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

Yared Beyene Yohannes [a,b,1], Yoshinori Ikenaka [a,1], Shouta M.M. Nakayama [a], Mayumi Ishizuka [a,*]

[a] Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo 060-0818, Japan

[b] University of Gondar, Faculty of Natural and Computational Science, Department of Chemistry, P.O. Box 196, Gondar, Ethiopia

[1] Both authors contributed equally in this manuscript

*Corresponding author

Tel.: +81 11 706 6949

E-mail address: ishizum@vetmed.hokudai.ac.jp

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

Abstract

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

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

1. Introduction

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

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

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

2. Materials and methods

2.1. Study site

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

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

2.2. Samples

Four bird and five fish species were collected between January 2011 and June 2012. In general, 23 bird individuals belonging to Hamerkop (*Scopus umbretta*, N = 5); African sacred ibis (*Threskiornis aethiopicus*, N = 7); Marabou stork (*Leptoptilos crumeniferus*, N = 6) and Great white pelican (*Pelecanus onocrotalus*, N = 5), and 105 fish specimens of *Oreochromis niloticus*, *Tilapia zillii*, *Carassius* spp., *Clarias gariepinus* and *Barbus intermedius* were collected. Information about the samples by species is given in Table 1. Muscle tissues were taken from the aforementioned species and stored at −20 °C until OCPs and stable isotopes analyses. For bird sampling, the Ethiopian Wildlife Conservation Authority (EWCA) issued a permit (Permission No. DA/31/284/012) allowing us to capture and sacrifice the above mentioned species of birds under the supervision of Veterinarian. All analyses were conducted at the Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan.

2.3. Stable isotope analysis

Dried muscle samples were lipid extracted using 2:1 (v/v) chloroform:methanol solution. Approximately 1 mg of each sample was loaded into tin capsule and analyzed using a Fisons NA1500 elemental analyzer equipped with a Finnigan MAT 252 isotope ratio mass spectrometer. Stable carbon and nitrogen isotope ratios were expressed in delta values as $\delta^{13}C$ or $\delta^{15}N$ (‰) = $[(R_{sample}/R_{standard}) - 1] \times 1000$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Pee Dee Belemnite carbonate and atmospheric nitrogen were used as standards for carbon and nitrogen, respectively. The analytical precision based on internal laboratory standards was with measurement precision of ±0.2‰ for both stable isotope ratios.

2.4. OCPs analysis

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

OCPs including DDTs (*o,p′*− and *p,p′*−DDT, DDE and DDD), hexachlorocyclohexanes (HCHs; α−, β−, γ− and δ−HCH), heptachlors (HPTs; heptachlor, *cis*− and *trans*−heptachlor epoxide), chlordanes (CHLs; *cis*−, *trans*− and oxy−chlordane, *cis*− and *trans*−nonachlor, drins (aldrin, dieldrin and endrin) and hexachlorobenzene (HCB) were analyzed using a Shimadzu Model 2014 gas chromatography micro electron capture detector (GC-μECD) equipped with a 30 m × 0.25 mm × 0.25 μm ENV-8MS capillary column. The initial oven temperature was held at 100 °C for 1 min; increased to 200 °C at 20 °C/min and then to 260 °C at 4 °C/min, which was held for 5 min. The injector and detector temperatures were set as 250 °C and 310 °C, respectively. Helium at a flow rate of 1.0 ml/min and nitrogen at 45 ml/min were used as carrier gas and make-up gas, respectively. One μl of each sample was injected in the splitless mode.

2.5. Quality control and quality assurance

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

2.6. Statistical analysis

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

3. Results and discussion

3.1. Stable isotope analysis

Significant differences of both $\delta^{13}C$ (F-ratio = 20.9; $p < 0.001$), and $\delta^{15}N$ (F-ratio = 25.2; $p < 0.001$) amongst bird and fish species were observed. $\delta^{13}C$ and $\delta^{15}N$ values of the studied species ranged from −24.8‰ to −15.3‰ and from 5.25‰ to 13.3‰, respectively (Fig. 2). In bird species, the aquatic bird, great white pelican showed significantly high $\delta^{15}N$ values compared to the other bird species ($p < 0.05$). Furthermore, this bird species also showed significant difference with the terrestrial bird species based on the $\delta^{13}C$ values (Fig. SI-2). The lowest and narrow range $\delta^{13}C$ values of great white pelican indicating the homogeneity of their feedings i.e., piscivorous feeding habits, whereas the wide range of $\delta^{13}C$ values for hamerkop, African sacred ibis and marabou stork showed the high heterogeneity of diet source for these bird species. Regarding fish species, the carnivorous fish species catfish and barbus showed significantly high $\delta^{15}N$ values (9.70‰ and 10.0‰, respectively) than planktivorous fish species tilapia (7.37‰), zillii (8.91‰) and carp (8.38‰) (Fig. SI-3).

3.2. Levels of OCPs

Of all target compounds analyzed, 10 OCPs were frequently detected in both bird and fish samples; *p,p′*–DDT, *p,p′*–DDE, *p,p′*–DDD, α–HCH, γ–HCH, *cis*– and *trans*–heptachlor epoxide, *cis*– and *trans*–chlordane, and *trans*–nonachlor. HCB and *o,p′*–DDT were detected only in bird and fish species, respectively (Table SI-1 and SI-2). Oxy−chlordane and β−HCH were rarely encountered but levels of drins (aldrin, dieldrin and endrin) were below detection limit (data not shown).

