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Contaminants of legacy and emerging concern in terrestrial passerines from a nature reserve in South China: Residue levels and inter-species differences in the accumulation

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ABSTRACT

Knowledge is limited about the bioaccumulation of persistent halogenated compounds (PHCs) in terrestrial wildlife. Several PHCs, including dichlorodiphenyltrichloroethane (DDT) and its metabolites (designated as DDTs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), decabromodiphenylethane (DBDPE) and 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), and stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) were analyzed in the muscle of four terrestrial passerines, *Parus major*, *Copsychus saularis*, *Pycnonotus sinensis* and *Pycnonotus jocosus*, from a nature reserve in South China. *P. major* had the highest PHC concentrations, with median values of 1060, 401, 92, 25 and 0.3 ng/g lipid weight for DDTs, PCBs, PBDEs, DBDPE and BTBPE, respectively. Fractions of DDT in *P. jocosus* and PCBs 153, 118 and 180 in *C. saularis* were higher compared with the other species. The inter-species differences in PHC concentrations and profiles could be attributed to the differences in trophic level, diet, living habits and metabolic capacity among the birds.

1. Introduction

Persistent halogenated compounds (PHCs), including dichlorodiphenyltrichloroethane (DDT) and its metabolites chlorodiphenyldichloroethylene (DDE) and dichlorodiphenyl dichloroethane (DDD) (the sum of DDT, DDE, and DDD is designated as DDTs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), are well-known for their persistence, bioaccumulation, long-range transport potential and toxicity (Lohmann et al., 2007; Kelly et al., 2007). Because of these characteristics, DDTs, PCBs and components of the Penta-BDE and Octa-BDE mixtures have been regulated by the Stockholm Convention on Persistent Organic Pollutants (UNEP, 2001, 2010). Deca-BDE has also been phased-out in Europe, and the production and its use in new products in the United States was discontinued in 2013 (USEPA, 2009). In response to PBDE bans or restrictions, some nonregulated brominated flame retardants (BFRs), such as decabromodiphenylethane (DBDPE) and 1, 2-bis (2, 4, 6-tribromophenoxy) ethane (BTBPE), are being used as replacements in some applications (Covaci et al., 2011; de Wit et al., 2010). Given that these alternative BFRs share some physiochemical properties similar to those of PBDEs, one might suspect that they might possibly be bioaccumulated in wildlife and humans, leading to exposure concerns (Covaci et al., 2011; de Wit et al., 2010).

Various studies conducted within the past decade have demonstrated the PHC contamination in South China (Zhang et al., 2002; Lin et al., 2009; Guo et al., 2009; Wu et al., 2010; Mo et al., 2012; Luo et al., 2009; Breivik et al., 2011; Liu et al., 2014; He et al., 2012). Because of the heavily historical use and the newly inputs, DDTs may still pose a threat to wildlife that resident in South China (Guo et al., 2009; Wu et al., 2012). In addition to DDTs contamination, fairly high concentrations of PCBs, PBDEs and some alternative BFRs were reported in wildlife from South China, particularly those from electronic waste (e-waste) recycling sites and electronics manufacturing and assembling centers in this

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region (Wu et al., 2010; Mo et al., 2012; Luo et al., 2009; He et al., 2012). All the bioaccumulation studies were almost exclusively carried out in aquatic ecosystems; studies on these chemicals in terrestrial ecosystems are scarce. Previous studies indicated that bioaccumulation of chemicals with high octanol–water partition coefficient (K_{OW}) and octanol–air partition coefficient (K_{OA}) (log K_{OW} >5 and log K_{OA} >6) may be differed between aquatic and terrestrial animals because of the different ability to absorb and eliminate these chemicals between water-respiring and air-breathing organisms (Kelly et al., 2007). Most of the PHCs fall the K_{OW}/K_{OA} ranges. Therefore, research focusing on accumulation characteristic of PHCs in terrestrial wildlife is essential to better understand the bioaccumulation behavior and impacts of these chemicals. Moreover, few studies have examined the occurrence of the PHCs in wildlife from the sites of ecological concern including nature reserves in this region (Mo et al., 2013). Occurrence of these chemicals in wildlife from key ecosystems is of concern given that most of the PHCs had shown toxicities to organisms (Hellou et al., 2013; George et al., 1988; Darnerud, 2003).

Birds, including terrestrial passerines, have been widely used to study environmental contamination and to evaluate the health of certain ecosystems (Furness, 1993). A few of these species spend their entire adult life in relatively small home ranges, territories and foraging areas. Additionally, they are widespread, sensitive to environmental changes and easily sampled. They are therefore particularly suitable for monitoring local contamination and for assessing the potential effects, often cumulative and non-linear, of many environmental contaminants acting simultaneously (Dauwe et al., 2003; Van den Steen et al., 2009).

The Shimentai National Nature Reserve (SNNR) is the largest nature reserve in South China. It is located in the northern part of the Guangdong Province and is close to some e-waste recycling sites and electronics manufacturing and assembling centers (Fig. 1). The SNNR is home to various kinds of vertebrates including 208 bird species and 68 other vertebrate species, some of which are rare or endangered (Kadoorie Farm and Botanic Garden, 2003). To date, few study have addressed the question of whether the SNNR wildlife have accumulated the PHCs and potentially been affected by these chemicals. The objective of the present study was to examine PHC residue levels and profiles in the muscle tissue of four terrestrial passerine bird species from the SNNR. Nitrogen and carbon stable isotopes in these birds were also analyzed to assess the influences of trophic level and diet on the bioaccumulation of PHCs.

