Journal of Hazardous Materials 163 (2009) 967–972

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Interspecies variability of Dioxin-like PCBs accumulation in five plants from the modern Yellow River delta

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ARTICLE INFO

Article history:
Received 10 March 2008
Received in revised form 17 June 2008
Accepted 10 July 2008
Available online 25 July 2008

Keywords:
Dioxin-like polychlorinated biphenyls (DL-PCBs)
Plants accumulation
Interspecies variance
Modern Yellow River delta

ABSTRACT

To investigate the interspecies variance of Dioxin-like polychlorinated biphenyls (DL-PCBs) in the plants from modern Yellow River delta, the concentrations of 12 DL-PCBs congeners were examined in five plant species and their associated soils. The DL-PCBs concentrations in plants (2.32–287.60 ng/kg dry weight) were low compared to most published literature, and the concentrations and ratios of DL-PCBs congeners in plants varied greatly among species. The properties of plants and PCBs were then studied to explore the factors affecting the interspecies variance of DL-PCBs accumulation. The plants with the smallest variance of morphological and physiological characteristics (*Imperata cylindrical* var. Major and *Phragmites australis* (Cav.) Trin. ex Steud) had the most similar accumulation patterns of DL-PCBs among the species tested. As the octanol–air partitioning coefficient (K_{oa}) of the DL-PCBs increased, interspecies variance decreased on the whole plant level. Interestingly, the correlation between the DL-PCBs concentrations in plants and $\log K_{oa}$ of congeners was found to be significant for annual plants, but for perennial plants it was not significant. Thus the patterns of uptake of DL-PCBs are different between annual and perennial plants.

1. Introduction

Polychlorinated biphenyls (PCBs) are a group of persistent, lipophilic, bioaccumulative and toxic semivolatile organic pollutants [1]. Due to the ease of long-range atmospheric transport, PCBs can be found even in places where they have never been used [2,3]. Current studies of PCBs influences have focused on human beings and other animals, whereas, studies on vegetation are less intense and widespread [4,5]. Furthermore, only few studies have been conducted on the Dioxin-like PCBs (DL-PCBs) in plants [6].

The patterns of PCBs accumulation in plants have been suggested to be different among species, and vary depending on the properties of both the compounds and the plants [7,8]. The octanol–air partitioning coefficient (K_{oa}), one of the most important physical–chemical properties of lipophilic compounds, can negatively decrease variance among species for PCBs accumulation [8]. Different factors such as leaf surface area, stomata density, cuticle chemical composition, etc., can affect the kinetics of air–plant exchange and the partitioning equilibrium achievement [8–14], hence leading to pronounced species difference for PCBs accumulation. The patterns of interspecies variability of plant accumulation of PCBs have been explored primarily among the grasses [8,12,15] and evergreen plants [13,16], but other species such as deciduous perennial plants have not been studied in detail.

The aims of the present study were to explore the interspecies variance including congener-specific variance in plants and the differences of DL-PCBs accumulation in annual and perennial plants from the modern Yellow River delta. The modern Yellow River delta is the widest and youngest wetland in the warm temperate zone of China. Particularly, it acts as an essential place for birds to rest during migration [17]. However, because of its unique location in the zone between inland and sea, this ecosystem of wetland is fragile and flexible. Hence, our study introduces more detailed chemical characteristics of the ecosystem which can be used to protect the delta.

2. Materials and methods

2.1. Sampling

Vegetation and soil samples were collected on October 21, 2006. The sampling site (N37°44′44″, E119°9′10″) is located in the modern Yellow River delta, which is by the inlet of Yellow River into the sea, in Dongying, Shandong Province, China (Fig. 1).

