ORIGINAL ARTICLE

Accumulation, root-shoot translocation and phytotoxicity of substituted polycyclic aromatic hydrocarbons in wheat

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Abstract

While pollution of the environment by polycyclic aromatic hydrocarbons has been rather well studied, the fate of substituted polycyclic aromatic hydrocarbons in ecosystems is less understood, notably in soil–plant systems. Here we hypothesized that substituted polycyclic aromatic hydrocarbons could enter wheat and induce phytotoxicity. We studied the accumulation, root-shoot translocation and phytotoxicity of α-tetralone, 1-nitronaphthalene, 1-nitropyrene, and 7,12-dimethylbenz[a]anthracene in wheat using hydroponic experiments. We deciphered the accumulation and translocation mechanisms by inhibition with sodium vanadate, glycerol and silver nitrate. Results show that pollutant concentrations increased rapidly in roots, reaching maximum of 38.3 μg/g for α-tetralone, 268.7 μg/g for 1-nitronaphthalene, 3566.1 μg/g for 1-nitropyrene, and 3632.7 μg/g for 7,12-dimethylbenz[a]anthracene. In contrast, the root-shoot translocation factors of α-tetralone, 1-nitronaphthalene, 1-nitropyrene and 7,12-dimethylbenz[a]anthracene were 2.4, 1.6, 0.1, and 0.1, respectively, thus decreasing significantly with increasing compound hydrophobicity. This could be explained by preferential adsorption for hydrophobic compounds, whereas less hydrophobic compounds could be partly translocated in a soluble form through plant water channels. The accumulated substituted polycyclic aromatic hydrocarbons in wheat induced the decrease of the total chlorophyll contents by 19.0–30.1%, yet did not cause lipid peroxidation damage. Overall, our findings indicates the bioaccumulation potential and toxicity of substituted polycyclic aromatic hydrocarbons in terrestrial plants.

Keywords Substituted polycyclic aromatic hydrocarbons · Plant · Root concentration factor · Translocation factor · Inhibitor · Oxidative stress

Introduction

Over the last century, contamination induced by polycyclic aromatic hydrocarbons (PAHs) has risen due to the intensified industrial activities, including fertilizers, insecticides, and pharmaceutical applications (Fan et al. 2021; Kang et al. 2010). On account of the mutagenicity, carcinogenicity, teratogenicity, and ubiquitous presence in various environmental media (Honda and Suzuki 2020; Wu et al. 2021), sixteen types of PAHs are defined as the priority contaminants in US Environmental Protection Agency (Diblasi et al. 2009). The ecotoxicity of PAHs in soil has been extensively reported (Titaley et al. 2016), owing to the fact that soil is the major sink for PAHs in the environment (Jesus et al. 2022; Sanchez-Pinero et al. 2022). Once PAHs enter the soil environment, they may undergo a series of abiotic and biotic processes, including chemical oxidation, photochemical transformation, and biodegradation (Krzyszczak and Czech 2021; Qiao et al. 2013). In these processes, PAHs could be converted into substituted polycyclic aromatic hydrocarbons (substituted PAHs) (Bandowe and Meusel 2017). Among them, three types of substituted PAHs, including oxygenated PAHs, nitrated PAHs, and methylated PAHs, are the

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most prevalent in the soil environment (Qiao et al. 2016). For instance, Brasher and Wolff reported that 14 types of oxygenated PAHs were detected in European soil, ranging from 88.0 to 2629.0 µg/kg (Brasher and Wolff 2004). Sun et al. (2017) reported that the contents of nitrated PAHs in the agricultural soil of eastern China were 50 ± 45 µg/kg. In addition, in comparison with PAHs, the higher toxicities of substituted PAHs were reported (Qiao et al. 2020). For example, the mutagenicity ability of substituted PAHs was higher than that of PAHs when tested against *Salmonella typhimurium* TA 98 and TA 100 (Lofroth et al. 1985). Meanwhile, the biotoxicity of substituted PAHs on barley (*Hordeum vulgare*) and mysid shrimp (*Americamysis bahia*) was also reported (Finch et al. 2017; Yun et al. 2019). Consequently, in consideration of the ubiquitous presence and potential risk of substituted PAHs in soil, it is critical to probe the environmental behaviors of substituted PAHs in the soil environment.

