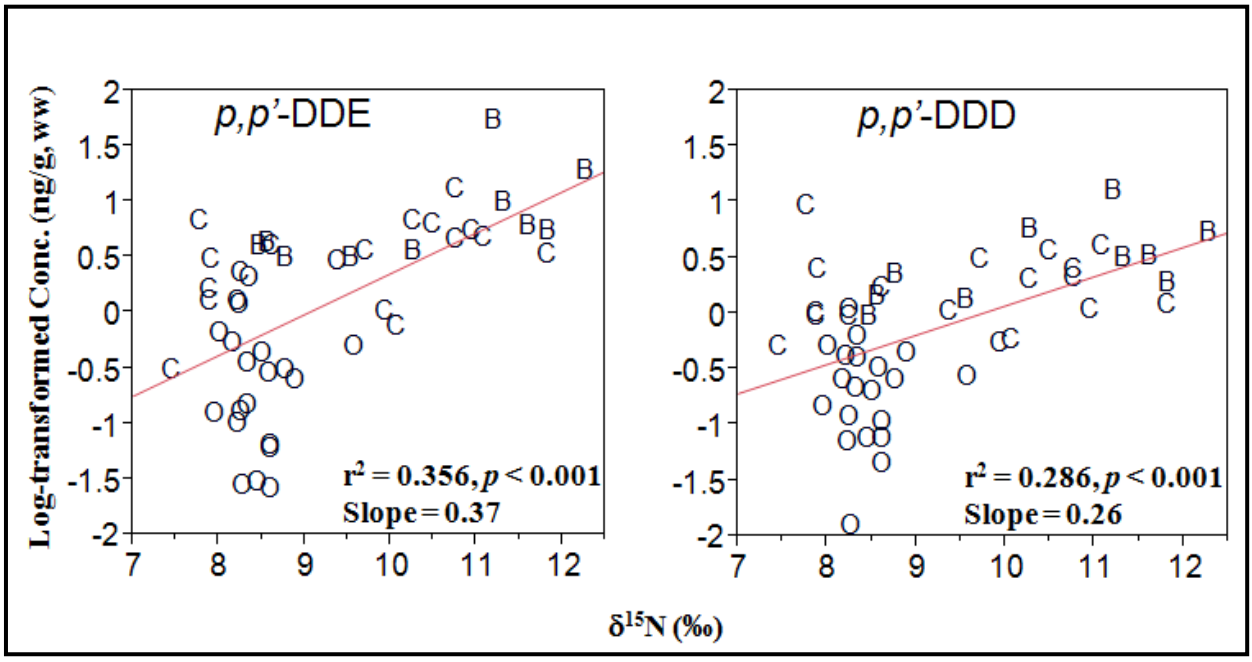

Fig. 4. Relationships between log-transformed concentration (ng/g wet weight) of *p,p′*–DDE and –DDD and $\delta^{15}N$ of individual fish in Lake Awassa, Ethiopia (O, *Oreochromis niloticus*; C, *Clarias gariepinus*; B, *Barbus intermedius*)

Interestingly, the slope for the regression equation of DDE (0.37) is higher than that of DDD (0.26) which implies that the congener DDE is abundantly accumulate in muscle. It might be attributed to its persistent nature and high rate of biomagnification nature along the food chain. This indicates that DDTs could be biomagnified in the food web of the lake which implies that increases as the trophic level increases. Significant biomagnification of ΣDDT through an aquatic food web has also been reported in many studies from different regions (Kidd et al., 2001; Hop et al., 2002; Hoekstra et al., 2003).

Relations between $\delta^{15}N$ and the log-transformed concentrations of heavy metals on wet weight basis in liver samples were examined and shown in Table 3. Significantly negative slopes were observed for log transformed Cd (–0.145), log Co (–0.247), log Cu (–0.129), log Ni (–0.203) and log Pb (–0.098).

Table 3. Linear regression equations for log-transformed metal concentration in liver vs $\delta^{15}N$ for three fish species from Lake Awassa

Variable vs. $\delta^{15}N$	N	Slope	Intercept	r^2	*p*-value	Notes
Log Zn	49	**0.122**	0.291	0.266	< 0.001	BM
Log Cd	49	**-0.145**	0.084	0.161	0.004	BD
Log Co	49	**-0.247**	1.587	0.311	< 0.001	BD
Log Cu	49	**-0.129**	2.931	0.102	0.025	BD
Log Ni	49	**-0.203**	0.916	0.167	0.003	BD
Log Pb	49	**-0.098**	-0.469	0.242	< 0.001	BD
Log Cr	49	0.045	-0.966	0.037	0.186	NS
Log Hg	49	0.058	-1.928	0.062	0.084	NS

N indicates sample number.

Notes indicate whether regressions support biomagnifications (BM), biodilution (BD), or not significant trends (NS).

Slopes with the significant difference ($p < 0.05$) are indicated in bold.

These results could be related to specific accumulation of these elements in lower trophic animals or show a consistent biodilution of those elements in liver tissue. On the contrary, an increasing relationship was observed between Zn concentrations (log-transformed) in the liver and $\delta^{15}N$ values (slope = 0.122, $p < 0.001$) (Table 3), which showed bioaccumulation trend in Lake Awassa food web. While non-significant ($p = 0.18$) slope was found for Cr. Even with respect to Hg, a trace metal that usually biomagnifies in higher trophic animals (Campbell et al., 2005; Ikemoto et al., 2008), no significant positive correlations ($p > 0.05$) were observed in this study. This lack of trend is probably related to the low Hg concentrations in *C. gariepinus* compared to *B. intermedius*, which might be due to its reliance on low-Hg prey items and to its fast growth rate that could result in growth biodilution (Desta et al., 2007).

Assessment of risk

Food guideline values for Cu (20 μg/g ww), Zn (50 μg/g ww), Cd (0.2 μg/g ww), Hg (0.3 μg/g ww) and Pb (2 μg/g ww) in edible part of fish have been summarized by the Ministry of Agriculture, Fisheries and Food (MAFF) in the UK (MAFF, 2000). Our results indicate the levels of metals in muscles were low. However, the concentration of Hg in *B. intermedius* showed high levels which exceeded the permissible limit (0.3 μg/g ww). This would indicate that consumption of these fish may be hazardous as Hg is readily absorbed and bound to protein in the organic form as methylmercury, which causes neurological impairment and kidney damage (Honda et al., 2006). Thus, to estimate individual exposure from fish, the Estimated Daily Intakes (EDIs) for Hg were calculated and compared with Tolerable Daily Intakes (TDIs). The data are on the assumption basis of 60 kg body weight and consumption of 150 g fresh fish per day as follows:

$EDI = (C \times FDC) / BW$

Where C is the concentration of the contaminants (μg/g), FDC stands for fish daily consumption (g/d) and BW represents the body weight (kg).

The EDI of Hg was calculated to be 0.65 μg/d/kg bw, which corresponds to 88% of the TDI value (0.7 μg/d/kg bw). Based on the maximum value, 0.59 μg/g, the daily intake of Hg would be 1.48 μg/d/kg bw, which was 2.1 fold higher than TDI value.

Conclusion

Significant differences of DDTs levels and profiles were found among the studied fish species. The species *B. intermedius* as being found at higher trophic level accumulated high DDTs levels, which demonstrates the bioaccumulation trend of persistent contaminants like DDTs. The accumulation of heavy metals varied among the species. Results showed that the *Oreochromis species* can accumulate most of the studied metals in liver tissues as compared to the other carnivorous species. Analysis of the potential hazardous levels for the health of human showed that Hg concentration levels in some *Barbus species* presented a relatively high risk. The results from this study, albeit small samples, call for further study on the level and extent of other inorganic and organic pollutant contaminations like methyl mercury, PCBs, etc. in the fresh water system as Lake Awassa continuously receives urban and industrial wastes from multiple sources.

- In previous part, the levels of heavy metals and OCPs such as DDTs, HCHs, chlordanes, drins, heptachlors and hexachlorobenzene were investigated in fish species from Lake Awassa. The result revealed the presence and dominance of DDTs among the other OCPs, and varied accumulation levels of heavy metals among the studied fish species.

- So to clarify more the distribution pattern and possible source of DDTs and heavy metals in Lake Awasssa, **surface sediments** were collected from the Lake. Sediments are recognized as the most important sink for contaminants, and often used to investigate the level of environmental pollution.

2.2.

Occurrence, distribution and ecological risk assessment of DDTs and heavy metals in surface sediments from Lake Awassa - Ethiopian Rift Valley Lake

Abstract

Dichlorodiphenyltrichloroethanes (DDTs) and heavy metals are ubiquitous contaminants with high bioaccumulation and persistence in the environment, which can have adverse effects on humans and animals. Although applications of DDTs have been banned in many countries, developing countries like Ethiopia are still using these for agricultural and medicinal purposes. In addition, heavy metals are naturally present in the aquatic environment and distributed globally. In this study, the occurrence, distribution, and ecological risk of DDTs and heavy metals in surface sediments from one of the Ethiopian rift valley lakes were studied. Twenty-five surface sediment samples from Lake Awassa, Ethiopia were collected and analyzed for DDTs and heavy metals. Results showed that concentrations of total DDTs ranged from 3.64 to 40.2 ng/g dry weight. High levels of DDTs were observed in the vicinity of inflow river side and coastal areas with agricultural activities. The heavy metals content were followed the order Zn>Ni>Pb>Cu>Cr >Co>As>Cd>Hg. Correlation analysis and principal components analysis demonstrated that heavy metals were originated from both natural and anthropogenic inputs. The levels of DDE and DDD in surface sediments exceeded the sediment quality guideline values, indicating that adverse effects may occur to the lake. A method based on toxic-response factor for heavy metals revealed that the calculated potential ecological risk indices showed low ecological risk for the water body.

Keywords: DDTs, Heavy metals, Sediment, Lake Awassa, Correlation analysis, Spatial distribution, Ecological risk assessment

Introduction

Sediments are known to be the ultimate sinks for contaminants discharged into the environment and become potential sources as they play a role in the remobilization of contaminants in aquatic systems (Doong et al., 2002; Malferrari et al., 2009). DDTs and heavy metals are ubiquitous contaminants in different compartments of the environment. These compounds are environmentally persistent, have high bioaccumulation potential, toxic and have adverse effects on humans and animals (Jones and de Voogt, 1999; Fernandez et al., 2000; Fatoki and Mathabatha, 2001). Although applications of DDTs have been banned in many developed countries, some developing countries are still used for agricultural and medicinal purposes (de Brito et al., 2002).

Ethiopia is one of the many African countries burdened by the problem of obsolete pesticides (Haylamicheal and Dalvie, 2009) and DDT has still been used for agricultural and public health programs (Amera and Abate, 2008). According to a report by Ethiopian Ministry of Health, Ethiopia uses approximately 400 tons of active-ingredient DDT for indoor residual spraying (IRS) per year (Biscoe et al., 2005). It has been used in many parts of the country especially on the rift valley, malaria epidemic prone region in Ethiopia. In addition, heavy metals are naturally present in the aquatic environment and distributed globally with a wide range of concentrations in sediments and biota (Ip et al., 2007).

The Ethiopian Rift Valley region which encompasses seven lakes is an important area for agricultural, commercial and industrial development of Ethiopia. It is a densely populated area with various agro industry enterprises and mechanized irrigation farms. At the same time, it is one of the most environmentally vulnerable areas in Ethiopia (Jansen and Hengsdijk, 2006). Anthropogenic sources of DDTs and heavy metals from urban and agricultural sources lead to unprecedented environmental contamination to the ecosystem. The construction of irrigation and drainage systems, clearing of forest, and use of fertilizers, herbicides and pesticides all contribute towards the damage of this indispensable but fragile system.

Studies carried out in Ethiopian Rift Valley Lakes have revealed the presence of heavy metals in water and sediment samples. The studies by Zinabu and Zerihun (2002) on the chemical composition of the effluent from Awassa textile factory and its effects on aquatic biota with a focus on phytoplankton and fried fish clearly showed high concentration of heavy metals and indicated the effect of pollutants due to effluent from factories. Other study on the concentration of heavy metals in Ethiopian rift valley lakes and their in-flows by Zinabu and Pearce (2003) showed the potential effect of hot springs especially as a source and means of transport of trace elements ultimately into the lakes. Recent study in Lake Awassa revealed significant differences of DDTs and heavy metals levels among three fish species (Yohannes et al., 2013a). However, no studies were carried out on DDTs in sediment in this ecosystem.

The objectives of the present study were (1) to investigate the concentration and distribution patterns; and (2) to identify the possible sources of DDTs and heavy metals in surface sediments from one of the Ethiopian Rift Valley Lake - the Lake Awassa. In addition, ecological risks of these contaminants in surface sediments by comparison with sediment quality guidelines (SQGs) were also assessed.

Materials and methods

Study site

Lake Awassa (6°33'–7°33' N; 30°22'–38°29' E; surface area: 90 km^2; mean depth: 11 m) is one of the many Ethiopian wet land resources situated in the middle of a series of Rift Valley lakes (Fig. 1). Without outlet, Lake Awassa is the smallest lake in the Ethiopian Rift Valley with the only perennial stream of the Tikur Wuha River at its northeastern shore. The lake lies to the west of Hawassa town and the existence and protection of this lake is very critical to the city and the population residing within the entire catchment. It supports commercial fishing activities and has a great potential for agricultural developments. However, the lake is presently faced with serious ecological problems due to deleterious anthropogenic activities in the catchment. The lake receives waste products from both industrial and domestic sources via Tikur Wuha River (Desta, 2003).

Sample collection

Surface sediment samples (n = 25) were collected from a number of sites in Lake Awassa using an Ekman grab sampler in January 2011. The sampling sites were located by a global positioning system and shown in Fig. 1. Each sediment sample was air-dried while covered with plastic sheets to avoid any contaminations and passed through a 2 mm sieve. The dried samples were stored at −20 ºC until analysis. The organic matter (OM) content was determined using loss on ignition (LOI) by heating the dry sediment at 550 ºC for 4 h. All analyses were done at the laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan.

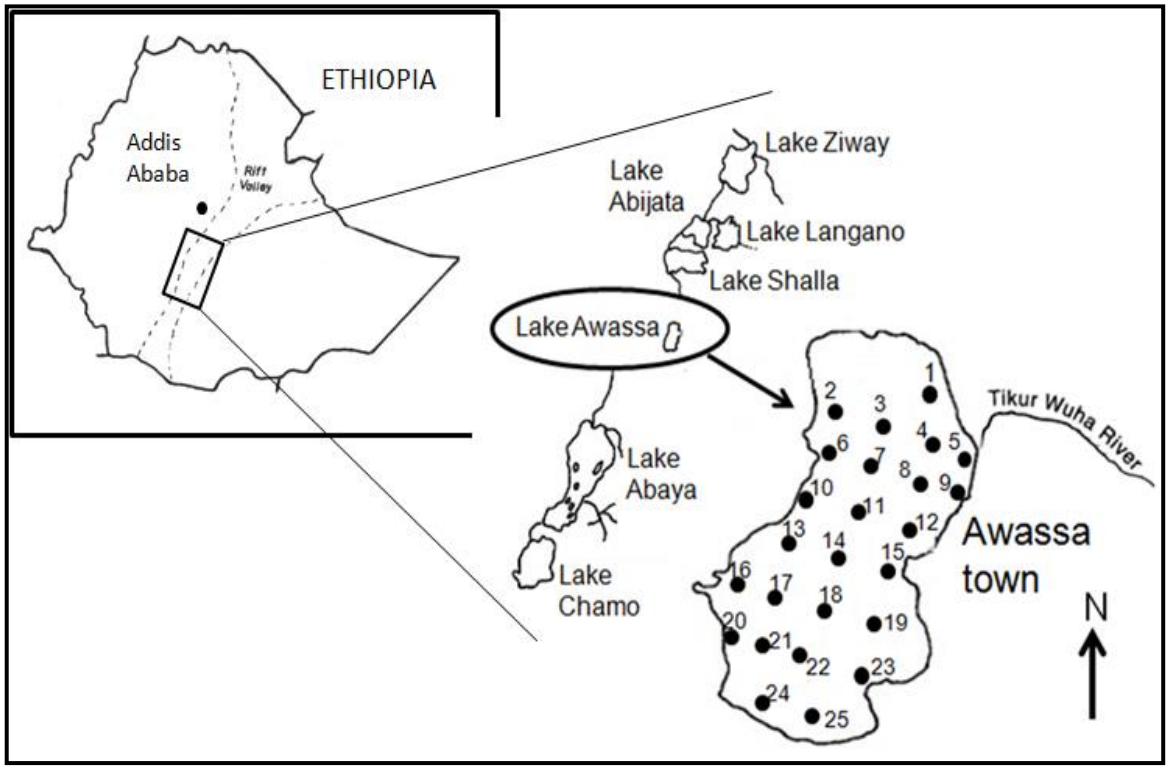

Fig. 1. Ethiopian Rift Valley lakes and location of sampling sites in Lake Awassa

Sample extraction and analysis of DDTs

Five grams of dried sediment samples were mixed with 15 g of anhydrous sodium sulfate and sonicated twice with 30 mL of hexane/acetone (1:1, *v/v*) in an ultrasonic bath for 20 min, with 2,4,5,6-tetrachloro-*m*-xylene (TC*m*X) added as surrogate standard. The extracts were combined, concentrated on a rotary vacuum evaporator and solvent-exchanged to hexane. Activated copper powder was added for desulphurization and a silica gel column containing 5 g of deactivated silica gel (5%) topped with anhydrous sodium sulfate was used for clean-up. The column was eluted with 100 mL of hexane/dichloromethane (7:3, *v/v*). The eluate was concentrated to about 2 mL and further to near dryness under gentle nitrogen flow. The extract was redissolved in 100 μL n-decane and transferred to GC-vials for analysis. A known amount of pentachloronitrobenzene (PCNB) as an internal standard was added prior to analysis.

The concentrations of DDTs (*p,p′*–DDT, *o,p′*–DDT, *p,p′*–DDE, *o,p′*–DDE, *p,p′*–DDD and *o,p′*–DDD) were determined by a gas chromatography equipped with ^{63}Ni micro-electron capture detector (GC-ECD: Shimadzu GC-2014, Kyoto, Japan). An ENV-8MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used for separation. The oven temperature was initially isothermal at 100 °C for 1 min and ramped at 20 °C/min to 180 °C, then at 4 °C/min to 260 °C and kept isothermal for 5 min. The temperatures of injector and detector were 250 °C and 320 °C, respectively. Helium as carrier gas with a flow rate of 1.0 mL/min and nitrogen as make-up gas at a flow rate of 45 mL/min were used. 1 μL sample was injected in the splitless mode for analysis.

Digestion and analysis of heavy metals

For total heavy metal analysis, an approximately 0.5 g of dry sediment was weighed into PTFE digestion vessels, followed by acid digestion using 8 mL of nitric acid (65%) in a closed microwave digestion system (Speed Wave MWS-2; Berghof, Germany). After cooling, samples were transferred into plastic tubes and made a final volume of 50 mL with Milli Q-water. A Hitachi atomic absorption spectrophotometer (AAS) (Model Z-2010, Tokyo, Japan) either with acetylene flame for copper (Cu) and zinc (Zn) or argon gas for arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), nickel (Ni) and lead (Pb) was used

for quantification.