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doi:10.1016/j.jhazmat.2008.07.081

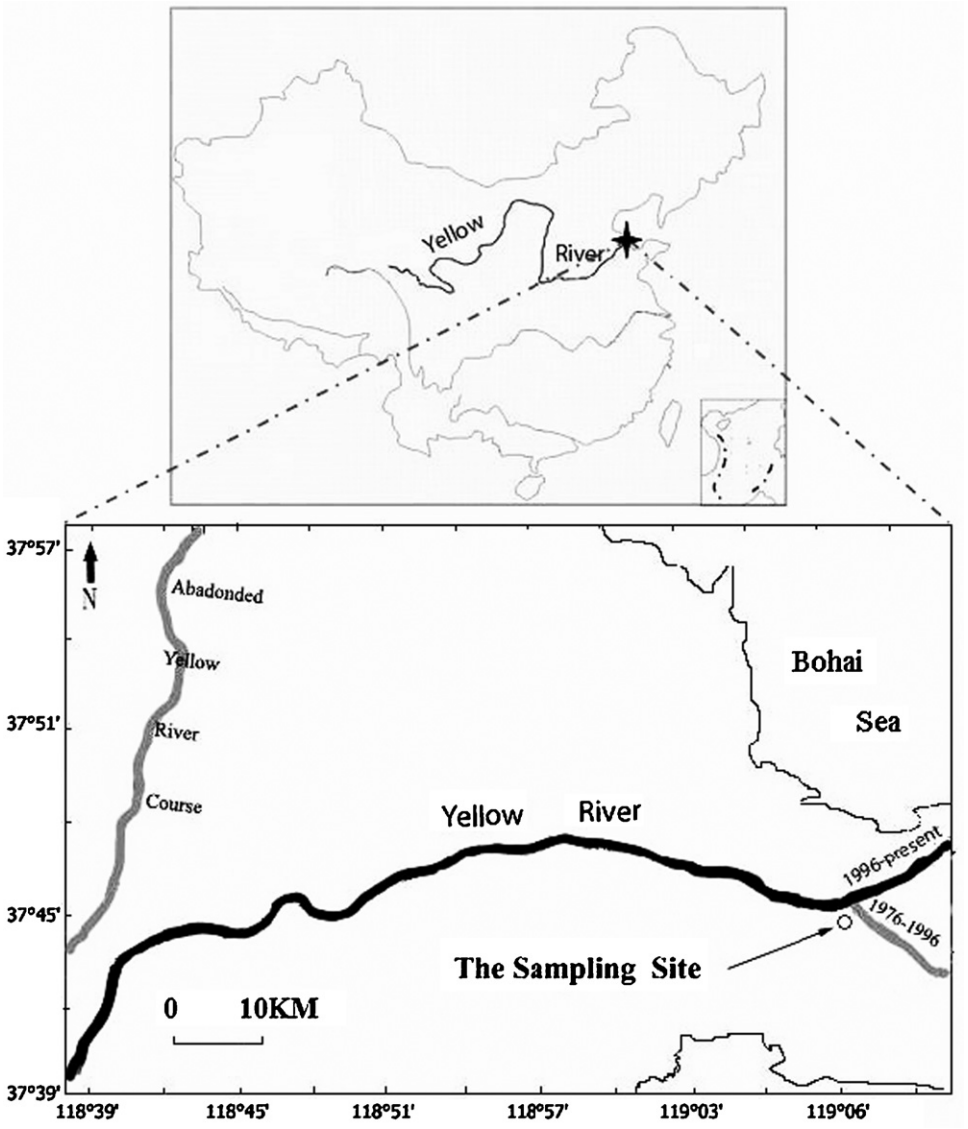

Fig. 1. Geographical location of the sampling site.

The plants in the modern Yellow River delta exhibit patch distribution and the species composition of the vegetation is very simple. Based on the vegetation survey of Xing et al. [18] and our field investigation, we selected five dominant species of the modern Yellow River delta for detailed study: congongrass (*Imperata cylindrical* var. major), reed (*Phragmites australis* (Cav.) Trin. ex Steud), suaeda salsa (*Suaeda Heteroptera*), saltcedar (*Tamarix chinensis* Lout.), and willow (*Salix matsudana* Koidz.). Considering the uniform atmosphere and composition of plant species, a 20 m × 20 m area which was dominated by the five plant species was chosen. At the beginning, the sampling sites were designed at 5 m intervals. As the plants in this area were not with high density but scattered, all five species were not always present on every site. *S. Heteroptera* was only available on three sites. Therefore, to get a comparable number of samples, each of the other four species were sampling at the three sites near *S. Heteroptera*. Thus together 15 sites were chosen finally. The whole plants (exclude willows, only the leaves and branches of the willows were sampled) and the associated 0–10 cm surface soil (about 500 g for each plant or soil sample) were sampled. All the plant samples were washed clean with deionized water, freeze-dried, homogenized, then ground into fine particles and passed through a 20 mesh sieve [6]. The soil samples were also freeze-dried and ground. The plant and soil samples were stored in glass bottles with lids at −18 °C.