It is widely known that organic contaminants of soil could be absorbed by plant roots, and then enter biological circulation through the food chain, posing a threat to human health (Liu et al. 2019; Yu et al. 2023). Thus, to scientifically avoid the negative effects induced by organic contaminants, a large number of studies explore the accumulation and translocation mechanism of organic contaminants in plants (Deng et al. 2018; Tao et al. 2009). Generally, organic contaminants could be absorbed by plant roots through an active process consuming energy and/or passive diffusive partitioning (Liu et al. 2021; Wan et al. 2017), and are subsequently transferred to plant shoots via the vascular system (Wild et al. 2005). For instance, previous studies indicated that the uptake pathways of plants, including, aquaporins and PAHs/H$^+$ cotransport system, played the critical role in accumulating and transferring PAHs (Barbier-Brygoo et al. 2000; Zhan et al. 2010). Meanwhile, the hydrophobicity of PAHs was also considered the main driving force in regulating the accumulation and translocation of PAHs in plant (Belykh 2009). The results of above researches imply that the accumulation and translocation processes of PAHs in plant are conjointly regulated by multiple factors. Comparatively, the physicochemical properties of substituted PAHs may be more complicated due to the presence of substituents, potentially resulting in the more diverse accumulation and translocation behaviors of substituted PAHs in the plant. However, to date, there are limited studies concerning the accumulation and translocation mechanism of substituted PAHs in plant tissues.

The accumulation of organic contaminants in plants potentially caused the phytotoxicity, showing the variation of intracellular reactive oxygen species levels and the photosynthesis pigments contents (Liu et al. 2009; Xu et al. 2023). Excessive reactive oxygen species could lead to reduced membrane activity and lipid peroxidation (Liu et al. 2020).

To maintain the plant health, the excessive reactive oxygen species content of plants is eliminated by antioxidant defense system in a synergistic manner, which primarily consisted of catalase, peroxidase, and superoxide dismutase (Kaushik and Aryadeep 2014). Paskova et al. (2006) reported the phytotoxicity induced by PAHs. However, as a more toxic derivative than PAHs, it is unclear whether substituted PAHs affect the antioxidant system and photosynthetic pigments of plants after being accumulated in plants, thereby hindering accurate assessment of their phytotoxicity.

In this study, to comprehensively understand the accumulation, translocation and phytotoxicity of substituted PAHs with different substituent groups in plants, wheat (*Xiaoyan 22*), as the common-cultivated crop, was used in hydroponic experiments. To this end, we first explored the variation of α-tetralone, 1-nitronaphthalene, 1-nitropyrene, and 7,12-dimethylbenz[a]anthracene concentrations in wheat roots and shoots with culture time; second, metabolic and aquaporin inhibitors were used to elucidate the mechanisms of wheat roots absorbing substituted PAHs; finally, the antioxidant defense system, lipid peroxidation and photosynthesis pigments of wheat were measured to disclose the phytotoxicity of substituted PAHs.

Experimental

Exposure experiments and analysis

After 14-day preincubation, wheat seedlings were exposed to the nutrient solution spiked with α-tetralone, 1-nitronaphthalene, 1-nitropyrene, and 7,12-dimethylbenz[a]anthracene. The seedlings were subsequently harvested at preselected intervals, and the nutrient solution was also sampled at the same time interval. The extraction and purification of substituted PAHs in wheat and nutrient solution were carried out according to the previous studies with minor modifications (Baroudi et al. 2020; Tfouni et al. 2018). Briefly, 20 mg (dry weight) of roots samples or 50 mg (dry weight) of shoots samples were extracted with acetonitrile. After purifying the extracts with magnesium sulfate, primary secondary amine sorbent and C18, the supernatant was further centrifuged at 12,000 rpm for 20 min, and analyzed by high-performance liquid chromatography (HPLC, Ultimate 3000, Thermo Scientific, USA). The extraction of substituted PAHs in nutrient solution was conducted by following the same procedure as described above except for the addition of purifying agent.

