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Enantioselective accumulation of chiral polychlorinated biphenyls in lotus plant (*Nelumbonucifera* spp.)

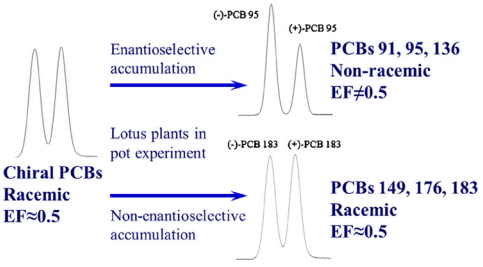
Shouhui Dai[a,c], Charles S. Wong[b], Jing Qiu[a,*], Min Wang[a], Tingting Chai[a], Li Fan[a], Shuming Yang[a]

[a] Institute of Quality Standard & Testing Technology for Agro-Products, Key Laboratory of Agro-product Quality and Safety, Chinese Academy of Agricultural Sciences; Key Laboratory of Agri-food Quality and Safety, Ministry of Agriculture, Beijing 100081, China
[b] Richardson College for the Environment, University of Winnipeg, Winnipeg MB R3B 2E9, Canada
[c] State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China

HIGHLIGHTS

- Enantioselective accumulation of chiral PCBs in lotus plants was investigated.
- The atropisomeric optical rotation of PCBs 91 and 95 were identified.
- Preferential accumulation of (−)-enantiomers of PCBs 91, 95 and 136 from sediment.
- Non-enantioselective accumulation for PCBs 149, 176 and 183 via root uptake.

GRAPHICAL ABSTRACT



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ABSTRACT

Enantioselective accumulation of chiral polychlorinated biphenyls (PCBs) 91, 95, 136, 149, 176 and 183 was investigated in lotus plants (*Nelumbonucifera* spp.) exposed to these chemicals via spiked sediment, to determine uptake and possible biotransformation for aquatic phytoremediation purposes. The concentrations of most PCBs were greatest in roots at 60 d (19.6 ± 1.51–70.6 ± 6.14 μg kg^{-1}), but were greatest in stems and leaves at 120 d (25.3 ± 6.14–95.5 ± 19.4 μg kg^{-1} and 17.4 ± 4.41–70.4 ± 10.4 μg kg^{-1}, respectively). Total amounts were greatest at 120 d and significantly higher in roots than those in stems and in leaves (1457 ± 220–5852 ± 735 ng, 237 ± 47.1–902 ± 184 ng and 202 ± 60.3–802 ± 90.2 ng, respectively), but represented less than 0.51% of the total mass of PCBs added to sediments, indicating that lotus plants were unlikely to remove appreciable amounts of PCBs from contaminated sediments. Racemic PCB residues in sediment indicate no enantioselective biodegradation by sedimentary microbial consortia over the entire experiment. Preferential accumulation of the (−)-enantiomers of PCBs 91, 95 and 136 were observed in roots, stems and leaves, but non-enantioselective accumulation was observed for PCBs 149, 176 and 183. These results indicate that aquatic plants can accumulate PCBs enantioselectively via root uptake, possibly by biotransformation within plant tissues as observed for terrestrial plants. This is also the first report to identify optical rotation of the atropisomers of PCBs 91 and 95.

1. Introduction

Polychlorinated biphenyls (PCBs) are a group of persistent organic pollutants that are of concern because of their persistence, toxicity and bioaccumulation [1]. Of the 209 PCB congeners, 19 have stable chirality from atropisomerism at ambient temperatures.

* Corresponding author at: 12 Zhongguancun South Street, Beijing 100081, China. Tel.: +86 10 82106560; fax: +86 10 82106500.
E-mail addresses: qiujing@caas.cn, jinn.qiu@hotmail.com (J. Qiu).

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These atropisomers (hereafter referred to also as enantiomers) exhibit identical physical and chemical properties in the environment, however, may have enantioselective biochemical processes and toxicities. For instance, they display different ability on changes in calcium uptake [2] that can lead to neurotoxicity [3], and induce amounts and activity of some enzymes. As a phenobarbital-type inducer, (+)-PCB139 can enhance aminopyrine *N*-demethylase, aldrin epoxidase and cytochrome P-450 (CYP) content more potently than (−)-PCB139 [4]. The (+)-enantiomers of PCBs 88 and 197 can induce much greater activity of ethoxyresorufin-*O*-deethylase (EROD) but the (−)-enantiomers are inactive for this [5]. Thus, evaluation of environmental fate and toxicity based on the racemate of chiral contaminants, including PCBs, is not thorough and accurate. Enantioselective biochemical processes would result to the change in enantiomer fraction (EF) of chiral chemicals, a good indicator of biological selectivity to trace enantiomer-specific metabolic processes and toxicological effects [6].