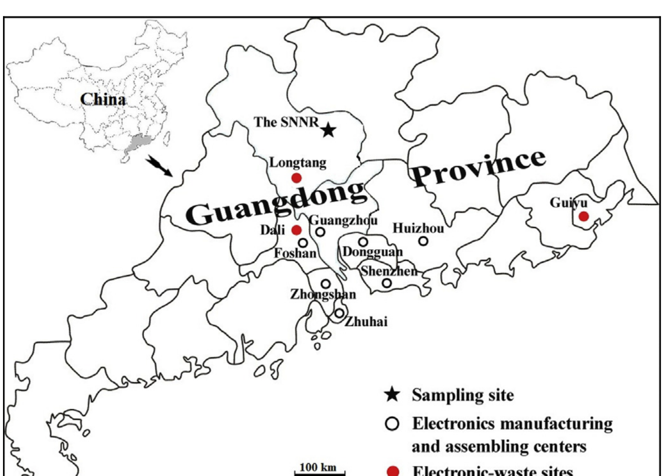

Fig. 1. Map of sampling site. SNNR = Shimentai National Nature Reserve.

2. Materials and methods

2.1. Sample collection

A total of 36 adult (fully grown) terrestrial passerine birds, including the great tit (*Parus major*) (GT) ($n = 18$), the oriental magpie-robin (*Copsychus saularis*) (OMR) ($n = 7$), the light-vented bulbul (*Pycnonotus sinensis*) (LVB) ($n = 5$) and the red-whiskered bulbul (*Pycnonotus jocosus*) (RWB) ($n = 6$), were collected from the SNNR between June 2012 and October 2013. The four bird species are all sedentary species. GT and OMR are insectivorous (Chen et al., 2012; Viney et al., 1994), while LVB and RWB are omnivorous (Peng et al., 2008). The birds were caught by plastic bird netting, approved by the Forestry Bureau of Guangdong Province, China. These birds were euthanized with N_2 and the pectoral muscle from each bird was excised. The muscle samples were stored at −20 °C until chemical analysis.

2.2. Sample extraction, cleanup, and analysis

The methods for sample extraction, cleanup and analysis were similar to those described elsewhere (Mo et al., 2012, 2013). Briefly, approximately 2–6 g of muscle tissue was homogenized and spiked with surrogate standards (CBs 30, 65 and 204 for DDTs and PCBs; $^{13}C_{12}$-BDE 209 and BDEs 77, 181, and 205 for PBDEs, BTBPE and DBDPE). The samples were then mixed with ashed anhydrous sodium sulfate and extracted in a Soxhlet apparatus for 48 h using 50% acetone in hexane. The extract was concentrated to 10 mL, and an aliquot of 1 mL was used for the determination of lipid content by gravimetry. Another aliquot of the extract was concentrated to 2–3 mL, and was then subjected to gel permeation chromatography (GPC) for lipid removal. The GPC fraction containing the target compounds was concentrated to 1–2 mL and purified by passage through a silica gel packed column (10-mm i.d.) which containing neutral activated silica (8 cm) and 40% sulfuric acid silica gel (8 cm). The final extracts were concentrated to near dryness under a gentle stream of purified nitrogen, and reconstituted in 50 μL of *iso*-octane. Known amounts of internal standards (CBs 24, 82 and 198 for DDTs and PCBs; BDEs 118 and 128 for PBDEs, BTBPE and DBDPE) were added to all extracts prior to instrumental analysis.

The extracts were injected into an Agilent 6890 gas chromatograph (GC) coupled to an Agilent series 5975B mass spectrometer (MS) for the determination of DDTs and PCBs. The MS was operated in an electron impact, selected ion monitoring mode (SIM). A DB-5 MS capillary column (60 m length, 250-μm i.d., 0.25-μm film thickness; J&W Scientific) was used for the separation of individual DDT or PCB isomers/congeners. The measurement of tri-to hepta-BDE congeners (BDEs 28, 47, 66, 85, 100, 99, 153, 154 and 183) was accomplished by use of an Agilent 6890 GC–5975 MS in an electron capture negative ionization (ECNI) mode. A DB-XLB capillary column (30 m length, 250-μm i.d., 0.25-μm film thickness; J&W Scientific) was used for the separation of congeners. The analysis of octa-to deca-BDE congeners (BDEs 196, 197, 202, 203, 206, 207, 208 and 209), DBDPE and BTBPE was performed using a Shimadzu Model QP2010 GC–MS using ENCI in the SIM mode. A DB-5HT capillary column (15 m length, 250-μm i.d., 0.1-μm film thickness; J&W Scientific) was used for separation. Details of the GC conditions and monitored ions have been described elsewhere (Mo et al., 2012, 2013).

2.3. Stable isotopic analysis

Nitrogen and carbon stable isotopes of the samples were analyzed using a previously described method (Wu et al., 2009). Briefly, subsamples of the muscle were freeze-dried, ground with a mortar and pestle and weighed (~1 mg) in tin capsules. Nitrogen and carbon stable isotopes ratios ($\delta^{15}N$ and $\delta^{13}C$) were analyzed using an isotope ratio mass spectrometer (Thermo Finigan Delta Plus XL) coupled to an elemental analyzer (Flash EA 112 series). The analytical precisions were ±0.5‰ for $\delta^{15}N$ and ±0.2‰ for $\delta^{13}C$. The results were expressed in δ notation as parts per mil (‰) according to the following equation:

$$\delta X_{sample} = \left[\left(R_{sample} / R_{reference} - 1\right)\right] \times 1000 \quad (1)$$

where X is ^{15}N or ^{13}C and R is the corresponding $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratio. References correspond to ammonium sulphate for $\delta^{15}N$ and carbon black for $\delta^{13}C$.

