Research Article

Organochlorine pesticides and polychlorinated biphenyls in carnivorous waterbird and fish species from Lake Hawassa, Ethiopia

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Abstract

Abstract Agricultural, vector-control and industrial activities around Lake Hawassa pose a risk of organochlorine contamination of the lake biota. To assess organochlorine contamination, we measured levels of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in 3 species of carnivorous waterbird and 3 species of fish. A total of 50 samples of fish and bird species sampled from Lake Hawassa in 2019. We investigated factors influencing accumulation of OCPs and PCBs. Reproductive risk associated with tissue levels of 4,4′-dichloro-diphenyl-dichloro-ethylene (*p,p′*-DDE) is also estimated. Results show that dichloro-diphenyl-trichloroethane (DDT) is the dominant contaminant found in both bird and fish species. *p,p′*-DDE is the dominant DDT metabolite in both bird and fish species. Geometric mean of *p,p′*-DDE varied from 49.8–375.3 and 2.2–7.7 ng g^{-1} ww in birds and fish, respectively. Average *p,p′*-DDE level in birds is 33.3 times higher than in fish. *p,p′*-DDE constitutes 93.4–95.2% of total DDTs in bird species. Degree of exposure, chemical stability, and resistance to environmental and biological degradation could explain higher levels of *p,p′*-DDE both in bird and fish species. There is significant variation in *p,p′*-DDE levels among bird and fish species owing to differences in feeding habits, foraging habitat, and lipid content. An increase in DDT levels with increasing size is observed in both bird and fish species. A significant positive association between log-transformed *p,p′*-DDE, and stable nitrogen isotope ratio ($\delta^{15}N$) values is found. There is no reproductive health risk in bird species as a result of the current levels of *p,p′*-DDE.

Article Highlights

- DDT is the dominant contaminant found in both bird and fish species
- There is interspecies variation in accumulation of *p,p′*-DDE among fish and bird species
- *p,p′*-DDE is biomagnified through food chain involving both bird and fish species

Keywords Biomagnification · Carnivorous waterbird · Fish · Organochlorine pesticide · PCB · Lake Hawassa

1 Introduction

Persistent organic pollutants (POPs) are synthetic chemicals with long environmental and biological half-lives, long-range transport capacity, and lipophilic property [1]. POPs accumulate in fatty tissues of organisms and biomagnify through the food chain reaching higher concentrations in organisms occupying higher trophic levels [2]. At present 30 POPs listed under the Stockholm convention including the first 12 legacy POPs [3]. Organochlorine

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pesticides (OCPs) and polychlorinated biphenyls (PCBs) constitute ten of the first 12 legacy pollutants listed under the Stockholm convention. Before they were banned by the Stockholm convention, OCPs and PCBs have been used for agricultural pest control and industrial purposes, respectively [4]. Despite their ban, OCPs and PCBs are still detected in virous environmental and biological matrices [5]. Dichloro-diphenyl-trichloroethane (DDT) is a prevalent environmental contaminant owing to its widespread use in the past as agricultural pest and disease vector control agent. DDTs have been shown to cause eggshell thinning that leads to reproductive failure in birds [6]. It also affects reproduction and growth in fish by interfering with the normal functioning of the endocrine system [7]. PCBs are known to cause impairments of reproductive behavior in birds, depressing courtship songs and nest attentiveness [8, 9].

Ethiopia signed and ratified the Stockholm convention in 2002 [10]. Even though the convention entered in to force in 2004 [4], it is only recently (in 2009) Ethiopia discontinued the use of DDT for agricultural purposes [11]. Moreover, the use of DDT is still continued for indoor residual spray (IRS) for the control of malaria vector mosquitoes [11]. OCPs environmental contamination also emanates from improperly stored obsolete pesticide stocks found distributed in different parts of the country [12]. Obsolete pesticide stocks are usually diverted to illegal distribution and use for agricultural purposes [12]. PCBs are also potential threats of environmental contamination due to the widespread presence of PCB-containing operational and worn-out high voltage transformers and capacitors [10]. Improper and open field storages of worn-out electrical equipment containing PCB are common in the country [13]

Aquatic systems are destinations to environmental pollutants through run-off water from agricultural lands and municipal waste dumping sites. Lake Hawassa is surrounded by both agricultural fields and Hawassa City. Runoff water from the surrounding vegetable, tobacco farms and municipal wastes could be sources of OCP and PCB contamination [14]. The lake is also surrounded by several industries releasing their effluents directly into the lake and into the tributary river. Referral hospital, breweries, beverages, and textile factories are some of the industries whose effluents end up into the lake [14–16]. Despite the presence of OCP and PCB exposure threats to the local biota, exposure and risk assessment studies are generally scarce and none for bird species.

Carnivorous waterbird species have been used as indicator species for biomonitoring of environmental POP contamination owing to their top trophic position [17]. Top-trophic level birds are susceptible for exposure and accumulation of POPs through biomagnification [18]. Declines in hatching success in carnivorous bird species as a result of high accumulation of 4,4′-dichloro-diphenyl-dichloro-ethylene (*p,p′*-DDE) have been documented [6]. In aquatic habitats and lake environments, the main route of contamination for carnivorous waterbird species is a diet of fish [19]. Fish as a possible source and route of DDT contamination to humans have been investigated [20–22]. However, fish as a key route for contamination of wildlife, particularly, carnivorous waterbird species have not been investigated from the present study site. To our knowledge, this is the first study to investigate levels of OCPs and PCB in carnivorous waterbird and fish species from Lake Hawassa.

The present study aims to assess the accumulation and risk associated with OCPs and PCB in carnivorous waterbird and fish species from Lake Hawassa. We test the following hypotheses: (1) Carnivorous waterbird species would accumulate higher OCP and PCB levels than fish species owing to their higher trophic position. (2) Birds and fish with larger sizes would accumulate higher levels of OCPs than those with smaller sizes.

