# Uptake, translocation and subcellular distribution of organophosphate esters in rice by co-exposure to organophosphate esters and copper oxide nanoparticle

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## HIGHLIGHTS

- Co-exposure of OPEs & CuONPs on rice was studied by hydroponics for the 1st time.
- CuONPs can hinder the OPE uptakes by rice root via competitive adsorption.
- CuONPs can change subcellular distribution of OPEs by affecting their adsorption.
- Root uptake of OPEs is a facilitated diffusion mediated by aquaporin channel.

## GRAPHICAL ABSTRACT
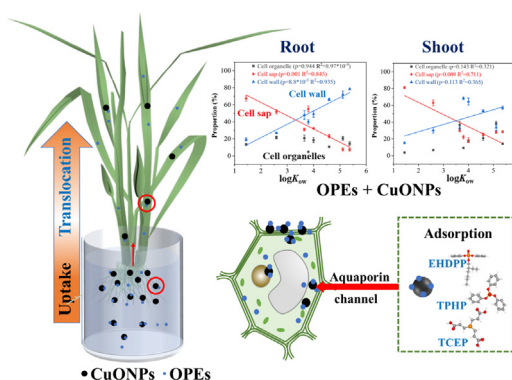


## ARTICLE INFO

## ABSTRACT

This study investigated the influence of copper oxide nanoparticles (CuONPs) and $Cu^{2+}$ on the uptake, translocation and subcellular distribution of organophosphate esters (OPEs) in rice seedlings using hydroponic experiments. The OPE concentrations in roots and shoots under the OPEs + CuONPs treatment were significantly lower than those with the OPEs + $Cu^{2+}$ (low level) or OPEs-only treatments, indicating that CuONPs can hinder the uptake of OPEs by root via competitive adsorption under short-term exposure. The plasma membrane permeability and antioxidant enzyme activity implied that CuONPs had a negligible impact on rice seedlings and could even reduce the toxicity of OPEs to rice root. A significant negative correlation between translocation factor and octanol-water partition coefficient was observed for the three treatments, implying an important role of hydrophobicity on the acropetal translocation of OPEs. Relatively hydrophobic OPEs were mainly adsorbed on cell wall, while hydrophilic OPEs were concentrated in cell sap. The subcellular distributions of OPEs in the OPEs + $Cu^{2+}$ (high level) or OPEs + CuONPs treatments slightly differed from the OPEs-only treatment, indicating that the coexistence of $Cu^{2+}$ or CuONPs with OPEs can influence the subcellular distribution of OPEs by affecting their adsorption or partitioning processes. Inhibition experiment suggested that root uptake of OPEs is a non-energy-consuming facilitated diffusion mediated by aquaporin channel, which can be slightly changed by the co-exposure of CuONPs. This study improved the understanding of uptake and translocation of OPEs by rice under the co-exposure to CuONPs.

# 1. Introduction

Organophosphate esters (OPEs) have been widely used as flame retardants and plasticizers in a variety of polymer-based industrial and commercial products. The production of OPEs has been extensively increased in demand in recent years as an alternative of PBDEs and other halogenated flame retardants due to their ban by the Stockholm Convention (Wang et al., 2020b). Worth mentioning, studies have indicated that OPEs have adverse health effects of carcinogenicity, neurotoxicity, contact irritability, and endocrine disruption on human (Behl et al., 2015; Carignan et al., 2017).

Organic chemicals can be absorbed by plants via either active or passive uptake processes (Collins et al., 2006), depending on their physicochemical properties. Active uptake is an energy-consuming process mediated by carrier proteins against the concentration gradient, while passive uptake, including simple diffusion and facilitated diffusion via carriers and channels, driven by mass flow or diffusion without energy consumption (Wang et al., 2020b). Absorption of non-ionized organic compounds by plant roots is generally considered as a simple passive diffusion process (Li et al., 2010). Uptake of OPEs by wheat was suggested to be facilitated diffusion mediated by aquaporin or anion channels, while uptake of OPE metabolites was considered as simple diffusion process (Gong et al., 2020). However, knowledge of the root uptake mechanisms of OPEs in crops, such as rice, is still limited, especially under the coexistence of other chemicals, e.g., nanoparticles.

Recent years, nano-enabled agrichemicals have raised concerns as surface-modified and the customized nanomaterials could control the rate of nutrient release Copper oxide nanoparticles (CuONPs) can be utilized as nano-fertilizers, as well as nano-insecticides due to their antibacterial properties (Reddy et al., 2021). Approximately 200 tons of CuONPs were produced in 2010, of which ~36 and 11 tons ended up in soil and water, respectively (Keller et al., 2013). Contribute to their widespread application and relatively high toxicity to biota, the potential pollution of CuONPs in agriculture may pose a threat to the ecosystem and human body. CuONPs can significantly reduce seedlings' length (Lee et al., 2008), and lead to the production and accumulation of reactive oxygen species (ROS) in plants (Shaw and Hossain, 2013).

Multiple pollutants usually co-existent in the real environment, especially in agricultural field irrigated with wastewater (Zhang et al., 2017). Co-existence of organic and inorganic pollutants in soil may lead to synergistic or antagonistic effects to plants. For instance, the co-exposure of PAHs and heavy metals decreased the uptake and translocation of PAHs by Pak Choi, possibly due to the commutative hindering effects on root adsorption or cation-π interactions (Deng et al., 2018). High concentration of Cu can inhibit the uptake and metabolism of BDE209 by pumpkin (Lu et al., 2013), but promote the uptake of BDE47 and BDE209 by corn due to the damage to the root cell membranes (Wang et al., 2016b). Since most studies focused on the co-exposure of organic chemicals and heavy metals on plants, researches on the joint efforts of organic chemicals and metal nanoparticles on plants are limited, especially on crops.

Rice, a global staple food, have extensive acreage and consumed by millions of people. A previous study (Zhang et al., 2016) suggested that rice ingestion is the main source for human exposure of OPEs in China. Here, we conducted a hydroponic experiment with a co-exposure of OPEs and CuONPs to explore the influence of CuONPs on the uptake, translocation, and subcellular distribution of OPEs in rice seedlings. Channel inhibitors were also applied to investigate the uptake mechanisms of OPE by rice with the impact of CuONPs. The present study will provide new information on the uptake and translation mechanisms of OPEs by rice with co-exposure of CuONPs.

# 2. Materials and methods

## 2.1. Plant cultivation

Japonica rice (*Oryza sativa* L. subsp. *Japonica*), a kind of rice widely cultivated in Northeast China, was chosen as the experimental plant. Seeds were sterilized with 3 % $H_2O_2$, rinsed with deionized water, and then germinated at 30 °C for 4 days in dark. After germination, seeds were transferred to half-strength Hoagland nutrient solution for hydroponic at 27/24 °C (day/night) with a 16/8 h (light/dark) cycle. Seedlings with similar size were selected for the hydroponic experiments.

## 2.2. Experiment design

### 2.2.1. Hydroponic experiments

The mixed standard solution of 5 OPEs, including TNBP, TCEP, TCIPP, TDCIPP and TPHP, was spiked to the nutrient solution to obtain a designed concentration of ~20 μg/L. The OPE-spiked nutrient solutions were dosed with 625 μmol/L CuONPs. Since the CuONPs can release ~2.5 % $Cu^{2+}$, a 15.6 μmol/L $Cu^{2+}$ treatment based on copper sulfate pentahydrate was used to simulate the effect of copper ion leaching from the CuONPs. Six bunches of seedlings with uniform height were cultured in each amber bottle with their root submerged in a full Hoagland nutrient solution and renewed every two days. Plants grown in nutrient solution without chemical-spiked ware also performed as the untreated control. Seedlings were collected for OPE analysis at intervals of 0, 48, 96, 120, 168 and 216 h after exposure. All treatments, including untreated controls (no contamination, only $Cu^{2+}$, and only CuONPs) and treated groups, were performed in triplicate.

