Research Article

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

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Abstract
This study presents the assessment of bioaccumulation and reproductive health risk associated with organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) exposure in carnivorous waterbird species. We investigated OCPs and PCBs exposure in muscle tissues of 4 species of carnivorous waterbird species from Lake Ziway, Ethiopia. The influences of trophic position and size on accumulation of organochlorine pollutants are investigated. The result shows that Dichloro-diphenyl-trichloroethanes (DDTs), Endosulfan and PCBs are detected. DDTs constitute the dominant contaminant among OCPs investigated. Trophic position and wing chord length are positively associated with levels of ∑DDTs. Mean levels of ∑DDTs and ∑PCBs vary from 143.9 to 1051.1 ng g^{-1} wet weight (ww) and not detected (ND)—3.5 ng g^{-1} ww, respectively. Mean levels of 4,4′-dichloro-diphenyl-dichloro-ethylene (p,p′-DDE), and 4,4′-dichloro-diphenyl-dichloro-ethane (p,p′-DDD) are significantly varied among the bird species. p,p′-DDE contribute 92.3–98.6% of total DDTs. About 26.7% of birds show p,p′-DDE levels above the minimum threshold to cause reproductive failures in birds. Generally, the findings of this study shows that DDT exposure in high trophic levels bird species from Lake Ziway could result in reproductive health risk. The present study may serve as a baseline for future comprehensive exposure and risk assessment studies.

Article Highlights

- *p,p′*-DDE is the dominant contaminant in muscle tissue of the investigated bird species
- DDT accumulation varies among the bird species investigated
- A quarter of the investigated birds are at risk of reproductive failure as a result of high *p,p′*-DDE levels

Keywords Biomagnification · Bird · Congener profile · DDT · Lake Ziway · PCB

1 Introduction

Persistent organic pollutants (POPs) are ubiquitous in the global environment due to their environmental persistence, resistance to degradation, and bioaccumulative characteristics [1]. Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) tend to accumulate in the fat tissue of organisms and biomagnified through food webs reaching higher concentrations in top-predators [2, 3]. Carnivorous waterbird species, due to their high trophic position in the local food web, are prone to exposure and possible adverse health effects of OCPs and PCBs. Bird population decline as a result of exposure and toxic effects of OCPs and PCBs have been reported [4]. Eggshell

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thinning is one of the main toxic effects of DDT resulting in breakage of eggshell before hatching, affecting reproduction [5]. PCB toxicity has been linked to several sublethal effects such as impaired reproductive behaviors [6], disruption of feather coloration [7], and impaired sound production [8]. Bird OCP and PCB exposure studies and possible associated health risks in the present study site are scarce.

OCPs, mainly dichloro-diphenyl-trichloroethanes (DDT), have been imported and used in Ethiopia for agricultural pest control purposes from 1960's to 2007 [9]. Beginning in 2009, the country has stopped the formulation of DDT and its use as agricultural pest control [10]. However, DDT is continued to be used for indoor residual spray (IRS) against malaria vector control [10]. Since the country's 75% of land area experiences malaria epidemic [11], there is also a widespread application of DDT for IRS purposes. The Ethiopian rift valley (ERV) is one of malaria prone regions where DDT application is widespread.

Although PCBs have not been produced in the country, electrical equipment including PCB containing high voltage operational and decommissioned electrical transformers and power capacitors were suggested as potential sources of PCB environmental contamination [12]. Sites of open-field storage of transformers and capacitors are common in the country. Poor management and handling of decommissioned transformers and capacitors would allow contamination of soils, water, and biota [13, 14]. Transformer oils and capacitors are known to contain PCB in various amounts [14], and in some regions, higher levels of contamination in birds have been associated with local density of transformers [14]. Ethiopia, as a party in Stockholm convention, is currently working towards elimination of PCB by 2025.

Lake Ziway is known for its bird diversity and as one of the main destinations serving as a wintering ground for several Palearctic migratory birds [15]. The lake is surrounded by Ziway town and intensive irrigated horticultural farming. Moreover, large-scale flower farms are operating in the vicinity of the lake. OCP and PCB contamination of the ERV region, particularly, Lake Ziway could occur as a result of leaching from agricultural lands, municipal, and industrial wastes [13, 16]. Prior studies in Ethiopia, a couple of years after the OCPs agricultural use was discontinued, have shown the occurrence of varying levels of OCP in birds and fish species [17, 18]. OCPs recorded include DDTs, PCBs, endosulfan, aldrin, dieldrin, hexachlorocyclohexanes (HCHs), heptachlor and chlordane [17, 18]. Among the DDT metabolites *p,p*′-DDT, 4,4′-dichlorodiphenyl-dichloro-ethane (*p,p*′-DDD), 4,4′-dichloro-diphenyl-dichloro-ethylene (*p,p*′-DDE) and *o,p*′-DDT have been recorded in fish and bird species [18]. The same studies have shown *p,p*′-DDE levels above threshold concentration to cause reproductive failures in birds. Despite the presence of threats, there have been no studies documenting the contemporary exposure levels of OCPs. Moreover, to our knowledge, the present study is the first to investigate the levels of PCB and associated health risks in carnivorous waterbird species from Lake Ziway. The findings of this study, therefore, will address an important data gap in understanding the present organochlorine pollutant exposure levels in bird tissues from Lake Ziway.

The objective of the present study is to assess the bioaccumulation and reproductive health risk associated with OCPs and PCBs in carnivorous waterbird species from Lake Ziway. We test the following hypotheses: (1) The contemporary OCP levels in investigated bird species would cause reproductive health risk. (2) The sizes of individual birds would be positively correlated with tissue levels of OCPs and PCBs.

In the next section, we consider the description of the study area, bird sampling, sample preparation for chemical analysis and chemical analysis. Section 3 presents the levels of OCPs and PCBs recorded in muscle tissues of bird species, and discusses factors affecting accumulation of OCPs and PCBs. This section also discusses reproductive risk associated with accumulation of *p,p*-DDE and PCBs. Section 4 shows the concluding remarks. Finally, Sect. 5 presents the list of references.