In the next section, we described the study area, bird and fish sampling, sample preparation and chemical analysis methods. Section 3 shows the levels of OCPs and PCBs in muscle tissues of bird and fish species, and discusses factors affecting accumulation of OCPs, including reproductive risk associated with accumulation of *p,p*-DDE. In Sect. 4, we present the concluding remarks of the study. Finally, Sect. 5 shows the literature cited.

2 Materials and methods

The samples used in the present work have been treated by the same method of lipid content determination, stable isotope analysis, sample preparation, chemical analysis and quality control methods as described in the authors published work [23]. Here we present the summary of the methods involved. The adoption of the methods is based on the similarity of target analytes, and tissue type.

2.1 Study area

Lake Hawassa is one of the Ethiopian Rift Valley (ERV) lakes found in Sidama region, Ethiopia. It is located along the side of Hawassa city some 275 km away from the capital city Addis Ababa. The lake lies between 7.0313° N latitude and 38.4219° E longitude (Fig. 1). The lake is situated at an elevation of 1,680 m above sea level and has a surface area ranging from 85 to 90 km square. The Lake has a maximum and minimum depth of 22 and 11 m, respectively. The lake receives inflow from the Tikur Wuha River but has no surface outflow. The lake provides economic benefits to local people through ecotourism, recreation, and fishery.

Large numbers of waterbird species, including local and Palearctic migrant birds occur in the Lake. The lake is known for harboring the largest population of Marabou Stork (*Leptoptilus crumeniferus*) in Ethiopia [24]. Six species of fish occur in the lake [25]. Nile tilapia (*Oreochromis niloticus*), African sharp-tooth fish (*Clarias gariepinus*), and African big barb (*Barbus intermedius*) constitute the most common fish species.

2.2 Sampling

Permission was obtained for bird capture and sampling from Ethiopian Wildlife Conservation Authority (EWCA), Addis Ababa (Ref. No.: Wl. 32/318/2011). A total of 20 individual birds belonging to three species and 30 individuals of fish belonging to three species were sampled from the site. The bird species sampled were marabou stork (*Leptoptilos crumeniferus*), African sacred ibis (*Threskiornis aethiopicus*), and hamerkop (*Scopus umbretta*). All bird species are carnivorous, mainly consuming fish and fish scrapes in the local habitat [27–29]. The fish species sampled were African big barb (*Barbus intermedius*), Nile Tilapia (*Oreochromis niloticus*), and African sharp-tooth fish (*Clarias gariepinus*). Birds were captured using traditional bird traps, then euthanized. Fish samples were purchased from the fresh catch of local fishermen. All bird and fish samples were transported to the laboratory for morphological measurement and excision of muscle samples. For birds, weight and wing chord length were recorded (Table 1). For fish, total length and weight measurements were recorded (Table 2). Bird morphological measurements were taken following the procedure described by Winker [30].

Individual bird and fish samples were dissected and 100 g of pectoral muscles and fillets were excised, respectively, for OCPs, PCBs, and stable isotope analysis. Excised bird and fish muscle samples were wrapped with aluminum foil, stored in a labeled zipper plastic bag and were frozen at − 18 °C. Then all bird and fish muscle samples

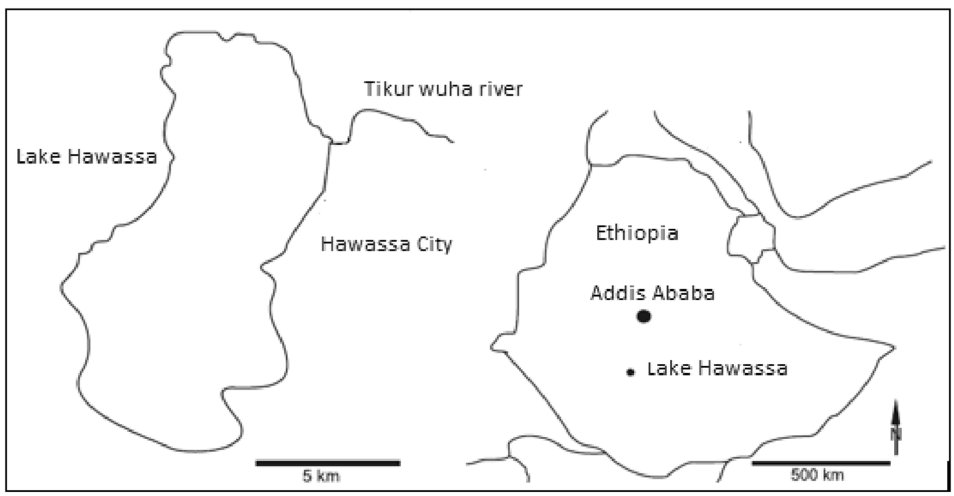

Fig. 1 Maps of Lake Hawassa (modified from Dsikowitzky [26])

Table 1 Weight and wing chord length of bird species sampled from Lake Hawassa

Bird species	N	Weight (g)			Wing chord length (cm)		
		M ± SD	Min	Max	M ± SD	Min	Max
L. crumeniferus	7	5164 ± 361	3990	6995	68.8 ± 4.2	62.6	75.5
T. aethiopicus	5	1222 ± 141	1050	1410	35.4 ± 1.4	31.1	37.3
S. umbretta	8	520 ± 131	390	800	31.6 ± 1.0	30.3	32.7

Table 2 Total length and weight of fish species sampled from Lake Hawassa

Fish spp	N	Total length (cm)			Weight (g)		
		M ± SD	Min	Max	M ± SD	Min	Max
C. gariepinus	10	37.3 ± 6.7	29.1	50.7	390 ± 215.4	181	929
O. niloticus	10	20.2 ± 0.6	19.5	21.5	145 ± 8.8	126	155
B. intermedius	10	28.3 ± 2.6	25.1	33.6	212 ± 55.7	158	341

were transported to the Norwegian Institute for Bioeconomy Research (NIBIO) laboratory for contaminant analysis.