2.4. Quality assurance and control

Quality assurance was performed by the surrogate standards spiking and regular analysis of procedural blanks, spiking blanks, and blind triplicate samples. A procedural blank was processed in each batch of 12 samples. Traces of BDEs 47, 206, 207 and 209, CBs 28/31, 118, 128, 153 and 180/193 and *p,p′*-DDE were detected in the procedural blanks (1.4–4.9, 0.3–3.9, and 15–19 ng/mL for PBDEs, PCBs, and DDTs, respectively), and the final reported concentrations were blank-corrected accordingly. Recoveries of the target compounds through the extraction, clean-up and fraction steps were checked by spiking known concentrations of BDEs 77, 181 and 205, ^{13}C-BDE 209, and CBs 30, 65 and 204 as surrogates in the samples, and spiking known amounts of *p,p′*-DDT, DDE and DDD, 20 major PCB congeners, and 10 major PBDE congeners in solutions which were run in parallel with the samples. The mean recoveries were 109% ± 5% (mean ± standard error) for BDE 77, 77% ± 2% for BDE 181, 83% ± 3% for BDE 205; 73% ± 5% for ^{13}C-BDE 209, 81% ± 2% for CB 30, 101% ± 2% for CB 65, and 92% ± 2% for CB 204. The mean recoveries of spiked PBDEs, PCBs, and DDTs in solutions ranged from 96%–103%, 81%–118%, and 88%–105%, respectively. The reported concentrations were not corrected by the recoveries. Muscle triplicates were analyzed, and the relative standard deviations (RSD) of the target compounds in the triplicate samples were less than 15%. Instrumental quality control was performed by regular injection of solvent blanks and standard solutions. On-column degradations of BDE209 and DDT were checked by daily injection of BDE 209 and DDT standard solutions before sample analysis. Degradation of BDE 209 and DDT on the GC liner was observed, but the degradation extent was <3%.

The method detection limits (MDLs) were estimated as the mean value of target compounds detected in procedure blanks plus 3 times of standard deviations. For the compounds undetected in the procedural blanks, the MDLs were estimated as a signal-to-noise ratio (S/N ratio) of 10. Based on an average of 0.03 g lipid in the muscle samples, the MDLs for DDTs, PCBs, and PBDEs ranged from 0.01–0.9, 0.1–3.8, and 0.2–0.93 ng/g lipid weight (lw), respectively. MDLs for DBDPE and BTBPE were 1.25 and 0.01 ng/g lw, respectively.

2.5. Data analysis

∑DDTs, ∑PCBs, ∑PBDEs, and ∑ABFRs are defined as the sum of *p,p′*-DDE, DDD, and DDT, the sum of 63 PCB congeners, the sum of 15 BDE congeners, and the sum of DBDPE and BTBPE, respectively. For samples with concentrations below the MDLs, half of the MDLs were used to calculate the mean concentrations. The concentration data in certain species were tested for normality using the Kolmogorov–Smirnov one-sample test with Lilifor's transformation. The data were found to be in violation of this assumption and therefore logarithmic transformations were employed. To evaluate the possible differences in contaminant concentrations between males and females of certain species and among the four bird species, two-way analysis of variance (ANOVA) was used. Possible differences in PBDE or PCB patterns among the four bird species investigated were assessed using principal components analysis (PCA). The probability level determining significance was set at $p < 0.05$.

3. Results and discussion

3.1. PHC levels

The concentrations of ∑DDTs in muscle of GT, OMR, LVB and RWB ranged from 220 to 5270, from 139 to 571, from 38 to 239, and from 30 to 358 ng/g lw, respectively (Table 1). To date, limited information on ∑DDTs in muscle of these four passerine species is available in the literature (summarized in Table 2). Most of the DDT data in GT is from eggs and the concentration in the muscle can be only found for nestlings. Dauwe et al. reported *p,p′*-DDE in the muscle of GT nestlings collected from four sites in Antwerp, Belgium, with geometric mean concentrations of 205, 159, 106, and163 ng/g lw (Dauwe et al., 2003). They are 3–7 times lower than that estimated in the present study (760 ng/g lw). The arithmetic mean value of ∑DDTs concentration in the muscle of the current GT (1770 ng/g lw) was also 2–5 times greater than those reported in GT nestlings sampled from two locations in Poland, and were approximately 30 times greater than that detected in GT nestlings from Odsmal, Sweden (Nyholm et al., 1995). ∑DDTs concentrations have also been reported in the muscle of adult LVB collected from rural, suburban, urban, and e-waste recycling sites in the Guangdong Province (Sun et al., 2014). The median ∑DDTs concentration in the muscle of the current LVB (128 ng/g lw) was 2 times greater than that reported in the species from a rural site and two suburban sites, but was comparable to or slightly lower than those reported in the species from an urban site and an e-waste recycling site. The results suggest that GT and LVB inhabiting the SNNR may be heavily contaminated by DDTs, although the accumulation of ∑DDTs in GT may be different between adults and nestlings. The SNNR is located in the tropical region of South China where pesticides are heavily used. Previous studies suggest the heavy contamination of DDTs in this region is due to the high rate of historical use, only a recent production ban, and new input sources of DDT (Zhang et al., 2002; Lin et al., 2009). The bioaccumulation of DDTs, especially DDE, is suggested to be linked to the population declines for several bird species because of their adverse affects on avian reproduction (Hellou et al., 2013). For the current birds, the highest DDE level (3.16 μg/g lw, 92 ng/g wet weight) are 1–3 orders of magnitude lower than those estimated to cause adverse affects, e.g., 20–1000 μg/g lw in the liver of cormorants (*Phalacrocorax carbo sinensis*) (Tanabe et al., 1998), 120 μg/g lw in the white-tailed sea eagle (*Haliaeetus albicilla*) eggs (Helander et al., 2002), and 1000 ng/g wet weight in eggs of the Little Egret (*Egretta garzetta*) and the Black-crowned Night Heron (*Nycticorax nycticorax*) (Connell et al., 2003). Thus, exposure to DDE may be unlikely to cause reproductive effects on the four bird species residing in the SNNR.

