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RESEARCH ARTICLE

Effects of film mulching on the distribution of phthalate esters in wheat grains from dryland

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Abstract

The application of plastic film in field crop production elevated the phthalate esters (PAEs) accumulation in wheat grains, which poses potential risks to human health. However, the variation of grain PAEs contents in different dryland areas is not clear, and the distribution of PAEs in different tissues of grains has not been studied yet. In the present study, field experiments in five sites (three provinces) with two treatments (soil with and without film mulching) were carried out to study the concentration and distribution of PAEs in grains and the effects of environmental factors on them. Results showed that the total PAE concentration (∑PAEs) in wheat grains ranged from 445 to 764 μg/kg, mainly in the forms of di-(2-ethylhexyl)-phthalate (DEHP), dibutyl phthalate (DBP), and diisobutyl phthalate (DIBP). Compared with control, total PAE concentrations in soils and wheat grains were significantly higher in treatments with film mulching. The effects of film on the proportion of PAEs in the flour and bran varied with experiment sites. Grain PAEs in the control groups presented significantly negative correlation with annual temperature, while there was a positive correlation between soil PAEs and bran PAEs in the film treatment. Results in this study are of great significance to comprehensively evaluate the effect of film mulching on grain safety in dryland wheat production.

Keywords Cereal · Film mulching · Arid area · Soil · Phthalates · Distribution

Introduction

Phthalate acid esters (PAEs, or phthalates), as a synthetic organic compound, have been extensively used in the production of plastics to increase the extensibility and plasticity of plastics (Benson. 2009, Colombani et al. 2009). The global plastic production exceeded 150 million tons in 2015, and the global annual use of PAEs reached approximately 6–8 million tons (Net et al. 2015). As environmental hormone disrupters, phthalates are not covalently bonded to the polymer (Abdel daiem et al. 2012) and can be migrated readily from plastic substrates to the environment (Chen et al. 2013, Colombani et al. 2009). Thus, six PAEs of them have been listed as priority pollutants in the United States Environmental Protection Agency (USEPA. 2013a). For example, in the United States, the highest concentration of Di-(2-ethylhexyl) phthalate (DEHP) allowed is 0.006 mg/L under the Safe Drinking Water Act (USEPA, 2013b), and the reference doses for DEHP recommended is 20 μg/(kg bw·d) (USEPA, 2005).

The wide use of plastic film in crop production has caused the accumulation of PAEs in soils and crops. PAEs have been detected in soils (Lü et al. 2018, Niu et al. 2014, Zeng et al. 2008), vegetables (Chen et al. 2017), wheat (Shi et al. 2019), and rice grains (Lu et al. 2016). The occurrence of PAEs in plants was reported to be affected by various factors, including soil PAEs, plant cultivars, and the physicochemical properties of PAEs. A pot experiment showed that vegetables mainly adsorbed PAEs from soil (Zeng et al. 2005). A long-term field experiment showed that there was a significant positive correlation of PAEs concentration between wheat and soil (Shi et al. 2019), while the results of the multisite investigation showed that there was no significant correlation between vegetable and soil PAEs (Li et al. 2016, Wang et al. 2015). Studies on rice genotypes

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showed that there were significant differences of DEHP concentrations in grains for different rice species (Cai et al. 2015). In addition, the PAE accumulation in crops is also affected by physicochemical properties of different PAE congeners. The PAE congeners with higher water solubility and lower octanol-water partition coefficient would be absorbed more easily by edible plants (Wang et al. 2015, Sun et al. 2015).

Wheat, as one of the main food crops, has been widely planted in the arid and semi-arid areas, where film mulching was extensively used in crop production. Our previous study showed that long-term film mulching could result in PAEs accumulation in wheat grains (Shi et al. 2019). However, the variation of grain PAEs content in different dryland areas is not clear, and the distribution of PAEs in different tissues of grains has not been studied yet. As an edible plant, wheat grains are often divided into bran and flour during processing, and flour will generally enter the human body as an edible part. Studying the proportion of PAEs in flour and bran of wheat grains, analyzing their differences, and the affecting factors among different sites are critical for assessing the health risk caused by plastic film mulching.

