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Hexabromocyclododecane in alpine fish from the Tibetan Plateau, China

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ABSTRACT

Hexabromocyclododecanes (HBCDs) has just been listed into Stockholm Convention as a persistent organic pollutant recently. This paper studied the HBCDs in 79 wild fish from high mountain lakes and rivers of the Tibetan Plateau. The $\sum$HBCDs in fish muscles ranged from non detectable levels to 13.7 ng/g lipid weight (lw) (mean value of 2.12 ng/g lw) with a high detection frequency of 65.8%. α-HBCD dominated among the isomers and accounted for 78.2% of the total burden. Concentrations of $\sum$HBCDs in the fish were significantly correlated with the lipid content. A decreasing trend was observed between α-HBCD and trophic level. Positive correlation was also noted between the HBCD levels in fish from lakes and the annual precipitation, and this implied the long-range atmospheric transport of HBCDs to the Tibet Plateau. This was the first work to widely explore HBCDs contamination in the aquatic ecosystems of the Tibetan Plateau.

1. Introduction

Hexabromocyclododecane (HBCDs) is the second highest volume brominated flame retardants used in Europe (Covaci et al., 2006). In China, the estimated domestic production of HBCDs was 18, 000 tons in 2011 (UNEP-Stockholm Covention, 2013a). HBCDs is manly used as an additive brominated flame retardant in the production of building insulation materials, i.e. expanded and extruded polystyrene foams, and upholstery textiles in percentages of 0.8–4%. The compound is also used in furniture, car interiors, electronic and housing of electrical equipment (Alaee et al., 2003). Because of non-covalent bond to the material, HBCDs is susceptible to transfer from the products into all environmental media (Covaci et al., 2006). The Stockholm Convention on Persistent Organic Pollutants at its 6th meeting listed HBCDs as a persistent organic pollutant (POP) for elimination due to its potential toxicity, environmental persistence, bioaccumulative tendencies, as well as long-range transport (UNEP-Stockholm Covention, 2013b). It makes the environmental occurrence of HBCDs to be a globally concerned issue.

Commercial HBCD mixture primarily consists of 3 diastereoisomers, α-, β-, and γ-HBCD with percentage of 10–13, 1–12 and 75–89%, respectively (Law et al., 2005). However, the relative abundance of the three diastereoisomers may change to 78, 13 and 9%, respectively, when the mixture is subject to thermal rearrangement at temperatures above 160 °C (Barontini et al., 2001). Because of the substantial dissimilarities in the structures, the isomers of HBCD (α, β, and γ) show different physicochemical properties, e.g. polarity, dipole moment and solubility. The water solubility of α-, β-, and γ-HBCD is 48.8, 14.7 and 2.1 μg/L, respectively (Covaci et al., 2006). These differing properties make the three diastereoisomers exhibit distinctive environmental behaviors. A number of studies reported that α-HBCD dominated the total HBCD concentration in biota while γ-HBCD dominated in the abiotic (Covaci et al., 2006).

Some POPs can undergo long-range atmosphere transport (LRAT) and be cold trapped in high mountain areas or high latitude regions (Daly and Wania, 2005; Tomy et al., 2009; Vorkamp et al., 2012). The Tibetan Plateau is one of the coldest and most remote regions in the world, with an average altitude over 4000 m above sea level. Sparse human population, minimal to nonexistent industrial activities, and its unique ecological condition, e.g. thin soil and sparse vegetation, make the Tibetan Plateau offer “natural experiments” of exposure to POPs through LRAT. Previous works have confirmed the altitudinal distributions of dioxins, polychlorinated biphenyls (PCBs) in soil and yaks (Pan et al., 2013), cold-trapping or cold condensation of organochlorine pesticides (OCPs) in conifer needles via LRAT (Yang et al., 2008). Since HBCDs has similar physicochemical properties to these of other POPs, it is

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supposed to conform to some transport mechanisms of other POPs in the Tibetan Plateau. However, the studies on the HBCDs in high mountain areas are very limited in the worldwide, especially in China. Up to now, there is no investigation on the distribution, environmental fate, and long range atmospheric transport of HBCDs in the environment of Tibetan Plateau.

Because of the low temperatures and oligotrophic status, the fish inhabiting Tibetan lakes or rivers always show very low growth rate and consequently accumulate higher concentration of pollutants (Kidd et al., 1995). This makes fish be more vulnerable to pollutants and thus become ideal study matrix to reveal the portions of total contaminant load in the Tibetan Plateau ecosystem (Demers et al., 2007). Yang et al. have detected high levels of heavy metals (Yang et al., 2011; Yang et al., 2007), OCPs (Yang et al., 2010, 2007), perfluorinated compounds (Shi et al., 2010), PCBs (Yang et al., 2010) and polybrominated diphenyl ethers (PBDEs) (Yang et al., 2011) in the fish from Tibetan lakes and rivers. However, to our knowledge, no information is available concerning the existence of HBCDs in the Tibetan Plateau. The objective of this work was to explore bioaccumulation, spatial distribution and patterns of HBCDs in fish of the Tibetan Plateau, thus to provide the first estimation of HBCDs contamination in the aquatic ecosystems of the Tibetan Plateau. The results will help us to better understand the source of HBCDs, the factors that influence the bioaccumulations of HBCDs in fish in high mountain lakes and the differences of the potential transport mechanisms of HBCDs from other organic pollutants in the Tibetan Plateau.

