Occurrence of Phthalate Acid Esters (PAEs) in Protected Agriculture Soils and Implications for Human Health Exposure

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Abstract
This study explored occurrence of phthalic acid esters (PAEs) in protected agriculture soils and assessed their potential health risks to humans. Results showed that DEHP and DBP were the most abundant PAEs congeners, with mean concentrations of 318.68 μg/kg and 137.56 μg/kg, respectively. DOP and BBP concentrations were relatively low, and DMP and DEP were not detected in all samples. DBP concentrations were higher than the allowable concentration standard value. Additionally, soil pH and organic matter were key environmental parameters which may play the vital roles to the occurrence of organic pollutants. Health risk assessment results indicated that dermal contact was the predominant human exposure route under non-dietary conditions, and children obtained higher health risk scores than adults. In summary, the overall health risk scores were at an acceptable level. These results provide insights for assessing soil environmental safety and ecological risks in protected agricultural soil.

Keywords Phthalic acid esters (PAEs) · Protected agriculture · Soil · Health risk assessment

Phthalic acid esters (PAEs) are anthropogenic plastic additives used to enhance the strength and plasticity of the target product, incorporated into various plastic products, such as toys, beverage containers, polyvinyl chloride (PVC) pipes, pharmaceutical, and personal care products (PPCPs), medical equipment, and agricultural films (Erythropel et al. 2014; Kang et al. 2021). PAEs present the hydrophobic properties and can easily dissociate from plastic products into different environmental matrices, owing to loose covalent bond between PAEs and plastic products (Song et al. 2019). Therefore, PAEs are ubiquitous in the environment and are alleged endocrine disruptors, posing a substantial threat to organisms, including human beings (Wang et al. 2021a, b; Chang et al. 2021). Due to concerns over the detrimental effects and possible health risks promoted by PAEs, six PAEs homologs including bis (2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), butyl benzyl phthalate (BBP), diethyl phthalate (DEP), di-n-octyl phthalate (DOP), and dimethyl phthalate (DMP) were nominated as priority pollutants by various countries, such as the United State, and China (USEPA 1980; Gao et al. 2018). Furthermore, DEHP was also listed in class 2B (possibly carcinogenic to humans) by the International Cancer Research Institute (ICRI) of the World Health Organization (WHO).

According to a recent study, approximately 300 million tons of PAEs are manufactured every year (Garcia and Robertson 2017). The global concern of PAEs contamination has become more conspicuous as emissions from product life cycles are ongoing (i.e., manufacture, usage, disposal) (Wang et al. 2013). In China, many protected agricultural areas (approximately 37,000 km²) were created and built to increase vegetable yield and quality. However, as they can improve flexibility, strength, and elasticity of plastic polymers, PAEs (20%–60%) were added to agriculture films (shed and mulch films), which were frequently used for agricultural activities (e.g., protecting crops) (Lü et al. 2018). As a result of this practice, PAEs leach from the plastic and enter the soil while crops are developing, possibly increasing the health risk to humans (Zhang et al. 2015; Shi et al. 2019). The exposure risk to humans promoted by PAEs should be given special attention since high temperatures and relative humidities are conditions maintained during protected agriculture activities, possibly enhancing PAE leaching to soils (Wang et al. 2013). Studies showed that
exposure risk of PAEs to human could evaluate by different methods and models. Wang et al. (2018) calculated the average daily dose of the human body under the non-dietary pathway through a health risk assessment model, and found that ingestion and dermal adsorption is the major pathways for human exposure to PAEs in soil. Additionally, the assessment of eco-toxicological effect of PAEs indicated that the environmental risk of PAEs in soil at different depths was acceptable (Wang et al. 2015).

At present, PAEs have gradually become the second largest environmental pollutants in the world (Lü et al. 2018), and they are also the most abundant semi-volatile organic compound (SVOCs) in agricultural soils in China (Cai et al. 2008). Therefore, the environmental pollution problems and risk effects caused by PAEs have attracted more and more attention and become a research hotspot in the environmental field. In this study, we investigated the spatial distribution of PAEs and the ratio of homologs in typical protected agricultural soils from northern China, and then studied environmental risk of PAEs in soil and the potential health risk of PAEs to the humans. In summary, the results of this study will provide a better reference for humans in soil cultivation and management.