Therefore, the objectives of this study were to clarify the distribution of PAEs in wheat grains and reveal their relations with film mulching and other environmental factors by conducting five field experiments simultaneously with two treatments (with and without film) in northwest China, including Shaanxi, Shanxi, and Gansu provinces.

Materials and methods

Chemicals and materials

Seven standard mixtures of PAEs, including DMP (dimethyl phthalate), DEP (diethyl phthalate), BBP (butyl benzyl phthalate), DIBP (diisobutyl phthalate), DBP (dibutyl phthalate), DEHP, and DNOP (Di-n-octyl phthalate) dissolved in acetonitrile with 2000 mg/L, were purchased from Reference Materials (Beijing, China). Benzyl benzoate was obtained from Dr. Ehrenstorfer (Augsburg, Germany). HPLC-grade acetonitrile was purchased from Tedia (Ohio, USA). Primary secondary amine (PSA, 40–60 μm) were obtained from Agela (Tianjin, China). Other chemicals (analytical purity), such as anhydrous magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl), were procured from Sinopharm Chemical Reagent (Beijing, China). $MgSO_4$ and NaCl were purified at 600 °C for 4 h in a muffle furnace. Ultrapure water was prepared from Millipore Corp. system (18.2 MΩ cm).

Design of sites for field experiment

Field experiments were carried out in five sites from three provinces, including Yongshou (YS) in Shaanxi, Hongtong (HT) and Wenxi (WX) in Shanxi, and Qingshui (QS) and Tongwei (TW) in Gansu during 2017–2018 in the northwest dryland area of China. Winter wheat was sown in September and harvested in June of the following year. Locations of field experiments were shown in Fig. 1. Soils in these locations are classified as silt loam by the USDA texture classification system (Soil Survey Staff, 1998). The basic physical and chemical properties of soil (0–20 cm) were exhibited in Table S1.

Two treatments with and without plastic film mulching were conducted for each field experiment, replicated three times per treatment. The local traditional mulching modes and management methods were adopted in this experiment. The local widely planted wheat cultivar was selected for each site. Planting patterns, wheat cultivars, and other field information were shown in Table S2. For the film mulching treatment (PM), the film used in the last growing season was removed and replaced with new one before next year's wheat sowing in four experiment sites (WX, HT, YS, and TW). In QS, the plastic film was not recovered but incorporated into the soil by tillage, and new film is used in the next growing season. For the control treatment (CK), wheat was planted in flat and the soil surface without mulching, and other conditions remain the same as the corresponding film mulching treatment in each site.

Sample collection and extraction

Grain and soil samples were collected in the harvest stage. During the sample collection, processing, and storage, plastic materials were avoided. Four wheat quadrats (1.0 × 1.0 m^2) representing the growth of whole plot were selected to determine the wheat grain yield.

For plant sample collection, 100 ears of wheat were selected randomly from each plot to ensure sample representativeness and threshed manually after air-drying. Some grains were rinsed three times with distilled water and deionized water, respectively, then divided into flour and bran, and finally ground into powder by using quadrumat junior mill (Brabender, Germany) and ball milling machine (Retsch, Germany). All processed powdered samples were wrapped in aluminum foil and stored at − 20 °C in refrigerator before analysis.

Soil samples were collected in the topsoil of 0–20 cm. Five cores in each plot were selected and collected by a steel soil auger, bulked, and then transferred to brown paper bags. All soil samples were ground and sieved through 60 mesh after air-dried and stored at − 20 °C in refrigerator before analysis.