2. Materials and methods

2.1. Sample collection

A total of seventy nine fish samples of eight different species (*Oxygymnocypris stewartii*, *Schizopygopsis younghusbandi*, *Schizothorax macropogon*, *Schizothorax o'connori*, *Schizothorax waltoni*, *Gymoncypris waddellii*, *Gymoncypris przewalskii* and *Racoma tibetanus)* were collected from 3 rivers (Yarlung Zangbo River, Lhasa River, and Niyang River) and 4 high mountain lakes (Yamdrok Lake, Qinghai Lake, Palgon Lake and Basum Lake) (Fig. 1). Of the seventy nine samples, thirty nine were taken in 2007 and forty in 2011. The Yarlung Zangbo River (YZR) is the largest river throughout the world with an average altitude of over 4000 m above sea level and originates from the Gemayangzong Glacier at elevation of 5200 m in south-central Tibet. The Lhasa River is the largest branch of the YZR and originates from south of Nyangqentanglha Mountain. Similarly, the Niyang River is also one branch of the YZR, which originates from the Mila Mountain, connects with the eastern of the Lhasa River and finally goes into the YZR. The Yamdrok Lake is a low concentration saline lake and located at the northern foot of the Himalayas. Qinghai Lake, lying in the northeast of Qinghai Province, is the biggest inland saline lake of China. Palgon Lake is an international lake, resting on the border of China and Kashmir, and the section within China is freshwater while within Kashmir is saline lake. As being adjacent to the city of Lhasa and the highway of Sichuan-Tibet, the freshwater Basum Lake is a famous tourist attraction.

All the fish samples belong to the family of *Cyprinidae* and subfamily of *Schizothoracinae*, which is unique in the Tibetan Plateau. The collected samples were preserved in insulation ice boxes (polyester) and immediately transported to the laboratory. The muscles of fish were then lyophilized, ground and kept at −20 °C until sample pretreatment. A portion of anhydrous sodium sulfate was included as a trip blank.

2.2. Trophic level calculation

Stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope ratios were used to characterize trophic information of the aquatic ecosystem in the Tibetan Plateau. Isotopes $\delta^{15}N$ and $\delta^{13}C$ were calculated according to the formulas $\delta^{15}N = [-1 + (^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{standard})] \times 1000$ and $\delta^{13}C = [-1 + (^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{standard})] \times 1000$. The trophic levels (TL) of fish were determined based on the nitrogen stable isotope analysis, as described by Fisk et al. (2001): $TL_{consumer} = 2 + (\delta^{15}N_{consumer} - \delta^{15}N_{zooplankton})/3.8$, where $TL_{consumer}$ is the trophic level of the target organism.

The condition factor (Bolger and Connolly, 1989) of the fish varied from 0.69 to 1.96 cg/cm^3 (100 g/cm^3) with a mean value of 1.10 cg/cm^3. The ranges of the lipid content and trophic level were 4.5–30.1% and 2.64–4.90, respectively. The average value of the water content of fish was 80.8%. Detail information was summarized in Table 1.

2.3. Sample pretreatment and instrumental analysis

The method of sample pretreatment and instrumental analysis was based on the previous published works (Feng et al., 2010; Zhu et al., 2012). Briefly, 1.5 g of dried fish muscles were converted to a free floating powder using anhydrous sodium sulfate and spiked with internal standards (^{13}C-labeled γ-HBCD) (Wellington Laboratories, Guelph, Canada). The samples were extracted using an accelerated solvent extractor (Dionex ASE 350) at 150 °C and a pressure of 1500 psi using a 1:1 *v*/*v* of *n*-hexane-dichloromethane mixture. The resultant extract was passed through a 30%, w/w of acidified silica to remove fat. The extract was reduced to 2 mL on a rotary evaporator and kept for purification.

Purification was achieved using a multi-layer silica gel column filled from bottom to top with 1 g of activated silica gel, 4 g of basic silica gel (1.2%, w/w), 1 g of activated silica gel, 8 g of acid silica gel (30%, w/w), 2 g activated silica gel and anhydrous sodium sulfate. The column was pre-washed with *n*-hexane, the extract was then added and was eluted with 100 mL dichloromethane. The resulting purified extract was concentrated to 2 mL and further reduced to about 50 μL using a stream of nitrogen. The extract was then transferred to 100 μL of methanol in a LC-micro-vial containing $^2H_{18}$-labeled γ-HBCD (Wellington Laboratories, Guelph, Canada) as recovery standards and then kept for instrumental analysis.

