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Title: Negligible Effects of TiO2 Nanoparticles at Environmentally Relevant Concentrations on the Translocation and Accumulation of Perfluorooctanoic Acid and Perfluorooctanesulfonate in Hydroponically Grown Pumpkin Seedlings (Cucurbita maxima × C. moschata)

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Keywords: TiO2 Nanoparticles; Pumpkin seedling; Perfluoroalkyl substances; Uptake; Accumulation

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Abstract: Titanium dioxide nanoparticles (TiO2-NPs) are widely distributed in the environment. It has been demonstrated that TiO2-NPs could modify the environmental fate and bioavailability of organic pollutants, which affects ecological risks of TiO2-NPs and organic pollutants. In this study, the uptake, translocation and accumulation of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) in pumpkin plants was investigated in the presence of TiO2-NPs. We reported for the first time the negligible effects of TiO2-NPs at environmentally relevant concentrations (0.05-5 mg/L) on the uptake and accumulation of PFOA and PFOS in hydroponically grown pumpkin seedlings regardless of root, stem and leaf. This phenomenon was independent of the initial concentrations of PFOA/PFOS and TiO2-NPs in the exposure solution. Also, seedling mass and contents of chlorophyll and anthocyanin were not affected by the co-exposure. Adsorption tests demonstrated the negligible adsorption of PFOA/PFOS on TiO2-NPs in the exposure solution. Moreover, uptake of PFOA/PFOS was insensitive to aquaporin inhibitor AgNO3 but significantly inhibited by niflumic acid (anion channel blocker) and 2,4-dinitrophenol (metabolic inhibitor) whereas Ti concentration in root was not affected by niflumic acid and 2,4-dinitrophenol but significantly decreased by AgNO3, indicating that transport of PFOA/PFOS and TiO2-NPs were via different routes into the pumpkin seedling. It was proposed that different pathways by which TiO2-NPs and PFOA/PFOS transported into the pumpkin seedling and negligible adsorption of PFOA/PFOS on TiO2-NPs contributed to the negligible effects of TiO2-NPs on the uptake, translocation and accumulation of PFOA/PFOS in pumpkin seedlings. In total, this work would improve our understanding of the ecological risks of TiO2-NPs in the environment.

Response to Reviewers: Dear Prof. Dr. Shuzhen Zhang,

The 'Response to Comments' has been uploaded. please see attach files.
Thank you very much.

To: Prof. Dr. Shuzhen Zhang

From: Prof. Xueping Zhao

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Dear Prof. Dr. Shuzhen Zhang,

Thank you very much for handling our manuscript entitled: 'Negligible Effects of TiO_2 Nanoparticles at Environmentally Relevant Concentrations on the Translocation and Accumulation of Perfluorooctanoic Acid and Perfluorooctanesulfonate in Hydroponically Grown Pumpkin Seedlings (Cucurbita maxima × C. moschata)' (**STOTEN-D-19-03789**). We are highly grateful to you and two reviewers for taking time to review our manuscript and providing valuable comments to revise and improve the manuscript. All the suggestions have been carefully considered in the revised manuscript and the improvements have been made as shown in the 'Response to Comments' attached to this letter.

All data are original and the work has not been considered elsewhere for publication.

We sincerely hope that you will find the revised version of the manuscript satisfactory for publication. Should there be any additional improvements necessary please do not hesitate to contact us.

Once again, we appreciate your guidance and suggestions, which are valuable in improving the quality of our manuscript.

With best regards,

Yours sincerely,

Prof. Xueping Zhao

Title Page

Negligible Effects of TiO_2 Nanoparticles at Environmentally Relevant Concentrations on the Translocation and Accumulation of Perfluorooctanoic Acid and Perfluorooctanesulfonate in Hydroponically Grown Pumpkin Seedlings (*Cucurbita maxima* × *C. moschata*)

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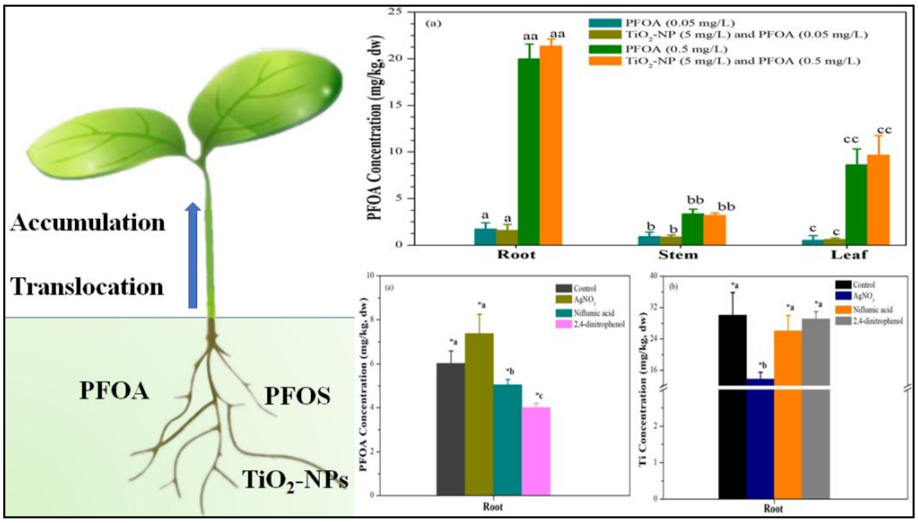
***Graphical Abstract**

Graphical abstract



***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

Highlights

1. Effect of TiO_2-NPs on accumulation of PFOA/PFOS in pumpkin seedling was explored.
2. Environmentally relevant concentrations of TiO_2-NPs were applied in this work.
3. The negligible effects were observed.
4. The negligible adsorption of PFOA/PFOS on TiO_2-NPs was proposed as one reason.
5. Different pathways of TiO_2-NPs and PFOA/PFOS into root were another reason.

