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Perfluoroalkyl Substances Accumulation in Lettuce: Effects of Cultivar, Growth Stage, and Cultivation Conditions on Food Safety

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Abstract

Poly- and perfluoroalkyl substances (PFAS) are environmentally persistent contaminants that pose growing food safety concerns due to their potential for accumulation in edible crops. This study investigated the uptake, translocation, and tissue distribution of 11 PFAS compounds in two hydroponically grown lettuce (*Lactuca sativa* L.) cultivars, Agila and Bonaly. Additionally, PFAS accumulation in Agila was assessed under field conditions in a PFAS-contaminated area. Under hydroponic conditions, lettuce plants at two developmental stages (28 and 56 days after sowing) were exposed to a mixture of PFAS at concentrations of 10 and 20 μg L^{-1} each. Under such conditions, Agila cultivar accumulated considerably higher levels of long-chain PFAS in both root and leaf tissues over time, whereas Bonaly cultivar demonstrated a more pronounced initial uptake and translocation of short-chain PFAS to leaves. Differently, Agila variety cultivated in a PFAS-polluted environment accumulated low concentrations of PFAS in leaf tissues, with only PFBA detected at minimal levels. The results emphasize the combined influence of plant variety, developmental stage, and cultivation methods on PFAS bioaccumulation, offering valuable guidance for food safety risk assessment and for developing targeted agricultural strategies in PFAS-contaminated areas.

Keywords: PFAS; *Lactuca sativa*; plant uptake; translocation; bioaccumulation; food safety; hydroponics; soil

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1. Introduction

Poly- and perfluoroalkyl substances (PFAS) constitute a class of more than 4700 synthetic compounds that are partially or fully fluorinated [1]. Starting from the 1950s, PFAS have been employed in the production of perfluorinated polymers, lubricants, paints, pharmaceuticals, healthcare products, cosmetics, and firefighting foams, as well as emulsifiers and surfactants attributable to their unique hydrophobic and oleophobic characteristics [2].

PFAS are characterized by strong carbon-fluorine (C-F) bonds possessing a dissociation energy of approximately 502 kJ mol^{-1}, one of the strongest chemical bonds in nature, and by the presence of various functional groups (e.g., carboxyl, sulfonic, hydroxyl) linked to the carbon chain [3]. The increasing PFAS use in industrial applications, and their environmental stability and mobility, have raised significant concern due to their

exceptional chemical stability, which makes them resistant to hydrolysis, photolysis, and biodegradation. This persistence allows PFAS to remain in the environment for extended periods, leading to their accumulation in living organisms and resulting in widespread xenobiotic contamination. Consequently, these compounds have received attention for their potential toxicity to human health and ecosystems [4].

Apart from occupational exposure, the predominant pathways of human exposure to PFAS are the ingestion of contaminated water, plant- and animal-based foods. This occurs as crops and livestock absorb PFAS from polluted soil or irrigation sources [5,6]. In polluted areas, additional sources of PFAS to crop plants are biosolids used as soil amendments and fertilizers, which lead to PFAS enrichment in soil [7].

PFAS uptake by largely consumed crop plants is of concern because epidemiological studies have established associations between exposure to PFAS and adverse health effects, primarily indicating an elevated risk of thyroid dysfunction, disruptions in lipid and insulin levels, hypercholesterolemia, immunotoxicity, diseases of the liver and kidneys, cancer, and unfavorable reproductive and developmental consequences [7–9]. The European Food Safety Authority (EFSA) has recognized these risks, establishing a tolerable weekly intake (TWI) of 4.4 ng kg^{-1} of body weight per week for four bioaccumulative compounds, including perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid, perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) [10].

The Veneto Region, in North-East Italy, is impacted by one of the largest known PFAS contamination areas [11,12], in which the surface water and groundwater display complex contamination profiles in an area of ca. 200 km^2, mostly under agricultural use. Since 2013, environmental monitoring programs have been showing that the PFAS concentrations in waters have levelled off to new baseline concentrations, particularly long-chain compounds such as PFOA and PFOS [11], triggering stringent regulatory measures such as the filtration of drinking water with granular activated carbon (GAC) and the limitation of groundwater from private sources [13]. Despite these interventions, concerns persist regarding the transfer of PFAS into the food chain, particularly via locally cultivated crops irrigated with polluted or marginally treated water sources.