2 Materials and methods

2.1 Study area

Lake Ziway is a naturally created water body situated in the central rift valley region of Ethiopia. It is located between 8.0073° N latitude, and 38.8415° E, longitude. It is situated at an elevation of about 1650 m a.s.l. The lake has an average surface area and total drainage area of 440 km^2 and 7488 km^2, respectively [19]. The lake is shallow with an average depth of 2.5 m [19]. The lake gets its major inflow of water from Katar and Meki rivers (Fig. 1). The outflow from the lake is through the river Bulbula. The lake gets its name from the town of Ziway. Ziway is a densely populated town situated on the west side of the lake. Various agricultural activities that are being carried out in the vicinity of the lake throughout the year, including vegetable and flower farming. Adami-Tulu pesticide factory is located about 7 km away from the Lake. The factory is known to formulate DDT for the country's consumption till 2009 [10].

Different habitat types occur surrounding the Lake such as the shore, riverine, woodland, and wet grassland. The shore supports more than 233 bird species, of which 8 are threatened [20]. The wetlands of Lake Ziway alone

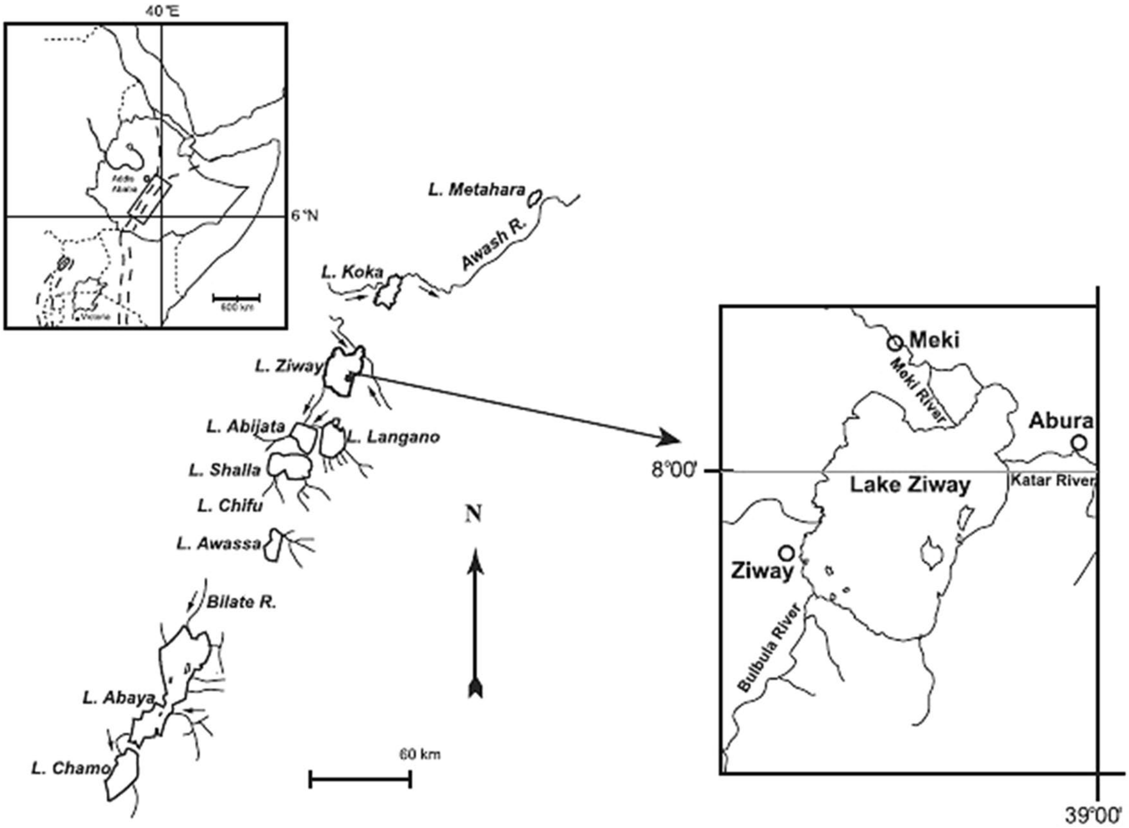

Fig. 1 Map showing Lake Ziway (Adopted and modified from Deribe et al. [16])

support over 20,000 waterbirds and as a result, the lake is recognized as one of the important bird areas in Ethiopia and qualifies as a Ramsar site [21]. The lake and the surrounding habitats provide foraging and breeding ground for many resident and migrant bird species.

2.2 Sample collection

Bird samples were collected from Lake Ziway in April and May of 2019. Samples were collected particularly from the west shores of Lake Ziway. Permission was obtained for bird sampling from Ethiopian Wildlife Conservation Authority (EWCA), Addis Ababa (Ref. No.: wl. 31/318/2011). A total of 30 birds belonging to four species were sampled. Bird species include great white pelican (*Pelecanus onocrotalus*), marabou stork (*Leptoptilos crumeniferus*), African sacred ibis (*Threskiornis aethiopicus*), and hamerkop (*Scopus umbretta*). These birds are so common [22] in the region that they can be used for biomonitoring of environmental pollution.

Birds were captured using traditional traps. Captured birds were immediately euthanized and transported to the laboratory. Biometric measurements including total body weight, and wing chord length were recorded (Table 1).

Each individual bird was dissected and about 100 g of pectoral muscle was measured. Each of the excised bird samples was wrapped with aluminum foil, stored in a labeled zipper plastic bag, and frozen at – 18 °C. Then, all bird muscle samples were transported to the Norwegian

Table 1 Weight and wing chord length of bird species

Bird species	Weight (g)				Wing chord length (cm)		
	N	M ± SD	Min	Max	M ± SD	Min	Max
P. onocrotalus	7	6407 ± 1268	4660	8545	64.1 ± 4.9	59.1	73.1
L. crumeniferus	8	7075 ± 1269	4450	8054	74.5 ± 4.0	66.6	78.4
T. aethiopicus	7	1524 ± 144	1320	1680	37.1 ± 1.4	35.7	39.0
S. umbretta	8	396 ± 56	320	490	31.0 ± 1.5	28.6	33.3

Institute for Bioeconomy Research (NIBIO) laboratory, for contaminant analysis.

