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

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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^{-1} 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 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

1. 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 ratio ($\delta^{15}N$) to characterize an organism's trophic position while stable carbon 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 is, 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 (ii) to estimate an indication of public health risk levels due to the pollutants associated with fish consumption.

2. Materials and methods

2.1. Study area and sample collection

Lake Awassa (surface area: 90 km^2; mean depth: 11m) 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).

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 °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.

2.2. Materials

A standard mixture (DDTs, HCHs, Chlordanes, Drins, Heptachlors and hexachlorobenzene (HCB) at 10 μg mL^{-1} was purchased from Dr. Ehrenstorfer GmbH, Germany. Florisil (60-100 mesh) from Kanto Chemical Corp. (Tokyo, Japan) was activated at 130 °C 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 for several times. For Hg analysis, the sample containers, quartz boats, were furnacing at 800 ^{o}C for 5 h.

2.3. Stable isotope analysis

Small sub-samples of muscle tissues were dried at 60 ^{o}C 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:

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

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 $\delta^{13}C$ and $\delta^{15}N$ measurements.

2.4. Analysis of organochlorine pesticides

Fish fillet of 10 g was homogenized with anhydrous sodium sulphate 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 sulphate. 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^{-1} to 180 °C; 4 °C min^{-1} to 240 °C, and finally at 10 °C min^{-1} 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^{-1} and nitrogen as the make-up gas at a flow rate of 45 mL min^{-1}.

2.5. Analysis of heavy metals

Approximately 1.5 g of individual samples were dried in an oven at 40 ^oC 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 ^oC (5 min hold); and increase to 190 ^oC (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 and DOLT-4) were used for calibration and analytical performance studies. Hg recoveries were between 90-105% for the certified standard materials. The method detection limit was determined as 0.2 ng g^{-1}.

2.6. 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^{-1} 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^{-1} for all OCPs.

For heavy metals, replicate blanks and the reference materials DORM-3 (Fish protein, the National Research Council, Canada) and DOLT-4 (Dogfish liver, the National Research Council, Canada) were used for method validation and quality control. Replicate analysis of these reference materials showed good accuracy, with recovery rates ranged from 80%-115%.

2.7. Statistical analysis

All the statistical analyses were performed using JMP 9 (SAS Institute, Cary, NC, USA) 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 ($p < 0.05$).

3. Results and discussion

3.1. 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.

3.2. 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^{-1} (mean 10.83 ng g^{-1} ww) and presented in Table1. 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 *Oreochromis niloticus* is an herbivorous feeding mode, mainly feeds on planktons and lives in pelagic areas; where as *Clarias gariepinus* and *Barbus 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 to *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).

3.3. Heavy metal concentrations

The concentration of heavy metals expressed as μg g^{-1} 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^{-1} 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^{-1} 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^{-1}, 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 move 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.

3.4. Relationships between stable isotope and concentration of pollutants

Stable isotopes of nitrogen (δ^{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 δ^{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 δ^{15}N values on wet weight bases, Fig. 4. 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 biomagnified in the food web of the lake which implies that increases as the trophic level increases. Significant biomagnification of Σ-DDT 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 δ^{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). 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 δ^{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).

3.5. *Assessment of risk*

Food guideline values for Cu (20 μg g^{-1} ww), Zn (50 μg g^{-1} ww), Cd (0.2 μg g^{-1} ww), Hg (0.3 μg g^{-1} ww) and Pb (2 μg g^{-1} 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^{-1} 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 (EDI) for Hg were calculated and compared with Tolerable Daily Intakes (TDI). The data are on the assumption basis of 60 kg body weight and consumption of 150 g fresh fish per day as follows:

$$EDI = (C \times FDC) / BW \qquad (2)$$

where C is the concentration of the contaminants (μg g^{-1}), FDC stands for fish daily consumption (g day^{-1}) and BW represents the body weight (kg).