The extraction and purification of PAEs were performed as in the previous study (Dong et al. 2017). For each sample extraction, 0.50-g sample was added into a 10-mL PTFE centrifuge tube. After the addition of 2.0 mL ultrapure water, the solution was acidified to pH 6.0 with 1 M hydrochloric acid, vortexed for 30 s, and then left to hydrate for 15 min. Next, 2.0 mL acetonitrile was added exactly to the mixture and shaken

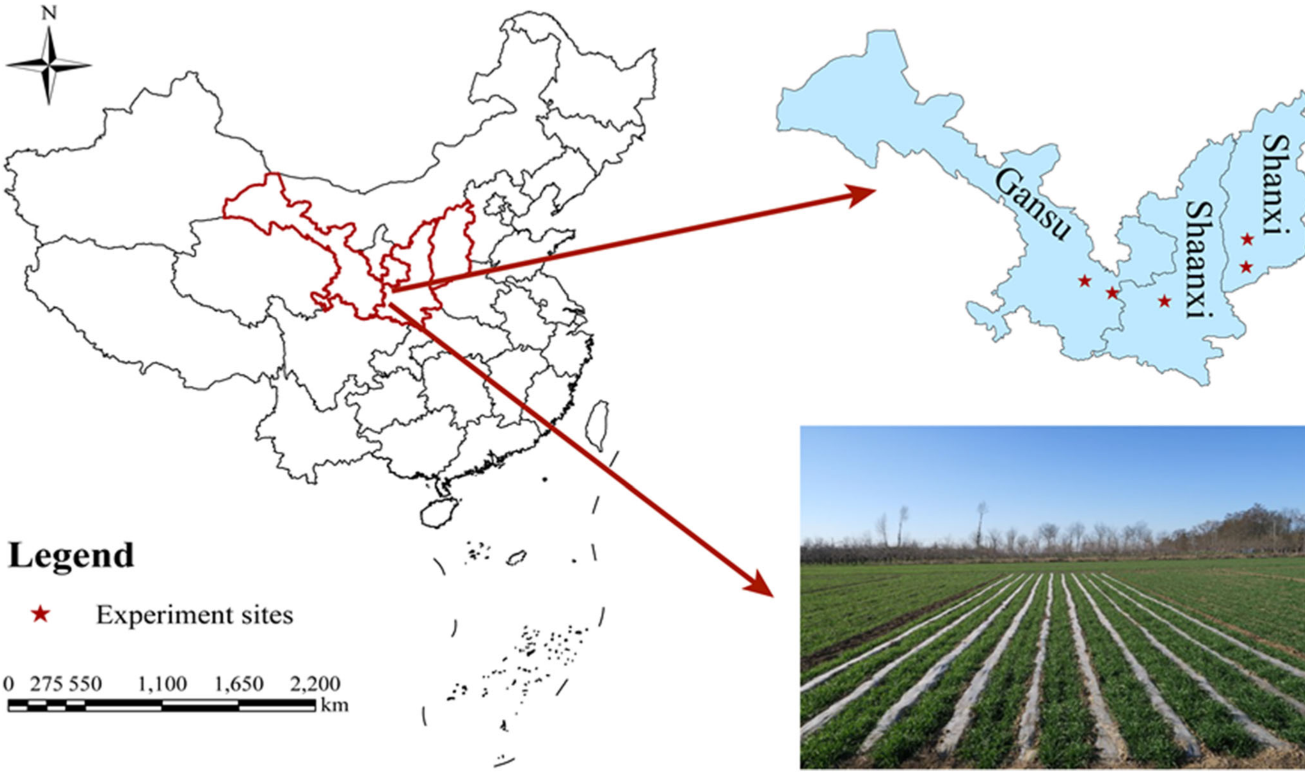

Fig. 1 Locations of field experiment sites in Gansu, Shaanxi, and Shanxi

violently by a vortex agitator for 2 min. Subsequently, 0.32 g of sodium chloride and 0.70 g of anhydrous magnesium sulfate were added into the mixture, vortexed for 30 s, and then centrifuged (Cence, China) at 6500 r for 5 min. One milliliter of the supernatant was added to a 2.0-mL PTFE centrifuge tube containing 150 mg of anhydrous $MgSO_4$ and 150 mg of PSA (40–60 μm). The mixture was vortexed for 1 min and centrifuged at 6500 r for 3 min. Finally, the extract was placed in a 2.0-mL brown vial and stored at − 20 °C for GC-MS analysis.