2.2. Analysis of DL-PCBs

The plant and soil samples were Soxhlet extracted in acetone and n-hexane (1:1, v/v) for 24 h, and copper was added to reduce the sulfur. Three duplicates of each plant or soil sample were dried at 80 °C for about 48 h to obtain the percent of water in the samples, and the dry weight of the extract was calculated. Then the extract was rotary evaporated to remove acetone and treated with sulfuric acid (USEPA method 3665A) [19]. The PCBs were separated using gel permeation chromatography with Bio-beads SX-3, followed by silica gel and Florisil columns. Activated carbon was used for the final DL-PCBs separations using the following eluents [20]: 15 ml of hexane (fraction A), 20 ml of hexane/toluene 99:1 (fraction B) and 20 ml of toluene (fraction C) under vacuum. Fraction B was analyzed for non-*ortho* PCBs: PCB77, 81, 126, 169, and fraction C was analyzed for mono-*ortho* PCBs, including PCB 105, 114, 118, 123, 156, 157, 167, 189. Then

Table 1
DL-PCBs congener concentrations (ng/kg d.w.) in the five plant species

	Plant species				
	I. cylindrica	*P. australis*	*S. Heteroptera*	*T. chinensis*	*S. matsudana*
PCB77	18.48	35.60	35.76	4.85	76.06
PCB81	2.32	28.46	29.85	167.93	4.64
PCB123	29.53	50.73	28.40	61.98	39.20
PCB118	54.06	90.00	140.55	46.50	78.26
PCB114	16.05	52.48	61.80	43.18	171.12
PCB105	9.19	34.90	8.52	39.90	8.18
PCB126	11.44	16.73	20.88	25.04	27.44
PCB167	21.14	13.60	30.57	26.06	15.71
PCB156	56.55	54.17	77.56	66.73	166.21
PCB157	18.43	55.24	37.92	49.32	27.84
PCB169	237.68	287.60	73.52	37.75	130.00
PCB189	100.61	99.72	185.58	151.15	72.69

the latter two fractions were concentrated to 1 ml for GC analysis.

The samples were analyzed by GC with an electron capture detector (ECD), using a modified dual-column method (USEPA method 8082A) [21]. This favored peak recognition when compounds co-eluted on one column. The injector was at 260 °C and detector was at 300 °C. The first column was a Rtx-5 fused-silica capillary column (30 m × 0.25 mm × 0.25 μm) from Restek (USA). The samples were injected (1 μl) in splitless mode with an autosampler. The carrier gas was nitrogen at a linear flow rate of 32.1 cm/s. The temperature program was as follows: initial temperature at 150 °C (held for 2 min), first ramp at 8 °C/min to 200 °C (held for 2 min), and second ramp at 2 °C/min to 290 °C (held for 15 min). The other column was a Rtx-1701 fused-silica capillary column (30 m × 0.32 mm × 0.25 μm) from Restek (USA). The nitrogen carrier gas' linear flow rate was 19.6 cm/s, and the GC oven temperature was initially 150 °C (held for 2 min), increased at 5 °C/min to 240 °C (held for 30 min). To further identify the DL-PCBs congeners, the plant and the paired soils of each species were analyzed by HRGC/HRMS (USEPA method 1668A) [22] with the first Rtx-5 fused-silica capillary column, in selected ion monitoring (SIM) mode.

A blank was analyzed together for each five samples, and each sample was replicated three times to ensure the accuracy of the results. Calibration standards were injected after every five samples. PCB 209 spiked into surrogates was used as recovery standard, and the recoveries ranged from 75.6 to 87.4%.