The analysis of HBCDs was performed on an Alliance 2695 high-performance liquid chromatograph (Waters, Milford, MA) coupled with a triple-quadrupole

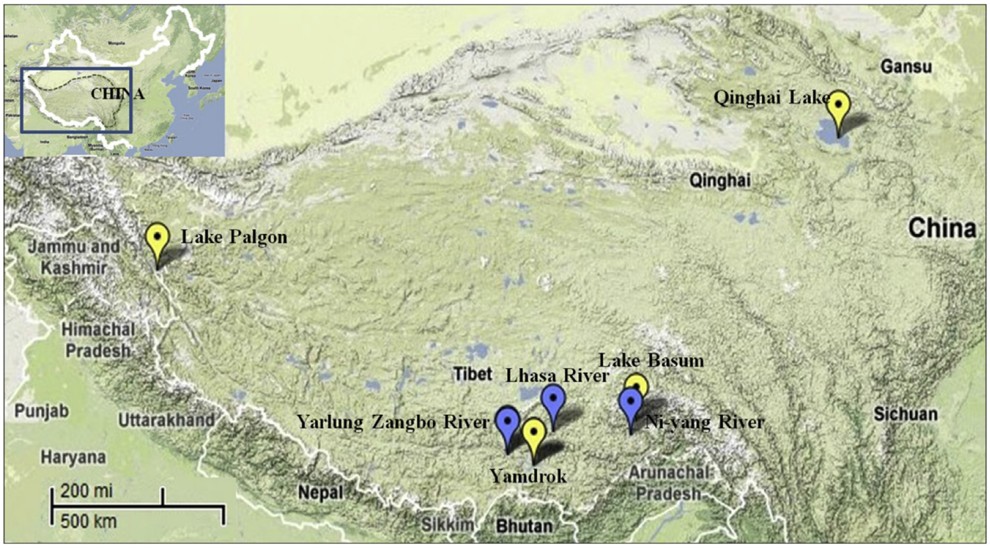

Fig. 1. Sampling sites in the Tibetan Plateau of China. (yellow and blue marks represent lake and river sampling sites, respectively). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Fish sample information and average concentrations of HBCD in fish muscles from river and lake sites of Tibetan Plateau (mean ± standard deviation).

Year	Sites	Altitudes (m)	Precipitation[a] (mm/yr)	Speices	No.	CF[b] (cg/cm^3)	TL	Lipid[c] (%)	α-HBCD	γ-HBCD	∑HBCD	Detection no.
									(ng/g dw)			
2011	Lhasa River	3580–5200	372–739	Oxygymnocypris stewartii	3	0.78 ± 0.14	3.62 ± 0.09	13.6 ± 3.8	0.24 ± 0.28	0.14 ± 0.24	0.38 ± 0.33	2
				Schizopygopsis younghusbandi	5	0.85 ± 0.14	2.75 ± 0.10	14.0 ± 2.0	0.26 ± 0.34	0.13 ± 0.15	0.40 ± 0.38	4
				Schizothorax macropogon	6	1.0 ± 0.47	3.01 ± 0.15	9.7 ± 3.9	0.17 ± 0.22	0.03 ± 0.04	0.20 ± 0.21	6
				Schizothorax o'connori	6	0.85 ± 0.14	2.95 ± 0.24	14.0 ± 6.5	0.62 ± 0.33	0.03 ± 0.08	0.65 ± 0.39	6
				Schizothorax waltoni	6	0.88 ± 0.12	3.29 ± 0.08	15.6 ± 7.8	0.27 ± 0.10	0.06 ± 0.10	0.33 ± 0.09	6
	Niyang River	2727– 5000	400–1000	Oxygymnocypris stewartii	1	1.02	3.82	11.43	n.d.[d]	n.d.	n.d.	0
				Schizopygopsis younghusbandi	1	1.46	3.30	7.80	n.d.	n.d.	n.d.	0
				Schizothorax macropogon	2	1.66 ± 0.29	3.13 ± 0.03	7.0 ± 2.6	0.46 ± 0.64	n.d.	0.46 ± 0.65	1
				Schizothorax o'connori	1	0.81	3.30	7.62	n.d.	n.d.	n.d.	0
				Schizothorax waltoni	3	1.38 ± 0.47	3.03 ± 0.99	12.5 ± 3.6	0.37 ± 0.30	n.d.	0.37 ± 0.30	3
	Yarlung Zangbo River	3600–5200	<300 to >4000	Oxygymnocypris stewartii	1	1.12	3.65	15.55	n.d.	n.d.	n.d.	0
				Schizothorax macropogon	1	1.72	3.22	14.40	0.33	n.d.	0.33	1
				Schizothorax o'connori	1	1.43	2.93	6.96	n.d.	n.d.	n.d.	0
				Schizothorax waltoni	1	1.21	3.33	15.44	0.37	n.d.	0.37	1
2007	Lhasa River	3580–5200	372–739	Oxygymnocypris stewartii	1	0.94	3.70	18.22	0.18	n.d.	0.18	1
				Schizopygopsis younghusbandi	1	1.14	3.03	15.73	0.26	0.12	0.38	1
				Schizothorax macropogon	1	1.14	3.12	13.75	0.28	n.d.	0.28	1
				Schizothorax o'connori	1	0.86	3.06	4.79	0.25	n.d.	0.25	1
				Schizothorax waltoni	1	1.23	3.22	17.64	0.43	n.d.	0.43	1
2011	Yamdrok Lake	4441	373	Gymoncypris waddellii	2	1.36 ± 0.31	3.38 ± 0.13	10.0 ± 4.3	0.12 ± 0.17	0.13 ± 0.18	0.25 ± 0.35	1
2007	Yamdrok Lake	4441	373	Gymoncypris waddellii	17	1.30 ± 0.12	3.54 ± 0.28	9.5 ± 3.5	0.01 ± 0.03	0.06 ± 0.10	0.07 ± 0.10	7
	Qinghai Lake	3225	337	Gymoncypris przewalskii	7	—[e]	4.02 ± 0.08	14.0 ± 6.5	0.05 ± 0.08	0.08 ± 0.10	0.13 ± 0.18	3
	Palgon Lake	4240	61	Racoma tibetanus	7	1.01 ± 0.10	4.63 ± 0.25	16.8 ± 6.4	0.01 ± 0.02	0.03 ± 0.05	0.04 ± 0.05	3
	Basum Lake	3538	646	Schizothorax o'connori	1	0.87	2.73	37.27	0.94	0.11	1.05	1
				Schizothorax waltoni	2	1.05 ± 0.09	3.25 ± 0.05	17.5 ± 2.1	0.84 ± 0.42	0.2 ± 0.28	1.04 ± 0.15	2

