Journal of Hazardous Materials Advances 7 (2022) 100131

Contents lists available at ScienceDirect

Journal of Hazardous Materials Advances

journal homepage: www.elsevier.com/locate/hazadv

Impacts of perfluorooctanesulfonic acid on plant biometrics and grain metabolomics of wheat (*Triticum aestivum* L.)

Polycarp C. Ofoegbu[a], Dane C. Wagner[a], Oluwasegun Abolade[a], Preston Clubb[a], Zachary Dobbs[a], Ian Sayers[a], Jenny E. Zenobio[b], Adeyemi S. Adeleye[b], Cyren M. Rico[a,*]

[a] Department of Chemistry and Biochemistry, Missouri State University, 901 S National Ave., Springfield, MO 65897, USA
[b] Department of Civil and Environmental Engineering, University of California, Irvine, CA 92697-2175, USA

ARTICLE INFO

Keywords:
Cereals
Grain quality
Perfluoroalkyl substances
PFOS
Stress
Toxicity

ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are utility chemicals that have become environmental contaminants of global concern due to their bioaccumulative and persistent nature. The accumulation and effects of perfluorooctanesulfonic acid (PFOS), a type of PFAS, on agricultural plants is a serious environmental concern that needs to be investigated. Wheat was exposed to soil amended with 0, 25, and 50 mg/kg PFOS and assessed for PFOS' impacts via agronomic measurements, biochemical assays, PFOS uptake, and ionomics and metabolomics analyses over the lifetime of the plant. PFOS was taken up in the roots and relocated to the grains, which accumulated PFOS at levels 12-18 times higher in treated plants than in control. Compared to control, 50 mg/kg PFOS reduced chlorophyll content by 49% and root biomass by 37% early on during the exposure. At the end of full life cycle exposure, PFOS did not affect the agronomic and productivity status of plants. However, PFOS impacted grain quality by reducing concentrations of macroelements (Mg, K, and P) and levels of important sugar metabolites (e.g., sucrose, glucose 6-phosphate, fructose 6-phosphate, trehalose). PFOS also decreased the abundance of non-polar proteinogenic amino acids but increased the levels of polar amino acids in grains. These findings highlight impacts of PFOS on an important agronomic crop.

Introduction

The impacts of climate change and environmental conditions on composition, food quality, and safety of grains have been widely investigated (Halford et al., 2014). However, similar research vigor on the effects of contaminants of emerging concerns on grain and food quality is lacking. Perfluorooctanesulfonic acid (PFOS) is a member of a class of compounds known as per- and polyfluoroalkyl substances (PFAS). PFAS are organic chemical compounds, which consist of a hydrocarbon framework, in which all or most of the hydrogen atoms have been substituted by fluorine atoms, and possess a functionalized head group such as sulfonate, carboxylate, phosphonate, and others (Buck et al., 2011). Over 5000 of these compounds have been identified, however, PFOS is prominent, being a pioneer member that was extensively utilized in various products, including firefighting foams, stain-resistant polishes, non-stick cookware, water-resistant clothing, and many others (Frisbee et al., 2010, Ahrens, 2011, Gobelius et al., 2017, OECD, 2018).

Although PFOS production has been phased out in the United States (US) and other Western countries (EPA, 2017), it is still considered an environmental contaminant of global concern with adverse health impacts in humans and animals (Gobelius et al., 2017). PFOS persists in the environment due to its strong carbon-fluorine bond (bond energy > 450 kJ/mol), low volatility and low vapor pressure (0.002 mmHg), high water-solubility (680 mg/L), and high boiling point (260°C) (EPA, 2017). PFOS is a strong acid that exists as an ion in the environment, and partitions strongly onto natural organic matter (Ahrens, 2011, EPA, 2017). Since PFOS does not degrade, it accumulates in environmental matrices (soil, water, air). Due to its unique physiochemical properties, PFOS has huge migration potential that it has been found in urban and most remote environment including the arctic (Giesy and Kannan, 2001). On one hand, PFOS adheres to particles resulting in long-range particle-bound transport in the atmosphere through gas-phase or sea spray (Ahrens, 2011). On the other hand, repeated biosolid application and reclaimed water usage are the major routes by which PFOS contaminates the terrestrial environment and agricultural ecosystem, especially the grain crops (Felizeter et al., 2012, Blaine et al., 2014b, Blaine et al., 2014a).

Plants can take up PFOS from contaminated soil and water and store it in their edible compartments (Blaine et al., 2014b, Felizeter et al., 2012, Wen et al., 2014, Mei et al., 2021, Ghisi et al., 2019). However, the impacts of PFOS on food crops, as well as its mechanisms of toxicity, are not well understood and remain a concern that needs to be addressed. Currently, there are three reports on metabolome changes

* Corresponding author.
E-mail address: CyrenRico@MissouriState.edu (C.M. Rico).

https://doi.org/10.1016/j.hazadv.2022.100131
Received 25 May 2022; Received in revised form 29 June 2022; Accepted 15 July 2022

in plants exposed to PFOS, and the studies were performed on leaves following short-term hydroponic exposure (Guo et al., 2020, Li et al., 2020a, Li et al., 2020b). These types of phytotoxicity study are needed to evaluate the ecological and human health effects of contaminants (Qu et al., 2010). However, PFOS toxicity on plants is a complex issue that can depend on factors such as plant species and physiology, root anatomy, PFOS concentration, exposure duration and growth stage (e.g., germinants, juvenile, full maturity), cultivation conditions (e.g., temperature, humidity, hydroponics vs soil), and soil/rhizosphere chemistry (e.g., pH, organic matter content, salinity) among others (Costello and Lee, 2020, Mei et al., 2021, Blaine et al., 2014a).

Wheat (*Triticum aestivum* L.) is a dietary staple for a large population around the globe, and an important source of carbohydrates, dietary fiber, phytochemicals, and vitamin B (Shewry and Hey, 2015). Costello and Lee (2020) identified 11 studies on wheat exposure to PFAS. These studies were based on short exposure scenarios focused on uptake, and conducted mostly in hydroponics (a few in soil) using a mixture of PFAS, with a limited understanding of phytotoxicity. While the rationale for combined exposure to a mixture of PFAS that reflects environmental conditions is understood, it is also important to conduct studies with individual PFAS members such as PFOS to understand PFOS-specific toxicity and related effects. Additionally, hydroponic studies may not be reflective of typical growth conditions for wheat, as they do not account for solute availability differences or contaminant sorption and modification obtainable in soil matrices that can modulate contaminant bioavailability or associated phytotoxicity in wheat (Costello and Lee, 2020, Dimkpa et al., 2013). Furthermore, the few soil studies conducted were predominantly focused on assessing factors that contribute to uptake and bioaccumulation of PFOS, but not to provide insights on toxicity.

The overarching goal of this study was to investigate physiological, phenological, biochemical, and metabolic responses, as well as PFOS storage in roots, shoots, and grains of wheat exposed to PFOS at shorter- and longer-term exposure scenarios in soil. The study tested the hypothesis that PFOS exposure will inhibit growth and productivity, promote stress, and reduce grain metabolite levels in wheat. The treatment concentrations (0, 25, and 50 mg/kg PFOS in soil) following those of Stahl et al. (2009) were adopted to provide insight on the kinds of stress and toxicity responses PFOS would induce in wheat that are otherwise not observed at low concentration. The results showed accumulation of PFOS in grains and modification of grain metabolite profile; but surprisingly wheat exhibited tolerance to PFOS toxicity in terms of agronomic performance and biomass productivity.

Materials and methods

Experimental design

Effects of wheat exposure to PFOS in soil over short-term (21 days) and full life-cycle (70 days) were investigated. The full life-cycle study was conducted to assess agronomic performance and productivity, PFOS and elemental uptake, and grain metabolome of wheat exposed to 0, 25, and 50 mg PFOS/kg soil; while a short exposure study focused on biomass production, plant height, chlorophyll content, enzyme activity, and lipid peroxidation. PFOS (CAS # 1763-23-1) was purchased from Sigma Aldrich (St. Louis, MO). Six pots, each containing 1.5 kg soil (a mixture of 2.2:1 (v:v) natural soil and sand), were prepared for each treatment. The amount of PFOS needed for each treatment was dissolved in water and poured into the pots. The treated soil was left in the dark for three days before transplanting wheat germinants. The plants were allowed to grow in a controlled condition in the greenhouse.

Plant cultivation and management

The wheat seeds, same source as that used in Rico et al. (2014), were sterilized with 5% bleach solution for 15 minutes. Seeds were distributed in autoclaved Petri dishes, and 3 mL of Millipore water was added. The Petri dishes were wrapped with aluminum foil and placed in a growth chamber (Percival, Perry, IA) set at 25°C and 70% humidity for four days. The germinants were allowed to grow for three more days before transplanting in the PFOS-treated soil (two seedlings per pot). These transplants were grown in the greenhouse at 16-h photoperiod, 25/15°C day/night temperature, 70% relative humidity, and 600 μmol m^{-2} s^{-1}. The plants were irrigated with Millipore water as needed, rotated every two days, and fed with 50 mL of Yoshida Nutrient Solution once a week within the first 40 days (Yoshida et al., 1971). Plant height was recorded every 15 days. At harvest, roots, shoots, and grains were collected and processed appropriately for the respective analysis to be carried out. Samples for elemental and PFOS uptake analyses were oven-dried and ground using a ball mill grinder (Retsch MM 200). Samples for metabolomics were flash-frozen in liquid nitrogen and stored at -80°C until analysis.

Short exposure biochemical assays

Chlorophyll content was determined by soaking clippings (100 mg) from the youngest leaves in 5 mL of 70% ethanol for 24 h in the dark. The absorbance of the supernatant was determined using a Cary 60 UV-visible spectrometer, and chlorophyll a and b contents were determined as described by Lichtenthaler and Welburn (1983). For lipid peroxidation, wheat leaves (0.5 g) were ground in liquid nitrogen and homogenized with 2 mL of 0.1% trichloroacetic acid (TCA). The homogenates were centrifuged at 0°C for 20 min at 5000 rpm. A solution of 1 mL supernatant, 1 mL of thiobarbituric acid (TBA), and 100 μL of butylated hydroxy toluene (BHT) was prepared and heated at 95°C for 30 min. The resulting mixture was centrifuged for 15 min at 5000 rpm. The absorbance of the supernatant was read, and the lipid peroxidation was calculated using the formula μM TBARS/g = $[(Abs_{532}\text{-}Abs_{600})/\varepsilon]$/mass of sample, where ε is the molar absorptivity coefficient (155 mol^{-1} cm^{-1} L), and Abs_{532} and Abs_{600} are absorbance readings at 532 nm and 600 nm, respectively.

Elemental uptake analysis

Ground samples and reference standard (peach leaves NIST 1547, Gaithersburg, MD, USA) were digested in 5 mL of plasma pure nitric acid (SCP sciences, Champlain, NY) using a microwave digester (CEM Mars 6^{TM}, Matthews, NC). The digestates were diluted to 2% HNO_3 before analysis using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Agilent Technologies 7900). Percent recoveries of reference standard were 90-110%.

