



Fate of Phthalic Acid Esters (PAEs) in Typical Greenhouse Soils of Different Cultivation Ages

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Abstract

An ultrasonic-assisted extraction methodology coupled with gas chromatography-mass spectrometer analytical technique was used to determine concentration of phthalic acid esters (PAEs) in typical greenhouse soil. The results showed that the developed method has a reliable recovery rate (80.78%–112.89%) and a low detection limit (10^{-4} mg/kg) which met the requirements of residue determination. The analysis of 32 soil samples revealed that except for dimethyl phthalate, the concentration of other five PAEs was detected and followed the sequence di-(2-ethylhexyl) phthalate > dibutyl phthalate > di-*n*-octyl phthalate > butylbenz phthalate > diethyl phthalate. Σ_6 PAEs concentrations ranged from 136.91 to 1121.74 $\mu\text{g}/\text{kg}$ (mean 319.59 $\mu\text{g}/\text{kg}$). PAEs was closely correlated with soil pH and organic matter, but not with cultivation ages which indicates that the increase of cultivation age is not the main reason for the change of soil PAEs concentration.

Keywords Phthalic acid esters (PAEs) · Greenhouse soil · Detection method · Cultivation age · Gas chromatography-mass spectrometry (GC-MS)

Phthalic acid esters (PAEs) are important plasticizers which are extensively used in industrial production due to their high performance and low cost. However, PAEs are readily desorbed from plastic products due to binding via hydrogen bonds and Van der Waals forces which may lead to the release of PAEs into the air (Lu et al. 2018), water (He et al. 2011) and soil (Xu et al. 2008), and their potential uptake into crops and vegetables (Zhao et al. 2015). PAEs may interfere with the endocrine system causing oxidative stress and cytotoxicity (Wang et al. 2017; Song et al. 2019). The United States Environmental Protection Agency (EPA) have listed six typical PAEs as priority pollutants including

dimethyl phthalate (DMP), dibutyl phthalate (DBP), diethyl phthalate (DEP), di-*n*-octyl phthalate (DOP), butyl benzyl phthalate (BBP) and di-(2-ethylhexyl) phthalate (DEHP). DMP, DBP and DEHP have also been included in the list of priority pollutants in China.

Studies assessing the concentration of PAEs in greenhouse soil have observed significant variability among different regions (Niu et al. 2014; Wang et al. 2015). For example, Σ PAEs concentration ranged from 1370 to 4900 $\mu\text{g}/\text{kg}$ in agriculture soil of northeastern China (Zhang et al. 2015) and 150–9680 $\mu\text{g}/\text{kg}$ in vegetable soil of Nanjing (Wang et al. 2013). In the other countries, PAEs in soil were also detected, such as Denmark of 50 $\mu\text{g}/\text{kg}$ (Jørgen Vikelsøe et al. 2002) and United Kingdom of 100 $\mu\text{g}/\text{kg}$ (Gibson et al. 2005).

PAEs are common organic contaminants in greenhouse soil, potentially originating from the use of plastic films in agriculture to reduce soil permeability. With the increase of cultivation ages, the amount of plastic film in soil may increase, and more PAEs will be released into the soil. In addition, extraction and detection of PAEs are difficult due to the complexity of the soil composition. Therefore, we hope to find a suitable analytical method to quantify the concentration of PAEs. Based on the above views, the objectives of this work were to (1) evaluate the efficiency and

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accuracy of an analytical approach for PAEs extraction and determination; (2) evaluate contamination status of PAEs in greenhouse soil and investigate the association of PAEs with cultivation ages and major soil properties.

Materials and Methods

Soil was sampled from three locations in Shouguang City, Shandong Province, China (Fig. 1). Thirty-two soil samples (SG1–SG32) were collected from a depth of 2–20 cm using a five-point sampling method. Subsamples were collected in sterilized brown ground glass jars, after which they were freeze-dried and stored at $-20\text{ }^{\circ}\text{C}$ prior to analysis. Six PAEs standards (liquid), including DMP (99.9%), DEP (97.6%), DBP (99.3%), BBP (99.9%), DEHP (99.7%) and DOP (99.9%) were purchased from Sigma-Aldrich. Solvents for PAEs extraction [acetone and hexane (high performance liquid chromatography/HPLC grade)] were purchased from Sigma-Aldrich and Tianjin Keromo Chemicals Limited respectively.