The median and range concentrations of total OCPs are summarized in Table 2. Levels of ∑OCPs in birds and fish ranged from 3.7 to 148.7 μg/g lw and 0.04 to 10.91 μg/g lw, respectively. Significant difference for ∑OCPs concentration was observed between the groups i.e., bird and fish species (F-ratio = 39.65, $p < 0.001$), whereas no significant differences were seen within each group (bird: F-ratio = 1.624, $p = 0.217$; fish: F-ratio = 1.163, $p = 0.332$). However, when individual OCP concentrations were compared among the bird species, significant difference was found only in levels of CHLs. Generally, the median concentrations of total OCPs were higher, for more than 10 times, in birds than in fish species (Table 2). Our result indicates moderate to high levels of OCPs in different bird and fish species. Marabou stork had the highest median concentrations of ∑OCPs as this bird species is a scavenger and having a wide range of feeding habits from both mainly terrestrial and aquatic food webs. In general, a large variability of pollutants levels especially in bird species was found within a single species. This might be attributed to different feeding ecology, age, habitat, condition of the birds, and seasonal variation of food compositions for terrestrial birds (Jaspers et al., 2006).

The relative proportions of ΣOCPs groups varied between bird and fish species are shown in Fig. 3. The OCP profile for all species was clearly dominated by DDTs, accounting for 52 to 76% in fish species and more than 99% in bird species. This result indicates the high degree of exposure to DDTs in biota from the Ethiopian Rift Valley region, which is most likely due to the recent use of DDT-IRS for malaria control (Van den Berg, 2009; WHO,

2007) as well as from illegal usage and contamination from past usage (Amera and Abate, 2008), and spills from obsolete pesticides (Haylamicheal and Dalvie, 2009). HCHs and CHLs were the next OCPs with highest concentrations followed by HPTs.

3.2.1. DDTs

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

3.2.2. Other OCPs

The α–, and γ–HCH isomers were detected in all samples except in great white pelican (Table SI-1 and Table SI-2), and γ–HCH (lindane) dominates in all samples. The predominance of γ–HCH in this study indicates the current usage of lindane in the region. A recent study in the Rift Valley region showed high concentrations of lindane in cattle liver tissues (highest level of 0.14 mg/kg wet wt) obtained from Holeta, Ethiopia (Letta and Attah, 2012). Maximum level of ΣHCHs was recorded in muscle tissue of African sacred ibis (0.18 µg/g lw) followed by marabou stork (0.13 µg/g lw) (Table 2). These bird species have a wide feeding habits in both aquatic and terrestrial food webs.

Cyclodiene insecticides, heptachlor epoxides and chlordanes were also detected in both fish and bird species with varying concentrations. Among the CHLs, *trans*–chlordane was the most abundant and dominant contributor to total chlordanes followed by *cis*–chlordane and *trans*–nonachlor as they are the major constituents in technical chlordane (Table SI-1 and Table SI-2), whereas oxy-chlordane was rarely encountered. Significantly high CHLs concentration (0.72 to 1.39 µg/g lw) was observed in great white pelican (F-ratio = 26.55, $p < 0.001$). According to the HPTs, *cis*– and *trans*–heptachlor epoxides were the predominant ones. The greatest median concentration of HPTs was detected in marabou stork (0.07 µg/g lw) followed by hamerkop (0.04 µg/g lw). In general, levels of HPTs in the muscle of the studied bird species ranged from 0.004 to 0.10 µg/g lipid wt (Table 2).

3.3. Profile differences among species

It is well known that differences in food habits, metabolic capacity and trophic position explains most of the variations in pollutant levels between different species. This study revealed that there were different bioaccumulation potentials of OCPs among the studied species. PCA was performed to carry out the comparison of OCPs profiles using frequently detected pollutants in both species and stable isotope values. The PCA revealed that 48% of the variation was accounted for the first principal component (PC1) and 13% by PC2 (Fig. 4). As observed from the loading plot (Fig. 4a), profiles of OCPs differ noticeably. PC1 was positively related to DDTs, HCB, *trans*–chlordane and stable isotopes, while HCHs, *cis*–chlordane, and *trans*–nonachlor had high loadings onto PC2. This indicates that PC1 increase significantly with increasing OCP levels, which likely is driven by high relative contribution of DDTs.

An interesting feature is also observed in the score plot (Fig. 4b). The bird and fish species separated along PC1 based on the loading pattern of OCPs. The plot clearly exhibited the species-specific differences in the levels of contaminants. The fish species are separated from the bird species, by having relative high levels of HCHs, *trans*–heptachlor epoxide and *o,p′*–DDT. Furthermore, there is a clear separation among the bird species along PC2. The aquatic bird species, great white pelican had high $\delta^{15}N$ values and showed unique loading plots associated with *trans*–chlordane that separated from the other bird species. As shown in Table SI-1, *trans*–chlordane was the most abundant contaminant measured in great white pelican. On the other hand, the terrestrial bird species, having a wide range of $\delta^{13}C$ values were strongly associated with *p,p′*–DDT, *p,p′*–DDD and *p,p′*–DDE. In general, this interspecific differences can be explained by differences in dietary habits and different exposure routes or metabolic efficiency of the studied bird species (Jaspers et al., 2006).

Great white pelican is an aquatic and piscivorous bird feeding primarily on fish. African sacred ibis is an insectivorous which feeds opportunistically on plowed lands and small preys such as small fishes, worms and eggs of birds while the marabou stork is a scavenger species feeds on everything it gets. The latter two bird species often occurs close to human habitation where DDT is sprayed for malaria control (Table 1).