### 2.2.2. Physiological indexes measurements

According to the negligible influence of the co-exposure of OPEs and low concentration of $Cu^{2+}$ in the time-dependent exposure, plasma membrane permeability (Wang et al., 2016b) and activities in superoxide dismutase (SOD) were detected to reveal the toxic effects and oxidative stress induced by three treatments: OPEs (20 μg/L), OPEs (20 μg/L) + CuONPs (625 μmol/L), and OPEs + high $Cu^{2+}$ (high concentration: 200 μmol/L). Seedlings were harvested at time intervals of 48, 120 and 216 h. Details are included in the S1.2, Supporting Information (SI).

### 2.2.3. Subcellular fractionation experiments

Eight OPEs were used in this experiment, i.e., TNBP, TCEP, TCIPP, TDCIPP, TBOEP, EHDPP, TPHP and TMPP. Seedlings were exposed to OPEs, OPEs + CuONPs and OPEs + high $Cu^{2+}$ ($Cu^{2+}$ concentration: 200 μmol/L) for 48 h exposure, and then the subcellular fractions of rice root and shoot cells were separated into three subcellular components: cell wall, cell organelles and cell sap (Lin et al., 2017). Briefly, fresh tissues were weighted and homogenized in an extraction buffer containing 50 mmol/L of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1.0 mmol/L DL-Dithiothreitol (DTT), 5.0 mmol/L ascorbic acid, 500 mmol/L sucrose and 1.0 % (w/v) polyvinylpolypyrrolidone cross-linked (PVPP). The homogenate was passed through a 60-μm sieve to separate the cell debris pellet. The supernatant was centrifuged at 4 °C and 500*g* for 10 min, and the residues and the cell debris pellet were combined, which were regarded as the cell wall fraction. The supernatant was then centrifuged at 4 °C and 10,000*g* for 30 min. OPEs in this precipitate were referred to as the cell organelles fraction, while OPEs in the supernatant were considered as the cell sap (water-soluble) fraction. After fractionation, cell wall and organelle were freeze-dried before OPE analysis, while cell sap was directly liquid-liquid extracted. The proportion (*P*) of OPEs in different subcellular fractions was calculated as followings:

$$P\ (\%) = m_{\text{subcell}}/m_{\text{total}} \times 100 \tag{1}$$

where $m_{\text{subcell}}$ is the mass (ng) of an OPE congener in a certain subcellular component, i.e., cell sap, cell walls and cell organelles, of root or shoot; $m_{\text{total}}$ is the total mass (ng) of this congener in the cell sap, cell walls and cell organelles (Gao et al., 2013).

### 2.2.4. Metabolic, aquaporin, $Ca^{2+}$ and anion channel inhibitors experiment

To identify the absorption pathways of OPEs, rice seedlings were exposed to 4 treatments for 24 h: OPEs, OPEs + CuONPs, OPEs + low $Cu^{2+}$

(concentration: 15.6 μmol/L) and OPEs + high Cu$^{2+}$ (concentration: 200 μmol/L). For each treatment, an inhibitor was added respectively, and totally 4 different inhibitors were tested. Carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP, 500 μmol/L) was applied as a metabolic inhibitor, glycerol (1 mmol/L) was used as an aquaporin inhibitor, 4,4-diisothio-cyanostibene-2,2-disulfonateas (DIDS, 10 μmol/L) was selected as an anion channel inhibitor, and LaCl$_3$ (250 μmol/L) was adopted for a Ca$^{2+}$ channel inhibitor.

### 2.3. Sample preparation and OPE analysis

Collected seedlings were first divided into root and shoot, and then washed with deionized water separately. Plant tissues were freeze-dried, cut into pieces (<1 mm) and stored at −20 °C. Plant or cell fraction samples were accurately weighed, added with surrogates (TCEP-$d_{12}$, TCIPP-$d_{18}$ and TPHP-$d_{15}$), extracted by ultrasonication with acetone/hexane/dichloromethane mixture (1:2:2, v/v) for 20 min and repeated for 3 times. The combined extracts were cleaned up using a silica gel column containing 0.5 cm sodium sulfate anhydrous, 1 cm of PSA and 3 cm silica gel (3 % deactivate).

Gas chromatograph-mass spectrometry (GC–MS, Shimadzu QP2020) applying a SH-RXI-5MS capillary column (30 m × 250 μm × 0.25 μm) was used for quantifying OPEs in an electron ionization (EI) mode. The temperature program started at 70 °C for 1 min and ramped up to 300 °C at 15 °C/min for 10 min.

### 2.4. Quality assurance and quality control

All glassware was baked at 450 °C for over 4 h and rinsed with *n*-hexane before use. A procedural blank was run alongside each batch of 10 samples to assess the potential contamination. Method detection limit (MDL) was set as the mean value in blanks plus three times the standard deviation. MDLs for OPEs were 0.003–0.42 ng/g (Table S3, SI). The average recoveries of TCEP-$d_{12}$, TCIPP-$d_{18}$ and TPHP-$d_{15}$ were 81.9.9 ± 18.2 %, 77.9 ± 18.5 % and 85.5 ± 21.6 %, respectively (Table S3). All samples were blank-corrected but not recovery-corrected. Results are expressed on a dry weight basis.

### 2.5. Statistical analysis

Data were analyzed and graphed using OriginPro 2022, Excel 2019 and SPSS 20.0. Multiple one-way analysis of variance (ANOVA) was conducted to assess the statistical difference. A *p*-value of 0.05 was taken as statistically significant.

## 3. Results and discussion

### 3.1. Time-dependent uptake and translocation of OPEs by rice

OPE concentrations in rice roots and shoots of 3 different treatments are shown in Fig. 1. Generally, the target OPEs in rice roots in these 3 treatments increased significantly at the beginning and reached the peak values after 168 h of exposure, except for TNBP, which increased continuously throughout the 216 h of exposure period. Meanwhile, OPEs in rice shoots in the 3 treatments generally increased throughout the whole exposure period, except for TPHP, which decreased after 168 h. The concentrations of OPEs in the roots or shoots of the OPEs + low Cu$^{2+}$ treatment were comparable to those of the OPE treatment, indicating that Cu$^{2+}$ released by CuONPs has a negligible effect on the uptake of OPEs by rice root. For the OPEs + CuONPs treatment, the concentrations of OPEs in roots or shoots were significantly lower (reduced by 20–48 %) than those of the OPE or OPEs + low Cu$^{2+}$ treatments, but their time-dependent increasing trends were resembled. Moreover, the difference between OPEs and OPEs + CuONPs treatments gradually increased with exposure time. Relatively low OPE concentrations in the rice of the OPEs + CuONPs treatment may be due to the adsorption of OPEs by the CuONPs. Uptake of CuONPs by rice root can be hindered by several barriers, e.g., mucilage, cuticle,

cell wall and blockage of pores of cell walls due to aggregation of NPs (Schwab et al., 2016). Chemicals adsorbed by the NPs can be hardly internalized by the receptor and thereby lowering the effective dose of the analyte in the exposure media and/or organism to reduce toxicity (Deng et al., 2017). Wu et al. (2016) reported a decreased toxicity of PBDEs to Chinese cabbage on a co-exposure to Ni/Fe NPs. To evaluate the adsorption influence of CuONPs on the concentration of OPEs in the nutrient solution, we conducted an adsorption experiment without rice seedings using a nutrient solution containing both OPEs (20 μg/L) and CuONPs (625 μmol/L). After mixing for 10 min and settling for 1 h, the solution were centrifuged at 4000 rpm/min, and then the supernatant was liquid-liquid extracted for OPE analysis. The results showed that 70.7–85.8 % OPEs (Table S4, SI) had been adsorbed by CuONPs due to their coexistence in the solution. The uptake process of nonionized chemicals from hydroponic solution into plant roots consists of two components: (1) "equilibration" of the aqueous phase in plant root with the concentration in solution; and (2) "sorption" of the chemical onto lipophilic root solids, such as lipids in membranes and cell walls (Paterson et al., 1991). CuONPs can prevent OPEs from entering into plant root through competitive sorption of OPEs with lipophilic root solids. The zeta potentials (Table S5, SI) and hydraulic diameters (Fig. S1, SI) of CuONPs in different solutions were analyzed. In the nutrient solution, adding OPEs increased the zeta potential (from −6.27 to −8.31 mV) and the hydraulic diameter (from 341 to 378 d·nm) of CuONPs, suggesting that OPEs adsorbed by CuONPs can slightly increase the hydraulic diameter of CuONPs and subsequently lead to a decreased uptake of CuONPs-adsorbed OPE by rice.