It is well established that the degree to which different PFAS accumulate in crops is determined by their molecular carbon chain length, functional group, the plant species, and the mode of environmental exposure [14]. Key plant traits influencing PFAS uptake include the root architecture, leaf morphology, growth duration, and tissue composition (e.g., water, lipid, and protein contents) [5,15], while the main soil properties that influence PFAS mobility and uptake are the organic matter (OM) content, texture, cation exchange capacity, and the pH value [5,14–16]. Plant and soil factors interact, creating site-specific exposure conditions responsible for the broad range of bio-concentration factors (BCFs) reported in the scientific literature [5], making it difficult to understand the prevailing factors leading to PFAS in soil/plant systems.

Lettuce (*Lactuca sativa* L.) is among the most consumed leafy vegetables, characterized by a short growth cycle, substantial water content, and accelerated nutrient absorption [17–19], and is globally cultivated in soil, substrate, and hydroponic systems. As a relatively fast-growing and relatively highly bio-accumulative plant, lettuce is also used as a model plant for assessing the potential phytoavailability of inorganic and organic contaminants and human dietary exposure risks. Although PFAS uptake in lettuce is well documented [20–22], limited data are available regarding the relative influence of cultivar, plant developmental stage, and cultivation system.

We hypothesized that (i) distinct lettuce cultivars absorb and accumulate PFAS differently when grown in hydroponics, (ii) PFAS uptake and translocation could differ between plants of different ages, and (iii) the PFAS accumulation profile of a lettuce species could

be different when grown in hydroponics or in soil. To test these hypotheses, we conducted controlled hydroponic experiments with two lettuce varieties and a field trial in PFAS-polluted soil. The findings from this study can provide new insights into PFAS behavior in lettuce cultivation and inform risk assessments for leafy vegetable consumption in contaminated regions.

2. Materials and Methods

2.1. Chemicals

Perfluorobutanoic acid (PFBA), perfluorobutane sulfonic acid (PFBS), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), and perfluorododecanoic acid (PFDoA) were used for the experiment (Sigma Aldrich, St. Louis, MS, USA). Isotopically-labeled standards ($^{13}C_4$-PFBA, $^{13}C_3$-PFBS, $^{13}C_5$-PFPeA, $^{13}C_6$-PFHxA, $^{13}C_4$-PFHpA, $^{13}C_8$-PFOA, $^{13}C_8$-PFOS, $^{13}C_9$-PFNA, $^{13}C_6$-PFDA, $^{13}C_7$-PFUnA, and $^{13}C_2$-PFDoA) were purchased from Wellington Laboratories (Guelph, ON, Canada) and employed as internal standards (IS) for mass spectrometry analysis.

2.2. Plant Growth and Treatment in Hydroponics

Hydroponic experiments were conducted in a growth chamber with the following environmental conditions: 25 ± 1 °C, 12-hour photoperiod, 250 µmol m^{-2} s^{-1} light intensity, and 40% relative humidity. Two cultivars of lettuce (*Lactuca sativa* L.), Agila and Bonaly, were selected for the study due to their agronomic relevance and different morphologies. Seed germination was initiated in sterile eppendorf tubes containing pre-soaked rockwool cubes. Each cube was pierced at the base to ensure optimal contact of roots with the Hoagland's nutrient solution (NS) and their uptake of water and nutrients. Seedlings were maintained under these conditions for four weeks, corresponding to BBCH scale 13 development stage, and then were transplanted into 1-L NS pots. Continuous oxygenation of the NS was ensured by air pumps equipped with fine-bubble diffusers, maintaining dissolved oxygen levels conducive to optimal root respiration and nutrient uptake.