3.4. Biomagnification of OCPs

In this study, 'biomagnification' was defined as the phenomenon of accumulating the chemicals through the food chain (e.g. accumulation of OCPs by birds through consumption of fish). The influence of trophic level on OCPs burden among the studied species was investigated by analyzing correlation between $\delta^{15}N$ values and mean OCPs concentration. The relationship between log transformed OCPs and $\delta^{15}N$ values is shown in Fig. 5. The regressions for DDTs (R^2 = 0.375) and CHLs (R^2 = 0.439) showed positive relationships ($p \leq 0.05$) between concentrations and $\delta^{15}N$ values. These results suggest the biomagnification potential of these compounds for the present lake Ziway food web. The slopes of the regression equations were 0.438 and 0.202, respectively. The slope of [OCPs] vs $\delta^{15}N$ gives an indication of the magnitude of biomagnification (Fisk et al., 2001; Borgå et al., 2001). The higher slope value observed for DDTs might be attributed due to their high hydrophobicity and recalcitrant nature. This finding was consistent with other reports on aquatic and terrestrial food chains (Borgå et al., 2001; Buckman et al., 2004). However, biomagnification was not observed for CHLs against $\delta^{15}N$ values when compared without great white pelican as this bird species had high levels of *trans*–chlordane (R^2 = 0.013; p = 0.814). Thus, it remains inconclusive whether chlordanes are actually biomagnified. A Negative linear relationship (R^2 = 0.682; slope = –0.294; p = 0.011) between $\delta^{15}N$ values and HPTs concentrations was found, indicating that heptachlors do not biomagnify through the food web, suggesting that the metabolic capability of HPTs in the studied species increase with the trophic level. Nevertheless HCHs showed no significant correlation with $\delta^{15}N$ values ($p = 0.518$), largely owing to their low octanol-water partition coefficients (log $K_{ow} \sim 4$) (Russ et al., 2002).

3.5. Comparison with other areas

Because of the absence of data concerning residue levels in same species and same matrices, the residue levels in muscle samples reported in other bird species were referred (Table 3). Data are from Asia, and Europe of which DDTs and HCHs are mostly detected. However, it is possible that the differences in the number of samples and sample types (captured alive or dead) might influence the outcome of this comparison. Being this, DDTs level in our study were higher than the concentration levels reported from southern China (Zhang et al., 2011), and India (Dhananjayan, 2012) at which DDT is still in use, and from Japan (Kunisue et al., 2003). However, they are lower than those in birds from Belgium (Jaspers et al., 2006), northern China (Chen et al., 2009), and Greenland (Jaspers et al., 2013). The HCHs concentration in the present study obviously lie at low end compared to those in muscle of various bird species collected from different areas (Table 3).

Concentration of CHLs in muscle of the aquatic bird, great white pelican was comparable to the concentrations reported in muscle of aquatic birds, grey horn and great crested grebe (0.014 to 2.5 μg/g lw) from Belgium (Jaspers et al., 2006), but lower than in the muscle of white-tailed eagles from west Greenland (Jaspers et al., 2013) (Table 3). On the other hand the levels of CHLs in hamerkop, African sacred ibis and marabou stork were uniformly low, indicating minimal exposure of CHLs to these birds. HPTs levels in muscle in our study are comparable with concentrations reported in the muscle of various bird species from northern China (non-quantifiable to 0.22 μg/g lw) (Chen et al., 2009), but lower than those in birds from India (1.1 to 91 ng/g ww) (Dhananjayan, 2012) (Table 3). HCB levels ranged from ND to 0.042 μg/g lw was by far lower than the concentration levels reported from Belgium and Green land (Jaspers et al. 2006; 2013) (Table 3).

3.6. Toxicological significance

The chemicals assessed in this study are toxic, persistent, can be biomagnified along the food chain and may adversely affect the health, survival and reproduction of birds. DDE is well known for its adverse effect on the health of wildlife especially birds associated with eggshell thinning and reduction in the survival of young birds (Connell et al., 2003). Average concentration of *p,p′*−DDE of 20−1000 μg/g lipid wt in livers of birds was suggested to pose a threat to individual bird reproduction (Tanabe et al., 1998). Moreover, the lowest observable effect concentration of 120 μg/g lipid wt in eggs was estimated for depressed productivity in white-tailed sea eagle (Helander et al., 2002). Thus, taking into consideration that lipid normalized *p,p′*−DDE concentrations measured in muscle were similar with liver tissues, the maximum concentration levels of DDE ranged from 38.7 to 138.5 μg/g lipid wt in bird species might be sufficient to cause adverse effects on reproduction which population declines are reported to occur. As far as heptachlor epoxides (4 to 100 ng/g lw) and HCB (ND to 42 ng/g lw) are concerned, the concentrations were much lower than 1.5 μg/g, at which associated with decreased reproduction rates in avian experimental study (Henny et al., 1993; Boersama et al., 1986). Therefore, there are indications that *p,p′*−DDE levels in the current study pose a threat in terms of toxicity (i.e., eggshell thinning and survival of young birds) to the bird species resides in the Rift Valley region because DDT is still using in the region. Therefore, future studies seem necessary.

4. Conclusion

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

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT), and from the Japan Society for the Promotion of Science (JSPS). We would also like to acknowledge the financial support provided by the Mitsui & Co., Ltd. Environment Fund, The Akiyama Life Science Foundation, and The Nihon Seimei Foundation. The authors sincerely gratitude to the Ethiopian Wildlife Conservation Authority (EWCA) for the permit to sample birds. We are most thankful to Mr. Yeneneh Teka (EWCA, director) and Dr. Fekede Regassa (EWCA, Wildlife veterinary senior expert) for collaboration with the collection of samples. The authors are also to thank Mr. Lemma Abera, Director of the Institute of Ziway Fisheries Resources Research Center laboratory for his kind indeed help in every aspect during sampling and for allowing us to perform dissections in the laboratory.

References

Aktar, W., Sengupta, D., Chowdhury, S., 2009. Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol 2(1), 1–12.

