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Variations in phthalate ester (PAE) accumulation and their formation mechanism in Chinese flowering cabbage (*Brassica parachinensis* L.) cultivars grown on PAE-contaminated soils

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ABSTRACT

Phthalate ester (PAE) accumulation in crops poses great risks to human health and has aroused great concern. Here, we investigated variations in di-*n*-butylphthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) accumulation by various Chinese flowering cabbage cultivars and revealed their variation mechanism. There were significant differences ($p < 0.05$) in shoot PAE concentrations of 28 cultivars. Moreover, significant positive correlations between DBP and DEHP concentrations in shoots of all cultivars indicated that they could be taken up simultaneously by various cultivars. Due to the lower translocation factor of low-PAE accumulator, its shoot PAEs concentrations were much lower than root compared to high-PAE accumulator. Further, subcellular distribution showed that PAE concentrations of root cell walls and organelles were much higher than those of shoots in low-PAE accumulator. Therefore, lower translocation from root to shoot and more PAEs accumulating in cell walls and organelles of root might act as main formation mechanism of low-PAE accumulator.

1. Introduction

Phthalate esters (PAEs) comprise the largest class of plastic additives used in industrial and consumer products. They are suspected endocrine disrupting chemicals (EDCs) and thus have effects on reproductive health and development, even at very low concentrations (Lottrup et al., 2006). China National Environmental Monitoring Center, United States Environmental Protection Agency (US EPA), and other international organizations have classified some phthalate compounds [including di-*n*-butyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP)] as priority environmental pollutants (Liao et al., 2010). In recent years, PAE levels in agricultural soils have increased dramatically due to the use of plastic film, wastewater irrigation and sewage sludge application in agricultural fields (Wang et al., 2013; Niu et al., 2014; Zhang et al., 2015). In China, elevated concentrations of PAEs, including DBP and DEHP, are frequently detected in agricultural soils, and higher concentrations of PAEs have been observed in the soils of vegetable fields (Cai et al., 2008a; Liu et al., 2010). Our previous study revealed total concentrations ($\sum_{PAEs}$) of six phthalate compounds listed as US EPA priority pollutants ranging from 0.073 to 11.2 mg kg^{-1} (dry weight, DW), with a mean value of 3.2 mg kg^{-1}, in 11 vegetable species collected from nine farms within the Pearl River Delta region (Mo et al., 2009). Contamination of agricultural soils by PAEs has become increasingly serious in southern China and may pose risks to human health through the food chain.

Recently, a novel alternative strategy of screening for or breeding antipollution crop cultivars has been proposed to reduce human exposure to soil contaminants through the food chain. Some cultivars accumulate specific pollutants at levels low enough for safe consumption even when grown in contaminated soil (Liu et al., 2010; Yu et al., 2006). In the past decades, numerous programs for breeding antipollution or low-accumulation crop cultivars targeting heavy metals (especially Cd) have been conducted in various crops, including rice (Ishikawa et al., 2012), wheat (Stolt et al., 2006), soybean (Hao et al., 2011), barley (Chen et al., 2007), Chinese cabbage (Liu et al., 2010), water spinach (Wang et al., 2009), and hot pepper (Xin et al., 2013). The mechanisms that lead to low accumulation of heavy metals in some crop cultivars

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have been investigated (Ueno et al., 2010; Xin and Huang, 2014), and key genes that limit Cd accumulation in rice have been identified (Ishikawa et al., 2012). However, little has been published on efforts to screen for or breed cultivars that accumulate low amounts of organic pollutants, especially PAEs. Although the uptake of organic compounds by plants has been widely studied in recent decades (Collins et al., 2006; Gao and Collins, 2009; Gao et al., 2013; Li et al., 2014), knowledge of the variations in uptake and accumulation of organic compounds among cultivars is still scarce.

The mechanisms of uptake and accumulation of organic pollutants by plants are governed by the physicochemical properties of the pollutants, environmental conditions, and the plant species (Collins et al., 2006). Recent studies have focused on the subcellular distribution of organic compounds in plants, a factor that may be related to pollutant uptake, translocation, and accumulation in plants (Gao et al., 2011, 2013). Cell walls were identified as the dominant storage compartments for organic contaminants, and they might be involved in the tolerance and detoxification of pollutants in plants (Ling et al., 2012). Gao et al. (2011) found that polycyclic aromatic hydrocarbons (PAHs) could pass through the cell wall boundary, dissolve in cell solution, and partition into organelles in arbuscular mycorrhizal roots of ryegrass (*Lolium multiflorum* Lam.). However, the subcellular distribution of organic compounds is typically reported at the species level, and intraspecies differences have rarely been investigated. Thus, information on the subcellular distribution of organic contaminants at the cultivar level is lacking.

Chinese flowering cabbage (*Brassica parachinensis* L.) is a very important vegetable in southern China, and is also exported to Europe, the Americas, Australia, and other regions. Our previous study revealed high levels of $\sum_{PAEs}$ in Chinese flowering cabbage (Mo et al., 2009). In the present study, 28 cultivars of Chinese flowering cabbage widely cultivated by farmers were planted in the soil spiked with PAEs to investigate variations in PAE accumulation and translocation among cultivars and to screen for low and high-PAE-accumulating cultivars. The distribution of PAEs at the subcellular level in low- and high-PAE-accumulating cultivars was also studied. This work is a basis for further studies on the mechanisms of low-PAE accumulation in Chinese flowering cabbage and the selection of low-pollution accumulators among cultivars of other crops.

2. Materials and methods

2.1. Chemicals and materials

DBP (98.7% purity) and DEHP (99.0% purity) were purchased from Aladdin Chemistry Co. (Shanghai, China). Their chemical structures and physicochemical properties are showed in Supplementary Fig. S1 and Table 1, respectively (Lu, 2009). Other chemicals were obtained from Damao Chemical Reagent Co. (Tianjin, China). The 28 cultivars of Chinese flowering cabbage used in this study were obtained from various research institutes (Supplementary Table S1).

Table 1
Selected physicochemical properties of PAEs.

PAEs	DBP	DEHP
Molecular weight (g mol^{-1})	278.3	390.6
Water solubility (mg L^{-1})	9.9	2.49×10^{-3}
log K_{OW}[a]	4.27	7.73
log K_{OA}[b]	8.45	10.13
log K_{AW}[c]	−4.23	−3.47

[a] K_{OW}, *n*-octanol–water partition coefficients.
[b] K_{OA}, *n*-octanol–air partition coefficients.
[c] K_{AW}, dimensionless air–water partition coefficient.