2.3 Stable isotope analysis

The analysis of nitrogen (^{15}N and ^{14}N) and carbon (^{13}C and ^{12}C) isotopes was performed following method described in Ayele et al. [23]. The analysis method generally included homogenization of 1 g muscle tissue and freeze-drying. Freeze-dried sample was combusted in a Flash Elemental Analyzer and stable isotopes were determined by a continuous Flow-Infrared Mass Spectrometer. The isotopic ratios ($^{15}N/^{14}N$ $^{13}C/^{12}C$) were expressed in delta-values as follows: $\delta^{15}N$ and $\delta^{13}C(‰) = \left[(RSample/RStandard) - 1\right]x1000$, where, $R = {}^{15}N/{}^{14}N$ for $\delta^{15}N$ or $R = {}^{13}C/{}^{12}C$ for $\delta^{13}C$ [21].

2.4 Lipid content determination

Lipid was extracted using SOXTEC Auto Lipid extractor as described in detail by Ayele et al. [23]. Summary of the extraction process described as follows. Lipid content was determined using pooled and homogenized five-gram sample. The sample was mixed with sodium sulfate powder and loaded in the extraction unit. The extraction solution was a mixture of ethyl acetate:cyclohexane with 1:1 ratio. After the completion of extraction lipid content was determined gravimetrically [23].

2.5 Reagents and chemicals

Mixture of POP standards, analytical grade acetonitrile and triphenyl phosphate were obtained from Dr. Ehrenstorfer GmbH Augsburg, Germany. Purified water was obtained from a Milli-Q Gradient A10 water system (Millipore, Bedford, MA, USA). Citrate buffering salts consist of 4 g magnesium sulfate, 1 g sodium chloride, 0.5 g sodium citrate dibasic sesquihydrate and 1 g sodium citrate tribasic dihydrate prepacked in a 15 mL tubes were supplied from Sigma-Aldrich GmbH, Germany. Primary Secondary Amine (PSA) clean-up tube consists of 150 mg of PSA and 900 mg $MgSO_4$ prepacked in 15 mL centrifuge tubes were supplied from Sigma-Aldrich GmbH, Steinheim, Germany. Enhanced Matrix Removal-lipid tubes (EMR-Lipid tube) and EMR-Lipid polishing tubes consist of $MgSO_4$ were supplied from Agilent technologies.

2.6 Sample preparation and chemical analysis

Sample preparation and chemical analysis were carried out at Norwegian Institute of Bioeconomy Research (NIBIO), Department of Pesticides and Natural Products Chemistry. Sample preparation was performed by the modification of the method described by Anastassiades et al. [31]. The modified method used is described in detail in Ayele et al. [23]. The summary of the method is as follows. 5 g of thawed muscle sample was measured into 50 mL extraction tube. 10 mL of Milli-Q water, 10 mL of acetonitrile and 50 ng (50µL) triphenyl phosphate internal standard were added. After homogenization, citrate buffering salt was added into the sample to aid phase separation between the tissue, water, and acetonitrile. Then the sample was cleaned using PSA clean-up tube and EMR-Lipid tube. Supernatant resulting from the cleaning processes was transferred to EMR-Lipid polishing tubed for the removal of traces of water from the acetonitrile. Finally, 15 µl of the aliquot of the supernatant was used for contaminant analysis using gas chromatography-mass spectrometry (GC-MS). The contaminants analyzed include DDTs (*p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD, *o,p′*-DDT, *o,p′*-DDE, *o,p′*-DDD), Oxychlordane, cis-chlordane, trans-Chlordane, Endosulpha-alpha, Endosulphan-beta, Endosulphan-sulphate, Aldrin and Dieldrin and PCBs (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, PCB180). The specifications and operating mode of the GC-MS used were as described in Ayele et al. [23].

2.7 Quality control

Quality control has been carried out following the method described in Ayele et al. [23]. Reagent blanks and spiked blanks analysis were performed for quality control. A mixture of 10 mL Milli-Q water and 10 mL acetonitrile, serving as reagent blank, treated through all of the analysis procedure. Analysis results showed no target analytes in the blank samples. A multi-level calibration curve was made by spiking a mixture of POP standards to a blank matrix of 5 g chicken and 5 g cod muscle. The coefficient of determination (r^2) for the calibration curve was ≥ 0.99. Five grams of cod and 5 g chicken muscles spiked at 10 ng g^{-1} of a mixture of POP standards showed recovery ranged from 80–100%, and 74–103% for all OCPs, respectively. Recovery for PCBs in cod and chicken muscle ranged from 70–88.4% and 62.1–82.2%, respectively. Relative standard deviation (RSD) was less than 14 for all contaminants investigated in both cod and chicken. The limit of detection (LOD) was 0.3 ng g^{-1} ww.

2.8 Risk assessments

Sublethal toxic effects from exposure to organochlorine pollutants are common and more important causes of wild avian population decline [32]. We performed reproductive health risk assessment in birds by comparing the present DDTs and PCBs tissue concentrations with minimum toxic threshold concentrations to cause reproductive failure in bird species from literature [19]. Due to the lack of toxic threshold data for the bird species investigated in the present study, toxic threshold concentrations recorded for other bird species were used. Care must also be taken during comparison due to differences in tissues types, sex, and developmental stages of birds investigated. Despite these shortcomings, the present avian reproductive risk assessment would provide a general reproductive risk estimation.

2.9 Statistical analysis

Comparison of mean levels of contaminants among species was performed using one-way analysis of variance (ANOVA) with Tukey (Kramer) HSD (Honestly Significant Difference) post hoc test. Pearson correlation was used to determine the association of DDT residue levels with morphological measurements in birds and fish. The association between log-transformed concentrations of sum DDTs (ΣDDTs) and $\delta^{15}N$ was used to determine the occurrence of biomagnification. The value of the slope of the regression line was used as a measure of the magnitude of bioaccumulation. Statistical analyses were performed at 0.05 significance level. SPSS statistical software (SPSS 20) was used in data analysis.