PFOS quantitation and plant uptake determination

The following chemicals were used in the determination of the actual concentration of PFOS used for the study as well as for the determination of PFOS uptake in wheat plants exposed to PFOS for 70 days: potassium perfluorooctanesulfonate (PFOSK, $C_8F_{17}SO_3K$, 98%) was purchased from Matrix Scientific (Columbia, SC), isotopically mass-labeled PFAS mixture (MPFAC-MXA) was obtained from Wellington Laboratories (Overland Park, KS), and HPLC-grade ammonium acetate, acetonitrile (ACN), and methanol (MeOH) were purchased from Fisher Scientific (Pittsburgh, PA). All the solutions were prepared with ultrapure water (18.2 MΩ-cm) by using a Millipore water purification system. PFOS and MPFAC-MXA stock solutions were prepared in methanol and stored at 4°C.

PFOS quantitation by HPLC/MS/MS

PFOS quantification was performed using a Waters Acquity UPLC system coupled with a Waters Quattro Premier XE triple-quadrupole

mass spectrometer (Waters Corp., Milford, MA). The mass spectrometer was operated using an electrospray source in negative ion mode. The UPLC/MS/MS system was operated by MassLynx 4.1 software with QuanLynx Application Manager. The separation and detection of analytes were achieved using a Waters Acquity UPLC BEH reversed phase C18 column (2.1 mm × 50 mm, 1.7 μm particle size, 130 Å pore size). The elution system consisted of 10 mM ammonium acetate in water (A) and 100% MeOH (B). The total run time was 5 min, and the flow rate was maintained constant at 0.3 mL per minute. Chromatography was carried out in gradient mode. The gradient conditions were 40% MeOH, ramped to 98% MeOH in 3 min before returning to 40% MeOH for 2 min to allow the columns to re-equilibrate. The injection volume was 10 μL and the column temperature was maintained at 50°C. Polypropylene vials of 300 μL (LEAP PAL, Raleigh, NC), 12 ng of the mass-labeled internal standard (MPFAC-MXA), and sample dilution of 1:1 MeOH/water were used to avoid matrix effects. For analysis, external calibration standards with concentrations ranging from 10 to 1000 μg/L was used. The limit of detection (LOD) and limit of quantification (LOQ) were estimated as 3 and 10 times the signal-to-noise ratio (S/N), respectively. The determined concentrations of PFOS are shown in the Supporting Information (SI) Table S1.

Extraction of PFOS from plant tissues

For the extraction tests, 25 mg/kg, or 50 mg/kg of PFOS was added to 100 mg of plant tissue (root, shoot, and grain) and equilibrated for 1 d at room temperature. After 1-d equilibration, samples were centrifuged for 20 min at 4500 rpm, and the supernatant was removed. The solids were sequentially extracted thrice with 5 mL of acetonitrile. For each extraction sequence, samples were vortexed for 30 s, sonicated for 1 h, heated at 50° C for another hour, and mixed overnight in a circular rotator at room temperature. The samples were then centrifuged at 4500 rpm, the supernatant decanted into a new 15 mL polypropylene tube, and the remaining solids were extracted again, following the same procedure. Samples were cleaned up in a 2 mL polypropylene vial using 25 mg of Envi-Carb powder and 0.75 mL of each extract. For cleaning, the suspensions were vortexed to mix, centrifuged for 20 minutes at 13,000 rpm, and a 50 μL aliquot of the supernatant was transferred into a 300 μL HPLC vial along with 50 μL of DI water, and 50 μL of the internal standard mixture stock. Based on the results (Table S2), acetonitrile efficiently extracted spiked plant tissues with excellent recoveries of PFOS from all plant compartments.

Metabolomics analysis

Metabolomics analysis of grains was carried out at the Fiehn Lab, West Coast Metabolomics Center, University of California, Davis. Sample preparation and analysis method were based on using gas chromatography-quadrupole time of flight-mass spectroscopy (GC-QTOF-MS). Data with less than 10% confidence level were excluded before subjecting data to chemometric analysis (Partial least squares-discriminant analysis, PLS-DA) in MetaboAnalyst 5.0 (https://metaboanalyst.ca), following a similar procedure as in a previous study (Huang et al., 2021). The PLS-DA assigned variable importance in projection values (VIP) to each metabolites which signifies their importance to group clustering or separation. A VIP value ≥ 1 ascribed to a metabolite is considered important in causing differences/separations between treatments (Chong et al., 2018). These metabolites were subjected to pathway analysis to determine biological pathways impacted by PFOS (Zeng et al., 2021).

Data analysis

Statistical analysis was performed using SAS statistical package (SAS Institute, Cary, NC). The data was tested for normality using univariate procedure in SAS followed by one-way analysis of variance using Least Significant Difference (LSD). Nonparametric test was performed on data that did not show normal distribution using Wilcoxon analysis and Kruskal-Wallis test followed by Dwass, Steel, Critchlow-Fligner (DSCF) multiple comparison analysis. Only grain Fe concentration exhibited non-normal distribution but showed differences in nonparametric DSCF analysis. All values were reported as mean ± standard error (SE) of six replicates.

Results and discussion

PFOS uptake in wheat

PFOS was absorbed in the roots and translocated to the aerial and edible compartments of wheat (Fig. 1, Table S3). The PFOS concentrations in the roots, shoots, and grains exposed to 50 mg/kg PFOS treatment were 2588-, 347-, and 12-folds more than those in the respective tissues of the control plants. Likewise, the concentrations of PFOS in the roots, shoots, and grains of the 25 mg/kg-treated plants were 1643-, 172-, and 18-folds more than those in the control plants. The amount of PFOS accumulated in roots and shoots was significantly different across treatments, whereas PFOS concentrations in grains of 25 and 50 mg/kg PFOS exposed plants (277 ± 59 and 192 ± 16 μg/kg, respectively) did not differ significantly from each other, but they were higher compared to the control (15 ± 0.5 μg/kg) (Table S3). Stahl et al. (2009) reported a much lower PFOS concentration in shoots and grains of wheat even at the same treatment levels used in this study, which could be due to uptake competition between the PFOS and PFOA (perfluorooctanoic acid) in the mixed treatment of Stahl et al. (2009) or differences in soil organic carbon, which strongly adsorbs PFOS. PFOS was also detected in control plants, probably due to the presence of PFOS in natural soils as already reported and discussed in several literature reviews and research publications (Ghisi et al., 2019, Kim et al., 2019, Choi et al., 2017). Background concentration of PFOS in soils with no known contamination was as high as 0.162 mg/kg (Brusseau et al., 2020).

Data obtained in the current data is consistent with literature reports showing PFOS accumulation in the storage organs (i.e., grain, ear) to a limited extent (wheat being more ready to accumulate PFOS than oats (*Avena sativa*) and maize (*Zea mays*)) (Stahl et al., 2009, Ghisi et al., 2019, Krippner et al., 2015, Wen et al., 2014), and mostly retained in non-edible or vegetative compartments of plants (roots and shoots) (Felizeter et al., 2012, Wen et al., 2014, Lechner and Knapp, 2011). PFOS is mostly adsorbed, rather than absorbed, in roots and the increasing biological complexity and multiple barriers prevent shoot-to-fruit relocation and partitioning of PFOS (Felizeter et al., 2012, Blaine et al., 2014a). PFOS was detected in the shoots of radish (*Raphanus sativus*), celery (*Apium graveolens*), tomato (*Lycopersicon lycoperiscum*), pea (*Pisum sativum*), and lettuce (*Lactuca sativa*), but not in the fruits of tomato and pea, when these plants were grown in soil amended with PFOS-contaminated biosolid (Blaine et al., 2014a, Blaine et al., 2014b). Liu et al. (2019) recently reported a similar finding in wheat grains and corn ears. The absence of PFOS in the fruits could be due to very low PFOS concentration in the soil (319.5 ng/g).

The mechanism for uptake and translocation of PFOS in plants is not fully known, but some insights have been discussed in recent reviews (Ghisi et al., 2019, Mei et al., 2021). Wen et al. (2013) and Blaine et al. (2014a) were perhaps the first to provide a systematic analysis on elucidating the uptake mechanisms of PFOS in plants. Wen et al. (2013) reported that water channels (aquaporins) or anion channels were the main routes of root entry in maize based on studies conducted in maize treated with aquaporin or anion channel blockers. Blaine et al. (2014a) proposed a general pathway for PFAS root uptake and relocation in plant organs, and discussed how plant physiology and root anatomy parameters such as Casparian strip, xylem, phloem, and cambium affect PFAS translocation. Climatic conditions (i.e., irradiance, temperature, humidity), which influence stomatal opening and transpiration rate, have been proposed to affect root-to-shoot relocation

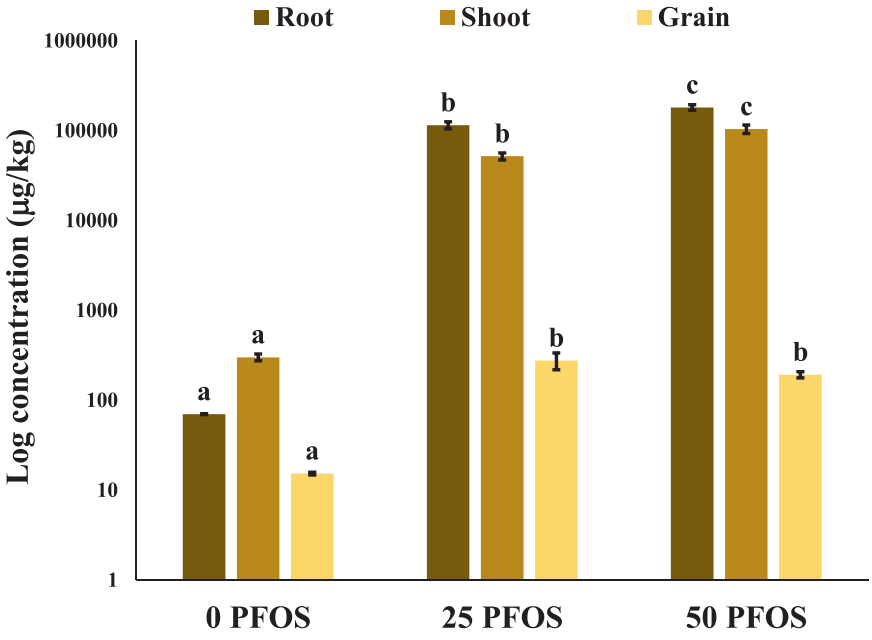

Fig. 1. PFOS concentration in wheat plants grown in soil supplemented with 0, 25, and 50 mg/kg PFOS. Values are mean ± SE ($n = 6$). Different letters indicate statistical difference between treatments ($p < 0.05$).

of PFOS (Ghisi et al., 2019, Blaine et al., 2014a, Zhao et al., 2013). Adsorption on root components, such as membrane proteins and lipids, or sequestration in soil organic matter are other factors that influence (block) root entry of PFOS (Felizeter et al., 2012, Zhao et al., 2011). Inside the root, the Casparian strip accounts for blocking PFOS movement to the shoots (Felizeter et al., 2012). Once it reaches the xylem, PFOS could move upward with root and shoot proteins, rather than lipids, primarily contributing to the translocation in plants (Wen et al., 2016). Finally, xylem-to-phloem movement presents another barrier (e.g., sorption into tissues, vascular cambium) for relocation to the final destination (i.e., storage organs such as grains, ears, and fruits) (Felizeter et al., 2012, Blaine et al., 2014a). Although there is no clear mechanism on PFOS uptake and translocation in plants, substantial evidence supports PFOS storage in the edible portions of plants and this presents a major route for trophic exposure, especially in humans (Ghisi et al., 2019).