Abstract

Titanium dioxide nanoparticles (TiO_2-NPs) are widely distributed in the environment. It has been demonstrated that TiO_2-NPs could modify the environmental fate and bioavailability of organic pollutants, which affects ecological risks of TiO_2-NPs and organic pollutants. In this study, the uptake, translocation and accumulation of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) in pumpkin plants was investigated in the presence of TiO_2-NPs. We reported for the first time the negligible effects of TiO_2-NPs at environmentally relevant concentrations (0.05-5 mg/L) on the uptake and accumulation of PFOA and PFOS in hydroponically grown pumpkin seedlings regardless of root, stem and leaf. This phenomenon was independent of the initial concentrations of PFOA/PFOS and TiO_2-NPs in the exposure solution. Also, seedling mass and contents of chlorophyll and anthocyanin were not affected by the co-exposure. Adsorption tests demonstrated the negligible adsorption of PFOA/PFOS on TiO_2-NPs in the exposure solution. Moreover, uptake of PFOA/PFOS was insensitive to aquaporin inhibitor $AgNO_3$ but significantly inhibited by niflumic acid (anion channel blocker) and 2,4-dinitrophenol (metabolic inhibitor) whereas Ti concentration in root was not affected by niflumic acid and 2,4-dinitrophenol but significantly decreased by $AgNO_3$, indicating that transport of PFOA/PFOS and TiO_2-NPs were via different routes into the pumpkin seedling. It was proposed that different pathways by which TiO_2-NPs and PFOA/PFOS transported into the pumpkin seedling and negligible adsorption of PFOA/PFOS on TiO_2-NPs contributed to the negligible effects of TiO_2-NPs on the uptake, translocation and accumulation of PFOA/PFOS in pumpkin seedlings. In total, this work would improve our understanding of the ecological risks of TiO_2-NPs in the environment.

Keywords

TiO_2 Nanoparticles; Pumpkin seedling; Perfluoroalkyl substances; Uptake; Accumulation

1. Introduction

In recent decades, titanium dioxide nanoparticles (TiO_2-NPs) have been extensively used in commercial products such as functional textiles, personal care products, food storage materials, house appliances and semiconductors due to the special physiochemical properties (Ai-Kattan et al., 2014; Shandilya et al., 2015; Morsella et al., 2016; Kaegi et al., 2017), which results in an accidental or intentional discharge of TiO_2-NPs into the natural environment during the production, usage and disposal of these products (Gondikas et al., 2014; Shandilya et al., 2015; Kaegi et al., 2017). On the basis of material flow models, predicted concentrations of TiO_2-NPs in the environment ranged from tens μg/L (e.g., water) to tens mg/kg (e.g., sediment and soil) (Sun et al., 2016; 2017). TiO_2-NPs have physicochemical properties including high insolubility and large surface area, which implies that they are prone to interact with other substances such as organic chemicals. With the co-existence of organic pollutants in the natural environment, TiO_2-NPs would modify the environmental fate and bioavailability of organic pollutants. The enhanced bioaccumulation of bisphenol A, perfluoroctanesulfonate (PFOS) and decabromodiphenyl ether in zebrafish was observed in the presence of TiO_2-NPs at environmentally relevant concentrations, and TiO_2-NPs acted as a carrier of chemicals due to the adsorption of chemicals on TiO_2-NPs (Wang et al., 2014; Qiang et al., 2015; Fang et al., 2016). To date, nevertheless few studies investigated the effect of TiO_2-NPs on the uptake, translocation and bioaccumulation of pollutants in plants. Given precautionary principles, some questions arise inevitably: What is the influences of TiO_2-NPs at environmentally relevant concentrations on the translocation and bioaccumulation of organic chemicals in plants? Can TiO_2-NPs at environmentally relevant concentrations enhance or moderate the uptake of organic chemicals in plant? And thus what is the potential reason for the phenomenon?

Perfluoroalkyl substances are a large group of anthropogenic persistent organic pollutants that have been attracted wide attention as global contaminants. Among them, perfluorooctanoic acid (PFOA) and PFOS are two of the most widely and extensively detected homologous in the natural environment (Butt et al., 2010;

Zareitalabad et al., 2013; Wang et al., 2015). Both PFOA and PFOS have shown high toxicity to aquatic organisms and plants (Du et al., 2010; Hagenaars et al., 2013; Nordén et al., 2016), and even neural toxicity was observed in recent (Yin et al., 2018). Plants such as pumpkin, soybean and wheat could accumulate PFOA and PFOS (Zhao et al., 2013; Bizkarguenaga et al., 2016; Xiang et al., 2018), being a potential risk for humans through food chains (de Vos et al., 2008; van Asselt et al., 2011). Foods of plant origin (e.g. fruit and vegetables) have been observed to be the most important for the dietary exposure to PFOA in some European countries despite relative low concentrations measured in these foods (Klenow et al., 2013). Obviously, it is of importance to understand whether uptake, translocation and accumulation of PFOA and PFOS in plants would be affected by TiO_2-NPs at environmentally relevant concentrations. Yet, no data on this issue has been available to date.