Inhibition experiments

Sodium vanadate (27 µM), glycerol (25 µM) and silver nitrate (0.1 µM) were applied to explore the uptake pathway of substituted PAHs by wheat roots. The detailed

experimental sections are provided in Text S1 of Supplementary Materials.

Determination of plant physiology

Approximately 0.5 g of fresh plant sample was frozen in liquid nitrogen and ground in a pre-cooled mortar with a pestle. After that, 4.5 mL phosphate buffer solution (67 mmol/L, pH = 7.4) was added to the mortar, thus obtaining the 10% plant homogenate. Malondialdehyde, peroxidase and superoxide dismutase were measured using a malondialdehyde kit, peroxidase kit and superoxide dismutase kit, respectively, (Suzhou Keming Biotechnology Co., Ltd., Jiangsu, China). The measurement was carried out according to the specifications of the detection kits.

Total chlorophyll content was determined by adopting the protocol of previously reported (Liu et al. 2020). Briefly, 0.5 g of fresh shoots was extracted with 10 mL ethanol: acetone (1:1, v:v). After 24 h of extraction, the mixture passed through a 0.45 μm membrane filter and then was measured by ultraviolet spectrophotometer (Evolution 201, Thermo Fisher Scientific) with 663 nm and 645 nm.

Results and discussion

Accumulation and translocation of substituted polycyclic aromatic hydrocarbons in wheat

The concentrations of substituted PAHs in the unspiked plant controls remained stable (Table S1), meaning that the losses of substituted PAHs caused by adsorption, degradation and evaporation could be neglected. In the unspiked substituted PAHs controls, four types of substituted PAHs were undetectable in the wheat (Table S1), confirming the negligible uptake of substituted PAHs from the air.

During the exposure period, the concentrations of α-tetralone, 1-nitronaphthalene, 1-nitropyrene, and 7,12-dimethylbenz[a]anthralene in wheat roots rapidly increased and, respectively, reached the maximum at 2, 2, 24, and 36 h of exposure time, which were 38.3, 268.7, 3566.1 and 3632.7 μg/g, respectively (Fig. 1a–d). The root concentration factor was used to characterize the accumulation capacity of substituted PAHs in wheat roots. The root concentration factor values of α-tetralone, 1-nitronaphthalene, 1-nitropyrene and 7,12-dimethylbenz[a]anthracite were 0.1, 0.3, 10.8 and 11.8, respectively (Table S2), consistent with the order of their hydrophobicity ($R^2 = 0.868$, $p < 0.05$, Fig. S1a). These results revealed the critical role of hydrophobicity in regulating the accumulation ability of substituted

