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Uptake and distribution of perfluoroalkyl substances by grafted tomato plants cultivated in a contaminated site in northern Italy

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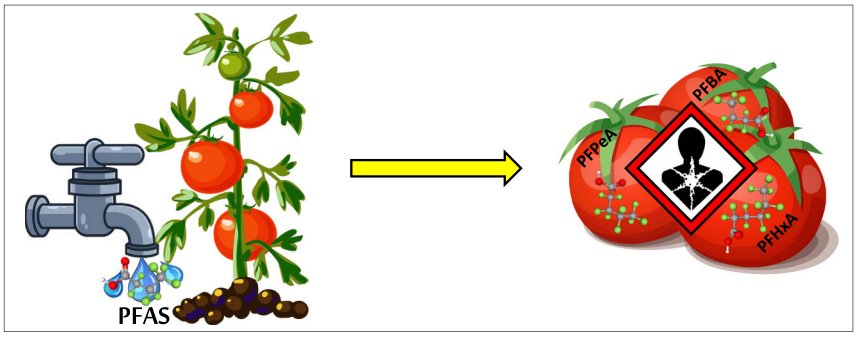
HIGHLIGHTS

- PFAS accumulated depending on the plant's vigor
- Only short chain PFAS translocated up to the fruits
- PFBS and PFOA were detected exclusively in the leaves.
- The health risk associated with the consumption of tomatoes is of concern.

GRAPHICAL ABSTRACT



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ABSTRACT

Poly- and perfluoroalkyl substances (PFAS) are highly persistent and mobile pollutants raising alarming concerns due to their capability to accumulate in living organisms and exert toxic effects on human health. We studied the accumulation of different PFAS in the leaves and fruits of tomato plants grown on a PFAS-polluted soil in North-East Italy. Tomato plants were grafted with different rootstocks characterized by different vigor, and irrigated with PFAS-polluted groundwater. Leaves and fruits of the first and sixth truss were analyzed at full plant maturity. All tomato varieties accumulated PFAS in leaves and fruits, with the highest concentrations detected in the most vigorous rootstock and reflecting the PFAS concentration profile of the irrigation water. PFAS with a chain length from 4 to 8 C atoms and with carboxylic and sulfonic functional groups were detected in plant leaves, whereas only carboxylic C4, C5, and C6 PFAS were detected in tomato fruits. A general trend of

Abbreviations: ASE, accelerated solvent extraction; C, concentration; CA, cellulose acetate; DI, daily intake; DW, dry weight; EFSA, European Food Safety Authority; EPA, United States Environmental Protection Agency; FW, fresh weight; HI, hazard index; LC-MS/MS, liquid chromatography tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; PCLs, protective concentration levels; PFAS, poly- and perfluoroalkyl substances; PFBA, perfluorobutanoic acid; PFBS, perfluorobutane sulfonic acid; PFDA, perfluorodecanoic acid; PFDoA, perfluorododecanoic acid; PFHpA, perfluoroheptanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFPeA, perfluoropentanoic acid; PFTeDA, perfluorotetradecanoic acid; PFUnA, perfluoroundecanoic acid; RfD, oral reference dose; SRM, selected reaction monitoring; TCEQ, Texas Commission on Environment Quality; THQ, target hazard quotient; TRRP, Texas Risk Reduction Program; TWI, tolerable weekly intake..

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decreasing PFAS concentrations in fruits upon increasing height of the plant trusses was observed. Calculation of the target hazard quotient (THQ) showed increasing values depending on the plant vigor. The hazard index (HI) values showed values slightly higher than 1 for the most vigorous plants, indicating potential risks to human health associated with the consumption of contaminated tomato fruits.

1. Introduction

Poly- and perfluoroalkyl substances (PFAS) are partially or fully fluorinated carbon compounds, respectively, with a functional terminal group, of which carboxylic and sulfonic groups are the most predominant. Collectively, PFAS are a class of > 4700 compounds (Koch et al., 2020), with both hydrophobic and lipophobic properties, and high chemical and thermal stability (Zhang et al., 2022). These peculiarities are ensured by the strength of the C-F bond, together with their molecular structure. Owing to their chemical properties, PFAS are anthropogenic pollutants highly persistent and mobile in the environment, spreading worldwide due to their intense use in various industrial applications (EEA, 2023). Once released into the environment, PFAS result resistant to chemical and biological degradation, and their movement in the watershed is mainly driven by the surface and groundwater flows (Mahinroosta et al., 2021; Raschke et al., 2022).

The main assessed routes of PFAS exposure for humans are ingestion of contaminated water, followed by inhalation of polluted air and dust, and dermal contact with contaminated media (Sunderland et al., 2019). The PFAS pollution of soils has been comparatively less studied with respect to surface and ground waters, because of technical difficulties in extraction and quantification of different PFAS. Additionally, in most of the PFAS-polluted areas there are neither threshold limits for soils nor mandatory monitoring surveys, due to the lack of a Soil Framework Directive like those protecting hydrosphere and atmosphere. Soils can be polluted by PFAS due to the advection of polluted groundwater or irrigation of agricultural soils with contaminated waters, by wet and dry deposition from the atmosphere (Gewurtz et al., 2019; Shimizu et al., 2021), and by soil amendment with polluted compost or sewage sludge (García-Valcarcel et al., 2012).

The PFAS-polluted soils can act as a secondary source for plants and agricultural crops, which can absorb and accumulate such chemicals in the aboveground organs (Ghisi et al., 2019; Lesmeister et al., 2021). Therefore, edible leaves, fruits, and vegetables produced in polluted areas can represent significant PFAS sources for humans, thus increasing the global risks to health. Significant association between PFAS exposure and adverse effects on human health has been proved by epidemiological studies (Sunderland et al., 2019; Fenton et al., 2021), with main evidence of increased risks of thyroid dysfunction, lipid and insulin dysregulation, hypercholesterolemia, immunotoxicity, liver and kidney disease, cancer, and adverse reproductive and developmental outcomes. New evidence of human exposure by crop produce is envisaged by the European Agency on Food Safety (EFSA), which has recently set provisional safety thresholds as a group tolerable weekly intake (TWI) from food of 4.4 ng/kg of body weight per week for four PFAS that accumulate in the human body, namely perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS). This new evidence-based policy applies to all agri-food products, including crop products, and for this reason it is of prime importance to evaluate PFAS bioaccumulation in different plant species cropped in polluted areas.

Among agricultural products, tomato (*Lycopersicum esculentum* Mill.) is one of the most important crops, and Italy is the first producer of tomato in Europe (EC, 2023). Its fruits are the major contributors of lycopene, a natural antioxidant molecule, and contain remarkable amounts of vitamins K, A, C, fiber, and carbohydrates (Khan et al., 2021). Moreover, tomato fruits are a source of minerals (Fe, K, P, S), and contain low levels of sodium, lipids, and calories (Khan et al., 2021). More than 50% of commercial tomato varieties are grafted because grafted plants are characterized by higher vigor, yields and product quality than ungrafted varieties (Kumar et al., 2015a; Kyriacou et al., 2020). Grafting is a widely spread and continuously increasing biotechnology of producing new horticultural plant varieties, resistant plants to pathogens and abiotic stress such as nutritional deficiencies and soil salinity, water excesses or deficits, and pollutants (Maurya et al., 2019; Xie et al., 2020; Yuan et al., 2021). Plants grafted on tomato rootstocks, particularly on Maxifort, were able to reduce the content of As in leaves, stems, and fruits (Stazi et al., 2016), suggesting that grafting could represent a strategy for reducing contaminants uptake in the commercial parts of tomato plants.

Due to their nutritional value, tomatoes are largely consumed worldwide, hence it is necessary to evaluate PFAS bioaccumulation in fruits and estimate the potential risks due to their occurrence in contaminated environments. In this regard, potential PFAS accumulation in tomato fruits has been reported from studies in hydroponics (Felizeter et al., 2014) and pot experiments (Blaine et al., 2013; Blaine et al., 2014). The PFAS accumulation data were reported from home-produced vegetables in areas close to fluorochemical industries (Bao et al., 2019), but information from field trials conducted in polluted agricultural areas, also accounting for different agronomic and environmental factors, is still scant.