[a] Data for alpine lake from reference(Xiang and Zheng, 1989), data for river from reference (Liu, 1999; Liu et al., 2012b; Xu, 2004).

[b] CF:Condition factor was determined according to the equation: $CF = (W/L^b) \times 100$, where W is the weight (g) of organism, L is the length (cm) of organism, and b is equaled to 3 which is generally used in other studies.

[c] Based on dry weight of fish muscles.

[d] Not detected.

[e] Not determined.

mass spectrometer (Quattro Premier XE, Micromass, Manchester, UK). Chromatographic separation of the analytes was performed on a ZORBAX C18 reverse-phase column (3 mm × 150 mm, 5 μm, Agilent, USA). A mixture of methanol (A), acetonitrile (B) and water (C) were selected as gradient mobile phase and programmed as follows: initial composition of 30:30:40 for A:B:C (*v/v/v*), ramped to 70:30:0 in 10 min, held for 4.9 min, returned to 30:30:40 in 0.1 min, and equilibrated for 5 min. For mass spectrometric analysis, an atmospheric pressure chemical ionization mode with negative ion mode was selected. Multiple reaction monitoring signals for quantification and confirmation ranged from m/z 640.6 → 79 and 640.6 → 81, respectively. More detailed descriptions of the instrumental analysis method can be found in our previous paper (Feng et al., 2010).

2.4. Quality assurance/quality control

One procedure blank of 15 g of anhydrous sodium sulfate was included for every batch of five samples. A trip blank was also analyzed for quality control. Results showed HBCDs were below the limits of detection (LODs) in all blanks. Isotope dilution method was used for procedure validation. The recovery of ^{13}C-labeled γ-HBCD internal standard in samples was 85.8 ± 15.0%. The mean method detection limit of α-, β-, and γ-HBCD was 113, 44 and 46 pg/g, respectively. The LOD was calculated as 3 times the signal-to-noise ratio on the corresponding mass trace of the analytes, while the limit of quantification was calculated as 10 times the signal-to-noise.