2.3 Lipid content determination

SOXTEC Auto lipid extractor (2050 SOXTEC™, FOSS® Analytical, Hilleroed, Denmark) was used in lipid content determination. Pooled and homogenized pectoral muscle samples were used in lipid content determination. The method of extraction was as described in Ayele et al. [23]. First, 5 g of homogenized muscle sample was mixed with sodium sulfate powder and transferred to cellulose extraction thimbles (Whatman™, China). Then the thimbles were loaded into the lipid extraction unit. Mixture of ethyl acetate:cyclohexane (1:1) was used as an extraction solution. During extraction process the samples were boiled in pre-weighed glass cups containing extraction solutions. After the samples gone through the extraction process involving boiling, rinsing, solvent recovery and predrying, the glass cups with residual lipid were weighed. Lipid contents were determined gravimetrically. Finally, percent lipid was determined using the following formula $\mathrm{Percent lipid} = (\mathrm{Weight of fat}/\mathrm{Weight of sample})\mathrm{x}100$.

2.4 Stable isotope analysis

Trophic position and carbon sources of individual birds were investigated using carbon and nitrogen stable isotopes. The stable isotope analysis was done as described in Ayele et al. [23]. The analysis method summarized as follows. One gram of pectoral muscle tissue was homogenized with 10 mL of water. One milliliter portion of the homogenized sample was transferred into a labeled plastic tube, covered with perforated parafilm and freeze-dried. 1 µg of the freeze-dried sample was packed in pre-weighed aluminum foil. Then, the packed sample was subjected to combustion in a Flash Elemental Analyzer until it turns into gas. Stable isotopes of Nitrogen (^{15}N and ^{14}N), and Carbon (^{13}C and ^{12}C) were determined by a Continuous Flow-Infrared Mass Spectrometer. The isotopic ratios (^{15}N/^{14}N and ^{13}C/^{12}C) were expressed as deviation from the standard as follows: δ^{15}N and δ^{13}C (‰) $= \left[(\mathrm{RSample}/\mathrm{RStandard}) - 1\right] \times 1000$; where R = ^{15}N/^{14}N for δ^{15}N or R = ^{13}C/^{12}C for δ^{13}C [17, 24].

2.5 Reagents and chemicals

POP standards, analytical grade acetonitrile, triphenylphosphate, ultra-purified water, citrate buffering salts, Primary Secondary Amine (PSA) clean-up tubes, Enhanced Matrix Removal (EMR)-lipid tube and EMR-Lipid polishing tubes were used in sample preparation and chemical analysis. Suppliers of these reagents and chemicals are as described in Ayele et al. [24]

2.6 Sample preparation

Sample preparation and chemical analysis were carried out at Norwegian Institute of Bioeconomy Research (NIBIO), Department of Pesticides and Natural Products Chemistry. Sample preparation for the analysis of organochlorines (OCs) was performed by a modification of method described in Anastassiades et al. [25]. Pectoral muscle sample was thawed and 5 g of the sample was measured into an extraction tube. 10 mL of Milli-Q water, 10 mL of acetonitrile and 50 ng (50 µL) triphenyl phosphate as an internal standard were added and homogenized using polytron homogenizer (P.T.3100, Kenematica, Switzerland). Citrate buffering salt (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) was added into the sample and centrifuged. Then, the sample was treated with dispersive clean-up methods using PSA clean-up tubes (consisting of 150 mg of PSA and 900 mg $MgSO_4$ prepacked in a 15 mL centrifuge tube) and EMR-Lipid tubes. 5 mL portion of the cleaned solution was added into an EMR-Lipid polishing tube (consisting of $MgSO_4$) for the removal of traces of water. Finally, 15 µl the supernatant was injected to gas chromatography-mass spectrometry (GC–MS) for OCPs and PCB analysis. The detail description and summary of the above method is found in Ayele et al. [23, 24], respectively.

2.7 Chemical analysis

PCBs (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, PCB180) and OCPs (DDTs (*p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD, *o,p′*-DDT, *o,p′*-DDE, *o,p′*-DDD), Oxychlordane, cis-chlordane, trans-Chlordane, Endosulfan-alpha, Endosulfan-beta, Endosulfan-sulphate, Aldrin and Dieldrin) were analyzed by gas chromffffffatography (Agilent 6890N). The specification of the gas chromatography-mass spectrometer, the inert gas used, the programmable temperature vaporizing (PTV) program and the operation modes were as described in Ayele et al. [23].

2.8 Quality assurance

Quality assurance was performed as described and summarized in Ayele et al. [23, 24], respectively. Quality assurance was performed by analysis of procedural reagent blanks and spiked blanks. Reagent blank, consisting of 10 mL Milli-Q water and 10 mL of acetonitrile, was run through the procedure. Results showed that no target analyte was detected in the blank samples. Calibration curve was made by spiking a mixture of POP standards to a blank matrix of 5 g chicken muscle for the determination linearity of instrumental analysis and quantification. The coefficient of determination (r^2) for the calibration curve was ≥ 0.99. Spiking experiments on three replicates (N = 3) of 5 g of chicken muscle spiked at 10 ng g^{-1} of a mixture of POP standards showed a recovery range from 74 to 103% for all OCPs. Recovery for PCBs ranged from 62.1 to 82.2%. Relative standard deviation (RSD) was less than 14% for all contaminants investigated suggesting precision and repeatability of the analysis process. The limit of detection (LOD) and limit of quantification (LOQ) were 0.3 and 0.9 ng g^{-1} ww for all OCPs and PCBs. Concentrations were expressed on a wet weight (ww) and lipid weight (lw) basis.

2.9 Risk assessments

In the wild condition, adverse toxic effects of OCPs and PCBs are more common than acute toxic effects. Adverse toxic effects have been associated with population decline in many wildlife species [26]. We estimated the reproductive health risks in the investigated bird species by comparing the present DDT and PCB tissue residue levels with minimum threshold concentrations to cause reproductive failure in bird species as described in Ayele et al. [24]. Minimum threshold concentration data recorded for other species was used due to the lack of such data for the investigated bird species [18]. During such comparison care must be taken as there might be differences in species, sex, developmental stage, tissue type of birds compared.

2.10 Statistical analysis

Comparison of mean levels of contaminants among species was done 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 between wing chord length and residue levels of contaminants. Linear regression was employed in the determination of biomagnification of contaminants in the food chain. The slope of the regression line between the log-transformed concentrations of DDTs and $\delta^{15}N$ was used as an indicator of bioaccumulation of ∑DDTs among bird species. Statistical analyses were performed at the significant level of 0.05. SPSS statistical software (SPSS 20) was used in data analysis.