Concentrations of OPEs in rice roots of the 3 treatments followed the same order: TDCIPP > TPHP > TNBP > TCIPP > TCEP. The relatively high concentrations of TDCIPP, TPHP and TNPB in roots may be due to their hydrophobicity, which indicates that root uptake of OPEs is primarily attributed to the partitioning to root organic components, particularly lipids. Similar trend of root uptake of OPEs by wheat were also reported by Wan et al. (2017). OPE concentrations in rice shoots of the 3 treatments followed the same order: TDCIPP > TCIPP > TCEP > TNBP > TPHP. It is interesting that TDCIPP with a moderate value of $K_{ow}$ had the highest concentration in both rice root and shoot, whereas TPHP with a high value of $K_{ow}$ showed a high level in root but the lowest level in shoot. The result suggests that the uptake and translocation of OPEs by rice root from solution are controlled by both hydrophilicity and hydrophobicity.

### 3.2. Effects of OPEs and CuONPs on rice physiology

To identify the toxic impacts of OPEs, Cu$^{2+}$ and CuONPs on rice seedlings, root plasma membrane permeability and activities of SOD in shoot and root were analyzed. Since the impact of the co-exposure of OPEs with low Cu$^{2+}$ concentration (15.6 μmol/L) was insignificant, we used the co-exposure of OPEs with a high Cu$^{2+}$ concentration (200 μmol/L) instead. Electrolytic leakage was used to determine the permeability of root cell membrane after exposure to OPEs, OPEs + high Cu$^{2+}$ and OPEs + CuONPs (Fig. 2A). A significant increase ($p < 0.05$) in the electrolytic leakage was only observed for the OPEs + high Cu$^{2+}$ treatment compared with the control. Cu$^{2+}$-induced changes in cell permeability can be attributed to a non-selective increase in electrical conductivity (Demidchik et al., 1997). Excess Cu$^{2+}$ can disrupt the root cell membrane, and break down the root exclusion mechanism, thereby disrupting the regulation of ion channel uptake (Maksymiec, 1997; Wang et al., 2016b). After 216 h exposure, the electrolytic leakage of rice root in the OPE treatment also increased significantly ($p < 0.05$), suggesting that long-term exposure of OPEs may damage the root cell membrane and exclusion mechanism. However, for the OPEs + CuONPs treatment, no significant difference was observed compared with the control during the whole 216 h exposure, indicating that short-term CuONPs exposure have a negligible toxic effect on the root cell membrane. Since CuONPs can be adsorbed onto root surface, like a shield, they can hinder the adsorption or absorption of OPE by root, thereby reducing the toxic effect of OPEs on rice root.
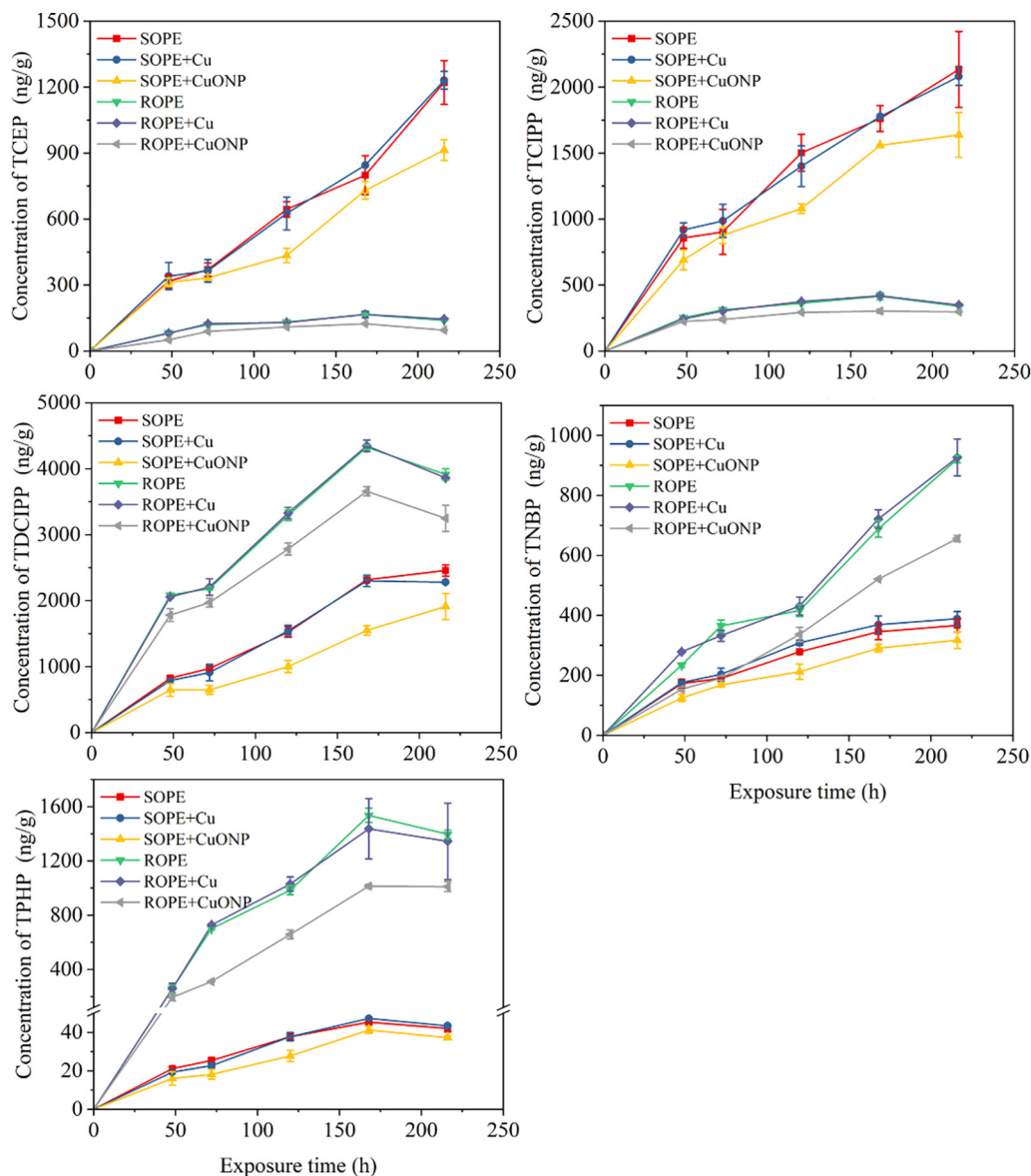
**Fig. 1.** Time-dependent concentrations of TNBP, TCEP, TCIPP, TDCIPP and TPHP in rice shoots and roots from the OPEs, OPEs + low $Cu^{2+}$ and OPEs + CuONPs treatments.