3 Results and discussion

3.1 Body measurements and lipid contents

Mean weight of birds varied from 520 g (*S. umbretta*) to 5164 g (*L. crumeniferus*). Mean wing chord length varied from 31.6 to 68.8 cm (Table 1). There was a strong statistically significant association between weight and wing chord length ($r = 0.99$; $p < 0.05$) suggesting both measurements could be used for gross determination of bird size [33]. Percent lipid contents for bird species were varied from 2.83 to 5.31. The maximum percent lipid content was recorded for *S. umbretta* followed by *T. aethiopicus* (2.90%). The mean total lengths of fish were varied from 20.2 to 37.3 cm. Mean weight of fish varied between 145 and 390 g. There was a significant association between total length and weight ($r = 0.9$; $p < 0.05$). Lipid contents of fish muscle samples varied from 1.05 to 1.72%. The maximum lipid content was recorded for *C. gariepinus* (Table 3).

3.2 OCPs and PCB tissue concentrations

Among the investigated OCPs and PCBs, DDT was the dominant contaminant detected in all bird and fish muscle samples. Except for dieldrin and PCBs, which were quantified only in one individual bird, the rest of the OCPs analyzed (Cis-, trans- and oxy-chlordane, Endosulfan-alfa, endosulfan-beta, endosulfan sulfate, and aldrin) were below the detection limit in all the samples (Table 3). The

Table 3 Geometric mean (GM) of DDTs (ng g^{-1} ww [ng g^{-1} lw]) and ranges of values in birds and fish species sampled from Lake Hawassa

Species	DDTs	Species	%Lipid	GM ± SD	Range
Bird	*p,p′*-DDE	*L. crumeniferus*	2.83	49.8 ± 1.8^{a} [1,759]	21.7–102.28 [767–3614]
		T. aethiopicus	2.9	135.6 ± 1.2^{b} [4,676]	107–172.57 [3707–5951]
		S. umbretta	5.31	375.3 ± 1.82^{c} [7,074]	137–752.52 [2577–14,172]
	p,p′-DDD	*L. crumeniferus*	–	2.5 ± 1.6 [88]	1.4–4.4 [50.2–156]
		T. aethiopicus	–	5.0 ± 1.79 [173]	2.7–11.4 [93.1–392]
		S. umbretta	–	17.5 ± 1.7 [329]	10.3–46.7 [193–880]
	ΣDDT	*L. crumeniferus*	–	53.0 ± 1.7^{a} [1,874]	24.9–103 [880–3,666]
		T. aethiopicus	–	145.2 ± 1.2^{b} [5,008]	128–184 [4411–6,342]
		S. umbretta	–	394.0 ± 1.8^{c} [7,427]	148–799 [2792–15,051]
	Dieldrin	*L. crumeniferus*	–	-	–
		T. aethiopicus	–	-	–
		S. umbretta	–	-	ND–15.3 [ND–289]
	ΣPCBs	*L. crumeniferus*	–	-	–
		T. aethiopicus	–	-	–
		S. umbretta	–	-	ND–5.5 [103.6]
Fish	*p,p′*-DDE	*C. gariepinus*	1.72	7.7 ± 1.9 [449]	3.4–29.6 [195–1720]
		O. niloticus	1.28	2.2 ± 2.0 [122]	0.7–4.3 [31–236]
		B. intermedius	1.05	6.9 ± 1.9 [656]	3.8–34.4 [366–3272]

Geometric mean values with different superscripted letters are significantly different ($p < 0.05$)

present finding of a higher proportion of DDTs is consistent with findings from previous studies [34, 35]. The predominance of DDTs in the present finding could be due to the widespread presence of DDT in the local environment [21]. DDT is widely used in the region for indoor residual spray (IRS) for malaria vector control [36]. There are also reports of illegal use of DDT for agricultural purposes especially by small-scale farmers [37] that could potentially contaminate the lake and the biota therein.

Among the DDT metabolites, *p,p′*-DDE was detected in all bird and fish species. 4,4′- dichloro-diphenyl-dichloroethane (*p,p′*-DDD) was detected in all bird species and one species of fish. 4,4′- dichloro-diphenyl-trichloro-ethane (*p,p′*-DDT) and 2,4′- dichloro-diphenyl-trichloro-ethane (*o,p′*-DDT) were not detected in all fish and bird muscle samples. The absence of *p,p′*-DDT may suggest the source of current environmental DDT exposure could be from the historic application. This is also substantiated by the value of the ratio of *p,p′*-DDE/*p,p′*-DDT. The ratio of *p,p′*-DDE/*p,p′*-DDT greater than 1.0 indicates past input, and the ratio less than 1.0 indicates fresh input [19]. The value of the ratio of *p,p′*-DDE/*p,p′*-DDT for both bird and fish samples was greater than 1.0 indicating historic use.

p,p′-DDE accounts for about 95.2% and 99.2% of all DDT metabolites in bird and fish species, respectively. The higher proportion of *p,p′*-DDE could result from its greater resistance to environmental transformation and high biomagnification potential [32]. Moreover, it could also result from the efficient biotransformation of parent *p,p′*-DDT [38] and long half-life of *p,p′*-DDE in fish tissue (7 years) [22]. The present finding of the predominance of *p,p′*-DDE is consistent with previous studies in birds [19], and in fish [21] from the same region.

The geometric mean of *p,p′*-DDE ranges from 49.8–375.3 and 2.2–7.7 ng g^{-1} ww in birds and fish, respectively. The geometric mean level of *p,p′*-DDD in birds ranges from 2.5 to 17.5 ng g^{-1} ww. The maximum mean levels of *p,p′*-DDE in birds is 48.6 to 169 times (average 33 times) higher than levels in fish. The relatively higher levels of *p,p′*-DDE in birds could be a result of its biomagnification in the local food web [18]. *p,p′*-DDE is the most resistant metabolite due to its long environmental and biological half-lives [32]. Its persistence together with its lipophilic property could contribute to the higher levels of *p,p′*-DDE in carnivorous waterbird species occupying high trophic position [18]. Accumulation of *p,p′*-DDE's in predator birds several folds (5–15 folds) higher than their prey have been documented [32]. Moreover, the predator bird's poorly developed oxidative detoxification system could also be responsible for high-level accumulation of lipophilic contaminants such as *p,p′*-DDE's. [35].