∑PCBs and ∑PBDEs concentrations in the muscle of the four passerine birds are presented in Table 1. Limited data can be available for PCB and PBDE concentrations in the muscle of the current four passerine birds (summarized in Table 2). ∑PCBs concentration in muscle of GT of this report (geometric mean, 454 ng/g lw; arithmetic mean, 570 ng/g lw) was higher than those examined in GT nestlings from Stanslawice, Poland and Odsmmal, Sweden (Nyholm et al., 1995), and was comparable to those detected in GT nestlings from a site near crematory and waste incinerator in Belgium (Dauwe et al., 2003) and from Klucze, Poland (Nyholm et al., 1995), but was lower than those estimated in GT nestlings from three PCB contaminated sites in Antwerp, Belgium (Dauwe

Table 1
The medians and ranges of lipid content (%) and concentrations (ng/g lipid weight) of persistent halogenated compounds in the muscle of four passerine birds collected from the Shimentai Nature Reserve (SNNR) in South China.

Species[a]	GT	OMR	LVB	RWB
n	18	7	5	6
Lipid	2.8 (1.7–4.7)	3.4 (2.2–4.9)	3.6 (2.7–4.2)	3.4 (2.6–4.3)
DDTs				
p, p′-DDE	711 (150–3160)	268 (101–482)	121 (34–228)	35 (28–331)
p, p′-DDD	233 (44–2404)	8 (3.5–62)	2.0 (0.1–3.4)	1.4 (0.01–4.1)
p, p′-DDT	42 (7.6–337)	23 (13–28)	4.4 (3.5–22)	13 (4–22)
ΣDDTs[b]	1060^{A} (220–5270)	291^{B} (139–571)	128 ^{BC} (38–239)	47^{C} (30–358)
PCBs[c]				
CB 99	16 (7.7–74)	6.2 (2.0–14)	3.7 (1.3–7.6)	1.5 (1.0–7.8)
CB 105	17 (7.1–60)	2.7 (0.7–5.0)	5.0 (1.5–8.5)	2.0 (1.4–5.8)
CB 118	39 (17–158)	7.0 (2.3–20)	11 (3.2–18)	4.0 (3.4–14)
CB 128	9.2 (3.2–35)	7.0 (2.1–10)	3.1 (1.8–9.7)	2.0 (1.6–5.0)
CB 138	40 (18–196)	24 (7.0–43)	8.4 (3.6–15)	4.6 (3.2–15)
CB 153/132	42 (19–216)	34 (12–72)	13 (4.6–27)	6.6 (4.5–22)
CB 180/193	24 (8.6–159)	17 (7.9–41)	7.4 (3.6–13)	4.0 (2.6–11)
ΣPCBs[d]	401^{A} (203–1770)	240^{B} (72–380)	108^{C} (45–183)	53^{C} (48–158)
PBDEs				
BDE 47	18 (6.2–69)	22 (1.7–45)	5.1 (3.0–5.6)	4.1 (1.8–7.8)
BDE 99	13 (6.7–63)	7.9 (2.3–135)	2.1 (0.7–5.3)	2.4 (1.2–29)
BDE 100	4.7 (2.5–20)	3.8 (1.6–68)	1.3 (0.3–1.9)	1.0 (0.6–5.2)
BDE 153	7.0 (1.5–31)	10 (3.3–54)	1.6 (0.9–3.0)	1.2 (0.6–3.2)
BDE 154	4.5 (1.7–17)	2.8 (2.0–29)	0.9 (0.2–1.1)	0.6 (0.5–1.6)
BDE 207	2.5 (0.2–16)	1.3 (0.8–26)	3.8 (0.7–7.7)	2.9 (1.2–5.0)
BDE 209	16 (2.3–57)	7.0 (2.0–226)	5.1 (1.9–7.0)	5.1 (4.1–30)
ΣPBDEs[e]	92^{A} (35–243)	81^{A} (29–340)	27^{B} (12–45)	30^{B} (11–51)
Alternative brominated flame retardants				
DBDPE	25 (14–125)	11 (2.7–82)	11 (4.5–19)	12 (3.6–26)
BTBPE	0.3 (0.1–1.2)	0.1 (0.02–0.3)	0.1 (0.01–0.2)	0.1 (0.02–0.1)
ΣABFRs[f]	25^{A} (4.6–125)	11 ^{AB} (2.8–83)	11^{B} (4.6–19)	12 ^{AB} (3.7–26)

Values sharing a letter represent the means do not differ significantly ($p > 0.05$).
[a] GT, the great tit (*Parus major*); OMR, the oriental magpie-robin (*Copsychus saularis*); LVB, the light-vented bulbul (*Pycnonotus sinensis*); RWB, the red-whiskered bulbul (*Pycnonotus jocosus*).
[b] Sum concentrations of *p,p′*-DDE, DDD, and DDT.
[c] Only the seven indicator PCBs are listed.
[d] Sum concentrations of 63 PCB congeners investigated.
[e] Sum concentrations of the 15 investigated PBDE congeners.
[f] Sum concentrations of DBDPE and BTBPE.