2.3. Statistical analysis

All data analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 10.0.

Correlation analyses were performed between DL-PCBs concentrations in plants and soils, $\log K_{oa}$ and DL-PCBs concentrations in plants. Pearson correlation was used to assess the relationships.

Hierarchical cluster analysis was employed among the data of DL-PCBs concentrations in five species. Before cluster analyses, the Kolmogorov–Smirnov test was used to confirm the normal distribution of data. The method used was linkage between-groups. The distances were calculated using square Euclidean distances. The similarities between the analyzed samples were presented in the dendrogram.

A one-way analysis of variance (ANOVA) was used to analyze the significant differences of DL-PCB concentrations between different species or congeners, followed by least-significant difference (LSD) tests ($p < 0.05$). The test of homogeneity of variances was performed before data analyses, and the data was transformed if the criteria were not met.

Table 2
DL-PCBs congener concentrations (ng/kg d.w.) in the associated soils of the five plant species

	Plant species				
	I. cylindrica	*P. australis*	*S. Heteroptera*	*T. chinensis*	*S. matsudana*
PCB77	19.09	17.88	12.57	19.02	16.91
PCB81	6.98	1.78	5.53	7.84	8.35
PCB123	23.66	16.97	19.66	23.93	20.88
PCB118	5.23	20.06	21.78	21.32	10.73
PCB114	167.84	103.58	52.96	38.49	133.46
PCB105	2.48	4.90	3.77	2.57	3.26
PCB126	0.77	16.55	16.14	14.08	16.38
PCB167	1.21	0.77	1.06	1.08	1.33
PCB156	7.53	15.47	3.16	7.43	9.09
PCB157	14.57	10.16	17.54	8.68	11.59
PCB169	1.69	0.57	1.74	1.16	1.73
PCB189	1.95	2.27	3.74	4.39	2.14

3. Results and discussion

3.1. Loadings and sources of DL-PCBs in plants

3.1.1. DL-PCB loadings

The DL-PCBs concentrations in the five species and associated soils were analyzed and the results are listed in Tables 1 and 2. Concentrations were all calculated on the dry weight basis. The average values of DL-PCBs congener concentrations measured in plants (2.32–287.60 ng/kg d.w.) and soils (0.57–167.84 ng/kg d.w.) were low compared to previously published reports [6].

3.1.2. Sources

To identify the sources of DL-PCBs in plants, correlation analyses between the concentrations, congener concentration ratios of DL-PCBs in plants and those in the corresponding soils were carried out (SPSS 10.0). The results show the concentrations and congener ratios of DL-PCBs in the plants were not significantly ($p > 0.05$) related to those in the corresponding soils (Table 3). This indicates that the source of DL-PCBs in plants from the modern Yellow River delta was not from soil, which is in good agreement with the findings that in areas not heavily polluted with PCBs, plants mainly take up PCBs from the atmosphere, and the uptake from soil can be negligible [23]. Cousins and Mackay also found that chemicals with $\log K_{oa} > 6$ entered plants mainly from the atmosphere, not from soil [24]. This is consistent with our results because for DL-PCBs, $\log K_{oa} > 8$.

3.2. Interspecies variance of DL-PCBs accumulation

The concentrations of the DL-PCBs among the five plant species were compared by one-way ANOVA analysis (SPSS 10.0). As Fig. 2 shows, the concentration of total DL-PCBs in *I. Cylindrical* was the lowest among the five species, and the other four species was not significantly different from each other ($p < 0.05$). The DL-PCBs congener concentrations of the five species were not uniform, however. PCB77, PCB81, PCB114, PCB123 and PCB156 (shown in Fig. 3) illustrate the pronounced differences among species. In the 12

Table 3
Pearson correlation between $\log K_{oa}$ and DL-PCBs concentrations in plants

Plant species	Pearson correlation	*p*-Level
I. cylindrical	−0.148	0.390
P. australis	−0.075	0.726
S. Heteroptera	−0.127	0.462
T. chinensis	−0.148	0.426
S. matsudana	0.525	0.080