The Veneto Region in North-East Italy hosts one of the largest and best characterized PFAS contamination clusters originated by a prolonged uncontrolled industrial discharge (ARPAV, 2023). The polluted area is in the order of 200 km^2, mainly under agricultural use, impacting > 100,000 inhabitants. Several prohibitions on the use of groundwater from private wells are currently in force, and activated carbon filters are installed for drinking water purification. Systematic monitoring of surface water and groundwater has been ongoing since 2013 with > 10,000 sampling wells analyzed so far (ARPAV, 2023), which shows relatively constant over time.

Based on the current knowledge, we hypothesized that i) tomato plants can accumulate high concentrations of PFAS in their fruits when cropped in a PFAS-polluted environment, ii) irrigation with contaminated water is the main PFAS source for PFAS plant uptake, iii) grafted plants may accumulate higher PFAS concentrations due to their greater vigor. We tested these hypotheses in a field trial mimicking the typical kitchen garden cultivation practice of residents of the PFAS-polluted area of the Veneto Region. In addition to the market and nutritional value, tomato is also a model plant allowing the study of PFAS accumulation in progressively ripening of fruits of different trusses, providing information on PFAS movement within the plant.

2. Materials and methods

2.1. Chemicals

Analytical standards of perfluorobutanoic acid (PFBA), perfluorobutane sulfonic acid (PFBS), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluorohexane sulfonic acid (PFHxS), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), and perfluorotetradecanoic acid (PFTeDA) were purchased from Wellington Laboratories (Canada). Identification and quantification of all PFAS were performed using the isotopically-labeled PFAS standards ($^{13}C_4$-PFBA, $^{13}C_3$-PFBS, $^{13}C_5$-PFPeA, $^{13}C_5$-PFHxA, $^{13}C_3$-PFHxS, $^{13}C_4$-PFHpA,

$^{13}C_8$-PFOA, $^{13}C_8$-PFOS, $^{13}C_9$-PFNA, $^{13}C_6$-PFDA, $^{13}C_7$-PFUnA, $^{13}C_2$-PFDoA, $^{13}C_2$-PFTeDA) from Wellington Laboratories, added at a fixed concentration as internal standards in LC-MS/MS analysis. UHPLC-MS grade water and methanol (> 99.95% purity) were obtained from Carlo Erba Reagents Srl (Italy).

2.2. Experimental design

The experimental trial was carried out in Vicenza, North-East Italy (45°53′95′′ N; 11°49′43″ E), in an area of PFAS-polluted soils falling within the high-risk zone defined by the Veneto Region (ARPAV, 2023). Geologically, the soil was characterized as sub-level colluvial surfaces (USDA classification – Typic Hapluderts fine, mixed, mesic; WRB – Haplic Vertisols) with a slope of less than 2% and a silty-clay texture. Soil texture was 64% sand, 17% silt, and 19% clay, with a pH value of 7.22, and soil organic matter (SOM) content of 1.86%. The average annual temperature and precipitation of the area are 17.6 °C and 1084 mm, respectively. The experimental area considered was 36 m^2, divided into three blocks of 12 m^2 each.

Concentrations of eight PFAS in the groundwater sampled from monitoring wells located in the radius of 2 km from the experimental site in the period 2013-2023 (ARPAV, 2023) are reported in Table 1, and the groundwater used for irrigating the plants was also analyzed.

Before transplanting the tomatoes, the soil was plowed, sampled for preliminary PFAS analysis, fertilized (150-100-200 kg/ha of N, P_2O_5, and K_2O mineral fertilizers), and refined by rotavator to bury the fertilizer. Within each block, a drip-irrigation system was set up using t-tapes with drippers placed every 0.3 m, with a flow rate of 1.4 L h^{-1} for each dripper. Subsequently, the soil was mulched with black polyethylene plastic film. The plants were transplanted with a planting distance of 0.50 m between rows and 0.50 m within rows.

For each block, four graft combinations were considered: an ungrafted control (cv Dominus F1 – ISI Sementi, Italy), and Dominus grafted onto Maxifort, Optifort and Dynafort rootstocks (De Ruiter, Bayer, Germany), with the three rootstocks characterized by decreasing vigor. The commercial variety Dominus F1 is a Midi-Plum tomato characterized by an early cycle, short internodes, and fruits weighing 30-35 g each. For each graft combination, four plants per block were grown, resulting in a total of 48 plants for the entire field experiment.

2.3. Sample collection, PFAS extraction and quantification by LC-MS/MS analysis

At the ripening of the first and sixth fruit trusses, six samples were taken for each treatment, considering both the whole truss and the two proximal leaves. Fully expanded leaves were sampled, and ripe fruits were harvested from the first and sixth trusses of each plant and immediately transported to the laboratory. Fresh (FW) and dry (DW) weights of both leaves and fruits were determined by weighing them before and after drying at 60 °C until constant weight. Dried leaves and fruits were ground into fine powder prior to PFAS extraction with an accelerated solvent extraction (ASE) system (Dionex ASE 350, Thermo Fisher Scientific, USA). For each replicate, 0.5 g and 1.0 g of leaves and fruits powder, respectively, were spiked with a ^{13}C-labeled PFAS internal standard mixture (Wellington Laboratories) and extracted. The ASE system is equipped with a PFAS-free tubing system, and operated at 125 °C and 1300-1500 psi, using methanol as solvent. Extracts (~ 17 mL) were then filtered using 0.22 μm cellulose acetate (CA) syringe membranes. Accuracy and recovery rates of the ASE extraction method were evaluated, and data are reported in Table S1. For the determination of PFAS concentration of the experimental site, irrigation water samples were spiked with the ^{13}C-labeled PFAS internal standard mixture (Wellington Laboratories) and filtered with 0.22 μm CA syringe membranes, whereas 1.0 g of soil was extracted by ASE and filtered as described above. All filtered samples were stored at 4 °C until LC-MS/MS analysis, without pre-concentration steps.

PFAS content was determined by LC-MS/MS analyses using a triple quadrupole mass spectrometer (TSQ Quantiva, Thermo Fisher Scientific, USA) coupled to ultra-high performance liquid chromatography (Ultimate 3000 UHPLC, Dionex, Thermo Fisher Scientific, USA). The instrument operated in selected reaction monitoring (SRM) mode (see Table S2 for the optimized parameters for SRM transitions), and a comprehensive description of the chromatographic method and mass spectrometer conditions is reported in detail by Sharma et al. (2020). A ^{13}C-labeled PFAS standard for each target compound allows for the direct comparison of the retention time thus reducing the risk of false positives. Leaves samples were diluted 1:1 with H_2O before injection. MS raw data were processed with Skyline MS software v. 21.2.0.425 (Adams et al., 2020) to quantify PFAS amounts in all tested samples.

2.4. Quality control and quality assurance

The linearity of the standard calibration curves for all thirteen PFAS was assessed between 0 and 40 μg L^{-1}. All molecules showed good linearity, with correlation coefficients $R^2 > 0.988$ (Table S3). PFAS contents were determined using the corresponding matrix-matched calibration curve. For the matrix-matched calibration curves, tomatoes of the same variety grown in uncontaminated soil of the same site and irrigated with rainwater were used. The PFAS content of the rainwater, soil, leaves, and fruits of such tomatoes was checked before selecting them as blank matrices for the calibration curves. The limit of detection (LOD) and the limit of quantification (LOQ) values were calculated for each target PFAS as the analyte peak with a signal-to-noise ratio of 3 and 10, respectively, and are reported in Table S3. Glassware and all materials potentially containing fluorinated polymers were avoided to prevent background contamination, and carry over and instrumental performance were monitored with blank injections (methanol spiked with the ^{13}C-labeled PFAS internal standard mixture (Wellington Laboratories)) every three sample injections.

