



Tracing the sources and microbial degradation of PCBs in field sediments by a multiple-line-of-evidence approach including compound-specific stable isotope analysis

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ABSTRACT

Comprehensive monitoring is crucial for tracing micropollutants in the natural environment. To better evaluate the sources and natural attenuation of polychlorinated biphenyls (PCBs), three composite sediment cores were sampled from a closed pond near e-waste recycling plants, and a multiple-line-of-evidence approach (MLEA) including quantification, enantiomer analysis, microbial community profiling, and compound-specific isotope analysis (CSIA) was used to investigate the fate of PCBs in sediment cores. The difference in the maximum PCB concentrations and associated depths between sites 1/2 and 3 and the corresponding significant ($p < 0.01$) difference in $\delta^{13}\text{C}$ values strongly indicated two different PCB inputs at sites 1/2 and 3. A significant ($p < 0.01$) negative correlation between the variation in chlorine per biphenyl (CPB) and Log the abundance of *Dehalococcoides*/total molar concentration of PCBs (Log Dhc/TPCB) along the cores suggested that different degrees of PCB degradation occurred and that *Dehalococcoides* likely participated in PCB degradation in these sediments. Nonracemic compositions and pronounced stable carbon isotope fractionation ($\Delta\delta^{13}\text{C} > 1\text{‰}$) of PCB congeners were observed, confirming that in situ degradation occurred in the sediment cores. The progressive enrichment in ^{13}C with increasing core depth suggested strengthened microbial degradation of the residual congener pools. The results of this study suggested that MLEA analysis of PCBs can provide reliable information to better monitor the sources and fate of these compounds in the environment.

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1. Introduction

Polychlorinated biphenyls (PCBs) are a classic group of persistent organic pollutants (POPs) that were mainly manufactured as dielectric fluids for capacitors and hydraulic fluids for transformers from 1930s to 1970s (Breivik et al., 2002). Although their production was banned in the 1970s due to their toxic, lipophilic and bioaccumulative characteristics, a large amount of PCBs was released into the aquatic environment, and eventually become enriched in sediments, which bioaccumulate through food chains

(Jepson and Law, 2016). Especially, that elevated PCB levels were found in worldwide e-waste recycling sites had caused a global problem that threatened ecology and human health (Ni and Zeng, 2009; Tue et al., 2010; Zeng et al., 2016; Zhang et al., 2012; Zhao et al., 2019). Therefore, monitoring the sources and transformation processes of PCBs in sediments are crucial for evaluating their risk to ecosystems and human health (Desforges et al., 2018).

Source apportionment and anaerobic dechlorination of PCBs has been studied extensively in field sediments sampling from many contaminated areas, including Lake Hartwell (Bzdusek et al., 2006a), the Sheboygan River (Bzdusek et al., 2006b), the Great Lakes (Soonthornnonda et al., 2011), the Fox River (Imamoglu et al., 2004), and so on. Most of these studies explored PCB sources and

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dechlorination mediated by microorganisms via the variation in homolog composition based on mass balance and multivariate receptor models, such as polytopic vector analysis (Magar et al., 2005), positive matrix factorization (Soonthornnonda et al., 2011), and anaerobic dechlorination models (Demirtepe et al., 2015). In the field, not only microbial degradation but also changes in sources and various physicochemical processes (absorption, desorption, and migration) can alter PCB homolog composition at different sediment intervals (Mai et al., 2005). Obviously, these mathematical modeling approaches cannot accurately distinguish microbial degradation from physical processes, which incapacitated them from tracing microbial degradation in field environment.

In recent decades, compound-specific stable isotope analysis (CSIA) has been developed as a robust tool for source apportionment and biodegradation discrimination of organic compounds (Schmidt et al., 2004). Isotope signature of a synthesized compound can be different due to the raw materials, the pathway, and the conditions, which means the isotope signature of one compound from different manufacturers can be different (Schmidt et al., 2004). Researchers have applied carbon isotope signatures to apportion sources of PCBs, polybrominated diphenyl ethers (PBDEs), and polycyclic aromatic hydrocarbons (Buczynska et al., 2013; Yanik et al., 2003; Zeng et al., 2013). During biodegradation of organic compounds, lighter isotopes generally exhibit faster reaction rates than their heavier isotopologues, leading to enrichment in heavier isotopes in the residual fractions, while physical processes such as dilution, sorption, transport, and volatilization do not typically lead to isotope fractionation (Elsner, 2010). To date, CSIA has been successfully used to evaluate the in situ microbial degradation of pesticides (Alvarez-Zaldivar et al., 2018; Bashir et al., 2015; Jin and Rolle, 2016), chlorinated hydrocarbons (Liu et al., 2017), chlorinated benzenes (Braeckevelt et al., 2007), and PBDEs (Huang et al., 2019). There is only one study that applied CSIA to assess microbial reductive dechlorination of PCBs in the laboratory and reported an absence of stable carbon isotope fractionation during dechlorination of PCB 61 to PCB 23 (Drenzek et al., 2001). Nevertheless, this absence of fractionation could not be extended to other microbial degradation scenarios because the magnitude of isotope fractionation could be influenced by many factors, including the masking of isotope fractionation, the type of bond broken (C–C or C–Cl), the metabolic enzyme involved, and the position of chlorines on the biphenyl rings (Liang et al., 2014a; Tang et al., 2017). Furthermore, when there was no isotope fractionation in the process of degradation, the intrinsic isotopic trend of ^{13}C depletion with increasing chlorine content in Aroclor mixtures suggested that CSIA could still be used to trace the in situ degradation signal of PCBs, as the reductive dechlorination of PCBs will create congeners with more depleted $\delta^{13}\text{C}$ values than native PCB congeners of similar chlorination (Horii et al., 2005).