2.2. Soil preparation and treatments

The levels of PAEs in the pot soil were set at 10 mg kg^{-1} and 100 mg kg^{-1}, respectively. After aging for 2 weeks in a dark environment, the initial soil concentrations of DBP and DEHP in the low-PAE (10 mg kg^{-1}) and high-PAE (100 mg kg^{-1}) treatments were measured at 8.27 mg kg^{-1} DBP and 9.24 mg kg^{-1} DEHP, and 81.35 mg kg^{-1} DBP and 85.92 mg kg^{-1} DEHP, respectively. The original soil, not treated with PAEs, was used as the control (CK). Next, ceramic pots (22 cm diameter × 18 cm height) were filled with the soils at 3.0 kg (DW) per pot and mixed with 6.0 g of chemical fertilizers consisting of urea and KH_2PO_4 (N:P:K = 4:3:4). Finally, 10 seeds of each cultivar of Chinese flowering cabbage were sown into the prepared pot. The soil properties and soil-mixed method were provided in Supplementary M1.

Three replicates were conducted for each treatment of the 28 cultivars (252 pots in total). The pots were randomly arranged in the greenhouse, grown at the natural temperature (25–32 °C), and watered daily with deionized water to maintain moderate soil moisture. During the seedlings grew, they were thinned gradually until five plants were left in each pot by day 15. Soil and plant samples were collected after 45 days of plant growth. The plant samples were washed orderly with tap water and deionized water, and then blotted with filter paper. The fresh plants were separated into roots and shoots with a stainless-steel knife, and both portions were weighed, respectively. The samples were kept frozen at −70 °C until PAE analysis and subsequent subcellular experiments.

2.3. Sample extraction and cleanup

The soil and plant samples were freeze-dried at −55 °C (Heto PowerDry LL3000; Thermo Scientific, MA, USA) and ground to pass through a stainless-steel sieve (0.4 mm). The procedures of sample ultrasonic-assisted extraction and cleanup using silicagel column were conducted following the method described in our previous study (Cai et al., 2007) with modifications (see Supplementary M1).

2.4. GC/MS analysis

Analysis of PAEs in the extracts was performed using gas chromatography coupled with mass spectrometry (GC/MS, Shimadzu QP2010 Plus, Japan) according to our previous method (Cai et al., 2007) with modifications (see Supplementary M1). The detection limits of DBP and DEHP in samples were 1.2 and 2.5 μg kg^{-1}, respectively. The surrogate recoveries in soil and vegetable samples ranged from 92.03% to 96.49% and 87.42%–107.17%, respectively. The detailed information about quality assurance/quality control (QA/QC) was provided in the Supplementary Materials.

2.5. Subcellular fractionation

The subcellular distribution of PAEs in shoots and roots of low and high accumulators was investigated following the method described in previous study (Gao et al., 2013) with modifications (see Supplementary M1). DBP and DEHP of the extracts were analyzed using GC/MS as noted above. The recoveries of DBP and DEHP from cell wall and organelle fractions ranged from 95.12% to 98.81% and 91.44%–107.92%, respectively.

2.6. Data analysis

Results are presented as means ± standard deviation (SD) on a

dry weight basis. Statistic analysis including one-way analyses of variance (ANOVA) followed by Duncan test were performed with Microsoft Excel 2003 and SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

To compare the relative responses of cultivars to the different levels of exposure to PAEs, the index of biomass response to stress (BRS) was calculated for each as follows (Wang et al., 2009):

$$BRS\ (\%) = (B_H - B_L)/BL \times 100$$

where B_H (g) and B_L (g) are the shoot biomasses (DW) with high- and low-PAE treatments, respectively.

3. Results

3.1. Response of biomass to PAE stress

Under each level of PAEs exposure, the variation in shoot biomass among some cultivars was significant ($p < 0.05$), with 3.19–4.71 g plant^{-1} (average of 3.93 g plant^{-1}, DW) for the control, 3.25–4.97 g plant^{-1} (average of 4.02 g plant^{-1}, DW) for the low-PAE level, and 3.05–4.60 g plant^{-1} (average of 3.90 g plant^{-1}, DW) for the high-PAE level (Supplemental Table S2), which suggests that the biomass variation resulting from genetic differences among cultivars was much greater than that from the soil levels of PAEs. Additionally, compared with the control, only the shoot biomasses of three cultivars (*Changhe60*, *Jianyesijiu*, and *Xishengsijiu*) were significantly lower ($p < 0.05$) with the low-PAE treatment (Supplementary Table S2), indicating that the low-PAE treatment was not phytotoxic to most cultivars. The BRS values showed that the shoot biomasses of 20 cultivars treated with low PAEs were higher than those of the corresponding high-PAE treatment, with seven differing significantly ($p < 0.05$). The other eight cultivars displayed a contrary trend, and three of them differed significantly ($p < 0.05$; Fig. 1). The results indicated that significant differences in the tolerance of PAEs existed among the various cultivars.

3.2. Accumulation of PAEs in different cultivars

Under PAE exposure, great variations were observed in the DBP and DEHP concentrations in shoots among the 28 cultivars within each treatment (Fig. 2a, b), with averages of 0.204 mg kg^{-1} (range 0.101–0.373) for DBP and 0.389 mg kg^{-1} (0.125–0.721) for DEHP under low-level exposure, and 1.261 (0.355–2.027) and 4.422 (0.783–8.527) mg kg^{-1} for DBP and DEHP, respectively, under high-level exposure. Hence, for shoot DBP accumulation among the various cultivars, the highest DBP concentrations within the low- and high-DBP treatments were 3.7- (*Changhe60* vs. *Lvbao70*) and 5.3-fold (*BaishunNo.2* vs. *CutiaoNo.31*) of the lowest ones, respectively. Similarly, the highest DEHP concentrations in shoots within the low- and high-DEHP treatments were 5.2- (*Huaguan* vs. *Lvbao70*) and 6.8-fold (*Shiyuehong* vs. *ChixinNo.4*) of the lowest ones, respectively. Moreover, significant positive correlations between the DBP and DEHP concentrations in shoots of all cultivars were found in both the low-PAE ($r = 0.833$, $p < 0.01$; Fig. 3a) and high-PAE treatments ($r = 0.762$, $p < 0.01$; Fig. 3b), indicating that DBP and DEHP could be taken up simultaneously by various cultivars of Chinese flowering cabbage without competition for accumulation. In order to explain the variation forming mechanism in PAE accumulation, *Lvbao70* was selected as a representative of low-PAE accumulator, showing lower DBP and DEHP concentrations (less by 50.49% and by 17.53% than DBP average values, and less by 67.87% and by 65.47% than DEHP average values, in low-PAE and