Physiological and productivity changes at short-term and full life-cycle exposures

The growth and yield performance of wheat was assessed at short-term and full life-cycle exposures to PFOS. Surprisingly, the data revealed that wheat could withstand stress from longer-term PFOS exposure at concentrations much higher than what is expected in the real environment. For example, plant height (data not shown), and root and grain biomass yields of wheat in the full life-cycle exposure scenario did not change significantly between treatments, except for shoot biomass which was significantly higher at 50 mg/kg PFOS compared to 25 mg/kg but neither treatment's shoot biomass was significantly different to control (Fig. 2A and B). However, there seems to be a visual physiological difference (e.g., emaciation) in the grains between PFOS treatments (Fig. 2A). In contrast to the result obtained in this study, Stahl et al. (2009) found significant reductions in plant height and grain yield of spring wheat exposed to 50 mg/kg mixture of PFOS and PFOA.

Physiological and biochemical indices (i.e., biomass, chlorophyll content, lipid peroxidation) were measured at the short exposure period (21 days) to complement the findings in the longer-term exposure experiments (Fig. 3). The data revealed that shoot biomass did not change between treatments, but root biomass in the 50 mg/kg PFOS treatment decreased by 37% compared to the control (Fig. 3A). Since the root biomass was not affected in the full life-cycle study, this finding in the short-term exposure suggests that plants probably directed energy resources to the roots during active plant growth, which limited the available resources for grain formation. When the plants reached the grain formation stage, photosynthates must have been depleted (since they have been diverted to promote root growth) and less resources were available for grain formation resulting in decreasing trend in grain biomass. Reports have shown that energy resources (e.g., photosynthetically fixed carbon) can be transferred to the roots for organic acids, amino acids, sugar, and protein production (Li et al., 2020c). The reduced root biomass observed in this short exposure study agrees with a previous work that showed inhibition of root elongation or reduced root biomass of wheat at ≥10 mg/L PFOS (Qu et al., 2010).

The chlorophyll a concentration also decreased by 49% in wheat plants exposed to 50 mg/kg PFOS relative to the control (Fig. 3B). The decreased chlorophyll concentration is also in good agreement with the work of Qu et al. (2010) that reported reduced chlorophyll content in wheat at >10 mg/L PFOS. Wheat grains are composed of approximately 70% of carbohydrates (Lasek et al., 2020), and reduced chlorophyll level could affect grain formation in wheat. In fact, the chlorophyll concentration and grain yield followed similar trends, although differences in grain biomass values were not statistically significant. Results also revealed a nonsignificant increasing trend in lipid peroxidation with increasing concentration of PFOS exposure (Fig. 3C). This data is contrary to the report that PFOS promoted electrolyte leakage in wheat seedlings (Qu et al., 2010).

There are conflicting reports on the impacts of PFOS in plant growth. Lechner and Knapp (2011) performed long-term (63-96 days) studies on PFOS (10-556 μg/kg) exposure to cucumber (*Cucumis sativus*), carrots (*Daucus carota* ssp. *Sativus*), and potato (*Solanum tuberosum*) in soil, and found no significant effects on whole-plant or fruit biomass. In a hydroponic study, Li et al. (2020a) exposed lettuce in mixed solutions (500-5000 ng/L) of PFOS and PFOA for 28 days, and also found no effects on the growth and biomass. In contrast, Yu et al. (2018) found reduced shoot biomass in nine cultivars of lettuce (*Lactuca sativa*) exposed to PFOS-amended soil (1.0 mg/kg) for 45 days. Another study showed that 200 mg/L PFOS decreased root and shoot lengths of wheat by 15.7% and 12.0%, respectively, compared to the control (Qu et al., 2010). Guo et al. (2020) also summarized the positive or negative effects PFOS induced in plants. The current study employed considerably high PFOS concentration in the exposure treatments (25 and 50 mg/kg PFOS), but adverse effects were only observed in root biomass and chlorophyll a

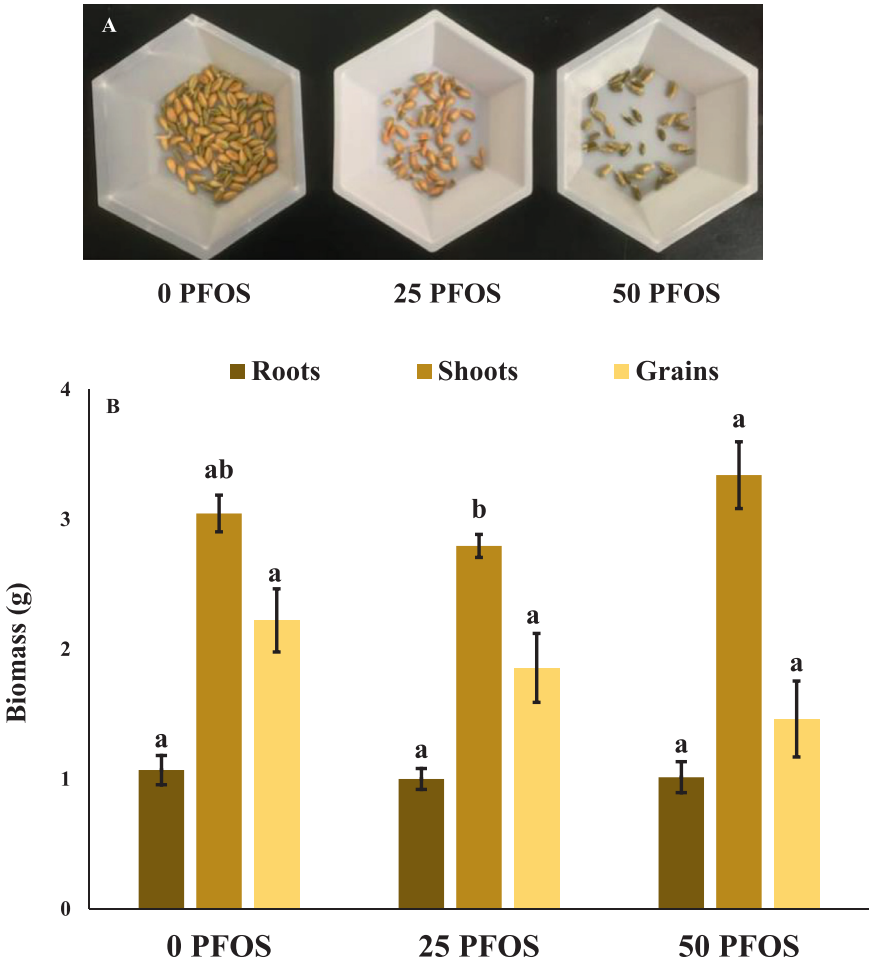

Fig. 2. (A) Grains and (B) biomass of wheat plants grown in soil supplemented with 0, 25, and 50 mg/kg PFOS for 70 days exposure. Values are mean ± SE (n = = 6). Different letters indicate statistical difference between treatments ($p < 0.05$).

during the short-term exposure studies. When the plants reached full maturity, we observed a nonsignificant dose-dependent decreasing trend in grain yield (with increasing PFOS exposure concentration), and visibly poor and emaciated grains at 50 mg/kg PFOS only (Fig. 2A and B). Together these sets of physiological and biochemical data demonstrate that wheat can tolerate high levels of PFOS in soil, which highly suggests that PFOS is not a threat to the agronomic performance and grain production of wheat at current realistic PFOS concentration in agricultural soils (e.g., ΣPerfluoalkyl acids = 0.29-4.28 μg/kg, of which 6% is PFOS) (Zhang et al., 2020).

Modification of elemental distribution

Elemental analysis revealed differences ($p < 0.05$) in grain Mg, P, K, and Fe concentrations (Fig. 4). Fig. 4A shows that 25 and 50 mg/kg PFOS increased Fe concentration by 208% and 250% in grains, respectively, compared to control. In contrast, the concentrations of Mg, P, and K in grains of 25 and 50 mg/kg PFOS decreased by 3-15%, 5-14%, and 5-14%, respectively, compared to control grains (Fig. 4B). There were no differences between the treated and control plants with respect to other elements (Ca, Mn, Cu) analyzed in the grains (Table S4). All the elements measured in the grains were also analyzed in the shoots and roots (Table S4). Relative to control, only 50 mg/kg PFOS decreased the concentrations of Mg and K in shoots, and decreased the concentration of P in roots (Table S4). In addition, Cu concentration decreased while Mn concentration increased in the roots of PFOS-treated plants compared to the control (Table S4).

The reduced Mg and K concentrations in the shoots treated with 50 mg/kg PFOS corroborates the significantly lower chlorophyll a in the short-term exposure. Mg is the central ion in chlorophyll and acts as a phosphorus carrier in plants, while K is involved in enzymatic processes related to photosynthesis, food formation, and transport of sugars to grains (Prajapati and Modi, 2012). The chlorophyll biosynthesis might have been impacted early on in the growth stage of wheat, which affected the grain production. The reduced concentration of Mg and K in the 50 mg/kg PFOS shoots also corroborates their lower concentrations in the grains. A related study on mixed PFOA and PFOS exposure in hydroponic media of lettuce showed a 14-24% decrease in Mg concentration in the leaves of treated plants (500-2000 ng/L) and a 8-10% decrease in K concentration in 1000-5000 ng/L treatment, compared to control (Li et al., 2020a). The same research group also reported decreases in Mg and K concentrations in roots of lettuce exposed to PFOS (500-2000 ng/L) compared to control (Li et al., 2020c). In contrast, Fe accumulation in grains markedly increased in PFOS treatments relative to control. The high level of Fe in grains is quite unusual as iron in plants is mostly localized in the chloroplast and its excessively high concentration in shoots is associated with iron toxicity (EPA, 2003). Mixed exposure to PFOS and PFOA (500-2000 ng/L) also enhanced Fe concentration in the shoots of lettuce compared to control (Li et al., 2020a). Current result indicates that PFOS modified Fe mobilization from shoot to grains, but it is difficult to identify the mechanism that could explain this data. Published reports have not explored changes in elemental concentrations in grains and a better understanding of current results in light of general trends is challenging to undertake. In additions, direct comparison to findings in available studies on elemental concentration in roots and shoots is not ideal since shoot-to-grain relocation of element is governed by biological processes different from those in root-to-shoot transport of elements.