In the present study, herein, a hydroponically grown pumpkin seedling (*Cucurbita maxima* × *C. moschata*) was used as a model plant to investigate the effect of TiO_2-NPs at environmentally relevant concentrations on the uptake, translocation and accumulation of PFOA and PFOS in plants. Three concentrations of TiO_2-NPs and two different dose of PFOA/PFOS were examined. Besides quantification the amount of titanium, PFOA and PFOS in different organs including root, stem and leaf, biomass and contents of chlorophyll as well as anthocyanin were determined. According to the results of adsorption experiments and effects of channel blockers and metabolic inhibitors on the uptake of PFOA and PFOS, we further proposed potential reasons for the observed phenomenon.

2. Materials and methods

2.1 Chemicals

Sodium hypochlorite, silver nitrate, niflumic acid, hydrochloric acid, 2,4-dinitrophenol, ethanol and chloroform were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Perfluorooctanesulfonic acid potassium salt (KPFOS >98%) and perfluorooctanoic acid (PFOA >96%) obtained from CNW Technologies GmbH (Shanghai, China) were used for hydroponic exposure experiments. Analytical reference standards of PFOA, PFOS, $^{13}C_8$-PFOS, $^{13}C_4$-PFOA and $^{13}C_8$-PFOA were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Acetonitrile and methanol of HPLC-MS grade were purchased from Fisher (Waltham, MA, USA). All reagents used in this study were of analytical or HPLC grade and used as received without any further treatment. Aqueous solutions were freshly prepared with ultrapure water which was from a Direct-Q-system (Millipore, Billerica, USA) with a resistivity of 18.2 MΩ/cm. Florisil SPE cartridges (1000 mg, 6 mL) were purchased from Applied Separations (Allentown, PA, USA). Oasis HLB SPE cartridges (60 mg, 3cc) were from Waters (Wexford, Ireland). Supelclean ENVI-Carb 120/140 was from Supelco (Bellefonte, PA, USA).

TiO_2-NPs (Aeroxide P25, 21 nm) were from Sigma-Aldrich (St. Luis, USA). The ζ potential and hydrodynamic size of TiO_2-NPs were determined using a Malvern Zeta sizer Nano Series dynamic light scattering (DLS) instrument. The morphology of TiO_2-NPs was observed through a JEOL JEM-2100F high-resolution transmission electron microscope (HRTEM) at 200 kV.

2.2 Materials

Pumpkin seeds (*Cucurbita maxima* × *C. moschata*, Taigu Yinong Seed Co., Ltd., Shanxi Province, China) were surface-sterilized by 70% (v/v) of ethanol for 5 min and then were soaked in 5% (v/v) sodium hypochlorite solution for 15 min. Afterward, seeds were washed thoroughly five times with ultrapure water and then soaked in ultrapure water for 8 h at 25^{o}C. Then, seeds were placed in Petri dishes in the dark at 25^{o}C to germinate for 3 d. The 3-day old pumpkin seedlings were then transferred to a vessel with wet sterile quartz sand and cultivated in an illumination growth chamber at 25^{o}C for a 16 h light period and at 22^{o}C for an 8 h dark period. Light was supplied by sets of fluorescent lighting with a light intensity of 150 - 200 μmol m^{-2} s^{-1}. After incubation of 7 d, seedling stems grew to 5~6 cm height, and healthy seedlings with similar strength were collected. The roots of seedlings were rinsed with ultrapure water before hydroponic exposures.

2.3 Hydroponic exposures

The reactors were 50 mL polypropylene Corning tubes. Two pumpkin seedlings were cultivated in each reactor, and each reactor was filled with 40 mL of ultrapure water with desired concentrations of TiO_2-NPs, PFOA or

PFOS. Three concentrations (0.05, 0.5 and 5 mg/L) of TiO_2-NPs and two dose (0.05 and 0.5 mg/L) of PFOA or PFOS were examined. After cultivation, two pumpkin plants in each reactor were combined into one sample for analysis. There were three parallel samples in each exposure group. Besides chemical exposures, plant blanks (with seedlings but without chemicals, three reactors), unplanted controls (without seedlings but with chemicals, three reactors) and TiO_2-NPs controls (seedlings single exposure to TiO_2-NPs, three reactors) were set-up simultaneously. The pumpkin seedlings were cultivated in an illumination growth chamber at 25^{o}C for a 16 h light period and at 22^{o}C for an 8 h dark period. Light was supplied by sets of fluorescent lighting with a light intensity of 150 - 200 μmol m^{-2} s^{-1}. The exposure was lasted for 8 d. All tubes were wrapped with aluminum foil to support root growth in the dark and to avoid possible photolysis of PFOA or PFOS. Here it should be noted that the plant was fixed by predrilled polypropylene foam snugly to make only roots emerge into the hydroponic solutions. Water loss via evaporation of water itself was determined by weighing the unplanted controls every day, indicating the negligible daily loss of water via volatilization. Also, water loss via transpiration from pumpkin seedlings was determined gravimetrically and replenished every day. After cultivation of 8 d, all pumpkin seedlings were manually collected and each seedling was sectioned into root, stem and leaf. Roots were ultrasonically rinsed with ultrapure water to remove possible chemicals adsorbed on the surface. The rinse water was combined with the exposure solution for the determination of chemicals in view of mass balance. All samples except those for chlorophyll and anthocyanin measurements were freeze-dried and stored at -20^{o}C before further treatment. The measurements of chlorophyll, anthocyanin, titanium, PFOA and PFOS were described in the Supplementary Material (Text S1, S2, S3 and S4 and Table S1).