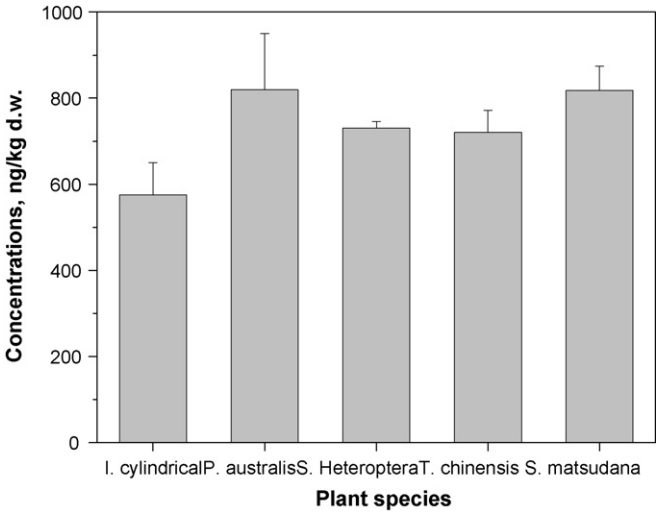

Fig. 2. Total DL-PCBs concentration in plants (error bars show the standard deviation of values).

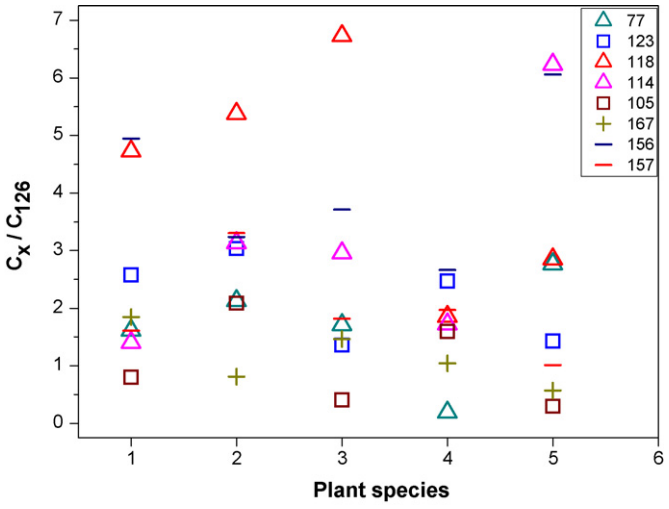

Fig. 4. Ratios of concentrations of PCB *X* (*X* = 77, 123, 118, 114, 105, 167, 156, 157): PCB126 in plants. 1: *I. cylindrical*; 2: *P. australis*; 3: *S. Heteroptera*; 4: *T. chinensis*; and 5: *S. matsudana*.

congeners of DL-PCBs, only the concentrations of PCB126 did not exhibit significant differences between any two of the five species. Thus, to investigate interspecies differences in accumulation of DL-PCBs on the congener level, PCB126 was later compared with the other DL-PCBs congeners. In summary, the accumulation of DL-PCBs in plants is greatly different among species on the congener level. Similar results were also found by Pier et al. [23].

In addition, the ratios of PCB 126 concentrations to other DL-PCBs congeners differed greatly among species (only part of the results were given), as Fig. 4 shows, though these plants had the same source congener ratios due to the uniform atmosphere. This suggests that different species have unique uptake kinetics towards specific congeners, perhaps related to properties of both the plants and the compounds.

3.3. *Factors affecting the interspecies variance of DL-PCBs accumulation*

To explore the factors affecting the interspecies variance of DL-PCBs accumulation in plants, the characteristics of plants and PCBs were investigated as follows.