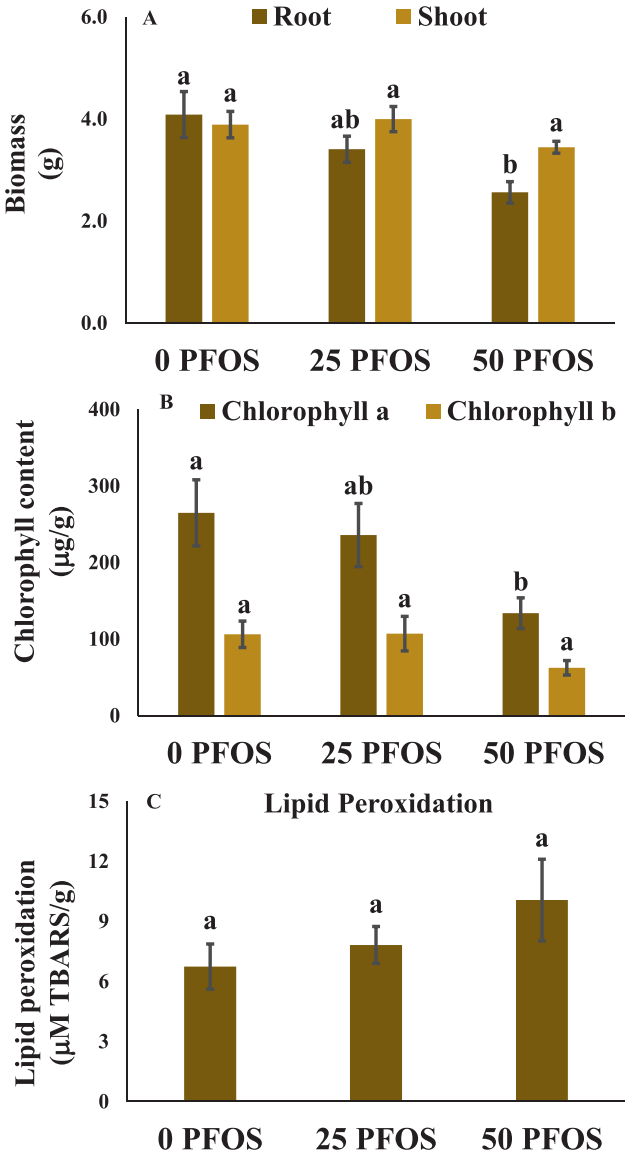

Fig. 3. (A) Root and shoot fresh biomass, (B) chlorophyll a and b, and (C) lipid peroxidation in wheat grown in soil supplemented with 0, 25, and 50 mg/kg PFOS for 21 days. Values are means ± SE (n = 6). Different letters indicate statistical difference between treatments (p < 0.05). TBARS = thiobarbituric acid reactive species.

The movement of elements from roots to shoots and ultimately in the grains is a complex physiological process in plants. The available literature reports attribute macro and micronutrient imbalances in plants exposed to PFOS on ROS-induced nutrient regulation or metabolic changes (Li et al., 2020a,c). However, other potential processes that contribute on how PFOS modulate elemental uptake in plants have not been explored. For example, microbial and enzyme activity, nutrient competition/antagonism, transporter proteins, and altered gene expression could contribute to changes in nutrients uptake in plants.

Metabolome changes in grains

Metabolomics was performed on grains since PFOS did not induce serious impairments on growth and productivity of wheat. For example, there were no changes in biomass or oxidative stress, but the most visible physiological impacts observed (i.e., decreasing trend in biomass and emaciated appearance of grains) as well as altered Mg, P, K, and Fe contents of grains could be a reflection of PFOS impacts on biochemical and metabolic processes in grains. Moreover, assessing grain metabolite composition will provide a better understanding on the impacts of PFOS on the nutritional quality of an important cereal.

Fig. 5 exhibits the effects of PFOS on the metabolite profile of wheat grains. The metabolomics analysis identified 185 metabolites, and partial least square discriminant analysis (PLS-DA) of these metabolites revealed that PFOS significantly modified the metabolite profile of wheat grains. Fig. 5A shows the PLS-DA plot obtained from MetaboAnalyst 5.0. Principal component 1 (PC1) and principal component 2 (PC2) explained 21.8% and 8.9% of the total variability, respectively. The data revealed that the 25 mg/kg PFOS overlaps with both the 0 mg/kg and 50 mg/kg PFOS treatments, while the 50 mg/kg PFOS treatment is well separated from the 0 mg/kg treatment which indicates that the 50 mg/kg PFOS treatment significantly impacted the metabolite profile of wheat grains much more than 25 mg/kg PFOS. There were 44 metabolites with variable importance in projection (VIP) scores ≥ 1 (Fig. 5B, Table 1); these differential metabolites were subjected to metabolic pathway analysis, which revealed that PFOS perturbed five metabolic pathways: arginine biosynthesis, starch and sucrose metabolism, alanine, aspartate and glutamate metabolism, arginine and proline metabolism, and linoleic acid metabolism (Table 2). The 44 differential metabolites were used for understanding the impacts of PFOS on wheat grains (Rico et al., 2020, Guo et al., 2020, Chong et al., 2018). Fig. 6 maps the metabolic pathway of the affected metabolites.

The literature does not report metabolomics of seed or grains from plants exposed to PFOS, based on the most recent reports and reviews on the topic (Li et al., 2022, Li et al., 2020a,b, Guo et al., 2020). As a result, comparative discussion of obtained results from the perspective of perfluoroakyl substances (PFAS) impacts on grain metabolome is limited. There are glaring differences on metabolite profiles of leaves exposed to PFOS (from other existing studies) compared to findings in grains in this study. In general, the abundances of sugar metabolites were upregulated (Li et al., 2020b, Guo et al., 2020), lipids and amino acids were downregulated (Guo et al., 2020, Li et al., 2020a,b), and the metabolites in the TCA pathway were altered (Li et al., 2020b) in plant leaves exposed to hydroponic solution containing PFOS. However, results from this study on the metabolome of wheat grains demonstrated that levels of sugar metabolites were downregulated, lipids were upregulated, amino acids regulation was varied, and the metabolites in the TCA pathway were not affected (Table 2, Fig. 6).

Changes in sugar metabolites

Starch and sucrose metabolism (i.e., sucrose, trehalose, glucose-6-phosphate, and fructose-6-phosphate) in grains was markedly disturbed by PFOS (Table 2, Figs. 5B, 6). Comparison between mean abundances revealed that sucrose, maltose, trehalose, glucose-6-phosphate, and fructose-6-phosphate decreased in abundance by up to 41%, 39%, 39%, 38%, and 50%, respectively, at PFOS-treated grains compared to control. Isomaltose decreased the most at 25 mg/kg PFOS compared to control. Other sugar metabolites that also decreased in abundance at PFOS treatments were 2-ketoglucose dimethylacetal, sophorose, and levoglucosan (up to 68%, 59%, 66%, respectively).

On the other hand, glucoheptulose and 6-deoxyglucitol levels increased (up to 99% and 100%, respectively) in PFOS-treated wheat grains compared to control, similar to its reported increased level in leaves or grains of wheat under drought or cerium oxide nanoparticles stress (Kang et al., 2019, Rico et al., 2020). The general decrease in sugar levels corroborates the PFOS dose-dependent decreasing trend in grain biomass (Fig. 2B), which could be significant because wheat grain contains 70% carbohydrates (Lasek et al., 2020). The current results indicate that PFOS decreased photosynthate production and/or storage in grains. In contrast, recent reports showed that PFOS increased sugar metabolites such as sucrose, maltose, fructose-6-phosphate, trehalose, and melibiose in leaves of *A. thaliana* and lettuce exposed to hydro-

Table 1
List of metabolites in wheat grains that showed VIP value ≥ 1.0. PFOS modified the abundance of these metabolites in the grains which caused differences or separations between PFOS treatments. Grains were harvested from wheat grown in soil amended with PFOS at 0, 25, and 50 mg/kg.

Sugars	Amino acids	Fatty acids	Organic acids	Nucleic acid	Others
Glucoheptulose	Asparagine	Oleic acid	Allantoic acid	Uracil	Ethanolamine
Isomaltose	Glutamine	Linoleic acid	Adipic acid	Guanine	Indole-3-lactate
Sucrose	Cysteine	Linolenic acid	3-aminoisobutyric acid	Uridine	Hydroquinone
Fructose-6-phosphate	Tryptophan	Lauric acid	Indole-3-propionic acid		Stigmasterol
Trehalose	Histidine	2-monoolein	2-isopropylmalic acid		1,3-diaminopropane
Glucose-6-phosphate	Ornithine		cis-gondoic acid		
Maltose	Urea		Galactonic acid		
6-deoxyglucitol			3,4-hydroxynamic acid		
2-ketoglucose			Glycolic acid		
dimethylacetal			Malonic acid		
Sophorose			Itaconic acid		
Levoglucosan			cis-sinapinic acid		
			Nicotinic acid		

Table 2
Perturbed biological pathways in wheat grains exposed to PFOS at 0, 25, and 50 mg/kg.

Sugars	p	Impact	Match status	Involved metabolites
Arginine biosynthesis	1.1×10^{-3}	0.28	4/18	Urea, Citrulline, Ornithine, Glutamine
Starch and sucrose metabolism	2.4×10^{-3}	0.24	4/22	Sucrose, Glucose 6-phosphate, Trehalose, Fructose 6-phosphate
Alanine, aspartate and glutamate metabolism	2.0×10^{-2}	0.19	3/22	Asparagine, Alanine, Glutamine
Arginine and proline metabolism	3.8×10^{-2}	0.29	3/28	Spermidine, Ornithine, Hydroxyproline
Linoleic acid metabolism	1.0×10^{-1}	1.00	1/4	Linoleate

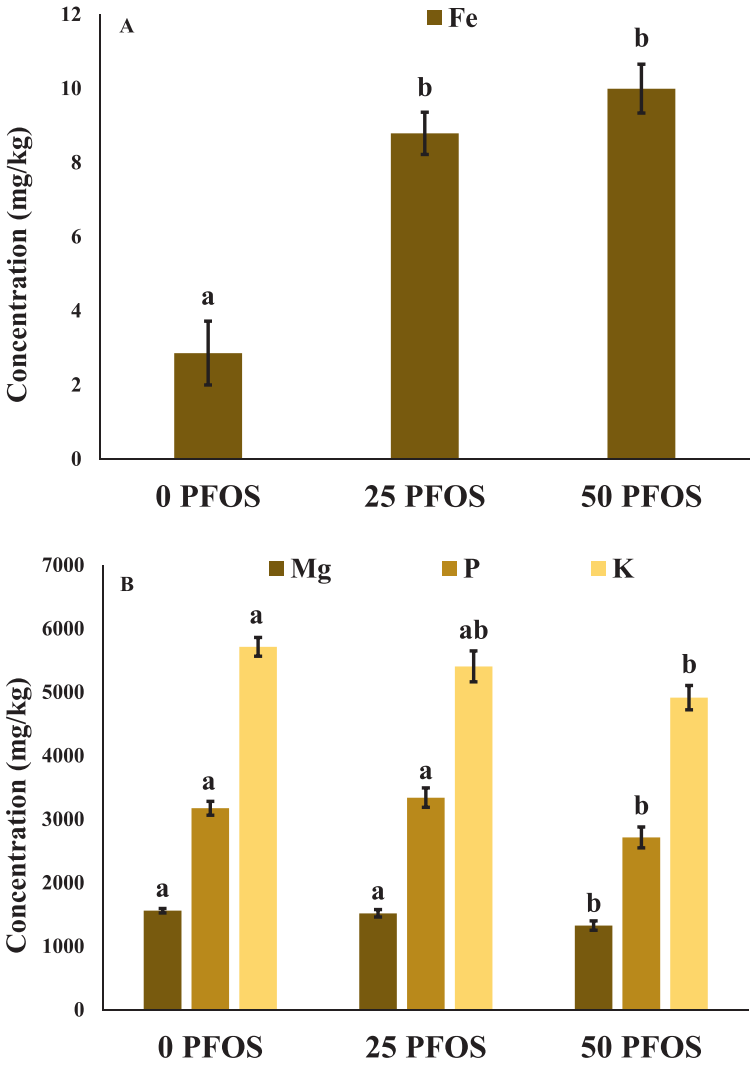

Fig. 4. Concentration of (A) Fe and (B) Mg, P, and K in wheat grains grown in soil supplemented with 0, 25, and 50 mg/kg PFOS. Values are expressed as mean ± SE ($n = 6$). Different letters indicate statistical difference between treatments ($p < 0.05$).