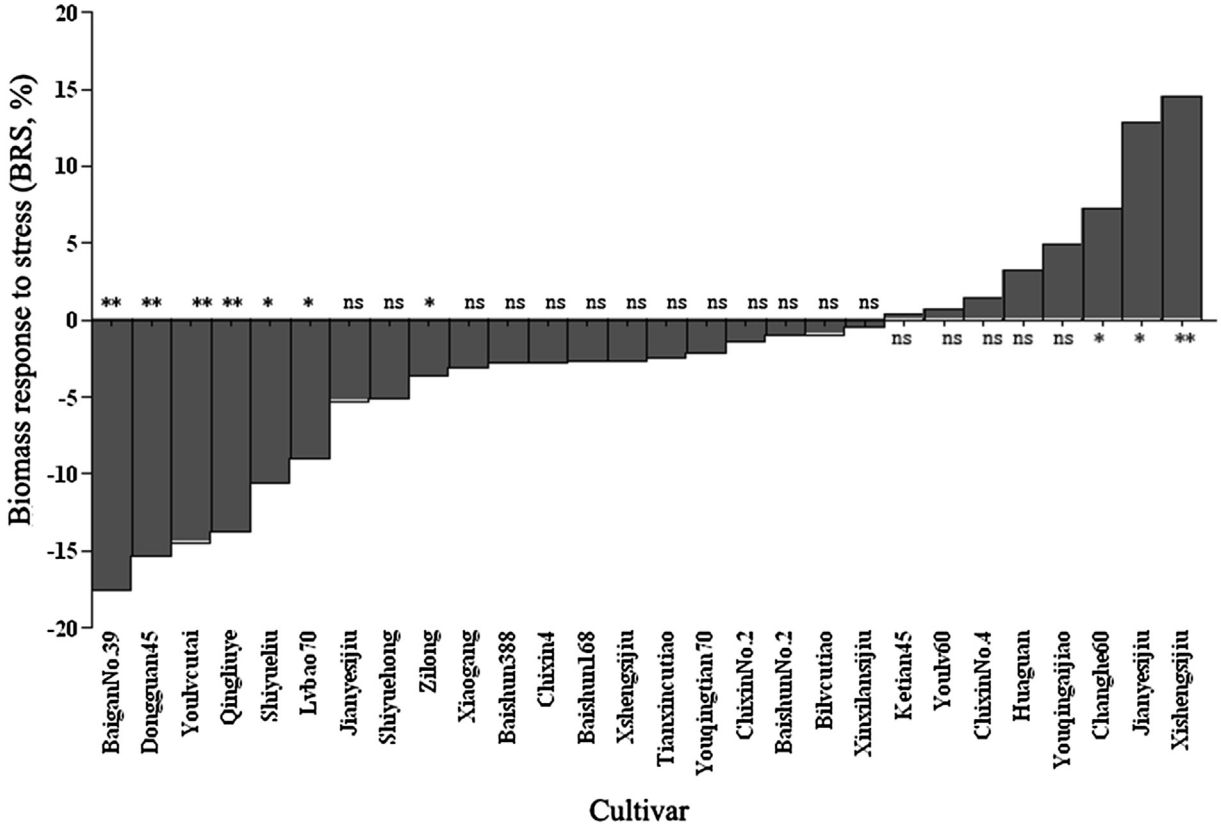

Fig. 1. Shoot biomass response to stress (BRS) of the 28 tested cultivars (*Note*: ns, *, and ** indicate that the differences of the shoot biomasses between the low- and high-PAE treatments were not significant, significant at the $p < 0.05$ level, and significant at the $p < 0.01$ level, respectively).