Although chiral PCBs were originally released to environment as racemates, they may be present in non-racemic proportions due to enantioselective biological processing. There are many reports of such enantioselectivity of chiral PCBs in animal species, such as mice and rats [7], fish, dolphins [8,9], spider [10], mysids [11], rainbow trout [12], and humans [13,14]. However, the few reports of accumulation and biotransformation of chiral PCBs in autotrophic species provide conflicting evidence as to the ability of these species to biodegrade such chemicals. There was no evidence that phytoplankton could biotransform PCBs, given observed racemic residues in some studies [15], or evidence of uptake from water of already non-racemic residues [16]. Likewise, non-racemic PCB 95 residues in grass reflected that volatilized from the underlying soil [17]. Zhai et al. [18] found that PCB 95, but not PCB 136, was enantioselectively removed in whole poplars hydroponically exposed to racemates of both congeners. Additionally, they also reported poplars as a potential plant for phytoremediation, as these trees can take up and translocate some lower chlorinated PCBs, and metabolize PCBs 33 and 77 to their hydroxlated PCBs [19]. Biotransformation capacity for PCBs was also confirmed in eucalyptus, particularly in leaves [20]. However, it is not clear what plants, if any, can be used for phytoremediation in the aquatic environment, to remediate contaminated sediments.

Lotus (*Nelumbonucifera* spp.) is an aquatic plant with a huge cultivated area of more than 2700 km^2 and a yearly output of 7.34 million tonnes in China [21]. Lotus roots have a number of culinary and medicinal uses. In addition, some studies have shown that lotus plants can accumulate pollutants such as pesticides and heavy metals from sediment and water [22,23]. Thus, lotus may accumulate PCBs and be usable for phytoremediation purposes, particularly if biotransformation of PCBs also occurs. This accumulation also may lead to contamination of food at contaminated sites for which phytoremediation was not intended. Biotransformation can be detected by shifts in enantiomeric composition of chiral chemicals [18]. Thus, the aim of this study was to investigate bioaccumulation of chiral PCBs in lotus plants and possible presence of enantioselectivity to assess its use for phytoremediation and capacity to contaminate food.

2. Experimental

2.1. Materials and chemicals

Racemic PCBs 91 (2,2′,3,4′,6-pentachlorobiphenyl), 95 (2,2′,3,5′,6-pentachlorobiphenyl), 136 (2,2′,3,3′,6,6′-hexachlorobiphenyl), 149 (2,2′,3,4′,5′,6-hexachlorobiphenyl), 176 (2,2′,3,3′,4,6,6′-heptachlorobiphenyl) and 183 (2,2′,3,4,4′,5′,6-heptachlorobiphenyl) (purities ≥ 97%) were obtained from AccuStandard (New Haven, CT, USA). Stock solutions were prepared in isooctane at 200 μg mL^{-1}, with working solutions prepared by dilution. All standards and solutions were stored in amber glass vials at 4 °C.

Isooctane, acetone and *n*-hexane were all of HPLC grade (J.T. Baker, Phillipsburg, NJ, USA). Analytical grade sulfuric acid was obtained from the Beijing Chemical Plant (Beijing, China). Chem tube-hydromatrix diatomite and florisil solid-phase extraction (SPE) cartridges (500 mg, 3 mL) were obtained from Agilent (Santa Clara, CA, USA) and used for extraction and cleanup, respectively. A Chirasil-Dex capillary column (25 m × 0.25 mm I.D. × 0.25 μm df) from Agilent was used to separate enantiomers of PCBs 91, 95, 136, 149 and 176. A BGB-172 capillary column (30 m × 0.25 mm I.D. × 0.18 μm d_f from BGB Inc., SWE) was used to separate enantiomers of PCB 183.