3. Results and discussion

3.1. HBCD diastereoisomer composition and concentrations in high mountain fish

HBCDs were detected in 52 fish out of the total 79 samples. Detailed data summaries of dry weight based and lipid normalized concentrations were summarized in Table 1 and Fig. 2, respectively. The sum of concentrations of the three congeners (∑HBCDs) in fish ranged from non detectable levels to 1.31 ng/g dw (dry weight, dw) (13.7 ng/g lw (lipid weight)), with mean value of 0.26 ng/g dw (2.12 ng/g lw). α-HBCD was the dominant congener with a detection frequency of 53.2% and an overall average concentration of 0.20 ng/g dw (1.53 ng/g lw). γ-HBCD had a detection frequency and mean concentration of 31.7% and 0.06 ng/g dw (0.59 ng/g lw), while β-HBCD was under LOD in all samples. It was observed that α-HBCD accounted for an average value of 78.2% of the total HBCD burden and even 100% in the samples from the Niyang River and the YZR. The composition was distinctly different from that in the commercial mixtures but consistent with the findings in most biota reported by other works (Harrad et al., 2009; Vorkamp et al., 2012). Though reasons for the composition difference between biota and commercial mixtures remain unclear, it is widely accepted that higher rate of biological uptake of α-HBCD due to its higher water solubility, lower rate of metabolism of α-HBCD, and the bioconversion from γ-HBCD to α-HBCD were the main explanation of enrichment of α-HBCD in biota (Yu et al., 2008). β-HBCD was detected at lower level with lower detection frequency and this could be mainly due to its low proportion in the HBCD mixture and/or its low potential of biomagnifications (Law et al., 2005).

Statistical analysis showed no significant species variation ($p > 0.05$) of the HBCD levels or the lipid contents among the five fish species (Oxygymnocypris stewartii, Schizopygopsis younghusbandi, Schizothorax macropogon, Schizothorax o'connori, and Schizothorax waltoni) collected from the rivers. With regarding to lake fish, as their species are unique for their own lakes, species variation analysis was not carried out. Insufficient data are available regarding HBCD levels of muscle tissues of fish in the general pollution areas, especially in China. So it is very difficult to assess the HBCD level of the fish in the Tibet Plateau. The very limited data showed the HBCD concentrations in this work was comparable to that in the marine fish (0.57–10.1 ng/g lw, mean value of 3.7 ng/g lw) along the Chinese coastline (Xia et al., 2011). However, it was significantly lower than those in freshwater fish from the polluted areas, such as the Yangtze River (11–330 ng/g lw) (Xian et al., 2008) and the electronic waste recycling area of China (199–728 ng/g lw) (Zhang et al., 2009). On a global scale, the levels of HBCD in the present study was slightly lower than those from the western Canadian Arctic (Tomy et al., 2009) and the eastern US lake (Tomy et al., 2004), and was 2–3 orders of magnitude lower than that from Europe (Janak et al., 2005) and Japan (Kakimoto et al., 2012) where the biggest HBCD markets in the world are. Compared to other POPs detected in the same fish from the same sampling sites based on our previous works, the HBCD level herein was at the lowest level. It was slight lower than PBDEs (Yang et al., 2011) and hexachlorobenzene (Yang et al., 2010). It was also about 10 folds lower than perfluorooctane sulfonate (Shi et al., 2010), PCBs (Yang et al., 2010), hexachlorocyclohexanes (Yang et al., 2010), about 100–1000 folds lower than dichlorodiphenyltrichloroethane and its metabolites (Yang et al., 2010) and mercury (Yang et al., 2011). On the whole, the atmospheric transport might be responsible for the relatively high concentration level and detection frequency of HBCDs in these high mountain fish, since no local source has been reported in most of the sampling sites based on the previous studies (Sheng et al., 2013; Wang et al., 2010), except for Basum Lake, which is a famous scene and it is located close to the residential areas comparing to the other sampling sites.

3.2. *Spatial and temporal variations of HBCDs in fish species*

Statistical analysis showed no significant difference between the HBCD concentrations or fish condition factors in fish from rivers and lakes ($p > 0.05$). The levels of HBCDs in descending order were Basum Lake > Yamdork Lake > Qinghai Lake > Palgon Lake. Thereinto, ∑HBCDs (1.04 ± 0.11 ng/g dw) and the detection frequency (100%) of fish from the Basum Lake was significantly ($p < 0.0001$) higher than those from the other sampling lakes (Fig. 2, Table 1). In a similar study, Yang et al. also reported remarkably higher PBDE concentrations in fish from Basum Lake (Yang et al., 2011). Furthermore, higher concentrations of OCPs and PCBs were also reported in fish from Basum Lake than the ones in the same matrices from Palgon Lake (Yang et al., 2010). Daly and Wania (Daly and Wania, 2005) have revealed precipitation as one of the ways in which POPs can be captured from air to other environmental medias. In this study, the concentration of HBCDs in fish from lakes was positively correlated with the annual precipitation of the sampling sites ($R = 0.950$, $p = 0.05$) (Fig. 2), which was quite in agreement with the relationship between the PBDEs levels in lake fish and the precipitation (Yang et al., 2011). This result implied that the potential sources of HBCDs in most sampling sites in this study came from the wet/dry deposition via the long range atmospheric transport. As mentioned above, besides the long range atmospheric transport, the more frequent anthropogenic activities around the sampling site Basum Lake might also influence the significantly higher fish concentrations of HBCDs and PBDEs in this lake. However, this baseless conjecture requires survey data to be verified.