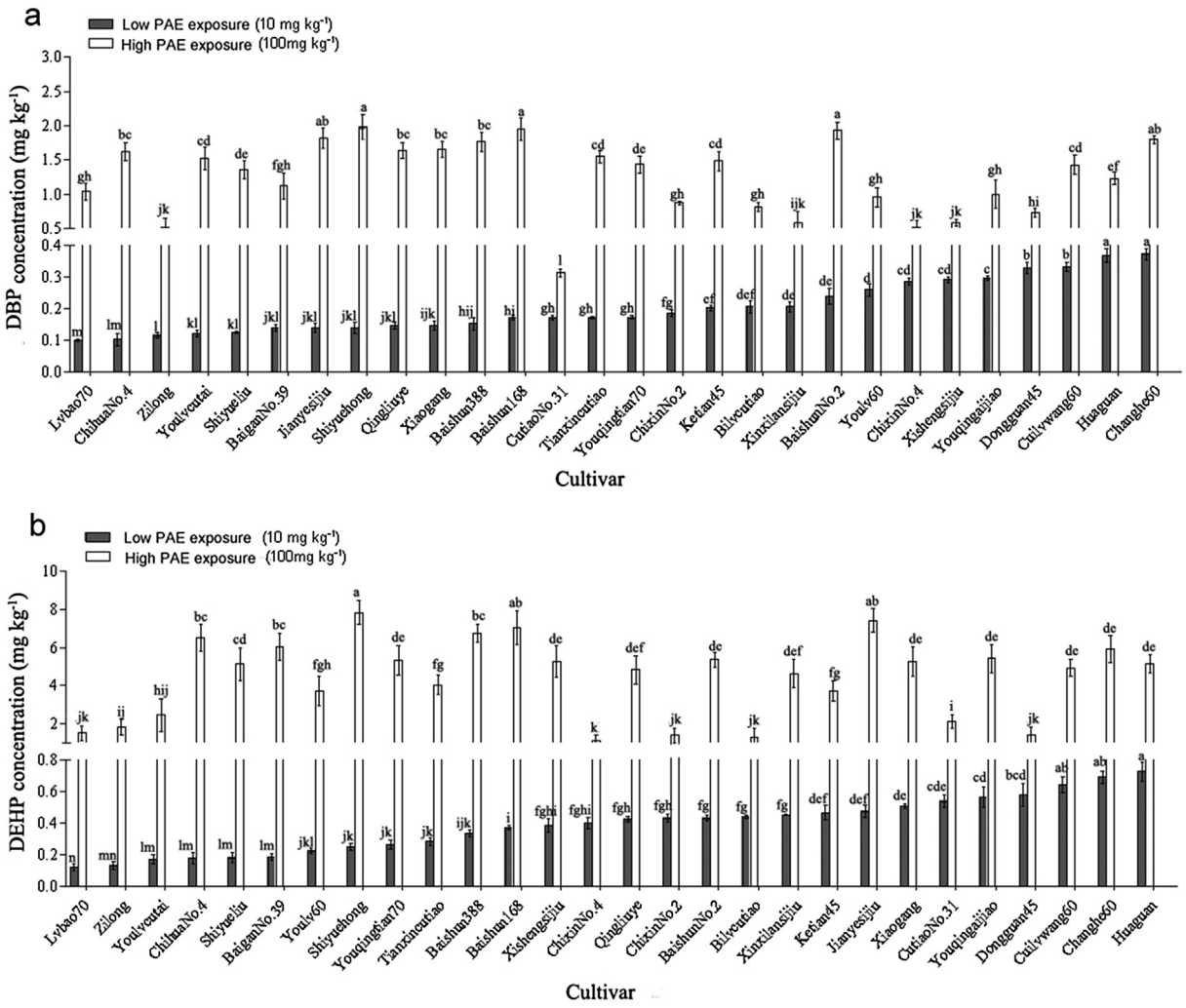

Fig. 2. Shoot DBP (A) and DEHP (B) concentrations (DW) of 28 tested cultivars under different PAE treatments. Different letters on the top of the histogram indicate significant difference at the same PAE-level treatment ($p < 0.05$).

high-PAE treatments, respectively) and higher shoot biomass. On the contrary, *Huaguan* was selected as a representative of high-PAE accumulator, showing higher DBP and DEHP concentrations (greater by 80.88% and by 2.22% than DBP average values, and greater by 78.15% and by 34.40% than DEHP average values, in low-PAE and high-PAE treatments, respectively) and higher shoot biomass.

3.3. Bioconcentration factor and translocation factor

The accumulation and translocation of PAEs by various cultivars of Chinese flowering cabbage were found to be different from each other (Table 2). Overall, no significant differences ($p > 0.05$) in bioconcentration factor (BCF, the ratio of PAE concentrations in plant roots to soil) were observed among the 28 cultivars in either the low- or high-PAE treatments. However, the translocation factor (TF, the ratio of PAE concentrations in plant shoots to roots) values of the 28 cultivars varied greatly and increased with the increase of shoot PAE concentrations. A significant difference ($p < 0.05$) in the TFs was observed between the lowest and highest accumulators in both the low- and high-PAE treatments. In the low-PAE treatment, the TFs of the highest accumulator (*Huaguan*) were higher by 5.1-fold (for DBP) and 2.6-fold (for DEHP) than those of the lowest accumulator *(Lvbao70*). In the high-PAE treatment, the TFs of the highest accumulator (*Shiyuehong*) were higher by 5.2-fold and 3.8-fold for DBP and DEHP, respectively, than those of the lowest accumulator (*ChixinNo.2* for DBP, and *Xishengsijiu* for DEHP; Table 2). These results indicate that the cultivars with higher TFs had a greater ability to accumulate PAEs in shoots despite their similar BCFs.

3.4. Subcellular distributions of PAEs in selected cultivars

In the low-PAE treatment, the PAEs in both shoots and roots were observed mainly in the cell walls (Table 3 and Fig. 4a), which was relatively insensitive to PAE stress. In shoots, the concentrations of PAEs in cell walls and organelles of *Huaguan* were significantly higher ($p < 0.05$) than in *Lvbao70*. However, the concentrations of PAEs in cell solutions of the two cultivars were not significantly different ($p > 0.05$; Table 3). In roots, the PAE concentrations in cell walls and organelles of *Lvbao70* and *Huaguan* showed a nonsignificant difference ($p > 0.05$). In *Lvbao70*, the PAE concentrations in root cell walls and organelles were much higher than those of shoots, whereas smaller differences were observed between the shoots and roots of *Huaguan* (Table 3). These results imply that PAEs taken up by roots of *Lvbao70* were retained more in

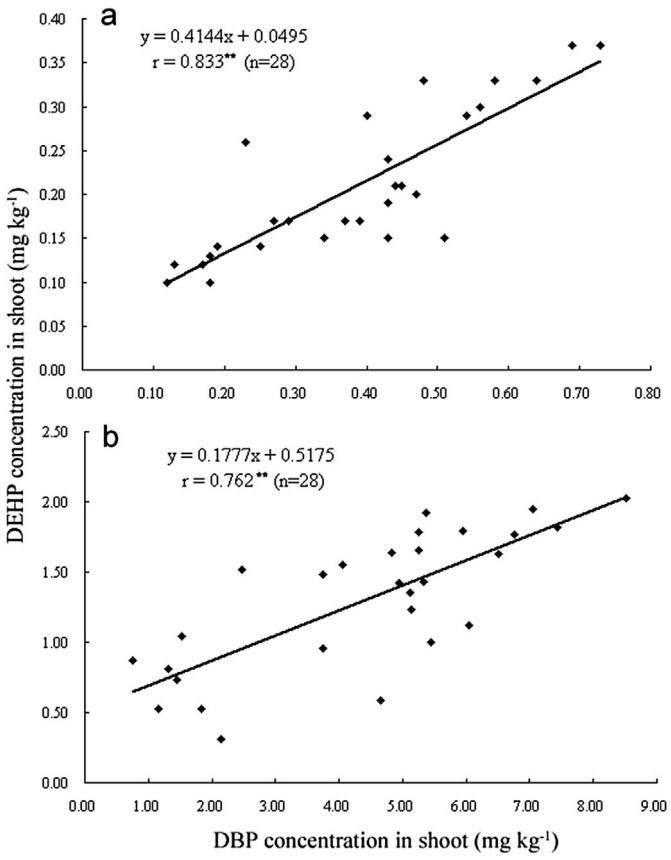

Fig. 3. Correlation between DBP and DEHP concentrations in shoots of 28 cultivars under low-PAE treatment (A) and high-PAE treatment (B), respectively (**$p < 0.01$).

the roots and thus translocated to shoots to a lesser extent. Therefore, the retention of PAEs in roots of *Lvbao70* was more effective than that of *Huaguan*, which might be one of main mechanisms leading to lower PAE concentrations in shoots of *Lvbao70*. Compared with other subcellular fractions, PAE levels in the cell solution (including the vacuole) were much lower because of the hydrophobicity of PAEs (Table 1). These aqueous cellular components, which were mainly composed of cell solution and were largely concentrated in the cell matrix between cells and organelles, were not inclined to enrich PAEs.