As a metalloenzyme, SOD is the first and most important line of defence for plant against reactive oxygen species (ROS), and can convert highly toxic ROS (i.e., superoxide radicals) into less toxic one (Wang et al., 2016a). Rice roots exposed to OPEs and OPEs + CuONPs showed no significant difference ($p > 0.05$) in the SOD activity compared with the control group during the 216 h of exposure, whereas roots exposed to OPEs + high $Cu^{2+}$ showed a significant increase ($p < 0.05$) in the SOD activity compared with the control and other two treatments, especially during the first 48 h (Fig. 2B). For shoot, only the OPEs + high $Cu^{2+}$ treatment showed a significant increase ($p < 0.05$) in the SOD activity compared with other treatments (Fig. 2C). Increased SOD activity in plant indicates an enhanced •$O_2^-$ production and oxidative stress tolerance (Thounaojam et al., 2014). Meanwhile, SOD activities generally decreased with the exposure time, which may be due to the damage of root exclusion mechanism.

### 3.3. Translocation of OPEs in rice

Translocation factor (TF), calculated as the ratio of chemical's concentration in shoot to that in root, represents the chemical's capability to translocate from root to above-ground organs. Translocation of OPEs by plant root can be affected by multiple factors, mainly depending on their physicochemical properties (Zhang et al., 2021). In the time-dependent experiment, the TFs of OPEs were calculated for different exposure intervals (Fig. 3). The median TFs of OPEs values in the OPEs, OPEs + low $Cu^{2+}$ and OPEs + CuONPs treatments were in the range of 0.0–8.77, 0.03–8.49 and 0.04–9.40 respectively, with TCEP > TCIPP > TNBP > TDCIPP > TPHP. The TFs of TCEP, TCIPP and TDCIPP gradually raised with the exposure time in three treatments, whereas the TFs of TNBP and TPHP decreased. The median TFs of OPEs were found to be significantly negatively correlated with their $\log K_{ow}$ values ($p < 0.05$) in all three treatments (Fig. S2), suggesting that OPEs with relatively low hydrophobicity were more prone to be translocated from root to shoot (Gong et al., 2020; Wan et al., 2017).

### 3.4. Subcellular distributions of OPEs in rice

The subcellular distribution of exogenous chemicals may affect their translocation, accumulation and metabolism in plant (Gao et al., 2013; Zhao et al., 2015). The subcellular distribution of OPEs in rice treated with OPEs, OPEs + high $Cu^{2+}$ and OPEs + CuONPs were investigated and showed in Fig. 4. Cell wall is a predominant sink in rice for hydrophobic OPEs ($\log K_{ow} > 4$), e.g., TNBP, TPHP, EHDPP and TMPP, regardless of
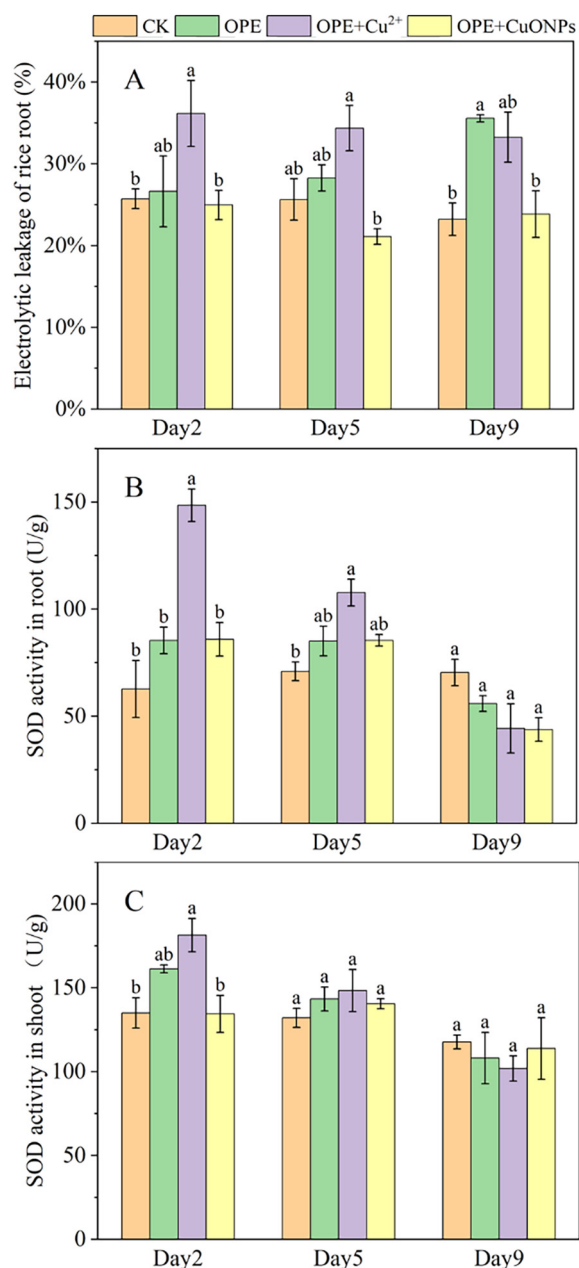
**Fig. 2.** Root electrolytic leakage (A) and SOD activities in root (B) and shoot (C) treated with 4 different treatments: control (CK), OPEs, OPEs + high $Cu^{2+}$ and OPEs + CuONPs. Bars with different letters within the same sampling period indicate significant differences ($p < 0.05$).

treatments and tissues (root or shoot), which accounted for 60.0–86.8 % of total OPE burdens in rice root and 33.5 %–69.7 % in rice shoot, respectively. A previous study (Zhao et al., 2015) also suggested that cell wall was the dominant storage compartment for hydrophobic phthalates in Chinese flowering cabbage. Cell wall, composed of cellulose, hemicellulose, pectin, lignin and proteins, acts as the first barrier of plants against exogenous pollutants (Guo et al., 2018; Zhao et al., 2015). Cell walls bear a negative charge (Miller et al., 2016), which may also facilitate the adsorption of OPEs on cell wall. A previous study (Ling et al., 2012) suggested that PAH concentration in cell can be greatly reduced due to the adsorption and binding of PAHs by cell wall, thereby their damages to many physiological and biochemical processes within the cell are thus prevented. Cell sap (water-soluble) is the dominant component for the relatively hydrophilic OPEs ($\log K_{ow} < 4$), such as TCEP, TCIPP, TBOEP and TDCIPP, with a proportion of 20.1–86.2 %.

Relationships between the subcellular distribution proportions of OPEs and their $\log K_{ow}$ were investigated and showed in Fig. 5. Organic compound with a higher water solubility and lower $\log K_{ow}$ value is enriched in the water-soluble fraction, while compound with a higher $\log K_{ow}$ and lower solubility is enriched in the cell solid phase, e.g., cell wall or organelles (He et al., 2017; Wang et al., 2019). The OPE proportions in cell sap and cell wall were significantly correlated with their $\log K_{ow}$ values with negative correlations for cell sap (root $p < 0.05$; shoot $p < 0.01$) and positive correlations for cell wall (root $p < 0.01$; shoot $p < 0.05$), except for the OPEs + CuONPs treatment. No significant correlations were found between the OPE component in cell organelles and $\log K_{ow}$ in rice root or shoot, except for the OPE treatment ($p = 0.034$), indicating a potential influence of $Cu^{2+}$ and CuONPs on subcellular distribution of OPEs. The result reveals that accumulation of OPEs in rice root and shoot and their subcellular distributions in cell are possibly controlled by the partitioning between cell wall and sap, depending on their physicochemical characteristics, e.g., hydrophobicity and lipophilicity (Wang et al., 2020a). For the exception of the OPEs + CuONPs treatment for cell wall or organelles, endocytosis is likely one transmembrane pathway for CuONPs to entry into plant cells (Wang et al., 2012), and the translocation of OPEs adsorbed by CuONPs may follow the transfer behavior of CuONPs within plant, which may, therefore, lead to a weak correlation between the distribution of OPEs in plant cell and $\log K_{ow}$.