Fig. 1 Time-dependent concentrations of α-tetralone (**a**), 1-nitronaphthalene (**b**), 1-nitropyrene (**c**), and 7,12-dimethylbenz[a]anthracene (**d**) in the wheat roots and shoots. The concentrations of all substituted polycyclic aromatic hydrocarbons in wheat roots and shoots rapidly increased during the exposure period. Error bars represent standard deviations from replicates (n = 3). The dw represents the dry weight
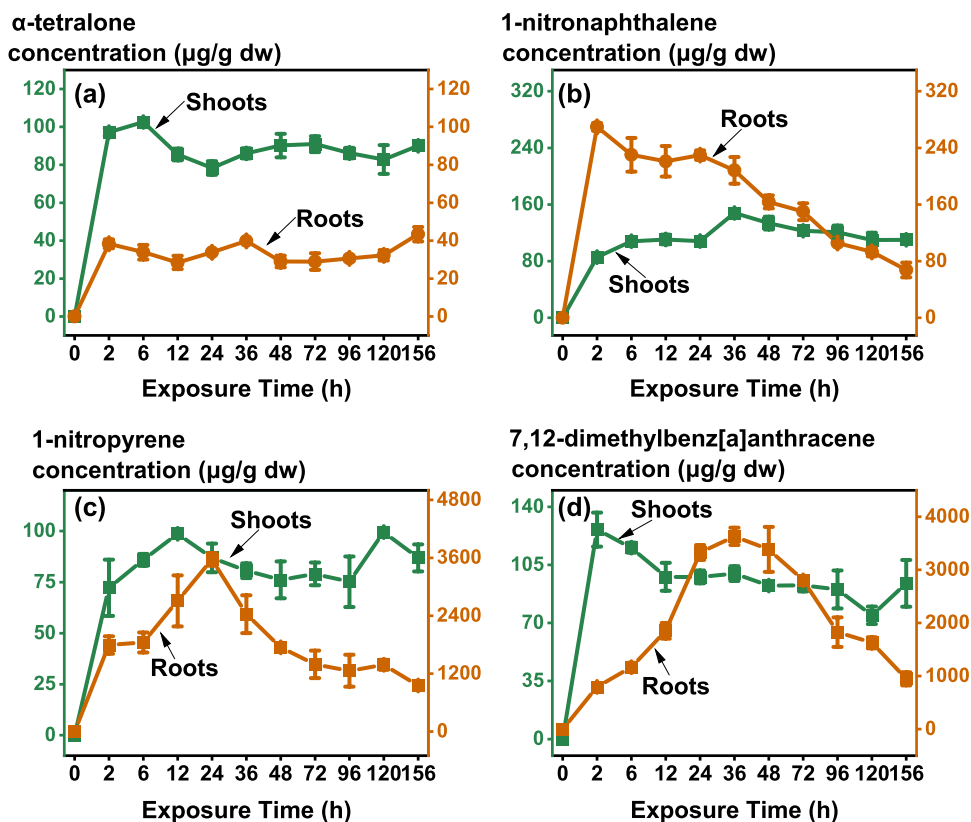
PAHs in wheat roots. To further decipher the accumulation mechanism of substituted PAHs in wheat roots, the mass balance of the substituted PAHs was estimated through comparing the total mass of each targeted compounds in the whole plant and the nutrient solution with their equivalent mass spiking in the solution. As shown in Table S3 and Fig. 2a–d, about 3.9%, 5.2%, 20.2%, and 20.3% of the initial mass of α-tetralone, 1-nitronaphthalene, 1-nitropyrene, and 7,12-dimethylbenz[a]anthracene were accumulated in the whole wheat, respectively, further demonstrating that the substituted PAHs with higher hydrophobicity were inclined to partition to wheat. Generally, lipid of plant is considered to be the main sites accumulating organic pollutants (Liu et al. 2019) and the organic pollutants with stronger hydrophobicity tend to closely bind with lipids (Wang et al. 2018; Zhan et al. 2018). Overall, we found that the accumulation ability of substituted PAHs in wheat depends on their hydrophobicity.

The accumulated substituted PAHs in the wheat roots could transfer to shoots. Translocation factor was calculated to assess the transfer ability of substituted PAHs from roots to shoots. As shown in Table S2, the translocation factor values of α-tetralone, 1-nitronaphthalene, 1-nitropyrene, and 7,12-dimethylbenz[a]anthracene were 2.4, 1.6, 0.1, and 0.1, respectively. The translocation factor values significantly declined with the increasing hydrophobicity

($R^2 = 0.798$, $p < 0.05$, Fig. S1b), implying that substituted PAHs with higher hydrophobicity were difficultly transferred to wheat shoots. Generally, organic chemicals absorbed by plants must pass the cortex of the root and Casparian strip of endodermis before reaching the shoots (Miller et al. 2016). However, organic compounds with higher hydrophobicity are difficult to pass through the cortex of root and Casparian strip of endodermis due to their macromolecular structure and strong binding affinity with lipids (Liu et al. 2019). Hence, the relatively lower transfer ability of 1-nitropyrene, and 7,12-dimethylbenz[a]anthracene from wheat roots to shoots might be attributed to their higher hydrophobicity and larger molecular weight (Felizeter et al. 2012). Overall, the substituted PAHs with higher hydrophobicity were more difficult to transport to aboveground tissues of wheat.