For total mercury (Hg), the samples were measured based on direct analysis system. The direct analysis incorporates the following sequence: thermal decomposition, catalytic conversion, gold amalgamation and atomic absorption spectrophotometry (Mercury analyzer, MA-3000; Nippon Instruments Corporation, Tokyo, Japan).

Quality control and quality assurance

DDT and its metabolites were identified by comparing their retention time with reference to the corresponding standard. The correlation coefficients (r^2) for the calibration curves were all greater than 0.995. Procedural blanks, spiked blanks and duplicate samples were analysed for every set of 10 samples to check for interference and cross contamination. The blank samples run contained no detectable amount of target analytes. In addition, surrogate standard (TC*m*X) was added to each of the sample to monitor procedural performance and the average surrogate recoveries were ranged from 82% to 110%. The quality of the analytical method was assured using the standard reference material SRM 1944 (New York/New Jersey Waterway Sediment). The average recoveries of the individual DDTs were ranged from 90% to 110% with relative standard deviation (RSD) less than 10%. Limits of detection calculated based on 3:1 signal versus noise value (S/N) were 0.1 ng/g. To minimize DDT degradation, the injection temperature was kept at 250 °C and the pre-column was cut for every ten samples to avoid adsorption in the injector port and prevent peak tailing.

For heavy metals, two reference materials SRM 1944 and BCR-320 (Channel Sediment, IRMM, Belgium) were used for method validation. Replicate analysis of these reference materials showed good accuracy with recovery rates ranged from 80% to 110%. The detection limits (μg/kg) of Cu, Zn, As, Cd, Co, Cr, Ni and Pb were 0.5, 0.1, 1.0, 0.2, 0.5, 0.5, 0.5 and 1.0, respectively. The detection limit of Hg was 2.0 pg total Hg. All of the results in the surface sediments were expressed on dry weight basis and were not corrected for recoveries.

Statistical analysis

All the statistical analyses (correlation analysis and PCA) were performed using JMP 9 (SAS Institute, Cary, NC, USA). Pearson's correlation with statistical significance was tested at $p < 0.05$ and $p < 0.01$. Principal components analysis (PCA) extracts eigenvalues greater than 1 and related loadings from the covariance matrix of original variables to produce new orthogonal variables, through varimax rotation. Spatial distribution of DDTs in sediments was performed by using Arc-GIS 9.3 software (ESRI, New York).

Results and discussion

Residue levels and spatial distribution of DDTs in surface sediments

Levels of DDTs varied depending on sampling sites indicating variable sources in different locations. Residual concentrations of DDTs in surface sediments are summarized in Table 1. The total concentrations of DDTs in surface sediments were found in the range of 3.64 to 40.2 ng/g with a mean/median concentration of 12.6/10.0 ng/g. Among the DDTs, *p,p′*–DDE was the predominant and its concentration was in the range of 1.15-18.1 ng/g with a mean/median value of 7.48/6.09 ng/g, followed by *p,p′*–DDD with a mean/median of 3.50/0.90 ng/g. When compared with other studies, levels of DDTs in surface sediments from Lake Awassa were higher than those in Singapore coastal areas (2.2–11.9 ng/g; Wurl and Obbard, 2005); Danshui River estuary, Taiwan (nd–9.85 ng/g; Hung et al., 2007); Lake Victoria, Uganda (0.41–8.07 ng/g; Wasswa et al., 2011) and Lake Manzala, Egypt (0.20–5.17 ng/g; Barakat et al., 2012a), but lower than other polluted areas such as River Lambro, Northern Italy (1.5–167 ng/g; Bettinetti et al., 2003); Casco Bay, Maine, USA (nd–455.5 ng/g; Wade et al., 2008) and Lake Maryut, Egypt (0.07–105.6 ng/g; Barakat et al., 2012b).

Table 1. Concentration of DDTs (ng/g dry weight), ratios of DDTs degradation product and contents of OM (%) in surface sediments of Lake Awassa

	Mean	SD	Minimum	Maximum	Median
***o,p'*-DDT**	0.87	0.60	0.20	2.63	0.70
***p,p'*-DDT**	0.97	0.76	nd	3.16	0.66
***p,p'*-DDD**	3.50	6.06	0.49	22.1	0.90
***p,p'*-DDE**	7.48	4.94	1.15	18.1	6.09
ΣDDT	12.6	9.42	3.64	40.2	10.0
(DDE+DDD)/ΣDDT	0.86	0.08	0.60	0.94	0.88
***p,p'*-DDE/*p,p'*-DDD**	7.08	6.04	0.15	19.0	4.78
***o,p'*-DDT/ *p,p'*-DDT**	1.08	0.40	0.40	1.86	1.10
OM (%)	13	7	3.8	23	11

nd = below detection limits.

The OM in surface sediment ranged from 3.8% to 23% (Table 1). There was no significant correlation between DDTs concentrations and OM content (r^2 = 0.08, p > 0.05) (data not shown), suggesting no potential influence of OM on the distribution of DDTs in surface sediments from Lake Awassa. It can be explained that besides OM, the intensity of OCP in the soil depends upon several other environmental factors such as temperature, pH, redox potential and moisture (Jiang et al., 2009).

The spatial distribution pattern and levels of DDTs in surface sediments of Lake Awassa are shown in Fig. 2 using contour map and bar graph. The highest levels of DDTs were found in the samples taken from three sites: 5, 13 and 20 with a value of 29.5 ng/g, 40.2 ng/g and 28.6 ng/g, respectively (Fig. 2). These high levels of DDTs were observed in the vicinity of the inflow Tikur Wuha river side (site 5) and from samples next to a village close to agriculture areas (sites 13 and 20).

The high levels of DDTs in the surface sediments could be primarily due to the influence of anthropogenic activities caused by inputs from agricultural and malaria campaigning activities. The sediment from sites 2, 4, 11, 12, 17, 18, 21, 22 and 23 were also found to contain relatively high concentrations in the range of 11.0 ng/g to 21.1 ng/g while concentrations of DDTs in the rest of the samples were below 10.0 ng/g dry wt.

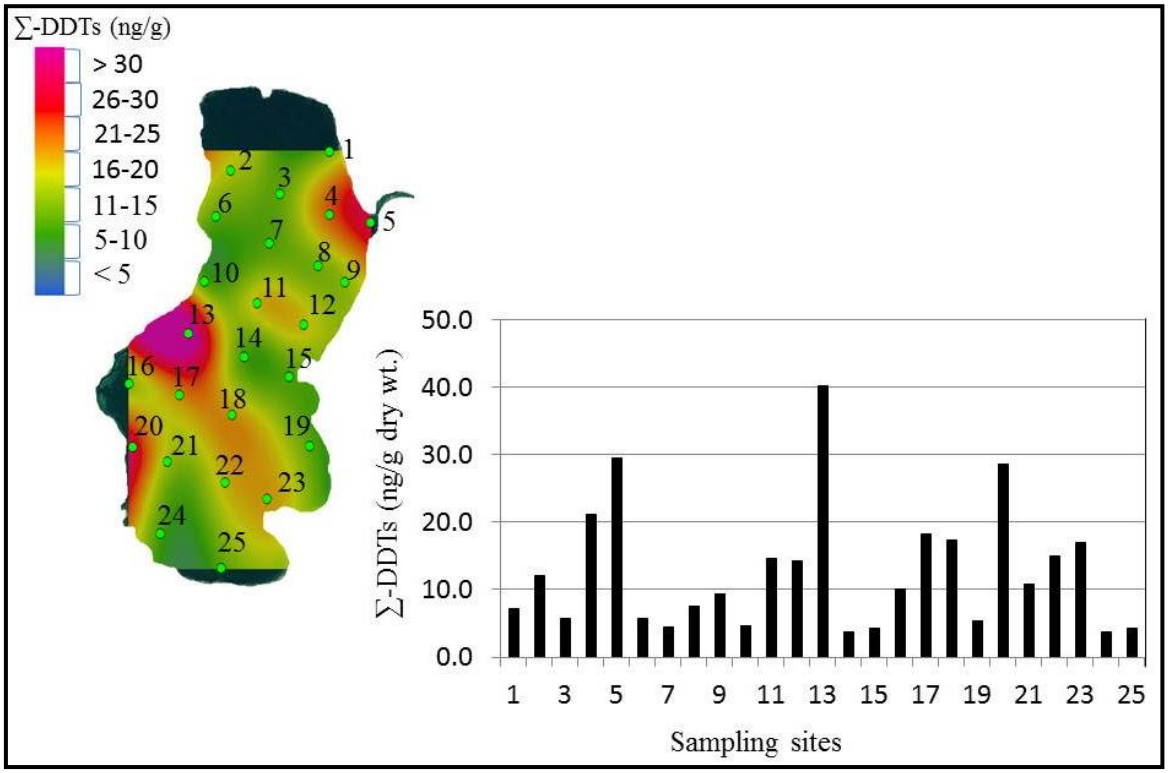

Fig. 2. Spatial distribution and total concentration profiles of DDTs in the surface sediments of Lake Awassa

Characterization of DDTs contamination in the surface sediments

The composition of DDT and its metabolites have been used to identify the possible sources of DDT as well as their fate in aquatic environment (Tao et al., 2007). The compositions of DDTs in the surface sediments are depicted in Fig. 3. In this study, the metabolites DDE and DDD occupied the predominant percentage. Sum of *p,p′*–DDE and *p,p′*–DDD were accounted for 60-94% of DDTs while percentage of the parent compounds (*o,p′* and *p,p′*–DDT) ranged from 0–40%. DDT can be biodegraded to DDE and DDD under aerobic and anaerobic conditions, respectively (Hitch and Day, 1992). Comparing the concentration of DDT and its metabolites can be inferred whether DDT's input are recent or not. Thus, ratios of (DDE + DDD)/ΣDDT can be used as indicator indices for assessing long term weathering (> 0.5) or recent introduction (< 0.5) of DDT to the environment (Hitch and Day,

1992) although this method is limited in regions where dicofol is used since this pesticide contains high levels of DDT as impurity (Qiu et el., 2005). In this study, ratios of (DDE + DDD)/ΣDDT ranged from 0.60 to 0.94 (Table 1), which suggested that the detected DDTs were originated from long term usages of DDTs although DDT is used in Ethiopia for agricultural and public health programs. This lack of fresh inputs might be happened due to biodegradation of DDT by micro-organisms and high temperature as Lake Awassa is a tropical lake. The DDE/DDD ratios in the surface sediments for most of the sampling sites in Lake Awassa were greater than 1 (Table 1), showing dominance of DDEs, which indicates the main degradation condition of DDT was aerobic. The ratio of *o,p′*–DDT/ *p,p′*–DDT can be also used to distinguish DDT pollution caused by technical DDT from that by dicofol (Qiu et el., 2005). The predominance of *o,p′*–DDT isomer over *p,p′*–DDT isomer (*o,p′*–DDT/*p,p′*–DDT = 1.08 ± 0.40; Table 1) suggests the application of dicofol in the catchment areas. A recent study in fish species from Lake Awassa also reported high *o,p'*–DDT/ *p,p′*–DDT ratio (Yohannes et al., 2013a).

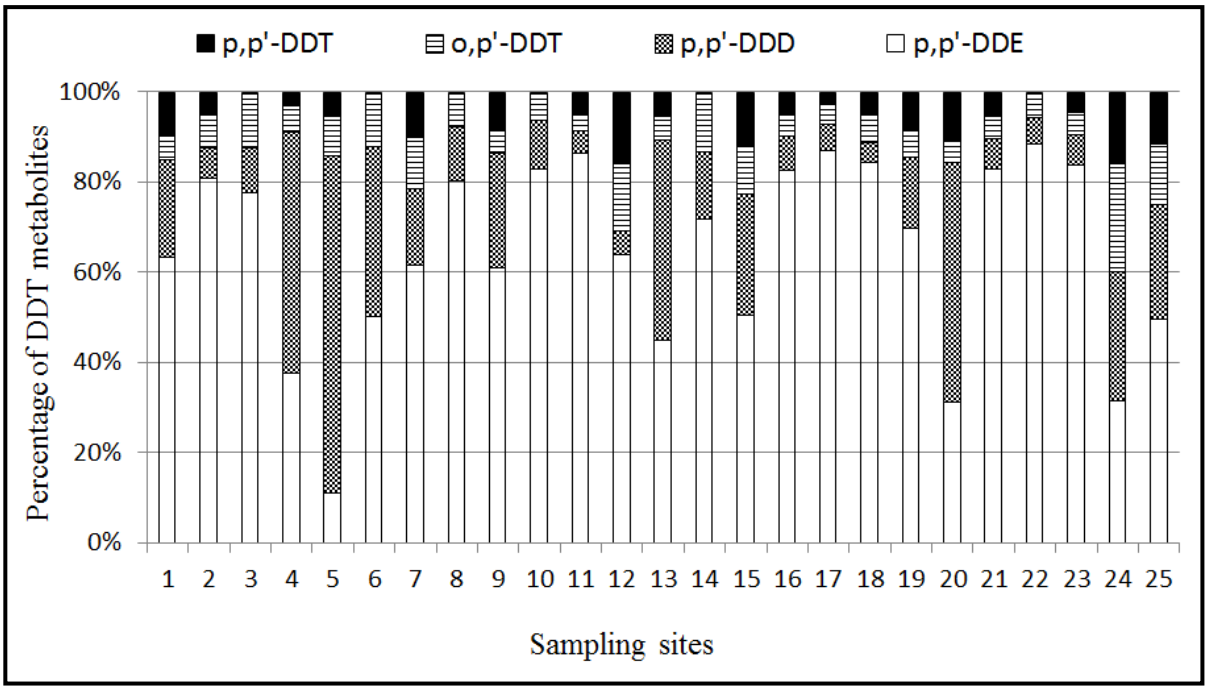

Fig. 3. Percentage composition of DDTs in surface sediments from Lake Awassa

Heavy metals concentration

Heavy metal concentrations in surface sediments from Lake Awassa are summarized in Table 2. The measured metal concentrations varied greatly and their mean values followed the order as Zn>Ni>Pb>Cu>Cr>Co>As>Cd>Hg. The micro nutrient, Zn presented highest level (mean 93.8 mg/kg dry wt.) followed by Ni (20.2 mg/kg dry wt.); whereas Cd and Hg presented the lowest values. The Kolmogorov-Smirnov test confirmed that the heavy metal concentrations in the samples were normally distributed with the exception of As, Co and Cr. According to sediment quality guidelines (SQGs) for freshwater ecosystems (CCME, 2002), almost all heavy metals levels did not exceed the interim sediment quality guidelines (ISQG) except for As (Table 2). Thus the raw data sets for Hg, Cd, Cr, Co, Cu, Pb, Ni and Zn conformed to the background population, whereas As presented anomalous values that was identified as an outlier as it had large variation of coefficient (Table 2).

Table 2. Descriptive statistics of heavy metal contents (mg/kg dry weight) in surface sediments of Lake Awassa

Metals	Mean ± SD	Median	Range	VC	Skewness	K-S test	ISQG	PEL
Hg	0.03 ± 0.02	0.03	0.01 - 0.08	0.58	0.76	**0.200**	0.17	0.486
Cd	0.21 ± 0.10	0.22	0.04 - 0.44	0.49	0.07	**0.138**	0.59	3.50
As	4.02 ± 3.70	2.65	0.84 - 18.2	0.92	1.10	0.012	5.90	17
Co	5.49 ± 1.94	6.34	1.28 - 7.40	0.35	-1.02	0.000	na	na
Cr	8.27 ± 2.58	9.81	1.95 - 10.2	0.31	-1.37	0.000	37.3	90
Cu	8.69 ± 3.75	9.60	2.40 - 13.6	0.43	-0.26	**0.086**	35.7	197
Pb	15.7 ± 6.53	17.7	2.65 - 24.2	0.41	-0.64	**0.104**	18	36
Ni	20.2 ± 11.1	19.8	2.46 - 37.2	0.55	0.05	**0.200**	na	na
Zn	93.8 ± 49.6	98.8	17.6 - 173.6	0.53	-0.19	**0.200**	123	315

Mean ± standard deviation.

VC: variation coefficient.

K-S test: Kolmogorov-Smirnov test.

Bold type means the data are normally distributed.

ISQG: interim sediment quality guideline; PEL: Probable Effect Level (CCME 2002).

na: not available.

Correlation among heavy metals

Correlation analysis was performed in order to check for the existence of correlations among metals at different sampling sites and to determine whether the said correlations existed between metals and organic matter. The results of Pearson's correlation coefficients are illustrated in Table 3. The analysis revealed significant ($p < 0.01$) correlations with high positive correlations between metals (e.g., Cr vs. Co, r = 0.920; Cu vs. Co, r = 0.866; Cu vs. Cr, r = 0.861; Ni vs. Cu, r = 0.921; Zn vs. Pb, Zn vs. Cu, r = 0.929; r = 0.837 and Zn vs. Ni, r = 0.942). These high correlations between specific heavy metal pairs in the sediments indicated that these metals had the same distribution characteristics or may reflect similar levels of contamination and/or release from the same source of pollution (Li et al., 2009; Yi et al., 2011). Similarly, Hg showed positive correlations with Cd, Co, Cr, Cu and Ni whereas arsenic (As) did not show any significant correlations with all metals (Table 3). The results of the Pearson correlation also indicated positive correlations ($p < 0.05$) between metals and OM except for As, meaning that organic matter had some influence on the distribution of metals in sediments (Amina et al., 1999).

Table 3. Pearson's correlation matrix between heavy metal concentrations and OM in surface sediments from Lake Awassa

	Hg	Cd	As	Co	Cr	Cu	Pb	Ni	Zn	OM
Hg	1	0.573**	0.297	0.505*	0.432*	0.527**	0.310	0.438*	0.311	0.847**
Cd		1	0.436*	0.631**	0.605**	0.680**	0.767**	0.615**	0.651**	0.634**
As			1	0.060	0.039	0.062	0.327	-0.127	-0.078	0.291
Co				1	0.920**	0.866**	0.715**	0.837**	0.860**	0.598**
Cr					1	0.861**	0.756**	0.801**	0.859**	0.575**
Cu						1	0.798**	0.921**	0.929**	0.655**
Pb							1	0.778**	0.837**	0.483*
Ni								1	0.942**	0.586**
Zn									1	0.514**
OM										1

Level of significance: *$p < 0.05$; **$p < 0.01$.

Correlations between As and other heavy metals were very low, suggesting that the pollution sources of As differed from those of other metals. The differences in correlations between the elements in sediment depends on physical, chemical and biological processes in the aquatic environment as well as discharging of pollutants through the sewage and other anthropogenic activities and their effects on the partitioning of elements in the aquatic system (Baeyens et al., 1998). Thus, in order to understand more about the distribution behavior of metals and possible source of pollution, principal component analysis (PCA) was applied.