2.2. Exposure experiment

Young lotus plants were grown in a PCB-free pond before they were used as the model plant in this work. Exposure control was executed in 20 small pots (50 cm diameter × 20 cm height) containing spiked sediment. Spiked sediment in each pot was created as follows: PCBs 91, 95, 149, 176 and 183 (1458 μg each) and PCB 136 (5833 μg) were dissolved in 100 mL acetone. This solution was mixed for 30 min into 1 kg of PCB-free sediment from a PCB-free pond in a Beijing suburb, and then diluted with 34 kg of PCB-free sediment to obtain spiked sediment (41.7 μg kg^{-1} nominal for all PCBs except 136 at 167 μg kg^{-1}) for exposure experiments. The healthy, actively growing, young, whole lotus plants were transplanted to pots of exposure and blank plant controls, with one plant per pot. Blank plant controls consisted of five pots containing PCB-free sediment with the same weight as spiked sediment. Dead plant controls were obtained from four dead lotus plants from the exposure controls after 30 d. After transplantation, 2 cm height of water was added, which was maintained over the experiment. No fertilizer was used.

Sediment (1000 g per pot), water (500 mL per pot), and quadriplicate plant tissue samples (roots, stems, leaves) in the exposure controls and blank plant controls, were collected at 30, 60, 90, 120 and 150 d. Root, stem and leaf samples were also collected from the dead plant controls at 30 d. Plant samples were rinsed with water to remove debris, dried with absorbent paper, weighed and then ground with a GM200 Grinder (Retsch Co., Germany) prior to extraction. All samples were kept in refrigerator at −20 °C until analysis.

2.3. Enantiomeric analysis

Extraction, cleanup and quantification of PCBs by enantioselective gas chromatography–mass spectrometry (GC–MS) were done as previously reported [24], with further details in Supplemental Information. Enantiomeric compositions were expressed as enantiomer fractions (EF) [25]:

$$EF = \frac{E1}{E1 + E2}$$

where E1 and E2 are the concentrations of the first-eluting enantiomers (E1) and the second-eluting enantiomers (E2), respectively.

Based on our previous report [26], the (−)-enantiomers of PCBs 136, 149 and 176 elute first on Chirasil-Dex column compared to their antipodes, while (−)-enantiomer of PCB 183 also elute first on BGB-172 column [27]. However, we are also able to identify the elution orders of the atropisomers of PCBs 91 and 95, which have not been identified until now. Individual enantiomers of both congeners were prepared using a Lux Cellulose-1 (Phenomenex Incorporation, USA) semipreparative cellulose-based chiral

column (250 mm × 10 mm with particle size of 5 μm) with 100% of *n*-hexane as mobile phase at a flow rate of 2.0 mL min^{-1}. Optical rotation was measured in *n*-hexane at 589.3 nm using a polarimeter. The (+)-enantiomer of PCB 91 and (−)-enantiomer of PCB 95 elute first on Chirasil-Dex. The average EFs of racemic PCB standards with standard deviations were 0.502 ± 0.005 for PCB 91, 0.499 ± 0.006 for PCB 95, 0.501 ± 0.002 for PCB 136, 0.501 ± 0.003 for PCB 149, 0.504 ± 0.004 for PCB 176 and 0.498 ± 0.002 for PCB 183 (n = 6).

All data are presented as mean ± standard deviation. Significant differences among were analyzed by one-way ANOVA with Tukey test at α = 0.05.

3. Results and discussion

3.1. PCB accumulation in lotus plants

PCBs were not detected in the sediment and young plants from the PCB-free pond, nor in any sample from the blank plant controls, indicating that the pots and lotus plants were not contaminated over the experimental period. After 30 d, PCBs were detected in the dead plant control (Fig. 1). For example, the concentration and total amount of PCB 91 after 30 d were 17.9 ± 2.17 μg kg^{-1} and 465 ± 51.1 ng, respectively. Abiotic partitioning of hydrophobic compounds, such as PCBs to organic-carbon-rich dead root material, is likely to account for the observed residues of PCBs in dead plant controls. Concentrations and amounts of PCBs in dead roots were significantly lower than those in living roots (Fig. 1). These results indicate that while physical sorption processes might occur in dead roots to accumulate PCBs, living roots have the ability to uptake or translocate contaminants such as PCBs. Because the dead roots began to rot after 30 d, the comparison between the dead and the living roots was not made thereafter.