In the high-PAE treatment, the percentages of PAEs in the cell solutions of shoots and roots of *Lvbao70* were higher than in those of *Huaguan* (Fig. 4b), which was the opposite of the results for plants grown in the low-PAE treatment (Fig. 4a, b). The variations in the concentrations of PAEs in cell solution might be one factor that caused the difference in BRS between *Lvbao70* and *Huaguan*. In both roots and shoots, more DEHP was accumulated in the cell wall and organelle fractions than DBP, owing to their different properties. Additionally, in the low-PAE treatment, the proportion of PAEs in the cell walls of both shoots and roots in *Lvbao70* was much higher than in the high-PAE treatment (Fig. 4a), but for *Huaguan*, such proportion was just slightly higher (Fig. 4b). That is, the capacity of the dominant storage compartment for PAEs in cell walls of *Lvbao70* was lower in the high-PAE treatment. The proportion of PAEs in the root cell walls of *Lvbao70* and *Huaguan* was similar in the high-PAE treatment (Fig. 4a(d) and Fig. 4b(d)), but that was obviously higher in *Lvbao70* than in *Huaguan* in the low-PAE treatment (Fig. 4a(c) and Fig. 4b(c)). In conclusion, the differing subcellular distributions might be an important factor that resulted in a smaller difference in PAE accumulation in shoots between *Lvabo70* and *Huaguan* under high-PAE exposure than under low-PAE exposure (Fig. 2). However, a smaller difference was noted in the PAE concentrations in the root cell solution of *Huaguan* between the low-PAE and high-PAE treatments than in *Lvbao70*, which might be responsible for a higher tolerance to PAEs of the former (Ling et al., 2012).

Table 2
Bioconcentration factors and translocation factors of 28 cultivars under different PAE treatments.

Cultivars	Bioconcentration factors (translocation factors)			
	10 mg kg^{-1} DBP	100 mg kg^{-1} DBP	10 mg kg^{-1} DEHP	100 mg kg^{-1} DEHP
Lvbao70	0.157 ± 0.029 (0.414 ± 0.057)	0.157 ± 0.034 (0.404 ± 0.043)	0.140 ± 0.024 (0.299 ± 0.038)	0.132 ± 0.025 (0.337 ± 0.049)
Zilong	0.160 ± 0.027 (0.454 ± 0.031)	0.164 ± 0.031 (0.478 ± 0.061)	0.147 ± 0.027 (0.291 ± 0.034)	0.140 ± 0.024 (0.520 ± 0.058)
Youlvcutai	0.148 ± 0.025 (0.606 ± 0.062)	0.166 ± 0.035 (0.597 ± 0.053)	0.144 ± 0.025 (0.346 ± 0.055)	0.143 ± 0.026 (0.182 ± 0.030)
ChihuaNo.4	0.136 ± 0.027 (0.645 ± 0.054)	0.170 ± 0.029 (1.529 ± 0.107)	0.139 ± 0.028 (0.355 ± 0.041)	0.152 ± 0.024 (0.468 ± 0.037)
Shiyueliu	0.141 ± 0.031 (0.655 ± 0.047)	0.150 ± 0.026 (1.215 ± 0.089)	0.146 ± 0.026 (0.343 ± 0.036)	0.144 ± 0.023 (0.432 ± 0.057)
BaiganNo.39	0.151 ± 0.028 (0.641 ± 0.039)	0.146 ± 0.028 (1.433 ± 0.094)	0.148 ± 0.025 (0.381 ± 0.051)	0.137 ± 0.025 (0.348 ± 0.033)
Youlv60	0.146 ± 0.030 (0.738 ± 0.067)	0.148 ± 0.027 (0.992 ± 0.075)	0.145 ± 0.029 (0.377 ± 0.046)	0.142 ± 0.025 (0.563 ± 0.067)
Shiyuehong	0.149 ± 0.034 (0.817 ± 0.061)	0.153 ± 0.028 (1.662 ± 0.110)	0.144 ± 0.027 (0.376 ± 0.040)	0.127 ± 0.025 (0.623 ± 0.062)
Youqingtian70	0.142 ± 0.026 (0.916 ± 0.072)	0.140 ± 0.025 (1.254 ± 0.073)	0.148 ± 0.027 (0.384 ± 0.047)	0.137 ± 0.027 (0.481 ± 0.063)
Tianxincutiao	0.144 ± 0.022 (0.940 ± 0.067)	0.148 ± 0.028 (0.988 ± 0.054)	0.151 ± 0.026 (0.378 ± 0.042)	0.142 ± 0.026 (0.532 ± 0.063)
Baishun388	0.133 ± 0.026 (1.145 ± 0.048)	0.144 ± 0.029 (1.589 ± 0.086)	0.159 ± 0.027 (0.387 ± 0.039)	0.155 ± 0.024 (0.511 ± 0.047)
Baishun168	0.139 ± 0.032 (1.296 ± 0.056)	0.148 ± 0.032 (1.659 ± 0.103)	0.167 ± 0.024 (0.400 ± 0.052)	0.151 ± 0.023 (0.562 ± 0.036)
Xishengsijiu	0.134 ± 0.026 (1.329 ± 0.044)	0.139 ± 0.028 (1.310 ± 0.058)	0.168 ± 0.026 (0.386 ± 0.039)	0.144 ± 0.022 (0.164 ± 0.072)
ChixinNo.4	0.142 ± 0.022 (1.301 ± 0.050)	0.161 ± 0.034 (0.324 ± 0.077)	0.148 ± 0.025 (0.409 ± 0.043)	0.150 ± 0.021 (0.517 ± 0.043)
Qingliuye	0.141 ± 0.027 (1.446 ± 0.034)	0.152 ± 0.031 (1.224 ± 0.057)	0.161 ± 0.025 (0.414 ± 0.047)	0.140 ± 0.026 (0.459 ± 0.039)
ChixinNo.2	0.131 ± 0.024 (1.559 ± 0.066)	0.168 ± 0.032 (0.320 ± 0.092)	0.168 ± 0.026 (0.420 ± 0.028)	0.147 ± 0.026 (0.280 ± 0.051)
BaishunNo.2	0.143 ± 0.024 (1.393 ± 0.073)	0.144 ± 0.028 (1.335 ± 0.071)	0.170 ± 0.026 (0.440 ± 0.053)	0.163 ± 0.027 (0.460 ± 0.042)
Bilvcutiao	0.143 ± 0.029 (1.507 ± 0.048)	0.156 ± 0.026 (0.385 ± 0.046)	0.169 ± 0.024 (0.452 ± 0.035)	0.145 ± 0.024 (0.272 ± 0.071)
Xinxilansijiu	0.129 ± 0.023 (1.585 ± 0.056)	0.149 ± 0.023 (1.193 ± 0.086)	0.164 ± 0.028 (0.459 ± 0.050)	0.135 ± 0.023 (0.194 ± 0.063)
Ketian45	0.131 ± 0.028 (1.582 ± 0.067)	0.147 ± 0.028 (0.989 ± 0.078)	0.160 ± 0.028 (0.518 ± 0.047)	0.129 ± 0.025 (0.618 ± 0.035)
Jianyesijiu	0.127 ± 0.025 (1.594 ± 0.079)	0.151 ± 0.030 (1.597 ± 0.097)	0.165 ± 0.028 (0.552 ± 0.047)	0.160 ± 0.028 (0.307 ± 0.045)
Xiaogang	0.131 ± 0.031 (1.619 ± 0. 081)	0.145 ± 0.025 (1.350 ± 0.069)	0.153 ± 0.025 (0.642 ± 0.075)	0.164 ± 0.029 (0.170 ± 0.027)
CutiaoNo.31	0.136 ± 0.027 (1.677 ± 0.064)	0.154 ± 0.027 (0.601 ± 0.054)	0.148 ± 0.028 (0.615 ± 0.066)	0.128 ± 0.028 (0.193 ± 0.039)
Youqingaijiao	0.148 ± 0.034 (1.652 ± 0.037)	0.146 ± 0.029 (1.322 ± 0.083)	0.161 ± 0.026 (0.631 ± 0.084)	0.147 ± 0.025 (0.323 ± 0.052)
Dongguan45	0.134 ± 0.025 (1.768 ± 0.046)	0.164 ± 0.031 (0.409 ± 0.036)	0.160 ± 0.027 (0.683 ± 0.046)	0.146 ± 0.026 (0.237 ± 0.058)
Cuilvwang60	0.123 ± 0.026 (1.909 ± 0.088)	0.144 ± 0.028 (1.275 ± 0.084)	0.147 ± 0.028 (0.706 ± 0.063)	0.126 ± 0.029 (0.478 ± 0.043)
Changhe60	0.113 ± 0.026 (2.127 ± 0.094)	0.145 ± 0.032 (1.437 ± 0.072)	0.154 ± 0.024 (0.746 ± 0.078)	0.134 ± 0.026 (0.396 ± 0.061)
Huaguan	0.115 ± 0.031 (2.115 ± 0.080)	0.144 ± 0.032 (1.318 ± 0.063)	0.150 ± 0.027 (0.767 ± 0.070)	0.148 ± 0.023 (0.578 ± 0.057)