Principal component analysis (PCA)

PCA has been applied to assist in identification of pollution by heavy metals from lithogenic action and/or anthropogenic sources (Zhou et al., 2007; Rodriguez et al., 2008). The results of PCA for heavy metal concentrations are listed in Table 4. According to these results, a two-component model, which accounted for 83.2% of all of the data variation, is used to group the elements. The initial component matrix indicated that Co, Cr, Cu, Ni, Zn and Pb were associated; displaying high values in the first component (F1, a contribution rate of 67.3%). The initial component matrix can be considered to be a lithogenic component, as the variability of the elements seems to be controlled by parent rocks as their concentration is low (Mico et al., 2006). These results imply that mineral weathering may be the main source of pollution for Co, Cr, Cu, Ni, Zn and Pb in the sediments of Lake Awassa. The Ethiopian Rift Valley region is known for a number of hot springs within the catchments of the lakes and these hot springs are sources and means of transport of metals from the rocks (Zinabu and Pearce, 2003). Cd, Hg and partially Pb have loading factors in the first and second principal components, indication of a mixed source from both lithogenic and anthropogenic inputs (e.g., agricultural practices, industrial activities, and fuel combustion or traffic). On the other hand, the second principal component matrix (F2, a contribution rate of 15.9%) showed high value for As, suggesting a pollution source of mainly anthropogenic sources.

Lake Awassa is likely to be affected by industrial effluents because its in-flow (Tikur Wuha River) receives effluents from the Awassa textile factory, ceramics and sisal factories (Zinabu and Zerihun, 2002; Desta, 2003). The rotation of the matrix show similar grouping with initial component matrix (contribution of 61.8%) for most of the metals. The second

component (F2, a contribution rate of 21.4%) on the rotation matrix showed much higher values for As than the other heavy metals, while Cd and Hg were grouped in both rotated component matrix.

Table 4. Total variance explained and component matrixes for heavy metals in surface sediments from Lake Awassa

Component	Initial eigen values			Extraction sum of squared loadings			Rotation sums of squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	6.06	67.3	67.3	6.06	67.3	67.3	5.56	61.8	61.8
2	1.43	15.9	83.2	1.43	15.9	83.2	1.93	21.4	83.2
3	0.734	8.16	91.4						
4	0.342	3.79	95.2						
5	0.200	2.22	97.4						
6	0.104	1.16	98.6						
7	0.079	0.880	99.4						
8	0.031	0.345	99.8						
9	0.021	0.230	100						

Elements	Component matrix		Rotated component matrix	
	F1	F2	F1	F2
Co	0.922	-0.108	0.906	0.201
Cr	0.910	-0.142	0.906	0.165
Cu	0.957	-0.100	0.937	0.219
Ni	0.922	-0.275	0.961	0.043
Zn	0.938	-0.273	0.976	0.050
Pb	0.878	0.131	0.787	0.412
Cd	0.794	0.426	0.611	0.663
Hg	0.559	0.432	0.386	0.592
As	0.150	0.923	-0.161	0.921

Extraction method: Principal component analysis; Rotation method: Varimax with Kaiser normalization.

Ecological risk assessment

Numerical sediment quality guidelines (SQGs) can be used to evaluate the degree to which the sediment-associated chemical status might adversely affect aquatic organisms and be designed to aid in the interpretation of sediment quality (Wenning and Ingersoll, 2002). To evaluate the eco-toxicological significance of DDTs contamination in surface sediments from Lake Awassa, the results were compared with the Canadian environmental quality guideline for fresh water sediment (CCME, 2002) and shown in Fig. 4.

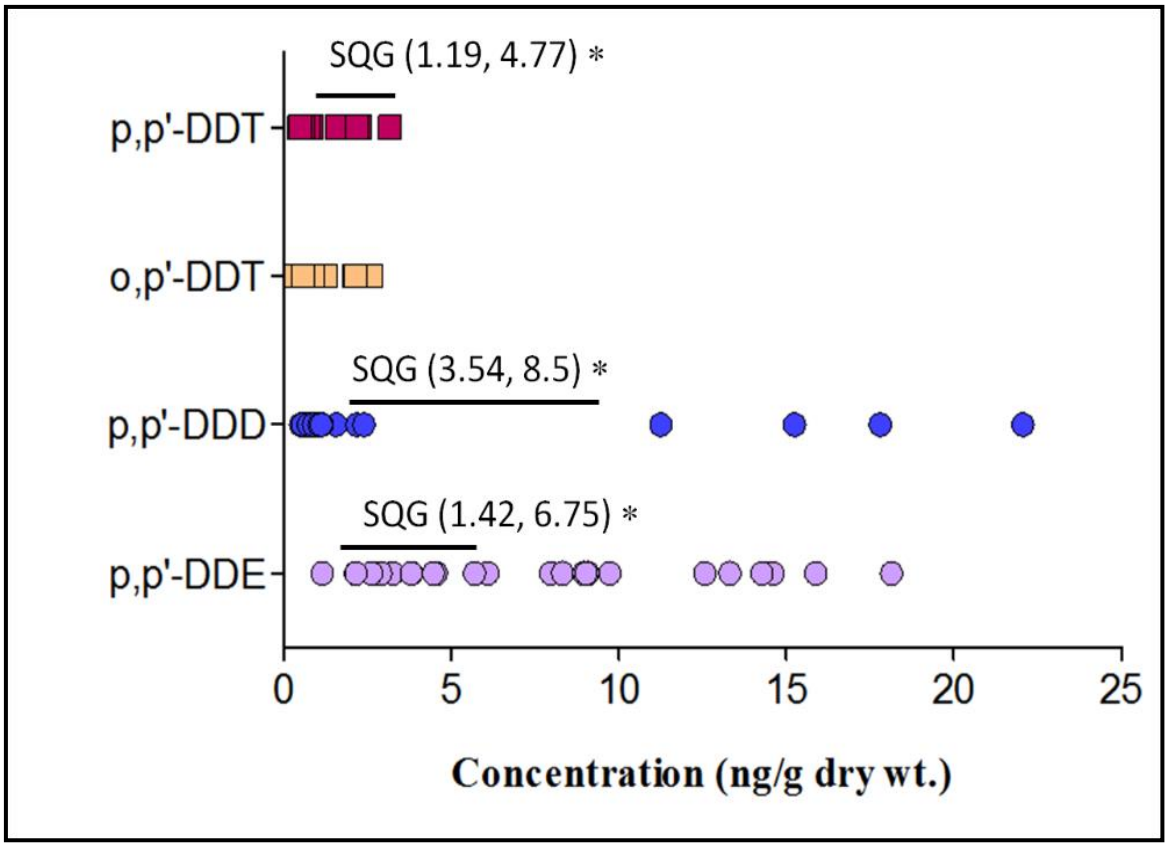

Fig. 4. Comparison of DDT concentrations with the Canadian Environmental Quality Guideline for Sediment (CCME 2002): ∗(ISQG, PEL); interim sediment quality guideline and probable effect level

The “probable effect level” (PEL) is used as it represents the concentration above which adverse effect would frequently occur. Concentrations of *p,p′*–DDE and *p,p′*–DDD in this study exceeded the PEL at 11 sites and 4 sites, respectively. This suggests that DDTs are of concern in Lake Awassa and it is necessary to continue monitoring the possible sources in the catchment area as they could cause problems for fish and wildlife through trophic transfer. Generally, further follow up studies on persistent organic pollutants (POPs) from biota of Lake Awassa are needed.

For sediment risk assessment, the potential ecological risk index (R_I) was used to assess the degree of heavy metal contamination in surface sediments proposed by Hakanson (1980) expressed as:

$R_I = \sum E_r^i = \sum T_r^i C_f^i$

$C_f^i = C_0^i / C_n^i$

Where R_I is the sum of potential risk of individual heavy metal; E_r^i is the potential risk factor of individual heavy metal; T_r^i is the toxic-response factor for a given substance which can reflect the level of trace element toxicity. The standardized response coefficients for the toxicity of trace elements are: Hg = 40, Cd = 30, As = 10, Cu = 5, Pb = 5, Cr = 2 and Zn = 1, respectively (Hakanson, 1980). C_f^i is the contamination factor; C_0^i is the present concentration of heavy metals in the sediment and C_n^i is a reference value for metals (e.g., Hg = 0.25, Cd = 1.0, As = 15, Cu = 50, Pb = 70, Cr = 90 and Zn 175 in mg/kg) (Hakanson, 1980). The potential ecological risk indices (E_r^i) were classified as: low ($E_r^i < 40$), moderate ($40 \leq E_r^i \leq 80$), high ($80 \leq E_r^i \leq 160$), very high ($160 \leq E_r^i \leq 320$) and serious ($E_r^i > 320$). Similarly, potential toxicity response indices (R_I) were classified as: low ($R_I < 150$), moderate ($150 \leq R_I \leq 300$), severe ($300 \leq R_I \leq 600$) and serious ($R_I > 600$). The calculated ecological risk indices (E_r^i and R_I) results of heavy metals in sediments of Lake Awassa are summarized in Table 5. The potential ecological risk indices were low. It is found that the potential ecological risk indices (E_r^i) of heavy metals were ranked in the order of Cd > Hg > As > Pb > Cu > Zn > Cr. All of the E_r^i values for the heavy metals studied in this investigation were lower than 40, suggesting a low ecological risk for the water body. The sediment R_I values ranged from 4.95 to 34.1 were also less than 150. These results indicate that surface sediments in Lake Awassa posed a low ecological risk.

Table 5. Summarized potential ecological risk indices and potential toxicity of heavy metals for surface sediments from Lake Awassa

	E_r^i							R_I
	Hg	Cd	As	Cu	Pb	Cr	Zn	
Mean	5.27	6.22	2.68	0.87	1.12	0.18	0.54	16.9
Minimum	1.12	1.29	0.56	0.24	0.19	0.04	0.10	4.95
Maximum	12.8	13.1	12.16	1.36	1.73	0.23	0.99	34.1

E_r^i: The calculated potential ecological risk indices of heavy metals.

R_I: The sum of potential risk of individual heavy metal.

Conclusions

The results obtained from this study are the first data on the levels and distribution of DDTs and heavy metals in surface sediments from Lake Awassa in Ethiopia. On the basis of investigations from 25 sampling sites, levels of DDTs varied depending on sampling sites indicating variable sources in different locations. GIS analysis demonstrated that high levels of DDTs were observed in the vicinity of the inflow river side and close to agriculture areas. Evaluation of eco-toxicological significance of DDT contamination showed a concern in Lake Awassa and it is necessary to continue monitoring the possible sources in the catchment area as they could cause problems in the biota of the lake. The PCA performed on nine heavy metals identified two principal components controlling their variability in the surface sediment. Levels of Co, Cr, Cu, Pb, Ni and Zn were associated in the first principal component, determined dominantly by soil parent rocks; lithogenic source while Cd and Hg have loading factors in both principal components, indication of a mixed source both from lithogenic and anthropogenic inputs. Analyses of the potential ecological risk of sediment heavy metal concentrations showed that the values of the potential ecological risk indices were low, suggesting a low ecological risk for the water body.

Chapter 3:

Ecological Risk Assessment of OCPs from Lake Ziway, Ethiopia

3.1.

Concentrations and human health risk assessment of organochlorine pesticides in edible fish species from a Rift Valley Lake – Lake Ziway, Ethiopia

Abstract

Fish consumption is known to have several health benefits for humans. However, the accumulation of organic pollutants, like organochlorine pesticides (OCPs) could pose health hazards. Thus, OCPs in edible fish species (*Oreochromis niloticus, Tilapia zillii*, *Carassius* spp., and *Clarias gariepinus*) from Lake Ziway, an Ethiopian Rift Valley Lake were investigated to assess the potential human health hazards of these contaminants. Dichlorodiphenyltrichloroethanes (DDTs), hexachlorocyclohexanes (HCHs), chlordanes, and heptachlors were observed with ΣOCPs concentration ranging from 1.41 to 63.8 ng/g ww. DDTs were the predominant contaminants (0.9 to 61.9 ng/g ww), followed by HCHs. The predominance of DDTs may be attributed to their current use in vector control and contamination from past usage. The estimated daily intakes (EDIs) of OCPs from all fish species were much lower than the acceptable daily intakes (ADIs), indicating that consumption of fish is at little risk to human health at present. However, the cancer risk estimates in the area of concern and the hazard ratios (HRs) of HCHs, DDTs, and heptachlors exceeded the threshold value of one, indicating daily exposure to these compounds is a potential concern. This may result in a lifetime cancer risk of greater than 1 in 10^6.

Keywords: Organochlorine pesticides, Fish, Lake Ziway, Risk assessment

Introduction

Organochlorine pesticides (OCPs) have been widely used and become a worldwide concern due to their persistence, bioaccumulative potential, chronic toxicity, and potential negative impacts on humans and wildlife (UNEP, 2001). It is known that most of the total intake of pesticide residues by human beings is through the food chain (Martinez et al., 1997). Fish are known to biomagnify pesticides from the surrounding environment (Mackay and Fraser, 2000), and transfer the pesticides to humans when consumed. Epidemiological studies indicate that some of these compounds may be associated with cancers in humans (Snedeker, 2001; Beard, 2006; IARC, 2008), and also influence the concentration of thyroid hormones (Meeker et al., 2007). Eskenazi et al. (2006) reported delays in neurodevelopment during early childhood due to the impacts of prenatal exposure to dichlorodiphenyltrichloroethanes (DDTs).

Although the use of OCPs has been banned or restricted, developing countries like Ethiopia still use them for agricultural and health purposes, and as a consequence they can be found in aquatic (Deribe et el., 2011; Yohannes et al., 2013a,b) and terrestrial ecosystems, for example in cow's milk (Gebremichael et al., 2013). Because it is landlocked, Ethiopia is highly dependent on lake aquatic environments for its economic development. The Ethiopian Rift Valley region, encompassing seven principal lakes, is a densely populated area confined with various agricultural activities where there is still an increasing trend of pesticide usage (Amera and Abate, 2008). Moreover, Ethiopia has implemented indoor residual spraying (IRS) with DDT for malaria control in the past few decades (WHO, 2007). Approximately 400 metric tons of active-ingredient DDT per year is used for IRS in many parts of the country including the Rift Valley, a malaria epidemic prone region (Biscoe et al., 2005; van den Berg, 2009). In addition, Ethiopia is one of the many African countries burdened with the problem of obsolete pesticides, which have been accumulated since the first imports in the 1960s (Haylamicheal and Dalvie, 2009). These were mostly organochlorine compounds such as chlordane, DDT, dieldrin and lindane that are banned or restricted in most countries. In this view, there is great likelihood that the Ethiopian Rift Valley ecosystem is exposed to large amounts of pesticides.

Lake Ziway, one of the Ethiopian Rift Valley lakes, is located in an area with many agricultural activities but few soil conservation efforts in its catchment area. Intensive agriculture in the proximity of the lake and municipal waste discharges are sources of pollution into this fresh water ecosystem (Jansen and Hengsdijk, 2006). It is therefore necessary to evaluate the current status of the OCPs in different fish species from Lake Ziway. A recent study on the lake examined only the levels and biomagnification of DDTs (Deribe et al., 2013). No other studies have been carried out on the levels and risk assessment of other OCPs in the lake.

Therefore, objectives of this study are to assess the accumulation levels of OCPs in edible fish species collected from Lake Ziway and to evaluate the potential risks to human health posed through dietary consumption of these fish. This study gives a comprehensive overview of OCPs' status in the fish species of different trophic levels in Lake Ziway and provides a basis for decision-makers to take effective measures aimed at mitigating potential health and ecological risks.

Materials and methods

Study area

The study area, Lake Ziway (surface area: 434 km^2) is a shallow freshwater lake located in the northern section of the Rift Valley (Fig. 1). It is fed by two inflowing rivers, the Meki River from the north-west and the Katar River from the east, and drains towards the Lake Abijata, through the Bulbula River. The lake has a large littoral zone containing emergent and submergent vegetation, which provides feeding, breeding and nursery habitats for fish (Admassu and Ahlgren, 2000; Erko et al., 2006). Lake Ziway contains different fish species including Nile tilapia (*Oreochromis niloticus*), Redbelly tilapia (*Tilapia zillii*), African big barb (*Barbus intermedius*), African sharptooth catfish (*Clarias gariepinus*), and Carp spp. (*Carassius carassius* and *Carassius auratus*) (Lemma, 2005). Fisheries on Lake Ziway are an open and easily accessible source of income and have always been a source of food and income for the people living on the shores of the lake. The landings of Lake Ziway used to be dominated by *O. niloticus*, but species of *C. gariepinus*, *T. zillii*, and *Carassius* spp. (*C. carassius* and *C. auratus*) are increasingly becoming a part of the catch. The potential yield

of all the species of the lake is estimated to range between 2,500 and 6,680 tons/yr (Spliethoff et al., 2009).

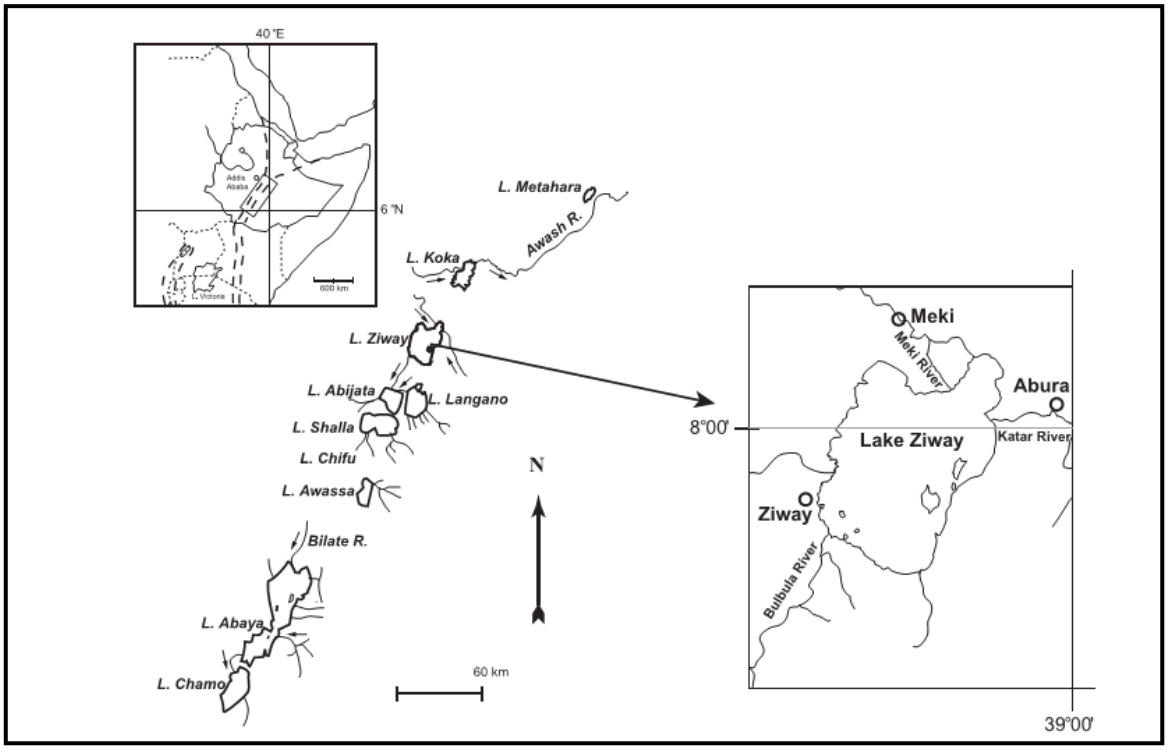

Fig. 1. Ethiopian Rift Valley lakes and the map of Lake Ziway (Deribe et al., 2013)

Sampling

A total of 100 individual fish belonging to *O. niloticus*, *T. zillii*, *Carassius* spp., and *C. gariepinus* fish species of Lake Ziway were purchased from the local fishermen in January 2011. Samples were transported to Ziway fisheries research laboratory where the body size and body weight were recorded. General information about the fish is given in Table 1. Fishes were dissected to obtain dorsal muscles and stored at −20 °C. The frozen samples were then transported to Japan for analysis. Each individual sample was lyophilized, homogenized separately and used for chemical analysis.

