Levels of selected pesticides and trophic transfer of DDTs through the aquatic food web in the Lake Ziway ecosystem

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Abstract

The levels of 27 selected pesticides and trophic biomagnification of DDT were investigated in biota samples of the Lake Ziway in the Rift valley region, Ethiopia. Pesticide residues were analyzed using gas chromatography coupled with mass spectrometry (GC MS). Carbon source and trophic position were calculated by using $^{13}$C and $^{15}$N stable isotopes, individually, and trophic magnification factors (TMFs) were inferred. Among pesticides analyzed, only DDT and its metabolites (o,p’-DDD, o,p'-DDE, p,p'-DDD, and p,p'-DDE) were quantified in biota samples. The most prominent metabolite was p,p'-DDE with mean concentration ranging from 0.22-7.7ngg$^{-1}$ wet weight. Moreover, the ratio of DDT/DDD+DDE in all the biota samples was less than 1 signifying historical DDT application. The trophic magnification factor (TMF) for p,p'-DDE, and ΣDDT were 1.18 and 1.19 respectively. Regression of log [ΣDDT] vs TL (trophic level) among all biota species showed a significant correlation, indicating that DDTs are biomagnifying along with the food web of Lake Ziway. The concentrations of DDTs and other organochlorine pesticides found in biota from Lake Ziway were, in general, lower than studies found in previous studies carried out in the same lake.

Keywords: Biomagnification, DDTs, Lake Ziway, Pesticides, Stable isotopes, Trophic level

1. Introduction

The use of pesticides within the agricultural sector in Ethiopia has contributed a significant advancement in agricultural technology and development. Also, it provides basic for agribusiness improvement, economic growth, and poverty reduction (Mengstie et al., 2017). Comprehensively, the use of agrochemicals in crop production shared with many farmers using pesticides for pest control to increase harvest and advance quality. The World Health Organization (WHO) reports that the two-hundredth of pesticide utilized in the world is focused on developing countries (PAN, 2012). For the last ten years, a quick increase in the amount and use of pesticides in the agriculture sector has been observed. This tendency is anticipated to continue for the future era due to social, economic, and innovative advancement (Greish et al., 2011).

In Ethiopia, for several years, organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs), Pyrethroid, and Carboxamide have been utilized for controlling pests on agricultural fields as well as for controlling malaria at the household level (Negatu et al., 2016). Recent agricultural growth in Ethiopia caused in higher demand for pesticides. However, there is no correct record of the particular volume of pesticides utilized in agriculture in Ethiopia.
Broad employment of pesticides for agricultural and non-agricultural activities has resulted in the prevalence of their residues in numerous environmental compartments including water, air, and soil (Schäfer et al., 2011). Organic pesticides are resistant to biological or chemical degradation. They are moreover mobile in the environment, robust ability for bioaccumulation in plants and animal tissues, and thus, directly or indirectly affect the health of personalities (Picó et al., 2007).

Lake Ziway is the freshwater lake inside the Rift Valley region in Ethiopia. The lake plays a significant part in providing improvement to the economy of society living around the lake and their livestock. Likewise, many frames use Lake water for irrigation (Meshesa et al., 2012). According to Desta et al. (2017), in addition to its economic and livelihood values, the lake supports unique ecological and hydrological characteristics. However, Lake Ziway is presently unprotected from numerous anthropogenic pressures because of the intensification of agricultural activities around the lake (Desta et al., 2017; Teklu et al., 2018). A study by Malefia, (2012) in Lake Ziway revealed that the increase in the intensive and unplanned uses of pesticides and fertilizers from small-scale farms and large-scale flower farms contributed to the growing deterioration of the lake’s water. Moreover, Jansen and Harmsen (2011) rumored that the lake water and the surrounding surface waters are polluted by the discharge of pesticide residue from the agricultural fields into the lake waters. Thus, the use of excessive pesticides around the lake is growing (Meshesa et al., 2012). As a result, the pH, turbidity, conductivity, and nutrient levels in this part of the lake have surpassed both the national and international standards (Malefia, 2012; Teklu et al., 2018). Levels of some organophosphorus pesticides (Deltamethrin, Diazinon, Spiroxamine) and organochlorine (endosulfan) were additionally detected in water (Tekilu et al., 2018). Moreover, the occurrence of residues of aldrin, DDTs, and its metabolites, chlordane, Edirne, endosulfans, dieldrin, methoxychlor, and heptachlor epoxide was reported in the fish samples (Deribe et al., 2013; Yohannes et al., 2014) of Lake Ziway, Ethiopia. These findings have given an alarm for the possibility of the occurrence of other frequently used pesticides such as organophosphorus pesticides (OPPs).

The concept of food web ecology is important to understand the structure and components of the community. Moreover, Constituents of the food web give imperative information about energy transfer within a community, predator-prey interactions, and competition between and within species (Morin, 2011). Biomagnification of persistent contaminants can impact the health of the food web within the ecosystem because the feeding habit is a major exposure route for many organic pollutants (LeBlanc and Buchwalter, 2010). Several studies including a study by Mazzoni et
al. (2020) investigated the trophic magnification factor (TMFs) of DDT and its metabolites in freshwater environments. Stable nitrogen is a common biomarker technique to investigate the biomagnification of organic pollutants, trophic level, and feeding preferences in aquatic food webs (Fisk et al., 2001; Borgå et al., 2011). Likewise, stable isotope examination has appeared as a tool in identifying the origin of food in the ecosystem and is used commonly to investigate aquatic ecology (Middelburg, 2014).

The determination of both stable C and N isotope ratios can provide valuable information into the feeding ecology of biota and its potential influence on the trophic enrichment of organic carbons (OCs) (Fisk et al., 2001; Hobson et al., 2002). Studies investigated levels of organochlorine pesticides in Lake Ziway have focused on fish species (Deribe et al., 2013; Yohannes et al., 2014). However, the biomagnification of organochlorine pesticide and the influence of trophic position on contaminant profiles within a quantified aquatic food web has not been addressed in this region. The objectives of this study were to investigate the levels of selected pesticide residues in the biota and examines the biomagnification and trophic transfer of DDTs in Lake Ziway.