Soil (10 g, 0.85 mm) sample was added to the mixed extractant (1:1, 30 mL) which was made of 30 mL of acetone and *n*-hexane (1:1), and then it was vortexed for 30 s and

allowed to stand overnight. The suspension was vortexed for 30 s and then sonicated in a sonicating waterbath for 30 min (300 W, $25\text{ }^{\circ}\text{C}$). The suspension was centrifuged (3000 r/min, 5 min, $25\text{ }^{\circ}\text{C}$) and the supernatant was filtered with the medium speed qualitative filter paper in a flat-bottomed flask. Added 20 mL of extractant to the glass centrifuge bottle, then sonicated and filtered according to the above procedure, and repeated this step once. At last, a total of 70 mL of extractant (30 mL + 20 mL + 20 mL) was consumed. The extract was reduced in volume to approximate 1 mL under nitrogen after which hexane (5 mL) was added. Then, the extract was reduced approximately to 1 mL (under nitrogen) again. At last, the final extract was brought to 1 mL with *n*-hexane prior to gas chromatography-mass spectrometer (GC-MS) analysis.

A Thermo Fisher Scientific TSQ 8000Evo GC triple-quadrupole mass spectrometer (equipped with AS1310 Autosampler) was used for the quantification of six PAEs. Specific analytical conditions were as follows: a TG-5MS (30 m \times 0.25 mm \times 0.25 μm) flexible silica capillary column and SRM mode detection were adopted, with high purity helium (99.999%) as the carrier gas. A flow rate of 1.2 mL/min, and an injection volume of 1 μL was used in non-pulsed injection mode, and the transmission line and ion source (EI)