Table 3
Concentrations of DBP and DEHP distributed in the cell walls, cell solution and organelles of shoots and roots under low-PAE treatments (mg kg^{-1}, DW).

PAEs	Cultivars	Shoots			Roots		
		Cell walls	Cell solution	Organelles	Cell walls	Cell solution	Organelles
DBP	LB	0.071 ± 0.011	0	0.034 ± 0.006	0.125 ± 0.032	0.011 ± 0.002	0.052 ± 0.027
	HG	0.236 ± 0.034	0.016 ± 0.007	0.119 ± 0.017	0.211 ± 0.039	0.009 ± 0.004	0.079 ± 0.006
	Difference[a]	3.32**	/ns	3.50**	1.69*	0.82 ns	1.52ns
DEHP	LB	0.213 ± 0.075	0	0.091 ± 0.018	0.419 ± 0.084	0.017 ± 0.013	0.157 ± 0.057
	HG	0.420 ± 0.049	0.018 ± 0.009	0.238 ± 0.026	0.462 ± 0.057	0.029 ± 0.002	0.202 ± 0.018
	Difference	1.97*	/ns	2.62**	1.10 ns	1.71 ns	1.29 ns

[a] Difference = HG/LB, ns,*, and ** indicate not significant, significance at the $p < 0.05$ level, and significance at the $p < 0.01$ level, respectively (n = 3).

4. Discussion

The effects of contaminants on the biomass of plants vary with the levels of contaminants, the plant species and cultivar, and the duration of the stress (Xin et al., 2013). In this study, the biomasses of some Chinese flowering cabbage cultivars under low-PAE stress were comparable to or even higher than those of the control, which might result from low concentrations of PAEs activating enzymes that accelerated plant growth (Hashmi et al., 2014). Although more than 50% of the tested cultivars showed a negative BRS under high-PAE stress (Fig. 1), only 7 of 28 cultivars indicated significant decrease ($p < 0.05$) in biomass. Farmers might not realize PAE contamination based on yield reduction and would likely continue to cultivate other cultivars with a negative BRS in the fields contaminated by PAEs. Hence, PAEs in field soils can be taken up by vegetables and thus do harm to human beings and other animals via the food chain (Mo et al., 2009).

We have found that crop cultivars vary greatly in the uptake, accumulation, and translocation of PAEs (Cai et al., 2006, 2008b; Zeng et al., 2005). Therefore, a possible cost-effective method to lower the concentrations of PAEs in vegetables is to screen for or breed low-contaminant accumulators. In this study, a significant difference ($p < 0.05$) was found in the shoot PAE concentrations among 28 cultivars of Chinese flowering cabbage in both low- and high- PAE treatments (Fig. 2). In the low-PAE treatment, the differences in the DBP and DEHP concentrations of shoots were up to 3.7-fold and 5.8-fold between high accumulators (*Huaguan*, *Changhe60*, and *Cuilvwang60*) and low accumulators (*Lvbao70*, *Zilong*, and *ChihuaNo.4*), respectively (Fig. 2). Moreover, the concentrations of DBP in shoots of the 28 cultivars were significantly correlated with those of DEHP (Fig. 3). The similar properties of these two PAE compounds probably accounted for their positive correlation in bioaccumulation (Chiou et al., 2001). Thus, screening for low-accumulating cultivars of Chinese flowering cabbage was possible for multiple PAEs.