The subcellular proportions of certain OPEs, such as TNBP, TCIPP and TBOEP, treated with OPEs + high $Cu^{2+}$ or OPEs + CuONPs slightly differed from those in OPEs treatment, but not for the other 5 OPEs. Generally, the proportions of TNBP and TCIPP increased with TBOEP decreased in the cell sap of the co-exposure treatments, indicating that the co-exposure of $Cu^{2+}$ or CuONPs may influence the subcellular distribution of OPEs by affecting their adsorption or partitioning processes. OPEs adsorbed by CuONPs may undergo the similar uptake pathways with CuONPs, such as passive diffusion through the permeable region of the cuticle and cell wall pores (Schwab et al., 2016). Since CuONPs (zeta potential in ultrapure water: $-8.58 \pm 0.23$ mV, nutrient solution: $-6.27 \pm 0.44$ mV, Table S5, SI) and cell walls both carry negative charges (Miller et al., 2016), it is difficult for CuONPs to be adsorbed or partitioned on cell wall due to the electrostatic repulsion, so do the adsorbed OPEs by CuONPs (OPEs + CuONPs zeta potential in ultrapure water: $-19.5 \pm 1.05$ mV, nutrient solution: $-8.31 \pm 0.64$ mV, Table S5). This may, to some extent, influence the subcellular distribution of OPEs, especially in cell walls. Meanwhile, the co-existence of OPEs and ions can increase the particle size of CuONPs (Fig. S1, SI), which can, therefore, affect the uptake and translocation of CuONPs-adsorbed OPEs in rice.

Cell sap is the primary storage space for hydrophilic organic chemicals with $\log K_{ow} < 2$, such as TCEP and TCIPP, which are prone to rapidly transfer to the xylem and transport to the shoots by the transpiration flow (Cousins and Mackay, 2001). Therefore, TCEP and TCIPP were found to have higher subcellular proportions in the shoot than root. An opposite trend was found for the hydrophobic OPEs, such as EHDPP and TMPP, which accounted for the majority in the root cell wall, thus obstruct their radial transfer to the shoot.

Subcellular translocation factors (STFs) were calculated as the ratio of chemical's concentrations (dividing the amount by the wet weight) in the same subcellular fraction in shoot to these in root (Fig. S3). Under the co-exposure of $Cu^{2+}$ or CuONPs, STFs of OPEs decreased in different degrees in cell walls and organelles compared to the only OPE exposure, in particular two chlorine OPEs, TCIPP and TDCIPP. Excess $Cu^{2+}$ may adversely disturb plant metabolism, affect the absorption and transport of essential elements (Cheng, 2003), as well as OPE translocation. Moreover, the adsorption of OPEs by CuONPs may also hinder OPE translocation. Interestingly, STFs of TCEP were much higher in the OPEs + CuONPs treatment, which may be attributed to its lowest $\log K_{ow}$. It is suggested that the toxic interactions between NPs and co-contaminant can be dominated by the properties of co-contaminant, e.g., hydrophilicity (Deng et al., 2017). Overall, translocation mechanism in the case of complex contamination may be related to a number of factors, and needs further investigation.
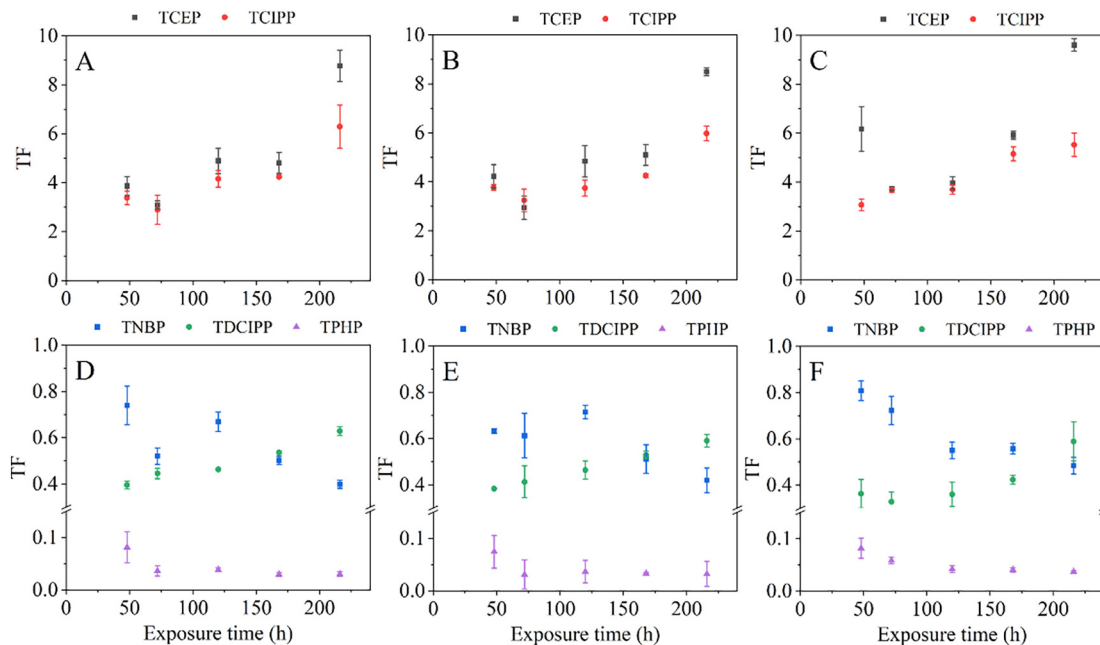
**Fig. 3.** *TFs of OPEs in the time-dependent experiment in the OPEs (A and D), OPEs + Cu²⁺ (B and E) and OPEs + CuONPs (C and F) treatments.*

### 3.5. Uptake mechanism of OPEs in rice

In order to study the uptake mechanism of OPEs by rice root, effects of different channel inhibitors on the uptake of OPEs were investigated. CCCP, glycerol, DIDS and LaCl$_3$ were applied as the metabolic, aquaporin, anion and Ca$^{2+}$ channel inhibitors, respectively. The result showed that the uptake of OPEs by rice root was significantly inhibited in different degrees by glycerol, but not for DIDS, CCCP and LaCl$_3$, and the inhibition differed