Nineteen of the 209 PCB congeners are axially chiral and form stable atropisomers in the environment (Kania-Korwel and Lehmler, 2016). In addition to CSIA, enantiomer analysis could also provide compelling proof of microbial PCB degradation in field sediments. Enantiomer analysis is based on the biochemical characteristics of chiral enantiomers that have identical chemical and physical properties but may have different biological properties, resulting in enantioselectivity only during biotransformation (Lu et al., 2019).

Compared to a single approach, comprehensive monitoring could provide a more sensitive and reliable evaluation of pollutant origins and biodegradation (Fischer et al., 2016). Therefore, in this study, a multiple-line-of-evidence approach (MLEA) was used to investigate the PCB sources and microbial degradation in field sediment cores. The PCB concentration, congener profiles, and dechlorinating bacterial community were analyzed in aquatic

sediments to elucidate PCB occurrence and transformation in situ. Then, the enantiomeric fraction (EF) of chiral PCBs and the carbon isotope composition of individual PCB congeners of several sediment cores were determined to characterize the environmental sources and conversion processes of PCBs.

2. Materials and methods

2.1. Sample collection

Three sampling sites, named site 1, site 2, and site 3, were selected from a closed pond near e-waste recycling plants in South China (23.6021 N, 113.0785 E) in 2016. The water depths of sites 1, 2, and 3 were approximately 1.6, 2.0, and 1.1 m, respectively. And the distances of site 1 to site 2, site 1 to site 3, and site 2 to site 3 were probably 15, 35, 25 m, respectively. The sampling region and methods have been described in our previous paper (Huang et al., 2019) and are briefly introduced as follows: at each sampling site, five sediment cores located within a 1 m radius were collected using a Plexiglas gravity corer. The diameter of the cores was 5 cm. A total of 15 sediment cores were collected and sliced into 3 cm segments using a stainless steel cutter; however, the surface layer was sliced into 5 cm segments. Then, the five individual sediment cores collected from each sampling site were pooled into one composite sediment core with the corresponding depth segments. Finally, three composite sediment cores with 13, 14, and 13 segments were obtained from sites 1, 2, and 3, respectively. After thorough incorporation, 5 g (wet weight) of sediment was taken from each sample for microbial community analysis, and the remaining part was used for PCB analysis. All samples were transported to the laboratory immediately and stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

2.2. Sample preparation

The microbial community composition and element (e.g., organic carbon, nitrogen, hydrogen, and sulfur) contents were reported previously (Huang et al., 2019). Quantitative real-time PCR (qPCR) (Bio-Rad CFX96 real-time PCR system; Bio-Rad, CA, USA) with a SYBR® green assay was performed in triplicate with sediment samples to quantify the abundance of *Dehalococcoides* (Wang and He, 2013). The analytical procedures were similar to those described in previous studies (Wang and He, 2013) and details could be found in the Supporting Information (SI). For PCB analysis, approximately 0.5 g and 100 g of dry sediment samples were used for quantification and determination of the carbon isotope composition of individual PCB congeners, respectively. The methods used to extract and clean up sediment samples for PCB quantification and CSIA were similar to those previously described (Zeng et al., 2013), with minor modifications, and are described in detail in the SI.