The cultivar variation in Chinese flowering cabbage was wide enough to allow for the screening of anti-pollution cultivars in field soil (Fig. 2). According to dietary guidelines for Chinese residents, the daily intake of vegetables for adult is no less than 400 g (Chinese Nutrition Society, 2007). Assuming a normal daily intake for this study, the DBP and DEHP exposure via ingestion of vegetable ranged from 0.687 to 2.460 and 0.833–4.807 μg kg^{-1} body weight (bw) per day, respectively, which were lower than the maximum DBP and DEHP reference doses of 100 and 20 μg kg^{-1} bw per day set by US EPA (Schecter et al., 2013), respectively. However, PAEs as endocrine disruptors may affect the hormone system at extremely low concentrations (Withgott, 2002; Lottrup et al., 2006). From a food safety perspective, and taking into account the biomass and TFs (TF < 1.0; Liu et al., 2010), the following cultivars could be suggested to be planted in low-PAE contaminated soils: *Lvbao70*, *Zilong*, *Shiyueliu*, *Youlv60*, *Shiyuehong*, and *Tianxincutiao*. Additionally, there were lower PAE residues in the rhizospheric soils of these low-PAE cultivars than that of high accumulators (*Huaguan*, *Changhe60*, and *Cuilvwang60*) (data not shown). These results implied that these low-PAE cultivars might enhance the removal of PAEs in the rhizospheric soil (Cai et al., 2008b), which may be partly responsible for their variation in PAE accumulation. Overall, these low-PAE cultivars could help remove contaminants from soil as well as accumulate PAEs at low enough level for safe consumption when grown normally in contaminated soils, which has important implications for future practical application to simultaneous production of safe food and bioremediation of soil polluted by PAEs.

BCF and TF values are usually used to evaluate contaminant uptake by roots from soil and translocation from roots to shoots (Pérez et al., 2013). The accumulation of PAEs in roots of the low accumulator (*Lvbao70*) was lower than that in the high accumulator (*Huaguan*; data not shown). Thus, the lower PAE concentrations in shoots of *Lvbao70* would typically be attributable to its decreased ability to take up PAEs into roots. However, the BCF value of *Lvbao70* was comparable to that of *Huaguan* (Table 2), and a similar phenomenon was observed in other plants (Cai et al., 2006; Mo et al., 2009). Although the uptake ability of PAEs by roots was not much greater in *Huaguan* than in *Lvbao70*, *Huaguan* showed a greater ability to translocate PAEs from roots to shoots (as shown by the TF; Table 2). Some studies have shown that the transportation of PAHs from roots to shoots was usually the major pathway for shoot accumulation (Gao and Zhu 2004; Gao and Collins, 2009). The transport ability of chemicals driven by the transpiration stream was mainly related with their solubility, $\log K_{OW}$ (logarithmic value of *n*-octanol–water partition coefficients) and $\log K_{OA}$ (logarithmic value of *n*-octanol–air partition coefficients), and could vary greatly in different plant species (Collins et al., 2006; Gao and Collins, 2009). The $\log K_{OW}$ values of DBP (~4.27) and DEHP (~7.73) both suggest a translocation potential from roots to shoots followed by accumulation in stem and leaf tissues (Ling et al., 2012). Compared with *Huaguan*, the transport ability of PAEs from roots to shoots was likely limited in *Lvbao70*. However, uptake from the atmosphere might also be an important pathway for chemicals with $\log K_{OA}$ values above 6 and $\log K_{AW}$ values above −6 ($\log K_{AW}$ = logarithmic value of dimensionless air–water partition coefficients) (Cousins and Mackay, 2001). Both DBP ($\log K_{OA}$ ~ 8.45 and $\log K_{AW}$ ~ −4.23) and DEHP ($\log K_{OA}$ ~ 10.13 and $\log K_{AW}$ ~ −3.47) exhibit these physicochemical properties (Table 1). Hence, the direct aerial deposition from volatile PAEs of polluted soils might contribute to the concentrations of PAEs in shoots. However, our previous study using rigid glass-compartment experiments showed that DBP and DEHP in shoots of Chinese flowering cabbage were derived mainly from root uptake, whereas the uptake by the leaves of DBP and DEHP volatilizing from contaminated soil was minor (Zeng et al., 2005). Therefore, the translocation of PAEs from roots to shoots may be a major difference between *Lvbao70* and *Huaguan* affecting shoot PAE accumulation.