In addition, to understand the kinetics of chemicals uptake by pumpkin seedlings, samples were also collected at certain intervals (e.g., 0 h, 4 h, 14 h, 1 d, 2 d, 3 d, 5 d and 8 d). Also, to examine potential uptake pathways of chemicals, different channel blockers such as $AgNO_3$ (0.5 mmol/L, water-channel blocker), niflumic acid (0.5 mmol/L, anion-channel blocker) and 2,4-dinitrophenol (0.5 mmol/L, metabolic inhibitor) were added into exposure solutions separately before cultivation (Wagatsuma et al., 1983; Wu et al., 2011; Devi et al., 2012). After 4 h exposure, samples were manually collected and treated according to the above-mentioned method. All the treatments were performed in three replicates.

2.4 Quality assurance and quality control (QA/QC)

To avoid contamination, polytetrafluoroethylene (PTFE) materials were not used. Fresh polypropylene tubes were used during the experiment. The isotope dilution method was applied to quantify PFOA and PFOS. Before sample extraction, internal standards of $^{13}C_8$-PFOS and $^{13}C_8$-PFOA were spiked. Before sample injection, $^{13}C_4$-PFOA was added. The ion ratios of qualifier to quantifier were checked for native compounds in each sample during the peak integration. The ratio was set as 26.5% for PFOA and 51.9% for PFOS with a maximum tolerance of ±30% (SANCO/12571/2013). The recoveries of $^{13}C_8$-PFOS, $^{13}C_8$-PFOA, PFOS and PFOA were in the ranges of 72-83%, 76-82%, 81-97% and 85-102%, respectively. The limit of detection (LOD) was defined as a value corresponding to a signal-to-noise ratio of 3 (S/N=3). The LODs of PFOA and PFOS were both 2 μg/kg. At least one procedure blank sample was prepared and analyzed with each batch of samples, indicating there was no contamination during the sample preparation. The levels of PFOA or PFOS found in the plant blanks and TiO_2-NPs controls were shown in Table S2. Since PFOA and PFOS concentrations in plant blank samples and TiO_2-NPs control samples were comparable, the reported results in this work were subtracted the plant blanks. One blank solvent was injected every 10 samples and no memory effect was observed. The amount of PFOA or PFOS recovered from exposure solutions and seedlings were 86-110% of their initial amount in the hydroponic solution. Titanium was not detected in plant blanks.

2.5 Statistical analysis

In this study, concentrations of PFOA, PFOS and Ti were shown on a dry weight basis. The normality of data was tested by using the Shapiro-Wilk Test (Origin 8.5), and the results showed that the data set for each treatment was normally distributed ($p < 0.05$). Then, student's t test (Origin 8.5) was used to evaluate possible differences of statistical significance between treatments. Statements of significant differences were based on $p < 0.05$.

3. Results and discussion

3.1 Characterization of TiO_2-NPs

According to HRTEM images, the average size of TiO_2-NPs used in this study was 24.3 ± 1.7 nm (Figure S1 in Supplementary Material), which is comparable to the size (21 nm) declared by the manufacturer (Sigma-Aldrich). DLS measurement showed the TiO_2-NPs (e.g., 5 mg/L) hydrodynamic size of 130 ± 3.2 nm, with a polydispersity index of 0.44 ± 0.21. Also, TiO_2-NPs showed a strongly negative charge, with a ζ potential of -33.3 ± 14 mV. Although TiO_2 is well-known to be relatively insoluble, dissolution kinetics as a function of exposure time (e.g., 0 h, 4 h, 14 h, 1 d, 2 d, 3 d, 5 d and 8 d) were examined in the TiO_2-NPs alone or mixture of TiO_2-NPs and PFOA/PFOS. No dissolved Ti was determined through the exposure period (data not shown), documenting the high stability of TiO_2-NPs in the aqueous solution, which is well consistent with the finding in previous studies (Bourgeault et al., 2015; Brzicova et al., 2019).

3.2 Effect of TiO_2-NPs on the uptake, translocation and accumulation of PFOA in pumpkin seedlings

The concentration of PFOA in pumpkin seedlings increased with the increasing concentration of PFOA in the exposure solution regardless of seedlings exposed to PFOA alone or mixture of PFOA and TiO_2-NPs (Figure 1a). However, no significant differences in concentrations of PFOA in seedlings were observed in the absence and presence of TiO_2-NPs (Figure 1a), suggesting negligible effects of TiO_2-NPs on the uptake and accumulation of PFOA in pumpkin seedlings. Moreover, this phenomenon was not affected by the initial concentrations of PFOA and TiO_2-NPs (Figure 1a, b). For example, the co-exposed seedlings showed comparable concentrations of PFOA regardless of the co-existed TiO_2-NPs at a concentration of 0.05, 0.5 or 5 mg/L (Figure 1b). This is different from the finding in a recent study about the effect of TiO_2-NPs on the accumulation of Pb in hydroponically grown rice seedlings. Cai et al. (2017) found that TiO_2-NPs at a concentration of 1000 mg/L reduced the uptake and bioaccumulation of Pb probably due to the obstruction effects of TiO_2-NP aggregates in the root tissues. Clearly, the concentration of TiO_2-NPs used in this study was far lower than that of the work operated by Cai et al. (2017), which would show more credible findings in our study due to the environmentally relevant concentrations of TiO_2-NPs.