Amera, T., Abate A., 2008. An assessment of the pesticide use, practice and hazards in the Ethiopian Rift Valley. African Stockpiles Programme (ASP).

Birdlife International, 2013. Country profile: Ethiopia http://www.birdlife.org/datazone/userfiles/file/IBAs/AfricaCntryPDFs/Ethiopia.pdf

Boersma, D. C., Ellenton, J. A., Yagminas, A., 1986. Investigation of the hepatic mixed-function oxidase system in herring gull embryos in relation to environmental contaminants. Environ Toxicol Chem 5, 309–318.

Borgå, K., Gabrielsen, G.W., Skaare, J.U., 2001. Biomagnification of organochlorines along a Barents Sea food chain. Environ Pollut 113, 187–198.

Buckman, A.H., Norstrom, R.J., Hobson, K.A., Karnovsky, N.J., Duffe, J., Fisk, A.T., 2004. Organochlorine contaminants in seven species of Arctic seabirds from northern Baffin Bay. Environ Pollut 128, 327–338.

Chen, D., Zhang, X., Mai, B., Sun, Q., Song, J., Luo, X., Zheng, E.Y., Hale, R.C., 2009. Polychlorinated biphenyls and organochlorine pesticides in various bird species from northern China. Environ Pollut 157, 2023–2029.

Connell, D.W., Fung, C.N., Minh, T.B., Tanabe, S., Lam, P.K.S., Wong, B.S.F., et al., 2003. Risk to breeding success of fish-eating ardeids due to persistent organic contaminants in Hong Kong: evidence from organochlorine compounds in eggs. Water Res 37, 459–467.

Deribe, E., Rosseland, B.O., Borgstrøm, R., Salbu, B., Gebremariam, Z., Dadebo, E., Norli, H.R., Eklo, O.M., 2011. Bioaccumulation of persistent organic pollutants (POPs) in fish species from Lake Koka, Ethiopia: the influence of lipid content and trophic position. Sci Total Environ 410-411, 136–145.

Dhananjayan V., 2012. Organochlorine pesticides and polychlorinated biphenyls in various tissues of water birds in Nalabana Bird Sanctuary, Chilika Lake, Orissa, India. Bull Environ Contam Toxicol 89, 197–201.

Donaldson, S.G., Van Oostdam, J., Tikhonov, C., Feeley, M., Armstrong, B., Ayotte, P., Boucher, O., Bowers, W., Chan, L., Dallaire, F., Dallaire, R., Dewailly, E., Edwards, J., Egeland, G.M., Fontaine, J., Furgal, C., Leech, T., Loring, E., Muckle, G., Nancarrow, T., Pereg, D., Plusquellec, P., Potyrala, M., Receveur, O., Shearer, R.G., 2010. Environmental contaminants and human health in the Canadian Arctic. Sci Total Environ 408, 5165–5234.

Drooge, B., Mateo, R., Vives, I., Cardiel, I., Guitart R., 2008. Organochlorine residue levels in livers of birds of prey from Spain: inter-species comparison in relation with diet and migratory patterns. Environ Pollut 153, 84–91.

Fisk, A., Hobson, K., Norstrom, R., 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the northwater polynya marine food web. Environ Sci Technol 35(4), 732–738.

Golubtsov, A.S., Dgebuadze, Y.Y., Mina, M.V., 2002. Fishes of the Ethiopian Rift Valley. In: Tudorancea, C., Taylor, W.D. (Eds.), Ethiopian Rift Valley Lakes. Backhuys Publishers, Leiden, pp. 167–258.

Haylamicheal, I., Dalvie, M., 2009. Disposal of obsolete pesticides, the case of Ethiopia. Environ Int 35, 667–673.

Helander, B., Olsson, A., Bignert, A., Asplund, L., Litzen, K., 2002. The role of DDE, PCB, coplanar PCB and eggshell parameters for reproduction in the white-tailed sea eagle (*Haliaeetus albicilla*) in Sweden. Ambio 31, 386–403.

Henny, C.J., Blus, L.J., Stafford, C.J., 1983. Effects of heptachlor on American Kestrels in the Columbian Basin, Oregon. J Wildlife Manage 47, 1080–1087.

Jaspers, V.L.B., Covaci, A., Voorspoels, S., Dauwe, T., Eens, M., Schepens, P., 2006. Brominated flame retardants and organochlorine pollutants in aquatic and terrestrial predatory birds of Belgium: levels, patterns, tissue distribution and condition factors Environ Pollut 139, 340–352.

Jones, K.C., de Voogt, P., 1999. Persistent organic pollutants (POPs): state of the science. Environ Pollut 100, 209–221.

Jaspers, V.L.B., Sonne, C., Soler-Rodriguez, F., Boertmann, D., Dietz, R., Eens, M., Rasmussen, L.M., Covaci, A., 2013. Persistent organic pollutants and methoxylated polybrominated diphenyl ethers in different tissues of white-tailed eagles (*Haliaeetus albicilla*) from West Greenland. Environ Pollut 175, 137–146.

Kunisue, T., Watanabe, M., Subramanian, A., Sethuraman, A., Titenko, A.M., Qui, V., Prudente, M., Tanabe, S., 2003. Accumulation features of persistent organochlorines in resident and migratory birds from Asia. Environ Pollut 125, 157–172.

Lam, J.C.W., Murphy, M.B., Wang, Y., Tanabe, S., Giesy, J.P., Lam, P.K.S., 2008. Risk assessment of organohalogenated compounds in water bird eggs from South China. Environ Sci Technol 42, 6296–6302.

Letta, B.D., Attah, L.E., 2013. Residue levels of organochlorine pesticides in cattle meat and organs slaughtered in selected towns in West Shoa Zone, Ethiopia. J Environ Sci Heal B 48, 23–32.