with treatments (Fig. 6). CCCP showed no significant effect ($p > 0.05$) on the root uptake of OPEs in the OPE treatment, suggesting that absorption of OPEs into rice root is likely to be a diffusion process without energy consuming (Gong et al., 2020). Diffusion via carriers or channels, such as water and anion channels, is recognized as non-energy-consuming uptake process, which is an important process for anthropogenic chemicals to enter plants. Glycerol is a low phytotoxic substrate for aquaporins (Wen et al., 2013), which can inhibit aquaporins by substrate competition (Meharg
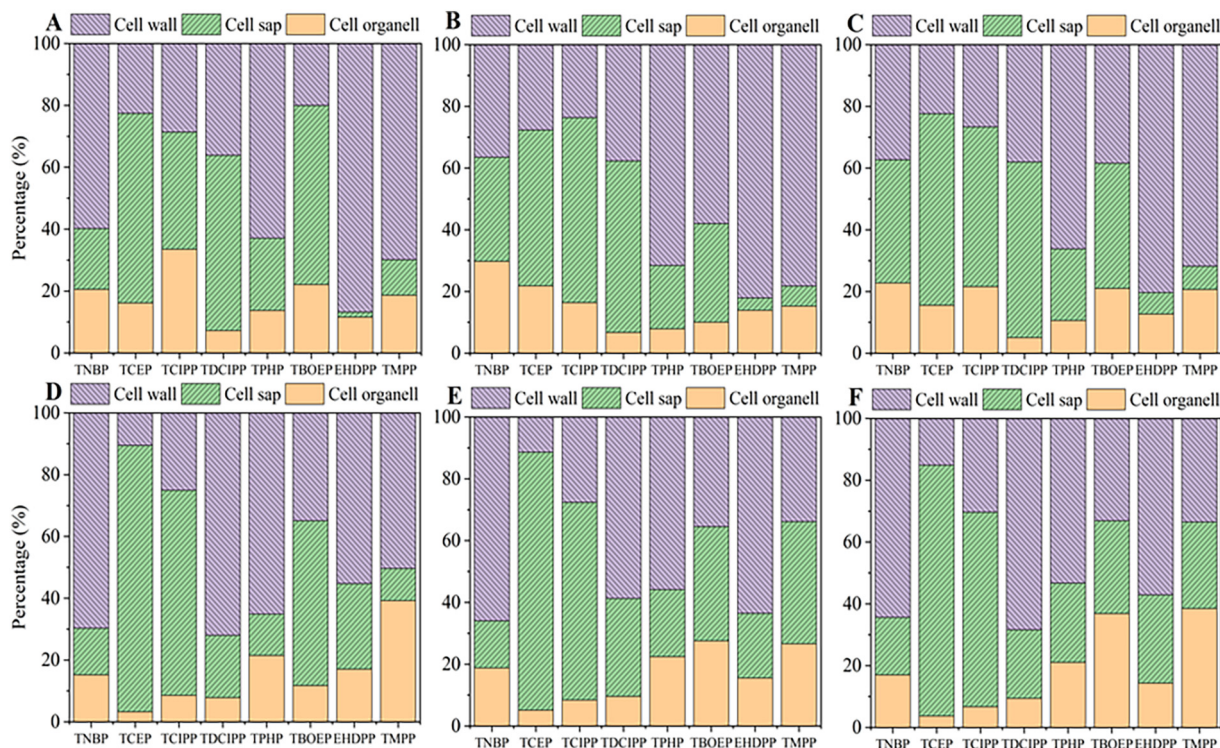


**Fig. 4.** Subcellular distributions of OPEs in cell walls, cell sap and cell organelles of rice root and shoot in 3 different treatments: OPEs (A root, D shoot), OPEs + high Cu$^{2+}$ (B root, E shoot) and OPEs + CuONPs (C root, F shoot).
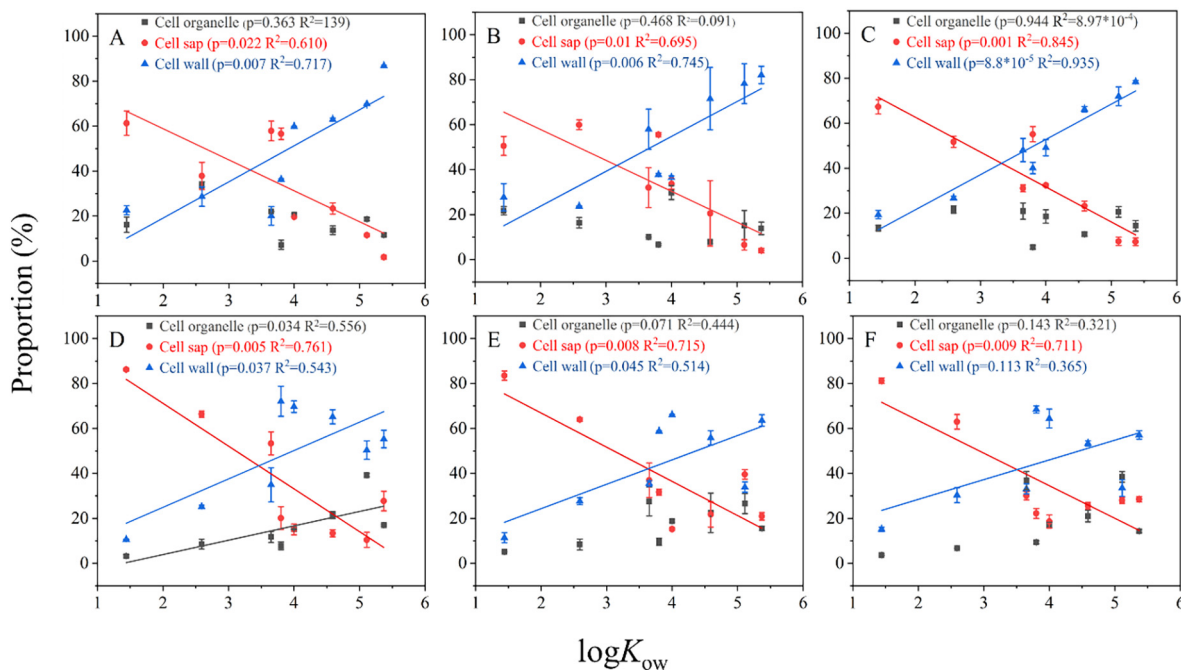
**Fig. 5.** Correlations between OPE proportions in rice root and shoot and their $\log K_{ow}$ in the treatments of OPEs (A root, D shoot), OPEs + high $Cu^{2+}$ (B root, E shoot) and OPEs + CuONPs (C root, F shoot).

and Jardine, 2003). In the OPE treatment, uptakes of TCEP, TPHP, EHDPP and TMPP by root were significantly inhibited ($p < 0.05$) by glycerol by 57.7 %, 66.8 %, 34.9 % and 43.6 % respectively compared with the control, whereas uptakes of TNBP, TDCIPP, TBOEP and TCIPP were also inhibited, but no significant by glycerol by 29.5 %, 26.8 %, 22.7 % and 9.4 % respectively. This result indicated that aquaporins may be at least partly involved in the root absorption of OPEs. Significant inhibition of glycerol to root uptake of perfluorooctanesulfonate (PFOS) by Lettuce (Yu et al., 2021) and maize (Wen et al., 2013) were also observed. However, this is different from the result of Gong et al. (2020), who found that uptake of TPHP by wheat root was significantly inhibited by glycerol (27 %), but not for

TNBP and TCEP uptake. This difference may be due to different plant species.

DIDS is a rapid-type anion channel inhibitor (Barbier-Brygoo et al., 2000), while $LaCl_3$ is a $Ca^{2+}$ channel inhibitor. Root uptake of OPEs was insensitive in response to DIDS and $LaCl_3$. Only insignificant inhibitions of TBOEP (29 %) and TDCIPP (20 %) were observed. Gong et al. (2020) also found that DIDS had no significant effects on root uptake of TNBP and TCEP, but a significant inhalation ($p > 0.05$, 30 %) on TPHP.