Any plastic products were avoided during the experiment. The intermediate standard solutions (10 mg/L) were prepared by diluting the standard substance and further diluting to the working standard solutions at the concentrations of 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 mg/L in acetonitrile, with the confidence interval of > 99%. For every 15 sample analyses, blank samples were analyzed. Benzyl benzoate was used to monitor the recovery as surrogate standards. The limits of detection of DMP, DEP, BBP, DIBP, DBP, DEHP, and DNOP ranged from 0.1 to 2.5 μg/kg, which was defined by a signal-to-noise ratio of 3:1.

Instrumental analysis

PAEs in the extracted compounds were analyzed with a TRACE 1310-ISQLT gas chromatography-mass spectrometry (GC-MS) system (Thermo Fisher Scientific, Waltham, MA, USA), which was operated using Xcalibur software. TG-5MS (5% phenyl methylpolysiloxane, 30 m × 0.25 mm × 0.25 μm) capillary column was used to complete chromatographic separation and the selected ion monitoring (SIM) mode was selected to quantify. The flow rate of high-purity helium was set 1.0 mL/min. The injection volume was 1 μL with splitless mode and the injector temperature was set 250 °C. The column temperature program was initiated at 60 °C for 1 min, increased to 220 °C at 20 °C/min, held for 5.0 min, raised to 280 °C/min at 5 °C/min, and ramped to 320 °C at 10 °C/min, maintained for 2.0 min. The temperature of ion source and transfer line was 300 °C.

Statistical analysis

All data for the two treatments (plastic film mulching and no mulching) in each site was analyzed by the SPSS Statistics 20.0 with independent samples *t* test ($P < 0.05$). Graphs were performed using the Origin 2017.

Results

Concentration of PAE congeners in soils

Phthalate acid esters were detected in all soil samples, and the ∑PAEs concentration of soil with film mulching was in the range of 331–1352 μg/kg with an average of 1103 μg/kg, which was higher than that in control (Fig. 2). Film mulching significantly increased soil PAEs in WX and QS mainly due to the increase of DEHP and DBP.

Of the seven tested PAEs, five PAEs congeners (i.e., DMP, DEP, DBP, DIBP, DEHP) were detected, but the proportion of PAE congeners in different sites were different. In all tested sites, the proportion of DBP was the highest with 87.8% in QS, 74.9% in YS, 60.7% in TW, 56.6% in WX, and 51.8% in HT. The proportion of DEHP was less than DBP, with 35.0% in TW, 34.4% in WX, 12.1% in YS, 3.1% in HT, and 2.5% in QS. In QS and HT, more DIBPs were detected than DEHP. Only traces of DEP (0.6%) and DMP (1.3%) were detected in all sites.

Concentration of PAE congeners in wheat grains

The proportions and concentrations of PAE congeners in wheat grains at different sites were presented in Fig. 3. The concentration of ∑PAEs in wheat grains (445–764 μg/kg) with plastic film (with the average of 645 μg/kg) was higher than that in control (with the average of 555 μg/kg). In all samples, DEHP, DBP, and DIBP were the most abundant PAE congeners, with the average concentration of 223, 214, and 145 μg/kg, respectively. Other congener profiles including DMP (2.9%) and DEP (0.2%) were detected in a small proportion. In particular, the concentration of grain DEHP in YS was the lowest among all sampling sites.