Root is the compartment where accumulated the highest concentration of PFOA. This phenomenon still remained in the presence of TiO_2-NPs (Figure 1a, b), indicating that the translocation of PFOA would not be affected by the co-existed TiO_2-NPs. Similarly, the uptake, translocation and accumulation of Ti in pumpkin seedlings were not affected by the co-existed PFOA regardless of the initial concentrations of TiO_2-NPs and PFOA in the exposure solution (Figure 1e, f), and the highest concentrations of Ti were also observed in root.

The uptake and accumulation kinetics of PFOA in different parts of pumpkin seedlings were investigated, showing different trends of PFOA among the root, stem and leaf (Figure 2a,b,c). The concentration of PFOA in root increased within the first 48 h, reaching the highest level of 20-25 mg/kg, followed by equilibrium afterwards (Figure 2a). The PFOA concentration in stem remained constant (~4 mg/kg) after a gradual initial increase within the first 48 h (Figure 2b). Similarly, the concentration of PFOA in leaf gradually increased toward 72 h, with the highest level of 12-14 mg/kg, and then kept constant concentration (Figure 2c). Moreover, the presence of TiO_2-NPs appeared not to affect the uptake and accumulation kinetics of PFOA. Interestingly, chlorophyll production of leaf was unaffected by the high level of PFOA accumulated (Table 1); the total chlorophyll of plant blanks was 13.0 ± 1.1 mg/g, with the PFOA-exposed or TiO_2-NPs and PFOA co-exposed seedlings having a value of 12.8 ± 1.6 or 13.3 ± 0.79 mg/g. Quantitation of anthocyanin production supported the negligible changes in chlorophyll as anthocyanin was considered as defense against chlorophyll loss (Jung et al., 2004; Garriga et al., 2014); exposure to PFOA alone or mixture of PFOA and TiO_2-NPs showed no impact on anthocyanin content (Table 1). Also, we found that seedling masses were not affected by the co-exposure (Figure S2).

3.3 Effect of TiO_2-NPs on the uptake, translocation and accumulation of PFOS in pumpkin seedlings

Besides PFOA, PFOS exhibited similar phenomena; as shown in Figure 1c, compared to exposure to PFOS alone, no significant changes in the concentration of PFOS accumulated in the seedling are observed in the presence of TiO_2-NPs (p<0.05), suggesting that co-exposure to TiO_2-NPs at environmentally relevant concentrations had no impacts on the uptake, translocation and accumulation of PFOS in pumpkin seedlings. This phenomenon was also independent on the initial concentrations of TiO_2-NPs and PFOS in the exposure solution (Figure 1c,d). We found that root showed the highest level of PFOS, suggesting that root could readily accumulate PFOS. Clearly, the PFOS concentrations (~200 mg/kg, Figure 1c) were an order of magnitude greater than those of PFOA (~20 mg/kg, Figure 1a) accumulated in root when seedlings were exposed to the same concentrations of PFOA and PFOS, indicating that PFOS would readily be accumulated by pumpkin seedlings compared to PFOA. Protein and lipid have found to play an important role in the accumulation and distribution of PFOA and PFOS in plants and protein would promote the root accumulation (Wen et al., 2016). The high PFOS in pumpkin root compared to PFOA might be attributed to the high affinity of PFOS to plant protein (Xia et al., 2013). A previous study conducted by Wen et al. (2013) also showed high levels of PFOS accumulated in the root of maize (*Zea mys* L. cv. TY2) than those of PFOA. The highest concentration of Ti was also observed in the root for the TiO_2-NP and PFOS co-exposure (Figure 1g, h), which was consistent with the phenomena of TiO_2-NP and PFOA co-exposure.

The uptake and accumulation kinetics of PFOS in different parts of pumpkin seedlings were examined (Figure 2d, e, f). PFOS concentrations in root, stem and leaf followed a comparable tendency; increasing to a maximum value, and then remaining constant. PFOS concentrations in root, stem and leaf were peaked at approximately 48, 48, and 72 h, respectively, with the highest concentrations in the ranges of 130-150, 10-15 and 50-60 mg/kg. It appears that TiO_2-NP exposure exerted negligible influence on the kinetics of PFOS accumulation in pumpkin seedlings, which is identical with observation of PFOA.

3.4 Potential mechanisms for negligible effects of TiO_2-NPs on PFOA/PFOS uptake and accumulation

(i) *PFOA/PFOS adsorption on TiO_2-NPs.* The data of this study showed that TiO_2-NPs at environmentally relevant concentrations had negligible impacts on the uptake, translocation and accumulation of PFOA and PFOS in the pumpkin seedlings, which is out of our expectation based on available studies. In order to understand the potential mechanisms, potential roles of PFOA/PFOS adsorption on TiO_2-NPs at concentrations in the hydroponic exposure experiments were examined, showing that negligible adsorption of PFOA/PFOS on the TiO_2-NPs in the tested exposure solution (Text S5 and Table S3), which might be related to the electrostatic repulsion between negative PFOA/PFOS molecules and negative-charged TiO_2-NPs in the exposure solution.