For the OPEs + high $Cu^{2+}$ treatment, the influences of the 4 inhibitors were similar to those in the OPE treatment with some exceptions. The inhibition effect of TNBP uptake by glycerol was significantly increased under
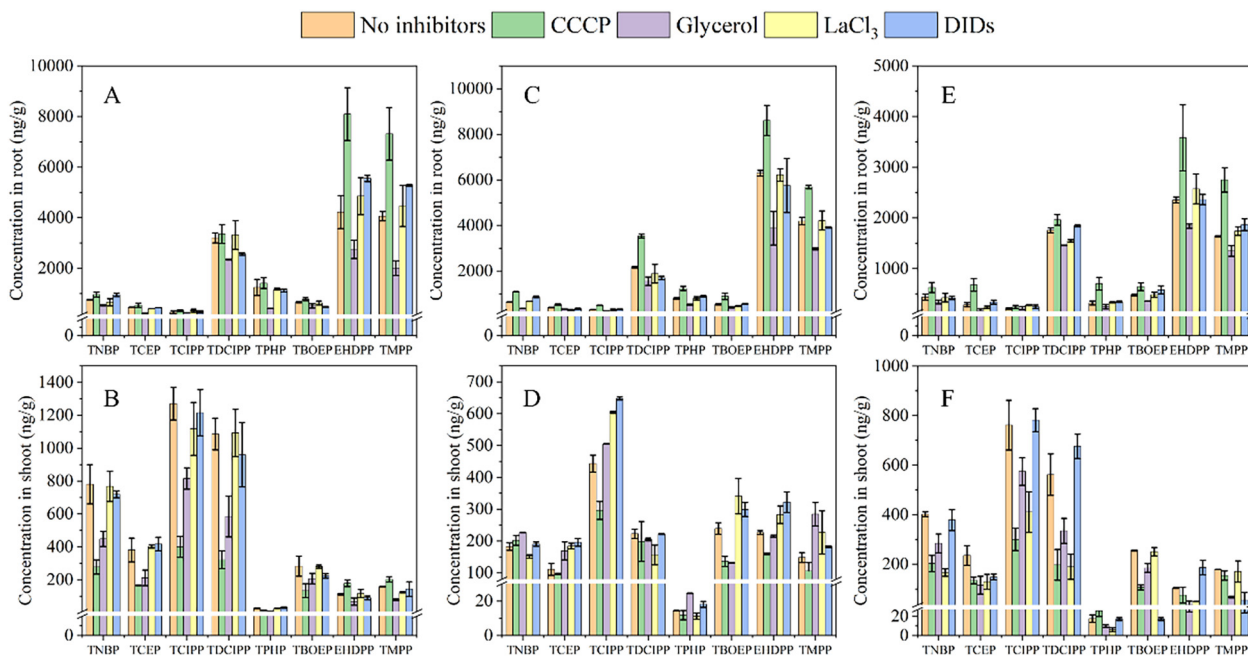


**Fig. 6.** Effects of inhibitors on OPE uptakes by rice in the OPEs (A root, B shoot), OPEs + high $Cu^{2+}$ (C root, D shoot) and OPEs + CuONPs (E root, F shoot) treatments.

the OPEs + high Cu$^{2+}$ treatment compared with the OPE treatment, while the effects of TCEP and TPHP were decreased. This may be due to the root damage by Cu$^{2+}$. However, inhibition effect of glycerol on OPE uptake was significantly reduced under the co-exposure of OPEs + CuONPs. NPs can be adsorbed onto root surface via mechanical adhesion and encapsulation by root secretions (Begurn et al., 2011), which may weaken the inhibitory effect of glycerin, and change the uptake pathway of CuONPs adsorbed OPEs. In the OPEs + CuONPs treatment, LaCl$_3$ also significantly inhibited the uptake of TNBP, TCIPP, TDCIPP and TPHP, which suggested that uptake of CuONPs adsorbed OPEs may be associated with competitive adsorption of Ca$^{2+}$. However, more investigations are needed to prove this mechanism.

## 4. Conclusion

We investigated the influence of the co-exposure of CuONPs or Cu$^{2+}$ on the uptake, transfer and subcellular distribution of OPEs by rice seedlings. The uptake and translocation of OPEs by rice was significantly reduced by short-term co-exposure to CuONPs due to the competitive sorption. The coexistence of Cu$^{2+}$ or CuONPs with OPEs can influence the OPE subcellular distributions by affecting their adsorption and partitioning processes. Hydrophobic OPEs were mainly distributed in cell walls and difficult to be transported to shoot. Root uptakes of OPEs are facilitated diffusion mainly mediated by aquaporin channel, which may be changed under the coexistence of CuONPs. However, this current study has some limitations. First, CuONPs and their potential conversion products in rice were not considered in this study. Second, the exposure time was relatively short, which may lead to a limited conclusion on the adverse impact of CuONPs. Moreover, influence of CuONPs on the degradation of OPEs in rice also needs our attention. Considering the complex contamination in the environment, more investigations are requested to explore the joint effects of co-exposure of organic chemicals and NPs on plants, especially crops.

## CRediT authorship contribution statement

**Yan Wang:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Project administration, Resources, Funding acquisition. **Die Wu:** Methodology, Investigation, Formal analysis, Data curation, Writing – original draft. **Fei Gao:** Investigation, Formal analysis. **Yue Xu:** Resources, Writing – review & editing. **Feng Tan:** Resources, Writing – review & editing.

## Data availability

Data will be made available on request.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.160664.

## References

Barbier-Brygoo, H., Vinauger, M., Colcombet, J., Ephritikhine, G., Frachisse, J.M., Maurel, C., 2000. Anion channels in higher plants: functional characterization, molecular structure and physiological role. Biochim.Biophys.ActaBiomembr. 1465, 199–218.

Begurn, P., Ikhtiari, R., Fugetsu, B., 2011. Graphene phytotoxicity in the seedling stage of cabbage, tomato, red spinach, and lettuce. Carbon 49, 3907–3919.

Behl, M., Hsieh, J.H., Shafer, T.J., Mundy, W.R., Rice, J.R., Boyd, W.A., et al., 2015. Use of alternative assays to identify and prioritize organophosphorus flame retardants for potential developmental and neurotoxicity. Neurotoxicol. Teratol. 52, 181–193.

Carignan, C.C., Minguez-Alarcon, L., Butt, C.M., Williams, P.L., Meeker, J.D., Stapleton, H.M., et al., 2017. Urinary concentrations of organophosphate flame retardant metabolites and pregnancy outcomes among women undergoing in vitro fertilization. Environ. Health Perspect. 125, 087018.

Cheng, S.P., 2003. Effects of heavy metals on plants and resistance mechanisms. Environ. Sci. Pollut. Res. 10, 256–264.

Collins, C., Fryer, M., Grosso, A., 2006. Plant uptake of non-ionic organic chemicals. Environ. Sci.Technol. 40, 45–52.

Cousins, I.T., Mackay, D., 2001. Strategies for including vegetation compartments in multimedia models. Chemosphere 44, 643–654.

Demidchik, V., Sokolik, A., Yurin, V., 1997. The effect of Cu2+ on ion transport systems of the plant cell plasmalemma. Plant Physiol. 114, 1313–1325.

Deng, R., Lin, D., Zhu, L., Majumdar, S., White, J.C., Gardea-Torresdey, J.L., et al., 2017. Nanoparticle interactions with co-existing contaminants: joint toxicity, bioaccumulation and risk. Nanotoxicology 11, 591–612.

Deng, S., Ke, T., Wu, Y., Zhang, C., Hu, Z., Yin, H., et al., 2018. Heavy metal exposure alters the uptake behavior of 16 priority polycyclic aromatic hydrocarbons (PAHs) by Pak Choi (Brassica chinensis L.). Environ.Sci.Technol. 52, 13457–13468.

Gao, Y., Zhang, Y., Liu, J., Kong, H., 2013. Metabolism and subcellular distribution of anthracene in tall fescue (Festuca arundinacea Schreb.). Plant Soil 365, 171–182.

Gong, X., Wang, Y., Pu, J., Zhang, J., Sun, H., Wang, L., 2020. The environment behavior of organophosphate esters (OPEs) and di-esters in wheat (Triticum aestivum L.): uptake mechanism, in vivo hydrolysis and subcellular distribution. Environ. Int. 135, 105405.