Table 1. Biometry data of fish species in this study from Lake Ziway

Fish species	N	Length (mm) Mean ± SD Min–max	Weight (g) Mean ± SD Min–max	Lipid content (%) Mean ± SD Min–max	Main food*
O. niloticus	27	213 ± 28 167–270	315 ± 111 178–554	[a] 0.75 ± 0.68 0.10–3.60	Blue green algae, detritus, macrophytes
T. zillii	19	174 ± 21 120–205	199 ± 59 111–312	[a] 0.90 ± 0.48 0.18–2.13	Macrophytes
***Carassius* spp.**	27	267 ± 39 160–332	585 ± 230 231–1199	[a] 0.87 ± 0.59 0.15–2.14	Macrophytes, detritus, green algae
C. gariepinus	27	353 ± 88 235–560	559 ± 454 154–1910	[a] 1.34 ± 2.52 0.23–5.3	Insect, fish eggs, fish, gastropods

N = number of samples.

[a] Means with different letter superscript are significantly different (Tukey test: $p < 0.05$).

* Reference: Deribe et al., 2013.

OCPs analysis

Samples were processed and analyzed using a method described by Yohannes et al. (2013a) with slight modifications. Approximately 10 g of muscle tissue from each fish was taken and mixed with anhydrous sodium sulfate. After spiking with the surrogate standard of 2,4,5,6-tetrachloro-*m*-xylene (TC*m*X), each sample was extracted using Soxtherm apparatus (S306AK Automatic Extractor, Gerhardt, Germany) with *n*-hexane:acetone (3:1, *v/v*) for 4 h. An aliquot of the extract (20%) was used for lipid measurement using gravimetric method. The remaining extract was applied to a column filled with 6 g florisil (activated at 150 °C overnight) for clean-up and eluted with a mixture of *n*-hexane:dichloromethane (7:3, *v/v*). The eluate was concentrated to 2 mL on rotary evaporator, and further to near dryness under gentle nitrogen flow. Finally, the extract was redissolved in 100 µL *n*-decane, and the internal standard pentachloronitrobenzene was added before instrumental analysis.

OCPs including DDTs (*o,p′*–DDT, *p,p′*–DDT, *o,p′*–DDE, *p,p′*–DDE, *o,p′*–DDD and *p,p′*–DDD), hexachlorocyclohexanes (HCHs; α–, β–, γ– and δ–HCH), heptachlors (HPTs; heptachlor, *cis*– and *trans*–heptachlor epoxide), chlordanes (CHLs; *cis*– and *trans*–chlordane, *cis*– and *trans*–nonachlor and oxychlordane), drins (aldrin, dieldrin and endrine) and hexachlorobenzene (HCB) were analyzed by gas chromatography equipped with an electron capture detector (Shimadzu GC–2014, Kyoto, Japan). An ENV–8MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with splitless injection was used to separate OCPs. One μL of each sample was injected. The column oven temperature was initially set at 100 °C for 1 min, increased to 180 °C at 20 °C/min and then to 260 °C at 4 °C/min, which was held for 5 min. The injector and detector temperatures were 250 °C and 310 °C, respectively. Helium at a flow rate of 1.0 mL/min and nitrogen at 45 mL/min were used as carrier gas and make-up gas, respectively.

Quality control and quality assurance

OCPs were identified by comparing their retention time with the reference to the corresponding standards. Multi-level calibration curves were created for the quantification and linearity ($R^2 \geq 0.995$) was achieved. Quality control was performed by analysis of procedural blanks and spiked blanks. Results showed that no target analysts were detected in blank samples and recoveries for spiked blanks ranged from 90% to 105%. The recovery rate of the surrogate, TC*m*X was 85 ± 11%. To check for the validity of the method used for the extraction and analysis of the samples, the standard reference material SRM 1947 (Lake Michigan Fish Tissue) was analyzed during the analysis of samples, and the recoveries ranged from 85% to 105% with RSD < 10%. The values reported here were not corrected for recoveries. Detection limits based on 3:1 signal to noise ratio (S/N) were between 0.05 and 0.1 ng/g for all OCPs. Concentrations were expressed on a wet weight (ww) basis.

Risk assessment

Various international organizations have subsequently established a series of standards and instructions to estimate the risks to human health from environmental pollutants in fish (USEPA, 2013). A straight forward risk assessment is performed through comparison with the levels set by laws and guidelines. However, this comparison was made without the consideration of factors like different eating habits and consumption rates. Thus, in this study, we investigated the risk assessment by two approaches. To comprehensively evaluate the health risk assessment, the 50^{th} and 95^{th} percentile measured concentrations were used.

Estimated daily intake (EDI)

Estimated dietary intakes of OCPs were calculated as follows:

$EDI = (C \times DR) / BW$

where C is the measured concentration of OCPs (ng/g ww), DR is average daily consumption rate of fish (g/day) and BW is body weight (kg), which was set at 60 kg (WHO, 2010). The average daily consumption rate was derived from FAO (2011). Though Ethiopians are traditionally meat-eaters, eating habits have been shifting in favor of fish in areas and communities where there is regular and sufficient supply. In those communities, annual fish consumption can exceed 10 kg/person (FAO, 2011). Thus, the DR was estimated at 30 g/day per person.

Potential carcinogenic risks

To assess public health risks posed through fish consumption, the cancer risk estimates and hazard ratios (HRs) were assessed on the basis of the guidelines of the United States Environmental Protection Agency (USEPA). Cancer risks associated with OCPs were estimated by combining the exposure dose and slope factor (USEPA, 2005). A public screening criteria for carcinogens is set at a carcinogenic risk level of 10^{-6}. Carcinogenic risks below 10^{-6} are considered acceptable, while carcinogenic risks above 10^{-4} are considered unacceptable. An area of concern is present between 10^{-6} and 10^{-4} (USEPA, 2005).

HR for cancer risks was assessed by comparing the EDI with the benchmark concentration (BMC) (Solomon et al., 2000; Jiang et al., 2005) using the following equation:

HR = EDI / BMC

The BMC for carcinogenic effects was derived from the cancer slope factor (CSF), which was obtained from the USEPA (USEPA, 2012). The BMC for carcinogenic effects represents the exposure concentration at which lifetime cancer risk is one in a million for lifetime exposure. A hazard ratio that is greater than one indicates that there is potential risk to human health (Dougherty et al., 2000).

Statistical analysis

Statistical analysis was performed using JMP 9 (SAS Institute, Cary, NC, USA). Descriptive statistics using one-way analysis of variance (ANOVA) were used to characterize the levels of OCPs in the studied fish species. Concentrations below the limit of detections were given a value of zero. Multiple comparisons among the fish species were tested using Tukey's HSD post hoc test. A significant level of $p < 0.05$ was used.

Results and discussion

The length and weight of the studied fish species varied from 120 to 560 mm and from 111 to 1910 g, respectively (Table 1). A continuous increase in length and weight was observed for all individuals with a significant and positive correlation (R^2 = 0.70, p < 0.001). The mean lipid content was in the range 0.75 ± 0.68% to 1.34 ± 2.52, and there was no significant difference (p > 0.05) among the studied fish species (Table 1). There was no significant correlation between the biometric data and lipid content (p > 0.05).

Levels of OCPs

OCPs were detected in muscle samples of all fish species, indicating their widespread contamination in Lake Ziway. DDTs, HCHs, HPTs, and CHLs were detected with varying concentrations (Table 2). The total concentrations of OCPs ranged from 1.41 to 63.8 ng/g ww, with a mean concentration of 7.72 ± 6.90 ng/g ww. The highest concentrations of OCPs were found in *C. gariepinus* (p < 0.05), which is a carnivorous fish and found at top trophic position. Among the OCPs analyzed, DDTs were the most commonly detected and were dominant in all samples. It accounted for 64.5 ± 10% (SD) (ranging from 52 to 78%) of the total OCPs. In general, the contamination pattern of OCPs in fish samples detected in this study was in the order of DDTs > HCHs > CHLs ≅ HPTs.

This result indicates the high degree of exposure to DDTs in biota from the Ethiopian Rift Valley region, which is most likely due to recent use of DDT for malaria control through IRS (Biscoe et al., 2005; van den Berg, 2009) as well as from past usage and spills from obsolete pesticides (Haylamicheal and Dalvie, 2009). It is also reported that DDT is still ongoing use by farmers in the region (Amera and Abate, 2008). Log transformed OCPs show a positive correlation with total length for all fish species (R^2 = 0.18; p < 0.001), whereas no significant correlation was found between lipid content and concentration of OCPs (R^2 = 0.00; p = 0.140).

Table 2. Levels of OCPs (ng/g wet weight) in muscle of four fish species from Lake Ziway

	O. niloticus	*T. zillii*	*Carassius* spp.	*C. gariepinus*
α-HCH	0.22 ± 0.06	0.19 ± 0.03	0.25 ± 0.09	0.27 ± 0.12
β-HCH	ND	0.31 ± 0.09	0.03 ± 0.11	ND
γ-HCH	0.67 ± 0.33	0.68 ± 0.52	0.22 ± 0.18	0.47 ± 0.42
δ-HCH	ND	0.27 ± 0.04	0.11 ± 0.21	ND
∑HCHs	[a] 1.26 ± 1.04	[a] 1.45 ± 0.61	[b] 0.61 ± 0.31	[b] 0.72 ± 0.47
*	029–5.10	0.91–3.54	0.16–1.85	0.27–2.01
Heptachlor	ND	ND	ND	ND
cis-heptachlor-epoxide	0.57 ± 0.27	0.08 ± 0.10	0.24 ± 0.11	0.23 ± 0.11
trans-heptachlor-epoxide	0.32 ± 0.09	0.20 ± 0.02	0.31 ± 0.23	0.42 ± 0.29
∑HPTs	[a] 0.90 ± 0.35	[c] 0.42 ± 0.11	[b,c] 0.59 ± 0.27	[b] 0.65 ± 0.28
*	0.44–2.27	0.19–0.69	0.20–1.52	0.34–1.56
oxy-chlordane	0.04 ± 0.04	0.16 ± 0.07	0.11 ± 0.06	0.10 ± 0.07
cis-chlordane	0.18 ± 0.04	0.26 ± 0.11	0.12 ± 0.07	0.16 ± 0.05
trans-chlordane	0.16 ± 0.05	0.20 ± 0.03	0.26 ± 0.21	0.29 ± 0.12
trans-nonachlor	0.03 ± 0.07	0.29 ± 0.10	0.37 ± 0.59	0.35 ± 0.23
∑CHLs	[b] 0.40 ± 0.10	[a] 0.91 ± 0.22	[a] 0.87 ± 0.82	[a] 0.90 ± 0.25
*	0.17–0.61	0.65–1.32	0.19–4.00	0.58–1.50
p,p′-DDE	1.32 ± 0.81	1.89 ± 2.02	2.42 ± 1.60	6.92 ± 11.47
o,p′-DDE	0.10 ± 0.08	0.35 ± 0.12	0.26 ± 0.36	0.12 ± 0.10
p,p′-DDD	0.40 ± 0.21	0.85 ± 0.41	0.58 ± 0.35	0.79 ± 0.68
o,p′-DDT	0.43 ± 0.17	0.53 ± 0.21	0.68 ± 0.74	0.43 ± 0.11
p,p′-DDT	0.31 ± 0.18	0.77 ± 0.66	0.57 ± 0.73	0.62 ± 0.40
∑DDTs	[b] 2.33 ± 1.09	[a,b] 4.38 ± 2.67	[a,b] 4.55 ± 2.80	[a] 9.0 ± 11.7
*	0.90–5.12	1.35–13.2	0.77–10.6	2.36–61.9
∑OCPs	[b] 4.89 ± 1.85	[a,b] 7.16 ± 2.63	[a,b] 6.62 ± 3.71	[a] 11.2 ± 11.7
*	2.46–10.9	3.59–15.2	1.41–15.0	4.00–63.8

ND = below detection limit.

Mean ± standard deviation.

* Min–max.

Values with different letters (a, b, c) within a row are significantly different at $p < 0.05$ level (Tukey test is applied).

DDTs

DDT and its metabolites were detected in all fish species (Table 2). Concentrations of DDTs in the muscle tissue are found at large variations ranging from 0.77 to 61.9 ng/g ww (mean concentration of 5.27 ± 6.73 ng/g ww). *C. gariepinus* with 9.0 ± 11.7 ng/g ww and *O. niloticus* with 2.33 ± 1.09 ng/g ww had the highest and lowest concentrations, respectively. This may be attributed to their different feeding habits because *C. gariepinus* is a carnivorous and *O. niloticus* is almost herbivorous fish species (Table 1). Overall, the concentrations of DDTs were higher than those of other OCPs. The possible reasons for the presence of high level of DDTs in the region may be its current use in vector control, illegal usage and contamination from obsolete pesticides (Haylamicheal and Dalvie, 2009; van den Berg, 2009). Reports from other African lakes also indicate much higher levels of DDT in aquatic organisms compared to other OCPs. In Lake Koka, Ethiopia DDT ranged from 0.05 to 72.53 ng/g ww and it was the predominant pesticide, with mean concentrations a factor of 10 times higher than other OCPs (Deribe et al., 2011) and in Lake Malawi DDT concentrations were up to 60 times higher than other OCPs has been reported for the biota (Kidd et al., 2001). Concentrations of DDTs found in this study (mean concentration of 2.33 to 9.0 ng/g ww) are higher than those found in Lake Victoria, Uganda (mean 1.39 to 1.67 ng/g ww) (Kasozi et al., 2006). However, they are lower than those in fish from Southern Lake Victoria, Tanzania (mean 15 and 20 ng/g ww) (Henry and Kishimba, 2006) and fish from Lake Burullus, Egypt (mean 2.76 to 45.13 ng/g ww) (Said et al., 2008), and comparable to fish from Lake Awassa, Ethiopia with ΣDDTs mean concentration of 1.80 and 9.0 ng/g ww for *O. niloticus* and *C. gariepinus*, respectively (Yohannes et al., 2013a). Direct comparisons should be made with caution since these studies were conducted on different species. With all the data pooled together, the concentration of DDTs (log transformed) was significantly correlated ($R^2 = 0.18$; $p < 0.001$) to total length of the fish, but not with % lipid content ($R^2 = 0.02$; $p = 0.139$).

The composition profiles of DDTs in the muscle tissue of the four fish species are shown in Fig. 2. Among the metabolites, *p,p′*–DDE was the predominant congener, accounting for 55% ± 15.72 (from 41 to 77%), followed by *p,p′*–DDT (15% ± 6.42), and *p,p′*–DDD (13% ± 4.80). The proportion of *p,p′*–DDE was higher in *C. gariepinus* than in the others,

comprising 77% of the mean DDT concentrations, showing that *C. gariepinus* found at high trophic level is more likely feeding on prey (both fish and invertebrates) and accumulates DDE, a more degraded form of DDT. In addition, this may be attributed to the more persistent nature of *p,p′*–DDE, and to its rate of biomagnification along the food chain in freshwater ecosystems (Rognerud et al., 2002). In contrast, the proportions of parent compounds (*o,p′*– and *p,p′*–DDT) in *O. niloticus* (29.8%), *T. zillii* (32.6%), and *Carassius* spp. (34.4%) were higher than in *C. gariepinus* (11.7%). This may be probably as a result of more efficient transfer of DDT to phytoplankton and macrophyte consuming herbivorous fish (Zhou et al., 2007).

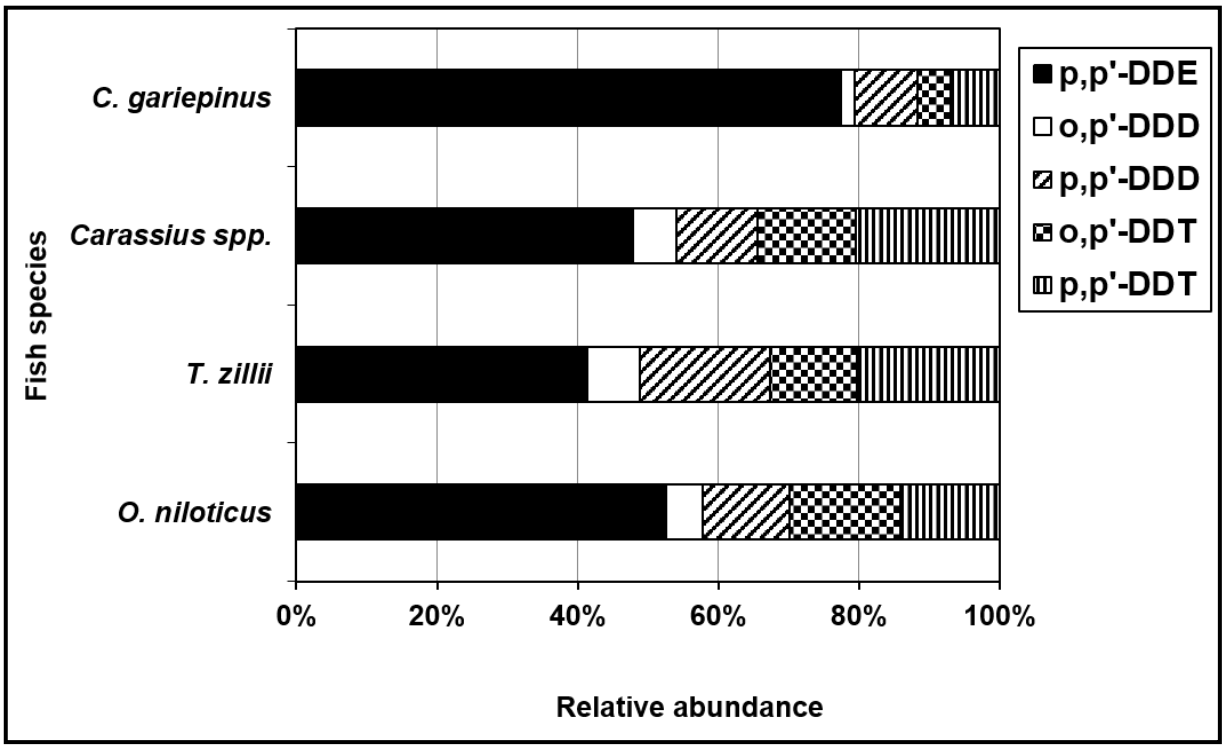

Fig. 2. Relative abundance of individual DDT components in four fish species from Lake Ziway

Technical DDT generally contains 75% *p,p′*–DDT, 15% *o,p′*–DDT, 5% *p,p′*–DDE, and <5% others. DDT can be metabolized into DDE under aerobic conditions or into DDD in anaerobic environments (Hitch and Day, 1992). Thus, the ratio of (*p,p′*–DDE + *p,p′*–DDD)/∑DDTs can indicate past or recent usage of technical DDT. A ratio greater than 0.5 generally indicates long term biotransformation of DDT, whereas a ratio of less than 0.5 may indicate recent input of DDT. In the present study, the ratio ranged from 0.55 to 0.87, suggesting that DDTs in fish from Lake Ziway were mainly due to historical use, and to its current use for vector control in the region since the Ethiopian government decided to continue using DDT because of the high incidence of malaria and the corresponding fatalities (Biscoe et al., 2005; WHO, 2007).