Materials and Methods

Fangcun is located on the Shandong Peninsula in northern China and has a temperate monsoon climate. The annual average temperature is 11–14°C, and the annual precipitation is 550–950 mm occurring primarily in summer. Fangcun is the largest tomato planting production base with many protected agriculture areas in the Shandong Peninsula. In this study, 12 representative soil samples (0–20 cm) were collected randomly from protected agriculture areas in Fangcun using a five-point sampling method (Table S1; Fig. S1). Stones, plant residues, and broken plastic films on the soil surface were removed during sample collection. The collected samples were stored in pre-cleaned brown glass bottles, sealed, and taken back to the laboratory for pretreatment. Samples were stored at −20°C until analysis to reduce errors.

Six PAEs standards (DEHP, DBP, DOP, BBP, DEP, and DMP) were obtained from Sigma-Aldrich, and the corresponding structure information were shown in the supplementary (Table S2). Acetone and n-hexane (High-performance liquid chromatography grade) were purchased from Tianjin Comio Chemical Reagent Co., Ltd., and other chemicals were of analytical grade.

The soil samples were air-dried, ground, and sieved through a stainless-steel sieve (20-mesh). For the extraction, 10.00 g of soil was placed in a glass conical flask, and six PAEs were extracted with 30 mL of a 1:1 mixture of acetone: n-hexane. An ultrasonic-assisted extraction method was applied in this study, as described in previous research (Li et al. 2020). A gas chromatography–mass spectrometry method with a TG-5MS (30 m × 0.25 mm × 0.25 µm) flexible quartz capillary column was used to quantitatively analyze the PAEs. Selected reaction monitoring (SRM) and splitless injection mode were used, with a flow rate and injection volume of 1.2 mL/min and 1 µL, respectively. The transmission line and electron impact (EI) ion source temperatures were 300°C, and the column temperature program was selected based on parameters previous studies (Li et al. 2020).

Soil organic matter (SOM), pH, alkali hydrolyzable nitrogen (AN), available phosphorus (AP), available potassium (AK), moisture content (SMC), soil texture (SCP), total nitrogen (TN), and the total salt content (SSA) of the soil samples were determined according to the methods in “Soil Agrochemical Analysis” (Lu 2000; Li et al. 2020).

The experimental materials used in this study consisted of stainless steel or glass to avoid contamination by plastic. Glass and stainless-steel instruments were strictly cleaned before the analysis by (1) ultrasonicication for 30 min, (2) drying, (3) soaking with a potassium dichromate lotion overnight, and (4) rinsing with deionized water for 30 min. Glass instruments without scales were baked at a high temperature (400°C) to remove impurities. Three replicates were determined for each trial, and the blank and spiked samples were measured concurrently to validate the accuracy of the experiment. Results showed that the mass spectral separation times of DMP, DEP, DBP, BBP, DEHP and DOP were 9.76, 10.97, 14.40, 18.48, 20.35 and 22.06 min (Fig. S2), respectively. The recovery rates of the six PAEs ranged from 80.78% to 112.89% (mean 97.61%), and the method detection limit was 0.01 µg/kg, validating detection stability and accuracy.

An assessment model, recommended by the United States Environmental Protection Agency (USEPA 2013), was used to estimate the non-carcinogenic and carcinogenic risks of PAEs to adults and children in protected agriculture areas. DMP, DEP, DBP, and DOP considered non-carcinogenic compounds, whereas DEHP and BBP were considered carcinogenic to humans. According to the assessment model, the average daily doses (ADD) of non-dietary intake (i.e., soil ingestion, dermal contact, and inhalation) of PAEs in adults and children were first studied, and then the total risk to human health of PAEs in soil was investigated. The detail of calculation, parameter selection and parameter factors of the health risk assessment model were listed in supplementary materials (Table S3).

The date was statistically analyzed using the Statistical Package for Social Sciences (SPSS 22.0). Pearson correlation and network analyses were used to evaluate the relationship between the PAEs and the physical and
chemical properties of soil. The results were presented as mean ± standard deviation.