Living lotus roots continually accumulated PCBs over time (Fig. 1). The total amounts in root were relatively stable from 120 d to the end of the experiment aside from a small decrease observed for PCB 176. However, PCB concentrations increased from 30 d to 60 d, but then quickly decreased after 60 d, except for PCB 183 (Fig. 1), likely from growth dilution. Concentrations and amounts of PCB 136 were significantly greater than those of the other congeners (by 2.1 to 4.1-fold and 2.2 to 4-fold, respectively) given its spiked concentration in sediment was four times greater than those of the other PCBs. Other plants also have been reported to be capable of accumulating organochlorine compounds from solution and soil. After being exposed hydroponically to PCBs 95 and 136 at concentrations of 0.003 mg L^{-1} and 0.002 mg L^{-1} respectively [18,19], poplars accumulated the greatest concentration of PCBs after 20 d. Lesser-chlorinated PCBs 3, 15, 28, 52 and 77 were initially sorbed to the root systems of whole hybrid poplar plants after hydroponic exposure [19]. Chlordane and DDT compounds in soil also can be sorbed by various strains of cucumbers [28].

The changes in PCB concentrations over time in living stem tissues (Fig. 2) were similar to those in roots, with the highest concentrations (25.3 ± 6.14–42.8 ± 8.28 μg kg^{-1} for all PCBs except 95.5 ± 19.4 μg kg^{-1} for PCB136) observed after 90 d and a decrease after 120 d. Concentrations in leaves also increased, with a maximum at 120 d (17.4 ± 4.41–39.0 ± 1.53 μg kg^{-1} for all PCBs except 70.4 ± 10.4 μg kg^{-1} for PCB136), but sharply declined afterwards (Fig. 3), showing changes from those in stems and in roots. The amount accumulated in roots had a similar trend as that of the PCB concentrations in stems and leaves. This observation suggests the rates of stems and leaves were similar to the rate they accumulated PCBs over 120 d. However, roots were very different, with rates of weight increase (data not shown) greater than that of PCB accumulation resulting growth dilution. PCB concentrations declined in stems and leaves rapidly but were stable in roots after 120 d, which may be related to plants having mature roots between 120–150 d but stems and leaves being dead. The total amount of PCBs in roots was the greatest, followed by that in stems (only 13–15% of roots), with the least in leaves. This reason may be related to uptake of PCBs by root tissue via exposure from the contaminated sediment.

There were no significant changes in the concentrations and amounts of any PCBs in sediments over the course of the experiment (Table S1), indicating no significant losses from the sediments by any process. PCB concentrations were in the range of 34.7 ± 2.78–37.0 ± 1.65 μg kg^{-1} for PCBs 91, 95, 149, 176 and 183, and 138 ± 10.7–146 ± 11.8 μg kg^{-1} for PCB 136. Approximately 93.0–96.5% of the mass of each congener added at the beginning of the experiment recovered from the exposure control at the end. The maximum total amount of PCBs accumulated in roots, stems and leaves was 1900–7600 ng at 120 d, which represents only 0.18–0.51% of the initial spiked amounts. Thus, while lotus plants can accumulate PCBs, they did not result in significant loss of PCBs from sediment. Other loss processes would include volatilization.

In water, PCBs were only detected at 30 d with very low concentrations (0.009 ± 0.004–0.027 ± 0.012 μg L^{-1}) (Table S1), and were not been observed after 30 d. Amounts for all congeners in water were significantly lower than those in plant tissues and in sediment at 30 d, respectively. Given these low concentrations in water, and the fact that lotus plants obtain nutrients and water mainly via the roots that were fully covered by contaminated sediment, it is likely that PCBs were accumulated from the sediment rather than from water.

3.2. Enantioselectivity in accumulation

The EFs of all congeners in sediment and water at all times were not significantly different from those of racemic standards (Table S1), indicating that no enantioselective process occurred in sediment and water over the course of the experiment. The EFs of PCBs 91, 95, and 136 (Fig. 4) in living plant tissues were significantly nonracemic, except for that at two time points for PCB 91, while those of all congeners in dead roots were racemic. The racemic residues in dead roots are consistent with PCB accumulation from physical absorption. It is also clear that translocation or biotransformation of PCBs by living lotus was enantioselective.