It was observed that some of the fish from Yamdork Lake and Qinghai Lake had only one isomer of HBCD i.e. γ-HBCD, unlike other lakes where α-HBCD was the dominant isomer. The reason for the isomer composition remains unknown, but could be the difference

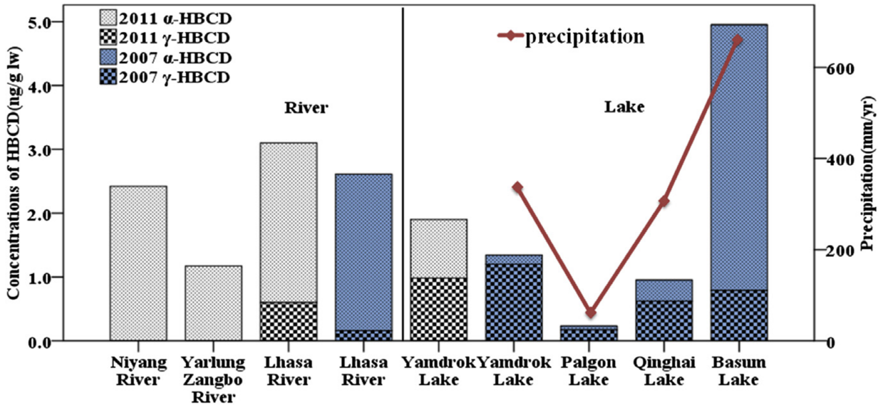

Fig. 2. Lipid normalized concentrations and composition profiles of HBCDs in rivers and lakes of Tibetan Plateau, and the annual precipitation in the lake sampling sites.

in fish species from the lakes. The fish in the Yamdork Lake and the Qinghai Lake both belong to the genus of *Gymoncypris* and can live in saline environment (Liu et al., 2012a). Their diet choices, exposure, and metabolic capabilities for HBCDs might be different from the other fish species living in the freshwater environment. Among the three sampling rivers, highest detection frequency (93.5%) and ∑HBCD level (0.39 ± 0.31 ng/g dw) was detected in the Lhasa River. For the Niyang River and the Yarlung Zangbo River, the detection frequencies were 50.0% and 50.0%, respectively.

The levels of the ∑HBCDs in fish collected in the year of 2007 and 2011 were used to investigate the temporal variations. As shown in Fig. 2, the ∑HBCD levels in fish from Lhasa River increased from 2.61 ng/g lw in 2007 to 3.11 ng/g lw in 2011, and from 1.34 ng/g lw in 2007 to 1.91 ng/g lw in 2011 for fish from Yamdrok Lake. It was observed that though the concentration variance was not significant, the detection frequency in 2011 (77.5%) was much higher than that in 2007 (53.9%). The average ratios of α-HBCD/γ-HBCD varied considerably from 14.6 in 2007 to 3.8 in 2011 for the Lhasa River, and from 0.3 in 2007 to 0.9 in 2011 for Yamdork Lake. Data regarding temporal trends in HBCD levels of biotic in other areas were very limited. Chen et al. (2011) reported an orders of magnitude increase of HBCD level in U.S. carp collected from 2006 to 2007 comparing to that of 1999–2002. Regarding to the polar region, a significant exponential time trend with an annual increasing rate of +6.1% for α-HBCD was found in ringed seals from East Greenland collected between 1986 and 2008 (Vorkamp et al., 2011). 2-fold increase of HBCDs concentrations in finless porpoises collected between 1990 and 2000 from South China Sea was also detected (Isobe et al., 2007). The temporal trend in the present study and other previous works supported the global perception that environmental levels of HBCDs might be increasing in recent years. Due to the wide altitude range of the rivers, the correlation between the concentrations of HBCD and the altitudes was only analyzed among the lake fish. No significant correlation was observed, which was same to the observation in Yang et al.'s work for PBDEs (Yang et al., 2011). However, fish concentrations of