3 Results and discussion

3.1 Morphological measurements and lipid contents

Mean weight of birds varied from 396 to 7075 g with minimum and maximum weights of 320 and 8545 g, respectively. Mean wing chord lengths were varied from 31.0 to 74.5 cm. Minimum and maximum wing chord lengths were 28.6 and 78.4 cm, respectively (Table 1). Lipid contents in the pooled muscle samples were varied from 2.57% to 6.62%. The lipid contents from the highest to lowest were as follows: *P. onocrotalus* (6.62%), *T. aethiopicus* (3.13%), *S. umbretta* (3.0%), and *L. crumeniferus* (2.6%).

3.2 Concentration of OCPs and PCBs

Mean ∑POPs ranging from 143.9 to 1053.0 ng g^{-1} has been recorded. The maximum ∑POPs were recorded for *P. onocrotalus* and the minimum as recorded for *T. aethiopicus*. OCPs were the predominant contaminants, two orders of magnitude higher than PCBs (Table 2). The dominant OCP analyte detected in bird tissues was DDT and its decomposition metabolites. The only other OCP detected was endosulfan sulfate and it was detected only in *S. umbretta*. DDTs were detected in all the tissue samples. PCBs were detected in 45% of the samples and quantified 36.7% of the samples. Cis-, trans- and oxy-chlordane, Endosulfan-alfa, endosulfan-beta, aldrin, and dieldrin were below the detection limit in all the samples. DDTs contribute from 99.4 to 99.8% of the sum of OCPs and PCBs indicating the contamination of Lake Ziway is mainly by DDTs. This finding is in agreement with previous findings from the same study site [18].

3.2.1 Concentrations of OCPs

Mean concentrations of ∑DDTs varied from 143.9 to 1051.1 ng g^{-1} ww, with total concentration ranging from 76.2 to 3197.2 ng g^{-1} ww. The highest and the lowest mean ∑DDTs were recorded in *P. onocrotalus* and *T. aethiopicus*, respectively. Among the six DDT metabolites investigated, *p,p'*-DDE, and *p,p'*-DDD were detected in all samples. However, *o,p'*-DDTs were not detected in all muscle tissue samples. *p,p'*-DDE, and *p,p'*-DDD were the main decomposition products of the parent compound *p,p*-DDT [27]. The concentration of *p,p*-DDT was below the detection limit in all samples except one individual of *P. onocrotalus* (1.09 ng g^{-1} ww). This finding is in line with findings

Table 2 Mean (M) value of *p,p′*-DDE, *p,p′*-DDD, ∑DDTs and ∑PCB (ng g-1 ww [ng g-1 lw])

POPs	Species	N	M ± SD	Min	Max
p,p′-DDE	*P. onocrotalus*	7	971.9 ± 921.6[a] [14,681 ± 13,921]	159.1[2,403.5]	2951.7[44,586.9]
	L. crumeniferus	8	461.8 ± 409.2 [17,762 ± 15,738]	59.4 [2,310.5]	1069.9 [41,630.7]
	T. aethiopicus	7	141.2 ± 60.1[b] [4,511 ± 1920]	74.8 [2,390.1]	241.4 [7,712.8]
	S. umbretta	8	663.3 ± 405.3[c] [22,110 ± 13,510]	152.5 [5,082.0]	1184.1 [39,471.3]
p,p′-DDD	*P. onocrotalus*		79.1 ± 76.5[d] [1,195 ± 1156]	9.3 [139.9]	245.6[3,709.2]
	L. crumeniferus		3.5 ± 1.6[e] [135 ± 62]	1.8 [69.3]	6.3 [244.4]
	T. aethiopicus		2.8 ± 1.1[e] [90 ± 35]	1.4 [44.1]	4.1 [131.6]
	S. umbretta		11.2 ± 6.9[e] [373 ± 230]	5.5 [182.0]	23.4 [779.3]
∑ DDTs	*P. onocrotalus*		1051.1 ± 996.9 [15,878 ± 15059]	168.4 [2,543.7]	3197.2 [48,295.9]
	L. crumeniferus		465.2 ± 410.2 [17,892 ± 15,777]	61.7 [2,402.0]	1074.3 [41,800.0]
	T. aethiopicus		143.9 ± 60.9 [4,597 ± 1946]	76.2 [2,434.2]	245.3 [7,836.7]
	S. umbretta		674.5 ± 409.1 [22,483 ± 13,637]	158.8 [5,294.3]	1204.8 [40,158.3]
∑PCBs	*P. onocrotalus*		1.5 ± 2.4 [23 ± 36]	ND	6.4 [96.2]
	L. crumeniferus		3.5 ± 7.0 [135 ± 269]	ND	20.5 [799.2]
	T. aethiopicus		–	ND	–
	S. umbretta		1.2 ± 2.38 [40 ± 79]	ND	6.9 [230.3]
Endosulfan-sulfate	*P. onocrotalus*		–	–	–
	L. crumeniferus		–	–	–
	T. aethiopicus		–	–	–
	S. umbretta		2.55 ± 1.33 [85 ± 44]	ND	11.13 [371]
∑POPs	*P. onocrotalus*		1053.0 ± 538.8 [15,906 ± 8139]	ND	3203.6 [48,392.2]
	L. crumeniferus		468.7 ± 264.7 [18,027 ± 10,181]	ND	1078.4 [41,962.7]
	T. aethiopicus		143.9 ± 80.7 [4,597 ± 2578]	ND	246.4 [7,872.8]
	S. umbretta		678.3 ± 327.7 [22,610 ± 10,923]	ND	1211.7 [40,388.7]

Mean values with different superscript letters are significantly different ($p < 0.05$)

∑DDTs: *p,p′*-DDE + *p,p′*-DDD

∑PCBs: PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, PCB180

in birds from Argentina [28]. Non-detection of *p,p*-DDT in 96.7% of investigated muscle samples may suggest that the current levels could be the result of past DDT use [28]. The value of the ratio of *p,p′*-DDE/*p,p′*-DDT, that is greater than 1.0, is also suggest the historic use [18, 29]. The discontinuation of the use of DDT for agricultural purposes since 2009 [10] could explain the absence of *p,p*-DDT in the majority of the samples. Studies have also pointed out partial replacement of DDT use for IRS by long-lasting insecticidal nets, use of carbamate and organophosphorus insecticides (such as propoxur and pirimiphos), and application of integrated pest and vector management since 2010 [9, 30], that could have contributed for the absence of *p,p*-DDT in investigated samples. Moreover, the elimination of obsolete pesticide stock is on progress in the country [9]. A decrease in the use of DDT in Ethiopia since 2009 have been documented [10]. The present finding is in contrast to earlier findings from the same study site which stresses the presence of fresh DDT release [16, 18]. That could probably be a result of exposure to the contemporary un-weathered DDT compounds [31]. The concentrations of ∑DDTs in the present study (4480–25,480 ng g^{-1} lw) have shown 38.3–74.2% decrease from that recorded in birds from the current study site in 2012 [18]. The decrease may be attributed to the decline and discontinuation of the use of DDT for IRs and agricultural purposes, respectively [10, 30].