Guo, J.-J., Tan, X., Fu, H.-L., Chen, J.-X., Lin, X.-X., Ma, Y., et al., 2018. Selection for Cd pollution-safe cultivars of Chinese kale (Brassica alboglabra L. H. Bailey) and biochemical mechanisms of the cultivar-dependent Cd accumulation involving in Cd subcellular distribution. J. Agric. Food Chem. 66, 1923–1934.

He, Y., Nie, E., Li, C., Ye, Q., Wang, H., 2017. Uptake and subcellular distribution of triclosan in typical hydrophytes under hydroponic conditions. Environ. Pollut. 220, 400–406.

Keller, A.A., McFerran, S., Lazareva, A., Suh, S., 2013. Global life cycle releases of engineered nanomaterials. J. Nanopart. Res. 15, 1692.

Lee, W.-M., An, Y.-J., Yoon, H., Kweon, H.-S., 2008. Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (Phaseolus radiatus) and wheat (Triticum aestivum): plant agar test for water-insoluble nanoparticles. Environ. Toxicol. Chem. 27, 1915–1921.

Li, X., Zhu, Y., Wu, T., Zhang, S., Christie, P., 2010. Using a novel petroselinic acid embedded cellulose acetate membrane to mimic plant partitioning and in vivo uptake of polycyclic aromatic hydrocarbons. Environ.Sci.Technol. 44, 297–301.

Lin, Q., Chen, S., Chao, Y., Huang, X., Wang, S., Qiu, R., 2017. Carboxylesterase-involved metabolism of di-n-butyl phthalate in pumpkin (Cucurbita moschata) seedlings. Environ. Pollut. 220, 421–430.

Ling, W., Lu, X., Gao, Y., Liu, J., Sun, Y., 2012. Polyphenol oxidase activity in subcellular fractions of tall fescue contaminated by polycyclic aromatic hydrocarbons. J. Environ. Qual. 41, 807–813.

Lu, M., Zhang, Z.-Z., Su, X.-L., Xu, Y.-X., Wu, X.-J., Zhang, M., 2013. Effect of copper on in vivo fate of BDE-209 in pumpkin. J. Hazard. Mater. 262, 311–317.

Maksymiec, W., 1997. Effect of copper on cellular processes in higher plants. Photosynthetica 34, 321–342.

Meharg, A.A., Jardine, L., 2003. Arsenite transport into paddy rice (Oryza sativa) roots. New Phytol. 157, 39–44.

Miller, E.L., Nason, S.L., Karthikeyan, K.G., Pedersen, J.A., 2016. Root uptake of pharmaceuticals and personal care product ingredients. Environ.Sci.Technol. 50, 525–541.

Paterson, S., Mackay, D., Bacci, E., Calamari, D., 1991. Correlation of the equilibrium and kinetics of leaf air exchange of hydrophobic organic-chemicals. Environ.Sci.Technol. 25, 866–871.

Reddy, P.V.G., Reddy, B.R.P., Reddy, M.V.K., Reddy, K.R., Shetti, N.P., Saleh, T.A., et al., 2021. A review on multicomponent reactions catalysed by zero-dimensional/one-dimensional titanium dioxide (TiO2) nanomaterials: promising green methodologies in organic chemistry. J. Environ. Manag. 279, 111603.

Schwab, F., Zhai, G., Kern, M., Turner, A., Schnoor, J.L., Wiesner, M.R., 2016. Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants - critical review. Nanotoxicology 10, 257–278.

Shaw, A.K., Hossain, Z., 2013. Impact of nano-CuO stress on rice (Oryza sativa L.) seedlings. Chemosphere 93, 906–915.

Thounaojam, T.C., Panda, P., Mazumdar, P., Kumar, D., Sharma, G.D., Sahoo, L., et al., 2014. Excess copper induced oxidative stress and response of antioxidants in rice (vol 53, pg 33, 2012). Plant Physiol. Biochem. 83 375-375.

Wan, W., Huang, H., Lv, J., Han, R., Zhang, S., 2017. Uptake, translocation, and biotransformation of organophosphorus esters in wheat (Triticum aestivum L.). Environ.Sci.Technol. 51, 13649–13658.

Wang, Z., Xie, X., Zhao, J., Liu, X., Feng, W., White, J.C., et al., 2012. Xylem- and phloem-based transport of CuO nanoparticles in maize (Zea mays L.). Environ.Sci.Technol. 46, 4434–4441.

Wang, F., Liu, J., Zhou, L., Pan, G., Li, Z., Zaidi, S.-H.-R., et al., 2016. Senescence-specific change in ROS scavenging enzyme activities and regulation of various SOD isozymes to ROS levels in psf mutant rice leaves. Plant Physiol. Biochem. 109, 248–261.

Wang, S., Wang, Y., Luo, C., Jiang, L., Song, M., Zhang, D., et al., 2016b. Could uptake and acropetal translocation of PBDEs by corn be enhanced following Cu exposure? Evidence from a root damage experiment. Environ.Sci.Technol. 50, 856–863.

Wang, W., Wan, Q., Li, Y., Xu, W., Yu, X., 2019. Uptake, translocation and subcellular distribution of pesticides in Chinese cabbage (Brassica rapa var. chinensis). Ecotoxicol. Environ. Saf. 183, 109488.

Wang, T.-T., Ying, G.-G., He, L.-Y., Liu, Y.-S., Zhao, J.-L., 2020a. Uptake mechanism, subcellular distribution, and uptake process of per fluorooctanoic acid and per fluorooctane sulfonic acid by wetland plant Alisma orientale. Sci. Total Environ. 733, 139383.

Wang, X., Zhu, Q., Yan, X., Wang, Y., Liao, C., Jiang, G., 2020b. A review of organophosphate flame retardants and plasticizers in the environment: analysis, occurrence and risk assessment. Sci. Total Environ. 731, 139071.

Wen, B., Li, L., Liu, Y., Zhang, H., Hu, X., Shan, X.-q., et al., 2013. Mechanistic studies of perfluorooctane sulfonate, perfluorooctanoic acid uptake by maize (Zea mays L. cv. TY2). Plant Soil 370, 345–354.

Wu, J., Xie, Y., Fang, Z., Cheng, W., Tsang, P.E., 2016. Effects of Ni/Fe bimetallic nanoparticles on phytotoxicity and translocation of polybrominated diphenyl ethers in contaminated soil. Chemosphere 162, 235–242.

Yu, P.-F., Li, Y.-W., Zou, L.-J., Liu, B.-L., Xiang, L., Zhao, H.-M., et al., 2021. Variety-selective rhizospheric activation, uptake, and subcellular distribution of perfluorooctanesulfonate (pfos) in lettuce (Lactuca sativa L.). Environ.Sci.Technol. 55, 8730–8741.

Zhang, X., Zou, W., Mu, L., Chen, Y., Ren, C., Hu, X., et al., 2016. Rice ingestion is a major pathway for human exposure to organophosphate flame retardants (OPFRs) in China. J. Hazard. Mater. 318, 686–693.

Zhang, S., Yao, H., Lu, Y., Yu, X., Wang, J., Sun, S., et al., 2017. Uptake and translocation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals by maize from soil irrigated with wastewater. Sci. Rep. 7, 12165.

Zhang, Q., Yao, Y., Wang, Y., Zhang, Q., Cheng, Z., Li, Y., et al., 2021. Plant accumulation and transformation of brominated and organophosphate flame retardants: a review*. Environ. Pollut. 288, 117742.

Zhao, H.-M., Du, H., Xiang, L., Chen, Y.-L., Lu, L.-A., Li, Y.-W., et al., 2015. Variations in phthalate ester (PAE) accumulation and their formation mechanism in Chinese flowering cabbage (Brassica parachinensis L.) cultivars grown on PAE-contaminated soils. Environ. Pollut. 206, 95–103.