2.5. Health risk assessment

To assess the health risk associated with PFAS-contaminated tomatoes consumption, the level of contamination of the first truss fruits was considered, since PFAS concentrations were generally higher than the sixth truss tomatoes. Assuming this as the worst-case scenario, the estimation of non-carcinogenic health hazards due to the consumption of tomato fruits was determined by calculating the target hazard quotient (THQ) for individual detected PFAS (i.e., PFBA, PFPeA, and PFHxA) with the following equation (Kavcar et al., 2009): $THQ_i = C_i \, x \, DI \, / \, RfD_i$, where C_i is the *i*-PFAS concentration (mg g^{-1} FW), DI is the average dietary daily intake per unit of body weight (BW) (g kg^{-1} BW day^{-1}), and RfD_i is the oral reference dose (mg kg^{-1} BW day^{-1}) of the *i*-PFAS. The DI of raw tomato fruits for Italian adult population (DI = 1.16 g kg^{-1} BW day^{-1}) was retrieved from the most recent version of the EFSA Comprehensive European Food Consumption Database, available on the *data.europa.eu* website (https://data.europa.eu/data/datasets/the-efsa-comprehensive-european-food-consumption-database?locale

Table 1
Average PFAS concentrations in the groundwater of the study area, detected during the institutional monitoring campaign of the ARPAV (2023) in the period 2013-2023. Concentrations are expressed in ng L^{-1} and reported as mean ± standard deviation (n = 11). PFHpS: perfluoroheptane sulfonic acid.

PFAS	PFBA	PFBS	PFPeA	PFHxA	PFHxS	PFHpA	PFHpS	PFOA	PFOS
Mean ± St. Dev.	382 ± 55	480 ± 79	228 ± 29	231 ± 23	26 ± 4	75 ± 17	5.0 ± 1.0	2044 ± 386	71 ± 12

=en), accessed in July 2023. The chronic RfD values for PFBA (0.001 mg kg^{-1} BW day^{-1}) and PFHxA (0.0005 mg kg^{-1} BW day^{-1}) were retrieved from the US EPA's IRIS Toxicological Review for PFBA and related salts, and the IRIS Toxicological Review for PFHxA and related salts, respectively (https://www.epa.gov/iris). The chronic RfD value for PFPeA (0.0005 mg kg^{-1} BW day^{-1}) was retrieved from the Texas Risk Reduction Program (TRRP) – Protective Concentration Levels (PCLs) tables (released on May 2023) by the Texas Commission on Environment Quality (TCEQ) (https://www.tceq.texas.gov/). The hazard index (HI) was calculated as the sum of individual THQs ($HI = THQ_{PFBA} + THQ_{PFPeA} + THQ_{PFHxA}$) (Alsafra et al., 2022). A $HI < 1$ indicates unlikely adverse effects due to PFAS exposure, while a $HI \geq 1$ indicates that there is a potential risk to observe chronic non-cancer adverse effects on human health.

2.6. Statistical analysis

Statistical analysis was conducted with RStudio software v. 2022.12.0 (RStudio Team, 2020), and differences were considered significant at $p \leq 0.05$. Potential outliers were tested by Grubbs's test and removed from the dataset prior to further statistical analyses. Left-censored data were analyzed by Wilcoxon test and Kruskal-Wallis test, using the functions available in the NADA package for R, according to Helsel (2012). The correlation between the PFAS average concentration in the irrigation water (average of values detected at the beginning and the end of the experiment) and the amount of PFAS detected in the leaves and fruits (average of the four rootstock combinations) was assessed by the calculation of the Pearson correlation coefficient.

3. Results

3.1. Level of PFAS contamination at the experimental site

The analysis of PFAS pollution of soil and irrigation water before the tomato transplant showed that only PFOA, PFOS, and PFDA were detected in the soil, whereas the other analyzed compounds were below the LOD or LOQ, with PFOA being the most abundant molecule followed by PFOS and PFDA (Table 2). The irrigation water was polluted by several PFAS, with PFOA and PFBS showing the highest concentrations (Table 2).

The PFAS contamination of soil and irrigation water was also assessed at the end of the experiment (Table 2). From the beginning to the end of the field trial, the soil showed accumulation of PFHxA, PFOA, and PFNA, PFDA concentration was constant, whereas PFOS concentration was reduced. The PFAS concentrations and profile of the irrigation water were consistent at the beginning and the end of the experiment, except for lower concentrations of PFBS and PFOA by ca. 34% and 29%, respectively, and PFOS concentration below the LOQ value at the end of the experiment.

3.2. PFAS accumulation in the leaves

Only PFBA, PFBS, PFHxA, PFHpA, and PFOA were detected, whereas the concentration of PFPeA, PFHxS, PFOS, PFNA, PFDA, PFUnA, PFDoA, and PFTeDA were below the LOQ in all samples (Fig. 1). All detected PFAS have a chain length between 4 and 8 carbon atoms and a carboxylic group as a terminal functional group, with the only exception of PFBS which has a sulfonic group. PFBA (Fig. 1A) accumulated in different amounts in the first truss depending on the tomato rootstock; similar quantities were measured in the sixth truss samples, apart from the ungrafted control that had a lower amount. Regarding PFBS (Fig. 1B), an increasing trend in concentration upon the increasing of truss height was observed, with the Maxifort rootstock accumulating more than other tomato varieties. On the contrary, the ungrafted plants showed no PFBS in the first truss, and low concentrations in the sixth truss (Fig. 1B). PFHxA displayed an accumulation trend similar to PFBA, with differences in the first truss samples related to the tomato grafting combination and comparable amounts in the sixth truss (Fig. 1C). Apart from the Maxifort rootstock, the concentration of PFHxA in the sixth truss was significantly higher than the first truss in all combinations (Table S4). PFHpA was detected only in the samples of the sixth truss of grafted tomato plants, with the exception of the Maxifort rootstock, where a comparable amount was detected only in the first truss samples (Fig. 1D). PFOA was detected in leaves of all tomato varieties, with no significant differences observed between first and sixth truss of all rootstock combinations (Fig. 1E). The Maxifort rootstock variety accumulated almost twice the amount of the other varieties (Fig. 1E).

Overall, PFAS concentrations in the leaves reflected the PFAS pollution profile of the irrigation water, with PFOA being the most abundant, followed by PFBS, PFBA, PFHxA, and PFHpA. In this regard, the Pearson correlation coefficients between the PFAS concentration in the irrigation water and the PFAS content in the leaves of the first and sixth trusses were 0.996 and 0.972, respectively (Table S5).

3.3. PFAS accumulation in the fruits

The analysis of PFAS content in the tomato fruits of the first and sixth truss showed that only PFBA, PFPeA, and PFHxA were translocated at concentrations higher than the LOQ in all samples. The PFAS uptake by fruits varied according to the molecule chain length, tomato rootstock, and truss height (Fig. 2, Table S6). PFBA was the most abundant, some differences were observed between fruits of the first and sixth truss, and with plants grafted on Dynafort rootstock accumulating almost twice the PFBA concentration in fruits of the first truss as compared to the other varieties (Fig. 2A). Concentration of PFPeA showed a decreasing trend in accumulation depending on the increasing truss height with -39% (Dynafort rootstock) up to -59% (Maxifort rootstock), with the exception of Maxifort rootstock and the ungrafted variety accumulating less than the grafted ones (Fig. 2B). PFHxA was detected only in the first truss samples of all grafting combinations, with the Dynafort, Optifort and the ungrafted control varieties accumulating less than the Maxifort one (Fig. 2C). The Pearson correlation between the PFAS concentration in

Table 2
PFAS concentrations detected at the experimental site at the beginning and at the end of the experiment. Values are expressed as ng kg^{-1} DW for soil and ng L^{-1} for water.