HCHs

HCHs were the second most prevalent OCP contaminants in the studied fish species and accounted for 17% (from 10% to 25%) of the total OCPs measured. The levels of HCHs in *T. zillii* (1.45 ± 0.61 ng/g ww) and *O. niloticus* (1.26 ± 1.04 ng/g ww) were significantly higher ($p < 0.05$) than that of *Carassius* spp. (0.61 ± 0.31 ng/g ww), and *C. gariepinus* (0.72 ± 0.47 ng/g ww) (Table 2). A negative relationship ($R^2 = 0.07$; slope = –0.02; $p < 0.01$) between log transformed ΣHCH and length of fish was found, whereas no significant relationship ($p > 0.05$) was found between lipid content and concentration of HCHs. Of the HCHs measured, α and γ–HCHs were frequently detected and the γ–isomer (lindane) was the predominant, accounting for 60% on an average of ∑HCHs in the muscle tissue. The higher γ–HCH concentrations in the samples indicate current usage of lindane around the lake. A recent study in the Ethiopian rift valley region also showed high concentrations of lindane in tissues taken from cattle with the highest level of 0.14 mg/kg ww in liver samples obtained from Holeta, Ethiopia (Letta and Attah, 2013). In general, the concentrations of HCHs in this study are lower than those in fish from Lake Taabo, Cote d'Ivoire (Roche et al., 2007), and fish from Lake Burullus, Egypt (Said et al., 2008).

CHLs and HPTs

With regard to the residual levels of CHLs, *trans*–chlordane, *cis*–chlordane and *trans*–nonachlor were detected in most of the samples as they are the dominant constituents in technical chlordane (Xu et al., 2004) whereas oxy-chlordane was rarely encountered. The presence of these compounds in the environment at relatively high concentrations as compared to oxy-chlordane likely indicates recent inputs of chlordane to the environment. The mean residual levels of CHLs in the muscle tissues in the present study ranged from 0.40–0.91 ng/g ww (Table 2). The use of chlordane is permitted in Ethiopia as a general insecticide (Ritter et al., 1995). Chlordane is imported to Ethiopia under the regulation of Ministry of Agriculture for termiticide usage only (EICDCR, 2004).

It was found that HPTs (*cis*–heptachlor epoxide and *trans*–heptachlor epoxide) were also present in most of the fish collected. The *cis* and *trans*–heptachlor epoxides predominated with a mean concentration of 0.28 ± 0.27 ng/g ww and 0.31 ± 0.21 ng/g ww, respectively (Table 2). The highest residual levels of HPTs (0.90 ± 0.35 ng/g ww) were found in *O. niloticus*, the herbivorous fish species.

Human health risk assessment

Fish consumption has been proven to be one of the major routes of human exposure to organic contaminants. To better understand the concentration levels, the concentrations of OCPs in the present study were evaluated against international existing limits. The EDI was calculated and compared with the acceptable daily intake (ADI) recommended by the Food and Agriculture Organization and the World Health Organization (FAO/WHO) Joint Meeting on Pesticide Residue (WHO, 2010). To comprehensively evaluate risk exposure, the 50th and 95th percentile EDIs of OCPs for each fish species were calculated. The EDIs of OCPs expressed as nanogram per kilogram body weight per day (ng/kg bw/d) through consumption of fish for the population are presented in Table 3. EDI of HCHs, HPTs, CHLs, and DDTs at both exposure levels were far below the ADI, indicating that consumption of fish at present would not pose a human health risk.

Table 3. Estimated daily intake values (ng/kg bw/d) of OCPs through the studied fish species by human

OCPs	ADI	50th (95th) percentile measured concentrations (ng/g ww)				50th (95th) estimated daily intakes			
		O. niloticus	*T. zillii*	*Carassius* spp.	*C. gariepinus*	*O. niloticus*	*T. zillii*	*Carassius* spp.	*C. gariepinus*
HCHs	5000 [a]	1.08 (3.39)	1.32 (2.60)	0.52 (1.20)	0.54 (1.53)	0.54 (1.70)	0.66 (1.30)	0.26 (0.66)	0.27 (0.77)
HPTs	100	0.86 (1.34)	0.42 (0.56)	0.53 (0.93)	0.60 (1.20)	0.43 (0.67)	0.21 (0.28)	0.27 (0.49)	0.30 (0.60)
CHLs	500	0.39 (0.60)	0.86 (1.26)	0.65 (2.41)	0.85 (1.41)	0.19 (0.30)	0.43 (0.63)	0.33 (1.20)	0.42 (0.72)
DDTs	10000	2.20 (4.49)	3.97 (8.23)	4.61 (9.21)	4.91 (21.21)	1.10 (2.24)	1.99 (4.12)	2.30 (4.61)	2.46 (10.61)

ADI = Acceptable daily intake (ng/kg bw/d).

[a] for γ-HCH. (WHO, 2010).

Table 4. Cancer risk estimates for HCHs, HPTs, CHLs and DDTs

OCPs	Cancer slope factor *	50th (95th) percentile cancer risks (x 10^{-4})			
	[per (mg/kg day)]	*O. niloticus*	*T. zillii*	*Carassius* spp.	*C. gariepinus*
HCHs	1.1 [a]	5.9 (18.7)	7.2 (14.3)	2.8 (6.6)	2.9 (8.4)
HPTs	4.5	19 (30)	0.9 (13)	12 (22)	13 (27)
CHLs	0.35	0.7 (1.0)	1.5 (2.2)	1.1 (4.2)	1.5 (2.5)
DDTs	0.34	3.7 (7.6)	6.7 (14.0)	7.8 (15.7)	8.4 (36)

[a] for γ-HCH.

*Cancer slope factors were from the United States Environmental Protection Agency (USEPA, 2012).

A carcinogenic risk assessment for OCPs was conducted using cancer risk estimates and HRs at the 50^{th} and 95^{th} percentile measured concentrations. As shown in Table 4, heptachlors showed much higher carcinogenic risk than other OCPs in all fish species. Regard to DDTs, the cancer risk for the 50^{th} exposure level ranged from 3.7 in *O. niloticus* to 8.4 x 10^{-4} in *C. gariepinus* suggested that a person would have a chances of about 4 and 8 in 10000 to develop cancer from DDTs, respectively. This carcinogenic risk increased from 7.6 to 36 x 10^{-4} on 95^{th} exposure level, which was unacceptable for human health. In general, the overall cancer risk estimates for all OCPs ranged from 0.7 x 10^{-4} to 36 x 10^{-4} on both the 50^{th} and 95^{th} exposure levels, and when compared to a target risk of >1 x 10^{-4}, are considered unacceptable. Thus, the carcinogenic risk of HCHs, HPTs, CHLs and DDTs among humans at present should be of concern.

HRs based on the 50^{th} and 95^{th} percentile exposure levels were assessed in each fish species and the results are shown in Fig. 3. HRs for cancer risk based on the 95th percentile concentrations of HCHs, HPTs, and DDTs were greater than one. The HRs for the OCPs followed almost the following sequence: HPTs > DDT ≥ HCHs > CHLs. For all fish species, the HRs for HPTs were greater than one, showing that consuming fish is harmful to humans. Based on landings, *O. niloticus* is the most caught fish in Lake Ziway. The carcinogenic risk due to HCHs for this fish species in also greater than one while for DDTs is less than one. However, for *T. zillii*, *Carassius* spp. and *C. gariepinus* the HRs for DDTs were greater than one. In general, cumulative daily exposure to OCPs because of fish consumption would yield a lifetime cancer risk of greater than one in a million. The results indicate that these compounds may be of particular concern because they are still in use.

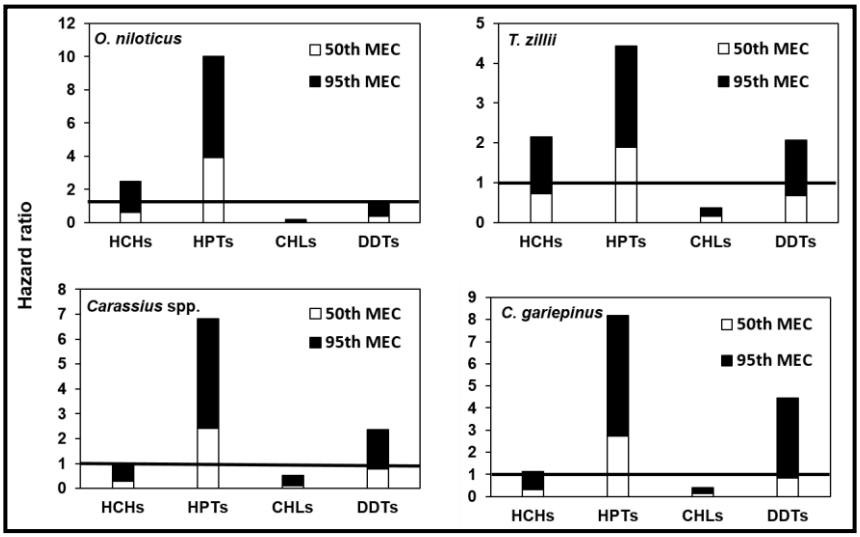

Fig. 3. Carcinogenic hazard ratios for daily consumption of fish from Lake Ziway, Ethiopia. (MEC: measured concentration & the horizontal line represents the hazard ratio of > 1, and any ratio higher than that indicates a risk)

Conclusion

This is the first study reporting on the levels and risk assessment of some OCPs in the most commonly caught fish species from the Ethiopian Rift Valley lake – Lake Ziway. The rift valley region is a populated area that is influenced by heavy pollution stemming from urban, agricultural and industrial activities. Our results indicated the presence of HCHs, HPTs, CHLs and DDTs with varying concentrations among the fish species. The overall conclusion of the evaluation is that DDTs were the main abundant pollutants, attributed to its current use in vector control and contamination from past usage. Dietary intakes estimated from the 50^{th} and 95^{th} percentile exposure level were far below ADIs. In contrast, the calculated cancer risk estimates and HRs of the studied fish species indicated that the consumption of most of the fish species could cause cancer as HR for cancer risk based on the 95^{th} percentile concentrations of HCHs, HPTs and DDTs was greater than one. In this study, only fish and some OCPs were investigated to assess the risk. The consumption of water, vegetables, and animal meat, and the levels of other environmental pollutants were not considered. Therefore, the actual health risk for local people through dietary intake could be higher.

- Varying concentration levels of HCHs, HPTs, CHLs and DDTs were found in the studied fish species from Lake Ziway, with DDTs being the predominant contaminant. The Eco-toxicological risk assessment showed a possible health concern from the daily consumption of fish.
- So this high levels of OCPs may also have an adverse effect on wildlife. In this view, the scope of the present study widens to understand the bioaccumulation levels and toxicological significance of these environmental pollutants in Lake Ziway.

Q. How are the levels of OCPs for the welfare of wildlife especially for birds?

- Thus **4 bird species** were collected for this purpose.

3.2.

Organochlorine pesticides in bird species and their prey (fish) from the Ethiopian Rift Valley region, Ethiopia

Abstract

Organochlorine pesticides (OCPs) and stable isotopes were measured in muscle from 4 bird and 5 fish species from the Ethiopian Rift Valley region where DDT is used for malaria control and vast agricultural activities are carried out. We investigated the bioaccumulation of OCPs such as DDTs, HCHs, chlordanes, and heptachlors between the species, and examined the potential risk posed by these compounds for bird species. Significant differences in contaminant profiles and levels were observed within the species. Levels of total OCPs ranged from 3.7 to 148.7 µg/g lipid in bird and 0.04 to 10.9 µg/g lipid in fish species. DDTs were the predominant contaminant, and a positive relationship between $\delta^{15}N$ and ΣDDT concentrations was found. The main DDT metabolite, *p,p′*–DDE was the most abundant and significantly greater concentrations in bird species (up to 138.5 µg/g lipid), which could have deleterious effects on survival and/or reproduction of birds.

Keywords: OCPs, DDTs, Bird, Bioaccumulation, Ethiopian Rift valley

Introduction

Organochlorine pesticides (OCPs) have been widely used and become worldwide concern due to their persistence, bioaccumulation ability through the food web, and potential negative impacts on humans and wildlife (Jones and de Voogt, 1999; Donaldson et al., 2010). Concentrations of OCPs are generally declining in developed countries, but levels in developing countries environment show an increasing level because they are still in use for agriculture and public health purposes. Especially, dichlorodiphenyltrichloroethane (DDT), which is highly persistent and toxic to biological functioning (Vasseur and Cossu-Leguille, 2006) is still using for malaria control in African countries (WHO, 2007). Ethiopia has been implementing indoor residual spraying (IRS) with DDT for malaria control, and uses approximately 400 metric tons of active-ingredient DDT per year (Sadasivaiah et al., 2007; van den Berg, 2009; WHO, 2007). It is used in many parts of the country including the Rift Valley, a malaria epidemic prone region. In addition, Ethiopia has one of the largest stockpiles of obsolete pesticides in Africa, which have been accumulated since the first imports in the 1960s (Haylamicheal and Dalvie, 2009). These were mostly organochlorine compounds such as chlordane, DDT, dieldrin and lindane that are banned or restricted in most countries. Therefore, high concentrations of OCPs can be found in top predators such as birds.

Birds have been used as sentinel species for environmental contaminants exposure owing to their higher trophic position, widespread distribution and sensitivity to environmental changes (Jaspers et al., 2006; Voorspoels et al., 2006). Thus, in Asia, Europe and North America they have been used intensively for monitoring contaminant concentrations (Drooge et al., 2008; Lam et al., 2008; Park et al., 2009). Studies have shown that contaminations from chlorinated insecticides have contributed to the decline of bird populations (Aktar et al., 2009; Mineau and Whiteside, 2013). Mortality of birds due to pesticide poisoning attributed to aldrin (Muralidharan, 1993) and monocrotophos (Pain et al., 2004) has been reported in India. One of the well-known sub-lethal effects caused by DDTs, particularly *p,p′*–DDE, is the thinning of eggshell thickness (Tanabe et al., 1998). However, despite the continuing usage of OCPs especially DDT in Africa, there is still a scarcity of data regarding the contamination status and ecological impacts of these

compounds in the surrounding ecosystems.

The Ethiopian Rift Valley Region is a densely populated area with various agricultural activities where there is still an increasing trend of pesticide usage (Amera and Abate, 2008). Current studies have revealed the contamination of the Rift Valley region environment (sediment and fish) by organochlorine chemicals (Deribe et al., 2011; Yohannes et al., 2013a, b). The results showed the predominance of DDTs compared to other organochlorine pollutants, attributing to its ongoing use in the Ethiopian Rift valley region. Nevertheless, no study has been conducted regarding to the levels and contamination status of these compounds on wildlife in general and birds in particular from Ethiopia. Therefore, the aims of this study were: (i) to assess the accumulation profiles of OCPs in four bird and five fish species, and (ii) to examine the potential risk posed by these compounds to delineate the bird species at risk.

Materials and methods

Study site

The Ethiopian Rift Valley region, which encompasses a series of lakes, streams and wetlands is an important area for agricultural, commercial and industrial development of Ethiopia. Lake Ziway, one of the Ethiopian Rift Valley lakes (surface area: 434 km^2) is a shallow freshwater lake situated in the northern section of the Rift Valley (Fig. 1). The lake is fed by a number of rivers, of which the Meki River from the north-west and the Katar River from the east are the most significant. Lake Ziway hosts population of different fish species including *Oreochromis niloticus, Tilapia zillii, Carassius auratus, Clarias gariepinus* and *Barbus intermedius* (Golubtsov et al., 2002). Lake Ziway is also known for its birds and hippos. The landings of Lake Ziway used to be dominated by these fish species and attracts a number of fish eating birds.

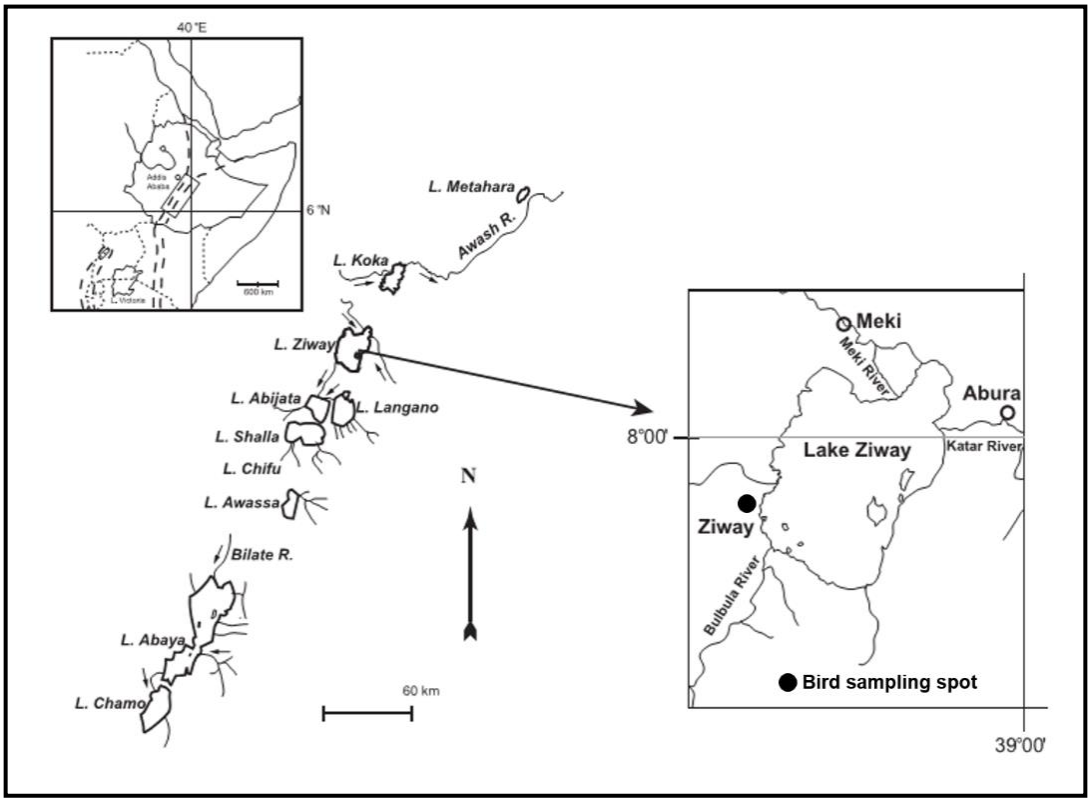

Fig. 1. Ethiopian Rift Valley lakes and the map of Lake Ziway

Lake Ziway supports over 20,000 water birds (Birdlife International, 2013). The most common species are *Pelecanus onocrotalus*, *Phalacrocorax lucidus*, *Scopus umbretta*, *Chroicocephalus cirrocephalus*, *Threskiornis aethiopicus*, *Chlidonias leucopterus*, *Leptoptilos crumeniferus*, *Haliaeetus vocifer*, etc. The Lake's ecosystem serves as breeding and wintering ground and as a migration stopover habitat for several resident and migratory bird species. It is one of the best sites in Ethiopia to see a diversity of bird species. However, most of the area around Lake Ziway has now been cleared for farmland, especially by large scale irrigated fields and floricultures. Therefore, the expansion of intensive agriculture (producing fruits, vegetables and flowers) and the IRS programme for malaria control has introduced pesticides and fertilizers into the ecosystem, and a decline in water birds and fish has been noted in recent years (Birdlife International, 2013).