2. Material and methods

2.1 Study Area

Lake Ziway is a shallow freshwater lake located in Rift Valley, in the southeast part of Ethiopia, at 1636 m a. s. l. (71°52’ N, 38°14.5’ E, Figure 1) which belongs to the Ziway–Shala basin and has a catchment area of about 7000km² and an average surface area of 490 km². The lake has an average volume of 1.8 km³ and a maximum depth of 9m (average depth, 2.5m) (Vallet-Coulomb et al., 2001). There are two inflowing rivers, the Meki River to the north-west, and the Ketar River to the east. The lake flows into Lake Abiyata, via the Bulbula River. The climate of the region is characterized by two types of rainfall with short duration distribution, highly variable, relatively low rainfall from February to June, and higher rainfall from July to September (Zegeye et al., 2006).

The pH of the lake varieties from 7 to 8 and the normal conductivity is around 400 μS cm⁻¹, which is low contrasted with most Ethiopian Rift Valley Lakes (Malefia, 2012). The lakeshore is secured with enormous scopes of sand and grass, infrequently trees, and shrubs that give path along a descending gradient to temporary and permanent wetland habitats such as shallow pools overwhelmed by grasses, lilies, and more profound reed beds dominated by Typha grass. The fish species of the lake include indigenous species like Nile tilapia (Oreochromis niloticus) and African big
barb (Barbus intermedius) and introduced species such as African sharp tooth catfish (Clarias gariepinus), common carp (Cyprinus carpio), Golden carp (Carassius auratus) and redbelly (Tilapia zillii) (Negassa and Getahun, 2003).

Figure 1 Map of Lake Ziway (adapted from Deribe et al., 2013)

2.2 Sample collection and preparation

Biological samples (n=62) were collected from Lake Ziway in December 2018. Aquatic organisms including phytoplankton and zooplankton were collected by pumping large volumes of water through plankton nets with different mesh sizes. Aquatic macrophytes (Typha latifolia and Aurudo donax) were collected by hand, thoroughly rinsed to remove organic matter and invertebrates, and placed into plastic bags and held on ice. Aquatic insects (Dipteral larvae) were sampled with 500-μm D frame nets by flipping rocks and from aquatic macrophytes and submerged substrates using a sweep net or searching by hand. Fish sampling was carried out by purchasing fish from the local fishermen upon landing. A total of 50 (30 females and 20 males) samples from four fish species (C. auratus, C. gariepinus, O. niloticus, and C. carpio) were sampled. The total length (cm), weight (g), and sex of each fish were documented. The contents of the gut were removed and preserved in 96 % ethanol. Muscle samples were taken from
each fish species, according to the procedures in the EMERGE protocol, as depicted by Rosseland et al. (2001) as referred to in Deribe et al. (2013) and frozen. The frozen biological samples were transported to Norway and were analyzed for stable isotopes of carbon ($^{13}$C and $^{12}$C) and nitrogen ($^{15}$N and $^{14}$N) to determine components of the food web, trophic pathways, and level of pesticides (selected OCPs, OPPs, carbamate, and pyrethroid derivatives). Assortment and preparation methods were like those of Pingram et al. (2014) and Penland et al. (2018).

### 2.3 Sample extraction and analysis

The pesticides were analyzed at the laboratory of the Norwegian Institute of Bio-economy Research (NIBIO): Pesticides and natural products chemistry, Norway. Frozen biota tissue (macrophyte, zooplankton (composite), invertebrate larvae, and fish tissue samples were slowly thawed in a refrigerator; were homogenized using a kitchen blender (Braun GmbH, Kronberg, Germany) into small pieces and mixed using Polytron (Kinematica AG) for 30 s with a speed of 2000–3000 rpm. Pesticides were extracted from accurately weighed 10 grams for macrophyte, 2 g for invertebrate, and 5 g fish samples in 50mL Teflon centrifuge tube following a modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) procedure (Norli et al., 2011). The samples were then rehydrated in 10 mL Milli-Q water (and extracted with 10 mL acetonitrile: glacial acetic acid (99:1 v/v) and shaken for 10 min using an "end over end" device. A tube holding pre-weighted amounts of 4 g magnesium sulfate (MgSO$_4$), 1 g sodium chloride, 0.5 g sodium citrate dibasic sesquishydrate, and 1 g sodium citrate tribasic dihydrate (CH$_3$COONa$_2$H$_2$O), supplied from SUPELCO, Bellefonte, PA, USA, a salt mixture was added and the tube then was shaken for 10 min using an "end over end" device. The mixture was used to help the partitioning of the organic and aqueous phases. After centrifugation (5 min at 3000 rpm) the aliquots of the resulting supernatant was decanted into a new tube containing 900 mg magnesium sulfate and 150 mg primary secondary amine (PSA) which is used to clean organic extract and shaken for 1 min, centrifuged at 3000 rpm for 5 min and 4 mL of the acetonitrile layer was transferred into 50 mL tube. Each sample solution was transferred to GC vials for pesticide analysis.

All targeted pesticides were measured using gas chromatography coupled with high-resolution mass spectrometry (GC-MS/MS). Pesticide concentrations were expressed in g. g$^{-1}$ (ww) and the limit of quantification (LOQ) with this method was 1 g.g$^{-1}$ (ww) with a measurement precision of 0.1 g.g$^{-1}$ when data were greater than the LOQ. In this study, pesticide concentration was not lipid-corrected as the maximal residue limit indicated by European regulations is expressed in wet weight.
2.4 Quality assurance and quality control

To remove the matrix effects on pesticide quantitation in the samples, calibration curves were created based on the spiked blank matrix and high linearity ($r^2 > 0.99$) was obtained for all target analytes. Method blanks and spiked control were included with each batch of 15 samples to check background contamination and monitor any instrument carryover. Three quality control standard solutions (20 ng/mL$^{-1}$ pesticide standards mixture) were run to monitor sensitivity drift along with each 8–12 real samples. Limit of detection (LOD) was set to three times the background noise (S/N = 3). Recovery tests were carried out in triplicate to evaluate the precision of the method. In biota samples, recoveries varied from 90% to 120% and precision was below 20% for all pesticides. Pesticide concentrations were measured through a comprehensive quality control scheme that included: laboratory blanks, matrix spikes, and triplicate samples. Blank contamination is the most common problem observed in the determination of pesticides at trace levels. Thus, precautions were taken to prevent contamination from personnel, organic solvents, equipment, and glassware. Blank assays were performed employing MilliQ water samples, to check for laboratory background levels of the studied compounds.