Beginning of the experiment													
	PFBA	PFBS	PFPeA	PFHxA	PFHxS	PFHpA	PFOA	PFOS	PFNA	PFDA	PFUnA	PFDoA	PFTeDA
Soil	< LOD	< LOQ	< LOD	< LOD	< LOD	< LOD	3756	1463	< LOD	1405	< LOD	< LOD	< LOD
Irrigation water	1630	3366	1049	1131	346	332	9867	523	< LOQ	98	< LOD	< LOD	< LOD
End of the experiment													
	PFBA	PFBS	PFPeA	PFHxA	PFHxS	PFHpA	PFOA	PFOS	PFNA	PFDA	PFUnA	PFDoA	PFTeDA
Soil	< LOD	< LOQ	< LOQ	2542	< LOQ	< LOD	5633	672	1962	1350	< LOD	< LOD	< LOD
Irrigation water	1769	2205	1148	1263	402	< LOD	7036	< LOQ	< LOQ	94	< LOD	< LOD	< LOD

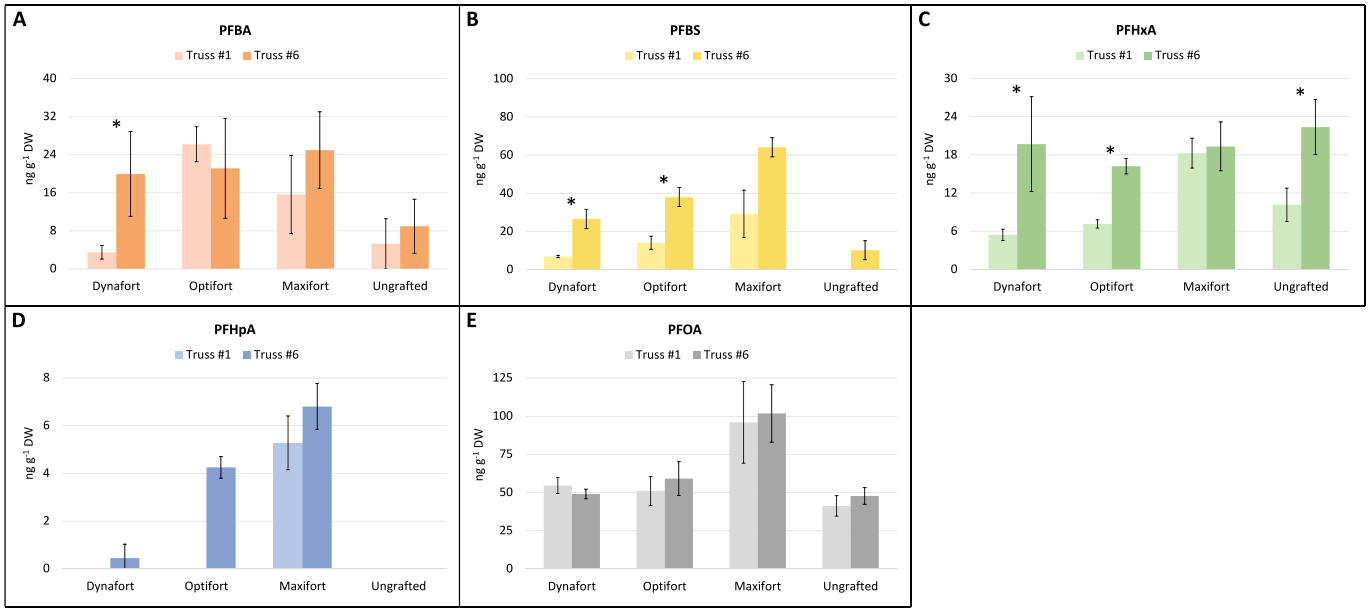

Fig. 1. PFAS content measured in the leaves expressed as ng g^{-1} DW: **A)** PFBA, **B)** PFBS, **C)** PFHxA, **D)** PFHpA, **E)** PFOA according to the grafting combination. In all plots, values indicate mean ± standard error ($n = 6$), * indicates $p \leq 0.05$ for statistical test on amounts of first (#1) and sixth (#6) truss of the same tomato rootstock combination.

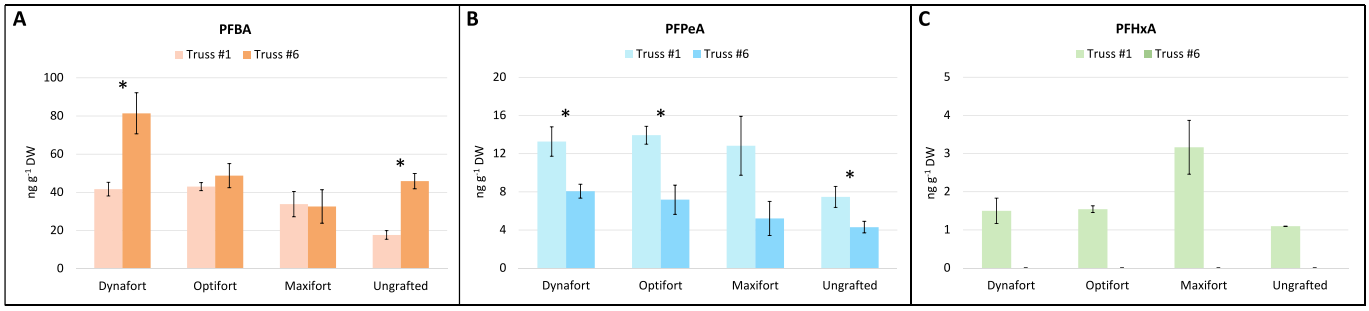

Fig. 2. Amounts of PFAS compounds quantified in the fruits expressed as ng g^{-1} DW: **A)** PFBA, **B)** PFPeA, **C)** PFHxA according to the grafting combination. In all plots, values indicate mean ± standard error (n = 6), * indicates $p \leq 0.05$ for statistical test on amounts of first (#1) and sixth (#6) truss of the same tomato rootstock combination.

the irrigation water and the PFAS content in the fruits of the first and sixth trusses showed coefficients of 0.895 and 0.966, respectively (Table S7).

3.4. Differences among tomato varieties

To evaluate the influence of grafting on the PFAS uptake by tomato plants, the PFAS content among varieties of the same truss was compared. Regarding the first truss leaves, the Optifort rootstock combination accumulated significantly higher amounts of PFBA and PFBS than the Dynafort one (Table S8). The amount of PFHxA was significantly higher in the Maxifort rootstock combination compared to Dynafort and Optifort. Differences among varieties for the other detected PFAS were not statistically significant. As for the sixth truss samples, no differences were observed for PFBA and PFHxA (Table S8). Differently, PFBS accumulated by the ungrafted control was significantly lower than those of the grafted varieties (Table S8). Maxifort rootstock combination accumulated more PFHpA than Dynafort and Optifort ones, and more PFOA with respect to Dynafort and the ungrafted control (Table S8).

Concerning fruits, PFAS detected in the ungrafted control were significantly lower compared to the grafted combinations (Table S9). In particular, PFBA and PFPeA levels detected in the first truss samples were significantly higher in Dynafort and Optifort with respect to the ungrafted plants, whereas PFHxA was significantly higher in Maxifort grafted plants compared to ungrafted tomato plants (Table S9). In the sixth truss, the concentration of PFBA accumulated by Dynafort was significantly higher than Maxifort and the ungrafted control, whereas the PFPeA amount was significantly lower in the ungrafted variety with respect to Dynafort rootstock combination (Table S9).

We hypothesized that the concentration of the short-chain PFAS below the LOQ values could be due to the fact that the soil was not irrigated with polluted water for many years before the beginning of the experiment, and the high mobility of the short-chain PFAS in the study area characterized by relatively humid climate which could have induced short-chain PFAS leaching from the soil. Differently, the PFAS with longer chains were retained in the soil owing to their lower environmental mobility.

3.5. Dietary exposure assessment

The health risk values associated with the consumption of raw PFAS-contaminated tomatoes for each tomato variety are reported in Table 3. The dietary risk assessment based on the average daily consumption of raw tomatoes by the Italian adult population (18-64 years) was conducted, and the associated non-carcinogenic chronic risk calculated.

Table 3
Target Hazard Quotient (THQ) of individual PFAS and Hazard Index (HI) calculated for the consumption of first truss tomato fruits of each variety.