Translocation mechanisms of substituted polycyclic aromatic hydrocarbons in wheat roots

To further decipher the translocation mechanism of substituted PAHs in wheat roots, energy metabolism and aquaporin inhibitors were used to explore their uptake pathways. Figure 3 illustrates the concentrations of the substituted PAHs in wheat roots in the presence of three kinds of inhibitors. The hydrophilic substituted PAHs, such as α-tetralone and 1-nitronaphthalene, were all sensitive to sodium

Fig. 2 Mass compositions of α-tetralone (**a**), 1-nitronaphthalene (**b**), 1-nitropyrene (**c**), and 7,12-dimethylbenz[a] anthracene (**d**) in the wheat at each exposure time (including 2, 6, 12, 24, 36, 48, 72, 96, 120, 156 h). After 156 h exposure, about 3.9%, 5.2%, 20.2%, and 20.3% of the initial mass of α-tetralone, 1-nitronaphthalene, 1-nitropyrene, and 7,12-dimethylbenz[a]anthracite were accumulated in the whole wheat
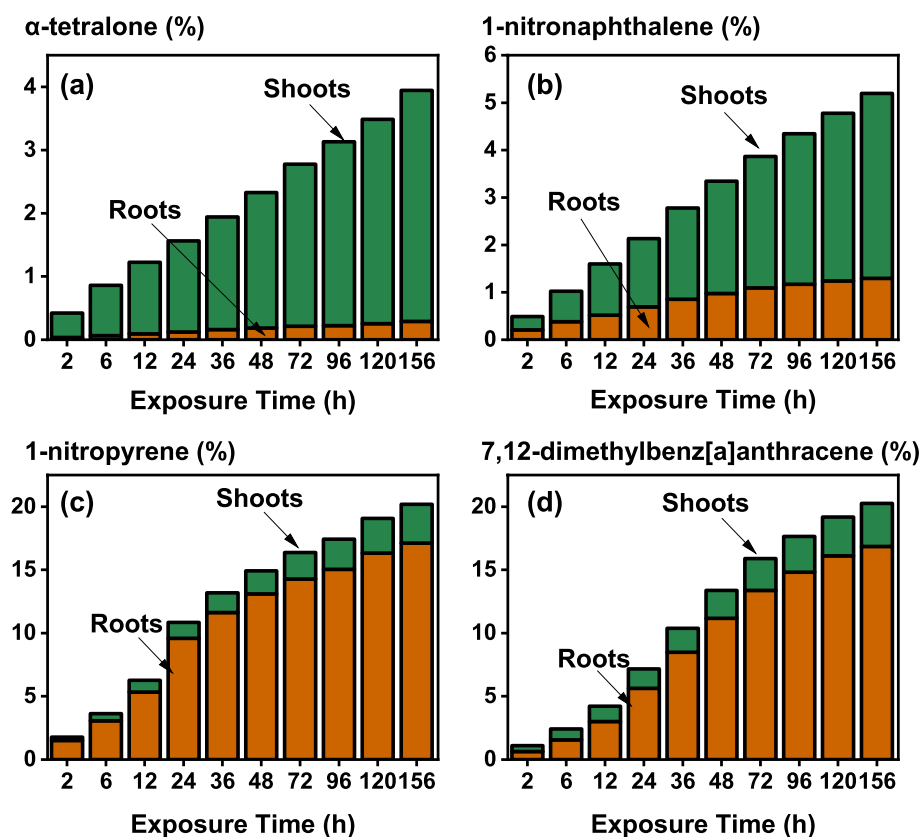
Fig. 3 Effects of metabolic and aquaporin inhibitors on the accumulation of α-tetralone (**a**), 1-nitronaphthalene (**b**), 1-nitropyrene (**c**), and 7,12-dimethylbenz[a]anthracene (**d**) by wheat roots. The observed concentrations of α-tetralone and 1-nitronaphthalene but not 1-nitropyrene, and 7,12-dimethylbenz[a]anthracite in wheat roots were significantly impacted by all inhibitors. Error bars represent standard deviations from replicates (n=3). Significant differences (ANOVA/Tukey HSD) are indicated by asterisks (*$p<0.05$). The dw represents the dry weight. All the results were normalized by control