**Results and Discussion**

In the present investigation, DEHP, DBP, DOP, and BBP were detected in all soil samples, while DMP and DEP were negligible. Table S4 shows that ∑6PAEs concentrations ranged from 350.11 to 767.10 μg/kg, with a mean value of 497.64 μg/kg. Additionally, DEHP was a predominant PAE, with concentration ranging from 199.82 to 564.04 μg/kg, and a mean value of 318.68 μg/kg, markedly higher than those of the other PAEs (Fig. 1). DBP concentrations were the second-highest among the homologs, with concentrations ranging from 110.47 to 166.31 μg/kg, and a mean value of 137.56 μg/kg. The DOP and BBP concentration were comparable in the present study, with an average value of 20.12 μg/kg and 21.29 μg/kg, respectively. Compared with the soil from other regions in China, ∑6PAEs in this study was slightly higher than that in agricultural soils from Zhongshan but significantly lower than that in vegetable soil from Beijing (Table S5). In addition, previous studies have shown that PAEs were detected in agricultural soils from other countries, such as Denmark and the Netherlands (Table S5). These results indicated that PAEs contamination varied spatially in agricultural soil within China, reflected by regional differences in concentration. Niu et al. (2014) found that PAEs contamination was relatively high in soils from densely populated and economically developed areas, suggesting that economic development, population density, soil utilization type, and agricultural film usage would affect PAEs concentrations in soil.

However, the actual degree of soil contamination cannot be entirely dependent on the total concentration of PAEs, and the concentration of phthalate monomer compounds should be considered. The relative contribution of each PAEs was studied in this work, and the results showed that DEHP had the highest proportion, accounting for more than 54% of the ∑PAEs concentration, followed (in decreasing order) by DBP>DOP>BBP. The latter three homologs collectively accounted for 46% the ∑PAEs concentration. These results suggested DEHP contamination in the protected agricultural soil may be more serious than that of the other PAEs measured in this study, consistent with measurements in various environmental matrices (Kong et al. 2013; Wang et al. 2013). Similar to protected agricultural soil, DEHP and DBP were also the most important PAE contaminations of in urban soils affected by intensive human activities (Yang et al. 2018; Zhao et al. 2018). PAEs are widely used synthetic additives that include many homologs with different properties, owing to their different alkyl chain lengths. DEHP and DOP, having long alkyl chains, are usually used as plasticizers in plastic products (Benjamin et al. 2015). In contrast, PAEs with short alkyl side chains (DMP and DEP) are mostly used as solvents for fertilizers and pesticides (Gao et al. 2014). Therefore, different PAE-containing products may affect the PAE homologs profiles in agricultural soils (Sun et al. 2016). More importantly, DEHP and DBP have higher molecular weights and octanol–water partition coefficients than DMP and DEP, reducing their mobility, enhancing their persistence, and rendering them resistant to degradation in soils (Li et al. 2012).

**Fig. 1** The concentrations (a) and distribution ratio (b) of congener PAEs of sample sites in protected agriculture soil

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As shown in Fig. 2, DEHP was the most abundant PAE, and correlation analysis showed that there was a significant positive correlation between DEHP and the ∑PAEs concentration. In addition, soil pH and organic matter (Table S6) had opposite effects on PAEs. Soil pH was negatively correlated with the ∑PAEs concentration and four homologs, whereas SOM was positively correlated with PAEs ($p < 0.05$, Fig. 2). Some studies have reported that soil pH and organic matter were the main factors affecting the chemical behavior of organic contaminants in soil (Li et al. 2016; Zheng et al. 2016). Soil pH affects the adsorption behavior of hydrophobic organic pollutants in soil (Venkata Mohan et al. 2007). For instance, the adsorption of relatively polar PAEs in the soil increased with a decreasing pH, but as the pH increased, the ionization degree of soil organic matter increased and the soil’s affinity for hydrophobic organics, such as phthalates, decreased, resulting in the desorption of adsorbed organic contaminants (Yang et al. 2013; Zheng et al. 2016). Furthermore, PAEs have low water solubility but can easily dissolve in organic solvents, such as acetone and n-hexane. Studies have shown that the presence of SOM affects the solubilization of PAEs (e.g., surface sites of humic acid bind PAEs), and the increase of organic matter content may increase the number of adsorption sites, enhancing PAE adsorption (Cousins. and Mackay 2000; Cui et al. 2010). The relationship between PAEs, soil pH, and organic matter was also investigated in previous studies (Li et al. 2016; Zheng et al. 2016). However, various biological and non-biological environmental factors in terrestrial soil ecosystems may affect the behavior of PAEs in soil. The use and inadequate cleaning of agricultural films and atmospheric deposition could affect the concentration of PAEs in soil (Wang et al. 2013). In addition, the application of pesticides and fertilizer impact soil properties, indirectly affecting the migration, transformation, and biodegradation. In summary, soil pH and organic matter may be the key mechanisms affecting the content of phthalates. Further study is warranted to improve understanding of the environmental fates of PAEs in the soil environment.