Table 2
Comparison of the concentrations (medians and ranges; ng/g lipid weight) of ∑DDTs, ∑PCBs, ∑PBDEs, and DBDPE in the muscle tissue of the great tit (GT), the oriental magpie-robin (OMR) and the light-vented bulbul (LVB) from the Shimentai National Nature Reserve with those reported in the literature.

Species	Country	Site description/Sample source	∑DDTs	∑PCBs	∑PBDEs	DBDPE	Reference
GT	China	SNNR, a nature reserve	1060 (220–5270) 28 (8.0–157)[a]	401 (203–1770) 12 (5.1–43)[a]	92 (35–243) 3.2 (1.4–5.9)[a]	25 (14–125) 0.7 (0.08–3.9)[a]	The present study
	Belgium	Fort 4, near printing works	205 (87–509)[b,c]	938 (439–2768)[b]	/		Dauwe et al., 2003
	Belgium	Fort 5, near printing works	159 (71–679)[b,c]	586 (283–1264)[b]	/		Dauwe et al., 2003
	Belgium	Fort 7, near crematory and waste incinerator	106 (68–216)[b,c]	461 (245–797)[b]	/		Dauwe et al., 2003
	Belgium	Fort 8, non-ferrous smelter	163 (61–917)[b,c]	1060 (310–3307)[c]	/		Dauwe et al., 2003
	Belgium	Fort 4, near printing works	5.87–8.1[a]	38–51[a]	0.82–1.23[a]		Dauwe et al., 2006
	Belgium	Fort 5, near printing works	5.59–7.15[a]	31–38[a]	0.69–0.92[a]		Dauwe et al., 2006
	Poland	Stanislawice, heavy metal polluted forest	350[d]	190[d]	/		Nyholm et al., 1995
	Poland	Klucze, heavy metal polluted forest	790[d]	590[d]	/		Nyholm et al., 1995
	Sweden	Odsmal, a backgroud site	60[d]	470[d]	/		Nyholm et al., 1995
OMR	China	SNNR, a nature reserve	291 (139–571)	240 (72–380)	81 (29–340)	11 (2.7–82)	The present study
	China	JX, a suburban site	/	/	120 (120–130)	8.2 (7.2–9.1)	Sun et al., 2012
	China	ZQ, a suburban site	/	/	240 (130–540)	15 (5.3–27)	Sun et al., 2012
	China	GZ, an urban site	/	/	840 (300–2600)	37 (13–92)	Sun et al., 2012
	China	QY, an electronic-waste recycling site	/	/	5200 (870–15,000)	25 (3.4–45)	Sun et al., 2012
LVB	China	SNNR, a nature reserve	128 (38–239)	108 (45–183)	27 (12–45)	11 (4.5–19)	The present study
	China	MM, a rural site	67 (53–82)	160 (150–180)	66 (53–69)	10 (7.5–17)	Sun et al., 2012, 2014
	China	ZQ, a suburban site	90 (39–1600)	370 (180–520)	97 (49–160)	8.5 (2.5–20)	Sun et al., 2012, 2014
	China	HS, a suburban site	69 (12–89)	260 (100–870)	170 (52–470)	22 (5.7–4.5)	Sun et al., 2012, 2014
	China	GZ, an urban site	120 (88–170)	250 (220–460)	330 (260–430)	51 (40–80)	Sun et al., 2012, 2014
	China	QY, an electronic-waste recycling site	170 (46–600)	7300 (3200–73,000)	1000 (630–4700)	12 (3.6–26)	Sun et al., 2012, 2014

[a] ng/g wet weight.
[b] Geometric mean concentrations.
[c] Only DDE.
[d] Arithmetic mean concentrations.

et al., 2003). Dauwe et al. (2006) reported ∑PCBs concentrations in GT nestlings from two PCB contaminated sites in Belgium, with median concentrations of 38–51 and 31–38 ng/g ww. These values were 3–4 times higher than that (12 ng/g ww) detected in the current GT. ∑PCBs concentrations were reported in the muscle of LVB from several sites in the Guangdong Province (Sun et al., 2014). The median ∑PCBs concentration detected in the current LVB (108 ng/g lw) was slightly lower than that examined in the species from a rural site, but was 2–3 times lower than those from two suburban sites and one urban site, 70 times lower than that detected in the species from an e-waste recycling site. ∑PBDEs concentrations were also reported in the muscle of GT, OMR and LVB (Table 2). The median concentration detected in the current GT (3.2 ng/g ww) was 3–4 times greater than those reported from two sites in Belgium (0.82–1.23 and 0.69–0.92 ng/g ww) (Dauwe et al., 2006). However, the median ∑PBDEs concentration estimated in the current OMR (81 ng/g lw) was 1.5–3 times lower than those detected in the species from two suburban sites, 10 times lower than that reported in the species from an urban site, and 60 times lower than that examined in the species from an e-waste site, in the Guangdong Province (Sun et al., 2012). The median ∑PBDEs concentration in the current LVB (27 ng/g lw) was also 2 times lower than that reported in the species from a rural site, 4–6 times lower than those detected in the species from two suburban sites, 11 times lower than that examined in the species from a urban site, and 37 times lower than that examined in the species from a e-waste recycling site, in the Guangdong Province (Sun et al., 2012). Taken together, ∑PCBs and ∑PBDEs concentrations detected in the current birds were at the lower end of the ranges reported in related species, although some e-waste recycling sites and electronics manufacturing and assembling centers are located in the vicinity of the SNNR (Fig. 1). The SNNR is located in a rural site and no known point-sources of PCB/PBDE exist in this zone. Most of the previously investigated passerine species were from suburban, urban, or PCB/PBDE contaminated sites, which may account for the lower PCB/PBDE residues in birds from the SNNR compared to those previously reported in related species. It was demonstrated that concentrations of industrial chemicals including PCBs and PBDEs in birds from urban sites were higher than those from rural sites (Van den Steen et al., 2009; Sun et al., 2012, 2014).