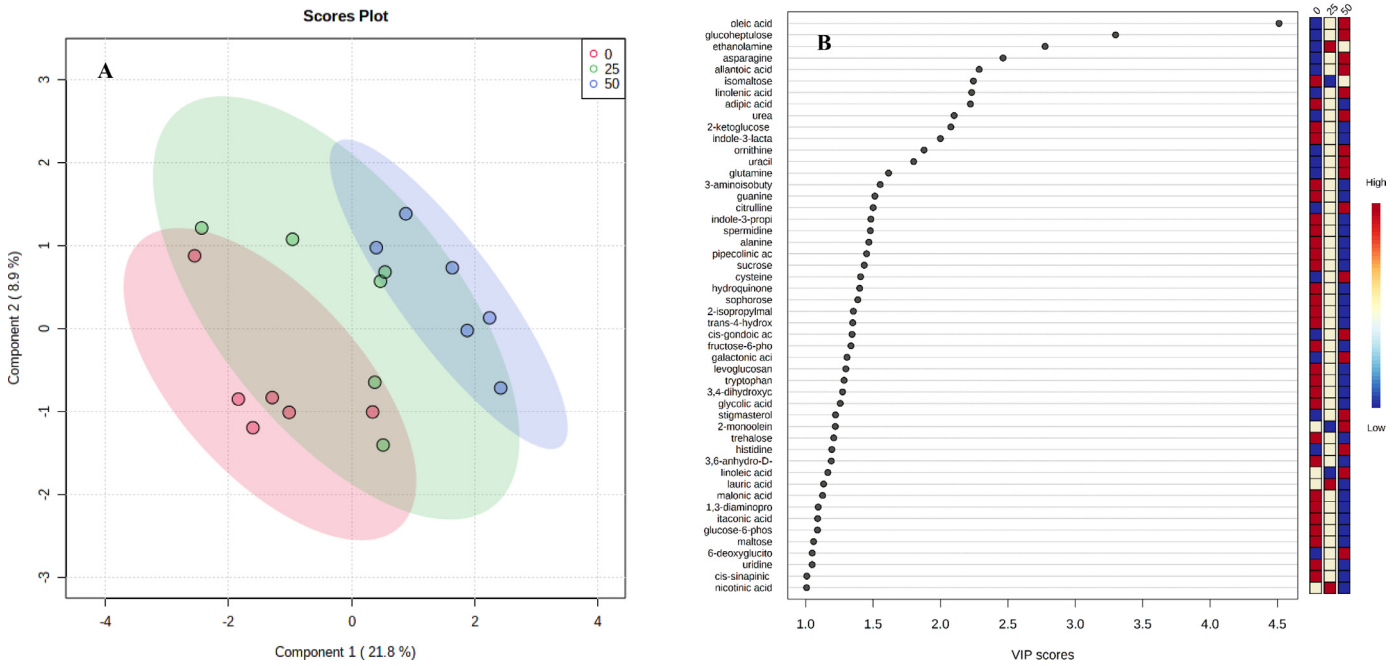

Fig. 5. (A) Partial least squares-discriminant analysis (PLS-DA) plot and (B) VIP score for metabolomics of wheat grains harvested from plants grown in soil supplemented with 0, 25, and 50 mg/kg PFOS for 70 days.

ponics solution of PFOS for a short period of time (Guo et al., 2020, Li et al., 2020a, Li et al., 2020b). There are studies showing varying effects of contaminants on grains: cerium oxide nanoparticles increased the levels of glucose-6-phosphate and fructose-6-phosphate in wheat grains whereas cadmium reduced the levels of carbohydrate metabolites in rice grains (Rico et al., 2020, Zeng et al., 2021). The current findings highlight the importance of analyzing grain metabolite of food crops exposed to PFAS and other anthropogenic contaminants.

Changes in amino acid metabolites

The amino acid synthesis in the grains was greatly affected as shown by the disturbed metabolic pathways: arginine biosynthesis; alanine, aspartate and glutamate metabolism; and arginine and proline metabolism (Table 2). Amino acids are important in plant physiological processes, and their accumulation can be considered a signal in modulating the metabolism of antioxidants for quenching the ROS induced by PFOS (Li et al., 2020c). Amino acids are major metabolites of plant primary metabolism and forerunners of synthetic signaling and defense-related components (Li et al., 2020c). The data showed both upregulation and downregulation of amino acids (Figs. 5B, 6). The abundances of proteinogenic amino acids alanine and tryptophan decreased (up to 46% and 50%, respectively), while those of asparagine, histidine, glutamine, and cysteine increased (up to 240%, 147%, 151%, and 122%, respectively) in PFOS treatments. Non-proteinogenic amino acids citrulline and ornithine, prominent metabolites from the arginine biosynthesis pathway that was significantly perturbed, were also upregulated up to 89% and 137%, respectively, in PFOS treatments compared to control. A closer look at the structures of these amino acids revealed that the downregulated proteinogenic amino acids are all non-polar, while the upregulated proteinogenic amino acids are polar. This could suggest that PFOS facilitates selectivity in altering essential amino acid metabolism in wheat. In addition, related studies have shown inverse relationships between sugar and amino acid contents (e.g., decreased levels of starch was accompanied by increased amounts of amino acids) in grains of wheat under stress (Halford et al., 2014, Wardlaw et al., 2002, Gooding et al., 2003), a trend similar to what was observed in this study.

The alteration in amino acid levels is consistent with the trend reported in the literature (Li et al., 2020c), and dysregulation of amino acids can have significant implications for stress in plants. For example, reduced concentration of valine, lysine, and methionine due to catabolism resulting from PFOS and PFOA exposure has been considered an adaptive strategy for coping with PFAS stress (Li et al., 2020c). The upregulation of cysteine is associated with the increased accumulation of iron in the grains because non-heme (Fe-S) iron is typically bound to the thiol group of cysteine. Glutamine and asparagine are involved in inorganic nitrogen fixation, and its upregulation implies that nitrogen fixation increased to adjust to PFOS exposure (Li et al., 2020c). Upregulation of glutamine was also observed in the metabolic response of lettuce and *A. thaliana* under PFOS exposure (Li et al., 2020c, Guo et al., 2020). Ornithine and citrulline are important amino acids for the urea cycle (Zhao et al., 2017), and their concerted increase with glutamine also supports the inference that nitrogen fixation increased to cope with PFOS-induced stress.

Allantoic acid and urea also showed PFOS concentration-dependent increase in abundances of up to 210% and 163%, respectively, relative to control (Figs. 5B, 6). The simultaneous increase in the metabolites in the arginine biosynthesis pathway (ornithine, citrulline, glutamine, allantoic acid, and urea) supports the assumption that there was increased nitrogen absorption to adapt to PFOS stress (Figs. 5B, 6). Allantoic acid is one of the ureides (acyl derivatives of urea, the other being allantoin) which are major storage forms of nitrogen in some plants (Streeter, 1979). Also the accumulation of ureides occurred concertedly with the accumulation of an intermediate e.g., asparagine (Serraj et al., 1999). Therefore, the concomitant increases in the metabolites of arginine biosynthesis; alanine, aspartate and glutamate metabolism; and arginine and proline metabolism corroborate the concerted metabolic activities to promote stress tolerance via N absorption, and amino acid and protein production (Wang et al., 2008).

Changes in fatty acid metabolites

Metabolites commonly associated with lipid/fatty acid metabolism such as oleic, linoleic, linolenic, and lauric acids were altered (Figs. 5B, 6), but linoleic acid metabolism was the only significantly perturbed