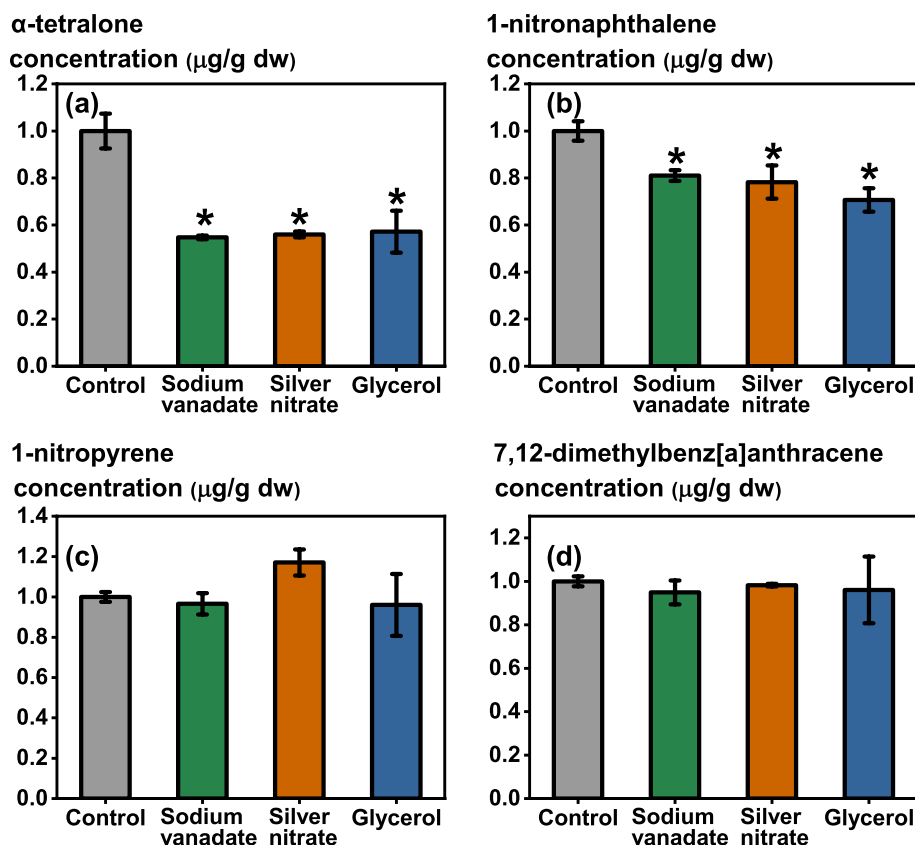
vanadate treatment. Their concentrations in the wheat roots with sodium vanadate treatment were obviously lower than the blank control; approximately 45.3% lower for α-tetralone and 19.0% lower for 1-nitronaphthalene (Fig. 3a, b). Sodium vanadate, a kind of energy metabolism inhibitor, could powerfully inhibit membrane P-type ATPases by competing the sites with phosphate on ATP (Zhan et al. 2015). Hence, the above results suggested that the absorption of α-tetralone and 1-nitronaphthalene was through active transport regulated by P-type ATPases in the wheat roots. Similar results were observed for glycerol and silver nitrate treatments, resulting in a decrease in the uptake rates of α-tetralone and 1-nitronaphthalene by 42.8–44.0% and 21.8–29.4%, respectively. (ANOVA, $p < 0.05$, Fig. 3a, b). Glycerol and silver nitrate are two typical water channel inhibitors with different inhibitory mechanisms (Meharg and Jardine 2003; Niemietz and Tyerman 2002). Among them, glycerol mainly inhibits aquaporin through substrate competition (Meharg and Jardine 2003), while silver nitrate could interact with the sulfur group on the cysteine residue of aquaporin to prevent water molecular from entering cells (Niemietz and Tyerman 2002). These results provided conclusive evidence that the water channel was an important way for wheat roots absorbing α-tetralone and 1-nitronaphthalene.