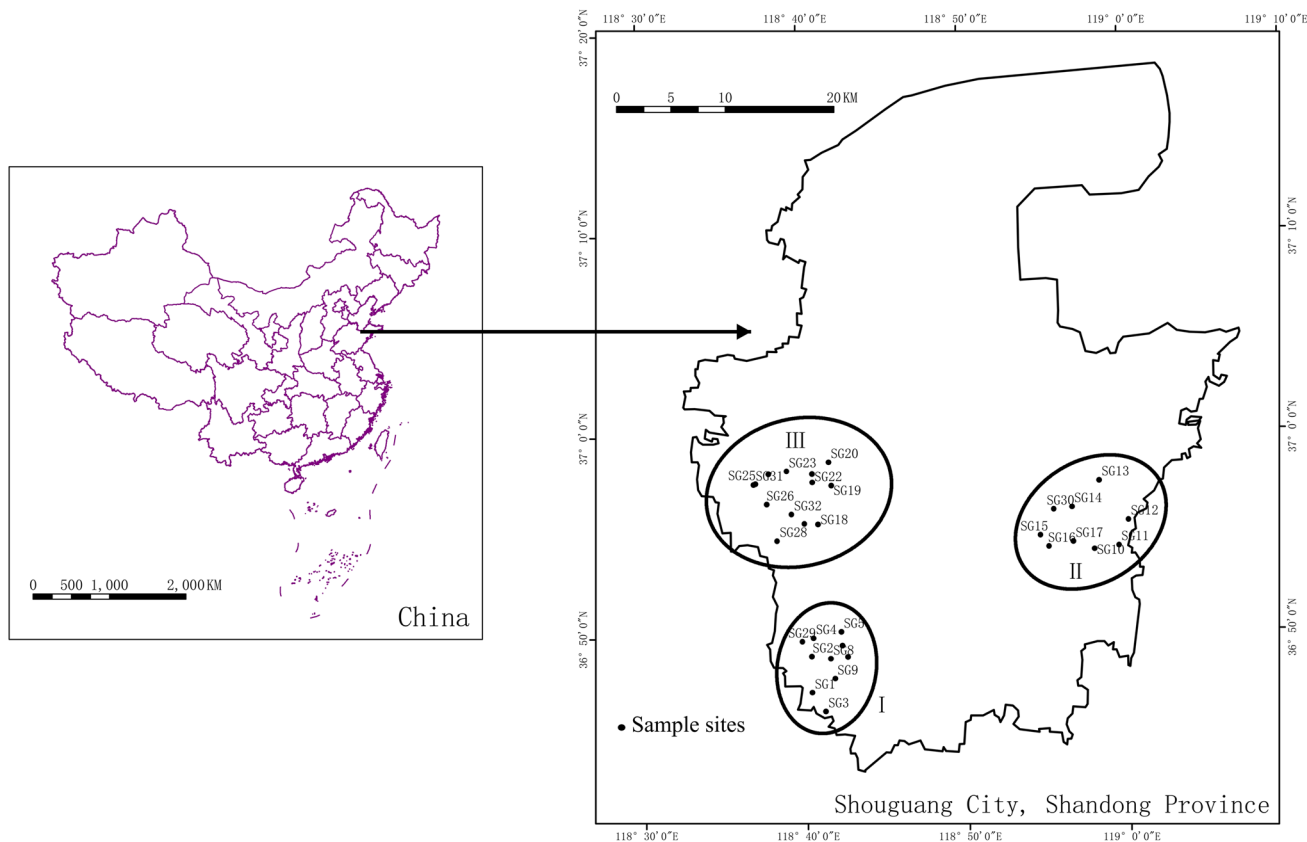


Fig. 1 Sampling sites of soils taken from 32 greenhouses in Shouguang city of China (SG Shouguang city, I, II, III three sampling areas)

temperatures were 300 °C. The column temperature program was initially at 50 °C for 1 min, increasing to 200 °C at 30 °C/min holding for 1 min, then increasing at 8 °C/min to 280 °C, holding for 3 min. Soil pH, organic matter (OM) and available nitrogen (AN) were determined by electrode method, potassium dichromate volumetric method and alkaline hydrolysis diffusion method, respectively (Wang et al. 2018).

All glass instruments were rinsed in potassium dichromate solution, and then dried at 400 °C. Organic solvents (*n*-hexane and acetone) were re-distilled before being used. For PAE analysis, blank samples and spiked samples were included for quality assurance and quality control. The experimental results were blank corrected and then analyzed. Spiked recoveries were established at four concentrations (50 µg/kg, 100 µg/kg, 1000 µg/kg, 10,000 µg/kg), with three replicates for each concentration. One-way analysis of variance (ANOVA) was performed out using the statistical software package SPSS 20.0. CANOCO software package for Windows 4.5 was used to conduct the redundancy analysis (RDA) to assess the relationship between physicochemical properties and PAEs accumulation in soil. Experimental data were expressed as mean ± standard deviation.

Results and Discussion

Six PAEs standard samples were detected by GC-MS. The ion chromatogram (Fig. S1) indicated that the separation of the six PAEs was satisfactory, and the peak shape of each PAE was optimal without impurity peaks. The detection time for a single sample was less than 25 min. Figure S2 showed that the six PAEs standard curves had excellent linearity ($R^2 > 0.999$) and the detection limit of the instrument reached 10^{-14} g. Thus, the PAEs in the soil extractant were analyzed with GC-MS after ultrasound-assisted extraction. To ensure the reliability of the extraction and determination of six major phthalate residues in the soil, the recovery

experiment was performed by spiking six PAEs monomers in the soil. Result showed that the detection limit of the method reached 10^{-4} mg/kg. Recoveries of the six PAEs ranged from 80.78% to 112.89% (Table 1), and the coefficient of variation (C.V) ranged from 0.57% to 9.77%. The recovery rates of the six PAEs in this study was higher than that of Zhang et al. (2008), Lu et al. (2016) and Li et al. (2017), which recovery rates in their study were 61.70%–97.80%, 77.62%–88.94% and 72.79%–96.46%, respectively. In addition, compared with the Soxhlet extraction (Cao et al. 2011) and accelerated solvent extraction (Zheng et al. 2016), the ultrasonic extraction method can shorten the extraction time and improve the extraction efficiency. On the other hand, the equipment used in this method was cheap, the operation was simple with high sensitivity detection instrument, and the results implied that the newly-developed PAEs extraction and detection method was satisfactory, which was recommended to be used to quantify PAEs in soil.