2.5 Stable isotope analyses

Stable isotope analyses were carried out at the Environmental Chemistry Section, Department of Plant and Environmental Sciences, Norwegian University of Life Sciences (NMBU). Stable-nitrogen ($\delta^{15}$N) isotope is often used to determine the trophic position of animals as the $\delta^{15}$N value increases with the TL (trophic level). Stable carbon ($\delta^{13}$C) isotope is often used to infer a particular food web since different photosynthesis mechanisms result in a typical $\delta^{13}$C pattern that changes slightly throughout the food chain. The stable isotopes of nitrogen ($^{15}$N and $^{14}$N) and carbon ($^{13}$C and $^{12}$C) were examined in homogenized and freeze-dried samples subjected to combustion in a Flash Elemental Analyzer (EA) as described in Deribe et al. (2013). $^{15}$N/$^{14}$N, and $^{13}$C/$^{12}$C were conveyed according to the following formula:

$$\delta R\% = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000$$

\textit{equation 1}

Where, $R = ^{15}$N/$^{14}$N for $\delta^{15}$N or $R = ^{13}$C/$^{12}$C for $\delta^{13}$C. Atmospheric nitrogen (N$_2$) was used as an R standard for nitrogen.

2.6 The trophic level calculation for biomagnification assessment
Based on the process of $^{15}$N enrichment in consumers over their prey (Post, 2002), the trophic position of the sampled organism was determined according to their relative abundance of $^{15}$N/$^{14}$N ($\delta^{15}$N). It was used the invertebrate larvae (spp.) as the baseline for TL $\delta^{15}$N estimation (mean value of invertebrate larvae; $\delta^{15}$N$_{baseline} = 8.7\%$) (Assumed to occupy a trophic level = 2) as the mean enrichment of $\delta^{15}$N per trophic level is 3.4 (Post, 2002), trophic level (TLs) for each species were estimated from raw $\delta^{15}$N value using the following equation:

$$TL = TL \text{ baseline} + \frac{(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}})}{3.4} + 2 \ldots \ldots \ldots \text{equation 2}$$

Where TL is the trophic level, $\delta^{15}$N$_{\text{consumer}}$ is the $\delta^{15}$N signature in the specified organism, $\delta^{15}$N$_{\text{baseline}}$ is the mean $\delta^{15}$N value for the dipteran larvae. (8.7%) and 3.4 is the trophic enrichment factor for $\delta^{15}$N in an aquatic food web recommended to be used for constructing food webs when a priori knowledge of $\Delta^{15}$N is unavailable (Hobson et al., 2002).

TMFs for DDTs were determined according to Walters et al. (2011). Trophic magnification factors (TMFs) were calculated to determine total DDT magnification in the food web. TMF calculation was performed for the lake food web (which included macrophytes, invertebrates, and fish) to determine the total DDTs that trophically magnified from macrophyte to fish. TMFs were derived from the plots of the natural log of $\Sigma$DDT concentrations (wet mass) versus TL:

$$\log_{10} [\Sigma\text{DDT}] = a + (b \times TL) \ldots \ldots \ldots \text{equation 3}$$

Where $a$ is the y-intercept (constant dependent on the background concentration) and $b$ is the slope of regression log$_{10}$ $[\Sigma\text{DDT}]$ function to TL calculated based on $\delta^{15}$N (indicating the biomagnification power of the contaminant). The above equation was used to calculate TMF (Fisk et al., 2001): The trophic magnification factor (TMF), also called food web magnification factor (FWMF), and is calculated from the slope using the following formula:

$$\text{TMF} = e^b \ldots \ldots \ldots \text{equation 4}$$

Contaminant with TMF greater than 1 is considered to biomagnify in the food chain while TMF values comprised between 0 and 1 indicate that the contaminant is not biomagnified in the food web. A TMF value inferior to zero indicates that the contaminant is excreted by the organisms in the food chain (Fisk et al., 2001).
2.7 Statistical analysis

The normality of data was tested with Shapiro-Wilk tests. Concentrations of pesticides were compared between species (macrophyte, zooplankton, invertebrate larvae, and fish species and) with one-way analyses of variance (ANOVA), followed by multiple comparison tests, performed with post hoc Tukey's honestly significant difference (HSD) test using Statistical Package For Social Sciences (IBM SPSS version 20). Simple linear regression analyses were used to examine the relationship between the logarithm of DDTs concentrations (log10 [DDTs]) and the trophic level of species ($\delta^{15}$N). Comparisons of the length and weight of each fish species were performed with Student's t-tests. Significance was set at $p = 0.05$. Microsoft Excel version, 2016 was used to perform descriptive statistics.