Mineau, P., Whiteside, M., 2013. Pesticide acute toxicity is a better correlate of U.S. grassland bird declines than agricultural intensification. PLoS ONE 8(2), e57457. doi:10.1371/journal.pone.0057457.

Muralidharan, S., 1993. Aldrin poisoning of sarus cranes (*Grus antigone*) and a few granivorous birds in Keoladeo National Park, Bharatpur, India. Ecotoxicology 2, 196–202.

Pain, D.J., Gargi, R., Cunningham, A.A., Jones, A., Prakash, V., 2004. Mortality of globally threatened sarus cranes *Grus antigon* from monocrotophos poisoning in India. Sci Total Environ 326, 55–61.

Park, J., Holden, A., Chu, V., Kim, M., Rhee, A., Patel, P., Shi, Y.T., Linthicum, J., Walton, B.J., Mckeown, K., Jewell, N.P., Hooper, K., 2009. Time-trends and congener profiles of PBDEs and PCBs in California peregrine falcons (*Falco peregrinus*). Environ Sci Technol 43, 8744–8751.

Ruus, A., Ugland, K.I., Espeland, O., Skaare, J.U., 2002. Influence of trophic position on organochlorine concentrations and compositional patterns in a marine food web. Environ Toxicol Chem 21, 2356–2364.

Sadasivaiah, S., Tozan, Y., Breman, J.G., 2007. Dichlorodiphenyltrichloroethane (DDT) for indoor residual spraying in Africa: how can it be used for malaria control? Am J Trop Med Hyg 77(Suppl 6), 249–263.

Tanabe, S., Senthilkumar, K., Kannan, K., Subramanian, A.N., 1998. Accumulation features of polychlorinated biphenyls and organochlorine pesticides in resident and migratory birds from South India. Arch Environ Con Tox 34, 387–397.

Van den Berg, H., 2009. Global status of DDT and its alternatives for use in vector control to prevent disease. Environ Health Perspect 117(11), 1656–1663.

Vasseur, P., Cossu-Leguille, C., 2006. Linking molecular interactions to consequent effects of persistent organic pollutants (POPs) upon populations. Chemosphere 62, 1033–1042.

Voorspoels, S., Covaci, A., Lepom, P., Jaspers, V.L.B., Schepens, P., 2006. Levels and distribution of polybrominated diphenyl ethers in various tissues of birds of prey. Environ Pollut 144, 218–227.

WHO (World Health Organization), 2007. Implementation of indoor residual spraying of insecticides for malaria control in the WHO African Region Report: Irs report 2007 Draft, pp. 20–22.

Yohannes, Y.B., Ikenaka, Y., Nakayama, S.M.M., Saengtienchai, A., Watanabe, K., Ishizuka, M., 2013a. Organochlorine pesticides and heavy metals in fish from Lake Awassa; Ethiopia: insights from stable isotope analysis. Chemosphere 91:857–863.

Yohannes, Y.B., Ikenaka, Y., Saengtienchai, A., Watanabe, K.P, Nakayama, S.M.M., Ishizuka, M., 2013b. Occurrence, distribution, and ecological risk assessment of DDTs and heavy metals in surface sediments from Lake Awassa-Ethiopian Rift Valley Lake. Environ Sci Pollut Res 20, 8663–8671.

Zhang X.L., Luo, X.J., Liu, J., Luo, Y., Chen, S.J., Mai, B.X., 2011. Polychlorinated biphenyls and organochlorinated pesticides in birds from a contaminated region in South China: association with trophic level, tissue distribution and risk assessment. Environ Sci Pollut Res 18, 556–565.

Figures legends:

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

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

Fig. 3. Relative proportion of OCPs in muscle tissues of bird and fish species from the Ethiopian Rift Valley region

Fig. 4. Principal Component Analysis based on log transformed contaminant concentrations (a) loading plot (b) score plot. Bird: Hamerkop (H), African sacred ibis (S), Marabou stork (M), Great white pelican (P); Fish: Tilapia (O), Zillii (Z), Carp (C), Catfish (G), Barbus (B)

Fig. 5. Mean ± SD of log-transformed OCPs (ng/g lw) vs. $\delta^{15}N$ values relationship in the present lake Ziway food web. Bird: Hamerkop (H), African sacred ibis (S), Marabou stork (M), Great white pelican (P); Fish: Tilapia (O), Zillii (Z), Carp (C), Catfish (G), Barbus (B)

Table 1.

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

Sample information and feeding habits of the species under study.

N: Number of samples

Table 2.
Median concentrations [range] (µg/g lipid weight) of OCPs in the muscle tissues of birds and fish species from Ethiopian Rift Valley Region