Samples

Four bird and five fish species were collected between January 2011 and June 2012. In general, 23 bird individuals belonging to Hamerkop (*Scopus umbretta*, *N* = 5); African sacred ibis (*Threskiornis aethiopicus*, *N* = 7); Marabou stork (*Leptoptilos crumeniferus*, *N* = 6) and Great white pelican (*Pelecanus onocrotalus*, *N* = 5), and 105 fish specimens of *Oreochromis niloticus*, *Tilapia zillii*, *Carassius* spp., *Clarias gariepinus* and *Barbus intermedius* were collected. Information about the samples by species is given in Table 1.

Muscle tissues were taken from the aforementioned species and stored at −20 ^{o}C until OCPs and stable isotopes analyses. For bird sampling, the Ethiopian Wildlife Conservation Authority (EWCA) issued a permit (Permission No. DA/31/284/012) allowing us to capture and sacrifice the above mentioned species of birds under the supervision of Veterinarian. All analyses were conducted at the Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan.

Table 1. Sample information and feeding habits of the species under study

Scientific name	Common name	Code	N	Habitat /Feeding habit	Reference
BIRD					
Scopus umbretta	Hamerkop	H	5	Mainly terrestrial / Predominantly of amphibians and small fish as well as crustaceans, worms and insects	http://www.birdlife.org
Threskiornis aethiopicus	African sacred ibis	S	7	Mainly terrestrial / Insectivorous, feeds opportunistically on plowed lands and also other small prey such as worms, molluscs, fish, frogs, lizards, small mammals, the eggs of birds and crocodiles, carrion	
Leptoptilos crumeniferus	Marabou stork	M	6	Mainly terrestrial / Carrion and scraps of fish, live fish, termites, locusts, frogs, lizards, snakes, rats, mice and birds	
Pelecanus onocrotalus	Great white Pelican	P	5	Aquatic / Entirely piscivorous, preferentially taking fish	
FISH					
Oreochromis niloticus	Tilapia	O	27	Zooplankton and blue green algae	
Tilapia zillii	Zillii	Z	19	Macrophytes	
***Carassius* spp.**	Carp	C	27	Zooplankton and blue green algae	
Clarias gariepinus	Catfish	G	27	Zooplankton, fish eggs, fish, gastropods	
Barbus intermedius	Barbus	B	5	Zooplankton, gastropods, larvae, fish eggs, fish	

N: Number of samples.

Stable isotope analysis

Muscle samples were dried for at least 48 h at 60 °C and ground to a fine powder. After grinding, samples were subjected to lipid extraction using 2:1 (v/v) chloroform:methanol solution. Approximately 1 mg of each sample was loaded into tin capsule and analyzed using a Fisons NA1500 elemental analyzer equipped with a Finnigan MAT 252 isotope ratio mass spectrometer. Stable carbon and nitrogen isotope ratios were expressed in delta values as $\delta^{13}C$ or $\delta^{15}N$ (‰) = $[(R_{sample} / R_{standard}) - 1] \times 1000$

Where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Pee Dee Belemnite carbonate and atmospheric nitrogen were used as standards for carbon and nitrogen, respectively. The analytical precision based on internal laboratory standards was with measurement precision of ±0.2‰ for both stable isotope ratios.

OCPs analysis

The extraction method and analysis were performed same as our previous study (Yohannes et al., 2013a) with modest modifications. Briefly, 10 g dorsolateral muscle of fish or 5 g pectoral muscle of bird was thawed, mixed with anhydrous sodium sulfate and extracted with hexane:acetone (3:1, v/v) in a Soxtherm apparatus (S306AK Automatic Extractor, Gerhardt, Germany) for 4 h. The surrogate 2,4,5,6-tetrachloro-*m*-xylene (TC*m*X) was spiked prior to extraction. An aliquot of the extract was used for gravimetrical determinarion of lipid content. The remainder was concentrated and cleaned up on a column filled with 6 g florisil (activated at 150 °C overnight), and eluted with n-hexane:dichloromethane (7:3, v/v). The eluate was concentrated to about 2 mL using rotary vacuum evaporator and then to near dryness under gentle nitrogen flow. The extract was reconstituted in 100 μL of *n*-decane and transferred to a GC vial.

OCPs including DDTs (*o,p′*– and *p,p′*–DDT, DDE and DDD), hexachlorocyclohexanes (HCHs; α–, β–, γ– and δ–HCH), heptachlors (HPTs; heptachlor, *cis*– and *trans*–heptachlor epoxide), chlordanes (CHLs; *cis*–, *trans*– and oxy–chlordane, *cis*– and *trans*–nonachlor, drins (aldrin, dieldrin and endrin) and hexachlorobenzene (HCB) were analyzed using a Shimadzu Model 2014 gas chromatography micro electron capture detector (GC-μECD) equipped with a 30 m × 0.25 mm × 0.25 μm ENV-8MS capillary column. The initial oven

temperature was held at 100 °C for 1 min; increased to 200 °C at 20 °C/min and then to 260 °C at 4 °C/min, which was held for 5 min. The injector and detector temperatures were set as 250 °C and 310 °C, respectively. Helium at a flow rate of 1.0 mL/min and nitrogen at 45 mL/min were used as carrier gas and make-up gas, respectively. One μL of each sample was injected in the splitless mode.

Quality control and quality assurance

For each batch of ten samples, procedural blanks and spiked blanks were consistently analyzed. Results showed that no target analytes were detected in blank samples and recoveries for spiked blanks ranged from 90% to 105%. The mean (± standard deviation) recovery of the surrogate standard (TC*m*X) was 85 ± 11% across all samples, and concentrations were not corrected for recovery. To further test the precision and accuracy of the analytical method, the standard reference material SRM 1947 (Lake Michigan Fish Tissue) was analysed using the same procedures, and the recoveries ranged from 85% to 105% with RSD < 12%. The limit of quantification set at 10:1 signal-to-noise ratio were 0.9 ng/g, 0.5–0.92 ng/g, 0.7–1.3 ng/g, 0.9 ng/g, and 0.6–1.5 ng/g for HCB, HCHs, DDTs, HPTs, and CHLs, respectively. Concentrations of OCPs were expressed as ng/g lipid weight (lw).

Statistical analysis

All the statistical analyses were performed using JMP 9 (SAS Institute, Cary, NC, USA). Statistical analyses were carried out on log-transformed concentrations to approximate a normal distribution of the data. Statistical differences were evaluated by one-way analysis of variance (ANOVA) accompanied with Tukey's test if necessary. Principal component analysis (PCA) based on log transformed concentrations was used to study inter correlations among species and concentrations below the LOQ were given a value of ½(LOQ). Linear regression models were used to examine associations between log transformed concentrations of OCPs with $\delta^{15}N$ values. The slope of the regression equation was used as index for bioaccumulation of OCPs among the studied species. The level of significance was set at $p < 0.05$.

Results and discussion

Stable isotope analysis

There were significant differences of both $\delta^{13}C$ (F-ratio = 20.9; $p < 0.001$), and $\delta^{15}N$ (F-ratio = 25.2; $p < 0.001$) amongst bird and fish species. $\delta^{13}C$ and $\delta^{15}N$ values of the studied species ranged from –24.8‰ to –15.3‰ and from 5.25‰ to 13.3‰, respectively (Fig. 2).

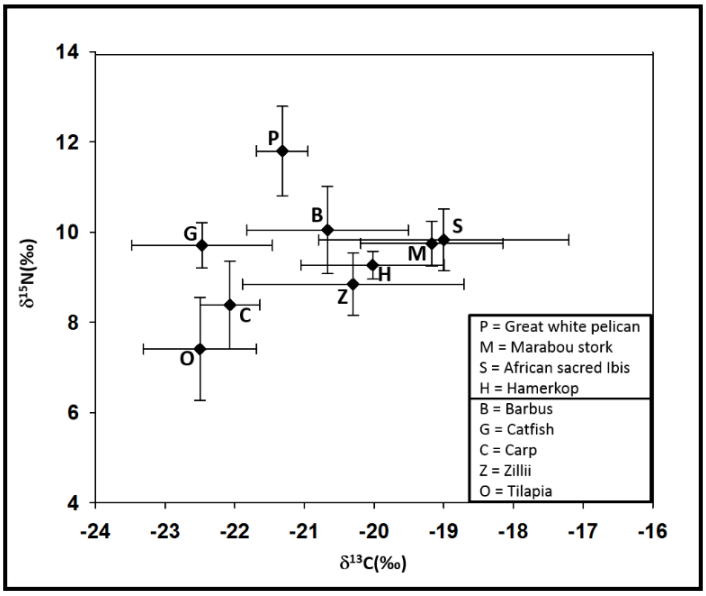

Fig. 2. Mean ± SD of isotope ratio of nitrogen and carbon ($\delta^{15}N$ and $\delta^{13}C$) of four birds and five fish species from Lake Ziway-Ethiopian Rift Valley region

In bird species, the aquatic bird, great white pelican showed significantly high $\delta^{15}N$ values compared to the other bird species ($p < 0.05$). Furthermore, this bird species also showed significant difference with the terrestrial bird species based on the $\delta^{13}C$ values (Fig. 3). The lowest and narrow range $\delta^{13}C$ values of great white pelican indicate the homogeneity of their feedings i.e., piscivorous feeding habits, whereas the wide range of $\delta^{13}C$ values for hamerkop, African sacred ibis and marabou stork means the high heterogeneity of diet source for these bird species. Regarding fish species, the carnivorous fish species catfish and barbus showed significantly high $\delta^{15}N$ values (9.70‰ and 10.0‰, respectively) than planktivorous fish species tilapia (7.37‰), zillii (8.91‰) and carp (8.38‰) (Fig. 4).

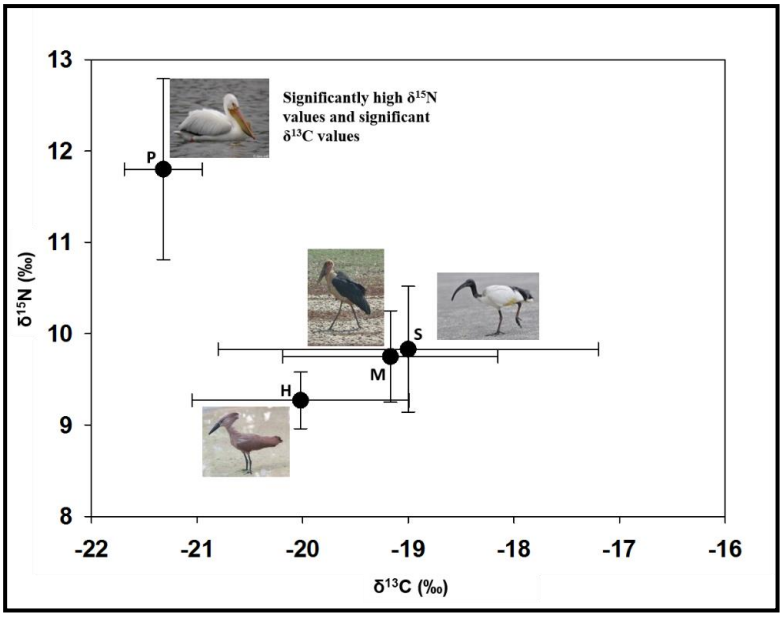

Fig. 3. Mean and standard deviation of isotope ratio of four bird species, Hamerkop (H), African sacred ibis (S), Marabou stork (M) and Great white pelican (P) from Ethiopia

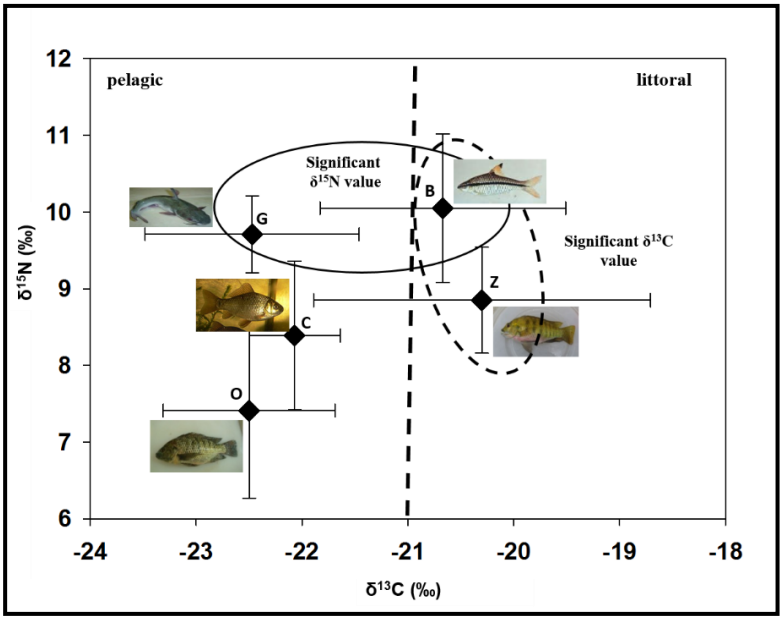

Fig. 4. Mean and standard deviation of isotope ratio of five fish species from Lake Ziway. Tilapia (O), Carp (C), Zillii (Z), Catfish (G) and Barbus (B)

Levels of OCPs

Of all target compounds analyzed, 10 OCPs were frequently detected in both bird and fish samples; *p,p′*–DDT, *p,p′*–DDE, *p,p′*–DDD, α–HCH, γ–HCH, *cis*– and *trans*–heptachlor epoxide, *cis*– and *trans*–chlordane, and *trans*–nonachlor. HCB and *o,p′*–DDT were detected only in bird and fish species, respectively (Tables 2 and 3). Oxy–chlordane and β–HCH were rarely encountered but levels of drins (aldrin, dieldrin and endrin) were below detection limit (data not shown).

Table 2. Statistical description on concentrations (ng/g lipid weight) of OCPs in muscle tissues of four bird species from Ethiopia

		α—HCH	γ—HCH	cis-Heptachlor-Epoxide	trans-Heptachlor-Epoxide	cis-Chlordane	trans-Chlordane	trans-Nonachlor	HCB	*p,p'*—DDE*	*p,p'*—DDD*	*p,p'*—DDT*
Hamerkop	Mean	4.98	67.29	37.10	ND	27.60	70.39	ND	11.40	31.86	1.23	0.03
	Std. Deviation	1.37	37.49	10.50		8.80	36.14		9.64	36.33	0.34	0.02
	Minimum	3.13	23.86	22.65		16.55	26.18		3.80	4.22	0.80	0.02
	Maximum	6.89	106.8	48.69		39.03	113.90		28.18	91.60	1.68	0.07
	Median	4.70	50.13	40.84		25.48	64.16		8.02	18.37	1.22	0.03
African sacred ibis	Mean	12.35	59.31	27.97	ND	38.42	52.78	21.55	5.70	17.85	2.16	3.41
	Std. Deviation	12.55	50.62	19.01		20.95	30.84	13.44	10.96	13.87	1.23	2.04
	Minimum	0.33	4.58	7.24		11.83	12.46	6.42	ND	2.23	0.69	0.79
	Maximum	29.97	153.7	52.73		69.62	98.48	44.99	29.74	38.85	4.35	6.32
	Median	10.21	61.83	21.84		37.39	59.43	20.79	0.56	11.28	2.24	3.59
Marabou stork	Mean	51.15	13.86	37.81	25.78	31.43	35.93	10.66	20.60	58.34	1.57	2.74
	Std. Deviation	39.48	7.38	18.25	11.84	14.40	21.26	6.75	18.59	52.08	0.86	1.94
	Minimum	5.84	3.59	9.61	10.91	18.77	11.50	3.50	ND	3.81	0.82	1.20
	Maximum	110.3	23.76	56.55	40.57	58.63	63.02	20.90	41.58	138.5	3.22	6.56
	Median	43.40	15.81	39.42	27.72	26.66	32.85	8.69	18.19	49.25	1.28	2.08
Great white pelican	Mean	ND	ND	8.73	ND	3.64	1002.0	3.15	14.08	23.30	3.17	1.50
	Std. Deviation			4.41		3.16	283.9	6.66	5.52	9.03	1.33	0.76
	Minimum			3.59		0.20	715.4	ND	7.98	16.06	2.03	0.94
	Maximum			14.41		8.46	1389.9	15.06	19.99	38.70	4.69	2.69
	Median			9.47		3.03	1009.5	0.34	13.30	20.41	2.46	1.03

ND = below detection limit.

*Concentrations were expressed as µg/g lw.