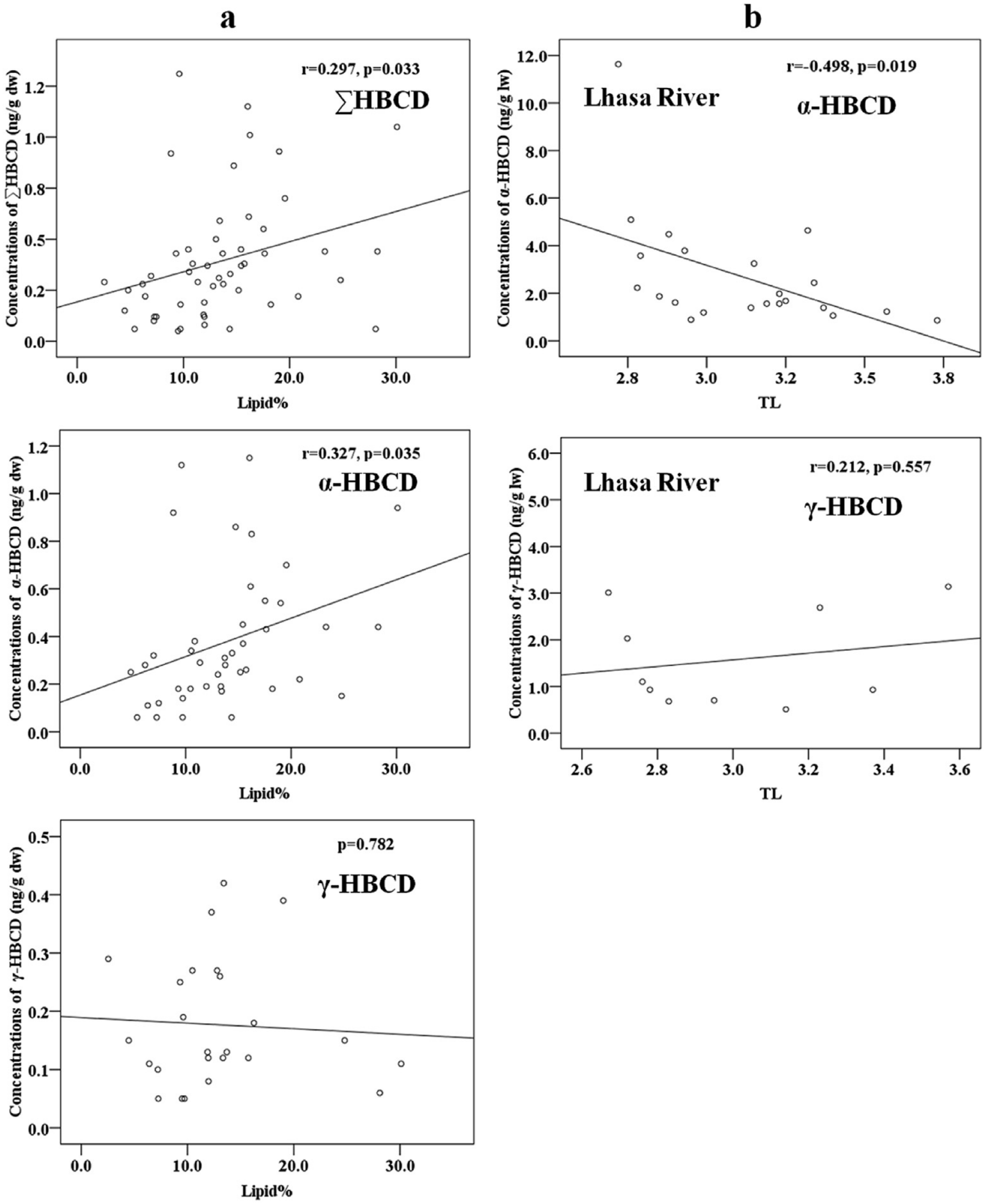

Fig. 3. (a) Correlations between HBCD concentrations and the lipid contents of fish muscles; (b) Correlations between HBCD concentrations and the trophic levels of fishes from the Lhasa River.

OCPs (Yang et al., 2010), heavy PCBs (Yang et al., 2010) and mercury (Yang et al., 2011) in those lakes increased with increasing altitude whereas that of perfluorooctane sulfonate (Shi et al., 2010) decreased with the increasing altitude.

3.3. Relationship between HBCDs levels and the lipid content and trophic level

Considering the lipid solubility of POPs, the lipid content of organisms is regarded as one of the factors that can influence bioaccumulation of POPs. Correlation analysis showed significant positive correlations ($p < 0.05$) between the HBCD levels (α-HBCD and $\sum$HBCDs) and the lipid content of fish (Fig. 3a), which was consistent with the results of mollusks collected from Chinese Bohai Sea (Zhu et al., 2012) and fish from the Lake Winnipeg, Canada (Law et al., 2006). A relatively high octanol-water partitioning coefficient (log K_{ow}) of 5.6 (Covaci et al., 2006) for HBCD commercial mixture might be the reason for this positive correlation. Though the log K_{ow} of γ-HBCD (5.47) (Hayward et al., 2006) was higher than the one of α-HBCD (5.07) (Hayward et al., 2006), the lower detection frequency made the correlation of γ-HBCD with lipid content not discernible. The body length and body weight were also concerned but no significant linear relationships were found between $\sum$HBCDs and these two growth indices of the fish ($p > 0.05$).