Statistically significant differences in mean levels of *p,p′*-DDE [$F(2,17) = 28.1$; $p < 0.05$] and *p,p′*-DDD [$F(2,17) = 26.3$; $p < 0.05$] were found among bird species. Maximum geometric mean *p,p′*-DDE level was recorded in *S. umbretta*. The variation in DDTs, particularly, *p,p′*-DDE (the main contributor to total DDTs) levels among bird species could be explained by lipid content [35], feeding habit [39], and foraging habitat [40, 41]. The high levels of ∑DDT *in S. umbretta* could be attributed to its highest mean lipid content [35]. In addition to that, high levels of *p,p′*-DDE in *S. umbretta* could be attributed to the bird species' preference to the diet of fish and fish scraps [29] and frequent foraging in aquatic habitats which allows a higher degree of exposure [18]. In the present study, *S. umbretta* accumulated 2.8 and 7.5 times higher *p,p′*-DDE than *T. aethiopicus* and *L. crumeniferus* and respectively. This finding is substantiated by the relatively narrower $\delta^{13}C$ value for the species with respect to the other two. On the other hand, the relatively lower levels of *p,p′*-DDE in *L. crumeniferus* and *T. aethiopicus* could be due to the inclusion of diets from terrestrial origins [28] and lower lipid contents.

Regarding fish species, there was a statistically significant difference in mean *p,p′*-DDE among fish species [$F(2,27) = 10.6$; $p < 0.05$] that could be due to difference in feeding habits [42], trophic position [43], lipid content [44] and specific habitats [39]. *O. niloticus, B. intermedius,* and *C. gariepinus* from ERV lakes have been shown to be herbivore, omnivore, and carnivore, respectively [21, 44, 45]. The highest *p,p′*-DDE level was found in *C. gariepinus* that could be attributed to its top trophic position, carnivorous feeding habit, and high lipid content than the rest species of fish. This finding is consistent with findings from earlier studies [22, 43, 44]. Moreover, *C. gariepinus'* bottom-dwelling habit predisposes it to a higher degree of exposure to DDTs associated with sediments [38]. *O. niloticus* had the lowest *p,p′*-DDE levels that could be explained by its low trophic position and herbivorous feeding habits [44].

3.3 Comparison with other studies

Muscle tissue concentrations of DDTs in the present study were compared with muscle tissue concentrations of birds from other studies around the world to determine the relative status of the present contamination. The concentrations of ∑DDT in the present study in birds (24.9–799 ng g^{-1} ww [880–15,051 ng g^{-1} lw]) were greater than values in herring gulls (*Larus argentatus*) from the Polish coastal zone of the Southern Baltic Sea (mean total DDT 0.1 ng g^{-1} ww) [22], in piscivorous birds from Argentina (1069–6480 ng g^{-1} lw) [46] and in Asian Openbill (*Anastomus oscitans*) from India (mean 96 ng g^{-1} ww) [47]. However, they were lower than values reported in piscivorous waterbird species from Ethiopia (3.7–148.3 µg g^{-1} lw) [19], in bird species from Iran (0.5–9040 ng g^{-1} ww) [35], in white-tailed eagles (*Haliaeetus albicilla*) from West

Greenland (0.7–530 µg g^{-1} lw) [48], in predator birds from Spain (141.6–24,772 ng g^{-1} ww) [17] and in *Catharacta* spp. of birds from Antarctica (287–1028 ng g^{-1} ww) [49]. However, the differences in sample size, study years, types of bird species in these studies make comparison difficult.

The tissue concentrations of ∑DDT in the present study in fish were greater than values reported in fish from Antarctica (up to 3 ng g^{-1}) [49], from Lake Ziway, Ethiopia (0.1–10.6 ng g^{-1} lw) [42] and in fish from Brazil (mean ∑DDT 2 ng g^{-1} ww) [22]. However, the levels in the present study were lower than values reported from Lake Hawassa, Ethiopia (7.8–172 ng g^{-1} ww, 6.82–73.3 ng g^{-1} ww) [21, 50], and in fish from Pakistan (16.9–402 ngg^{-1} ww) [20]. The present levels were also lower than values in fish from Lake Ziway and Lake Koka (0.9–172 ng g^{-1} ww, 0.1 to 72.5 ng g^{-1} ww), respectively [43, 44], and from South African Lakes (73 and 893 ng g^{-1} wet mass) [38], (1.9 to 5643 ng g^{-1} ww) [51], (< LOQ–61 ng g^{-1} ww) [52].

3.4 Association of DDT tissue concentration with bird and fish size

Accumulation of OCPs depends on age and size of birds [35, 53]. Due to difficulty in determination of the age of birds in the wild, we use morphological measurements as a gross representation of size. Since the weights of birds vary depending on gut fullness, we use wing chord length as an index of bird size [33]. The investigation of the relationship between wing chord length (cm) and log-transformed ∑DDTs (ng g^{-1} ww) showed a positive association for all species of birds (Fig. 2). However, all associations were not statistically significant. Contrary to the general rule, the absence of significant association between bird size and accumulation of DDTs among individuals of each of *T. aethiopicus* and *S. umbretta* species could result from the small variation in wing chord length (range = 31.1–37.3, 30.3–32.7, respectively) that could lead to similar periods of exposure to DDT (Table 1). In individuals of *L. crumeniferus,* however, there was a relatively large difference between the minimum and maximum wing chord length (range = 62.6–75.5). The non-significant association could result from the higher proportion of juveniles (personal observation & implication from wing chord length) with relative lower percent lipid content, shorter periods of exposure [34]. In addition to the above, small sample size may play a role in the non-significant associations found. The positive influence of age and lipid content on the accumulation of DDT have been documented [35, 53].