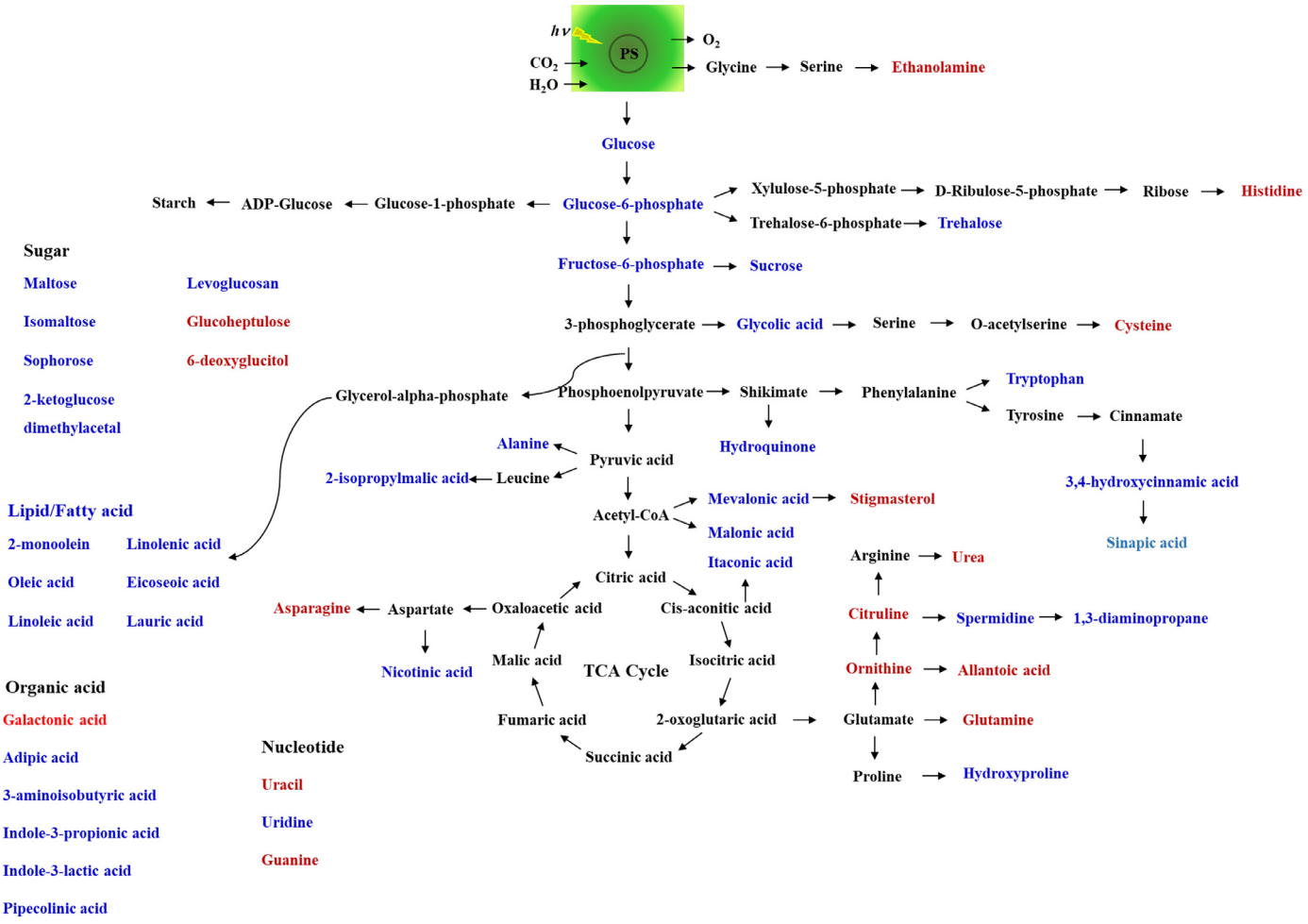

Fig. 6. Metabolic pathway depicting the dysregulation of metabolites (VIP score ≥ 1) in wheat grains harvested from plants exposed to PFOS for full life cycle. Metabolites in red or blue font signifies upregulation or downregulation in PFOS treatments, respectively, compared to control.

metabolic pathway (Table 2). The abundance of oleic and linolenic acids increased up to 216% and 310%, respectively, in a PFOS concentration-dependent manner. The unsaturated fatty acid cis-11-eicosenoic acid (or cis-gondoic acid) also increased in level (up to 93%) at PFOS treatments, consistent with increases in levels of major unsaturated fatty acids (oleic and linolenic acids). In contrast, the levels of linoleic and lauric acids were altered differently. Compared to that of the control, the linoleic acid level increased by 81% when wheat was exposed to 50 mg/kg PFOS, and decreased by 9% when exposed to 25 mg/kg PFOS. On the other hand, the lauric acid level in wheat plant exposed to 50 mg/kg PFOS decreased by 44% relative to that of the control. Increased levels of oleic, linoleic, and linolenic acids are indicators of biotic and abiotic stress in plants as they function as antioxidants, membrane ingredient modulators in glycerol lipids, carbon and energy reserve in triacylglycerols, and precursors of bioactive molecules (e.g., the stress hormone jasmonic acid) (He et al., 2020). Oleic acid also facilitates the interaction between salicylic acid and jasmonic acid signaling pathways against pathogen invasion (He et al., 2020). Increased levels of oleic, linoleic, and linolenic acids in wheat grains exposed to 50 mg/kg PFOS suggest a greater perturbation in fatty acid synthesis in this treatment compared to the 25 mg/kg PFOS treatment (Zeng et al., 2021). Alternatively, increased accumulation of fatty acids could be due to PFOS inhibiting fatty acid breakdown due to its structural similarity with fatty acids (Zeng et al., 2019). Given that PFOS could have profound effects on fatty acid profile of grains, particularly the polyunsaturated fatty acids which play an important role in energy metabolism and stress regulation, we suggest that researchers pay more attention to this issue (Halford et al., 2014, Zeng et al., 2021).

Changes in organic acid metabolites

The organic acids that had VIP scores ≥ 1.0 were all downregulated in PFOS treatment compared to control, except for galactonic and allantoic acids (Figs. 5B, 6), which indicates that PFOS strongly impacted energy metabolism and other cell metabolic pathways during grain formation. The abundances of adipic acid, 3-aminoisobutyric acid, indole-3-propionic acid, indole-3-lactic acid, pipecolinic acid, 2-isopropylmalic acid, glycolic acid, 3,4-hydroxycinnamic acid, sinapic acid, itaconic acid, and malonic acid decreased as the concentration of PFOS increased. Compared to that of the control plants, the nicotinic acid level was higher by 32% in 25 mg/kg PFOS-treated grains, but lower by 37% in 50 mg/kg PFOS-treated grains. Interestingly, all of these altered organic acids were not included in the list of common organic acids identified in grains of 19 wheat cultivars reported in Rodríguez et al. (2012). Both levels of galactonic acid and allantoic acid increased in PFOS-treated grains. The increased level of galactonic acid was not consistent with the decrease in levels of sugar which serves as precursor for galactonic acid. The increased abundance of allantoic acid was consistent with increased level of ornithine. Likewise, the decrease in level of malonic acid in PFOS treatments agreed with the reductions

in levels of its precursor alanine. The metabolite 2-isopropylmalic acid is an intermediate in pathway involving leucine biosynthesis and in the conversion of oxaloacetate to α-ketoglutarate via cis-aconitate and isocitrate intermediates in the TCA cycle (Katz et al., 2004). Malonic acid is also indirectly involved in the TCA cycle wherein it is synthesized via the malate branch or citrate/acetyl-CoA pathway of the TCA cycle (Igamberdiev and Eprintsev, 2016, Chen et al., 2011). Similarly, itaconic acid is produced from aconitic acid of the TCA cycle (Klement and Büchs, 2013, Goldberg and Rokem, 2009). Together, changes in levels of 2-isopropylmalic, malonic, and itaconic acids suggest that the TCA cycle in grains was remotely affected by PFOS. Nicotinic acid, a metabolite derived from pyridine nucleotide synthesis from aspartic acid (Cleaves and Miller, 2001, Ashihara et al., 2015), is an important coenzyme in hundreds of cellular redox reactions, although high concentration could also be toxic for cell division, in plants (Gerdes et al., 2012, Sasamoto and Ashihara, 2014).

The literature indicates that most of these downregulated organic acids serve in plant resistance to biotic and abiotic stresses or constitute grains' nutritional metabolites. For example, high concentrations of indole-3-propionic acid, indole-3-lactic acid, or pipecolinic acid betaines in humans were found to be directly correlated with high intake of whole grains (e.g., wheat, rye) (de Mello et al., 2017, Kärkkäinen et al., 2018, Zhu et al., 2016). The decrease in abundances of both indole-3-propionic acid and indole-3-lactic acid matched the decrease in tryptophan level; perhaps, because they are tryptophan-derived metabolites (Ehrlich et al., 2020, Konopelski and Mogilnicka, 2022), and their decreased levels could have played a role in grain biomass since they are involved in promoting grain yield in wheat (Raheem et al., 2018). The metabolites, 3,4-hydroxycinnamic acid and sinapic acid, play important roles in defense against biotic and abiotic stresses, pest deterrence, disease protection, etc. (Nair et al., 2004). Similarly, pipecolinic acid and 3-aminoisobutyric acid help in plant resistance to stress (Das et al., 2017, Gouffi et al., 2000, Lenk et al., 2019). Together, the decreases in abundances of organic acids decreased resistance to stress.

Changes in DNA/RNA metabolites

The abundance of nucleobase, uracil, was upregulated by up to 130%; and the levels of nucleobase, guanine, and nucleoside, uridine, were downregulated by up to 19% and 15%, respectively, in PFOS-treated grains compared to those of the control (Figs. 5B, 6). The increase in uracil level could be connected to the increased iron content in the grains because iron is important for RNA synthesis of which uracil is a major component (Khodour et al., 2019). On the other hand, the decrease in uridine could be due to decrease in sugar levels in grains (Fig. 6). Other studies also reported impacts of PFOS on DNA/RNA metabolites: increased levels of these metabolites or their significant regulations indicate plants response to repair DNA damage (Li et al., 2020a, Guo et al., 2020, Li et al., 2020b). Significant dysregulations of DNA/RNA metabolites have also been observed in wheat grains exposed to cerium oxide nanoparticles (Rico et al., 2020). A related study proposed that modifications in levels of nucleotide metabolites are associated with reduced metabolic activities of seeds including nucleic acid degradation and de novo biosynthesis of purines and pyrimidines (Liu et al., 2016).

Changes in other metabolites

The other dysregulated metabolites in PFOS treated grains include hydroquinone, spermidine, 1,3-diaminopropane, stigmasterol, and ethanolamine (Figs. 5B, 6). These molecules also aid plants to adjust to various stresses (Liu et al., 2015, Aboobucker and Suza, 2019, Chen et al., 2018) or hold potential health benefits for human diet (Giner et al., 2022, Jiménez-Escrig et al., 2006, Madeo et al., 2018). Hydroquinone, a very potent antioxidant, is present in wheat grains (in decreasing abundance: germ > bran > whole grains) as β-glucosides of hydroquinone (arbutin) (Schendel, 2019, Zhokhov et al., 2010). Ethanolamine is produced by direct decarboxylation of serine or as a by-product of choline synthesis in plants (Rontein et al., 2001). The metabolite 1,3-diaminopropane is the product of cleaving aminopropyl side-chain in spermidine (Asthir et al., 2002), and its decrease correlates with the decrease in spermidine abundance. Stigmasterol is a plant sterol that plays a role in signaling activities to promote tolerance to biotic and abiotic stresses during plant development (Aboobucker and Suza, 2019).

Conclusion

Agronomic measurements, biochemical assays, bioaccumulation assessment, and ionomics and metabolomics analyses were employed to evaluate the effects of exposure to PFOS (0-50 mg/kg) on wheat plant grown in contaminated soil. PFOS promoted stress that affected grain quality more than plant biometric parameters (e.g., height, biomass). Grain yield exhibited a nonsignificant PFOS dose-dependent decreasing trend, which resulted in grains having low levels of macroelements and major sugars (sucrose, maltose, trehalose, glucose-6-phosphate, fructose-6-phosphate). PFOS was also absorbed in the roots and accumulated in the aerial plant parts, including the grains. These findings suggest that cultivation of wheat in PFOS-contaminated soil could affect the nutritional value of the grains. In addition, the ability of PFOS to bioaccumulate in the human body makes consumption of PFOS-laden wheat unsafe. To the best of the authors knowledge, this is the first report showing that PFOS affects grain quality in ways that may have unknown implications to human health and ecological integrity. Future studies should focus on continuous exposure of plants to PFOS at realistic environmental concentrations.

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.

Funding Information

The authors have no funding information to declare.