Preferential enrichment of the (−)-enantiomers for PCBs 91, 95 and 136 were observed in the various living plant tissues. Significant differences were observed between EFs of PCBs 91 and 136 in leaf and in root, and in leaf and in stem, but not in stem and in root for both congeners. These observations indicate that enantioselectivities in root and in stem were more pronounced than those in leaf. The enantioselectivity of PCB 136 observed in the current study contrasts with the near-racemic residues in tissues of whole poplar plants, in which accumulation of the (+)-enantiomer (E2-PCB 95) was observed especially in the middle and bottom xylem [18]. Our observation of (−)-PCB 95 (E1-PCB 95) enrichment in lotus roots is similar to that previously observed in grass [17]. Preferential enrichment of (−)-enantiomers were observed for PCBs 95 and 136 in eucalyptus leaves [20]. Species-specific differences in bioprocessing of PCBs have been observed in various plant and animal species [26,29,30]. Differences in the extent and direction of enantioselectivities may be depend on factors such as growth environment, accumulation, translocation among tissues and enzymatic metabolism.

As with PCBs 91 and 136, more pronounced enantioselectivity was also observed for PCB 95 in roots and in stems compared to that in leaves. Additionally, EFs of PCB 95 in roots and stems were significantly different (α = 0.05) from those of PCBs 91 and 136, indicating

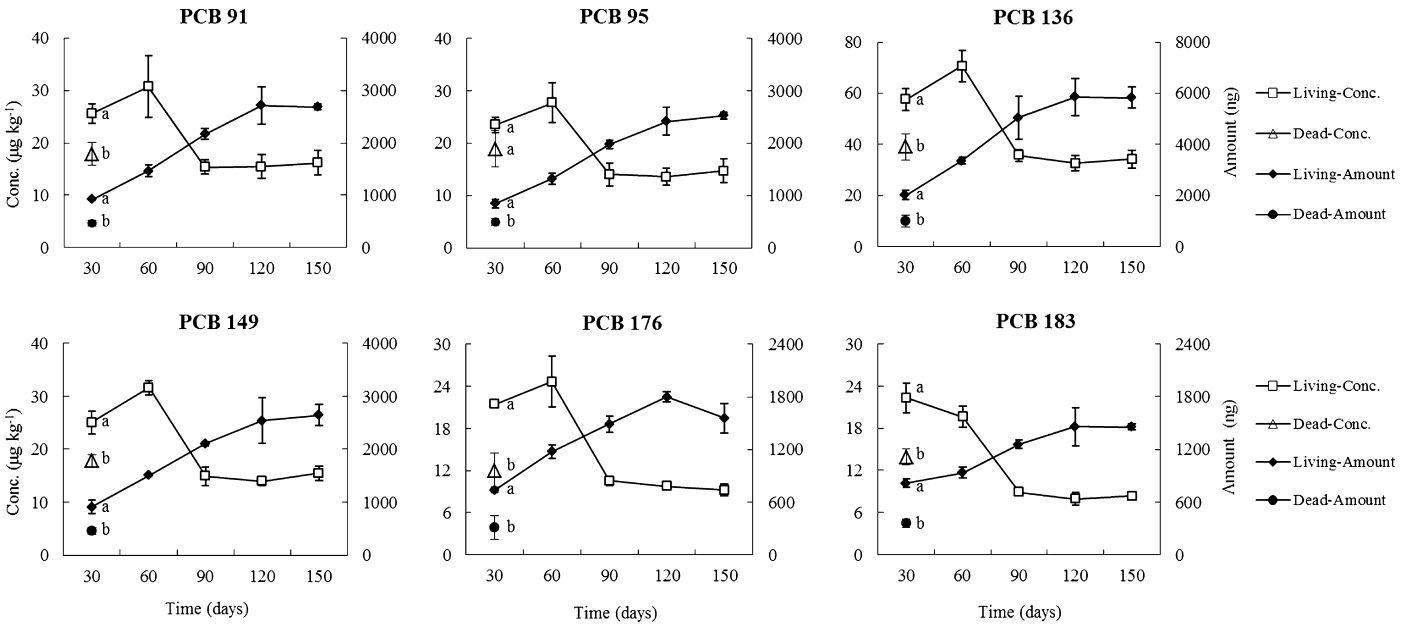

Fig. 1. Concentrations and amounts of PCBs in the roots of living and dead lotus plants over time. Significant differences among concentrations or amounts indicated by different letters by one-way ANOVA.

greater enantioselectivity compared to the latter PCBs. However, the enantioselectivities of all PCBs did not significantly change with growth, because no significant differences ($\alpha = 0.05$) among EFs at any time point between 30–150 d were observed in all tissues for PCBs 91, 95 and 136, respectively.