3. Results

3.1 Pesticide concentrations in biological samples

Organochlorine pesticides (Aldrin, Chlordane-cis, Chlordane-trans o p'DDD, p p'DDD o p'DDE, p p'DDE, o p'DDT, p p'DDD, Dieldrin, $\alpha$-Endosulfan, $\beta$-Endosulfan, Endosulfan-sulphate, HCB $\alpha$-HCH, $\beta$HCH, $\gamma$-HCH, heptachlor, heptachlor epoxide cis, heptachlor epoxide trans, metalaxyl, methoxychlor, oxychlordane), Organophosphorus pesticides (chlorpyrifos, diazinon) and other pesticides (bupirimate, fenarimol, tetradifon), and Pyrethroid (deltamethrin), Carboxamide (boscalid) were analyzed in biota samples (macrophyte, zooplankton, invertebrate(Dipteral larvae), and fish species,) from Lake Ziway (Table 1). Among the pesticides analyzed in biological samples, only DDT and its metabolites (o,p'-DDD, o,p'-DDE, p,p'-DDD, and p,p'-DDE) were quantified in 86.7% of the samples. Including DDTs, none of the pesticide analyzed was detected in macrophyte samples ((Typha latifolia and Aurudo donax). A predominant metabolite was p,p'-DDE which was detected in 85% of the samples analyzed. p,p' DDT, p,p' DDD, o p' DDT, o,p' DDE, and o,p' DDD were detected in 23.3%, 15%, 10%, 11.7% and 1.7% of total samples, respectively. The metabolite, o,p' DDE, and o,p' DDD were only detected in Clarias gariepinus.
The maximum mean concentration of Σ DDT (sum of p,p′ DDT, p,p′ DDE, p,p′ DDD, o,p′ DDT, o,p′ DDE, and o,p′ DDD), was recorded in Clarias gariepinus (12 ng·g⁻¹ wet weight). Mean concentration of ΣDDT was present in the order: Clarias gariepinus (12 ng·g⁻¹ ww) > Carassias auratus (5.92 ng g⁻¹) > Oreochromis niloticus (2.43 ng g⁻¹ ww) > Cyprinus carpio (2.3 ng g⁻¹ww), Invertebrate (1.44 ng g⁻¹ww), and Zooplanktons (0.71 ng g⁻¹ww).
Significantly different DDTs levels were found among the species. A significantly higher ΣDDT level was observed between *C. gariepinus* and all other biota (*P* < 0.05). Significantly higher p,p′-DDE were found in *O. niloticus* than in zooplankton (*p* < 0.001) and significantly lower than *C. gariepinus* (*P* < 0.006) and *C. auratus* (*P* < 0.015). However, p,p′-DDE levels found in *O. niloticus* were not significantly different from invertebrate (*P* > 0.05) and *C. carpio* (*P* = 0.74). The relative percentage of DDTs is shown in Figure 2. Among the metabolites of DDTs, p,p′ DDE contributed on average 67.3% to the Σ DDT, followed by p,p′-DDT, which is accounted for 24.3% on average.

In the present study, o,p′-/p,p′-DDT ratios were lower than the technical DDT mixture which is 0.2. The concentration of p,p′ DDE was higher than p,p′- DDT in each biota analyzed and contributed 30 to 100% of the total DDT concentration.

*Figure 2: Composition of individual DDT components (to Σ-DDT ng/g WW) in biota (*Clarias gariepinus*, *Carassius auratus*, *Oreochromis niloticus*, *Cyprinus carpio* Invertebrate, and Zooplanktons) in Lake Ziway*
Table 2: A comparison of DDTs and other organochlorine pesticides concentrations (Mean with minimum and maximum values) by different authors of Lake Ziway fish species

| Sampling period | Species      | p,p'-DDT (ng/g) | p,p'-DDE (ng/g) | p,p'-DDD (ng/g) | Σ-DDT (ng/g) | ΣHPTsn | ΣHCHsn | CHls | Reference          |
|-----------------|--------------|-----------------|-----------------|-----------------|--------------|--------|--------|------|--------------------|
| 2008            | C. gariepinus| 6.22 (3.28-17.37) | 17.81 (3.8-122.77) | 3.98 (0.37-30.6) | 28.78 (7.8-171.96) | *      | *      | *    | Deribe et al., 2013 |
|                 | C. aurtus    | 12.49 (4.94-56.49) | 7.10 (1.53-20.3) | 2.84 (0.35-14.12) | 18.33 (3.65-76.17) | *      | *      | *    |                    |
|                 | O. niloticus | 5.3 (2.86-8.95) | 5.67 (1.00-19.31) | 4.87 (0.77-56.87) | 18.93 (4.78-139.01) | *      | *      | *    |                    |
|                 | T. zilli     | -               | 4.7 (0.89-40.73) | 1.42 (0.33-12.73) | 6.04 (0.89-53.47) | *      | *      | *    |                    |
| 2011            | C. gariepinus| 0.62 (2.6-61.9) | 6.92 (0.34-1.56) | 0.79 (0.27-2.01) | 9.0 (0.58-1.50) | 0.65 (0.59) | 0.72 (0.61) | 0.90 (0.87) | Yohannes et al., 2014 |
|                 | C. aurtus    | 0.57 (0.77-10.6) | 2.48 (0.20-1.52) | 0.58 (0.16-1.85) | 4.55 (0.19-4.00) | 0.59 (0.61) | 0.87 (0.40) |        |                    |
|                 | O. niloticus | 0.31 (0.90-5.12) | 1.32 (0.44-2.27) | 0.40 (0.29-5.10) | 2.33 (0.17-0.61) | 0.90 (1.26) | 1.90 (0.40) |        |                    |
|                 | T. zilli     | 0.77 (0.42-1.45) | 1.89 (0.19-0.69) | 0.85 (0.91-3.94) | 4.38 (0.65-1.32) | 4.38 (1.35-13.2) | 1.89 (0.91-3.94) |        |                    |
| Present study   | C. gariepinus| 2.3 (12.00) | 7.70 (100-30) | 1.30 (nd-6.00) | 12 (1.00-5.2) | n.d (n.d) | n.d (n.d) | n.d   | Our study          |
|                 | C. aurtus    | 1.92 (nd-7.00) | 4.00 (1.00-9.00) | n.d (n.d) | 5.92 (1.00-16) | n.d (n.d) | n.d (n.d) | n.d   |                    |
|                 | C. carpio    | n.d (nd-6.00) | 2.3 (nd-6.00) | n.d (n.d) | 2.30 (nd-6.00) | n.d (n.d) | n.d (n.d) | n.d   |                    |
|                 | O. niloticus | 0.93 (nd-9.00) | 1.00 (nd-6.00) | n.d (n.d) | 2.43 (nd-15.50) | n.d (n.d) | n.d (n.d) | n.d   |                    |

n.d = below detectable limit, * = not analyzed
3.2 Lipid content

The lipid content of the fish species from Lake Ziway was within the range of 0.10–0.91 percent (Table 3). The highest mean lipid content was recorded in *C. gariepinus*, followed by, *C. auratus, C. carpio*, and *O. niloticus*. A significant difference between mean lipid content (%) was found among fish species (*P* < 0.001). However, the mean lipid content of *C. carpio* was not significantly different from *C. auratus* (*P* > 0.05). Mean concentration of p,p’DDE and p,p’DDD show a positive correlation with lipid content for all fish species (*R*² = 0.12; *P*, 0.014 and *R*² = 0.18; *P*, 0.003 respectively), whereas no significant correlation was found between lipid content and concentration of p,p’DDT (*R*² = 0.07; *p* = 0.18).