The association between log-transformed *p,p′*-DDE (ng g^{-1} ww), and total length in fish species was investigated to determine the influence of age on *p,p′*-DDE accumulation. Moderate (r = 0.62) and weak (r = 0.15) positive associations were found in *C. gariepinus* and *B. intermedius,* respectively. In *O. niloticus* the association

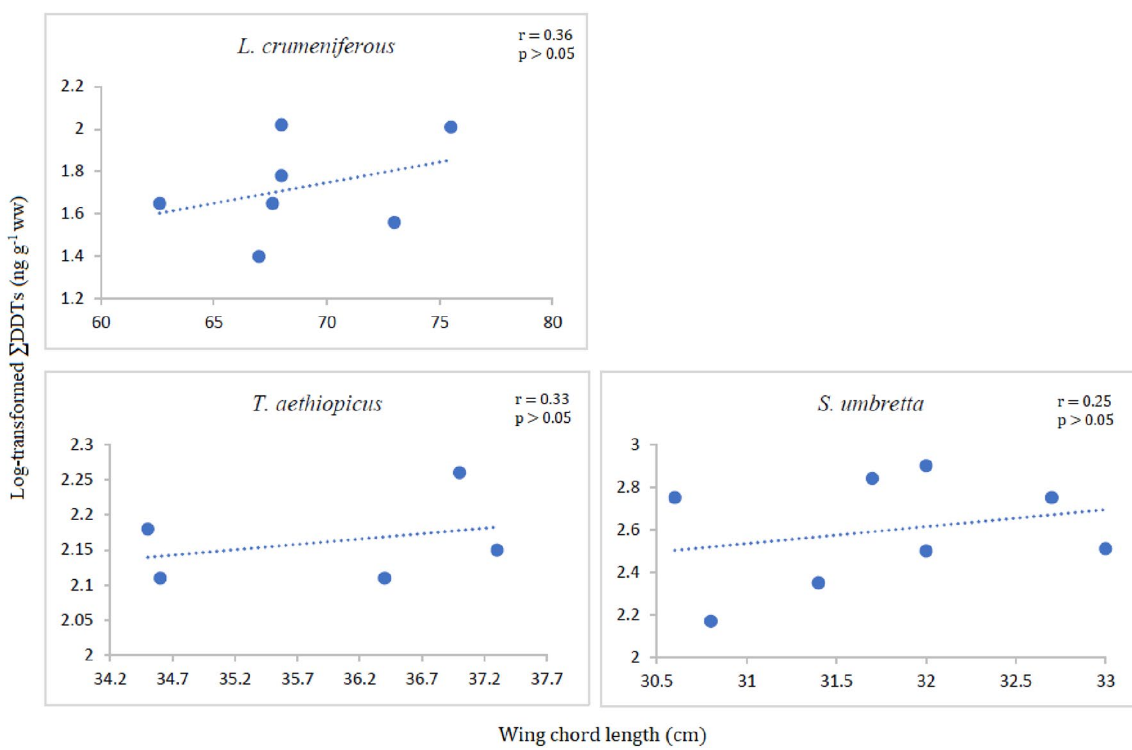

Fig. 2 Association between log-transformed ∑DDTs (ng g^{-1} ww) and wing chord length (cm)

was negative (r = – 0.43; p > 0.05). All associations between *p,p′*-DDE and total length were not statistically significant (Fig. 3). Despite the presence of greater variation in total length among individuals of each of *C. gariepinus* (range = 29.1–50.7) and *B. intermedius* (range = 25.1–33.6), the absence of significant positive association is most likely a result of exposure to constant levels and shorter time period *p,p′*-DDE took to reach a steady-state tissue concentration as a result of its higher biomagnification potential [32]. Once a steady-state is reached, extended exposure periods would bring an insignificant increase in *p,p′*-DDE levels. To be certain, however, this needs further investigation. The negative association in *O. niloticus* could be a result of biodilution that in turn could be caused by faster growth in tropical fish [54]. The phenomenon of biodilution of contaminants in fish from Lake Hawassa was documented [50]. In addition to that, the negative association in *O. niloticus* could result from the combined effect of very small variation in total lengths (range = 19.5–21.5) [43] and small sample size.

3.5 Stable isotopes analyses

Trophic position and carbon sources of bird and fish species were determined using carbon and nitrogen stable isotopes. Trophic relationships among species of birds and fish are given in Fig. 4. In all investigated bird species, the mean stable nitrogen isotope ratio ($\delta^{15}N$) and stable carbon isotope ratio ($\delta^{13}C$) values varied from 9.6 to 12.6‰ and – 23.1 to – 22.3‰, respectively. In fish species, the mean $\delta^{15}N$ and $\delta^{13}C$ values varied from 0.6–5.8‰ and – 25.1 to – 23.9‰, respectively. The level of $\delta^{15}N$ values in bird species in the present study generally correspond to birds utilizing diets of aquatic origin (preys of planktivorous and piscivores fish) [55]. The $\delta^{15}N$ values in birds were significantly higher than values in all the fish species [$F(1,22) = 23.7$; $p < 0.05$], indicating their higher trophic position relative to fish species. Considering the trophic fractionation factor of 3‰ [56], the difference between the maximum and minimum mean $\delta^{15}N$ suggests all species of birds occupy the same trophic position. In fish species, the difference of 5.2‰ between the maximum and minimum mean $\delta^{15}N$ values indicates that the fish species occupy two trophic levels. The carnivorous fish *C. gariepinus* occupies the highest trophic position relative to the other two fish species. The lowest $\delta^{15}N$ values were recorded for *O. niloticus* and *B. intermedius* indicating their lowest trophic position in the local food web. The low trophic position of *O. niloticus* and *B. intermedius* is in agreement with their herbivore and omnivore diets, respectively [42, 44].