PCBs 149, 176 and 183 were accumulated non-stereoselectively (Table S1). There is no evidence that lotus plants could biotransform these congeners. This result contrasts that of other reports for other species. Non-racemic signatures were observed for PCB 149 in grass [17] and eucalyptus leaves [20], liver of harbour porpoises (*Phocoena phocoena*) [14], mullet and sea trout [8], PCBs 176 and 183 in grass shrimp, sliver perch, mullet, sea trout, silver perch and dolphin [8], PCBs 149 and 183 in egg yolk and in the blood plasma of adult glaucous gulls [29]. (+)-PCB 149 was preferentially enriched in water snakes and barn swallows but its antipode was enriched in fish, bivalves and crayfish [26].

The enantioselectivities of chiral PCBs in eucalyptus leaves and pine needles were influenced via root uptake from soil and subsequent translocation into the foliage [20]. However, as our data shows, PCBs 91, 95 and 136 had apparent enantioselective residues in lotus plants but were racemic in sediment and water. It is likely that the non-racemic enantioselectivity observed was due to stereoselective processes to PCBs during uptake and/or transformation within roots, although the exact mechanisms are unclear.

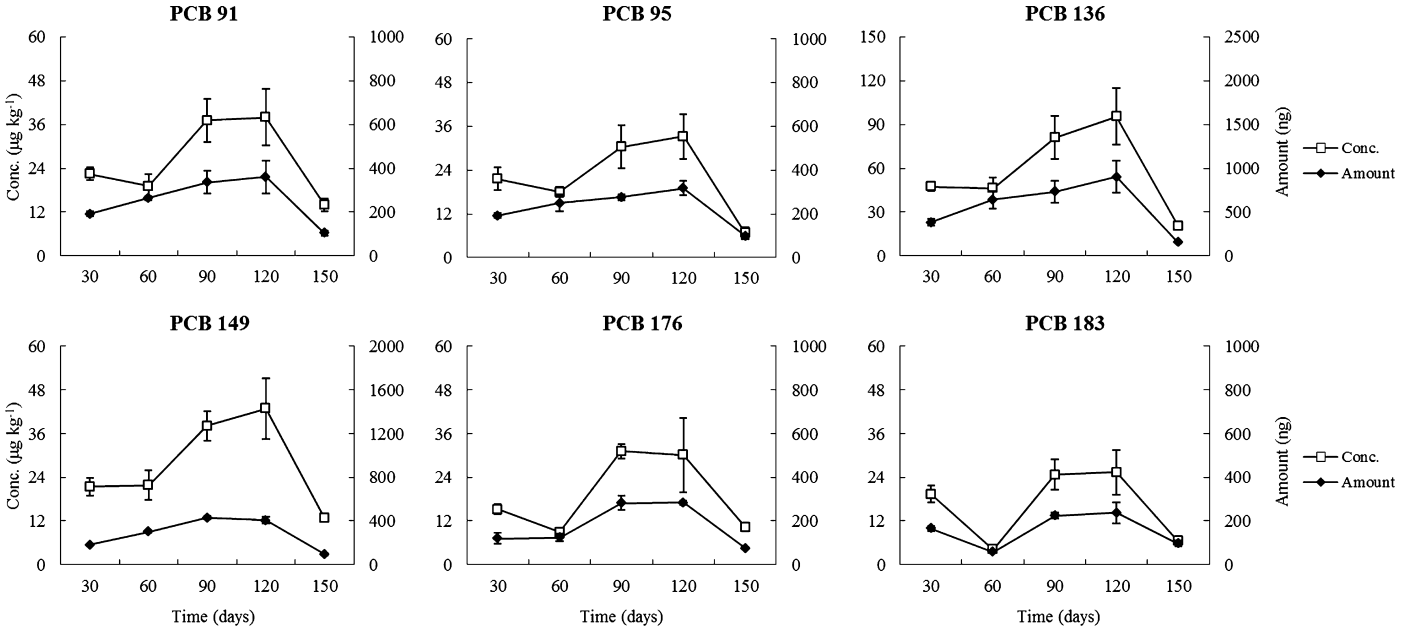

Fig. 2. Concentrations and amounts of PCBs in lotus stems over time.