The EDI of Hg was calculated to be 0.65 μg day^{-1} kg^{-1} bw, which corresponds to 88 % of the TDI value (0.7 μg day^{-1} kg^{-1} bw). Based on the maximum value, 0.59 μg g^{-1}, the daily intake of Hg would be 1.48 μg day^{-1} kg^{-1} bw, which was 2.1 fold higher than TDI value.

4. 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… in the fresh water system as Lake Awassa continuously receives urban and industrial wastes from multiple sources.

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

Species (common name)	n		Standard length (cm)	Weight (g)	Lipid (%)	δ^{15}N (‰) Mean±SD (Range)	δ^{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).

Table 2. Mean and range of heavy metal concentrations (μg g^{-1} 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.

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.

Figures caption:

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

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

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

Fig. 4. Relationships between log-transformed concentration (ng g^{-1} 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*)

Fig. 1.

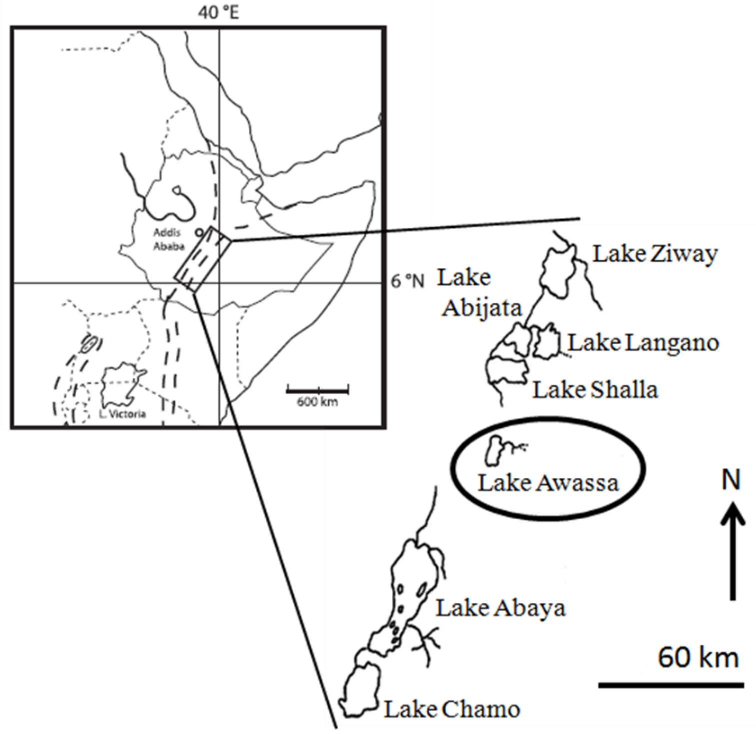
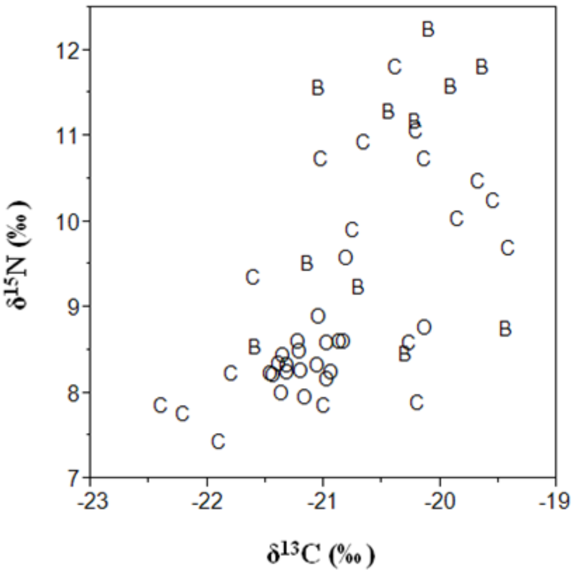
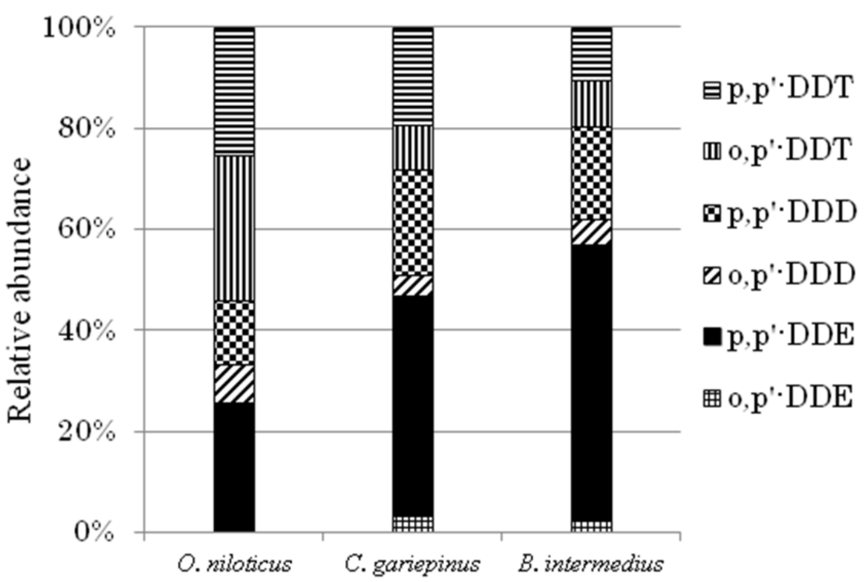