The maximum ∑DDTs levels recorded in the present study (3197.2 ng g^{-1} ww [48,295.9 ng g^{-1} lw] were higher than the values in muscle tissue of Kentish Plovers *(Charadrius alexandrines)* (3416 ng g^{-1} lw) from China [32], feather of *Strigidae* family birds (15–295 ng g^{-1} feather) from Iran [33], and in muscle tissue of Eurasian spoonbill (*Platalea leucorodia*) (822 ng g^{-1} ww) from India [34]. On the other hand, the present levels were lower than maximum values in muscle tissues of various bird species from Greenland (510,000 ng g^{-1} lw) [35], from Ethiopia (3700–148,300 ng g^{-1} lw) [18] and in whole-body homogenate of white-breasted waterhen (*Amaurornis phoenicurus*) (24–9000 ng g^{-1} ww) from North Vietnam [36]. However, comparison is difficult due to differences in size, study year, type of tissue, and bird species in these studies.

Mean concentrations of *p,p′*-DDE varied from 141.2 to 971.9 ng g^{-1} ww with minimum and maximum values of 59.4 and 2951.7 ng g^{-1} ww, respectively (Table 2). *p,p′*-DDE constitute 92.3–98.6% of total DDTs (*p,p′*-DDE + *p,p′*-DDD). The high proportion of *p,p′*-DDE to total DDT could be due to its higher lipophilic property ($logK_{ow}$ = 6. 02) than *p,p′*-DDD ($logK_{ow}$ = 5.69) enhancing its bioaccumulation capability [16]. Moreover, high degree of exposure to weathered DDT resulted from the historic application of DDT, long environmental (10–15 years in soil) [37], and biological half-lives may explain its high concentration and proportion in bird tissues [36]. Predominance of *p,p′*-DDE among DDT metabolites have been documented in several studies [18, 35, 38].

Mean levels of *p,p′*-DDE, and *p,p′*-DDD were significantly varied among the bird species ($F(3,26) = 5.93$; $p < 0.05$, $F(3,26) = 20.86$; $p < 0.05$), respectively. The highest mean *p,p′*-DDE and *p,p′*-DDD were found in *P. onocrotalus* (971.9 ng g^{-1} ww, 79.1 ng g^{-1} ww, respectively) followed by *S. umbretta* (663.3 ng g^{-1} ww, 11.2 ng g^{-1} ww, respectively). Species variation in *p,p′*-DDE accumulation could be a result of differences in trophic level, lipid content [39], feeding habit [28], and foraging habitat [36].

In the present study, *P. onocrotalus* occupies the highest trophic position (Fig. 4). The high levels of *p,p′*-DDE, and *p,p′*-DDD in *P. onocrotalus* can be attributed to its top trophic position, higher lipid content and total dependence on fish diets that are frequently shown to accumulate DDTs [16]. Moreover, *P. onocrotalus′* diet consists of 90% large fish [20] which predisposes the species to a high degree of exposure as larger and older fishes accumulate more DDTs than smaller ones [17]. Next to *P. onocrotalus*, the highest *p,p′*-DDE in *S. umbretta* could be explained by its increased dependence on diets from aquatic sources than terrestrial sources. This was substantiated by the lower and narrower $\delta^{13}C$ values in *S. umbretta* that is similar to *P. onocrotalus* (Fig. 4) which may suggest greater degree utilization of aquatic carbon source and specialist feeding behavior. It is noted that organochlorine pollutants are more available in aquatic than terrestrial habitats [40] as a result of leaching from agricultural lands [41]. In addition, *S. umbretta* species prefers and frequently consumes fish scraps from the local fish processing sites [42] that could contribute to higher levels of DDTs. The relatively greater frequency of foraging in aquatic habitats than terrestrial areas distinguish *P. onocrotalus* and *S. umbretta* from *L. crumeniferus* and *T. aethiopicus,* and probably the reason for the accumulation of 1.6–5.6 times higher DDT levels in the former than the later ones. Further investigation is needed to quantify the proportion of time spent foraging in aquatic and terrestrial habitats for a better understanding of the influence of foraging habitat on organochlorine accumulation in birds.

3.2.2 Concentrations of PCBs

The levels of ∑PCBs were varied from (Not detected) ND—20.5 ng g^{-1} ww. Maximum mean ∑PCBs were recorded for *L. crumeniferus* (3.5 ng g^{-1} ww), followed by *P. onocrotalus* (1.5 ng g^{-1} ww) and *S. umbretta* (1.2 ng g^{-1} ww) (Table 2). However, there was no statistically significant variation in mean ∑PCBs concentration among the species ($F(3,26) = 1.33$; $p > 0.05$). Even though there is no significant variation in PCB concentration among bird species, the maximum value recorded in Marabou stork (*L. crumeniferus*) could be attributed to its nesting locations and feeding habit. Marabou storks mostly nest on large trees found in Ziway town [43] and feed on a wide range of terrestrial prey from municipal waste dumping sites [43] that might contribute to the relatively higher PCB levels [14, 38, 44]. An earlier study from the current study site revealed that terrestrial diets could constitute up to 60% of *L. crumeniferus′* diets [43]. Studies have also shown that higher levels of PCB in birds attributed to urbanization, generalist feeding habits [44], foraging at e-waste dumping sites [38], and nesting in the vicinity of high voltage electrical transformers [14].