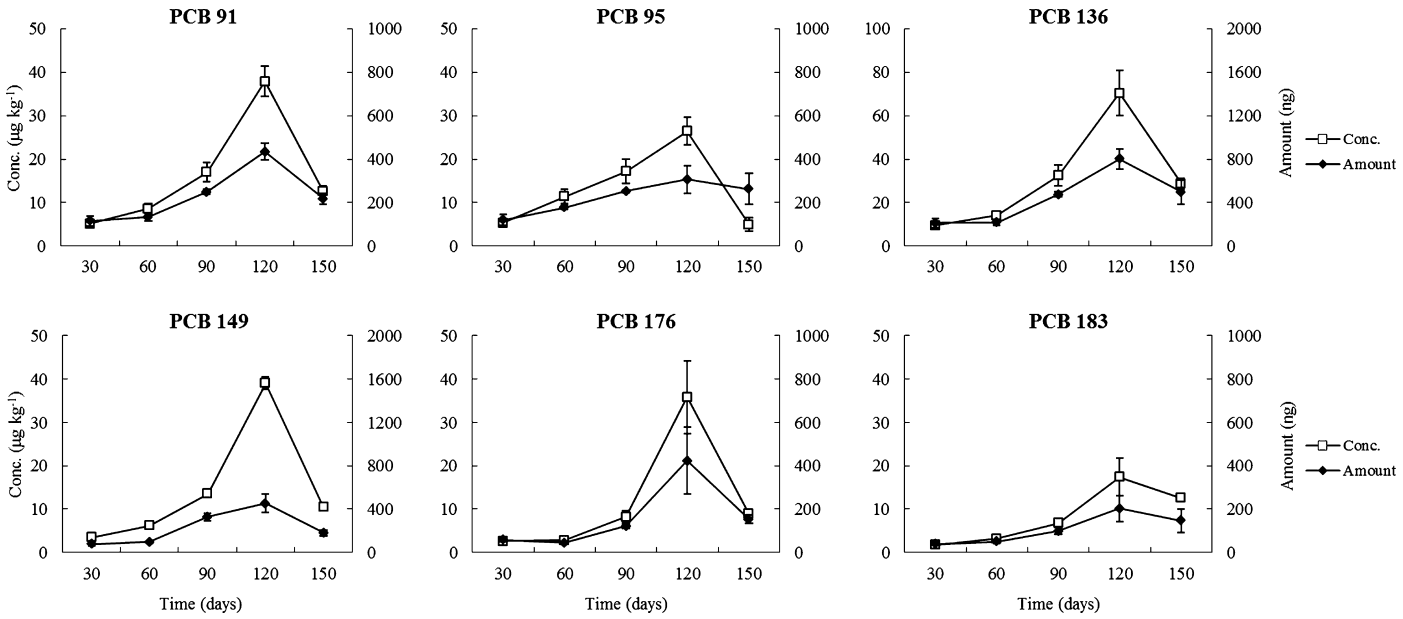

Fig. 3. Concentrations and amounts of PCBs in lotus leaves over time.

The uptake process is not likely to be enantioselective, as has previously been observed for uptake of non-racemic PCBs 95 and 136 to grass from soil [17], attributed predominantly to foliar uptake of volatile emissions from soil [31]. Further changes in the EFs of PCBs in plant tissues may be due to enzymatic metabolism, affinity of PCBs with macromolecules such as proteins and DNA, or other actions between the individual enantiomers. For example, in tissues of mice, preferential binding of (+)-PCB136 to CYP2B and CYP3A in hepatic microsomes may result to the enantioselective enrichment of (+)-PCB136 [32].