PAEs proportion of in wheat bran and flour

For all treatments, the main PAE congeners in bran and flour were DEHP, DBP, and DIBP, but their concentrations varied greatly among different sites (Fig. 4). Compared to control, film mulching increased the

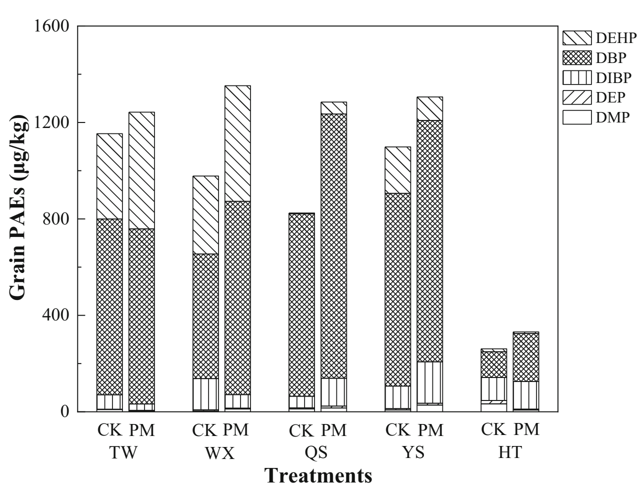

Fig. 2 Concentration of PAE congeners in soils at different sites. CK, control without mulching; PM, film mulching

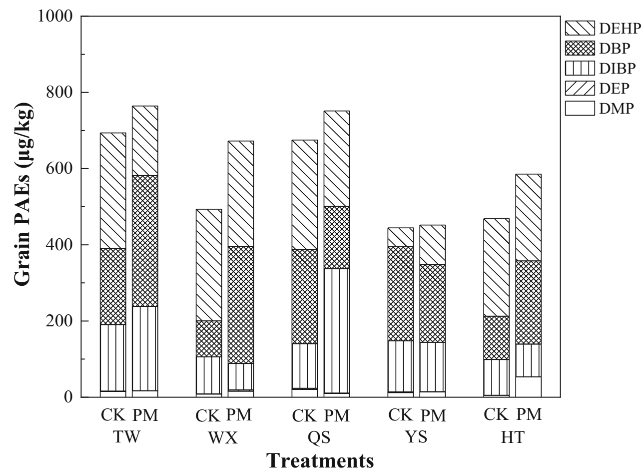

Fig. 3 Concentration of PAE congeners in wheat grains at different sites. CK, control without mulching; PM, film mulching

∑PAEs concentration of the bran in WX and enhanced the ∑PAEs concentration of flour in all sites. For control groups without film (Fig. 4, CK), the concentration of ∑PAEs in the bran was higher than that in the flour for each site. The concentration of ∑PAEs concentration in the bran was in the range of 503–1108 μg/kg, with an average of 777 μg/kg, while that in the flour ranged from 223 to 675 μg/kg, with an average of 481 μg/kg. For PM treatment with film mulching (Fig. 4, PM), the concentration of ∑PAEs in the bran was higher than that in the flour in the experiment site WX, QS, and YS, but opposite results were found in TW and HT. In TW and HT, a higher concentration of ∑PAEs was observed in the flour, which was mainly due to the increase of DBP and DIBP. The concentration of ∑PAEs in the bran ranged from 399 to 1165 μg/kg, with the average of 838 μg/kg. However, the ∑PAEs concentration in the flour was in the range of 230–789 μg/kg, with an average of 581 μg/kg.

Relations between grain PAEs and environmental factors

For control groups without film, there was a negative correlation between grain PAEs and annual temperature, but no significant relationship was found between grain (or flour, or bran) PAEs and other environmental factors. For PM treatments with plastic film, only a significantly positive correlation was found between bran PAEs and soil PAEs, while there was no significant relationship between PAEs in the bran/flour/grain and other environmental factors. The correlation analysis also showed that there was no significant relationship between soil and wheat grains for PAE congeners (DBP, DIBP, and DEHP) or total PAEs (Fig. 5 and Fig. S1).