The levels of ∑PCBs in the present study (ND—20.54 ng g^{-1} ww [ND—799.22 ng g^{-1} lw]) were higher than values in eggs of Cattle egret *(Bubulcus ibis)* (∑PCBs = 46 ng g^{-1} lw) from South Africa reported by Polder [45] and (1.9–9.6 ng g^{-1} ww) by Bouwman [46]. However, the present PCB levels were lower than values in muscle tissue of Kentish Plovers (*Charadrius alexandrines*) (15.7–1466 ng g^{-1} lw) from China [32], in muscle tissue of Great Tit (*Parus major)* (461–1060 ng g^{-1} lw) from Belgium [47] and in liver tissues of Balearic shearwater *(Puffinus mauretanicus)* (150–800 ng g^{-1} ww) from Portugal [48]. The current levels are also lower than values in eggs of purple heron *(Ardea purpurea)* from Spain (520–1400 ng g^{-1} ww) [49].

3.2.3 PCB congener profile

In the entire sample analyzed for PCBs highly chlorinated congeners, PCB-101, -118, -138, 153, and -180, were detected (Table 3). The order of mean concentrations of PCB congeners from high to low was as follows: PCB-153 (3.0 ng g^{-1}) > PCB-138 (1.3 ng g^{-1}) > PCB-180 (1.1 ng g^{-1}) > PCB-118 (0.8 ng g^{-1}) > PCB-101 (0.2 ng g^{-1}) > PCB- 52 (ND) & PCB-26 (ND). The aforementioned order could be attributed to differences in chlorine content, lipophilic property, and biomagnification capacity among the congeners [50]. The present finding is in

Table 3 Mean levels of PCB congeners (ng g-1 ww [ng g-1 lw]) with minimum and maximum values

PCBs	Species	N	M ± SD	Min	Max
PCB-101	*P. onocrotalus*	7	–	ND	–
	L. crumeniferus	8	0.2 ± 0.5 [6.6 ± 19.2]	ND	1.35 [52.5]
	T. aethiopicus	7	–	ND	–
	S. umbretta	8	–	ND	–
PCB-118	*P. onocrotalus*		–	ND	–
	L. crumeniferus		0.7 ± 1.6 [26.1 ± 61.5]	ND	4.61 [179.4]
	T. aethiopicus		–	ND	–
	S. umbretta		0.1 ± 0.4 [4.3 ± 13.3]	ND	1.05 [35.0]
PCB-138	*P. onocrotalus*		0.3 ± 0.84 [4.8 ± 12.7]	ND	2.22 [33.5]
	L. crumeniferus		0.7 ± 1.61 [29.6 ± 61.9]	ND	4.31 [167.7]
	T. aethiopicus		–	ND	–
	S. umbretta		0.2 ± 0.64 [7.7 ± 21.3]	ND	1.82 [60.7]
PCB-153	*P. onocrotalus*		0.7 ± 1.02 [10.9 ± 15.4]	ND	2.64 [39.9]
	L. crumeniferus		1.6 ± 2.28 [63.4 ± 87.7]	ND	6.94 [270.0]
	T. aethiopicus		–	ND	–
	S. umbretta		0.6 ± 0.97 [21.3 ± 32.3]	ND	2.55 [85.0]
PCB-180	*P. onocrotalus*		0.4 ± 0.73 [6.5 ± 11.0]	ND	1.51 [22.8]
	L. crumeniferus		0.5 ± 1.26 [16.7 ± 48.5]	ND	3.32 [129.2]
	T. aethiopicus		–	ND	–
	S. umbretta		0.2 ± 0.56 [7.0 ± 18.7]	ND	1.49 [49.7]

concordance with the order of congeners that was found in the eggs of Northern gannets (*Morus bassanus*) [51]. PCB-118, PCB-138, PCB-153 and PCB-180 constitute about 97.8% of all congeners. This finding is also consistent with earlier findings by Costa [48] in Balearic shearwater (*Puffinus mauretanicus*).

Carnivorous waterbird species generally occupy higher trophic positions. The higher proportion of higher chlorinated congeners in the studied birds may probably be a result of primarily exposure to higher chlorinated congeners from their diet [52]. It also could occur as a result of high chlorinated congeners' resistance to biotransformation [3] and their higher biomagnification capacity [40].

PCB-153 predominates the PCB congener profile in all samples that could be due to its higher log K_{ow} value than most of the rest of PCB congeners analyzed [53]. PCB congener profile in *P. onocrotalus, L. crumeniferus* and *S. umbretta* in decreasing order were PCB-153 > PCB-180 > PCB-138, PCB-153 > PCB-138 > PCB-118 > PCB-180 > PCB-101 and PCB-153 > PCB-138 > PCB-180 > PCB-118, respectively (Fig. 2). The slightly varying congener profile observed may be attributed to variation in trophic level, feeding habit, metabolic capacities, and species-specific response [3, 40].

3.3 The influence of lipid contents and size on organochlorine pollutant concentrations

The relationship between mean DDTs levels and percent lipid content of pooled muscle samples was investigated. All *p,p′*-DDE, *p,p′*-DDD, ∑DDTs and ∑PCB showed positive relationship with percent lipid content ($r = 0.78$; $p > 0.05$), ($r = 0.99$; $p < 0.05$), ($r = 0.81$; $p > 0.05$) and ($r = 0.37$; $p > 0.05$). However, except for *p,p′*-DDD, all associations were not