Rootstock combination	THQ PFBA	THQ PFPeA	THQ PFHxA	HI
Dynafort	0.605	0.385	0.044	1.03
Optifort	0.605	0.392	0.043	1.04
Maxifort	0.440	0.333	0.082	0.86
Ungrafted	0.254	0.215	0.032	0.50

Values of THQ were highest for PFBA for fruits of all tomato varieties, compared to values of PFPeA and PFHxA (Table 3). Dynafort and Optifort rootstock combinations showed similar THQ values, the Maxifort showed intermediate values, whereas fruits from the ungrafted plants scored the lowest values (Table 3). The HI values associated with the consumption of tomatoes of Dynafort and Optifort rootstock combinations showed values slightly higher than 1, whereas the HI values associated with fruits of the Maxifort and ungrafted plants were 0.86 and 0.50, respectively.

4. Discussion

4.1. PFAS uptake, translocation, and accumulation in plant organs

The analysis of the water used for the tomato plants irrigation showed PFAS concentrations comparable to those detected in the groundwater from the surrounding wells monitored in the institutional environmental survey (Table 1). Nevertheless, the soil where the cultivation field trial was conducted showed accumulation of only PFOA, PFOS, and PFDA, at concentrations comparable to those previously reported for the soils of the study area by the Environmental Protection Agency of the Veneto Region (ARPAV, 2020). No accumulation of shorter chain PFAS in the studied soil confirms their low tendency to accumulate in soil (Brusseau et al., 2020), and this could also be due to the fact that the water of the polluted well was not used for a few years before the establishment of the field trial. In this period, the shorter chain PFAS could have been lost through seepage into groundwater due to the high mean precipitations in the area, whereas the calcareous nature of the soil may have caused the retention of the longer chain PFAS, as reported by Cai et al. (2022).

The PFAS accumulation in tomato leaves paralleled the PFAS profile of the irrigation water since those compounds were likely absorbed through the water mass flow driven by the plant's evapotranspiration potential. Accumulation of PFAS in leaves of various agricultural plants has been previously reported (Ghisi et al., 2019; Zhou et al., 2021), and the results of our study confirm that PFAS are taken up from the soil solution (Zhao et al., 2018). In particular, the shortest and most hydrophilic PFAS tend to accumulate to a higher extent than the long-chain compounds (Lesmeister et al., 2021), which are often associated with the plant root apparatus (Lin et al., 2020). However, while the accumulation of PFAS in plants has been mainly attributed to its concentration in soils (Wen et al., 2014; Lesmeister et al., 2021), our results demonstrated that PFAS accumulation in leaves could be significantly correlated to the use of polluted irrigation water, with no apparent retention or selection operated by the soil. Our results confirmed those reported by McDonough et al. (2021), who monitored the PFAS plant uptake after experimental additions to river waters, and reported that their movement in the water phase accounted for a significant share of PFAS uptake by tomato plants.

Our results also provided insights into the movement and partition of PFAS across plant tissues. In fact, their concentrations in the leaves increased with the increasing truss height, whereas the opposite trend was observed for fruits, where only C4-C6 carboxylic PFAS were detected. For example, PFOS and PFDA were present in comparable amounts both in soil and irrigation water, however, they were not translocated up to leaves or fruits in significant quantities. Differently, PFOA, which was also detected in both soil and water, even if in considerably higher concentrations, accumulated in a relevant amount in the leaves, but not in the fruits. Short-chain compounds (up to PFHpA) were all present in the irrigation water, and a positive correlation was observed between the initial concentration in the water and their amounts in the plant tissues. Although PFHxS content in the irrigation was comparable to PFHpA, it was found neither in the leaves nor in the fruits of any tomato variety. PFPeA was detected only in the fruits but not in the leaves, even if its amount in the irrigation water was remarkable and it has been found to accumulate in the leaves of other plant species (Ghisi et al., 2019; Zhou et al., 2021).

The PFAS distribution and translocation in horticultural plants occur via apoplastic, symplastic, or transmembrane pathways (Miller et al., 2016). In the case of PFAS, it has been reported that transport mechanisms of different perfluorinated compounds vary depending on the plant species (Mei et al., 2021), and hydroponic experiments conducted with lettuce, tomato, zucchini, and cabbage have shown that PFAS uptake by roots relies on two mechanisms: (i) uptake through the transpiration stream, particularly for short-chain compounds, and (ii) sorption to root surface tissue for long-chain PFAS (Felizeter et al., 2012; Felizeter et al., 2014). Though PFAS translocation in plants commonly occurs via both passive and active mechanisms (Mei et al., 2021), research conducted on maize (Wen et al., 2014), wheat (Zhang et al., 2019), *Alisma orientale* (Wang et al., 2020), *Chrysanthemum coronarium*, cabbage, and cucumber (Gu et al., 2023), showed that aquaporins and ionic channels could represent PFAS entry points as specific inhibitors for aquaporins and for anion channels reduced PFAS uptake in various plants (Wen et al., 2014; Zhang et al., 2019; Wang et al., 2020; Gu et al., 2023). Our findings are in line with previous results of PFAS distribution in leaves and fruits and suggest that diverse transporters are responsible for the selective entry of different perfluorinated compounds, acting at the Casparian strip in roots for entering the xylem stream. Moreover, a thick tap root system typical of tomato might allow larger contaminants to cross the epidermis into the apoplast, being retained at the root level, as suggested by Blaine et al. (2014).

In our experiment, PFBA, PFBS, PFHxA, PFHpA, and PFOA were found in the leaves, whilst PFBA, PFPeA, and PFHxA only were detected in the fruits. Based on the available literature data and our results, we hypothesize that differences in detected PFAS between leaves and fruits could be explained by considering the different pathways followed by nutrients to reach the target plant organs. In the case of leaves, PFAS entered into roots are translocated to leaves through the transpiration stream across the xylem, leading to their accumulation as the water evaporates (Felizeter et al., 2014). In the fruits, the transpiration stream is low as the nutrients for their development and ripening are transported via phloem sap, and the phloem uploading requires specific transporters in the companion cells, which could be more selective for shorter and more hydrophilic compounds. This hypothesis could explain why PFPeA, but not PFBS, was translocated to fruits, as the sulfonic group of the latter makes the molecule more hydrophobic compared to PFPeA.

Future research should test and validate the proposed transport mechanisms for PFAS compounds, to better predict the potential agri-food contamination produced in PFAS-polluted environments.

4.2. Impact of tomato variety on PFAS uptake

While plant grafting is an ever-increasing used biotechnology for improving plant yields and quality and concerns ca. 50 % of tomato cultivation in Europe and Asia (Raymond, 2013), to our best knowledge this is the first report focusing on the effect of plant rootstock on the accumulation of PFAS. Our results showed significant increases in PFAS uptake in grafted tomato plants owing to their greater vigor, in contrast to previous results showing that grafting could reduce the uptake of metallic contaminants including As, Cd, and Ni (Savvas et al., 2013; Stazi et al., 2016; Kumar et al., 2015a; Kumar et al., 2015b; Yuan et al.,

2019; Xie et al., 2020). The Maxifort rootstock, characterized by high vigor, significantly increased the content of PFBS, PFHpA, and PFOA in the leaves compared to other grafting combinations and the control. These responses can be attributed to the rootstock's greater exploratory capacity, as reported by Kumar et al. (2017) and Maurya et al. (2019). This aspect allowed for an enhanced ability to intercept PFAS from the soil and water supplied through irrigation. The accumulation gradient is evident when comparing the Dynafort and Maxifort rootstocks for many perfluoroalkyl compounds. Only the concentration of PFBA and PFHxA in the sixth truss fruits did not significantly differ among the rootstocks. This behavior may be due to a physiological translocation effect in the plant's distal tissues, which are more active during the crop cycle.