2.3. Instrumental analysis

PCB congeners were determined by gas chromatography/mass spectrometry (GC/MS) (Agilent 7890A/5975C MSD, Agilent Technology, CA) with an electron impact (EI) ion source in selective ion monitoring (SIM) mode. A DB-5 MS column (60 m \times 0.25 mm i.d. \times 0.25- μm film thickness) was used to separate the 209 PCB congeners. PCB quantification was based on calibration curves constructed from eight concentrations of internal standard solutions. Chiral PCB atropisomers were separated using two chiral columns, a Chirasil-Dex column (25 m \times 0.25 mm i.d. \times 0.25- μm film thickness) and BGB-172 column (30 m \times 0.25 mm i.d. \times 0.18- μm film thickness). Detailed descriptions of the GC conditions and

the ions monitored for PCB quantification and enantiomer separation are given in the SI. EFs were calculated as follows:

$$EF = \frac{A}{A + B}$$

where, for PCB 91, 95, 132, 135, 136, 149, 174, 176, and 183, A and B represent the peak areas of the (+)- and (-)-enantiomers, respectively, and for PCB 45, A and B represent the peak areas of the first-eluting (E1) and second-eluting (E2) enantiomers, respectively, as the elution order of the PCB 45 atropisomers were unknown.

CSIA of PCBs was performed using a method that was similar to that used in our previous studies (Tang et al., 2018; Zeng et al., 2013), with minor modifications, and is described in detail in the SI. Briefly, the extracts used for CSIA were first analyzed to determine the purity, the distribution pattern, and the retention times of the target compounds using the same GC/MS system and chromatographic column in full scan mode as those used for quantification. The stable carbon isotope composition ($\delta^{13}\text{C}$ value) of individual PCB congeners was determined by isotope ratio mass spectrometry (IRMS; Trace GC UltraSoLink Delta V Advantage, Thermo-Fisher Scientific, Waltham, MA).

2.4. Quality assurance and quality control (QA/QC)

A complete set of QA/QC samples was subjected to the quantitative analysis and CSIA procedures. During quantitative analysis, four laboratory blank samples were processed along with a sediment extract batch to monitor background interferences and contamination. Trace amounts of a few PCBs (PCB 28/31, 117, 118, 175, 195) were detected in the method blanks, and the concentrations ranged from 0.81 to 3.90 ng/g dry weight (dw). The recoveries of surrogate standards [$n = 41$; mean \pm standard deviation ($AV \pm SD$)] were as follows: $93.09 \pm 5.81\%$ (PCB 30), $98.54 \pm 3.72\%$ (PCB 65), and $95.15 \pm 7.54\%$ (PCB 204). The background values detected in the method blanks were subtracted, and no surrogate corrections were made to the final concentrations. For CSIA, a PCB standard mixture was analyzed to verify instrument stability. No significant isotope fractionation was observed for any of the congeners in the PCB standard mixture (Table S1). A co-injected standard, 2,4,6-trichlorobiphenyl (PCB 30), obtained from Ultra Scientific (North Kingstown, RI, USA) was added to the sample extracts prior to CSIA. The $\delta^{13}\text{C}$ of the co-injected standard was determined off-line by IRMS (Flash 2000 EA-Delta V Plus, Thermo-Fisher Scientific, USA), and the differences between the ratios in the co-injected standard measured online (-29.17 to -28.88%) and off-line (-28.80%) in each sample were less than 0.5% , indicating the reliability of the carbon isotope data. Each extract was analyzed at least three times, and the data were considered only when the standard deviation of $\delta^{13}\text{C}$ values was less than 0.5% .

2.5. Statistical analysis

Statistical analysis was performed using SPSS 21.0 and Origin 8.0 software for Windows. All of the data were verified for normality using the one-sample Kolmogorov-Smirnov test prior to other statistical analyses. Pearson's correlation analysis was employed to test the statistical correlations between the chlorine per biphenyl (CPB) and Log the abundance of *Dehalococcoides*/total molar concentration of PCBs (Log DhC/TPCB) in three sediment cores. Independent-samples t-tests were performed to test for significant differences in the EFs compared to standard values (0.5). The variation in $\delta^{13}\text{C}$ with sediment depth was also analyzed using Pearson's correlation analysis. The differences in $\delta^{13}\text{C}$ between sites 1/2 and site 3 were evaluated by one-way analysis of variance

(ANOVA) with a post hoc Tukey's honest significant difference test.

3. Results and discussion

3.1. PCB concentrations and composition profiles

A total of 159 congeners were detectable, with 146 target peaks separated. The PCB concentrations ($\sum_{159}\text{PCBs}$: sum of 159 congeners) ranged from 100 to 71,000, 120 to 28,000, and 400–19,000 ng/g, dw at sites 1, 2, and 3, respectively (Table S2). As shown in Fig. 1, the PCB concentrations clearly increased and then decreased from the deepest segment to the surface of the cores, which was significantly different from the trend observed for PBDEs in the same cores, which generally decreased with the core depth (Huang et al., 2019). This difference likely results from the different dismantlement histories and inputs of PCBs and PBDEs in the study area. PCBs were mainly used as dielectric fluids in capacitors and hydraulic fluids in transformers, and the production and commercial use of PCBs were banned by the EPA in 1979 (Breivik et al., 2002; Erickson and Kaley, 2011; Ross, 2004), while PBDEs were widely used in commercial electronic equipment and building materials and only recently added to the list of POPs in the Stockholm Convention (Abbasi et al., 2015; UNEP, 2017).