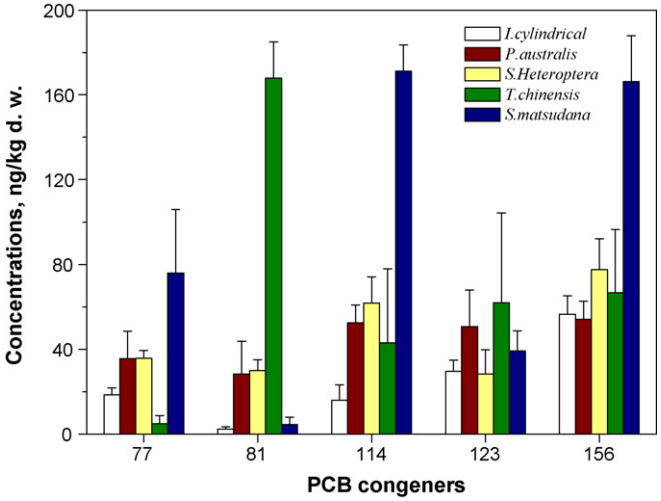

Fig. 3. The concentrations of selected PCBs congeners in the five plants (error bars show the standard deviation of values).

Hierarchical cluster analysis of the five plant species labeled by the concentrations of DL-PCBs congeners was conducted by SPSS 10.0. The results (Fig. 5) show that *I. cylindrical* and *P. australis*, which belong to the same family—Gramineae, had the most similar patterns of DL-PCBs accumulation among the five species. Pier et al. also found that PCBs accumulation in plants varied among different genera [23]. Thus, the accumulation patterns of PCBs in plants appear to be grouped according to the plant taxonomy. This is because the accumulation of PCBs in plants is greatly affected by morphological and physiological characteristics, which have resulted in different resistance to uptake of PCBs [8–12]. For instance, plants with larger surface area and higher lipid content tend to accumulate more PCBs [4,5]; the orientation and shape of leaves influence the kinetics of persistent organic pollutants entering plants [5]; and the amount and composition of epicuticular waxes greatly affect a plant's capacity to take up PCBs [14]. The morphological and physiological characteristics vary among individual plants of the same species, and are increasingly different for different plant genus and types [5]. Consequently, because only *I. cylindrical* and *P. australis* belong to the same family in this study, they have more similar physiology and biochemical characteristics compared to the other three plants. As a result, the variance of their accumulation patterns was the smallest among the five species.

Many studies have showed that the physical–chemical properties of the PCBs, mainly their octanol–air partitioning coefficient, K_{oa}, greatly affected the accumulation of PCBs in plants [5,8,23]. Hence in our study, the correlation of interspecies variance (represented by the ratios of DL-PCBs concentrations in the other four plants to that in *I. Cylindrical*) with log K_{oa} of DL-PCBs was investi-

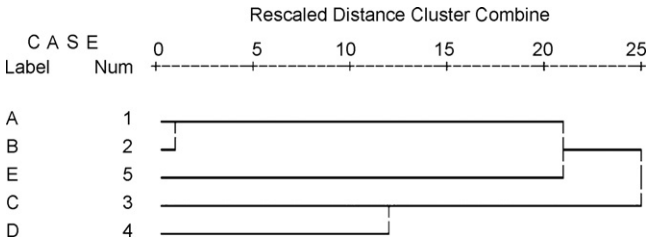

Fig. 5. Dendrogram of the five plants by hierarchical cluster analysis labeled by the DL-PCBs concentrations in the plants. A: *I. cylindrical*; B: *P. australis*; C: *S. Heteroptera*; D: *T. chinensis*; and E: *S. matsudana*.

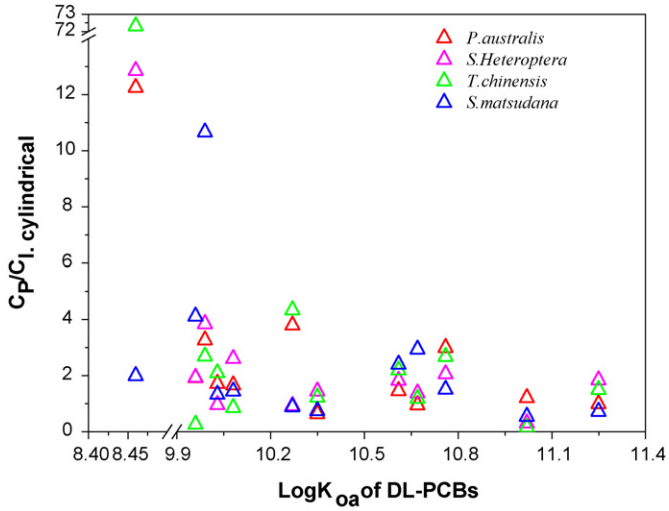

Fig. 6. The ratios of DL-PCBs concentrations (plant: *I. cylindrical*) related to log K_{oa}.