Different from α-tetralone and 1-nitronaphthalene, the observed concentrations of 1-nitropyrene, and

7,12-dimethylbenz[a]anthracene in wheat roots were not significantly impacted by all inhibitors (ANOVA, $p > 0.05$, Fig. 3c, d). This might be due to the relatively large hydrophobicity and molecular weight of 1-nitropyrene, and 7,12-dimethylbenz[a]anthracene, resulting in the accumulation of 1-nitropyrene, and 7,12-dimethylbenz[a]anthracene in wheat roots mainly through adsorption rather than absorption (Zhou et al. 2020). Overall, wheat roots showed multiple pathways for the accumulation of substituted PAHs with low hydrophobicity, whereas substituted PAHs with high hydrophobicity predominantly accumulated in wheat roots through adsorption.

The correlations between root concentration factor, translocation factor and inhibition percentages were generally used to characterize the translocation mechanism of organic contaminants in plants (Zhou et al. 2020). Interestingly, in the treatments with sodium vanadate, glycerol and silver nitrate, their inhibition percentages all increased with the increasing translocation factor values (Fig. S2a–c), but all declined with the increasing root concentration factor values (Fig. S3a–c). These results showed that not all the accumulated substituted PAHs in wheat roots could migrate to shoots, while only the absorbed part that crossed at least one lipid bilayer into the absorbable portion of the xylem was capable of translocation (Mueller et al. 2016; Zhou et al. 2020). Hence, since α-tetralone and 1-nitronaphthalene

could be absorbed by wheat roots in the form of water channels and H^+ gradients, then becoming available for subsequent translocation. Conversely, 7,12-dimethylbenz[a]anthracene with the highest hydrophobicity was strongly adsorbed on root epidermis, resulting in difficult to transport to aboveground tissues via xylem.

Effects of exposure to substituted polycyclic aromatic hydrocarbons on enzymes activities and chlorophyll in wheat

Yun et al. (2019) reported that nitrated PAHs in barley could cause phytotoxicity, such as increase in intracellular reactive oxygen species levels. The excessive reactive oxygen species could lead to lipid peroxidation (Liu et al. 2020). To maintain the plant health, the activities of superoxide dismutase and peroxidase are regulated by plants to sustain the balance of intracellular reactive oxygen species levels (Kaushik and Aryadeep 2014). To investigate whether substituted PAHs caused phytotoxicity, the activities of superoxide dismutase and peroxidase, as well as malondialdehyde content in wheat roots, were measured during all preselected intervals (Fig. 4). Except for 2 h time point, peroxidase activity of exposed wheat decreased by 2.9–26.2% within 156 h compared to the control group (Fig. 4a). Similarly, the superoxide dismutase activity of wheat exposed to substituted PAHs decreased by 2.0–32.4% within 156 h,

except at 120 h (Fig. 4b). The decreased activities of superoxide dismutase and peroxidase indicated that substituted PAHs exposure did not induce a significant accumulation of reactive oxygen species in wheat. This result was further proved by the fact that the malondialdehyde content did not insignificantly increase in the exposed group compared to the blank group (Fig. 4c), implying that the lipid peroxidation damage of wheat root cells was not apparent under the substituted PAHs stress.

Chlorophyll content of plants, as a tracer agent, could indicate electrons transformation activities during photosynthesis, and it is sensitive to oxidative damage throughout the growth period of wheat (Kurade et al. 2016; Lan et al. 2018). As shown in Fig. 4d, in the treatments with substituted PAHs, the total chlorophyll contents of wheat decreased by 19.0–30.1% compared to the control within 36 h of exposure. Greenberg et al. (1997) reported that the main mechanism by which PAHs and other toxic substances exert stress on plants was through the inhibition of photosynthetic processes. Therefore, the decrease in chlorophyll content induced by the addition of substituted PAHs might inhibit the growth of wheat by reducing its light-use efficiency (Ali et al. 2019; Gao et al. 2019). However, the insignificant variation of total chlorophyll contents after 36 h of exposure potentially attributed to the activated tolerance and detoxification at its subsequent growth (Seth et al. 2008). Overall, the photosynthetic pigments rather than the