Enantioselective metabolism of PCBs by lotus plants might be one another important reason for the observed enantiomer compositions. A major mechanism for changing EFs of PCBs is from stereoselective metabolism by CYPs [33]. For example, enantioselective biotransformation of chiral PCBs in the plant leaves may possibly be due to metabolism by CYP enzymes there [20]. The following rules were used to explain the rates of metabolism of PCBs by CYP isoenzymes [34]. Transformation rates of PCBs decrease with increasing molecular weight (1), and increase with increasing number of meta-para hydrogen atoms (2). Therefore, differences in molecular weight among the PCBs studied could account for the amount of biotransformation, and perhaps EF changes in lotus plants. The order of enantioselectivity in roots at 120 d was PCB 95 > PCBs 91 and 136 > PCBs 149, 176 and 183. Rule (1) may account for this order, but rule (2) does not account for the differences in enantioselectivity because there is only one set of vicinal meta, para hydrogen atoms in PCBs 149, 176 and 183 but two sets for the other two PCBs. Lesser-chlorinated congeners such as PCBs 91 and 95 often show greater enantioselectivity because they can be readily biotransformation by CYPs compared to higher-chlorinated PCBs [35].

CYP2B isozymes more readily biotransform PCB congeners with ortho-meta vicinal H-atoms, which can explain why PCB 183 was accumulated in racemic amounts. Other studies have observed stereoselective biotransformation of PCBs by CYPs, although we cannot determine specific details of this likely process in lotus plants at the current time. For example, (+)-PCB 136 could be preferentially metabolized to 5- and 4-OH-PCB 136 enantiomers, and resulting to enrichment of (−)-PCB 136. The formation of neurotoxic metabolites like 4-OH-PCB 136 may play a role in the developmental neurotoxicity of PCBs [7]. PCBs 91, 95 and 136 can be stereoselectively biotransformed to OH-PCB and dihydroxylated metabolites (diOH-PCB) by rat CYP2B1 *in vitro*, with significantly higher biotransformation activities of (+)-PCB 91 (E1-PCB 91), (+)-PCB 95 (E2-PCB 95), and (+)-PCB 136 but no apparent stereoselectivity for PCB 149 [33]. The formation rate of 5-OH-PCBs formation is congener-specific for these four PCBs. *In vivo*, tissue levels of OH-PCBs increased with increasing dose of racemic PCB 95 in adult female mice following subchronic exposure [36].

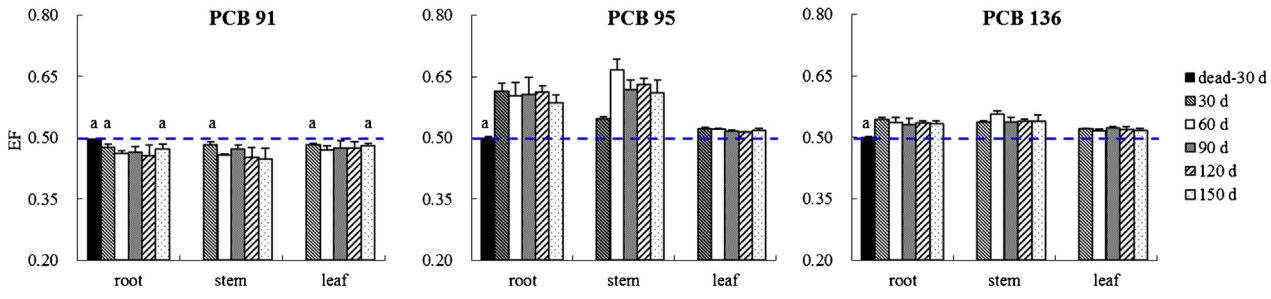

Fig. 4. Comparison of EFs for PCBs 91, 95 and 136 in tissues of dead and living lotus plant over time (significant difference of EFs with standard except "a" by one-way ANOVA).

4. Conclusions

This paper investigated enantioselective accumulation of six chiral PCBs in lotus plants (*Nelumbonucifera* spp.) after being exposed to contaminated sediments. The results found that the highest concentrations of most PCBs were observed at 60 d in roots but at 120 d in stems and leaves, and significantly greater amounts of PCBs at 120 d in roots. However, this accumulation was not enough for phytoremediation of PCB-contaminated sediments. Enantioselective accumulation was observed for PCBs 91, 95 and 136 in roots, stems and leaves with preferential accumulation of the (−)-enantiomers, but PCBs 149, 176 and 183 were racemic in all plant tissues, and all PCBs were racemic in sediment over the entire experiment. These results indicate that chiral PCBs can be enantioselectively accumulated by aquatic plants via root uptake, and then likely biotransformed within plant tissues. To the best of our knowledge, this is the first report of enantioselective accumulation of chiral PCBs in an aquatic plant. While enantioselective accumulation and translocation were observed, future work should clarify what factors may affect these processes.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2014.08.034.

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