(ii) *Uptake channels of PFOA/PFOS and TiO_2-NPs.* Effects of $AgNO_3$ (aquaporin inhibitor), niflumic acid (anion channel blocker) and 2,4-dinitrophenol (metabolic inhibitor) on uptake and accumulation of PFOA/PFOS were examined in the co-exposure solutions. Uptake of PFOA/PFOS was insensitive to aquaporin inhibitor $AgNO_3$; compared to control, no significant differences in the concentration of PFOA/PFOS in the root were observed (Figure 3a,c), indicating that aquaporins may not be a major channel for PFOA/PFOS transport into pumpkin seedling root. In contrast, treatments with niflumic acid and 2,4-dinitrophenol significantly inhibited the uptake of PFOA in root by 16 and 33% respectively ($p < 0.05$, Figure 3a). Comparably, the uptake of PFOS was depressed by niflumic acid and 2,4-dinitrophenol significantly ($p < 0.05$, Figure 3c), and in comparison with control, 81% and 79% of PFOS were recovered in root. Obviously, these findings suggested that transport of PFOA and PFOS into pumpkin seedling was similar, and it was primarily an energy-dependent process and mediated by anion channels, which is well consistent with the finding in previous study on uptake of PFOA in maize (Wen et al., 2013). The *pKa* values of PFOA and PFOS were reported to be -0.2 and -3.27, respectively (Brooke et al., 2004; Steinle-Darling et al., 2008), and thus PFOA and PFOS were in the anionic form in hydroponic solution with pH of approximately 7.3 (data not shown). This might be the reason why the transport pathway of PFOA/PFOS into pumpkin root was related with anion channels.

The Ti concentration in root was not affected by the niflumic acid and 2,4-dinitrophenol (Figure 3b,d), suggesting that transport of TiO_2-NPs into pumpkin seedling root might be independent of energy process and anion channels. Furthermore, we found that aquaporin inhibitor $AgNO_3$ significantly decreased the uptake of Ti by 51-54% in root ($p < 0.05$, Figure 3b,d), demonstrating that the aquaporins would be involved in uptake of TiO_2-NPs into pumpkin seedling root. Clearly, transport of PFOA/PFOS and TiO_2-NPs were via different routes into the pumpkin seedling, indicating that there was no competitive uptake between PFOA/PFOS and TiO_2-NPs. Taken together, it was reasonable to understand the negligible effects of TiO_2-NPs at environmentally relevant concentrations on the uptake, translocation and accumulation of PFOA/PFOS in the pumpkin seedling.

4. Conclusions

The production, usage and disposal of commercial products containing TiO_2-NPs or PFOA/PFOS would result in their occurrence in the aquatic environment. Although previous studies documented either the enhanced accumulation of chemicals by TiO_2-NPs in aquatic organisms like zebra fish or the moderated uptake of chemicals by TiO_2-NPs in hydroponically grown plants like rice seedlings, this study reported for the first time that TiO_2-NPs at environmentally relevant concentrations had negligible effects on the uptake, translocation and accumulation of PFOA and PFOS in hydroponically grown pumpkin seedlings. The observed phenomenon was probably resulted from the different pathways by which TiO_2-NPs and PFOA/PFOS transported into the pumpkin seedling root and negligible adsorption of PFOA/PFOS on TiO_2-NPs.

To investigate the impact of TiO_2-NPs at environmentally relevant concentrations on the chemical accumulation in plants is of vital importance for evaluating its ecological risks. The results in the present study is contradictory to previous work, which suggests that more work should be conducted on the effect of TiO_2-NPs on the chemical accumulation in plants in future, including clarifying the potential mechanisms. Meanwhile, it is worth investigating whether other engineered nanomaterials at environmentally relevant concentrations also have negligible impacts on the transportation and accumulation of organic pollutants in plants in future, to comprehensively understand the environmental risks of engineered nanomaterials.

Notes

The authors declare no competing financial interest.

Acknowledgements

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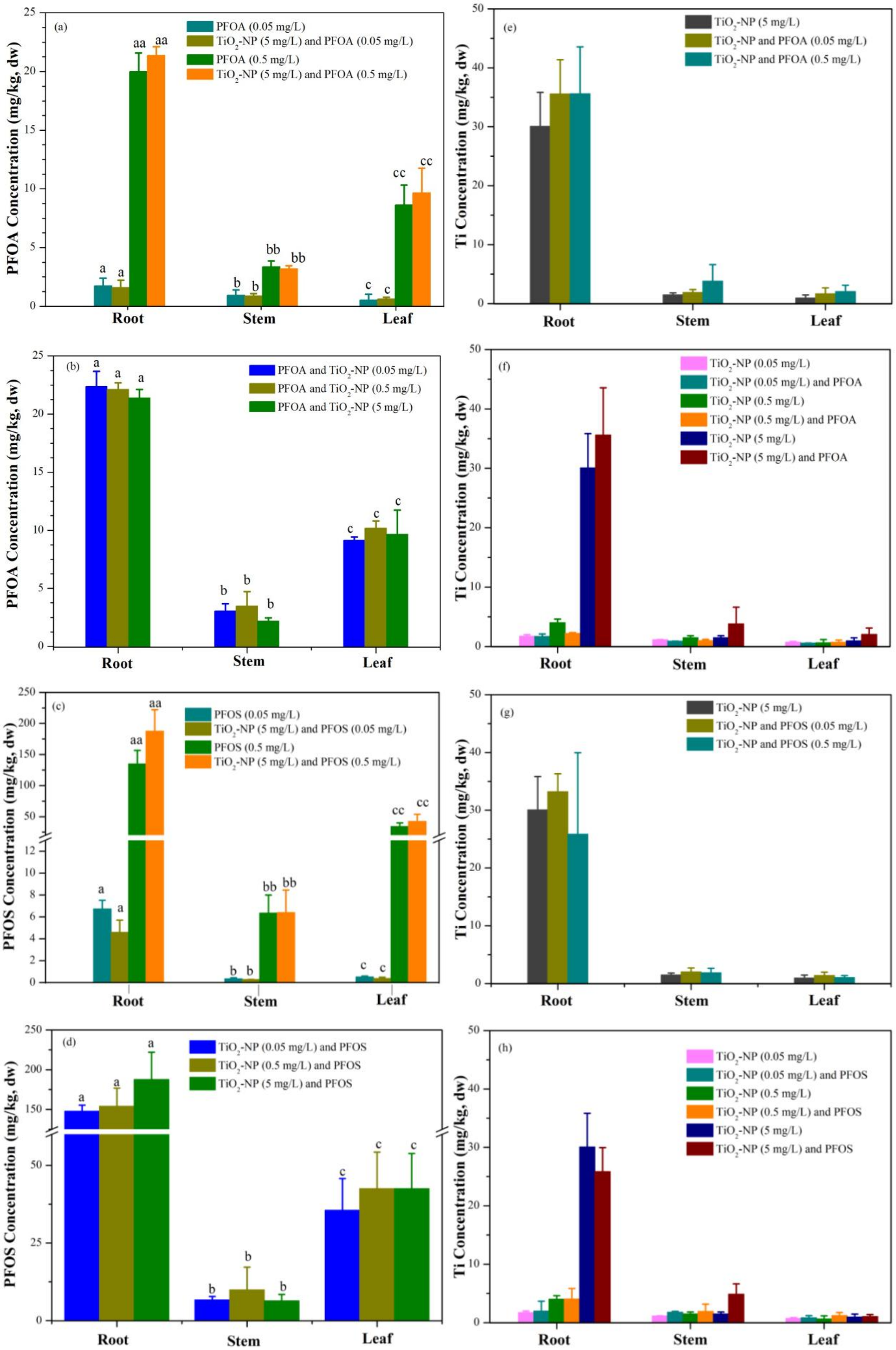