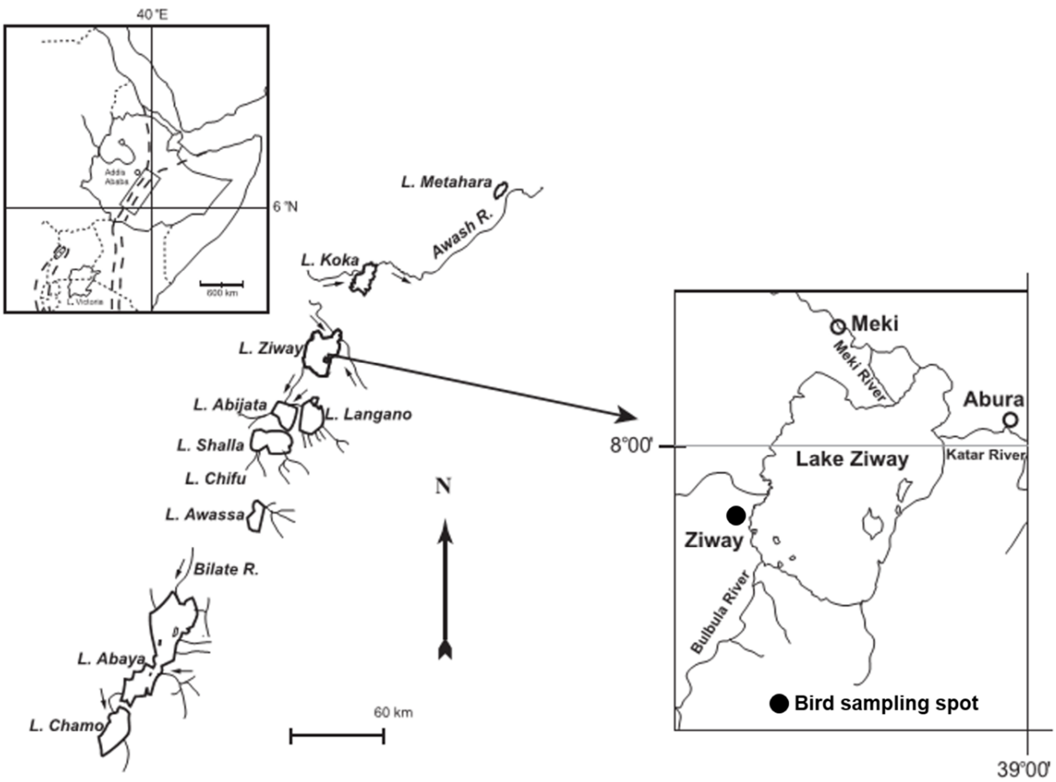
Species (Code)	*N*	Lipid %*	∑HCHs	∑HPTs	∑CHLs	∑DDTs	∑OCPs
Hamerkop	5	1.84 ± 0.49	0.05 [0.03–0.11]	0.04 [0.02–0.05]	0.10 [0.05–0.14]	19.4 [5.0–93.3]	19.6 [5.1–93.6]
African sacred ibis	7	1.43 ± 1.06	0.07 [0.005–0.18]	0.02 [0.01–0.05]	0.13 [0.03–0.19]	17.5 [3.7–40.8]	17.8 [3.7–40.9]
Marabou stork	6	1.58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

N = Number of samples
ND: Not detected or below detection limit
*Data showed as mean ± standard deviation

Table 3.
Comparison of concentrations of OCPs (range, µg/g lipid weight) in muscle of four bird species from Ethiopia with those in other bird species

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

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



40 °E
6 °N
Addis Ababa
600 km
L. Victoria
L. Metahara
Awash R.
L. Koka
L. Ziway
L. Abijata
L. Langano
L. Shalla
L. Chifu
L. Awassa
Bilate R.
L. Abaya
L. Chamo
N
60 km
Meki
Meki River
Abura
8°00'
Lake Ziway
Katar River
Ziway
Bulbula River
Bird sampling spot
39°00'

Fig. 1

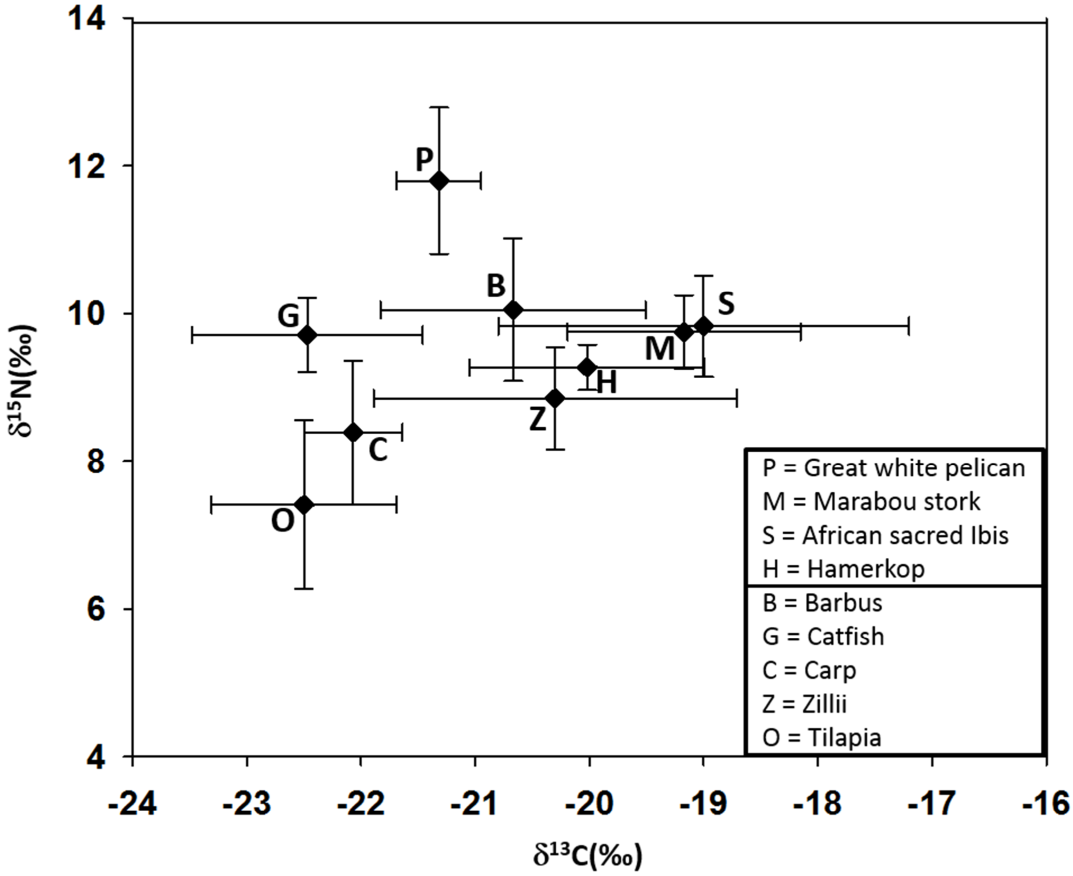

14
12
10
8
6
4
δ15N(‰)
-24
-23
-22
-21
-20
-19
-18
-17
-16
δ13C(‰)
P
B
G
S
M
H
Z
C
O
P = Great white pelican
M = Marabou stork
S = African sacred Ibis
H = Hamerkop
B = Barbus
G = Catfish
C = Carp
Z = Zillii
O = Tilapia