3.3 Concentrations of DDTs and fish size

The mean total length of fish samples from Lake Ziway was 38.1, 27.7, 27.5, and 18.2 cm for *C. gariepinus, C. auratus, C. carpio, and O. niloticus* respectively. The mean weight for the fish samples was 639, 428.4, 278.9, and 118.8 g for *C. gariepinus, C. auratus, C. carpio, and O. niloticus* respectively (Table 3). The concentration of DDTs shows a positive correlation with fish size (Figure 3). The mean concentration of p,p’-DDE was significantly related to total length for *Clarias gariepinus* (*F* = 41.94, df =1, *P* < 0.001), *Carassius auratus* (*F* = 6.47, df=1, *P* = 0.027) and *Oreochromis niloticus* (*F* = 5.54, *P* = 0.037). No significant relation was observed between the concentration of p,p’-DDE, and the total length of *Cyprinus carpio* species. Also the concentration of p,p’-DDD (*F* = 28.76, df =1, *P* < 0.001), was significantly related to total length for *C. gariepinus*. However, the concentration of p,p’-DDT (*P* > 0.05) was not significant at all for all fish species. There was a significant relationship between concentration p,p’-DDE (*F* = 29.45, df =1, *P* < 0.001), and weight of *C. gariepinus*. The concentration of p,p’-DDD (*F* = 19.33, df = 1, *P* = 0.001) were positively correlated with weight of *C. gariepinus*. However, the concentration of p,p’-DDT (*P* > 0.05) was not significant at all for all fish species.
Table 3: Mean standard deviation, minimum and maximum values of total length(cm), weight(g), stable isotope ratios of nitrogen (δ¹⁵N, ‰), and carbon (δ¹³C, ‰), number of samples(N), and lipid content of the examined biota from Lake Ziway, Ethiopia, sampled in 2018. * Means with different letter superscript are significantly different with in column (Tukey test is applied; p < 0.05). * Trophic level calculated according to equation 2

| Species      | N   | Length (cm) | Weight (g) | Lipid content (%) | δ¹⁵N (‰) | Δ¹³C (‰) | Trophic (TL)* |
|--------------|-----|-------------|------------|-------------------|----------|----------|---------------|
| C. gariepinus| 13  | 38.1(4.6)   | 637(900)   | 0.74(0.13)        | 14.72 (0.95) | -21.7(0.44) | 3.6           |
|              |     | 26, 76      | 145.4, 3430| 0.55, 0.91        | 13, 16   | -22, -21 |
| C. carpio    | 10  | 27.5(1.3)   | 278.95(45.3)| 0.32(0.03)       | 13.8 (0.79) | -22.9(0.88) | 3.3           |
|              |     | 25, 30      | 210, 376.4 | 0.26, 0.36        | 13.15    | -25, -23 |
| C. auratus   | 13  | 27.4(6.8)   | 428.4(337.1)| 0.34(0.06)       | 13.38 (0.77) | -22.3(0.63) | 3.2           |
|              |     | 20, 38      | 128.7,1099.8| 0.28, 0.51       | 12.15    | -23, -21 |
| O. niloticus | 14  | 18.2(2.1)   | 118.5(39.5)| 0.16(0.05)       | 10.43 (1.9) | -22.6(1.87) | 2.4           |
|              |     | 15, 22.5    | 69.6, 206.5| 0.10, 0.26        | 8, 13    | -17, -19 |
| Invertebrate | 5   |             |            | 8.74              |          |          | 2.0           |
|              |     |             |            | -26.5(0.71)       |          |          | -27, -26     |
| T. latifolia | 5   |             |            | 2 (1.8)           |          |          | 0.2           |
|              |     |             |            | -26.6(0.89)       |          |          | -28, -26     |
| A. donax     | 5   |             |            | 2(1.4)            |          |          | 0.2           |
|              |     |             |            | -26.8(0.84)       |          |          | -28, -26     |
Figure 3: The relationship between p,p′-DDE concentrations in ng g⁻¹ ww and length in cm for C. auratus, O. niloticus, C. gariepinus, and C. carpio (A, B, C, D respectively) from Lake Ziway
3.4 Stable isotope and Trophic level

The δ¹⁵N values of the biota ranged from 2% to 16% (Table 3). The mean values of the δ¹⁵N were larger for *C. gariepinus* (14.7%) indicating the higher trophic level of the lake food web which is followed by *C. carpio* (13.8%), *C. auratus* (13.4%), *O. niloticus* (10.4%), invertebrate larvae (8.7%), *T. latifolia* (2%), and *A. donax* (2%). The δ¹⁵N of *C. gariepinus* and *C. carpio* were statistically different from all the other biota (*p* < 0.05). *C. gariepinus* had the highest mean δ¹⁵N and shared the top trophic level with *C. carpio* (Tukey HSD, *P* > 0.05); their δ¹⁵N levels were significantly different from the rest of the biota analyzed (*P* < 0.05) (Table 3).

Concerning stable δ¹³C isotope signatures, ranged from -28 to -19.0% in the biota sampled from the Lake Ziway food web of the Rift valley region, show the species of the lake utilized carbon sources with both littoral and pelagic origin (Figure 4). *C. gariepinus* had the greatest δ¹³C enrichment of any biota (mean δ¹³C value of -21.8%). While the invertebrate larvae, C sources were likely derived from macrophyte. There were no statistical differences in δ¹³C among the fish species analyzed (Tukey HSD, *p* > 0.05). The three fish (*C. auratus*, *C. carpio*, and *C. gariepinus*) relied on C sources that were more benthic in origin (and more enriched in δ¹³C). Interestingly, *O. niloticus* δ¹³C content was not significantly different from that of any other fish species analyzed, indicating a broad diet (Table 3). The lake invertebrate larvae, the herbivorous, have δ¹³C is more negative than any of the food web components analyzed. As a strict herbivore, invertebrate species were the most ¹⁴N depleted.