Although the three sediment cores had similar PCB concentration trends, the maximum PCB concentrations appeared at different sediment depths at the three sites (Fig. 1). The maximum concentrations appeared at 9–14 cm depth at sites 1 and 2 but at 21–23 cm depth at site 3. The maximum concentrations at two different depths at sites 1/2 and 3 may suggest at least two periods of massive capacitor and transformer dismantling and consequent PCB inputs (Fig. 1). Other factors besides dumping waste, like aquatic organisms or even heavy rain, may also affect the concentration of PCBs at the top layers, especially with shallow water sites. However, these factors could not be the main causes of the maximum PCB concentrations that appeared at different sediment depths at the three sites. If the top layers are affected a lot by the aquatic disturbance, then the increasing trends of PBDE concentration in the top sediments should also be disturbed as well as for PCBs. Oblique accumulation of dismantling waste in the same direction could also cause the maximum concentrations to appear in

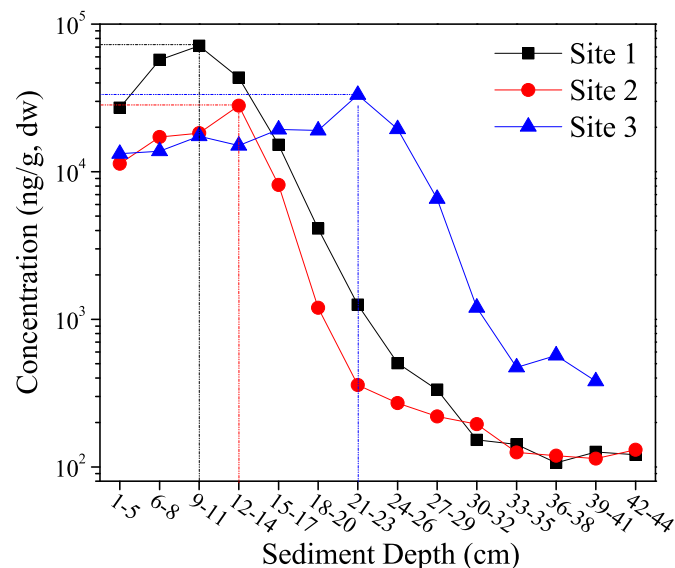


Fig. 1. The variation in total PCB levels ($\sum_{159}\text{PCBs}$, ng/g, dw) in the three composite sediment cores. (The ordinate is expressed as the logarithmic transformation form.)

different depth intervals. In this case, sites whose maximum concentrations appeared much earlier should be closer to the sources and have much higher concentrations of contaminants, such as PBDEs, in the three sediment cores (Huang et al., 2019). However, this case did not apply to PCBs because the maximum PCB concentrations at site 3 appeared in deeper intervals than those at site 1 but were lower than the PCB concentrations at site 1 (Fig. 1).

The average PCB congener profiles (mol %, 1–29 cm sediment interval) in the three composite sediment cores are shown in Fig. S2. Sediment intervals below 29 cm were excluded from the contribution analyses due to the low PCB concentrations at these depths. The PCB congener profiles at the three sampling sites were generally similar and dominated by PCB 28/31, 52, 110, and 118 (Fig. S2). Although there were no apparent differences in the transverse direction, significant vertical variations in congener distributions were found in the sediment cores, particularly for the molar fractions of di-/tri-PCBs and penta-/hexa-PCBs. The former increased with increasing sediment depth at sites 1 and 2 but decreased and then increased at site 3 (Fig. S3). For penta-/hexa-PCBs, the variation tendencies were opposite (Fig. S3). Two factors that might have a contribution to the homolog proportion variation. One was microbial degradation of PCB from Penta-/Hexa-PCB to Tri/Di-PCB because anaerobic PCB dechlorination could convert more chlorinated PCB congeners to less chlorinated ones. The molar proportion of more highly chlorinated PCBs decreased, and those of less chlorinated PCBs increased with increasing sediment depth, as reported previously (Magar et al., 2005). Another one was the upward/downward diffusion/migration of PCBs from the maximum concentration intervals because the less chlorinated PCBs (mono- to tri-PCBs) had much greater mobility because of their lower octanol-water partition coefficients and higher solubility (Mai et al., 2005). Therefore, we needed more information to clarify whether microbial dechlorination contributed to the variation in PCB profiles.