Fig. 4 Dynamic changes in peroxidase activity (**a**), superoxide dismutase activity (**b**), malondialdehyde content (**c**) in wheat roots, and total chlorophyll content (**d**) in wheat shoots. The accumulated substituted polycyclic aromatic hydrocarbons did not increased malondialdehyde content and autioxidant enzyme activities, but increased the total chlorophy content of wheat. Error bars represent standard deviations from replicates (n = 3). Significant differences (ANOVA/paired-samples *t* test) between exposure treatment and blank control at the same time point are indicated by an asterisk (*$p < 0.05$)
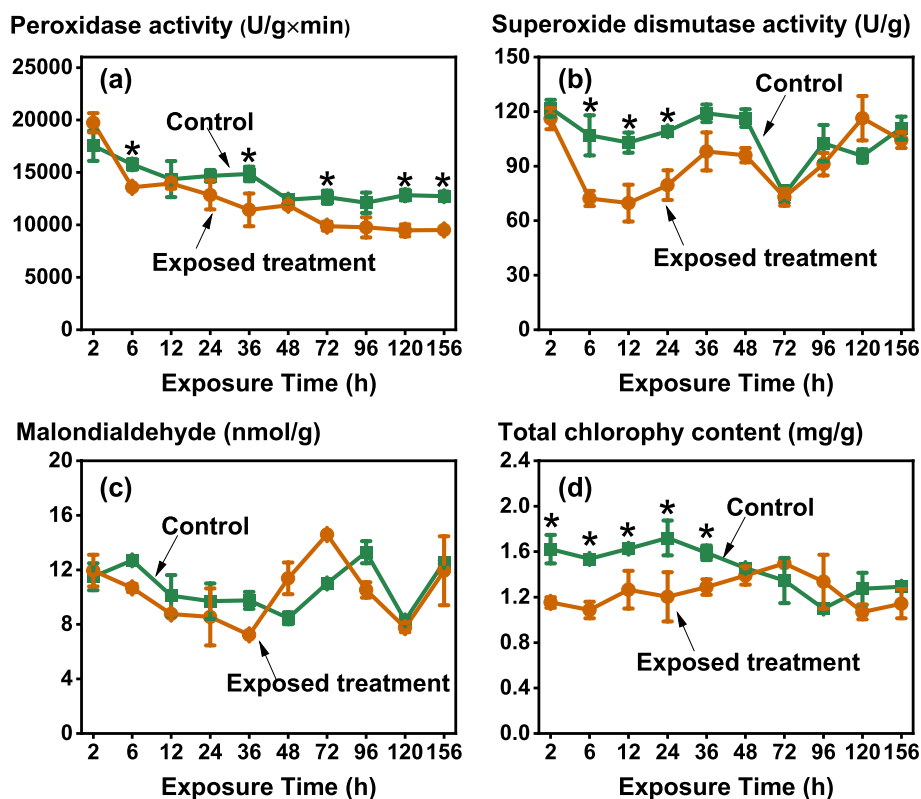
antioxidant system of wheat were primarily impacted under the substituted PAHs stress.

Conclusion

Substituted PAHs in soil may finally enter the human body via the food chain and cause adverse impacts to humans, but the migration of substituted PAHs in soil–plant system is poorly understood. This study investigated the accumulation and translocation behaviors of substituted PAHs in wheat, and found that the four types of substituted PAHs could be significantly accumulated on wheat root epidermis, driven by their hydrophobicity. However, not all substituted PAHs accumulated in plant roots could migrate to the plant shoots. The inhibition experiment indicated that only hydrophilic substituted PAHs absorbed by wheat roots in the form of water channels and H^+ gradients could easily translocate from roots to shoots. The accumulated substituted PAHs in wheat may finally enter the human body via the food chain. Hence, further investigations are needed to consider the accumulation capacity of substituted PAHs in wheat grain and the potential risk of substituted PAHs on human body through ingestion.

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Declarations

Conflict of interest The authors declare no competing interests.

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