Average trophic levels (TLs) calculated based on δ¹⁵N for each organism were 0.2, 0.26, 2.0, 2.4, 3.2, 3.3, and 3.6 for *T. latifolia*, *A. donax*, Invertebrate, *O. niloticus*, *C. auratus*, *C. carpio*, and *C. gariepinus* respectively (Table 3). It was assumed that the dipteral larvae samples collected during this study represented the TL 2.0, as this genus is known to graze on primary producers (mainly macrophytes). Fish species, except *O. niloticus* (TL 2.4), generally represented the third trophic level. *C. auratus* (TL 3.2) and *C. carpio* (TL 3.3) feed on detritus and invertebrate. *C. gariepinus* (TL 3.6) prey on various fish and benthic invertebrates and as a result have a more ¹³C enriched (benthic) signature relative to other fish species. This species had slightly higher δ¹⁵N signatures compared to other fishes due to predation on other fish species and (or) feeding on benthos, which is typically more δ¹⁵N enriched relative to pelagic biota (Figure 4). The stable isotope profile of *O. niloticus* (TL 2.4) indicates that these specimens were feeding mainly on macrophytes or primary producers, which is consistent with the dietary profile of this species.
Concentrations of DDTs and trophic position

The $\delta^{15}$N of *O. niloticus* were statistically different from all the other fish species (Tukey HSD, $p < 0.001$). *C. gariepinus* had the highest mean and *O. niloticus* occupied the lowest ranks of the fish food web, nearly one trophic level less than *C. auratus*. The $\delta^{15}$N of *C. gariepinus* were not statistically significant from *C. auratus* and *C. carpio* (Tukey HSD, $p > 0.05$).

The concentrations of $\Sigma$DDT and $p,p'$-DDE showed a positive correlation with $\delta^{15}$N ($F = 17.5$, df = 1, $P < 0.001$, N = 62 and $F = 11.7$, df = 1, $P = 0.001$, N = 62 respectively), however, the relationship between the concentrations of the other DDT metabolites ($p,p'$-DDT, $o,p'$-DDT, $o,p'$ DDE and $o,p$-DDD) and $\delta^{15}$N was not significant ($P > 0.05$).

**Figure 4**: Relative trophic position of biota (*C. gariepinus, C.auratus, O. niloticus, C. carpio Invertebrate, and Zooplanktons*), sampled in December 2018 from Lake Ziway, values are based on the mean of the stable isotope ratios of nitrogen ($\delta^{15}$N, %) and carbon ($\delta^{13}$C, %). Horizontal and vertical lines indicate the SDs of the mean values.

**Figure 5** illustrates the biomagnification of $\Sigma$DDTs with the trophic level in biota from Lake Ziway. A significant relationship was found between log-transformed concentrations of $\Sigma$DDT ($\log_{10}\{\Sigma$DDT$\}$) versus TL calculated based on $\delta^{15}$N signatures of organisms in Lake Ziway ($r^2 = 0.23$, $P < 0.001$). Trophic magnification factors (TMF) were calculated based on the slope of the linear regression model. TMF calculated was higher than 1 (TMF = 1.19). When all species were pooled together, the relationship between concentration $p,p'$-DDE and TL based on $\delta^{15}$N was significant ($F = 23.2$, df = 1, $P < 0.001$, N = 62), with TMF of 1.18.
Figure 5: The relationship between log<sub>10</sub>-transformed, concentration for DDTs (ng g<sup>-1</sup>), and isotopically determined trophic level (TL) from Eq. (2). Mean values for each species macrophyte, invertebrate, and four fish species.

4. Discussion

4.1 Pesticides concentrations

Among 27 pesticides analyzed in biological samples, only DDT and its metabolites (o,p′-DDD, o,p′-DDE, p,p′-DDD, and p,p′-DDE) were quantified. Interestingly, all other pesticides analyzed were below the detectable limit in all biological samples (Table 1). The absence of organochlorine pesticides in the biota sample, such as dieldrin, endosulfan, HCB, HCH, chlordane was probably because, no current use of these pesticides by the farmers around the Lake Ziway, in their farming activities. Our recent survey study also revealed that small-scale vegetable farmers around the littoral zone of Lake Ziway in Ethiopia, do not use the most hazardous pesticides of WHO class 1a and 1b and banned pesticide such as notorious DDT, dieldrin, HCB, HCH, chlordane, and Endosulfan (Mergia et al. 2021). The decreasing trend of concentration of organochlorine pesticides in the study area, Lake Ziway biota could be related to the effectiveness of the National Implementation Plan (NIP) for the Stockholm Convention in Ethiopia. The main objective of the NIP is to prepare a comprehensive and realistic action plan for the effective management of POPs chemicals in the Ethiopian context and to reduce, and ultimately eliminate, the use and release of POPs following the
requirements of the Stockholm Convention and national sustainable development objectives and strategies such as the Environmental Policy and the Plan for Accelerated and Sustainable Development to End Poverty (FEPA, 2006). Moreover, the presence of DDTs residues in Lake Ziway biota was expected based on the previous history of heavy use and long environmental persistence. The present study showed that the organochlorine levels in both fish and another biota were substantially lower than those recorded in other environmental and biological samples (Mzoughi et al., 2016; Unyimadu et al., 2018).

None of the analyzed organophosphorus pesticides (chlorpyrifos, diazinon), Carboxamide (boscalid), pyrethroids (deltamethrin), and other pesticides (bupirimate, fenarimol, tetradifon) were detected in any of the samples analyzed despite all of them being registered in Ethiopia and some are used heavily by small scale vegetable farmers along the littoral zone of Lake Ziway, especially chlorpyrifos, dimethoate, and diazinon used in vegetable farms (applied on onion, cabbage, and tomato), and chlorpyrifos-methyl in wheat (Negatu et al., 2016; Mengstie et al., 2017). These pesticides are known as non-persistent compounds of low water solubility (Aislabie et al., 1997), and therefore, the absence of their residues was as expected. This may be because they rapidly degrade, depending on their formulation, the rate and method of application, and climatic factors. Further, high solubility and relatively short-life in the environment are factors in the degradation (Abd El-Gawad et al., 2014). The deltamethrin pesticide was commonly used in the study area for pest control on wheat and vegetables. But being non-persistent (Aislabie et al., 1997), below detectable levels in biota would indicate fast degradation in the environment. Since most irrigated vegetable schemes were located close to the Lake Ziway, their contaminated drainage system would directly or indirectly discharge into the Lake watercourse. Windblown dust, drift during active spray seasons, or discharge from shallow underground water may also contribute to Lake water contaminations with pesticides.