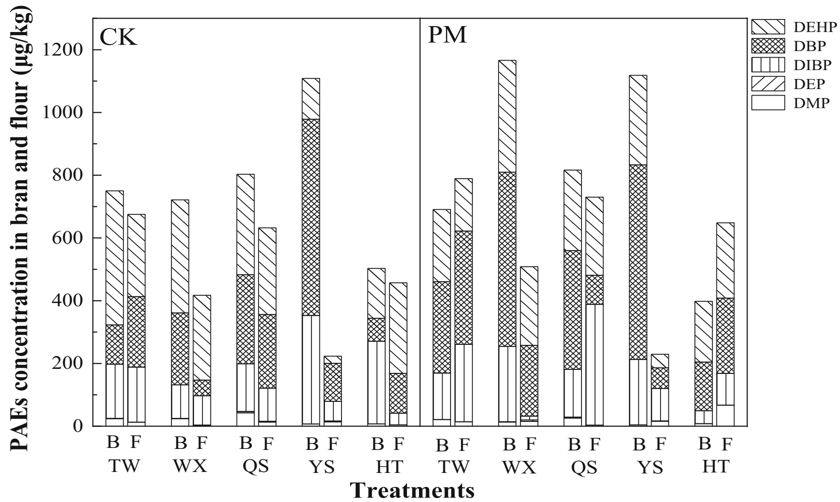

Fig. 4 Concentration of PAE congeners in the brans and flour of wheat grown at different sites. CK, control without mulching; PM, film mulching. B, bran; F, flour

Discussion

PAEs in the soil

In the present study, plastic film mulching significantly increased the content of PAEs in soil, and different levels of PAEs were also detected in the soil of control groups (Fig. 2). Soil PAEs in control groups may come from pesticide (Li. 2018), fertilizer applied (Mo et al. 2008), or atmospheric deposition (Zeng et al. 2010). Compared with the control group, the increased values of soil PAEs were quite different in the film mulching group for different sites (Fig. 2), which could be the result of a combination of factors. The concentration of soil PAEs depended on the release rate of PAEs from film (He et al. 2014), the degradation rate in soil (Steinmetz et al. 2016), and the physicochemical properties of PAE monomers (Net et al. 2015). Plastic materials could release PAEs into the environment during aging process, and the release rates are closely related to the environment microbial activity (Cai et al. 2018, Paluselli et al. 2019, Wang et al. 2016). In addition, primary degradation half-lives of PAE monomers with different molecular weight in soils range from several days to several months (Staples et al. 1997). Future researches should be conducted to explore the environmental behavior (especially the mechanisms of release and degradation) of PAEs in different soils with residual plastic film.

PAEs in the wheat grain

No significant correlation was found between grain PAEs and soil PAEs in control groups, while only a significant positive correlation was found between bran PAEs and soil PAEs in the film treatments (Fig. 5 and Fig. S1). These results could be related to the uptake pathways of PAEs by winter wheat. It has been reported that both roots and leaves may adsorb semivolatile organic pollutants in the environment, but the role of root and foliar uptake of PAEs in its accumulation in wheat has not been developed yet (Gao et al. 2017, Wang et al. 2012). Potted experiments showed that *Brassica parachinensis L.* adsorbed PAEs mainly through the roots (> 70%), and less than 30% of PAEs in the plant was from the foliar uptake of PAEs volatilized from soil (Zeng 2004).