3.2. Variation in CPB and indigenous putative PCB-Dechlorinating bacteria

In most PCB dechlorination studies (Pakdeesusuk et al., 2005) has been used as an indicator to track the degree of dechlorination (the number of removed chlorines relative to total chlorines). In this study, CPB along the core was calculated (equation (1)) and is shown in Fig. 2.

$$CPB = \frac{\sum_{i=1}^n \text{Number of Chlorines}_i \times \text{molar concentration}_i}{\sum_{i=1}^n \text{molar concentration}_i} \quad (1)$$

where n is the number of compounds.

The CPB trends were similar at sites 1 and 2 but different at site 3 in the top segments. Significant decreases in CPB from the upper core to the deeper core at sites 1 and 2 were observed (site 1: $r = -0.734$, $p < 0.01$; site 2: $r = -0.719$, $p < 0.01$), while CPB increased in the top five intervals and then significantly decreased with core depth at site 3 (1–17 cm: $r = 0.712$, $p > 0.05$; 18–41 cm: $r = -0.786$, $p < 0.05$). These CPB profiles might indicate different degrees of PCB dechlorination in the three sediment cores. To clarify the role of microbial dechlorination in the CPB variation, qPCR was utilized to determine the abundance of the obligate organohalide-respiring bacteria (OHRB) – *Dehalococcoides* (Zhen et al., 2014).

As shown in Fig. S4, the abundance of *Dehalococcoides* ranged from 3×10^5 to 1.5×10^6 , 9×10^4 to 6×10^5 , and 5×10^4 to 1×10^7 copies/g (dw) at sites 1, 2, and 3, respectively. Although the abundance changes of *Dehalococcoides* in the 1–20 cm intervals at sites 1 and 3 were in line with the CPB changes in the corresponding sediment segments (Figs. 2 and S4), these abundance data were not suitable to be directly used to explain the variation in CPB. In fact, the dechlorination degree of PCBs (as estimated by CPB) was related to not only the abundance of PCB dehalogenators but also the PCB concentrations. Therefore, it was more reasonable to normalize the abundance of *Dehalococcoides* by the total molar concentration of PCBs (TPCB) for CPB evaluation. As expected, statistically significant ($p < 0.01$) negative correlations between CPB and Log Dhc/TPCB were observed at all of the three sites (Fig. 2). Contrary to the trend of CPB, the Log Dhc/TPCB had a significantly increasing trend along the sediment depth at sites 1 and 2 (site 1: $r = 0.955$, $p < 0.01$; site 2: $r = 0.913$, $p < 0.01$), while it decreased followed by increasing with the core depth (1–17 cm: $r = -0.902$, $p < 0.05$; 18–41 cm: $r = 0.743$, $p < 0.05$). Based on the above analyses, three conclusions could be tentatively drawn: 1) *Dehalococcoides* likely participated in PCB dechlorination in sediment; 2) CPB could characterize the degree of PCB dechlorination; 3) except for the top several segments at site 3, the significant correlation of decrease of CPB and increase of Log Dhc/TPCB with sediment depth suggested that the PCB dechlorination degree increased along the sediment depth. Overall, the influence of PCB or other POP

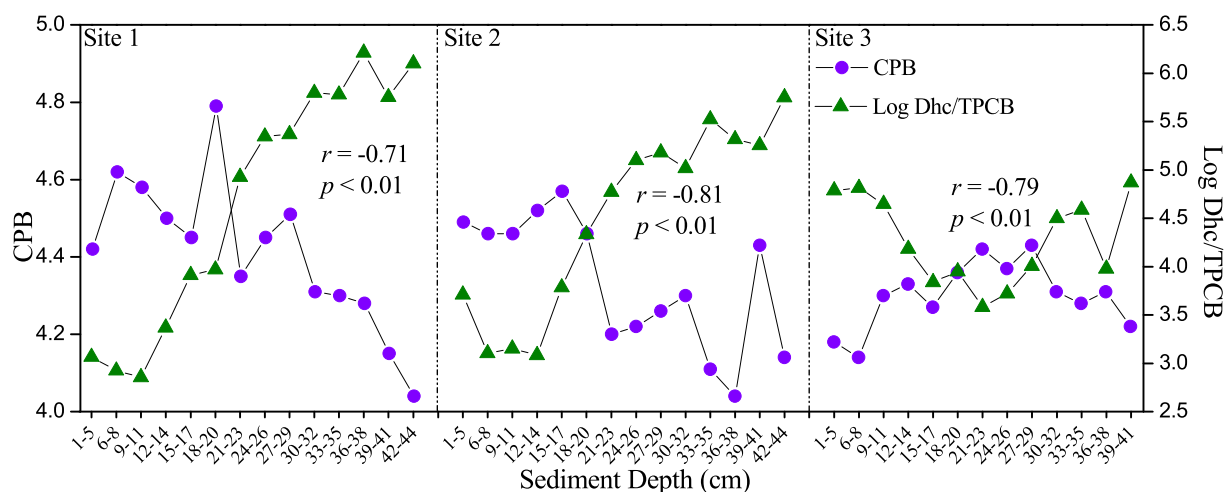


Fig. 2. The correlation between CPB and Log Dhc/TPCB in the three sediment cores. (Violet circles represent CPB, corresponding to the left ordinate; olive triangles represent Log Dhc/TPCB, corresponding to the right ordinate.). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