It was found through the analysis of 32 soil samples that Σ_6 PAEs concentration was ranged from 136.91 to 1112.74 µg/kg (Fig. 2 and Table 1). The concentration of Σ_6 PAEs in SG1, SG8, SG23 and SG31 was higher than that in other samples. According to previous reports, the concentration of PAEs in the greenhouse soil in different regions of China was quite different. The results showed that PAEs concentration in soil in Shouguang was higher than that observed in vegetable producing locations in Anhui (204–484 µg/kg. Wang et al. 2016), but lower than that in Guangzhou (3250–8050 µg/kg. Zeng et al. 2008) and Shenyang (520–1730 µg/kg. Li et al. 2017).

All PAEs monomers except DMP were detected in this study. A high concentration of DEP was found in SG3, SG10 and SG11 which was 15.48 ± 11.11 µg/kg, 18.76 ± 10.82 µg/kg and 19.00 ± 9.15 µg/kg, respectively. All samples had similar BBP concentration, which ranged from 19.06 to 20.12 µg/kg. DOP was detected in 34.38% of surveyed sites, having the concentration ranging from 19.96 to 37.68 µg/kg. DEHP was the most abundant PAEs monomer (Fig. 2)

Table 1 Detection and concentration of six PAEs in soil of Shouguang city and soil allowable concentrations and cleanup objective value of PAEs compounds in US (µg/kg) (Wang et al. 2013)

PAEs	Min	Max	Mean	Standard deviation (SD)	Method detection limit (MDL)	Recovery (%)	Allowable concentration	Cleanup objective value
DMP	–	–	–	–	0.10	80.78–97.91	20	2000
DEP	–	19.00	17.75	0–11.11	0.10	85.22–104.34	70	7100
DBP	36.27	198.6	98.88	0.71–18.90	0.10	87.94–107.05	81	8100
BBP	18.95	20.12	19.06	0.0024–0.19	0.10	106.82–112.89	1220	50,000
DEHP	77.75	1004	192.37	3.35–31.73	0.10	95.33–98.05	4350	50,000
DOP	–	37.68	23.68	0–3.47	0.10	97.78–104.09	1200	50,000
Σ_6 PAEs	136.91	1112.74	315.95	2.30–93.56	–	–		

– means not available

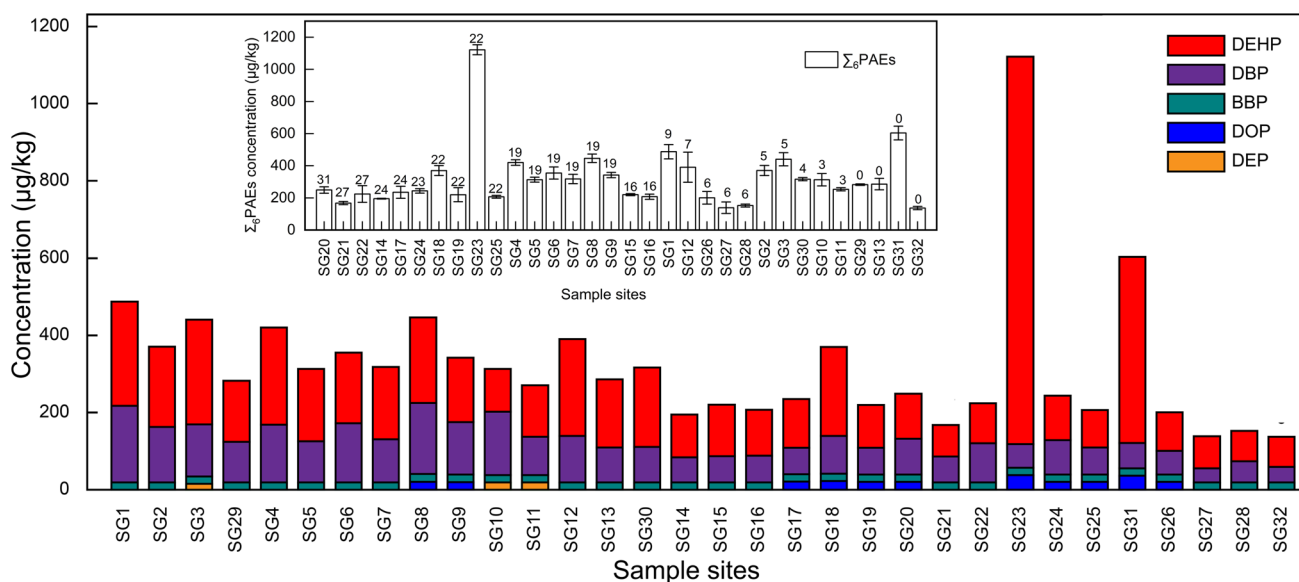


Fig. 2 Concentration of PAEs in greenhouse soil in Shouguang city (Note the Arabic numerals in the figure represent the age of cultivation of soil samples, unit is 'year')