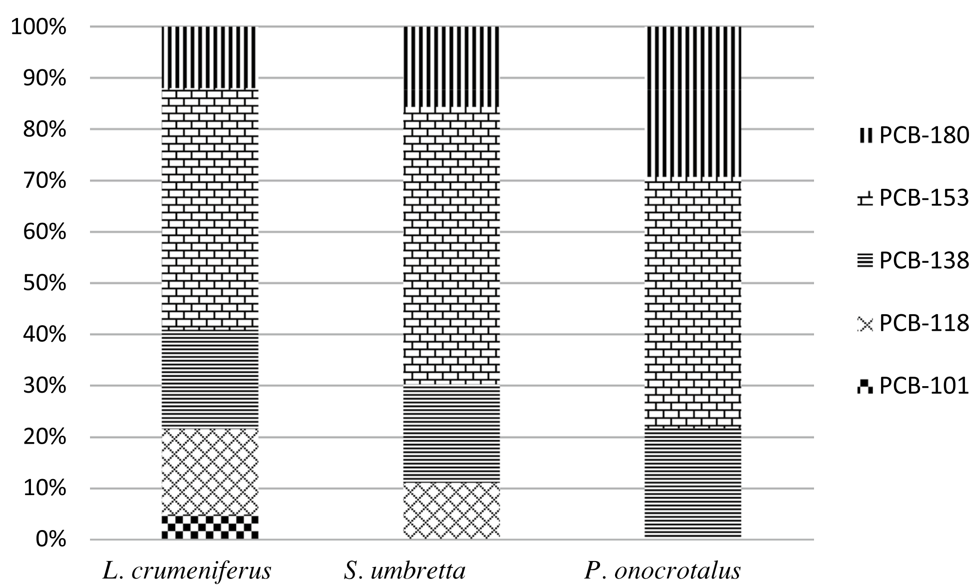

Fig. 2 The relative concentration of PCB congeners in three species of birds

statistically significant. The positive association may suggest that lipid content could have influence in the accumulation of DDTs and PCBs [39].

Organochlorine pollutants attain higher tissue concentration in birds as a result of the biomagnification process [18]. The longer the period of exposure to these pollutants, the higher will be body concentration of these pollutants [35]. As a result, the more aged individual would have a longer period of exposure and expected to have a higher organochlorine pollutant burden [35]. We have investigated the relationship between bird size and DDTs accumulation in birds. Since weight of birds vary based on gut fullness, we used wing chord length as a measure of bird size to avoid bias [54]. Results showed that, in all bird species, a positive association between log-transformed ∑DDT (ng g^{-1} ww) and wing chord length (cm) was found (Fig. 3). However, association was statistically significant only in *L. crumeniferus* ($r = 0.87$; $p < 0.05$). The lack of statistically significant association in the rest of the bird species could be due to a very small variation in wing chord lengths among the individuals of each species (Table 1). The positive association between log-transformed ∑DDT and wing chord length may suggest that size is an important variable explaining the accumulation of OCPs

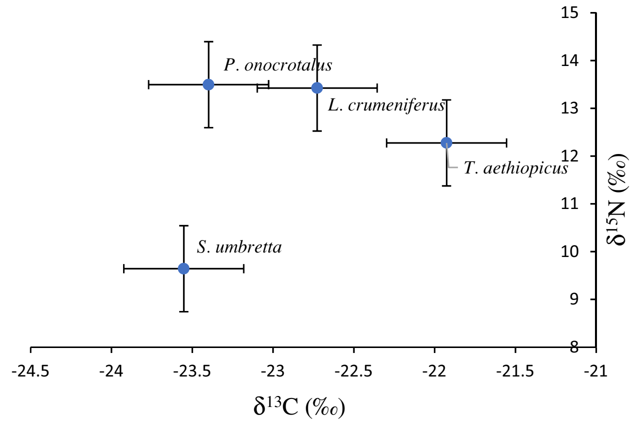

Fig. 4 Trophic position of bird species sampled from Lake Ziway

assuming large birds tend to be aged [39]. This finding is in agreement with earlier findings by Jaspers [35].

Regarding PCBs, there was positive association between log-transformed ∑PCB and wing chord length in *P. onocrotalus* ($r = 0.91$, $p > 0.05$). However, in *L. crumeniferus* ($r = 0.00$, $p > 0.05$) there was no clear relationship and in *S. umbretta* ($r = -0.55$, $p > 0.05$) the association was negative (Figure not shown). The absence of clear association in *L.*

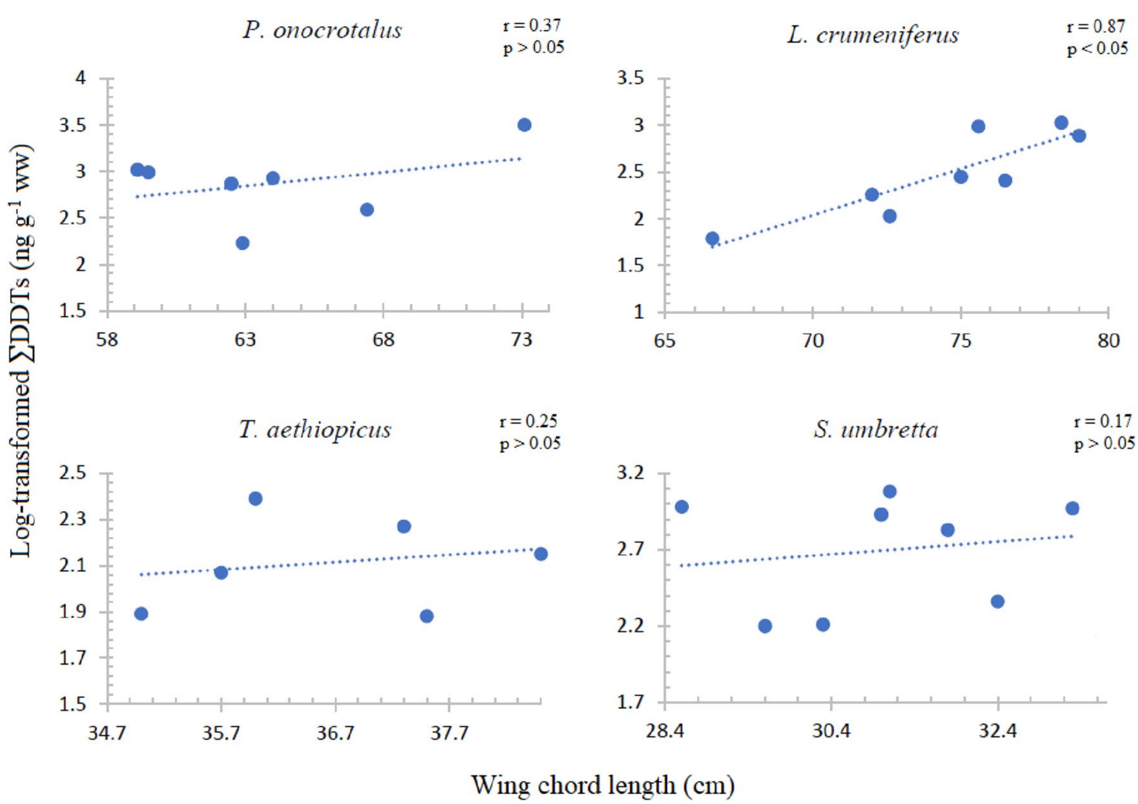

Fig. 3 Relationship between log-transformed ∑DDTs (ng g^{-1} ww) and wing chord length (cm)

crumeniferus could be due to the effect of a small sample size. The negative association in *S. umbretta* could result from the effect of small sample size and less quantification frequency of PCBs (36.7%) affecting the regression analysis results. The correlation between wing chord length and PCBs was not performed for *T. aethiopicus* since PCBs were below the limit of quantification.