It is well known that terrestrial ecosystems become the depository of heavy metals and deleterious organic matter from human activities. Various contaminants are incorporated into the soil and promote detrimental effects on soil organisms (e.g., earthworms and vegetables) and humans through skin contact and oral inhalation. As the example, recent studies have shown that the concentration of PAEs in agricultural soils in some areas of China was relatively high and significantly exceeded the allowable concentrations recommended (Tables S5 and S7), and PAEs in agricultural soil with film was significantly higher than that in open-air soil, which also indicated that there was a higher environmental risk in protected agriculture (Wang et al. 2021a, b).

In this study, we assessed the environmental risks of six priority phthalate substances, and the results showed that DMP and DEP were not observed in any of the soil samples, indicating that the concentration of these congeners

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**Fig. 2** Correlation analysis between PAEs and soil physical and chemical properties (a: blue means positive correlation while red means negative correlation, circle size means absolute value of correlation; b: brown means negative correlation while red means positive correlation, the thickness of the line means the absolute value of the correlation)
did not exceed the allowable concentration standards, which meant that they presented low environmental risk to human health. For other samples, the highest concentrations were 564.04 μg/kg (DEHP), 166.31 μg/kg (DBP), 27.20 μg/kg (DOP), and 22.16 μg/kg (BBP), respectively. It was also observed that DEHP, DOP, and BBP in soil did not exceed the allowable concentration standard value, while DBP concentrations ranged from 110.47 to 166.31 μg/kg, significantly exceeding the allowable concentration standard value of 81 μg/kg in all samples (i.e., exceedance rate of 100%), which was similar to results of Shouguang (Zheng et al. 2016) and Shenyang (Li et al. 2017), but higher than those of Zhongshan (93.85%) (Li et al. 2015) and Shantou (6.30%) (Wu et al. 2015). However, it should be noted that the concentration of DBP was far lower than the “cleanup objective” value and environmental risk limits (ERLs) that were derived using data on ecotoxicology and environmental chemistry (van Wezel et al. 2000).

Farming activities in protected agriculture (e.g., sowing, fertilization, harvest, etc.) may increase the probability of human exposure to PAEs pollutants. In addition, these contaminants may pose potential long-term exposure health risks to humans through multiple pathways. Since there were not issued relevant standards for PAEs pollutants in agricultural soil in China, in this study, we calculated the carcinogenic and non-carcinogenic risks of different PAE homologs in protected agricultural soil for different populations of people (adults and children) according to a risk assessment method recommended by the US EPA. Results showed that dermal contact was the major exposure pathway for adults and children to ingest PAEs, accounting for more than 75% of the total intake, followed by soil ingestion, accounting for 20.01%–24.61% of the total intake. While studies indicated that PAEs were also present in the air (Ma et al. 2020), the proportion of PAEs inhaled in this study was low, accounting for only 0.02%–0.14% (Table 1). It is worth considering about that although adults were mainly involved in agricultural production, children’s intake of PAEs was significantly higher than that of adults, suggesting that children may be more likely to ingest contaminants from the soil environment. In addition, Fig. 3 shows the non-carcinogenic and carcinogenic risks of PAEs form protected agriculture to adults and children. The results indicated that the hazard quotient values of the four PAE monomers were all less than 1, suggesting that their non-carcinogenic risk was relatively low. Furthermore, the hazard quotients of DEHP and DBP were higher than those of DOP and BBP, implying that these two pollutants have relatively higher health risks. Among the four PAE homologs, DEHP and DBP were considered potentially carcinogenic (Ji et al. 2014). In the present study, the carcinogenic risk of DEHP and DBP estimated was very low because their carcinogenic risk scores were lower than 10⁻⁶ (Fig. 3). These results indicated that PAEs in protected