The mean DDT concentrations ranged from 0.31-12.00 ng.g⁻¹ wet weight (ww) obtained in this study from Lake Ziway for biota samples were generally lower than previous studies by Deribe et al. (2013) from the same lake. Deribe et al. (2013) determined that the mean concentration of ΣDDT ranging from 6.04 to 28.78 ng.g⁻¹ wet weight (ww). Similar previous studies from fish samples by Yohannes et al. (2014) revealed that the concentration of ΣDDT ranging from 2.33 to 9.00 ng.g⁻¹ wet weight (ww) which is lower than Deribe et al. (2013) investigation, however no significantly different from the current study. This shows the decreasing trend of DDTs in Lake Ziway biota (Table 2). The possible explanation for the decrease could be no current use of DDTs and other banned pesticides by local
farmers for agriculture and no use of DDTs for the control of the mosquito malaria vector (Mergia et al., 2021, accepted for publication, our study). The level of DDT metabolites and forms in biota samples in Lake Ziway may suggest an indication of their widespread usage before becoming regulated since they degrade slowly and are persistent enough to accumulate in the water environment. DDT is a highly persistent pesticide in the environment with a half-life of 2–15 years (USEPA, 1989).

Concentrations of o,p’-isomers (o,p’-DDT, DDD, and DDE) were rarely detected in our samples which usually are less than the limit of detection. Whereas the p,p’-DDT, p,p’-DDE, and p,p’-DDD were the most commonly detected compounds. In the current study, the p,p’DDE was the most commonly detected metabolite, with a mean concentration ranging from 0.22 to 7.70 ng.g-1ww. In agreement with our study, investigations from the same lake by Deribe et al. (2013) and Yohannes et al. (2014) showed that DDE and DDD occupied the dominant concertation in fish samples. Likewise, many studies have found Σ-DDTs to be the highest in concentration compared to other OCPs measured in freshwater fish with p,p’ DDE the most abundant and persistent of the metabolites analyzed (Wepener et al., 2012; Mzoughi et al., 2016). The studies by Hiller et al. (2011) and Mzoughi et al. (2016) have shown that p,p’-DDE is resistant against degradation so that it persists in the environment and living organisms. Degradation of p,p’ DDT by the process of mixed-function oxidases to the metabolite p,p’ DDE could be a good explanation for high levels and accumulation of p,p’ DDE in biota (Schmitt et al., 1990). Also, the biological transformation of DDT to DDE may contribute to the higher levels of p,p’ DDE in biota samples(Wepener et al., 2012).

In the present study, the ratio of DDT/DDD+DDE in all the biota samples was less than 1 signifying aged or historical DDT application(Unyimadu, et al., 2018). Besides under hot, dry climatic conditions, DDT can be degraded at a high rate as revealed by Jiries et al. (2002) which is typical of the tropical environment. Moreover, the ratio of DDD/DDE can reveal the degradation pathways of DDT, since DDE and DDD are aerobic and anaerobic degradation products of DDT, respectively (Aislabie, 1997). A ratio of DDD/DDE less than one (<1) shows aerobic degradation and higher than 1 (>1) shows anaerobic degradation (Hiller et al., 2011). In the present study zooplankton and fish (Clarias gariepinus) samples where DDD and DDE were simultaneously detected, the DDD/DDE ratio in the biota sampled was much less than 1 in all cases. These results are indications that the degradation pathways in the Lake Ziway biota were aerobic.
4.2 Concentration DDTs and fish size

As reported by Rognerud et al. (2002) the length and weight (size) of fish are important variables for explaining the concentration of DDTs. In the present study, a significant positive relationship between the concentrations of p,p'-DDE, and total length was found in Oreochromis niloticus, Carassius auratus, and C. gariepinus. A study from the same lake (Lake Ziway) by Deribe et al. (2013) is in agreement with the present study, which reported that is significantly related to total length for O. niloticus, C. auratus, and C. gariepinus but not for T. zillii. The progressive increase in concentrations of p,p'-DDE in C. gariepinus, C. auratus, and O. niloticus, as well as the increase of p,p'-DDE, and p,p'-DDD in C. auratus with an increasing total weight of the fish is most probably a result of bioaccumulation of DDTs with age. The length distribution of C. carpio analyzed in this study was very narrow and represents mainly the same size individuals, and may explain why the concentrations of p,p'-DDE, p,p'-DDT and p,p'-DDD in this species did not increase significantly with increasing total length and weight.

4.3 Relationships between stable isotope and concentration of pollutants

The DDTs concentrations in biota from the Lake Ziway food web increased from macrophytes to invertebrate, to the various fish species analyzed in this study and are similar to those reported in other studies from the same lake and Lake Hawassa Ethiopia (Deribe et al., 2013; 2014; Yohannes et al., 2014). Stable isotopes of nitrogen (δ¹⁵N) 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; Ouédraogo et al., 2015). Hence, relations between δ¹⁵N and log-transformed concentration of DDTs were examined to investigate the trophic level dependent accumulation of the pollutant among the studied, macrophyte, invertebrate, and fish species. The degradation of metabolites, p,p'-DDE was detected in all species and used to study DDT bioaccumulation. Concentrations of the metabolites showed a significant increase (p < 0.001) with increasing δ¹⁵N values on wet weight bases.