and its concentration ranged from 77.75 to 1003.54 $\mu\text{g}/\text{kg}$, accounting for 35.46%–89.46% of $\Sigma_6\text{PAEs}$. The occurrence of PAEs in the soil of Shouguang was similar to that reported by Wang et al. (2013). DEHP is the most widely used plasticizer, accounting for approximately 50% of total production (He et al. 2018). Besides, the use of fertilizers (Zhang et al. 2015), pesticides and plastic films (Chen et al. 2018) may also be a source of DEHP. In the present study, excepted the SG10 which DBP accounted for 52.41% of $\Sigma_6\text{PAEs}$, DEHP was the most prevalent PAEs in soil. In addition, the concentration of DBP ranged from 36.27 to 198.63 $\mu\text{g}/\text{kg}$ ($98.88 \pm 41.13 \mu\text{g}/\text{kg}$), which was weaker than that of DEHP (except for SG10). The results are similar to those observed by Net et al. (2015) that $\Sigma_6\text{PAEs}$ concentration was dominated by DEHP and DBP in soil samples.

According to the United States Environmental Protection Agency (USEPA), the concentration of five PAEs monomers (DEHP, DEP, BBP, DOP, DMP) did not exceed their respective control standards (4350 $\mu\text{g}/\text{kg}$, 71 $\mu\text{g}/\text{kg}$, 1215 $\mu\text{g}/\text{kg}$, 1200 $\mu\text{g}/\text{kg}$, 20 $\mu\text{g}/\text{kg}$. Table 1). DBP concentration in 19 samples exceeded the allowable concentration (81 $\mu\text{g}/\text{kg}$), but did not exceed the cleanup objective value (8100 $\mu\text{g}/\text{kg}$). In this study, DBP concentration was lower than $44 \pm 20 \mu\text{g}/\text{kg}$ (Zheng et al. 2016), and DMP was not detected. Previous studies have demonstrated that the concentration of PAEs in soils of different regions in China was generally between several $\mu\text{g}/\text{kg}$ and dozens of mg/kg , with significant difference (Kong et al. 2012). In Xinjiang, Guangdong, Fujian and Taiwan provinces, the pollution level of PAEs was the highest, followed by central part of China (such as Henan, Chongqing and Hubei provinces), and the concentration of

PAEs was ranged from 75 to 6369 $\mu\text{g}/\text{kg}$ (Niu et al. 2014). In these areas, the industry was developed or the plastic agricultural film was widely used. In addition, DEHP and DBP were the main contributors of PAEs, and the detection rate and concentration level of other PAEs homologues were relatively low (Li et al. 2015; Chai et al. 2014). Generally, $\Sigma_6\text{PAEs}$ concentrations in soil of Shouguang were generally lower than those reported in other regions of China, which was similar to the study of Guo et al. (2011).

Soil properties can strongly influence the behavior of organic pollutants in soil. Therefore, it is necessary to find out the relationship between soil properties (Table S2) and PAEs concentration. The correlation of $\Sigma_6\text{PAEs}$ and six PAEs with soil properties was analyzed by correlation analysis and redundancy analysis (RDA). RDA results showed that DEHP had the highest contribution to $\Sigma_6\text{PAEs}$, which was significantly relevant (Fig. 3). It is well known that DEHP is the most widely used plasticizer in production and use, and its construction is stable. This may result in DEHP being the most abundant PAEs in soil, air and water (Wang et al. 2015; Zhang et al 2008; Lu et al 2018). Although DOP was detected in only limited samples, its contribution to $\Sigma_6\text{PAEs}$ was second only to DEHP. The correlation coefficient between DBP and $\Sigma_6\text{PAEs}$ was 0.269, which was lower than the concentration of DEHP and its proportion contribution to $\Sigma_6\text{PAEs}$.