Acknowledgement

This material is based upon work supported by the National Science Foundation under Grant No. 1828069. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. C.M. Rico acknowledges funding from the Missouri State University Summer Faculty Grant, and the contribution of CHM462/662 Spring 2021 students that participated in Course-based Undergraduate Research Experience (CURE) which produced data for the short exposure studies. J. Zenobio would also like to acknowledge the UC Chancellor's Fellowship for financial support.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hazadv.2022.100131.

References

Aboobucker, SI, Suza, WP, 2019. Why do plants convert sitosterol to stigmasterol? Front. Plant Sci. 10.

Ahrens, L, 2011. Polyfluoroalkyl compounds in the aquatic environment: a review of their occurrence and fate. J. Environ. Monit. 13, 20–31.

Ashihara, H, Ludwig, IA, Katahira, R, Yokota, T, Fujimura, T, Crozier, A, 2015. Trigonelline and related nicotinic acid metabolites: occurrence, biosynthesis, taxonomic considerations, and their roles in planta and in human health. Phytochem. Rev. 14, 765–798.

Asthir, B, Duffus, CM, Smith, RC, Spoor, W, 2002. Diamine oxidase is involved in H2O2 production in the chalazal cells during barley grain filling. J. Exp. Bot. 53, 677–682.
Blaine, AC, Rich, CD, Sedlacko, EM, Hundal, LS, Kumar, K, Lau, C, Mills, MA, Harris, KM, Higgins, CP, 2014a. Perfluoroalkyl acid distribution in various plant compartments of edible crops grown in biosolids-amended soils. Environ. Sci. Technol. 48, 7858–7865.
Blaine, AC, Rich, CD, Sedlacko, EM, Hyland, KC, Stushnoff, C, Dickenson, ERV, Higgins, CP, 2014b. Perfluoroalkyl acid uptake in lettuce (Lactuca sativa) and strawberry (Fragaria ananassa) irrigated with reclaimed water. Environ. Sci. Technol. 48, 14361–14368.
Brusseau, ML, Anderson, RH, Guo, B, 2020. PFAS concentrations in soils: Background levels versus contaminated sites. Sci. Total Environ. 740, 140017.
Buck, RC, Franklin, J, Berger, U, Conder, JM, Cousins, IT, De Voogt, P, Jensen, AA, Kannan, K, Mabury, SA, Van Leeuwen, SP, 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr. Environ. Assess. Manag. 7, 513–541.
Chen, H, Kim, HU, Weng, H, Browse, J, 2011. Malonyl-CoA synthetase, encoded by ACYL ACTIVATING ENZYME13, is essential for growth and development of Arabidopsis. Plant Cell 23, 2247–2262.
Chen, W, Taylor, MC, Barrow, RA, Croyal, M, MASLE, J, 2018. Loss of phosphoethanolamine n-methyltransferases abolishes phosphatidylcholine synthesis and is lethal. Plant Physiol. 179, 124–142.
Choi, G-H, Lee, D-Y, Jeong, D-K, Kuppusamy, S, Lee, YB, Park, B-J, Kim, J-H, 2017. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) concentrations in the South Korean agricultural environment: a national survey. J. Integr. Agric. 16, 1841–1851.
Chong, J, Soufan, O, Li, C, Caraus, I, Li, S, Bourque, G, Wishart, DS, Xia, J, 2018. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucleic Acids Res. 46, W486–W494.
Cleaves, HJ, Miller, SL, 2001. The nicotinamide biosynthetic pathway is a by-product of the RNA world. J. Mol. Evol. 52, 73–77.
Costello, MCS & Lee, LS 2020. Sources, Fate, and plant uptake in agricultural systems of per- and polyfluoroalkyl substances. Current Pollution Reports.
Das, A, Rushton, PJ, Rohila, JS, 2017. Metabolomic profiling of soybeans (Glycine max L.) reveals the importance of sugar and nitrogen metabolism under drought and heat stress. Plants 6, 21.
De Mello, VD, Paananen, J, Lindström, J, Lankinen, MA, Shi, L, Kuusisto, J, Pihlajamäki, J, Auriola, S, Lehtonen, M, Rolandsson, O, Bergdahl, IA, Nordin, E, Ilanne-Parikka, P, Keinänen-Kiukaanniemi, S, Landberg, R, Eriksson, JG, Tuomilehto, J, Hanhineva, K, Uusitupa, M, 2017. Indolepropionic acid and novel lipid metabolites are associated with a lower risk of type 2 diabetes in the Finnish Diabetes Prevention Study. Sci. Rep. 7, 46337.
Dimkpa, CO, Mclean, JE, Martineau, N, Britt, DW, Haverkamp, R, Anderson, AJ, 2013. Silver nanoparticles disrupt wheat (Triticum aestivum L.) growth in a sand matrix. Environ. Sci. Technol. 47, 1082–1090.
Ehrlich, AM, Pacheco, AR, Henrick, BM, Taft, D, Xu, G, Huda, MN, Mishchuk, D, Goodson, ML, Slupsky, C, Barile, D, Lebrilla, CB, Stephensen, CB, Mills, DA, Raybould, HE, 2020. Indole-3-lactic acid associated with Bifidobacterium-dominated microbiota significantly decreases inflammation in intestinal epithelial cells. BMC Microbiol. 20, 357.
EPA 2003. Ecological Soil Screening Level for Iron Interim Final. Office of Solid Waste and Emergency Response Directive 9285. 7-69.
EPA, U, 2017. Technical Fact Sheet – Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA) Technical Fact Sheet - PFOS and PFOAEPA 505-F-17-001.
Felizeter, S, Mclachlan, MS, De Voogt, P, 2012. Uptake of perfluorinated alkyl acids by hydroponically grown lettuce (Lactuca sativa). Environ. Sci. Technol. 46, 11735–11743.
Frisbee, SJ, Shankar, A, Knox, SS, Steenland, K, Savitz, DA, Fletcher, T, Ducatman, AM, 2010. Perfluorooctanoic acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: results from the C8 Health Project. Arch. Pediatr. Adolesc. Med. 164, 860–869.
Gerdes, S, Lerma-Ortiz, C, Frelin, O, Seaver, SMD, Henry, CS, De Crécy-Lagard, V, HANSON, AD, 2012. Plant B Vitamin Pathways and their Compartmentation: a Guide for the Perplexed. J. Exp. Bot. 63, 5379–5395.
Ghisi, R, Vamerali, T, Manzetti, S, 2019. Accumulation of perfluorinated alkyl substances (PFAS) in agricultural plants: a review. Environ. Res. 169, 326–341.
Giesy, JP, Kannan, K, 2001. Global distribution of perfluorooctane sulfonate in wildlife. Environ. Sci. Technol. 35, 1339–1342.
Giner, RM, Ríos, JL, Máñez, S, 2022. Antioxidant activity of natural hydroquinones. Antioxidants 11, 343.
Gobelius, L, Lewis, J, Ahrens, L, 2017. Plant uptake of per- and polyfluoroalkyl substances at a contaminated fire training facility to evaluate the phytoremediation potential of various plant species. Environ. Sci. Technol. 51, 12602–12610.
Goldberg, I, Rokem, JS, 2009. Organic and fatty acid production, microbial. In: SCHAECHTER, M. (Ed.), Encyclopedia of Microbiology, third ed.. Academic Press, Oxford.
Gooding, MJ, Ellis, RH, Shewry, PR, Schofield, JD, 2003. Effects of restricted water availability and increased temperature on the grain filling, drying and quality of winter wheat. J. Cereal Sci. 37, 295–309.
Gouffi, K, Bernard, T, Blanco, C, 2000. Osmoprotection by pipecolic acid in Sinorhizobium meliloti: specific effects of D and L isomers. Appl. Environ. Microbiol. 66, 2358–2364.
Guo, Q, He, Z, Liu, X, Liu, B, Zhang, Y, 2020. High-throughput non-targeted metabolomics study of the effects of perfluorooctane sulfonate (PFOS) on the metabolic characteristics of A. thaliana leaves. Sci. Total Environ. 710, 135542.
Halford, NG, Curtis, TY, Chen, Z, Huang, J, 2014. Effects of abiotic stress and crop management on cereal grain composition: implications for food quality and safety. J. Exp. Bot. 66, 1145–1156.
He, M, Qin, C-X, Wang, X, Ding, N-Z, 2020. Plant unsaturated fatty acids: biosynthesis and regulation. Front. Plant Sci. 11.
Huang, X, Cervantes-Avilés, P, Li, W, Keller, AA, 2021. Drilling into the metabolomics to enhance insight on corn and wheat responses to molybdenum trioxide nanoparticles. Environ. Sci. Technol. 55, 13452–13464.
Igamberdiev, AU, Eprintsev, AT, 2016. Organic acids: the pools of fixed carbon involved in redox regulation and energy balance in higher plants. Front. Plant Sci. 7.
Jiménez-Escrig, A, Santos-Hidalgo, AB, Saura-Calixto, F, 2006. Common sources and estimated intake of plant sterols in the spanish diet. J. Agric. Food Chem. 54, 3462–3471.
Kang, Z, Babar, MA, Khan, N, Guo, J, Khan, J, Islam, S, Shrestha, S, Shahi, D, 2019. Comparative metabolomic profiling in the roots and leaves in contrasting genotypes reveals complex mechanisms involved in post-anthesis drought tolerance in wheat. PLoS One 14, e0213502.
Kärkkäinen, O, Lankinen, Ma, Vitale, M, Jokkala, J, Leppänen, J, Koistinen, V, Lehtonen, M, Giacco, R, Rosa-Sibakov, N, Micard, V, Rivellese, Aaa, Schwab, U, Mykkänen, H, Uusitupa, M, Kolehmainen, M, Riccardi, G, Poutanen, K, Auriola, S, Hanhineva, K, 2018. Diets rich in whole grains increase betainized compounds associated with glucose metabolism. Am. J. Clin. Nutr. 108, 971–979.
Katz, JE, Dumlao, DS, Wasserman, JI, Lansdown, MG, Jung, ME, Faull, KF, Clarke, S, 2004. 3-isopropylmalate is the major endogenous substrate of the saccharomyces cerevisiae trans-aconitate methyltransferase. Biochemistry 43, 5976–5986.
Khodour, Y, Kaguni, LS, Stiban, J, 2019. Iron-sulfur clusters in nucleic acid metabolism: varying roles of ancient cofactors. Enzymes 45, 225–256.
Kim, H, Ekpe, OD, Lee, J-H, Kim, D-H, Oh, J-E, 2019. Field-scale evaluation of the uptake of Perfluoroalkyl substances from soil by rice in paddy fields in South Korea. Sci. Total Environ. 671, 714–721.
Klement, T, Büchs, J, 2013. Itaconic acid – a biotechnological process in change. Bioresour. Technol. 135, 422–431.
Konopelski, P, Mogilnicka, I, 2022. Biological effects of indole-3-propionic acid, a gut microbiota-derived metabolite, and its precursor tryptophan in mammals' health and disease. Int. J. Mol. Sci. 23, 1222.
Krippner, J, Falk, S, Brunn, H, Georgii, S, Schubert, S, Stahl, T, 2015. Accumulation potentials of perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs) in maize (Zea mays). J. Agric. Food Chem. 63, 3646–3653.
Lasek, O, Barteczko, J, Barć, J, Micek, P, 2020. Nutrient content of different wheat and maize varieties and their impact on metabolizable energy content and nitrogen utilization by broilers. Animals 10, 907.
Lechner, M, Knapp, H, 2011. Carryover of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) from soil to plant and distribution to the different plant compartments studied in cultures of carrots (Daucus carota ssp. Sativus), potatoes (Solanum tuberosum), and cucumbers (Cucumis Sativus). J. Agric. Food Chem. 59, 11011–11018.
Lenk, M, Wenig, M, Bauer, K, Hug, F, Knappe, C, Lange, B, Timsy, Häußler, F, Mengel, F, Dey, S, Schäffner, A, Vlot, AC, 2019. Pipecolic acid is induced in barley upon infection and triggers immune responses associated with elevated nitric oxide accumulation. Mol. Plant-Microbe Interact. 32, 1303–1313.
Li, J, Sun, J, Li, P, 2022. Exposure routes, bioaccumulation and toxic effects of per- and polyfluoroalkyl substances (PFASs) on plants: a critical review. Environ. Int. 158, 106891.
Li, P, Oyang, X, Xie, X, Guo, Y, Li, Z, Xi, J, Zhu, D, Ma, X, Liu, B, Li, J, Xiao, Z, 2020a. Perfluorooctanoic acid and perfluorooctane sulfonate co-exposure induced changes of metabolites and defense pathways in lettuce leaves. Environ. Pollut. 256, 113512.
Li, P, Oyang, X, Xie, X, Li, Z, Yang, H, Xi, J, Guo, Y, Tian, X, Liu, B, Li, J, Xiao, Z, 2020b. Phytotoxicity induced by perfluorooctanoic acid and perfluorooctane sulfonate via metabolomics. J. Hazard. Mater. 389, 121852.
Li, P, Xiao, Z, Sun, J, Oyang, X, Xie, X, Li, Z, Tian, X, Li, J, 2020c. Metabolic regulations in lettuce root under combined exposure to perfluorooctanoic acid and perfluorooctane sulfonate in hydroponic media. Sci. Total Environ. 726, 138382.
Lichtenthaler, K, Welburn, AR, 1983. Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11, 591–592.
Liu, C, Ding, F, Hao, F, Yu, M, Lei, H, Wu, X, Zhao, Z, Guo, H, Yin, J, Wang, Y, Tang, H, 2016. Reprogramming of seed metabolism facilitates pre-harvest sprouting resistance of wheat. Sci. Rep. 6, 20593.
Liu, J-H, Wang, W, Wu, H, Gong, X, Moriguchi, T, 2015. Polyamines function in stress tolerance: from synthesis to regulation. Front. Plant Sci. 6.
Liu, Z, Lu, Y, Song, X, Jones, K, Sweetman, AJ, Johnson, AC, Zhang, M, Lu, X, Su, C, 2019. Multiple crop bioaccumulation and human exposure of perfluoroalkyl substances around a mega fluorochemical industrial park, China: Implication for planting optimization and food safety. Environ. Int. 127, 671–684.
Madeo, F, Carmona-Gutierrez, D, Kepp, O, Kroemer, G, 2018. Spermidine delays aging in humans. Aging 10, 2209–2211.
Mei, W, Sun, H, Song, M, Jiang, L, Li, Y, Lu, W, Ying, G-G, Luo, C, Zhang, G, 2021. Per- and polyfluoroalkyl substances (PFASs) in the soil–plant system: sorption, root uptake, and translocation. Environ. Int. 156, 106642.
Nair, RB, Bastress, KL, Ruegger, MO, Denault, JW, Chapple, C, 2004. The Arabidopsis thaliana REDUCED EPIDERMAL FLUORESCENCE1 gene encodes an aldehyde dehydrogenase involved in ferulic acid and sinapic acid biosynthesis. Plant Cell 16, 544–554.
OECD, 2018. Toward a new comprehensive global database of per- and polyfluoroalkyl substances (PFASs): summary report on updating the OECD 2007 list of per- and polyfluoroalkyl substances (PFASs). ENV/JM/MONO 7.
Prajapati, KB, Modi, HA, 2012. The importance of potassium in plant growth - a review. Indian J. Plant Sci. 1, 177–186.
Qu, B, Zhao, H, Zhou, J, 2010. Toxic effects of perfluorooctane sulfonate (PFOS) on wheat (Triticum aestivum L.) plant. Chemosphere 79, 555–560.