The subcellular distribution of organic contaminants has

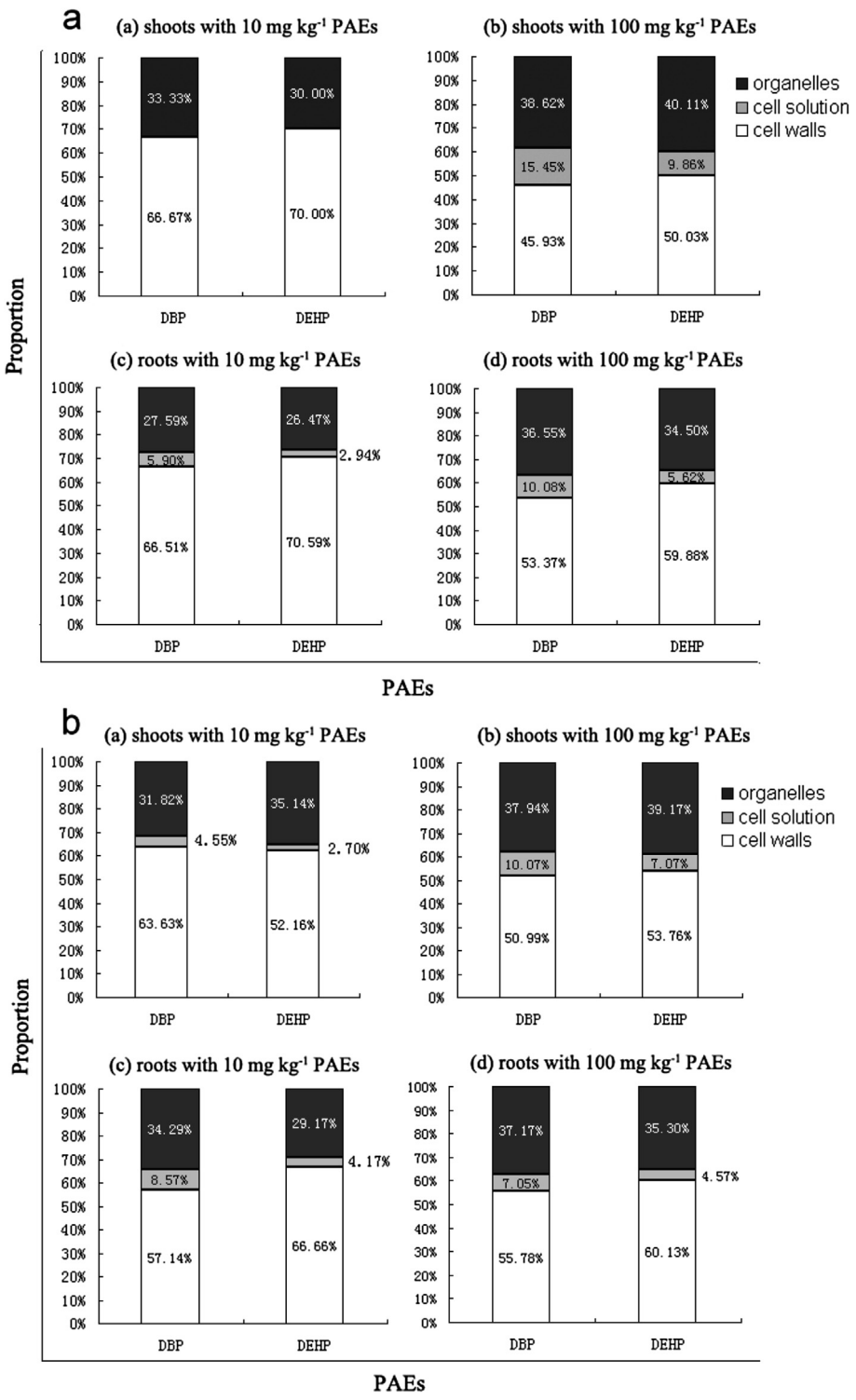

Fig. 4. Proportions of PAE distributed in three subcellular fractions of shoots and roots for *Lvbao70* (A) and *Huaguan* (B). (a), shoots with 10 mg kg^{-1} PAEs treatment; (b), shoots with 100 mg kg^{-1} PAEs treatment; (c), roots with 10 mg kg^{-1} PAEs treatment; (d), roots with 100 mg kg^{-1} PAE treatment. The proportions of DBP and DEHP were calculated from their measured amounts in each fraction to their total amounts in plant cells.

important effects on their accumulation, translocation, and metabolism in different plant species and cultivars (Gao et al., 2011, 2013; Kang et al., 2010). The first barrier that protects the protoplast against contaminant toxicity, plant cell walls, can bind organic contaminants due to cell wall components such as pectin, cellulose, hemicellulose, and lignin, restricting transport of the contaminants across cell membranes (Kang et al., 2010). In the present study, cell walls and organelles were found to be the dominant storage compartments for PAEs in Chinese flowering cabbage (Table 3 and Fig. 4). Similar reports have shown that the adsorption and binding of organic contaminants (e.g., PAHs) by cell walls greatly decreased their concentrations in the cytosol and enhanced their removal, thereby preventing damage to many physiological and biochemical processes in cells (Ling et al., 2012). In the low-PAE treatment, as

compared to *Huaguan*, *Lvbao70* consistently had a lower proportion of PAEs in other cell fractions of the roots, with up to 66.51–70.59% of the PAEs in the cell wall fraction (Fig. 4), which was helpful for decreasing the transport of PAEs into the shoot, as demonstrated by the TFs (Table 2). Additionally, the higher lipid content of the organelle fraction was responsible for the greater accumulation of organic lipophilic compounds (Gao et al., 2013; Kang et al., 2010). In this study, the concentrations of PAEs in shoot organelles were significantly lower ($p < 0.01$) in *Lvbao70* than in *Huaguan* (Table 3), which corresponds to the lower PAE concentrations in shoots of *Lvbao70*. Reducing the concentrations of contaminants in the cytosol is one defense strategy used by plants against contaminant-induced toxicity and is achieved by various mechanisms (Wu et al., 2005). In the low-PAE treatment, the concentrations of PAEs in the cell solutions of both roots and shoots were very low (<0.03 mg kg^{-1}) and not significantly different ($p > 0.05$) between the two cultivars (Table 3). The lower levels of PAEs in soluble cellular components may be due to the hydrophobicity of PAEs and their degradation by abundant enzyme systems in the soluble fraction (Gao et al., 2011, 2013). The effects of lipid contents and enzyme systems on the subcellular distribution of PAEs in Chinese flowering cabbage, and their variation between cultivars, need to be investigated further.

The uptake and accumulation of organic contaminants by plants are also influenced by their physicochemical properties (Calderon-Preciado et al., 2012). Gao and Collins (2009) reported that the logK_{OW} of a compound can be a determinant of the plant uptake, translocation, or partitioning process. However, little information is available on the relationship between the subcellular distributions of organic contaminants with their physicochemical properties (e.g., logK_{OW}). In the present study, DEHP accumulated to a greater extent in the cell wall fraction due to its higher logK_{OW}, whereas DBP, with higher water solubility (S_w) and a lower logK_{OW} than DEHP, entered the cell solution in greater proportions (Fig. 4). In a previous study, the uptake and translocation of other organic contaminants in plant tissues were driven by the transpiration flux and found to be related to the solubility of the individual compound (Gao and Collins, 2009). Thus, driven by the transpiration flux, DBP might be more easily liberated from the constraints of cell walls and translocated through the symplastic or apoplastic space into other tissues (Gao et al., 2011). This theory explains the higher TF for DBP than for DEHP in Chinese flowering cabbage, which would be supported by the transpiration stream concentration factor relationship reported by others, in which the transport from roots to shoots declined with increasing logK_{OW} (Burken and Schnoor, 1998; Gao and Collins, 2009). However, higher concentrations of DEHP were observed in the shoots of all cultivars than of DBP, which might result from the greater metabolic transformation of DBP within plant tissues. Metabolic processes and rates have been shown to be specific to particular chemicals and plant species (Gao et al., 2013). In addition, low-molecular-weight or short-chain organic contaminants (e.g., DBP) may be metabolized more easily in plant bodies than high-molecular-weight or long-chain ones (e.g., DEHP) (Kvesitadze et al., 2014). Unfortunately, little is known about the metabolic pathways of EDCs, such as PAEs in plants; this area is worth further investigation.

5. Conclusions

A low-PAE accumulator of Chinese flowering cabbage (*Lvbao70*) was identified that not only could grow normally in PAE-polluted soil but might also be safer for consumption. Moreover, variations in the accumulation and translocation of PAEs among 28 cultivars were investigated, and furthermore, the variation mechanism was revealed by studying the subcellular distribution of PAEs in low and high accumulators (*Huaguan*). Therefore, this study laid a foundation for a strategy to control organic contamination in agricultural soils.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2015.06.008.

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