concentrations and homolog-specific vertical migration of PCBs on the variations in CPB and Log Dhc/TPCB could not be excluded. Therefore, further analytical methods need to be applied to confirm the biotransformation, such as enantiomer analysis and compound-specific stable isotope analysis.

3.3. Enantiomer analysis

Enantiomer analysis of chiral PCBs could provide a promising approach to trace microbiologically mediated processes in complex sediment media (Pakdeesusuk et al., 2003; Wong et al., 2001). In the present study, EFs were obtained for 10 PCB congeners: PCB 45, 91, 95, 132, 135, 136, 149, 174, 176, and 183 in interval 1–20 cm, 1–17 cm, and 1–32 cm for sites 1, 2, and 3, respectively. All EFs were corrected with chiral PCB standards and Aroclor mix standards (Aroclor 1242:1248:1254:1260 = 1:1:1:1). As shown in Fig. 3, three sediment cores had significantly nonracemic EFs for most detected chiral PCB congeners. Nonracemic EFs were determined for PCB 45 at site 3 and PCB 149 at site 1, and PCB 91, 132, 135, and 176 in the three sediment cores had EFs significantly different from 0.5 (t -test, $p < 0.05$) (Fig. 3). The nonracemic composition observed in the sediment cores is strong evidence of stereoselective biotransformation or generation mediated by microorganisms (Wong et al., 2007). The racemic or near-racemic EFs of PCB 45 at sites 1 and 2; PCB 149 at sites 2 and 3; and PCB 95, 136, 174, and 183 at sites 1, 2, and 3 were also observed in the present study. Some of the observed sediment enantiomer profiles in this study were consistent with those in previous studies, but others were not. Wong et al. reported nonracemic EFs for PCB 91, 95, 132, 136, 149, 174, 176, and 183 in Hudson River, Housatonic River and Lake Hartwell sediments (Wong et al., 2001, 2007). Yu et al. also found enantioselective dechlorination of PCB 174, 149, and 132 in laboratory microcosm studies (Yu et al., 2018). As shown in Fig. S5, all of the chiral congeners had the same concentration variations with the total PCBs in the three sediment cores (Fig. S5 and Fig. 1). The EF values of these four chiral PCBs exerted similar trends with increase following by decrease and increase along with the core at sites 1 and 2, respectively, while the EF values of PCB 91 and 176 generally decreased with the core depth and the opposite trends were found for PCB 132 and 135 at site 3 (Fig. S5). Specifically, it was interesting to note that the EF values followed the concentration variations for nonracemic PCBs at site 1, suggesting that concentrations might affect the EF values via changing mass transport or degradation

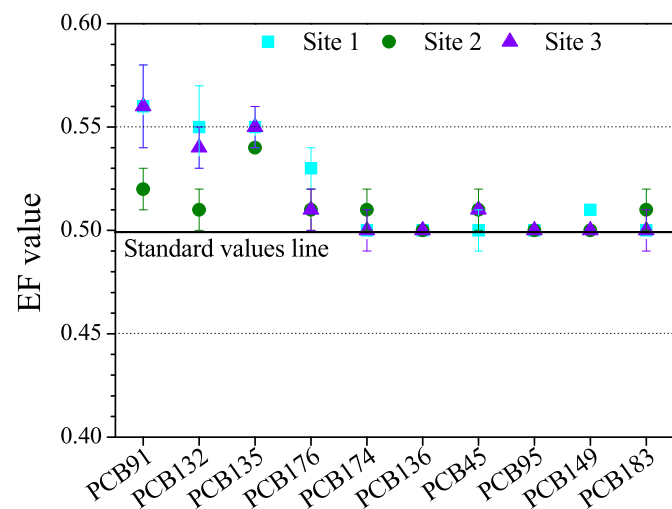


Fig. 3. The EFs of chiral PCB congeners in the sediment cores.

rates of chiral PCBs. Liu et al. reported that enantiomer fractionation could be changed with different degradation rates or different extent mass transfer limitation of enantiomers (Liu et al., 2019). Further relevant researches were needed to clarify the potential effects of the concentration on the enantiomer fractionation.