Different soil physicochemical properties showed different relationships with PAEs and monomers. Soil moisture content and clay percentage were positively correlated with DEP, DEHP and BBP, but negatively with DOP, DEHP and ΣPAEs (Fig. 3). Clay percentage had significantly

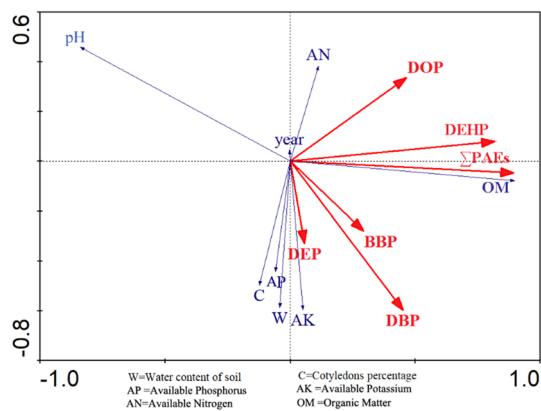


Fig. 3 The results of canonical redundancy analysis (RDA) between PAEs concentration and physicochemical properties of soils

influence on the concentration of DEP, DEHP and BBP in soil. Besides, soil pH was significantly and negatively correlated with DBP, DEHP, Σ PAEs, which may be attributed to the greater affinity of soil for PAEs at lower pH. This was consistent with the results found by Shailaja et al. (2007), who suggested that the amount of PAEs adsorbed in soil decreased as pH increased which may be because if the soil environment is alkaline, carboxyl and hydroxyl of the organic group is reduced, and the charge field is changed, thereby reducing the accumulation of organic matter on the soil agglomerates. In addition, it was a negative correlation between PAEs and pH in this study, which was similar to the results of Zheng et al. (2016) and Mohan et al. (2007). PH could also affect the activity of soil microorganisms, thus affecting the degradation rate of PAEs. PAEs may affect the structure and metabolic diversity of soil microbial communities and disturb soil enzyme activity (Xie et al. 2010). Soil organic matter (OM) can also change the environmental behavior of PAEs in soil (Li et al. 2016). The results of this study revealed that soil OM showed positive correlations with DBP, DEHP and Σ PAEs. This was possibly because PAEs in soil were dominated by DBP and DEHP with larger molecular weight and greater organic carbon distribution coefficients, which promotes binding with soil organic matter. Based on this study, soil pH and OM were important factors that impacted PAEs accumulation in greenhouse soil. In addition, soil nutrients (N, P and K) showed a poor correlation with the content of PAEs in the soil, as shown in Fig. 3 and Table S3.

Since plastic film is a major contributor to PAEs, cumulative use of films over the years may result in increased concentration of PAEs in soil. Therefore, PAEs concentration of soil may be correlated with ages of cultivation. However, in the present study, there were no obvious trend of PAEs concentration changes. The concentration of PAEs was higher in 5 years, 7–9 years and 19–22 years,

and decreased after 22 years. PAEs concentration in greenhouse soil did not increase linearly with the increase of cultivation age, which agreed with several previous studies (Zheng et al. 2016). In addition, the correlation analysis also confirmed the above points. Results indicated that cultivation age of facility agriculture soil was poorly correlated ($r = 0.001$) with Σ_6 PAEs. Accumulation and degradation of PAEs in soils were closely related to soil tillage patterns, agricultural film types, irrigation and temperature conditions which was also supported by Wang et al. (2013), Li et al. (2016), Ma et al. (2015) and Zhang et al. (2015). With the increase of planting age, farmers choose different cultivation methods and remove the residual agricultural film in time, which will have a positive effect on soil PAEs concentration (Wang et al. 2015). At the time of our investigation, almost all farmers regularly removed the remaining film in the soil when the vegetable planting was over. In addition, PAEs not only accumulated in the soil, but were also transferred and degraded. PAEs in the soil may be biodegraded rapidly because the greenhouse soil has high temperature, abundant moisture and intense microbial activity. All these may lead to the changes of PAEs in soil.

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