Despite the fact that PBDEs were the most predominant BFRs in the samples analyzed, DBDPE and BTBPE were detectable in all specimens examined (Table 1). DBDPE concentrations in GT, OMR, LVB, and RWB ranged from 14 to 125, 2.7–82, 4.5–19, and 3.6–26 ng/g lw, respectively. Compared with DBDPE, BTBPE concentrations were lower, with values of 0.1–1.2, 0.02–0.3, 0.01–0.2, and 0.02–0.1 ng/g lw in GT, OMR, LVB, and RWB, respectively. This may indicate that the use of BTBPE is lower than other alternative BFRs such as DBDPE in electrical and electronic products, or that it doesn't accumulate as readily in these species. Data on DBDPE in passerines were scarce (summarized in Table 2), and no data can be available for BTBPE. Median DBDPE level (11 ng/g lw) in the current OMR was comparable to those detected in the same species collected from two suburban sites in Guangdong Province, but was lower than those reported in the species from an urban site and an e-waste recycling site in this region (Sun et al., 2012). The median value (11 ng/g lw) in the current LVB was comparable to those estimated in the species from a rural sites and a suburban site in the Guangdong Province (Sun et al., 2012), but was 2–5 times lower than those detected in the species from another suburban site, an urban site, and an e-waste site in the Guangdong Province (Sun et al., 2012). The high detection frequency and elevated concentrations of DBDPE in birds from the SNNR suggest the widespread occurrences of this compound in the local environment. DBDPE and BTBPE have been reported as alternatives for PBDEs in some applications such as electrical equipments, and DBDPE is the second highest used additive BFR in China after PBDEs (Covaci et al., 2011). A vast number of electronics manufacturing and assembly centers is located approximately 200–400 km south of the SNNR (Fig. 1). DPDPE and BTBPE released from these centers could have been transported to the SNNR where they could have accumulated in the current passerines. Recently, many factories and plants including electronics manufacturing in the Guangdong Province have been relocated to the places (including the sampling area) where the rental rates are lower and labor cost is cheaper (Liu et al., 2014). The shift in industry in the Guangdong Province may also have contributed to the DBDPE and BTBPE exposure of the current birds. With the

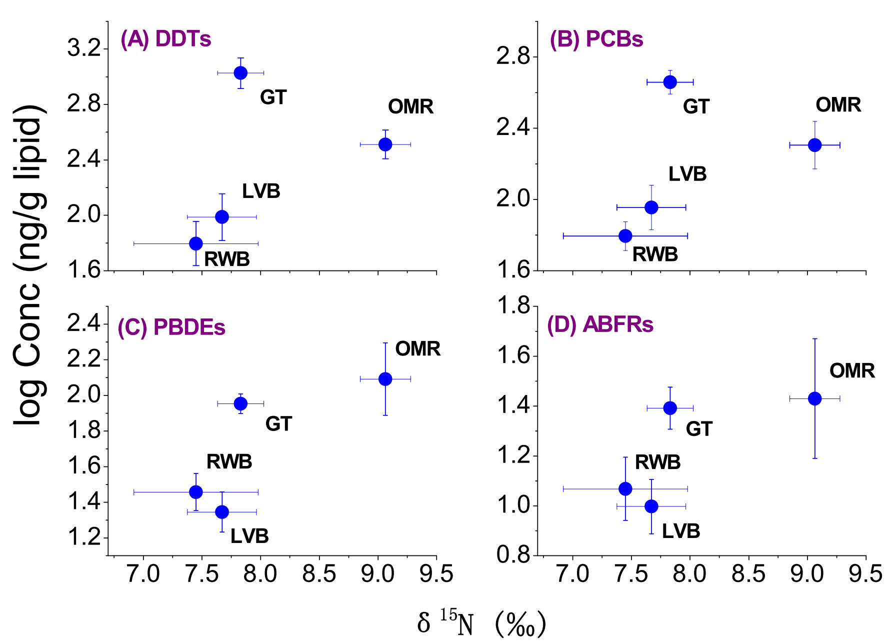

Fig. 2. Relationship between the $\delta^{15}N$ values and the concentrations of (A) DDTs, (B) PCBs, (C) PBDEs, and (D) ABFRs in the muscle of four passerines from the SNNR. GT = the great tit, OMR = the oriental magpie-robin, LVB = the light-vented bulbul, RWB = the red-whiskered bulbul.

increasing demand for alternatives for PBDEs, it is likely that environmental levels of DBDPE and other alternative BFRs will be markedly increased in the near future. The environmental fate and potential toxic effects of these emerging pollutants should be further investigated.

3.2. Concentration difference among species

There was no significant difference in PHC concentrations between females ($n = 9$) and males ($n = 9$) of the great tit (two-way ANOVAs, $p > 0.05$). When concentrations were compared among bird species, differences were found in levels of ∑DDTs, ∑PCBs, ∑PBDEs, and ∑ABFRs, with the highest median concentration in GT and the lowest in RWB (Table 1). Statistic analysis also revealed that significantly higher concentrations were observed in GT compared to OMR, LVB and RWB for ∑DDTs and ∑PCBs, and compared to LVB and RWB for ∑PBDEs ($p < 0.05$; Table 1). Further, significantly higher concentrations were found in OMR compared to RWB for ∑DDTs ($p < 0.05$; Table 1), and compared to LVB and RWB for ∑PCBs and ∑PBDEs ($p < 0.05$; Table 1). Finally, significantly higher concentrations were measured for ∑ABFRs in GT in comparison to RWB ($p < 0.05$; Table 1).