Within the fruits, only PFHxA concentration showed an accumulation gradient between Dynafort and Maxifort, whereas for the other identified compounds responses varied depending on the complexity of the involved molecule. Generally, longer chain PFAS showed reduced accumulation in the sixth truss compared to the first truss, which resulted in a more easily reachable metabolic sink. The greater mobility of short-chain PFAS has also been reported for other leafy vegetables such as lettuce and red chicory (Adu et al., 2023; Gredelj et al., 2020a; Gredelj et al., 2020b). Overall, our results of PFAS accumulation and profile confirm that rootstocks conferring greater plant vigor increase root uptake (Wang et al., 2016) as compared to non-grafted plants.

4.3. Assessment of health risk

The evaluation of the health risk related to ingestion of PFAS-contaminated raw tomatoes assessed by the THQ and HI values showed potential hazards for the Italian adult population based on the EFSA data on average daily intake of raw tomato fruits. The PFAS impacts on human health are increased risk of thyroid disruption (Mokra, 2021), liver toxicity (Nian et al., 2019), immunotoxicity (Ehrlich et al., 2023), reproductive impairment (Chambers et al., 2021), and neurodevelopment adverse outcomes (Luo et al., 2022). Most of the research focused on PFOA and PFOS, since they are the most previously used and abundant compounds that occur in the environment and biota, whereas less is known about the short-chain PFAS toxicity. Because of the high probability of co-occurrence, and consequently co-exposure, potential harmful effects of short- and long-chain perfluorinated compounds have been evaluated together in most cases (Chambers et al., 2021). Similarly, we searched for the reference dose value of the detected PFAS compounds on the EFSA archive, however, no data are available since the PFAS tolerable weekly intake (TWI) considered by the EFSA refers to PFOA, PFNA, PFOS, and PFHxS only. However, as shown in this work and in previously published data, these four long-chain compounds are less translocated to the epigeal part of plants, especially in fruits, and even less if they contain a sulfonic group (PFOS and PFHxS). Thus, according to the current EFSA guidelines, the consumption of tomato fruits obtained by our field trial could cause no risks to human health, whereas a potential risk was highlighted by considering the concentration of short-chain compounds such as PFBA, PFPeA, and PFHxA in tomato fruits. We therefore suggest adopting the reference dose values reported by the US EPA's IRIS Toxicological Reviews, to estimate the risks for human health more realistically.

5. Conclusions

The presented work confirmed that tomato plants take up and concentrate various PFAS in their leaves and fruits when cultivated in PFAS-polluted sites. Leaf concentrations of PFAS paralleled those of the irrigation water, with no apparent 'filtering' effects by the soil. Fruits accumulated mainly C4-C6 carboxylic PFAS, whereas neither longer chain nor sulfonated PFAS were translocated to fruits, allowing us to hypothesize specific control mechanisms exerted by the phloem loading cells. Grafted tomato varieties characterized by higher vigor accumulated the same PFAS as the non-grafted variety, but at generally higher concentrations owing to their water uptake and soil exploration potential. Unlike the case of potentially toxic elements, these results showed that grafting could not reduce the absorption capacity of PFAS.

The health risk assessment unveiled that the produced tomato could be considered 'safe-to-eat' according to the current EFSA guidelines on PFAS TWI since PFOA, PFNA, PFOS, and PFHxS were not detected in the fruits. However, by considering the impact of PFBA and PFHxA based on the IRIS Toxicological Reviews, the results of this work demonstrated that the health risks derived from the consumption of the tomato produced in the field trial could not be considered absent. We suggest updating the current guidelines by including more mobile and short-chain PFAS that can accumulate at high concentrations in cropped plants cultivated in polluted environments, to improve the level of health protection of populations living in PFAS-contaminated areas.

CRediT authorship contribution statement

Ilaria Battisti: Formal analysis, Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing. **Anna Rita Trentin:** Investigation, Resources, Validation. **Emma Franzolin:** Investigation. **Carlo Nicoletto:** Methodology, Resources, Writing – original draft, Writing – review & editing. **Antonio Masi:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **Giancarlo Renella:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

Adams, K.J., Pratt, B., Bose, N., Dubois, L.G., St John-Williams, L., Perrott, K.M., Ky, K., Kapahi, P., Sharma, V., MJ, MacCoss, Moseley, M.A., Colton, C.A., BX, MacLean, Schilling, B., Thompson, J.W., Alzheimer's Disease Metabolomics Consortium, 2020 Apr 3. Skyline for small molecules: a unifying software package for quantitative metabolomics. J. Proteome Res. 19 (4), 1447–1458. https://doi.org/10.1021/acs.jproteome.9b00640.

Adu, O., Ma, X., Sharma, V.K., 2023 Apr 5. Bioavailability, phytotoxicity and plant uptake of per-and polyfluoroalkyl substances (PFAS): a review. J. Hazard. Mater. 447, 130805. https://doi.org/10.1016/j.jhazmat.2023.130805.

Alsafra, Z., Scholl, G., De Meulenaer, B., Eppe, G., Saegerman, C., 2022 Aug 14. Hazard ratio and hazard index as preliminary estimators associated to the presence of furans and alkylfurans in Belgian foodstuffs. Foods 11 (16), 2453. https://doi.org/10.3390/foods11162453.

ARPAV, 2020. Inquinamento da sostanze perfluoralchiliche (PFAS). Approfondimento in merito alla presenza di PFAS nei suoli e nei sedimenti delle zone interessate dalla contaminazione delle acque superficiali. In: Giandon, P., Rosa, A. Dalla, Obber, S. (Eds.), Environmental Protection Agency of Veneto Region. Available at. https://www.arpa.veneto.it/temi-ambientali/suolo/file-e-allegati/relazione-suoli–pfas-2020.pdf/@@display-file/file. Accessed on July 25, 2023.

ARPAV, 2023. Concentration of perfluoroalkyl substances (PFAS) in waters sampled by the Environmental Protection Agency of Veneto Region (ARPAV). Database available at. https://www.arpa.veneto.it/dati-ambientali/open-data/idrosfera/concentrazione-di-sostanze-perfluoroalchiliche-pfas-nelle-acque-prelevate-da-arpav. Accessed on July 25, 2023.

Bao, J., Yu, W.J., Liu, Y., Wang, X., Jin, Y.H., Dong, G.H., 2019 Apr 30. Perfluoroalkyl substances in groundwater and home-produced vegetables and eggs around a fluorochemical industrial park in China. Ecotoxicol. Environ. Saf. (171), 199–205. https://doi.org/10.1016/j.ecoenv.2018.12.086.

Blaine, A.C., Rich, C.D., Hundal, L.S., Lau, C., Mills, M.A., Harris, K.M., Higgins, C.P., 2013 Dec 17. Uptake of perfluoroalkyl acids into edible crops via land applied biosolids: field and greenhouse studies. Environ. Sci. Technol. 47 (24), 14062–14069. https://doi.org/10.1021/es403094q.

Blaine, A.C., Rich, C.D., Sedlacko, E.M., Hundal, L.S., Kumar, K., Lau, C., Mills, M.A., Harris, K.M., Higgins, C.P., 2014 Jul 15. Perfluoroalkyl acid distribution in various plant compartments of edible crops grown in biosolids-amended soils. Environ. Sci. Technol. 48 (14), 7858–7865. https://doi.org/10.1021/es500016s.

Brusseau, M.L., Anderson, R.H., Guo, B., 2020 Oct 20. PFAS concentrations in soils: background levels versus contaminated sites. Sci. Total Environ. 740, 140017 https://doi.org/10.1016/j.scitotenv.2020.140017.

Cai, W., Navarro, D.A., Du, J., Ying, G., Yang, B., McLaughlin, M.J., Kookana, R.S., 2022 Apr 15. Increasing ionic strength and valency of cations enhance sorption through hydrophobic interactions of PFAS with soil surfaces. Sci. Total Environ. (817), 152975 https://doi.org/10.1016/j.scitotenv.2022.152975.

Chambers, W.S., Hopkins, J.G., Richards, S.M., 2021 Nov 22. A review of per- and polyfluorinated alkyl substance impairment of reproduction. Front. Toxicol. (3), 732436 https://doi.org/10.3389/ftox.2021.732436.

EEA, 2023. Cross-Cutting Story 3: PFAS. European Environmental Agency. Available at. https://www.eea.europa.eu/publications/zero-pollution/cross-cutting-stories/pfas.