Pollutant concentrations (ng g⁻¹ ww) of ΣDDT, in the biota of the Lake Ziway food web, were significantly correlated with their trophic position (Figure 5). We found the overall relationship between ΣDDT and trophic position to be described by the model I regression equation log₁₀ [ΣDDT] (ng g⁻¹ ww) = 0.19(TLδ¹⁵N) – 0.062. The slope of this regression (i.e., 0.19) is an estimate of the biomagnification power of DDTs in this food web and it is analogous to a study by Hobson et al. (2002). Lake Ziway biota showed a clear pattern of δ¹⁵N enrichment with trophic level from largely dipteran larva to planktivorous Nile Tilapia (O.niloticus) to carnivorous C. gariepinus (TL 3.6). Similar to the
The present study *C. gariepinus* prey on various small fish and benthic invertebrates (Tadiso et al., 2011; Deribe et al., 2014), and as a result, have a more $^{13}$C enriched (benthic) signature relative to other fish species. This finding was comparable with other studies that have examined this relationship between pollutants biomagnification and trophic status, as described by $\delta^{15}$N. For example, Hoekstra et al. (2003) reported a significant correlation between ΣDDT concentrations and $\delta^{15}$N in the Arctic marine food web from the southern Beaufort–Chukchi Seas. They determined that this correlation supported their results that $\delta^{15}$N could be used as a measure of trophic position. Moreover, Kidd et al. (1995) described a significant relationship between $\delta^{15}$N and both the log wet weight and lipid-corrected concentrations of DDT, toxaphene, and hexachlorocyclohexane in biota from a freshwater food web in Lake Laberge, Yukon Territory, and suggested that the slope of these regressions may be correlated to the biomagnification potential of these specific hydrophobic contaminants.

The biomagnification power (0.19) of ΣDDT observed in Lake Ziway food web is comparable in magnitude to that measured in freshwater fishes (i.e., 0.2–0.3) from various lakes by Kidd et al. (1995), who also investigated trophic position using $\delta^{15}$N values in muscle tissue. Kidd et al. (1995) described DDT biomagnification only in fish species whereas the biomagnification power of 0.19 found in our study is the overall biomagnification of DDTs through macrophytes, invertebrates, and fish. Conversely, Lake Ziway exhibited lower biomagnification DDT compare to studies conducted in other areas, such as the Southern Beaufort- Chukchi Seas, in the Arctic (Hoekstra et al., 2003), subarctic lakes in Yukon Territory (Kidd et al. 1998), marine food web from south-eastern Norway (Russ et al., 1999), Mekong Delta, South Vietnam (Ikemoto et al., 2008), as well as Lake Malawi, East Africa (Kidd et al., 2001).

According to Bouillon et al. (2011), pollutants having a trophic magnification factor (TMF) greater than 1 are considered to biomagnify in the food chain, while a TMF value ranged between 0 and 1 shows that the pollutant is not biomagnified. In the present study, the TMF value for total DDTs exceeded 1, indicating that the level of DDT bioamplify along with the food webs. In this study, the TMF values were 1.18 and 1.19 for p,p’DDE, and ΣDDT respectively which is in agreement with studies reported by Kidd et al. (2001) in African lake food web (TMF for DDT = 1.16, TMF DDE = 1.25,), also constant with marine food web investigation by Dromard et al. (2018), with TMF of chlordane ranged from 1.07-1.25. However, the TMF values of ΣDDT in this study were lower than those reported by Sun et al. (2017) in South China mangroves with TMF of 2.61 and 2.76 for DDT and PCB respectively. Likewise, a study in Arctic food webs by Jarman et al., (1996) reported TMF of 2.41 and 2.20 for PCB and DDT.
respectively, in the Congo Basin River, TMF of DDT was 1.7 (Verhaert et al., 2013). However, the value of biomagnification factors has been greatly influenced by the length of the food chain. Studies conducted in the Arctic or cold sea regions generally incorporate a larger scale of trophic levels (from primary producers to marine mammals and sea birds) than in other ecosystems. Although there are many possible reasons for the lower biomagnification rate, the main explanation is probably due to the complex food web, the species diversity and species abundance in tropical lakes result in different food resources with variable DDT burdens. Moreover, the greater DDTs level in fish compared to zooplankton and invertebrate is also an indication of the feeding ecology and following dietary exposure as well as pollutants bioaccumulation and possible biotransformation (Borgå et al., 2001; Fisk et al., 2001).

Conclusions

Our findings demonstrated that except DDT and its metabolite the other pesticides analyzed are below the detectable limit in the Lake Ziway ecosystem. Concentrations, ‘fingerprint’ ratios of certain DDTs, high concentrations of p,p’DDE, and low p,p’DDT levels in biota suggest that DDTs have not been used recently in agriculture around the Lake Ziway after their ban. Unlike previous studies banned pesticides such as dieldrin, endosulfan, HCB, HCH, chlordane were below detectable in biota samples from Lake Ziway. The DDTs levels in both fish and other biota were substantially lower than those recorded in other environmental and biological samples. Likewise, the concentrations of DDTs and other organochlorine pesticides found in biota from Lake Ziway were, in general, lower than studies found in previous studies carried out in the same lake. Even though pesticides such as OPPs, Pyrethroid, deltamethrin, boscalid, and other pesticides such as bupiriminate, fenarimol, and tetradifon being registered in Ethiopia and used heavily by small scale vegetable farmers along the littoral zone of Lake Ziway, none of them have detected in any of the samples analyzed. The TMF data derived in Lake Ziway showed clearly that biomagnification occurs for DDT, with TMFs equal to 1.19 and the study demonstrates biomagnification of persistent contaminants (e.g. DDTs) in tropical lakes, although this is lower than in other areas.

Acknowledgment

This research was funded by the Institutional Collaboration program between Hawassa University and the Norwegian University of Life science (NMBU-HU ICOP –IV). The lab work was assisted by staff members at Bioforsk, Pesticide Chemistry Section. The authors are extremely grateful to Hans Christian Teien and Marit Nanrups for their help in the
analysis of isotopes at the Environmental Chemistry Section, Norwegian University of Life Sciences. We thank Ziway Fishery research center staff for their help during sampling.

Consent for publication

The authors declare that they agree with the submission and eventual publication of the Journal of Ecotoxicology

Conflict interest

The authors declare that there is no conflict of interest associated with the subject of the article

Funding

The authors have no relevant financial nonfinancial interest to disclose

Availability of data and materials

All data generated or analyzed during this study are included in the manuscript

Consent of publication

Not applicable

Author’s contribution

Weldemariam, Eklo, and Yimer designed and supervised all stages of the study. Mergia and Weldemariam participated in samples collection and data collection monitoring. Mergia prepared the datasets, wrote the first draft of the manuscript, and performed the preliminary statistical analyses. All authors have read and approved the final manuscript
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