Birds also have shown significantly higher and wider $\delta^{13}C$ values than values recorded for all species of fish

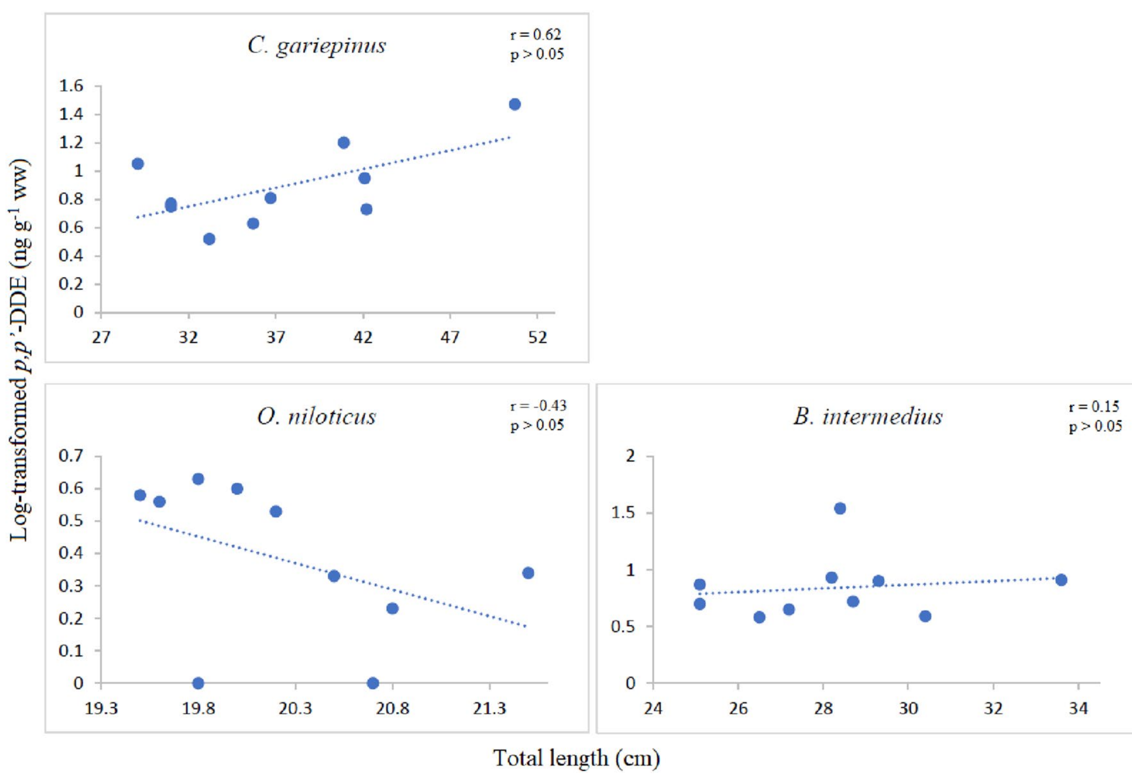

Fig. 3 Correlation between log-transformed *p,p′*-DDE (ng g^{-1} ww), and total length (cm)

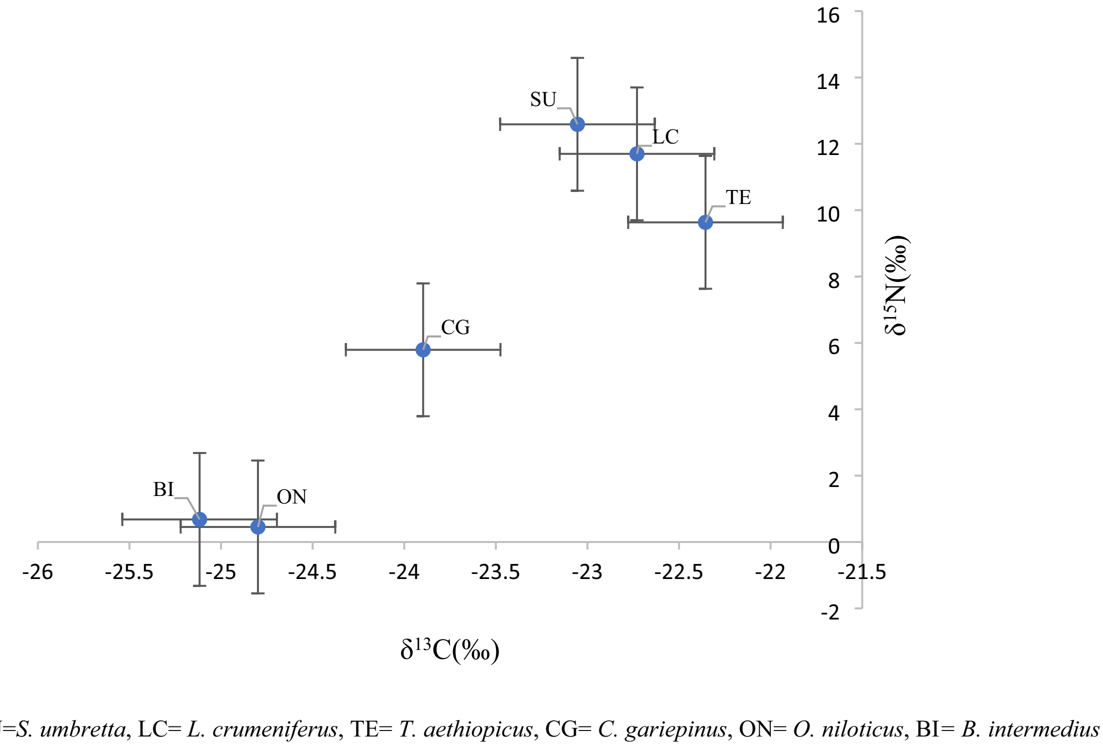

Fig. 4 Trophic levels of bird and fish species sampled from Lake Hawassa, Ethiopia

[$F(1,22) = 49.7$; $p < 0.05$]. The higher $\delta^{13}C$ values in birds indicate that the birds are generalist in their feeding habit as a result of the utilization of a wide range of aquatic food sources [27, 28]. Moreover, fish scrape (that is composed of different species) constitute diets of the studied bird species at Lake Hawassa that could diversify prey items. *B. intermedius* and *O. niloticus* have shown the lowest and narrowest $\delta^{13}C$ values implying utilization of a narrow range of food sources from the pelagic zone [21]. With respect to *B. intermedius* and *O. niloticus*, *C. gariepinus* showed relatively higher value of $\delta^{13}C$ ratio. This could suggest the utilization of carbon sources from the littoral zone.