Difference in dietary habits of the bird species investigated may contribute to the observed interspecies variances in PHC residues. GT and OMR are insectivorous, preying primarily on insects and arthropods (Chen et al., 2012; Viney et al., 1994); while LVB and RWB are omnivorous (Peng et al., 2008). The diet of LVB and RWB includes mainly plant food such as berries, soft fruits, tender leaves, and vegetables (Peng et al., 2008). A diet investigation revealed that the volume rate of plant food reached 75% and 76% for LVB and RWB, respectively (Peng et al., 2008). Nitrogen stable isotope data further revealed that the insectivorous GT ($\delta^{15}N = 7.83 \pm 0.20$, mean‰ ± SE) and OMR ($\delta^{15}N = 9.06 \pm 0.21$) generally occupied higher trophic levels in the food chain compared to the omnivorous LVB ($\delta^{15}N = 7.67 \pm 0.29$) and RWB ($\delta^{15}N = 7.45 \pm 0.53$) (Fig. S1 of the Supplementary Matereials, "S" indicates figures in the Supplemental Material afterwards), and are therefore at greater risk of accumulating PHCs than the later two bird species. Studies on the influence of feeding habits of birds on their pollutant burdens also reported an association between PHC residues and feeding habits, with higher levels in the insectivorous relative to the omnivorous and the phytophagous (Ramesh et al., 1992). Relationship between PHC concentrations and $\delta^{15}N$ values showed that PHC concentrations were generally increased with the increasing $\delta^{15}N$ values (Fig. 2), suggesting biomagnification of PHHs in the current birds. However, the levels of DDTs and PCBs were observed to be higher in GT compared with OMR, although the $\delta^{15}N$ values of GT are smaller than OMR (Fig. 2). The avid characteristic in diet of GT may lead to their higher PCB and DDT exposure compared to OMR. It was reported that GT can consume more than 90% of their body weight each day (Yang and Ru, 2000). In contrast, OMR contained ∑PBDEs and ∑ABFRs at similar levels to GT (Fig. 2). OMR often lives close to human habitations (Viney et al., 1994), where PBDEs and alternative BFRs are present at high levels, possibly resulting in higher burdens of these chemicals in their body compared to the other species.

3.3. Contaminant patterns

Overall, ∑DDTs were predominant among the PHCs analyzed, followed by ∑PCBs then ∑PBDEs and ∑ABFRs. ∑DDTs, ∑PCBs, ∑PBDEs, and ∑ABFRs contributed 37%–61%, 31%–40%, 6%–19%, and 3%–9% to the total PHCs, respectively. Contaminant patterns of PHCs including DDTs, PCBs and PBDEs in passerine birds from South China have not been previously reported. However, a similar accumulation pattern with the current study was observed in an aquatic bird, the common kingfisher (*Alcedo atthis*), collected from a rural site in South China (Mo et al., 2013), and may indicate an environmental hot spot of DDT contamination.

For DDTs, *p,p'*-DDE was most frequently detected in the four passerine bird species, constituting more than 68% of ∑DDTs in all species (Fig. S2). Among other DDT compounds, *p,p'*-DDT and *p,p'*-DDD were frequently detected, but were observed at lower fractions than *p,p'*-DDE. However, a relatively high fraction (average of 20%) of *p,p'*-DDT was detected in RWB, suggesting that a recent scattered input of DDT might exist in the sampling area. Several other studies have also indicated an ongoing fresh input of DDT in South China, mostly due to the illegal applications of technical DDT, the use of the pesticide dicofol, and several types of antifouling paints which contain high concentrations of DDT (Zhang et al., 2002; Lin et al., 2009). Concerning DDTs profiles among species, significant difference was observed in the GT, with a higher fraction of *p,p'*-DDD in that species (average of 26%) than those in the other three species (in averages of 1%–6%) (Fig. S2). This difference in DDTs profiles could be related to dietary differences between GT and the other three species as indicated by the carbon stable isotope signatures: GT was