The variation EF trends along with the core and the discrepancies similarities and differences in EF patterns between the present study and previous studies may have resulted from differences in the microbial composition. Even the same microbial consortia may behave mutative growth under different environmental conditions, such as energy sources, temperatures, pH, and osmolality (van Elsas et al., 2011). These variations of microbe growth could change the expression of chiral degradation functional enzymes, and then affected the degradation kinetics of individual enantiomers, resulting in variable EF patterns (Liu et al., 2019). Another possibility is that the enantiomer compositions of PCBs in the field may be influenced by generation from more than one chiral or prochiral parent and/or by biotransformation to more than one daughter congener, resulting in an increase or decrease in the EF. Thus, the dechlorination pathways are much more complicated in situ, and enantiomer analysis is useful only for stereoselective pathways. Other techniques are necessary to study PCB biotransformation within sediments.

3.4. Compound-specific stable carbon isotope signatures

To elucidate the degradation process of the most dominant PCB congeners, which are usually achiral compounds, the compound-specific stable carbon isotope signatures of PCBs were measured. In this study, the $\delta^{13}\text{C}$ values of 24 congeners in total were determined in the 1–20 cm and 1–29 cm intervals at sites 1/2 and 3, respectively. The GC-C-IRMS chromatograms of these PCB congeners in sediment samples are given in Fig. S6. Except for several congeners that coeluted with non-PCB compounds or were not accurately quantified due to a low concentration, the detailed PCB carbon isotope data are given in Tables S3 and S4. The $\delta^{13}\text{C}$ values of these PCB congeners ranged from -28.66 to -24.87‰ , -29.18 to -25.33‰ , and -29.41 to -25.60‰ at sites 1, 2, and 3, respectively.

Generally, the $\delta^{13}\text{C}$ values of most PCBs at sites 1 and 2 were comparable and significantly higher than those from the corresponding layers at site 3 ($p < 0.05$) (Fig. 4 and Table S5). These results suggested that the sediment cores at sites 1 and 2 likely had the same PCB source that was different from the source of PCBs at site 3, as different manufactured PCB products have different carbon isotope compositions (Hori et al., 2005). These isotope data further supported the occurrence of two massive PCB input events implicated by the different maximum concentrations and associated depths in the three sediment cores. Of course, different extent of microbial transformation might also have a contribution to the isotopic difference of PCBs at three sampling sites.

Significant carbon isotope composition variations ($\Delta\delta^{13}\text{C} > 1\text{‰}$), defined as the greatest difference in the isotopes of the designated congeners in the same sediment core ($\Delta\delta^{13}\text{C} = \delta^{13}\text{C}_{\text{max}} - \delta^{13}\text{C}_{\text{min}}$), were observed for PCB 22, 42, 64, 87, 91, 97, 99, 101, 118, 107/149, and 153/132 at site 1; for PCB 8/5, 22, 44, 48/47, 64, 70/66/95, 87, 91, 97, 101, 118, 107/149, and 153/132 at site 2; and for PCB 18, 22, 31/28, 42, 44, 49, 52, 64, 87, 91, 97, 99, 101, 105, 110, 107/149, and 153/132 at site 3 (Table S5). All of these congeners' $\delta^{13}\text{C}$ values also significantly differed among the different intervals of the same sediment core relative to the values from repeated analysis of the same sample ($p < 0.05$) (SI Table S6). These compound-specific isotope variations suggested that significant PCB microbial degradation occurred in the sediment cores (Fischer et al., 2016).