Ehrlich, V., Bil, W., Vandebriel, R., Granum, B., Luijten, M., Lindeman, B., Grandjean, P., Kaiser, A.M., Hauzenberger, I., Hartmann, C., Gundacker, C., Uhl, M., 2023 Feb 22. Consideration of pathways for immunotoxicity of per- and polyfluoroalkyl substances (PFAS). Environ. Health 22 (1), 19. https://doi.org/10.1186/s12940-022-00958-5.

European Commission''s Agricultural and Rural Development Portal, Tomatoes Statistics, 2023. Available at. https://agriculture.ec.europa.eu/data-and-analysis/markets/overviews/market-observatories/fruit-and-vegetables/tomatoes-statistics_en. Accessed on July 28, 2023.

Felizeter, S., McLachlan, M.S., de Voogt, P., 2012 Nov 6. Uptake of perfluorinated alkyl acids by hydroponically grown lettuce (Lactuca sativa). Environ. Sci. Technol. 46 (21), 11735–11743. https://doi.org/10.1021/es302398u.

Felizeter, S., McLachlan, M.S., De Voogt, P., 2014 Apr 16. Root uptake and translocation of perfluorinated alkyl acids by three hydroponically grown crops. J. Agric. Food Chem. 62 (15), 3334–3342. https://doi.org/10.1021/jf500674j.

Fenton, S.E., Ducatman, A., Boobis, A., DeWitt, J.C., Lau, C., Ng, C., Smith, J.S., Roberts, S.M., 2021 Mar. Per- and polyfluoroalkyl substance toxicity and human health review: current state of knowledge and strategies for informing future research. Environ. Toxicol. Chem. 40 (3), 606–630. https://doi.org/10.1002/etc.4890.

García-Valcarcel, A.I., Miguel, E., Tadeo, J.L., 2012 Jun. Determination of ten perfluorinated compounds in sludge amended soil by ultrasonic extraction and liquid chromatography-tandem mass spectrometry. Anal. Methods 4, 2462–2468. https://doi.org/10.1039/C2AY25387A.

Gewurtz, S.B., Bradley, L.E., Backus, S., Dove, A., McGoldrick, D., Hung, H., Dryfhout-Clark, H., 2019 Aug 6. Perfluoroalkyl acids in great lakes precipitation and surface water (2006-2018) indicate response to phase-outs, regulatory action, and variability in fate and transport processes. Environ. Sci. Technol. 53 (15), 8543–8552. https://doi.org/10.1021/acs.est.9b01337.

Ghisi, R., Vamerali, T., Manzetti, S., 2019 Feb. Accumulation of perfluorinated alkyl substances (PFAS) in agricultural plants: a review. Environ. Res. 169, 326–341. https://doi.org/10.1016/j.envres.2018.10.023.

Gredelj, A., Nicoletto, C., Polesello, S., Ferrario, C., Valsecchi, S., Lava, R., Barausse, A., Zanon, F., Palmeri, L., Guidolin, L., Bonato, M., 2020 Jun 10. Uptake and translocation of perfluoroalkyl acids (PFAAs) in hydroponically grown red chicory (Cichorium intybus L.): growth and developmental toxicity, comparison with growth in soil and bioavailability implications. Sci. Total Environ. 720, 137333 https://doi.org/10.1016/j.scitotenv.2020.137333.

Gredelj, A., Nicoletto, C., Valsecchi, S., Ferrario, C., Polesello, S., Lava, R., Zanon, F., Barausse, A., Palmeri, L., Guidolin, L., Bonato, M., 2020 Mar 15. Uptake and translocation of perfluoroalkyl acids (PFAA) in red chicory (Cichorium intybus L.) under various treatments with pre-contaminated soil and irrigation water. Sci. Total Environ. 708, 134766 https://doi.org/10.1016/j.scitotenv.2019.134766.

Gu, Q., Wen, Y., Wu, H., Cui, X., 2023 Mar 1. Uptake and translocation of both legacy and emerging per- and polyfluorinated alkyl substances in hydroponic vegetables. Sci. Total Environ. 862, 160684 https://doi.org/10.1016/j.scitotenv.2022.160684.

Helsel, D.R., 2012. Statistics for Censored Environmental Data Using MINITAB® and R, 2nd edition. John Wiley & Sons.

Kavcar, P., Sofuoglu, A., Sofuoglu, S.C., 2009 Mar. A health risk assessment for exposure to trace metals via drinking water ingestion pathway. Int. J. Hyg. Environ. Health 212 (2), 216–227. https://doi.org/10.1016/j.ijheh.2008.05.002.

Khan, U.M., Sevindik, M., Zarrabi, A., Nami, M., Ozdemir, B., Kaplan, D.N., Selamoglu, Z., Hasan, M., Kumar, M., Alshehri, M.M., Sharifi-Rad, J., 2021 Nov 19. Lycopene: food sources, biological activities, and human health benefits. Oxid. Med. Cell. Longev. 2021, 2713511. https://doi.org/10.1155/2021/2713511.

Koch, A., Aro, R., Wang, T., Yeung, L.W.Y., 2020. Towards a comprehensive analytical workflow for the chemical characterisation of organofluorine in consumer products and environmental samples. Trends Anal. Chem. 123, 115423 https://doi.org/10.1016/j.trac.2019.02.024.

Kumar, P., Edelstein, M., Cardarelli, M., Ferri, E., Colla, G., 2015a. Grafting affects growth, yield, nutrient uptake, and partitioning under cadmium stress in tomato. HortScience 50 (11), 1654–1661. https://doi.org/10.21273/HORTSCI.50.11.1654.

Kumar, P., Rouphael, Y., Cardarelli, M., Colla, G., 2015b. Effect of nickel and grafting combination on yield, fruit quality, antioxidative enzyme activities, lipid peroxidation, and mineral composition of tomato. J. Plant Nutr. Soil Sci. 178 (6), 848–860. https://doi.org/10.1002/jpln.201400651.

Kumar, P., Rouphael, Y., Cardarelli, M., Colla, G., 2017 Jun 30. Vegetable grafting as a tool to improve drought resistance and water use efficiency. Front. Plant Sci. 8, 1130. https://doi.org/10.3389/fpls.2017.01130.

Kyriacou, M.C., Colla, G., Rouphael, Y., 2020. Grafting as a sustainable means for securing yield stability and quality in vegetable crops. Agronomy 10 (12), 1945. https://doi.org/10.3390/agronomy10121945.

Lesmeister, L., Lange, F.T., Breuer, J., Biegel-Engler, A., Giese, E., Scheurer, M., 2021 Apr 20. Extending the knowledge about PFAS bioaccumulation factors for agricultural plants - a review. Sci. Total Environ. 766, 142640 https://doi.org/10.1016/j.scitotenv.2020.142640.

Lin, Q., Zhou, C., Chen, L., Li, Y., Huang, X., Wang, S., Qiu, R., Tang, C., 2020 Jun. Accumulation and associated phytotoxicity of novel chlorinated polyfluorinated ether sulfonate in wheat seedlings. Chemosphere 249, 126447. https://doi.org/10.1016/j.chemosphere.2020.126447.

Luo, F., Chen, Q., Yu, G., Huo, X., Wang, H., Nian, M., Tian, Y., Xu, J., Zhang, J., Zhang, J., Cohort, Shanghai Birth, 2022 Jul 7. Exposure to perfluoroalkyl substances and neurodevelopment in 2-year-old children: a prospective cohort study. Environ. Int. 166, 107384 https://doi.org/10.1016/j.envint.2022.107384.

Mahinroosta, R., Senevirathna, L., Li, M., KrishnaPillai, K., 2021 Mar. A methodology for transport modelling of a contaminated site with perfluorooctane sulfonate due to climate interaction. Process. Saf. Environ. Prot. 147, 642–653. https://doi.org/10.1016/j.psep.2020.12.034.

Maurya, D., Pandey, A.K., Kumar, V., Dubey, S., Prakash, V., 2019. Grafting techniques in vegetable crops: a review. Int. J. Chem. Stud. 7 (2), 1664–1672.