Fig. 2

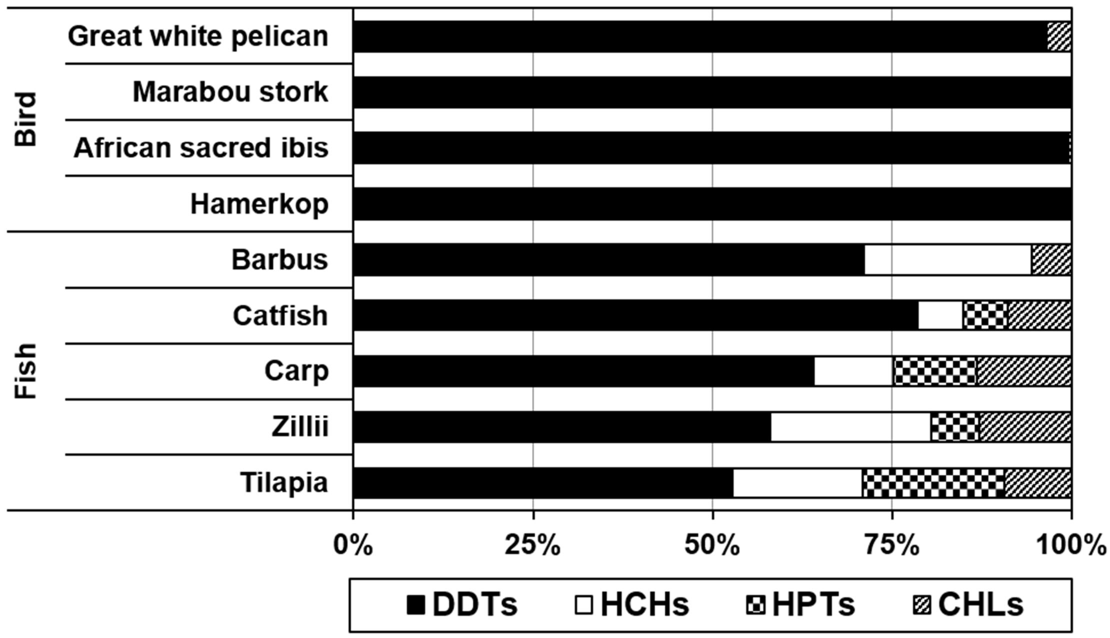

Bird
Great white pelican
Marabou stork
African sacred ibis
Hamerkop
Fish
Barbus
Catfish
Carp
Zillii
Tilapia
0%
25%
50%
75%
100%
DDTs
HCHs
HPTs
CHLs