Table 3. Statistical description on concentrations (ng/g lipid weight) of OCPs in muscle tissues of five fish species from Lake Ziway, Ethiopia

Species		α–HCH	γ–HCH	cis-Heptachlor-Epoxide	trans-Heptachlor-Epoxide	cis-Chlordane	trans-Chlordane	trans-Nonachlor	*p,p′*–DDE	*p,p′*–DDD	*o,p′*–DDT	*p,p′*–DDT
Barbus	Mean	21.06	222.2	ND	ND	13.21	20.90	22.46	592.6	54.23	29.24	33.45
	Std. Deviation	10.88	132.4			11.66	9.93	10.84	348.3	25.62	23.85	16.74
	Minimum	10.43	48.58			ND	9.35	7.78	198.8	24.77	ND	12.03
	Maximum	34.61	372.5			25.25	31.13	36.44	1028.3	81.64	54.78	51.48
	Median	18.28	205.7			11.04	20.97	24.00	698.8	61.86	23.51	27.31
Catfish	Mean	49.32	75.57	46.30	73.50	33.73	52.87	63.30	1152.1	137.9	89.36	107.6
	Std. Deviation	32.56	88.08	39.00	56.08	27.34	43.81	51.29	2087.6	209.2	70.16	93.98
	Minimum	1.58	2.57	ND	1.89	1.09	1.86	1.50	12.48	4.31	4.66	6.01
	Maximum	125.7	396.5	176.2	252.8	95.45	219.2	250.1	10195.7	1126.1	286.1	493.2
	Median	39.98	50.29	42.28	61.46	28.52	37.88	48.12	501.40	87.73	79.16	97.95
Carp	Mean	42.81	46.33	39.91	71.59	23.24	36.69	50.90	329.7	82.47	110.4	113.2
	Std. Deviation	32.44	74.01	31.03	122.4	28.37	21.67	63.78	243.0	53.13	115.9	93.86
	Minimum	13.30	2.20	10.24	9.05	ND	10.20	ND	58.89	20.19	ND	ND
	Maximum	140.4	308.0	150.5	554.8	117.5	92.62	200.1	1140.9	226.7	450.1	319.0
	Median	30.37	17.68	31.03	30.31	17.06	31.87	19.02	243.68	69.52	51.28	84.16
Zillii	Mean	29.72	116.0	14.81	29.88	40.53	ND	40.97	251.9	121.9	77.68	108.3
	Std. Deviation	23.53	133.3	24.98	20.85	28.25		21.33	184.4	71.52	48.11	100.8
	Minimum	9.91	13.46	ND	9.09	ND		14.96	25.78	21.78	18.88	ND
	Maximum	110.1	543.1	93.41	97.50	117.7		105.5	642.4	308.8	201.2	351.0
	Median	23.77	66.00		28.30	34.92		40.30	238.9	116.5	71.09	68.29
Tilapia	Mean	46.44	136.4	124.9	72.01	41.46	32.88	11.99	248.8	75.00	111.3	56.52
	Std. Deviation	31.54	114.4	95.97	54.53	39.21	24.54	35.30	175.1	44.98	149.6	39.48
	Minimum	5.00	9.17	10.63	6.44	3.86	ND	ND	10.30	6.41	11.80	ND
	Maximum	155.6	487.1	423.6	277.8	209.9	122.4	161.1	792.1	205.5	800.2	134.3
	Median	38.74	101.8	106.1	57.56	28.89	26.80	0.00	220.2	66.16	72.51	47.26

ND = below detection limit.

The median and range concentrations of total OCPs are summarized in Table 4. Levels of ∑OCPs in birds and fish ranged from 3.7 to 148.7 μg/g lw and 0.04 to 10.91 μg/g lw, respectively. A significant difference for ∑OCPs concentration was observed between the groups i.e., bird and fish species (F-ratio = 39.65, $p < 0.001$), whereas no significant differences were seen within each group (bird: F-ratio = 1.624, $p = 0.217$; fish: F-ratio = 1.163, $p = 0.332$). However, when individual OCP concentrations were compared among the bird species, a significant difference was found only in levels of CHLs. Generally, the median concentrations of total OCPs were higher, for more than 10 times, in birds than in fish species (Table 4). Our result indicates moderate to high levels of OCPs in different bird and fish species. Marabou stork had the highest median concentrations of ∑OCPs as this bird species is a scavenger and having a wide range of feeding habits from both mainly terrestrial and aquatic food webs. In general, a large variability of pollutants levels especially in bird species was found within a single species. This might be attributed to different feeding ecology, age, habitat, condition of the birds, and seasonal variation of food compositions for terrestrial birds (Jaspers et al., 2006).

Table 4. Median concentrations [range] (µg/g lipid weight) of OCPs in the muscle tissues of birds and fish species from Ethiopian Rift Valley Region

Species	N	Lipid %*	∑HCHs	∑HPTs	∑CHLs	∑DDTs	∑OCPs
Hamerkop	5	1.84 ± 0.49	0.05	0.04	0.10	19.4	19.6
			[0.03–0.11]	[0.02–0.05]	[0.05–0.14]	[5.0–93.3]	[5.1–93.6]
African sacred ibis	7	1.43 ± 1.06	0.07	0.02	0.13	17.5	17.8
			[0.005–0.18]	[0.01–0.05]	[0.03–0.19]	[3.7–40.8]	[3.7–40.9]
Marabou stork	6	1.58 ± 0.53	0.05	0.07	0.07	52.7	53
			[0.02–0.13]	[0.02–0.10]	[0.03–0.14]	[5.8–148.3]	[5.9–148.7]
Great white pelican	5	3.55 ± 1.16	ND	0.01	1.0	23.8	24.8
				[0.004–0.014]	[0.72–1.39]	[19.2–45.1]	[19.9–46.5]
Tilapia	27	0.76 ± 0.69	0.14	0.17	0.07	0.41	0.81
			[0.02–0.58]	[0.02–0.70]	[0.01–0.49]	[0.05–1.94]	[0.10–3.44]
Zillii	19	0.83 ± 0.45	0.16	0.06	0.12	0.62	0.87
			[0.05–0.88]	[0.01–0.30]	[0.03–0.39]	[0.10–1.61]	[0.26–2.98]
Carp	27	0.89 ± 0.60	0.06	0.06	0.10	0.49	0.81
			[0.02–0.58]	[0.03–0.56]	[0.03–0.43]	[0.12–1.34]	[0.23–2.58]
Catfish	27	1.34 ± 2.52	0.09	0.10	0.14	0.80	1.22
			[0.004–0.52]	[0.003–0.34]	[0.004–0.41]	[0.03–10.6]	[0.04–10.9]
Barbus	5	1.66 ± 1.29	0.24	ND	0.05	0.90	0.89
			[0.06–0.40]		[0.02–0.10]	[0.27–1.18]	[0.37–1.58]

N = Number of samples.

ND: Not detected or below detection limit.

*Data showed as mean ± standard deviation.

The relative proportions of ΣOCPs groups varied between bird and fish species are shown in Fig. 5. The OCP profile for all species was clearly dominated by DDTs, accounting for 52 to 76% in fish species and more than 99% in bird species. This result indicates the high degree of exposure to DDTs in biota from the Ethiopian Rift Valley region, which is most likely due to the recent use of DDT-IRS for malaria control (van den Berg, 2009; WHO, 2007) as well as from illegal usage and contamination from past usage (Amera and Abate, 2008), and spills from obsolete pesticides (Haylamicheal and Dalvie, 2009). HCHs and CHLs were the next OCPs with highest concentrations followed by HPTs.

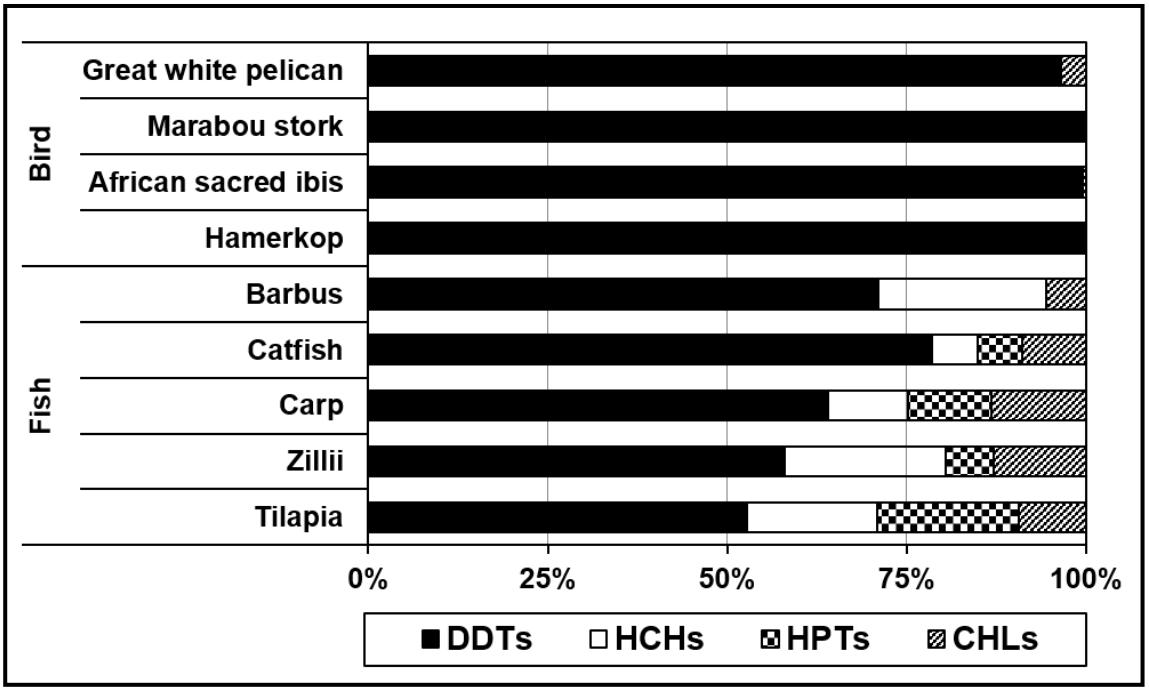

Fig. 5. Relative proportion of OCPs in muscle tissues of bird and fish species from the Ethiopian Rift Valley region

DDTs

DDTs were the most prominent organochlorine pollutants detected in the investigated samples. The levels of ΣDDTs ranged from 3.7 to 148.3 µg/g lw in bird species and from 0.03 to 10.6 µg/g lw in fish species. The highest DDTs concentrations were observed in marabou stork (median 52.7 µg/g lw) followed by great white pelican (median 23.8 µg/g lw) (Table 4). Ecological and feeding habit of marabou stork may be probably a plausible explanation for elevated DDTs. This bird species often occurs close to human habitation where DDT is sprayed for malaria control in addition to sewage ponds and agricultural areas, and is scavenger, eats everything what it gets (Table 1). In agreement with other studies (Tanabe et al., 1998; Chen et al., 2009; Dhananjayan, 2012), *p,p′*–DDE was the most abundant isomer and had significantly high burden in all samples studied in the lake Ziway food web (Table 2). It accounted for 87% on average (from 76 to 96%), followed by *p,p′*–DDD (7% on average) in bird species (Fig. 6). This may be explained by high chemical stability and persistence, and biomagnification potential of *p,p′*–DDE in the environment and in living organisms. Other DDT compounds, *o,p′*–DDT, *p,p′*–DDT and *p,p′*–DDD were observed at much lower levels (i.e., 1–2 orders of magnitude lower than *p,p′*–DDE). The mean ratios of *p,p′*–DDT/*p,p′*–DDE for the studied bird species were < 1.0, suggesting mainly contamination by old DDT. The ratios were 0.001, 0.046, 0.064, and 0.191 for hamerkop, marabou stork, great white pelican and African sacred ibis, respectively. This result indicates the difference in dietary habit, DDT exposure period and the metabolic capacity of the bird species. Nonetheless, *p,p′*–DDT was detected in all bird species, indicating the exposure to a “fresh” source of DDT.

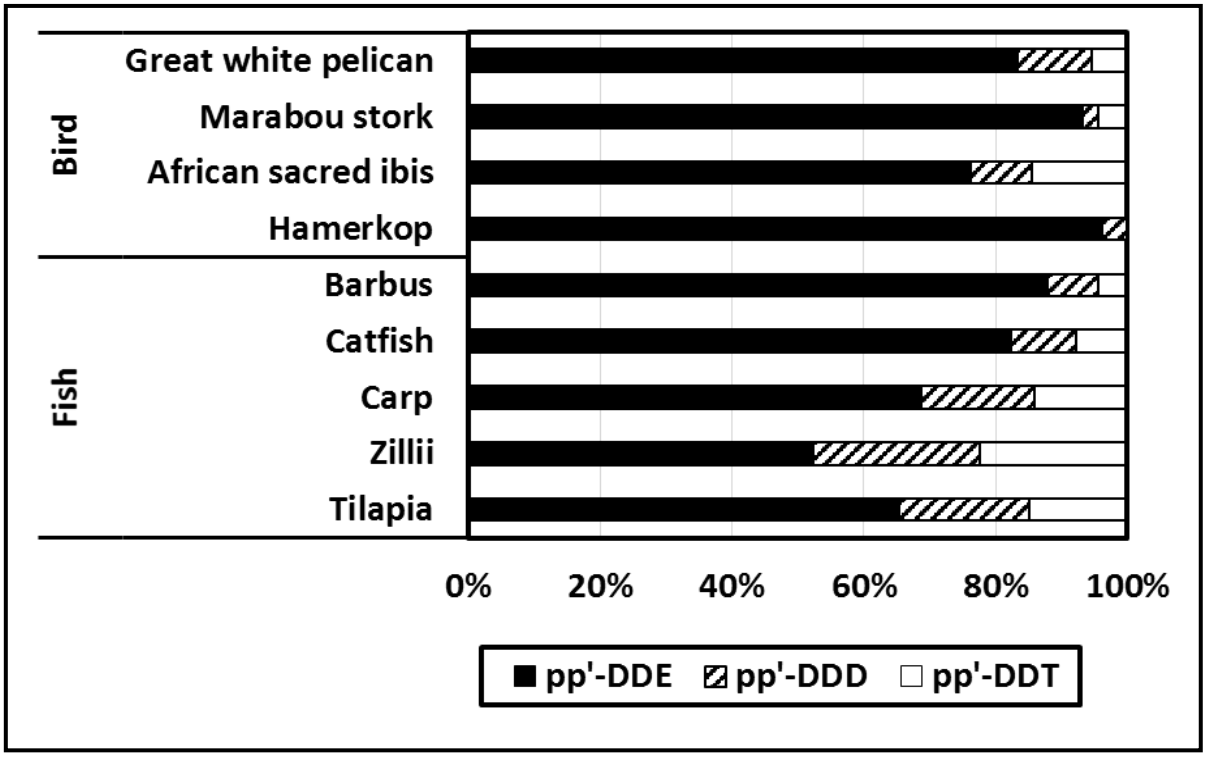

Fig. 6. Relative abundance of individual DDT components in four bird and five fish species from Ethiopian Rift Valley region

Other OCPs

The α–, and γ–HCH isomers were detected in all samples except in great white pelican (Tables 2 and 3), and γ–HCH (lindane) dominates in all samples. The predominance of γ–HCH in this study indicates the current usage of lindane in the region. A recent study in the Rift Valley region showed high concentrations of lindane in cattle liver tissues (highest level of 0.14 mg/kg wet wt) obtained from Holeta, Ethiopia (Letta and Attah, 2013). Maximum level of ΣHCHs was recorded in muscle tissue of African sacred ibis (0.18 µg/g lw) followed by marabou stork (0.13 µg/g lw) (Table 4). These bird species have a wide feeding habits in both aquatic and terrestrial food webs.

Cyclodiene insecticides, heptachlor epoxides and chlordanes were also detected in both fish and bird species with varying concentrations. Among the CHLs, *trans*−chlordane was the most abundant and dominant contributor to total chlordanes followed by *cis*−chlordane and *trans*−nonachlor as they are the major constituents in technical chlordane (Tables 2 and 3), whereas oxy-chlordane was rarely encountered. Significantly high CHLs concentration (0.72 – 1.39 µg/g lw) was observed in great white pelican (F-ratio = 26.55, $p < 0.001$). According to the HPTs, *cis*− and *trans*−heptachlor epoxides were the predominant ones. The greatest median concentration of HPTs was detected in marabou stork (0.07 µg/g lw) followed by hamerkop (0.04 µg/g lw). In general, levels of HPTs in the muscle of the studied bird species ranged from 0.004 to 0.10 µg/g lipid wt (Table 4).

Profile differences among species

It is well known that differences in food habits, metabolic capacity and trophic position explains most of the variations in pollutant levels between different species. This study revealed that there were different bioaccumulation potentials of OCPs among the studied species. PCA was performed to carry out the comparison of OCPs profiles using frequently detected pollutants in both species and stable isotope values. The PCA revealed that 48% of the variation was accounted for the first principal component (PC1) and 13% by PC2 (Fig. 7). As observed from the loading plot (Fig. 7a), profiles of OCPs differ noticeably. PC1 was positively related to DDTs, HCB, *trans*−chlordane and stable isotopes, while HCHs, *cis*−chlordane, and *trans*−nonachlor had high loadings onto PC2. This indicates that PC1 increase significantly with increasing OCP levels, which likely is driven by high relative contribution of DDTs.

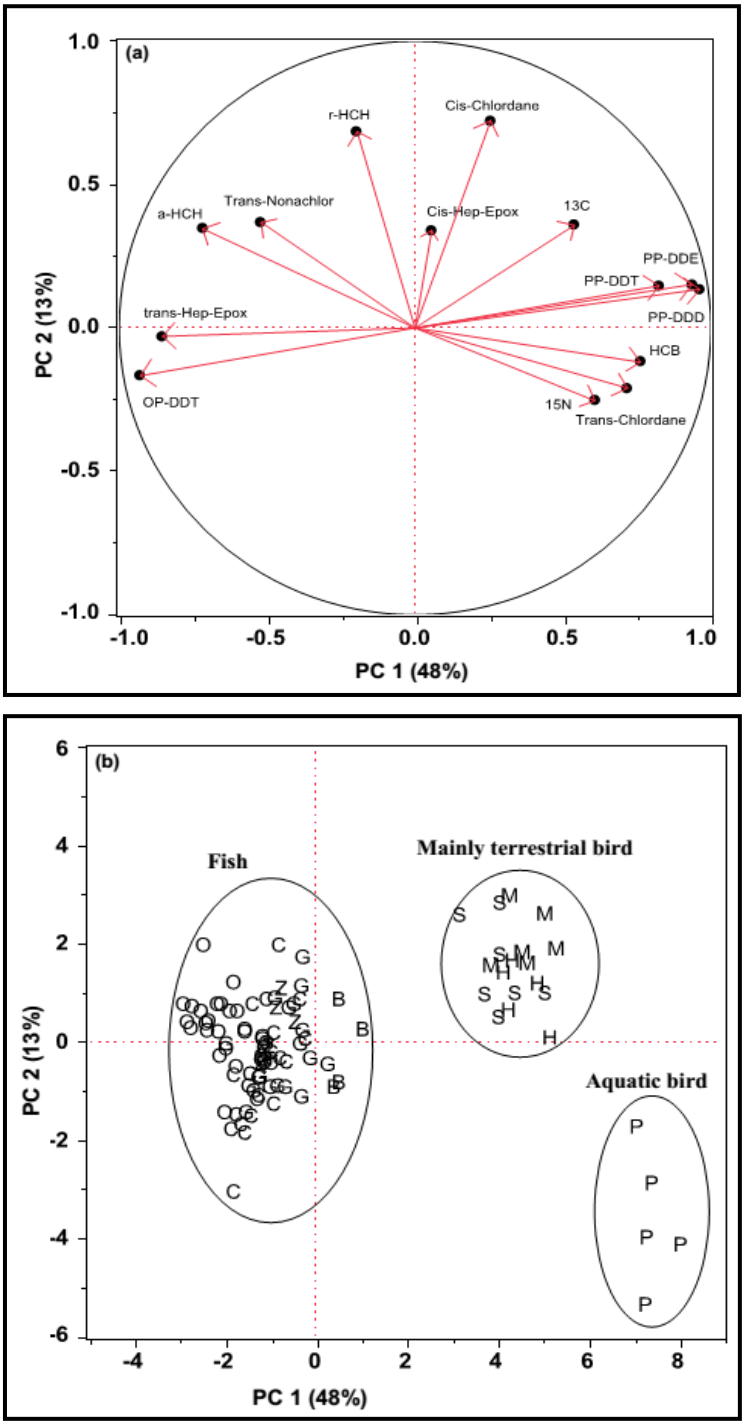

Fig. 7. Principal Component Analysis based on log transformed contaminant concentrations (a) loading plot (b) score plot. Bird: Hamerkop (H), African sacred ibis (S), Marabou stork (M), Great white pelican (P); Fish: Tilapia (O), Zillii (Z), Carp (C), Catfish (G), Barbus (B)

An interesting feature is also observed in the score plot (Fig. 7b). The bird and fish species separated along PC1 based on the loading pattern of OCPs. The plot clearly exhibited the species-specific differences in the levels of contaminants. The fish species are separated from the bird species, by having relative high levels of HCHs, *trans*–heptachlor epoxide and *o,p′*–DDT. Furthermore, there is a clear separation among the bird species along PC2. The aquatic bird species, great white pelican had high $\delta^{15}N$ values and showed unique loading plots associated with *trans*–chlordane that separated from the other bird species. As shown in Table 2, *trans*–chlordane was the most abundant contaminant measured in great white pelican. On the other hand, the terrestrial bird species, having a wide range of $\delta^{13}C$ values were strongly associated with *p,p′*–DDT, *p,p′*–DDD and *p,p′*–DDE. In general, this interspecific differences can be explained by differences in dietary habits and different exposure routes or metabolic efficiency of the studied bird species (Jaspers et al., 2006). Great white pelican is an aquatic and piscivorous bird feeding primarily on fish. African sacred ibis is an insectivorous which feeds opportunistically on plowed lands and small preys such as small fishes, worms and eggs of birds while the marabou stork is a scavenger species feeds on everything it gets. The latter two bird species often occurs close to human habitation where DDT is sprayed for malaria control (Table 1).