Biomagnification of *p,p′*-DDE through the local food chain involving fish and bird species was determined from a regression line between log-transformed *p,p′*-DDE concentrations against $\delta^{15}N$ values (Fig. 5). The outcome of the regression analysis indicates a significant positive association between *p,p′*-DDE and $\delta^{15}N$ [$F(1,22) = 27.9$; $p < 0.05$] suggesting the occurrence of biomagnification of *p,p′*-DDE through the local food web. The value of the slope of the regression line (slope = 0.11; $R^2 = 0.56$) indicates rate of biomagnification of *p,p′*-DDE ([log*p,p′*-DDE] = $0.11 * \delta^{15}N + 0.65$). Biomagnification of DDTs in the food web involving fish [50] and bird and fish species were documented [19]. The order of *p,p′*-DDE levels among fish and birds (*S. umbretta* > *T. aethiopicus* > *L. crumeniferus* > *C. gariepinus* > *B. intermedius* > *O. niloticus*) substantiates the variation in *p,p′*-DDE levels was a result of the difference in trophic levels. The biomagnification of DDTs in a food web involving fish and birds is consistent with other findings [2].

3.6 Risk assessments

DDTs, particularly, *p,p′*-DDE, have been known to affect bird reproduction and survival of young [6, 57]. The risk associated with the levels of *p,p′*-DDE in birds was assessed by comparing tissue concentrations with toxicity thresholds from literature. Considering lipid normalized *p,p′*-DDE muscle tissue concentrations are comparable with liver concentrations [19], the maximum *p,p′*-DDE levels in the present study (3614–14,172 ng g^{-1} lw) were below the minimum threshold average concentration (20,000 g g^{-1} lw) in liver, which was linked with impairment in individual bird reproduction [58]. Assuming 20% maternal DDT transfer rate into eggs [59], the current levels of *p,p′*-DDE were also below the minimum threshold concentration of 3 µg g^{-1} ww that was suggested to cause reproductive failure in brown pelican (*Pelecanus occidentalis*) [57]. However, caution must be taken due to the presence of interspecies differences in sensitivity to *p,p′*-DDE [60].

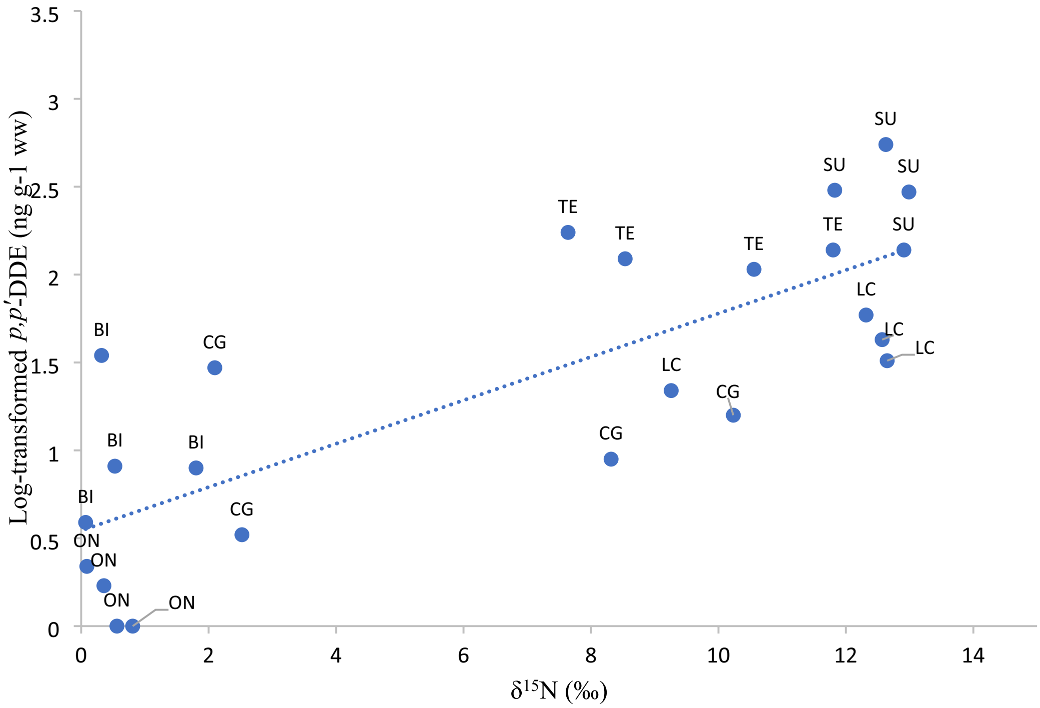

Fig. 5 Regression between log-transformed *p,p′*-DDE, and $\delta^{15}N$ values shows biomagnification of *p,p′*-DDE through the local food web

4 Conclusions

The carnivorous waterbird species have accumulated an average of 33 times higher levels of *p,p′*-DDE than the fish species. Generally, larger individuals of each species of birds and fish have accumulated relatively higher levels of *p,p′*-DDE than those with smaller sizes. *p,p′*-DDE constitute the main exposure threat in Lake Hawassa. Trophic position, feeding habits, and lipid contents are the main factors influencing the accumulation of *p,p′*-DDE in both fish and carnivorous waterbird species. There is no reproductive health risk associated with the current levels of *p,p′*-DDE in carnivorous waterbird species. The presence of organochlorine exposure threats in the area suggests the need for further investigation of contamination in other waterbird species. The present study may serve as a baseline for further investigation of organochlorine contamination in waterbird and fish species from the ERV lakes.

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Data availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Research involving human and animals participants International and national guidelines for the care and use of animals were followed during the present research.

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