Fig. 3

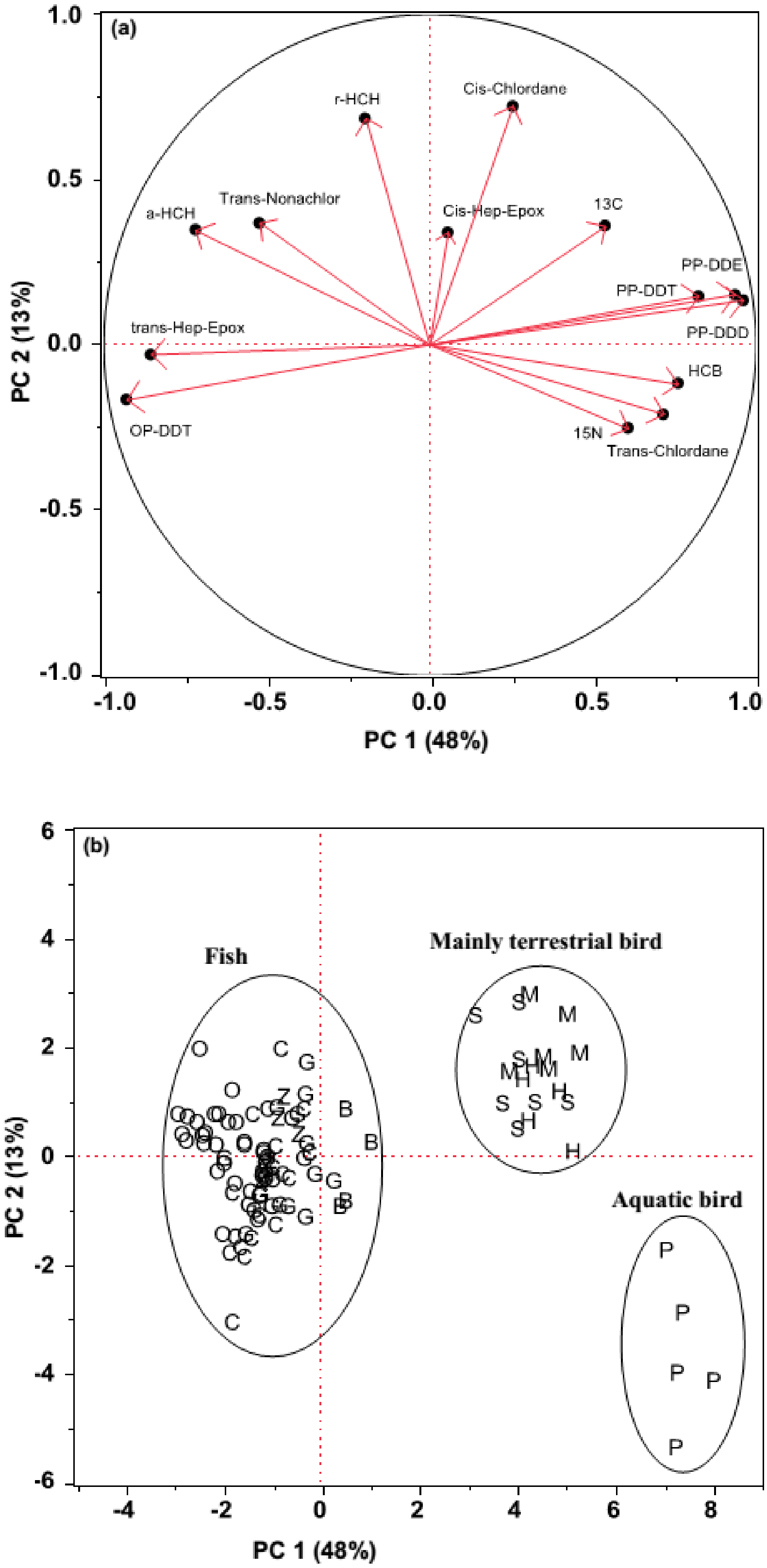

(a)
r-HCH
Cis-Chlordane
Trans-Nonachlor
a-HCH
Cis-Hep-Epox
13C
PP-DDE
PP-DDT
PP-DDD
trans-Hep-Epox
HCB
OP-DDT
15N
Trans-Chlordane
PC 2 (13%)
PC 1 (48%)
(b)
Fish
Mainly terrestrial bird
Aquatic bird
PC 2 (13%)
PC 1 (48%)

Fig. 4

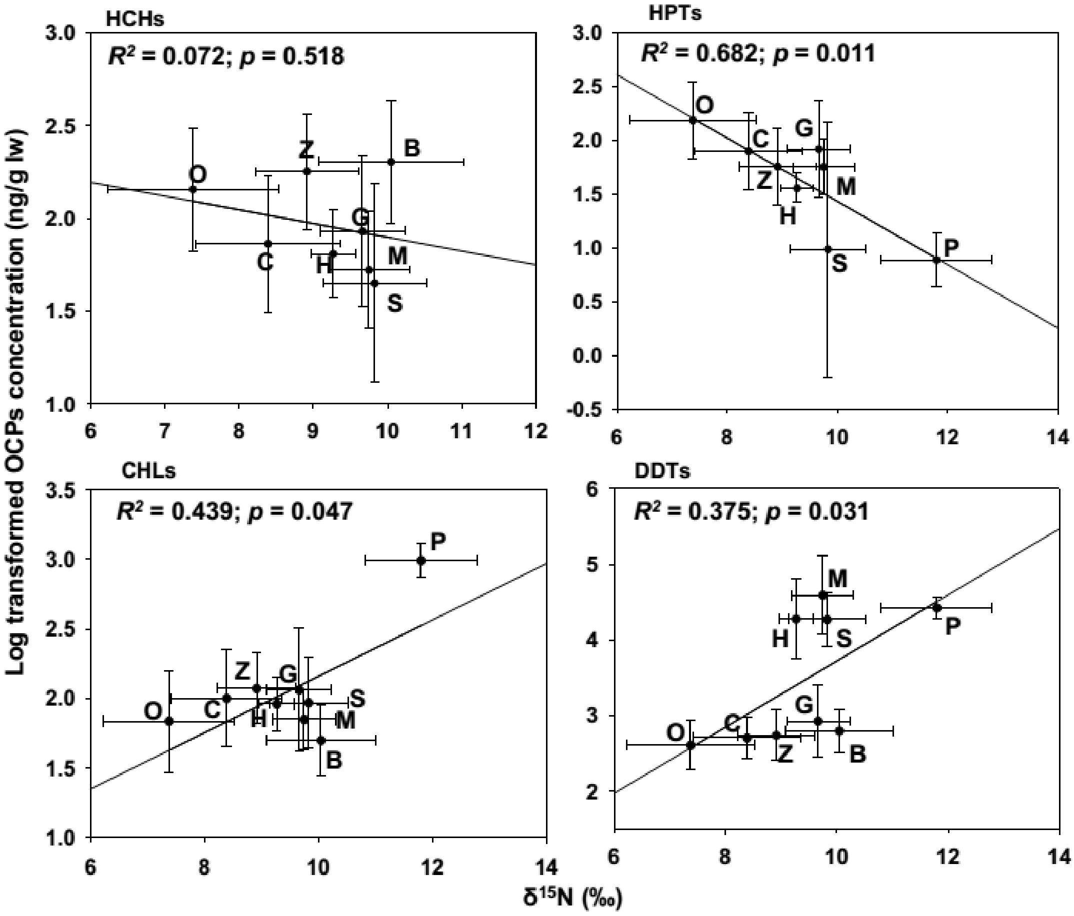

HCHs
R^2 = 0.072; p = 0.518
HPTs
R^2 = 0.682; p = 0.011
CHLs
R^2 = 0.439; p = 0.047
DDTs
R^2 = 0.375; p = 0.031
Log transformed OCPs concentration (ng/g lw)
$\delta^{15}N$ (‰)

Fig. 5