3.4 Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$)

The mean $\delta^{15}N$ and $\delta^{13}C$ ratio values were varied from 9.6 to 13.5‰ and − 23.5 to − 21.9‰, respectively. The difference of 3.9‰ between the maximum and minimum mean values of $\delta^{15}N$ indicates that the birds occupy two trophic levels assuming a mean trophic enrichment factor of 3.4‰ [55]. The highest mean $\delta^{15}N$ ratio values found in *P. onocrotalus* and *L. crumeniferus* may suggest both species occupy a higher trophic position with respect to the rest. The lowest mean $\delta^{15}N$ values were recorded for *S. umbretta* and *T. aethiopicus* (Fig. 4) may indicate the species' lower trophic position.

P. onocrotalus and *S. umbretta,* had the lowest and narrow mean $\delta^{13}C$ values, which suggest the utilization of a similarly narrow range of food sources (Fig. 4). This is corroborated by the preference and dependance of both species on fish diet [42, 56]. In addition to that, during the dry season, when the level of the lake water decreases, the availability of fish as prey increases due to increased visibility. As a result, *S. umbretta* could increase its dependence on a fish diet that may result in low and narrow $\delta^{13}C$ values that otherwise is generalist in its feeding habit [18]. (N.B. Bird samples were collected in May, the last month of the dry season). The relatively higher and wider $\delta^{13}C$ values of *T. aethiopicus* and *L. crumeniferus* indicate that these species are generalist in their diet and utilize a wide range of food sources from both terrestrial and aquatic habitats [43, 57]. This finding is consistent with a study by Yohannes et al. [18].

The influence of trophic level on tissue concentration of OCPs and PCBs was investigated using values of regression coefficients and slope of the regression line between log-transformed ∑DDTs and $\delta^{15}N$. The result shows a positive association between log-transformed ∑DDTs and $\delta^{15}N$, which could suggest the occurrence of biomagnification of DDT in the local food web (Slope = 0.01; r^2 = 0.002). The slope of the regression line (slope = 0.01) revealed a smaller rate of biomagnification than previously reported (slope = 0.48) from the same study site [18]. The order of levels of DDTs among species of birds appeared as follows: *P. onocrotalus* > *S. umbretta* > *L. crumeniferus* > *T. aethiopicus.* Despite the presence of minor deviation, the variation in the levels of DDTs among species could be explained by the trophic positions of birds (Fig. 4).

Regarding PCBs, a negative association between log-transformed ∑PCBs and $\delta^{15}N$ (slope = -0.03; $r^{2\cdot}$ = 0.04) was found suggesting PCBs tissue concentrations do not increase with an increase in trophic level. PCBs are lipophilic and resistant to biotransformation [3] and as consequence, they biomagnify through food webs [49]. The negative association between PCB levels and trophic position could result from the combined effect of small sample size and low detection frequency (36.7%).

3.5 Risk assessments

One of the well-known effects of *p,p'*-DDE in birds is eggshell thinning which results in eggshell breakage before hatching and abnormal gas exchange [58, 59]. Assuming lipid normalized *p,p'*-DDE levels in muscle and liver tissues are comparable, the maximum concentration of *p,p'*-DDE in the present study (7,712.8–44,586.9 ng g^{-1} lw) partially overlaps with the average liver concentrations of *p,p'*-DDE (20–1000 µg g^{-1} lw) which were associated with reproductive impairment in individual birds [60]. As a result of this overlap, 26.7% of muscle tissue specimens (1 from *P. onocrotalus*, and 3 from *L. crumeniferus,* and 4 from *S. umbretta*) have shown concentrations well above the minimum threshold concentration of 20 µg g^{-1} lw. This suggests the present levels of *p,p'*-DDE could pose the risk of reproductive impairment in carnivorous waterbird species.

Various sublethal effects have been associated with PCB exposure [50]. PCBs toxicities were linked to decreased nest attentiveness [6], disruption of feather coloration [7], and impairment of sound production in birds [8] that may culminate in an impairment of reproductive behaviors. Varying threshold levels of PCB in various tissues have been linked to reproductive impairments in birds. Egg concentration of PCBs varying from 1 to 25 µg g^{-1} ww have been implicated in a decreased number of hatchings in cormorants and eagles [61]. Blood residue of 243 ng g^{-1}ww was associated with an adverse effect on reproductive behavior in Glaucous Gull (*Larus hyperboreus*) [6]. Egg concentration of PCBs ranging from 1.6 to 10 µg g^{-1} ww have been linked with reproductive failure in Cormorants and heron species [50]. Assuming 20% maternal to egg transfer [62], the ∑PCB levels found in the present study were well below the lowest threshold egg concentration (1–25 µg g^{-1} ww) [61] to cause reproductive failure in studied birds. However, comparing toxicity thresholds is very difficult due to differences in the number of PCBs investigated, degree of chlorination, the toxicity of congeners, and species-specific responses they elicit.

4 Conclusions

The current levels of DDT recorded in the investigated species cause reproductive health risks. There is a positive correlation between the size of individual birds and the levels of OCPs in muscle tissues. Generally, the findings of the present study suggest that DDTs are the most dominant and prevalent contaminant in the investigated carnivorous waterbird species from Lake Ziway. The current levels of DDT in the studied birds have shown an average decrease of 60% compared to that recorded a decade ago. Trophic position is an important variable influencing DDT accumulation. PCB levels were dominated by higher chlorinated congeners. The levels of PCB measured in studied birds were too low to cause reproductive impairments in studied birds. The findings of this study may contribute relevant information for the protection of carnivorous waterbird species. The present study focused only few species of carnivorous waterbird species. Future POP monitoring and health risk studies involving more species of carnivorous waterbirds is recommended.

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

Research involved in human or animal rights International and national guidelines for the care and use of animals were followed in the present research that have involved animals.

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