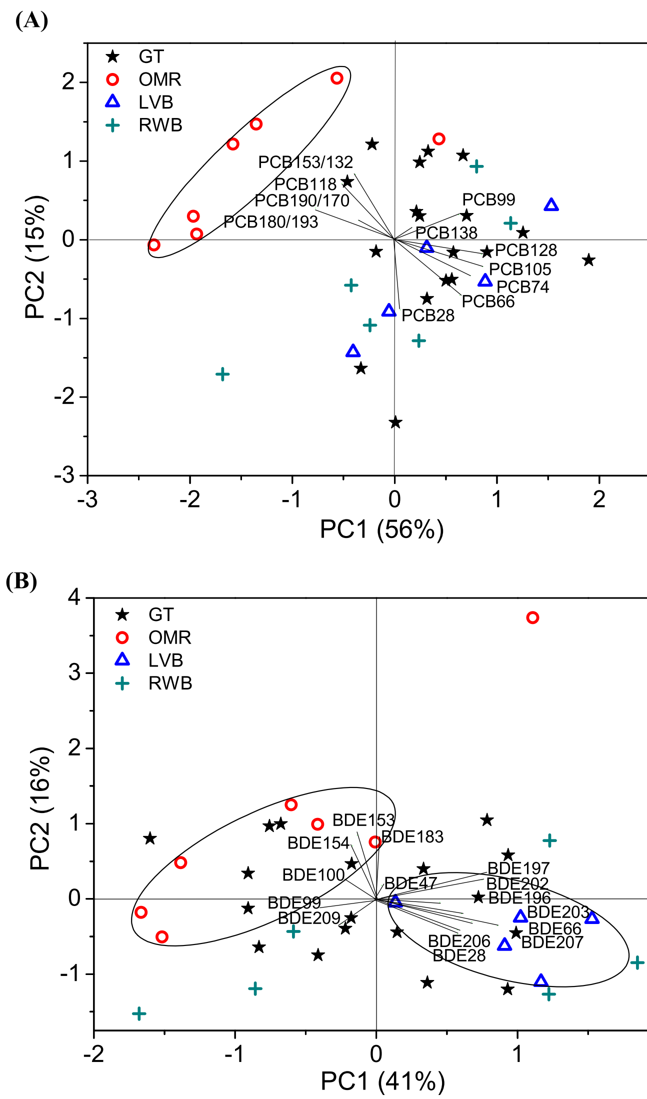

Fig. 3. Biplot from principal component analysis based on the fractional composition of (A) PCB and (B) PBDE congeners. The full line originating from the center of the plot represents the factor loadings and the figure legends represent the factor scores for all birds analyzed. GT = the great tit, OMR = the oriental magpie-robin, LVB = the light-vented bulbul, RWB = the red-whiskered bulbul.

significantly ^{13}C-depleted ($\delta^{13}C = -28.0$‰) compared to the other species (−24.7‰, −26.7‰, and −26.9‰ for OMR, LVB, and RWB, respectively) (Fig. S1). Alternatively, the difference in DDTs profiles could probably be attributed to differences in the metabolic capacity for DDT among the investigated bird species.

For PCBs, congeners with 5–7 chlorines dominated, with CBs 153/132, 138, 118 and 180/193 being the most abundant congeners (Fig. S3). Together, they constituted 35%–42% of the $\sum$PCBs. This pattern resembled many of those reported in diverse bird species worldwide, being due to the large fraction of these congeners in technical PCB mixtures and their high persistence and great bioaccumulation potential (Chen et al., 2009). However, PCA analysis showed that the exact contribution of each congener was different among the four bird species investigated, with higher fractions of CBs 153, 118, and 180/193 in OMR compared to the other three species (Fig. 3A). This difference could be related to the distinctive feeding and living habits of OMR, as indicated by the nitrogen and carbon stable isotope signatures: OMR was significantly ^{13}C- and ^{15}N-enriched compared to the other species investigated (Fig. S1).

The major PBDE congeners in the four passerine bird species were BDEs 209, 47, 99, 207, and 153, collectively contributing 71%–79% to the $\sum$PBDEs (Fig. S4). It is worth noting that 32%–49% BDE congeners in the present birds had 8 or more bromine atoms, which have only been scarcely reported in wildlife to date. The predominance of these higher brominated congeners has also previously reported in terrestrial birds from China environment (Chen et al., 2007; Sun et al., 2012), indicating large use of Deca-BDE mixture (BDE 209 is the major congener) in China, and great bioaccumulation potential of these congeners in terrestrial food webs. When compared the PBDE profiles among the four species, it showed that OMR and LVB showed different patterns from the other species: OMR exhibited a significant higher fraction of BDE 153 and LVB showed higher fractions of BDEs 206 and 207 (Fig. S4). The PCA analysis further revealed the different patterns of the two bird species (Fig. 3B). The different PBDE profile in OMR may be attributed to its distinctive feeding and living habits, as aforementioned. For LVB, the higher fractions of BDEs 206 and 207 may originate from biodegradation of BDE 209. This is further suggested by the depressed PBDE concentrations in the general trend predicted by the $\delta^{15}N$ values (Fig. 2C). Although biodegradation of BDE 209 has not been reported in LVB, previous studies revealed that BDE 209 can be biotransformed via debromination into lower brominated congeners including BDEs 206 and 207 in other terrestrial birds, e.g., European starlings (*Sturnus vulgaris*) and American kestrels (*Falco sparverius*) (Van den steen et al., 2007; Letcher et al., 2014).

To illustrate if there are some differences in the bioaccumulation of PCBs and PBDEs between air-breathing and water-respiring organisms, we compared the PCB and PBDE congener profiles in the current birds with two fish species, mud carp (*Cirrhinus molitorella*) and northern snakehead (*Channa argus*) (Figs. S5 and S6; unpublished data). A greater relative abundance of congeners containing more than eight bromines or 6 chorines, particularly BDE 209 and CBs 153 and 180, were observed in the terrestrial passerines compared to the fish species. The greater fractions of the higher halogenated congeners in the birds compared to the fish species may be due to the biomagnification of these chemicals (normally having a Log K_{OW} of ~4–~8 and Log K_{OA} > 8.2) in the birds which generally does not occur in aquatic organisms, as indicated by the food web magnification models proposed by Kelly et al. (2007).

4. Conclusions

Despite a number of studies investigating PHC contamination in South China, there are limited data for some ecosystems of ecological concern including nature reserves. Furthermore, there are only limited studies that have addressed the occurrence of PHCs in wildlife from terrestrial food webs. Our results demonstrated the overall bioaccumulation of DDTs, PCBs, PBDEs, and two alternative brominated flame retardants in terrestrial passerines residing in the SNNR. Our findings also revealed the influences of trophic level, feeding and living habits and the metabolic capacity of certain species on the bioaccumulation of PHCs in the terrestrial birds. Although several e-waste recycling sites and electronic assembling centers are located near the SNNR, the concentrations of PCBs and PBDEs in the current birds are not very high. It should be keeping monitoring at reasonable scale and frequency to make sure these levels do not increase. Considering the elevated levels of DDTs detected, more sensitive endpoints may be necessary to confirm that there are no health effects of DDTs on the birds and other wildlife of the SNNR.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2015.03.037.

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