Figure 1. Concentrations of PFOA, PFOS and Ti in the root, stem and leaf of pumpkin seedlings after 8-d

exposure (bars: average value (n=3), lines: standard deviations). The same normal letter indicates non-significant differences among treatments at 0.05 level.

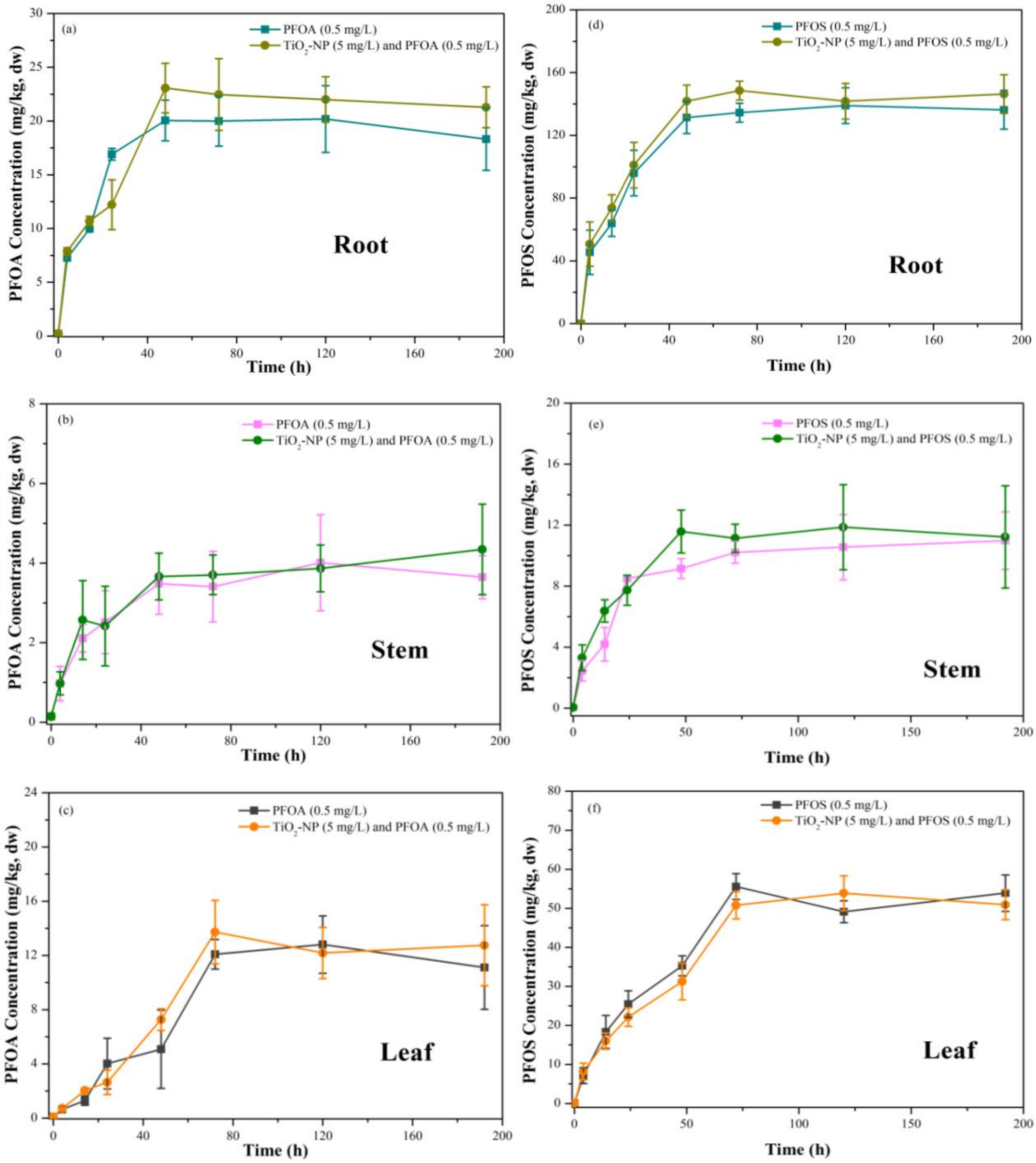

Figure 2. Kinetics of PFOA/PFOS accumulation in different compartments (root, stem and leaf) of pumpkin seedlings in single or co-exposure treatments (dot and square: average value (n=3), lines: standard deviations).