Many POPs can biomagnified/bioaccumulated in biota via trophic level or food chain. To explore the biomagnification potential of HBCD in fish, lipid-normalized HBCD concentrations and the trophic levels were correlatively analyzed. TLs of the fish in the present study ranged from 2.64 to 4.90, with the highest mean value of 4.63 for *Racoma tibetanus* from the Palgon Lake and the lowest one of 2.84 for *Schizopygopsis younghusbandi* from the Lhasa River. Because the number of the fish samples in which detectable HBCDs existed was no more than 3 in most of the sampling sites, and there was only one fish species collected in the Yamdork Lake, correlation analysis was only carried out among the data from the Lhasa River. The result indicated the concentration of α-HBCD in the fish from the Lhasa River was negatively correlated with TL ($r = -0.498$, $p = 0.019$) whereas there was no such trend for γ-HBCD (Fig. 3b). This negative relationship indicated potential trophic dilution rather than magnification in the portion of the river food web involving fish. Though Tomy et al. (2008) reported the trophic dilution of HBCD existed in marine food webs from eastern Canada and Shaw et al. (2012) indicated the biomagnification factors (BMFs) for HBCDs was smaller than 1 in the food webs from northwest Atlantic coast, their results are still some different from the investigation in the present study. In their work, Tomy et al. reported the trophic magnification of α-HBCD and trophic dilution of γ-HBCD in a food web. Shaw et al. found a low biomagnification of α-HBCD from prey fish to livers of harbor seals (BMF 1.0–3.0) but no biomagnification of α-HBCD from the prey fish to blubber of harbor seals (BMF 0.54–0.89). Nevertheless, the biomagnification potential of HBCDs has been confirmed based on the investigations of HBCD concentrations in the world wide, especially of α-HBCD in the aquatic food web (Law et al., 2006; Wu et al., 2010) and the arctic marine food web (Frederiksen et al., 2007; Jenssen et al., 2004; Tomy et al., 2008).

For an in-depth insight into trophic relationships among the fish species of the food web in the Lhasa River, δ^{15}N and δ^{13}C isotope analysis related to the tissue concentrations of α-HBCD were carried out (Fig. 4). The δ^{13}C ranged from −22.0‰ in *Schizothorax macropogon* to −11.7‰ in *Schizopygopsis younghusbandi* while δ^{15}N ranged from 6.7‰ in *Schizopygopsis younghusbandi* to 10.8‰ in *Oxygymnocypris stewartii*. *Schizopygopsis younghusbandi* (SY) was the most enriched fish species with a wide variation in terms of δ^{13}C signatures, occupying a relatively lower trophic level and higher α-HBCD level (2.97 ng/g lw). The clear separation of SY from the other fish species in the δ^{13}C signatures suggested the different carbon source of SY from the others (Campbell et al., 2000) and that SY might belong to a different food chain from other species. Among the other four species, *Oxygymnocypris stewartii* (OS) was in the highest trophic position whereas *Schizothorax o'connori* (SC) occupied a wide variation of trophic position coupled with a lowest mean value, which was consistent with the fact that OS belongs to carnivorous fish feeding on other fish and aquatic insects while SC is herbivorous. On the contrary, α-HBCD level of SC was quite higher than the one of OS, suggesting no biomagnifications exist in this food web.

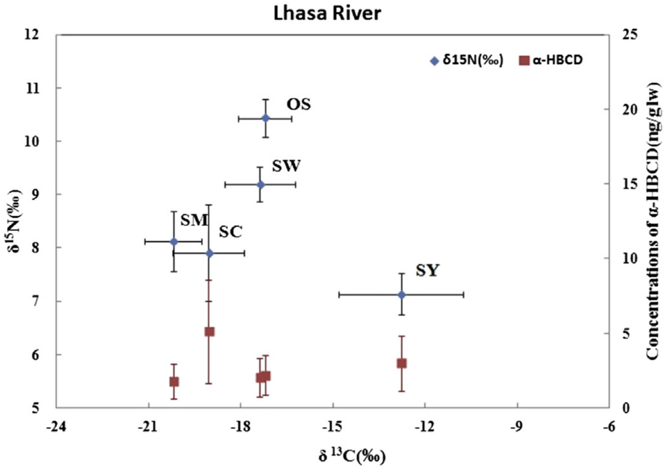

Fig. 4. Relationship of δ^{13}C and δ^{15}N (α-HBCD concentrations) of fish species of the aquatic food web from the Lhasa River. (OS, SW, SM, SC and SY are the simplified readers of fish species, and represent *Oxygymnocypris stewartii*, *Schizothorax waltoni*, *Schizothorax macropogon*, *Schizothorax o'connori* and *Schizopygopsis younghusbandi*).

4. Conclusions

This study confirmed the wide occurrence of HBCD in the aquatic ecosystems of the Tibetan Plateau, and indicated that the long-range atmospheric transport of HBCDs in the Tibet Plateau preferred to dependent on the dry/wet deposition rather than cold-trapping or cold condensation in this high mountain area compared to other contaminants such as PBDEs and organic mercury. Combined with other reports on the biomagnification of HBCDs, the negative correlation between α-HBCD concentration and TL in the Lhasa River aquatic food web suggested that more comprehensive researches about HBCDs biomagnification should be carried out.

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