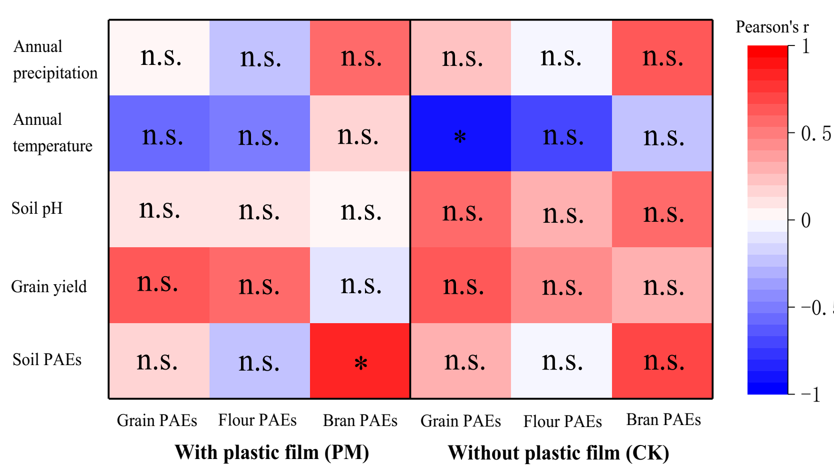

Fig. 5 Relationships between grain PAEs, flour PAEs, bran PAEs, and environment factors (soil PAEs, grain yield, soil pH, annual temperature, and annual precipitation) in treatments with plastic film or not. * and ns indicate the difference is significant ($p < 0.05$) and not significant ($p > 0.05$), respectively. CK, control without mulching; PM, film mulching

Therefore, it is hypothesized that grain PAEs in control without film might be related to the foliar uptake.

In addition to PAEs concentration in the environment, the accumulation of PAEs in grains was also affected by the wheat cultivars. A previous study on 20 rice cultivars through pot experiment showed that there was genotypic variation of rice in the accumulation of PAEs in grains (Cai et al. 2015); similar results also reported on the study of Chinese flowering cabbage (*Brassica parachinensis L.*) (Zhao et al. 2015).

The distribution of PAEs in bran and four

Results showed that for treatments with film, proportions of PAEs in the bran and flour with film were different in different sites (Fig. 4), and a significant positive correlation was found between bran PAEs and soil PAEs in the film treatments. PAEs in soil released from film may be adsorbed by the roots and then transported into the grain of wheat. However, our knowledge on the mechanisms of PAEs uptake and grain loading in the wheat is currently still limited. It has been reported that some of the organic contaminants were fixed in the lipid of the roots, while some entered the cellular tissues through the epidermis of roots and then transported upward through the xylem (Köstner et al. 1998, Shi et al. 2017, Sun et al. 2015). However, the unloading pathways of PAEs to grain are still unclear, and how phthalates are translocated to the crease, pericarp tissues, and flour needs to be further studied.

In the present study, most of the grain PAEs increased as a result of film mulching went into flour instead of bran for most experiments sites, but only one exception for WX where the increased PAEs in bran far exceeded that in flour (Fig. 4). In addition, results showed that environmental factors (such as soil pH, annual precipitation, and temperature) had little effects on the PAEs concentrations in the bran or flour (Fig. 5). Therefore, we assumed that the distribution of PAEs in bran and flour of wheat grains may be attributed to differences in PAEs uptake and grain loading among different wheat cultivars. Significant variation in DEHP concentrations has also been reported in the roots, stems, leaves, and grains of most rice cultivars among the rice cultivars (Cai et al. 2015). Plastic film mulching cultivation plays a crucial role in food production due to its increased yield in dryland, but the contamination of PAEs in soil and crops cannot be ignored. Up to now, screening for low-PAE accumulating wheat cultivars, especially in wheat flour, is an effective way to resolve the contradiction between efficient production and food safety.

Conclusions

Multisite field experiments showed film mulching significantly increased the concentrations of PAEs in soil and wheat grains. The concentration of ∑PAEs in wheat grains ranged from 445 to 764 μg/kg, and soil PAEs ranged from 261 to 1352 μg/kg, mainly in the forms of DEHP, DBP, and DIBP. The increased proportion of PAEs in flour and bran caused by film mulching varied among different experiment sites. A positive correlation was found between soil PAEs and bran PAEs in the treatments with film. Further researches should be carried out to elucidate the pathways of uptake and translocation of PAEs in wheat and the variations among different wheat cultivars.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-021-12406-x.

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Authors contributions Yingying Sun: Sampling; formal analysis; writing—original draft
Chao Li: Writing—review
Xinxin Zhang: Formal analysis
Mei Shi: Supervision; resources; writing—review
Zhaohui Wang: Supervision; resources; and writing—review

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Data availability The data for this study are available on reasonable request.

Compliance with ethical standards

Competing interest The authors declare that they have no competing interest.

Ethical approval Not applicable.

Consent to participate Not applicable.

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