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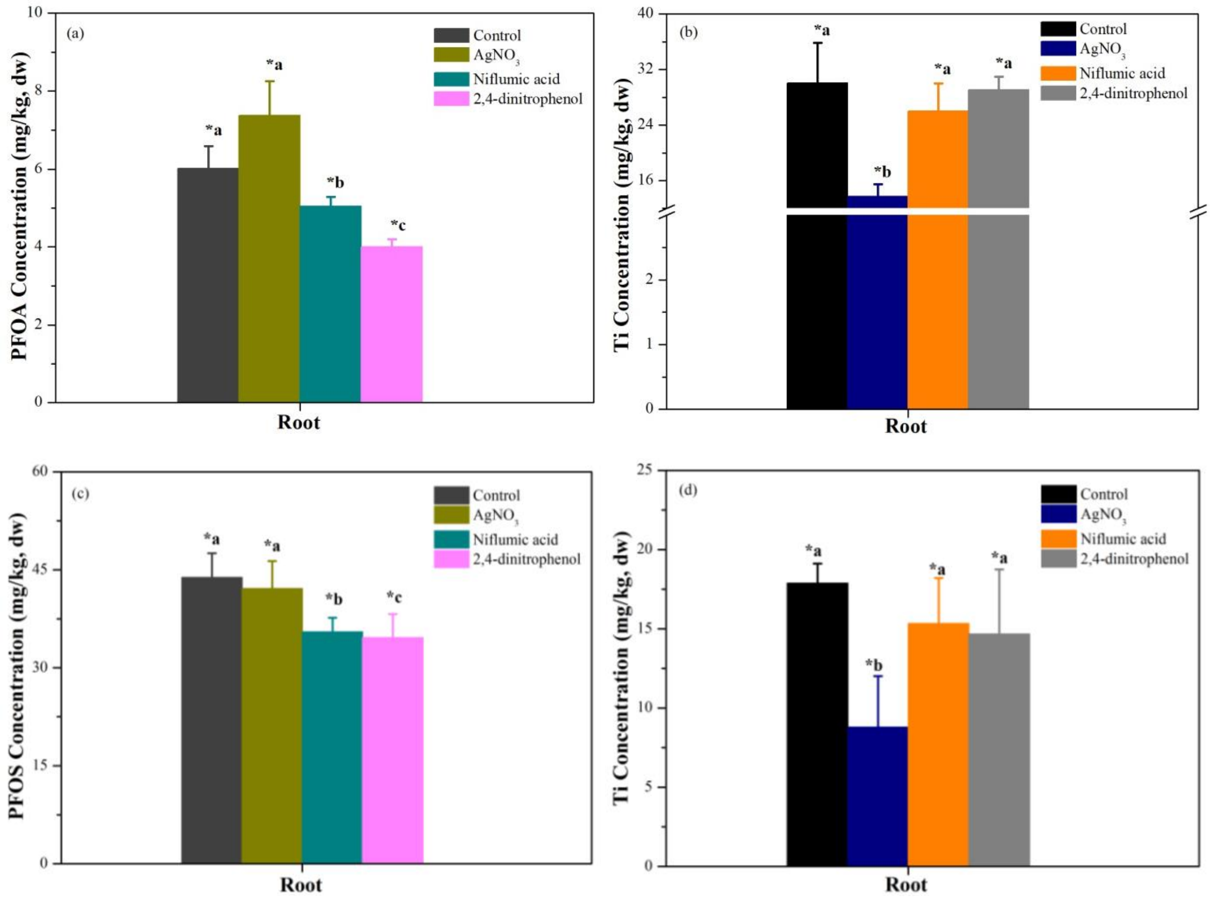

Figure 3. Effect of $AgNO_3$, niflumic acid and 2,4-dinitrophenol on the concentrations of PFOA/PFOS and Ti in the root of pumpkin seedling ($^{*}p < 0.05$ compared with the control). (a) and (b) results were from the co-exposure of TiO_2-NPs and PFOA. (c) and (d) results were from the co-exposure of TiO_2-NPs and PFOS.

Table 1. Contents of chlorophyll and anthocyanin in the leaf of pumpkin seedlings collected from different treatments.[a]

	Plant blanks	TiO_2-NPs controls	PFOA	PFOS	TiO_2-NPs + PFOA	TiO_2-NPs + PFOS
Chlorophyll contents (mg/g)	13.0 ± 1.1	13.5 ± 1.4	12.8 ± 1.6	13.4 ± 1.9	13.3 ± 0.79	12.7 ±1.2
Anthocyanin contents (mg/g)	0.11 ± 0.012	0.11 ± 0.013	0.11 ± 0.012	0.11 ± 0.021	0.11 ± 0.00	0.12 ± 0.013

[a] Here concentrations of TiO_2-NPs, PFOA and PFOS were 5.0, 0.5 and 0.5 mg/L respectively for pumpkin seedlings exposure.

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