40 °E
Addis Ababa
6 °N
L. Victoria
600 km
Lake Ziway
Lake Abijata
Lake Langano
Lake Shalla
Lake Awassa
N
Lake Abaya
60 km
Lake Chamo

Fig. 2.

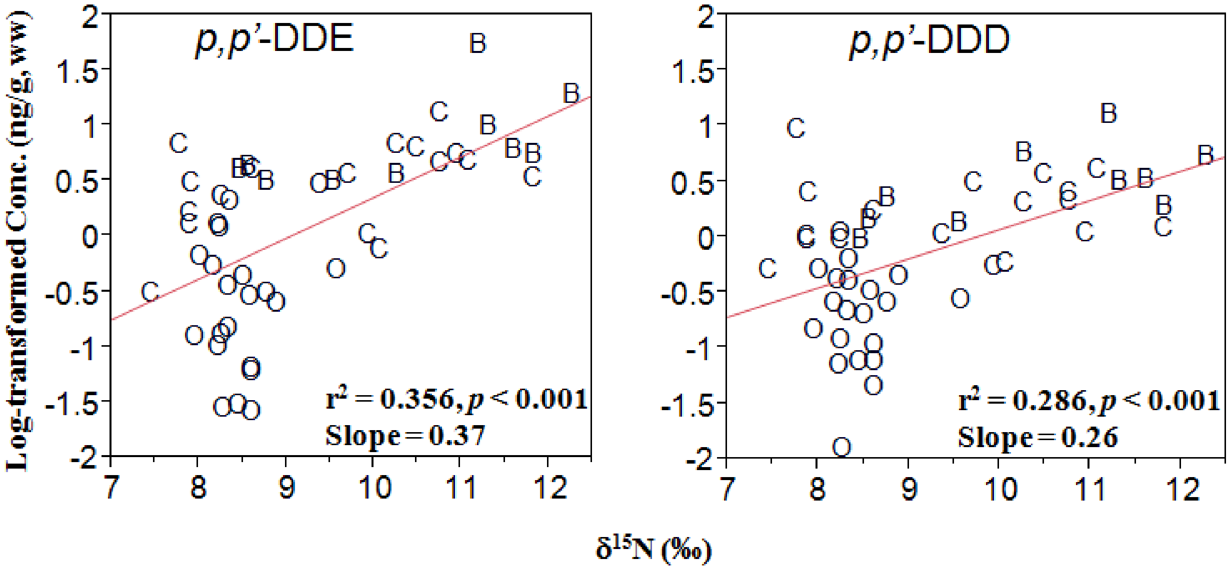

δ15N (‰)
12
11
10
9
8
7
-23
-22
-21
-20
-19
δ13C (‰)
B
C
O

537 Fig. 3.



538

539 Fig. 4.



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