gated. As Fig. 6 shows, the interspecies variance of plants decreased with increasing log K_{oa} of DL-PCBs. Similar results were obtained by Böhme et al. [8], but they studied the leaves of different annual plants, and our research focused on whole plants. Therefore we can draw the conclusion that the properties of PCBs affect the plant interspecies variance not only on the foliage level, but also on the whole plant level. The mechanism of uptake and redistribution of PCBs by plant tissues except foliage is not yet known, and we are continuing to research how different plant tissues respond to PCBs exposure.

3.4. Uptake variance between annual and perennial plants

To explore the accumulation patterns of PCBs among plants of different types, correlation analysis of log K_{oa} and DL-PCBs concentrations in the five plant species was carried out (SPSS 10.0). A positive correlation between log K_{oa} and DL-PCBs concentrations of three annual species (*I. cylindrical*, *P. australis and S. Heteroptera*) was found (Table 4), with Pearson correlation coefficients of 0.390, 0.464 and 0.354 ($p < 0.05$), respectively. But in the perennial species (*T. chinensis* and *S. matsudana*), the correlation between log K_{oa} and DL-PCBs concentrations was not significant ($p < 0.05$). The results can be explained by considering the mechanism of uptake of PCBs. The uptake of PCBs by the plant foliage can be considered as a two-compartment system: plant cuticles absorb airborne PCBs, and PCBs come into inner plant cells [15]. Hung et al. found that in the latter compartment, the uptake of PCBs by grasses was related to log K_{oa} of PCBs, while in the former compartment, no relationship was found [25]. Because the latter compartment is much slower than the former one, the final results of the two compartments were related to K_{oa} [25], which was consistent with our results for the three annual plants, but not consistent with the results of the two perennial species. This inconsistency can be attributed to the deciduous property of perennial species. When the perennial plants took up PCBs, the foliage behaved as a two-compartment system [25], but the perennial tissues of plants perhaps exhibit a different pattern. Thus, when the accumulation is measured as a combination of leaves and other perennial tissues, the effect of foliage performance is considerably reduced because the leaves are refreshed every year. Overall, we suggest that on the whole plant level, annual and perennial deciduous plants have different PCBs accumulation characteristics—annuals are related to log K_{oa} and perennials are independent of log K_{oa}.

Table 4
Pearson correlation between DL-PCBs concentrations in plants and soils

Plant species	Pearson correlation	*p*-level
I. cylindrical	0.464*	0.004
P. australis	0.354**	0.034
S. Heteroptera	0.390**	0.019
T. chinensis	−0.267	0.115
S. matsudana	0.294	0.082

* Correlation is significant at the 0.01 level.
** Correlation is significant at the 0.05 level.

4. Conclusions

The DL-PCBs accumulated in five plants of the modern Yellow River delta showed four main characteristics: (i) the plants and soils in the sampling site of the modern Yellow River delta were not heavily polluted, and the DL-PCBs congener concentrations and ratios in plants differed greatly among species; (ii) *I. cylindrical* and *P. australis* had the smallest variance among the five species for accumulating DL-PCBs, because only these two plants belong to the same family among the five species; (iii) interspecies variance of plants increased with increasing log K_{oa} of PCBs on the whole plant level; and (iv) accumulation of DL-PCBs for annual plants was related to log K_{oa}, while for perennial plants it was independent of log K_{oa}.

Future studies of PCBs accumulation patterns in plants should be widely conducted among different types of plants, such as comparison of evergreen plants and deciduous plants, to further explore the models developed from laboratory experiments.

Acknowledgments

Financial support for this study was supplied by the National Scientific Foundation of China (20577030). We thank Zaifeng Wang in Jinan environment monitoring station for suggestions on methods and the Dongying environmental protection agency for field sampling help. We also appreciate Dr. Pamela Holt for revising this paper.

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