The carbon isotope trends with sediment depth were analyzed

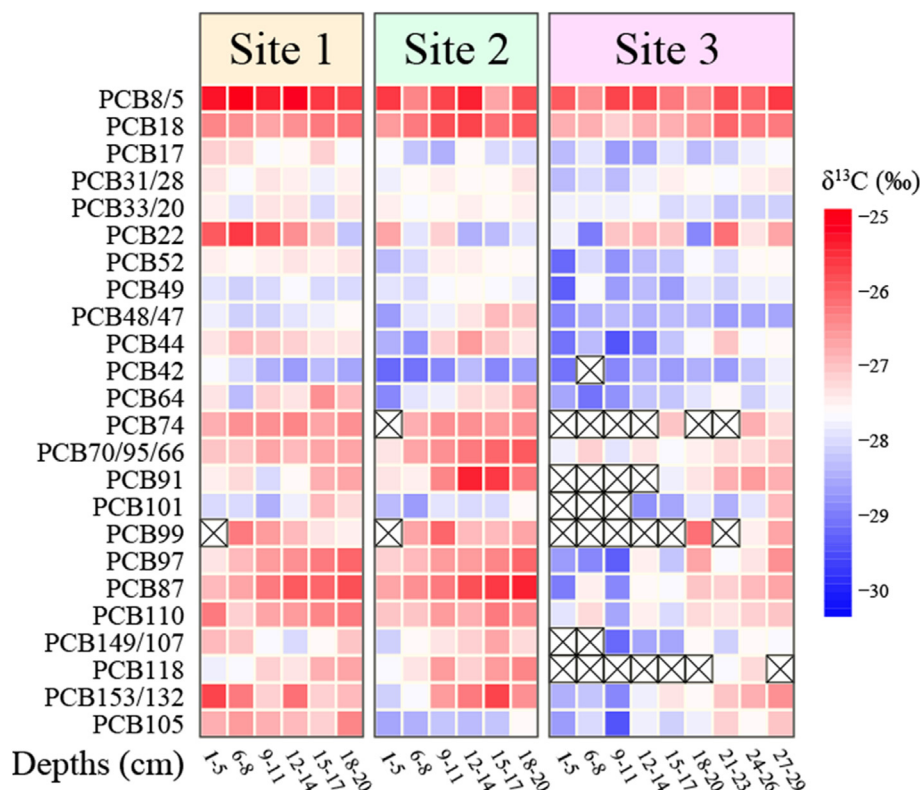


Fig. 4. The $\delta^{13}\text{C}$ values of individual PCB congeners in the sediment cores (The symbol "X" represents that the $\delta^{13}\text{C}$ values were not detected).

(Table S5). Most PCB congeners with significant isotope fractionation effects ($\Delta\delta^{13}\text{C} > 1\text{‰}$) showed ^{13}C enrichment from the surface to the bottom layers (Figs. 4 and S7). In particular, PCB 97, 87, and 118 at site 1; PCB 48/47, 64, 70/95/66, 97, 87, 118, 107/149, and 153/132 at site 2; and PCB 18, 31/28, 52, 44, 42, 64, 101, 97, 87, 105, 107/149, and 153/132 at site 3 had significant ^{13}C enrichment with increasing sediment depth (Fig. S8). As the sediment depth increased, the ^{13}C enrichment of these PCB congeners was approximately in agreement with the decrease in CPB and increase in Log Dhc/TPCB in the sediment cores (Figs. 2 and 4), demonstrating that these PCB congeners underwent significant microbial degradation in the sediment cores because degradation generally results in ^{13}C enrichment in the native congeners (Tang et al., 2017). A similar degradation process that caused ^{13}C enrichment along the core was previously found for PBDEs in the same sediment cores (Huang et al., 2019). However, there were inconsistent trends between the $\delta^{13}\text{C}$ values and CPB (or Log Dhc/TPCB) in the top intervals at site 3 (Figs. 2 and 4). The reasons for this might include the following: 1) Various physical processes, such as the migration discussed above, could alter CPB values but not isotope composition (Elsner, 2010). 2) In addition to dechlorination, other microbial PCB degradation processes that create non-PCB products could also occur in field sediments (Liang et al., 2014b; Rhee et al., 1993), resulting in isotope fractionation and complicated CPB variations.

^{13}C depletion with depth was also observed in the sediment cores, including PCB 22, 42, 99, and 153/132 at site 1 and PCB 8/5 and 22 at site 2 (Figs. 4 and S7). These compounds were likely created by microbial dechlorination of higher chlorinated PCB congeners and inherit more ^{12}C from metabolic substrates (Drenzek et al., 2001). As shown in Fig. 4, the same PCB congener (e.g., PCB 22, 42, 99, and 153/132) may have experienced two antipodal carbon isotope enrichments in different sediment cores, suggesting that it underwent various microbial degradation

processes mediated by multiple PCB-degrading bacterial species in field sediments. Given the complexity of the microbial degradation process in the field, PCB congeners may simultaneously undergo generation and degradation mediated by microorganisms, leading to ^{13}C depletion and enrichment. The $\delta^{13}\text{C}$ values observed in Fig. 4 essentially describe the end-isotope compositions of PCB congeners that synthesize the ^{13}C depletion and enrichment since the original isotope composition.

4. Conclusions

- The difference in the maximum PCB concentrations and associated depths between sites 1/2 and 3 and the corresponding significant ($p < 0.01$) difference in $\delta^{13}\text{C}$ values were observed.
- The variation in CPB had a significant negative correlation with Log Dhc/TPCB along the cores.
- Nonracemic compositions and pronounced stable carbon isotope fractionation ($\Delta\delta^{13}\text{C} > 1\text{‰}$) of PCB congeners were detected.
- The MLEA could provide insight into PCB source identification and postdepositional mobility and transformation, including in situ microbial PCB degradation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.watres.2020.115977>.

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