Biomagnification of OCPs

In this study, 'biomagnification' was defined as the phenomenon of accumulating the chemicals through the food chain (e.g. accumulation of OCPs by birds through consumption of fish). The influence of trophic level on OCPs burden among the studied species was investigated by analyzing correlation between $\delta^{15}N$ values and mean OCPs concentration. The relationship between log transformed OCPs and $\delta^{15}N$ values is shown in Fig. 8.

The regressions for DDTs (R^2 = 0.375) and CHLs (R^2 = 0.439) showed positive relationships ($p \leq 0.05$) between concentrations and $\delta^{15}N$ values. These results suggest the biomagnification potential of these compounds for the present lake Ziway food web. The slopes of the regression equations were 0.438 and 0.202, respectively. The slope of [OCPs] vs $\delta^{15}N$ gives an indication of the magnitude of biomagnification (Borgå et al., 2001; Fisk

et al., 2001). The higher slope value observed for DDTs might be attributed due to their high hydrophobicity and recalcitrant nature. This finding was consistent with other reports on aquatic and terrestrial food chains (Borgå et al., 2001; Buckman et al., 2004). However, biomagnification was not observed for CHLs against $\delta^{15}N$ values when compared without great white pelican as this bird species had high levels of *trans*–chlordane (R^2 = 0.013; p = 0.814). Thus, it remains inconclusive whether chlordanes are actually biomagnified. A negative linear relationship (R^2 = 0.682; slope = –0.294; p = 0.011) between $\delta^{15}N$ values and HPTs concentrations was found, indicating that heptachlors do not biomagnify through the food web, suggesting that the metabolic capability of HPTs in the studied species increase with the trophic level. Nevertheless HCHs showed no significant correlation with $\delta^{15}N$ values (p = 0.518), largely owing to their low octanol-water partition coefficients (log K_{ow} ~ 4) (Russ et al., 2002).

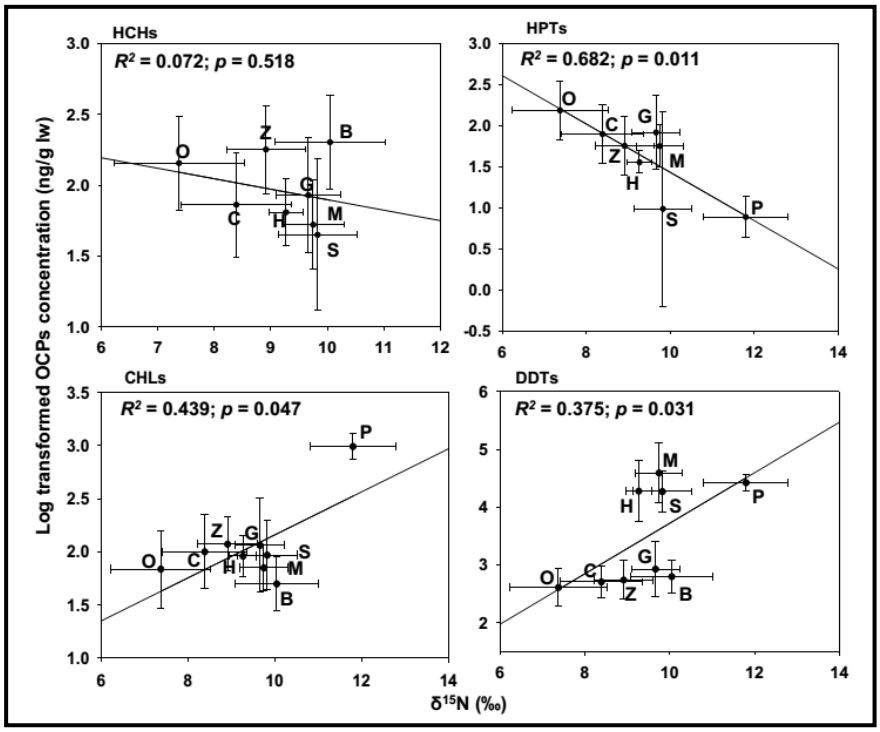

Fig. 8. Mean ± SD of log-transformed OCPs (ng/g lw) vs. $\delta^{15}N$ values relationship in the present lake Ziway food web. Bird: Hamerkop (H), African sacred ibis (S), Marabou stork (M), Great white pelican (P); Fish: Tilapia (O), Zillii (Z), Carp (C), Catfish (G), Barbus (B)

Comparison with other areas

Because of the absence of data concerning residue levels in same species and same matrices, the residue levels in muscle samples reported in other bird species were referred (Table 5). Data are from Asia, and Europe of which DDTs and HCHs are mostly detected. However, it is possible that the differences in the number of samples and sample types (captured alive or dead) might influence the outcome of this comparison.

Being this, DDTs level in our study were higher than the concentration levels reported from southern China (Zhang et al., 2011), and India (Dhananjayan, 2012) at which DDT is still in use, and from Japan (Kunisue et al., 2003). However, they are lower than those in birds from Belgium (Jaspers et al., 2006), northern China (Chen et al., 2009), and Greenland (Jaspers et al., 2013). The HCHs concentration in the present study obviously lie at low end compared to those in muscle of various bird species collected from different areas (Table 5).

Concentration of CHLs in muscle of the aquatic bird, great white pelican was comparable to the concentrations reported in muscle of aquatic birds, grey heron and great crested grebe (0.014 – 2.5 µg/g lw) from Belgium (Jaspers et al., 2006), but lower than in the muscle of white-tailed eagles from west Greenland (Jaspers et al., 2013) (Table 5). On the other hand the levels of CHLs in hamerkop, African sacred ibis and marabou stork were uniformly low, indicating minimal exposure of CHLs to these birds. HPTs levels in muscle in our study are comparable with concentrations reported in the muscle of various bird species from northern China (non-quantifiable to 0.22 µg/g lw) (Chen et al., 2009), but lower than those in birds from India (1.1 – 91 ng/g ww) (Dhananjayan, 2012) (Table 5). HCB levels ranged from ND to 0.042 µg/g lw was by far lower than the concentration levels reported from Belgium and Green land (Jaspers et al. 2006; 2013) (Table 5).

Table 5. Comparison of concentrations of OCPs (range, µg/g lipid weight) in muscle of four bird species from Ethiopia with those in other bird species

Location	Species (N)[1]	Collection year	DDTs	HCHs	Heptachlors	Chlordanes	HCB	Reference
Ethiopia	4	2012	3.7–148.3	ND–0.18	0.004–0.10	0.03–1.39	ND–0.042	This study
	*		114–1599	ND–1.90	0.12–1.56	0.76–39.4		
Northern China	7	2004–2006	0.2–1000	0.1–24.1	ND–0.22			Chen et al., 2009
Belgium	7	2003/2004	0.12–860	0.007–5.6		0.007–37	0.02–14	Jaspers et al., 2006
Greenland	1	1997–2009	0.7–530	0.02–3.7		0.36–160	0.1–10	Jaspers et al., 2013
Asia/Japan	2	1998/1999	0.34–33	0.02–0.69				Kunisue et al., 2003
India	7*	2006	6–822	ND–157	1.1–91			Dhananjayan 2012
Southern China	8*	2005–2007	1.6–140	0.9–67				Zhang et al., 2011

ND: Below detection limit.
*Values were expressed as ng/g ww.
N: Number of samples analyzed.
[1]Species: **Ethiopia**: Hamerkop(5), African sacred ibis (7), Marabou stork (5), and Great white pelican (5); **North China**: Kestrel (6), Sparrowhawk (Eurasian (11) and Japanese (6)), Owl (scops (6), long-eared (6) and little (6)), and Buzzard (6) (common and upland); **Belgium**: Common buzzard (16), Kestrel (3), Eurasian sparrowhawk (5), Owl (long-eared (6) and barn (7)), Grey heron, Great crested grebe; **Greenland**: White tailed eagle (17); **Japan**: Crow (carrion (5) and jungle (15)), **India**: Northern shoveler (2), Northern pintail (2), Garganey (2), Lesser sand plover (1), Brown-headed gull (2), Eurasian spoonbill (1), and Ruff (1); **South China**: Chinese-pond heron (5), Common Snipe (3), White-breasted waterhen (11), Slaty-breasted rail (5), Water cock (2), Ruddy-breasted crack (5), Common moorhen (1), Oriental turtle dove (2).

Toxicological significance

The chemicals assessed in this study are toxic, persistent, can be biomagnified along the food chain and may adversely affect the health, survival and reproduction of birds. DDE is well known for its adverse effect on the health of wildlife especially birds associated with eggshell thinning and reduction in the survival of young birds (Connell et al., 2003). Average concentration of *p,p′*–DDE of 20–1000 µg/g lipid wt in livers of birds was suggested to pose a threat to individual bird reproduction (Tanabe et al., 1998). Moreover, the lowest observable effect concentration of 120 µg/g lipid wt in eggs was estimated for depressed productivity in white-tailed sea eagle (Helander et al., 2002). Thus, taking into consideration that lipid normalized *p,p′*–DDE concentrations measured in muscle were similar with liver tissues, the maximum concentration levels of DDE ranged from 38.7 to 138.5 µg/g lipid wt in bird species might be sufficient to cause adverse effects on reproduction which population declines are reported to occur. As far as heptachlor epoxides (4 – 100 ng/g lw) and HCB (ND to 42 ng/g lw) are concerned, the concentrations were much lower than 1.5 µg/g, at which associated with decreased reproduction rates in avian experimental study (Henny et al., 1983; Boersama et al., 1986). Therefore, there are indications that *p,p′*–DDE levels in the current study pose a threat in terms of toxicity (i.e., eggshell thinning and survival of young birds) to the bird species resides in the Rift Valley region because DDT is still using in the region. Therefore, future studies seem necessary.

Conclusion

This study is the first report of OCPs contamination in birds and their prey of the Ethiopian Rift valley region and constitutes a starting point for future studies that evaluate temporal changes of OCPs in birds in this region. An overall appraisal of the OCPs concentrations suggested that DDTs were the most prominent contaminants, which is most likely due to their recent use for IRS as well as contamination from present illegal usage, past usage and spills from obsolete pesticides. Recent releases of γ–HCH (lindane) and technical chlordane were also observed in the region. The main DDT metabolite, *p,p′*–DDE was by far the most important compound in all samples and had significantly high burden in bird species, which may be sufficient to cause adverse effects on reproduction. Generally, the results from this study, albeit limited samples, call for a further study to evaluate the level and adverse effects of POPs on avian populations in the Rift Valley region.

Chapter 4:

Concluding Remarks and Future Perspectives

The purpose of this doctoral thesis was to investigate the contamination of Ethiopian Rift Valley aquatic ecosystem with OCPs and heavy metals using sediment, fish and birds. The bioaccumulation levels in fish and bird species were analyzed associated with stable isotope ratio. In addition the ecotoxicological risk assessment was evaluated for the well faring of humans and wildlife particularly in birds.

Historically, the usage of chemical pesticides in Ethiopia was low. However, the recent developments in increased food production and expansion in agricultural activities in the Rift Valley region have resulted in higher consumption of pesticides. The impacts of pesticides in Ethiopia are much more aggravated by the limited knowledge among users on safe practice, toxicological and chemical properties of these substances. Therefore, there is a need to conduct an assessment on the adverse effects of these pollutants (pesticides) in the terrestrials and aquatic ecosystems. In this study, the ecotoxicological risk assessment of pesticides was assessed in the Rift Valley aquatic ecosystem. The key concerns were related to OCPs and the major findings are:

- This study provided up-to-date information on OCPs and heavy metals concentrations in aquatic ecosystems (sediment, fish and birds) from the Rift Valley region, Ethiopia.

- It was found the presence of DDTs, HCHs, heptachlors, and chlordanes with varying concentrations in the aquatic ecosystem samples. In general, the contamination is dominated by DDT and its metabolites, attributing to its current use for malaria control and agriculture activities, and also contamination from past usage and spills from obsolete pesticides.

- Significant difference of DDTs levels (0.63 to 73.28 ng/g ww) were found among the studied fish species from Lake Awassa, and positive relationships between $\delta^{15}N$ values and metabolites of DDT (DDE and DDD) were found.

- The concentration ranges of DDTs in surface sediments from Lake Awassa were between 3.64 and 40.0 ng/g dry weight, and the levels varied depending on sampling sites. The levels of DDE and DDD from the surface sediments showed ecological concern on Lake Awassa because DDT is still being used in the area.

- ΣOCPs ranging from 0.04 to 10.9 µg/g lipid and 3.7 to 148.7 µg/g lipid in 5 fish and 4 bird species, respectively. Significant interspecies differences on OCPs levels were observed among the fish species. The carnivorous fish species presented higher concentrations of OCPs.

- The carcinogenic hazard ratios of OCPs for most of the studied fish species were greater than the threshold value **1**, suggesting that daily exposure to OCPs through consumption of fish would result a lifetime cancer risk of greater than one in a million.

- High burden of DDTs due to its use in agriculture and public health may cause toxic effects (i.e., eggshell thinning and survival of young birds) to Ethiopian bird species.

In general, our findings call for urgent action to reduce the level of OCPs' exposure and their effects on wildlife and human health. The low level of awareness in the study area and the public health and environmental consequence resulting from the misuse of pesticides is alarming. Therefore, there should be an integrated effort from governmental and non-governmental organizations in order to plan, tackle and control the use of pesticides effectively under the requirements of the Stockholm Convention, especially in relation to the misuse of DDT in agriculture. Although DDT is a low cost antimalarial tool, the possible adverse ecotoxicological effects through IRS must be carefully weighed against the benefits to malaria control.

- ☐ Focus on the awareness raising of farmers on proper pesticide management related issues.

- ☐ Provision of information and education to the public in order to minimize the exposure to pesticides.

- ☐ Food items should be routinely monitored for their concentrations of OCPs.

Therefore, Routine ecotoxicological risk assessment of persistent organic pollutants in the Ethiopian Rift Valley region seems necessary.

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Levels and Effects of Organochlorine Pesticides and Heavy Metals in Aquatic Ecosystem from the Rift Valley Region, Ethiopia

(エチオピア・リフトバレーにおける有機塩素系農薬と重金属による汚染状況の解明と水圏生態系への影響評価)

概要　Abstract in Japanese

有機リン系殺虫剤(OCPs)および重金属類は、水圏生態系における深刻な環境汚染物質であり、環境中長期残留性、生物濃縮性および内分泌かく乱や発がんなどの強い毒性から、これらによる環境汚染の対策は世界的に解決しなければならない問題の一つとなっている。水中に存在する OCPs や金属類の中には、食物連鎖の高次栄養段階に位置する野生動物あるいはヒトに高濃度に濃縮・蓄積するものがある。人工的な化学物質である OCPs の使用は、多くの先進国では禁止されているが、エチオピアなどの発展途上国においては未だに使用されているのが現状である。特に、安価かつ多様な用途に用いることができる DDT (Dichloro-diphenyl-trichloroethane)は、農業やマラリアなどの感染症を媒介するベクターコントロールの目的に依然として使用されている。さらに、エチオピアにおいては、現在では使用されていない農薬(Obsolete Pesticides)が大量に貯蔵され、未整備の貯蔵施設や不適切な管理のため、これらの農薬の環境への拡散も問題視されている。

アフリカ東部に位置し、7 つの湖から成るエチオピア・リフトバレーは、エチオピアの農業および工業の主要地域である。一方で、これらの地域産業の著しい発展は、同地域における農薬や金属類による環境汚染を引き起こしている可能性も無視できない。そこで、本博士論文では、底質・魚類・鳥類における OCPs と重金属類の蓄積濃度解析を行うことで、(1)リフトバレー地域の 2 つの湖(Awassa 湖と Ziway 湖)における、OCPs と重金属類の生物濃縮機構の解明、(2)環境毒性学的観点からのヒトや野生動物に対するリスク評価を目的とした。

Awassa 湖における研究結果より、DDT 類および重金属類の蓄積レベルには魚類間での種差が確認された。特に、高次栄養段階の魚類種において、より高濃度の DDT 類が検出され、DDT 類の生物濃縮性が認められた。また、湖内の全域（25 地点）から採取した底質の DDT 類の水平濃度分布解析により、流入河川付近あるいは湖岸の農業地域において高濃度の DDT 類が蓄積していることが明らかになった。以上の結果より、Awassa 湖周辺のヒトおよび野生動物に対する DDT 類をはじめとした農薬類汚染の影響が懸念された。

さらに Ziway 湖における研究では、魚類および鳥類における OCPs の濃度レベルおよびリスクアセスメントを行った。対象とした化学物質（HCHs、heptachlors、chlordanes、DDTs）の濃度は生物種間での差が認められた。これらの化学物質の中では、DDT 類が主に蓄積していることが明らかになり、上記のベクターコントロールのための DDT 使用や、Obsolete Pesticides の流出・拡散などが原因として考えられた。発がん性危険率の解析を算出したところ、この地域に生息する魚類筋肉の恒常的な摂食は、発がん性のリスクを有している可能性が明らかになった。また、野生鳥類種においても、個体の生存および繁殖に影響を及ぼす可能性のある高濃度の DDT 類が蓄積していることを解明した。

以上の研究結果から、エチオピア・リフトバレー地域における継続的なモニタリングや汚染に対する有効な対策の必要性が明らかになった。