Raheem, A, Shaposhnikov, A, Belimov, AA, Dodd, IC, Ali, B, 2018. Auxin production by rhizobacteria was associated with improved yield of wheat (Triticum aestivum L.) under drought stress. Arch. Agron. Soil Sci. 64, 574–587.

Rico, CM, Lee, SC, Rubenecia, R, Mukherjee, A, Hong, J, Peralta-Videa, JR, Gardea-Torresdey, JL, 2014. Cerium oxide nanoparticles impact yield and modify nutritional parameters in wheat (Triticum aestivum L.). J. Agric. Food Chem. 62, 9669–9675.

Rico, CM, Wagner, D, Abolade, O, Lottes, B, Coates, K, 2020. Metabolomics of wheat grains generationally-exposed to cerium oxide nanoparticles. Sci. Total Environ. 712, 136487.

Rodríguez, LH, Morales, DA, Romero, CD, Rodríguez, ER, 2012. The organic acid profile in wheat cultivar grains. Int. J. Food Sci. Technol. 47, 627–632.

Rontein, D, Nishida, I, Tashiro, G, Yoshioka, K, Wu, WI, Voelker, DR, Basset, G, Hanson, AD, 2001. Plants synthesize ethanolamine by direct decarboxylation of serine using a pyridoxal phosphate enzyme. J. Biol. Chem. 276, 35523–35529.

Sasamoto, H, Ashihara, H, 2014. Effect of nicotinic acid, nicotinamide and trigonelline on the proliferation of lettuce cells derived from protoplasts. Phytochem. Lett. 7, 38–41.

Schendel, RR, 2019. 10 - phenol content in sprouted grains. In: FENG, H., NEMZER, B, DEVRIES, J.W. (Eds.), Sprouted Grains. AACC International Press.

Serraj, R, Vadez, V, Denison, RF, Sinclair, TR, 1999. Involvement of ureides in nitrogen fixation inhibition in soybean1. Plant Physiol. 119, 289–296.

Shewry, PR, Hey, SJ, 2015. The contribution of wheat to human diet and health. Food Energy Secur. 4, 178–202.

Stahl, T, Heyn, J, Thiele, H, Huther, J, Failing, K, Georgii, S, Brunn, H, 2009. Carryover of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) from soil to plants. Arch. Environ. Contam. Toxicol. 57, 289–298.

Streeter, JG, 1979. Allantoin and allantoic acid in tissues and stem exudate from field–grown soybean plants. Plant Physiol. 63, 478–480.

Wang, L, Zhong, M, Li, X, Yuan, D, Xu, Y, Liu, H, He, Y, Luo, L, Zhang, Q, 2008. The QTL controlling amino acid content in grains of rice (Oryza sativa) are co-localized with the regions involved in the amino acid metabolism pathway. Mol. Breed. 21, 127–137.

Wardlaw, If, Blumenthal, C, Larroque, O, Wrigley, CW, 2002. Contrasting effects of chronic heat stress and heat shock on kernel weight and flour quality in wheat. Funct. Plant Biol. 29, 25–34.

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

Wen, B, Li, L, Zhang, H, Ma, Y, Shan, Xq, Zhang, S, 2014. Field study on the uptake and translocation of perfluoroalkyl acids (PFAAs) by wheat (Triticum aestivum L.) grown in biosolids-amended soils. Environ. Pollut. 184, 547–554.

Wen, B, Wu, Y, Zhang, H, Liu, Y, Hu, X, Huang, H, Zhang, S, 2016. The roles of protein and lipid in the accumulation and distribution of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in plants grown in biosolids-amended soils. Environ. Pollut. 216, 682–688.

Yoshida, S, Forno, DA & Cock, JH 1971. Laboratory Manual for Physiological Studies of Rice, Los Baños, Philippines.

Yu, P-F, Xiang, L, Li, X-H, Ding, Z-R, Mo, C-H, Li, Y-W, Li, H, Cai, Q-Y, Zhou, D-M, Wong, M-H, 2018. Cultivar-dependent accumulation and translocation of perfluorooctanesulfonate among lettuce (Lactuca sativa L.) cultivars grown on perfluorooctanesulfonate-contaminated soil. J. Agric. Food Chem. 66, 13096–13106.

Zeng, T, Fang, B, Huang, F, Dai, L, Tang, Z, Tian, J, Cao, G, Meng, X, Liu, Y, Lei, B, Lu, M, Cai, Z, 2021. Mass spectrometry-based metabolomics investigation on two different indica rice grains (Oryza sativa L.) under cadmium stress. Food Chem. 343, 128472.

Zeng, Z, Song, B, Xiao, R, Zeng, G, Gong, J, Chen, M, Xu, P, Zhang, P, Shen, M, Yi, H, 2019. Assessing the human health risks of perfluorooctane sulfonate by in vivo and in vitro studies. Environ. Int. 126, 598–610.

Zhang, M, Wang, P, Lu, Y, Lu, X, Zhang, A, Liu, Z, Zhang, Y, Khan, K, Sarvajayakesavalu, S, 2020. Bioaccumulation and human exposure of perfluoroalkyl acids (PFAAs) in vegetables from the largest vegetable production base of China. Environ. Int. 135, 105347.

Zhao, H, Chen, C, Zhang, X, Chen, J, Quan, X, 2011. Phytotoxicity of PFOS and PFOA to brassica chinensis in different Chinese soils. Ecotoxicol. Environ. Saf. 74, 1343–1347.

Zhao, H, Guan, Y, Zhang, G, Zhang, Z, Tan, F, QUAN, X, Chen, J, 2013. Uptake of perfluorooctane sulfonate (PFOS) by wheat (Triticum aestivum L.) plant. Chemosphere 91, 139–144.

Zhao, L, Hu, J, Huang, Y, Wang, H, Adeleye, A, Ortiz, C, Keller, AA, 2017. 1H NMR and GC–MS based metabolomics reveal nano-Cu altered cucumber (Cucumis sativus) fruit nutritional supply. Plant Physiol. Biochem. 110, 138–146.

Zhokhov, SS, Broberg, A, Kenne, L, Jastrebova, J, 2010. Content of antioxidant hydroquinones substituted by β-1,6-linked oligosaccharides in wheat milled fractions, flours and breads. Food Chem. 121, 645–652.

Zhu, Y, Wang, P, Sha, W, Sang, S, 2016. Urinary biomarkers of whole grain wheat intake identified by non-targeted and targeted metabolomics approaches. Sci. Rep. 6, 36278.