McDonough, A.M., Bird, A.W., Freeman, L.M., Luciani, M.A., Todd, A.K., 2021 Sep 15. Fate and budget of poly- and perfluoroalkyl substances in three common garden plants after experimental additions with contaminated river water. Environ. Pollut. 285, 117115 https://doi.org/10.1016/j.envpol.2021.117115.

Mei, W., Sun, H., Song, M., Jiang, L., Li, Y., Lu, W., Ying, G.G., Luo, C., Zhang, G., 2021 Nov. Per- and polyfluoroalkyl substances (PFASs) in the soil-plant system: sorption, root uptake, and translocation. Environ. Int. 156, 106642 https://doi.org/10.1016/j.envint.2021.106642.

Miller, E.L., Nason, S.L., Karthikeyan, K.G., Pedersen, J.A., 2016 Jan 19. Root uptake of pharmaceuticals and personal care product ingredients. Environ. Sci. Technol. 50 (2), 525–541. https://doi.org/10.1021/acs.est.5b01546.

Mokra, K., 2021 Feb 21. Endocrine disruptor potential of short- and long-chain perfluoroalkyl substances (PFASs)-a synthesis of current knowledge with proposal of molecular mechanism. Int. J. Mol. Sci. 22 (4), 2148. https://doi.org/10.3390/ijms22042148.

Nian, M., Li, Q.Q., Bloom, M., Qian, Z.M., Syberg, K.M., Vaughn, M.G., Wang, S.Q., Wei, Q., Zeeshan, M., Gurram, N., Chu, C., Wang, J., Tian, Y.P., Hu, L.W., Liu, K.K., Yang, B.Y., Liu, R.Q., Feng, D., Zeng, X.W., Dong, G.H., 2019 May. Liver function biomarkers disorder is associated with exposure to perfluoroalkyl acids in adults: isomers of C8 health project in China. Environ. Res. 172, 81–88. https://doi.org/10.1016/j.envres.2019.02.013.

Raschke, A., Pouyan Nejadhashemi, A., Rafiei, V., Fernandez, N., Shabani, A., Li, S.G., Asce, F., 2022 Jul. Opportunities and challenges of integrated large-scale PFAS modeling: a case study for PFAS modeling at a watershed scale. J. Environ. Eng. 148 (9), 05022005. https://doi.org/10.1061/(ASCE)EE.1943-7870.0002034.

Raymond, G., 2013. Grafting Market Developments. Available at. http://www.vegetablegrafting.org/wp/wp-content/uploads/2013/11/session-4-raymond-scri-vege-grftg-symp-nov13.pdf. Accessed on August 21, 2023.

RStudio Team, 2020. RStudio: Integrated development for R. RStudio, PBC, Boston, MA. Available at. https://posit.co/products/open-source/rstudio/.

Savvas, D., Ntatsi, G., Barouchas, P., 2013 Jan. Impact of grafting and rootstock genotype on cation uptake by cucumber (Cucumis sativus L.) exposed to Cd or Ni stress. Sci. Hortic. 149, 86–96.

Sharma, N., Barion, G., Shrestha, I., Ebinezer, L.B., Trentin, A.R., Vamerali, T., Mezzalira, G., Masi, A., Ghisi, R., 2020 Mar 15. Accumulation and effects of perfluoroalkyl substances in three hydroponically grown *Salix* L. species. Ecotoxicol. Environ. Saf. 191, 110150 https://doi.org/10.1016/j.ecoenv.2019.110150.

Shimizu, M.S., Mott, R., Potter, A., Zhou, J., Baumann, K., Surratt, J.D., Turpin, B., Avery, G.B., Harfmann, J., Kieber, R.J., 2021 Apr. Atmospheric deposition and annual flux of legacy perfluoroalkyl substances and replacement perfluoroalkyl ether carboxylic acids in Wilmington, NC, USA. Environ. Sci. Technol. Lett. 8 (5), 366–372. https://doi.org/10.1021/acs.estlett.1c00251.

Stazi, S.R., Cassaniti, C., Marabottini, R., Giuffrida, F., Leonardi, C., 2016. Arsenic uptake and partitioning in grafted tomato plants. Hortic. Environ. Biotechnol. 57, 241–247. https://doi.org/10.1007/s13580-016-0036-6.

Sunderland, E.M., Hu, X.C., Dassuncao, C., Tokranov, A.K., Wagner, C.C., Allen, J.G., 2019 Mar. A review of the pathways of human exposure to poly- and perfluoroalkyl

substances (PFASs) and present understanding of health effects. J. Expo. Sci. Environ. Epidemiol. 29 (2), 131–147. https://doi.org/10.1038/s41370-018-0094-1.

Wang, Y., Thorup-Kristensen, K., Jensen, L.S., Magid, J., 2016 Jun 16. Vigorous root growth is a better indicator of early nutrient uptake than root hair traits in spring wheat grown under low fertility. Front. Plant Sci. 7, 865. https://doi.org/10.3389/fpls.2016.00865.

Wang, T.T., Ying, G.G., He, L.Y., Liu, Y.S., Zhao, J.L., 2020 Sep 1. Uptake mechanism, subcellular distribution, and uptake process of perfluorooctanoic acid and perfluorooctane sulfonic acid by wetland plant Alisma orientale. Sci. Total Environ. 733, 139383 https://doi.org/10.1016/j.scitotenv.2020.139383.

Wen, B., Li, L., Zhang, H., Ma, Y., Shan, X.Q., Zhang, S., 2014 Jan. 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. https://doi.org/10.1016/j.envpol.2013.09.040.

Xie, Y., Tan, H., Sun, G., Li, H., Liang, D., Xia, H., Wang, X., Lao, M., Deng, H., Wang, J., Tang, Y., 2020. Grafting alleviates cadmium toxicity and reduces its absorption by tomato. J. Soil Sci. Plant Nutr. 20, 2222–2229. https://doi.org/10.1007/s42729-020-00289-9.

Yuan, H., Sun, L., Tai, P., Liu, W., Li, X., Hao, L., 2019 Feb 20. Effects of grafting on root-to-shoot cadmium translocation in plants of eggplant (Solanum melongena) and tomato (Solanum lycopersicum). Sci. Total Environ. 652, 989–995. https://doi.org/10.1016/j.scitotenv.2018.10.129.

Yuan, H., Tai, P., Gustave, W., Xue, F., Sun, L., 2021. Grafting as a mitigation strategy to reduce root-to-shoot cadmium translocation in plants of Solanaceae family. J. Clean. Prod. 319, 128708 https://doi.org/10.1016/j.jclepro.2021.128708.

Zhang, C., McElroy, A.C., Liberatore, H.K., Alexander, N.L.M., Knappe, D.R.U., 2022 May 17. Stability of per- and polyfluoroalkyl substances in solvents relevant to environmental and toxicological analysis. Environ. Sci. Technol. 56 (10), 6103–6112. https://doi.org/10.1021/acs.est.1c03979.

Zhang, L., Sun, H., Wang, Q., Chen, H., Yao, Y., Zhao, Z., Alder, A.C., 2019. Uptake mechanisms of perfluoroalkyl acids with different carbon chain lengths (C2-C8) by wheat (Triticum acstivnm L.). Sci. Total Environ. 654, 19–27. https://doi.org/10.1016/j.scitotenv.2018.10.443.

Zhao, H., Guan, Y., Qu, B., 2018 Jan 2. PFCA uptake and translocation in dominant wheat species (Triticum aestivum L.). Int. J. Phytoremediation 20 (1), 68–74. https://doi.org/10.1080/15226514.2017.1337066.

Zhou, Y., Zhou, Z., Lian, Y., Sun, X., Wu, Y., Qiao, L., Wang, M., 2021 Jul 1. Source, transportation, bioaccumulation, distribution and food risk assessment of perfluorinated alkyl substances in vegetables: a review. Food Chem. 349, 129137 https://doi.org/10.1016/j.foodchem.2021.129137.