| Congener | Adults | Children | Adults | Children | Adults | Children | Adults | Children |
|----------|--------|----------|--------|----------|--------|----------|--------|----------|
| DEHP     | 2.74×10⁻⁷ | 7.73×10⁻⁷ | 1.09×10⁻⁶ | 3.08×10⁻⁶ | 1.74×10⁻⁶ | 1.44×10⁻⁵ | 3.03×10⁻⁹ | 1.74×10⁻⁹ |
| DBP      | 2.34×10⁻⁷ | 6.59×10⁻⁷ | 4.14×10⁻⁶ | 2.02×10⁻⁵ | 6.86×10⁻⁶ | 5.12×10⁻⁵ | 2.03×10⁻⁹ | 1.01×10⁻⁹ |
| DOP      | 1.29×10⁻⁷ | 1.49×10⁻⁷ | 1.88×10⁻⁶ | 1.16×10⁻⁵ | 9.60×10⁻⁷ | 8.90×10⁻⁵ | 1.90×10⁻⁹ | 1.00×10⁻⁹ |
| BBP      | 2.69×10⁻⁷ | 3.18×10⁻⁷ | 3.72×10⁻⁶ | 2.49×10⁻⁵ | 1.16×10⁻⁶ | 1.06×10⁻⁵ | 2.59×10⁻⁹ | 1.31×10⁻⁹ |

Table 1 The average daily dose for adults and children via non-dietary pathways
agricultural soil posed an insignificant health risk to humans as they did not exceed the acceptable level. However, children exhibited a higher non-carcinogenic risk and carcinogenic risk than adults, illustrating that the toxic properties of PAEs may be more deleterious in children than in adults, possibly because detoxification and metabolism functions are weaker for children than those of adults.

PAEs are typical environmental endocrine-disrupting substances. Studies have reported that the levels of T3 and T4 in adult blood are negatively correlated with PAE metabolites in urine (Park et al. 2017), and positive correlation between urinary PAE levels and overweight/obesity found in children (Xia et al. 2018). Thus, these studies indicated that human health could be threatened by PAE exposure, possibly for extended time. In this study, the risk assessment may be slightly underestimated because the health risks of PAEs under dietary routes were not considered. The ecological and health assessment of PAEs through the food chain requires further attention. Furthermore, studies on PAE toxicity to mammals are primarily focused on rats or mice (Ha et al. 2016), and more experimental data on PAE toxicity (and their metabolites) to other animals after long-term exposure are needed to fully understand the health risks and mechanisms of PAEs.

In general, the results showed that there were four ubiquitous PAE homologs detected in the protected agricultural soil of Fangcun. In this study, the concentration of DEHP was the highest, followed by DBP. Concentration of DOP and BBP were similar and relatively low, indicating that DEHP and DBP in protected agricultural soil dominated the total PAE concentration. Among the four PAEs detected, only DBP exceeded the allowable concentration standard with a 100% exceedance rate, but it did not exceed the “cleanup objective” value or ERLs. Correlation analysis indicated that soil pH had a significant negative correlation with PAEs, whereas the soil organic matter might dramatically promote PAE adsorption. Furthermore, dermal contact was identified as the primary route of PAE exposure under non-dietary conditions, followed by soil intake, while inhalation intake was almost negligible. The intake of PAEs by children was higher than that of adults, suggesting that children may have a higher risk of exposure. Risk assessment results showed that carcinogenic and non-carcinogenic risks were at an acceptable level, but the risk assessment scores of children were significantly higher than those of adults, warranting further attention. These research results will provide evidence for the ecological risk assessment of PAEs and enrich basic data for the establishment of PAE soil pollution standards and pollution restoration.

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