Plantlets of both cultivars were equally divided into three groups: one group grown in a PFAS-free NS, and the other two treated with a mixture of 11 PFAS at two concentrations, 10 µg L^{-1} and 20 µg L^{-1} for each molecule. The experiment was carried out using plants at two growth stages: 28 and 56 days after sowing (DAS), representing young and mature developmental stages, respectively, to highlight age-dependent differences in PFAS uptake and translocation, and plant physiological response. For each condition (cultivar × concentration × age), five biological replicates were grown (n = 5).

At the end of the exposure period, plants were carefully harvested and separated into roots and shoots to assess the bioaccumulation of PFAS in plant tissues.

2.3. Field Cultivation Trial

The open field experiment was carried out in Vicenza, North-East Italy (45°53′95″ N; 11°49′43″ E), to address the pressing issue of PFAS pollution affecting the area, as documented by the survey of the Veneto Region [11]. The experimental area was 36 m^2, divided into three blocks of 12 m^2 each, was previously described by Battisti et al. [23]. Soil properties are reported in Table S1, and soil PFAS contamination is described in Table S2. Lettuce seedlings of Agila variety were transplanted with a spacing of 0.30 m between and within rows for optimal air circulation and nutrient utilization, resulting in a total of 48 plants for the entire field experiment. Regular irrigation with PFAS-contaminated water was ensured by a drip-irrigation system with drippers placed every 0.3 m, using a flow rate of 1.4 L h^{-1}

for each dripper. The PFAS content of the irrigation water is reported in Table S2. Plants were harvested at full maturity (8 weeks), and leaves were analyzed for their PFAS content to assess their safety for the human diet. Agila variety was chosen because the hydroponic experiments showed it was the most accumulative variety.

2.4. PFAS Sampling, Extraction, and Quantification by LC-MS/MS Analysis

At the end of the exposure period, roots and fully expanded leaves of lettuce plants grown under hydroponic conditions were sampled to assess the accumulation of PFAS. Roots were gently washed with Milli-Q water and blotted with clean tissue paper. Fresh weight of tissues was determined, then plant tissues were freeze-dried to constant weight to determine dry weight (DW). Dried samples were then finely ground using a planetary ball mill to obtain a homogeneous powder suitable for PFAS extraction. For each replicate, 110 mg and 350 mg of powdered root and leaf tissue, respectively, were weighed and spiked with IS prior to PFAS extraction with methanol by Accelerated Solvent Extraction (ASE) as previously described by Battisti et al. [23]. Recovery and accuracy data are reported in Table S3.

Leaves of lettuce plants grown in open field were harvested and immediately transported to the laboratory. Fresh (FW) and dry (DW) weights were determined by weighing before and after drying at 60 °C until constant weight. Dry samples were then ground in liquid nitrogen, and 600 mg of tissue powder were spiked with IS prior to PFAS extraction by ASE.

PFAS quantification was carried out by targeted liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), using a TSQ Quantiva triple quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), interfaced with a UHPLC system (Dionex Ultimate 3000, Thermo Fisher Scientific, Waltham, MA, USA). Data were acquired in selected reaction monitoring (SRM) mode (optimized parameters for SRM transitions are reported in Table S4). A full description of chromatographic separation and instrument conditions is reported by Sharma et al. [24]. All samples were diluted at a ratio of 1:1 with H_2O prior to injection.

Raw data were processed using Skyline MS software version 21.2.0.425 [25] to quantify PFAS contents in all the samples. PFAS bioconcentration factor (BCF), which is defined as the ratio of the concentration of PFAS in the plant tissue to the initial PFAS amount supplied in the medium, was calculated for the hydroponics experiment using the equation reported by Battisti et al. [26].

2.5. Quality Control and Quality Assurance

Glassware and fluoropolymer-containing substances were avoided along the whole analytical workflow to prevent any background contamination. Carry-over and instrumental performance were monitored with blank injection (methanol spiked with IS) every three injections.

The linearity of the matrix-matched standard calibration curves for each PFAS was assessed between 0 and 40 μg L^{-1}. All molecules showed good linearity, with correlation coefficients $R^2 > 0.99$ (Table S5). Control plants from hydroponic experiment were used for the matrix-matched calibration curves. For each target PFAS, the limit of detection (LOD) and the limit of quantification (LOQ) values were calculated as the analyte peak with a signal-to-noise ratio of 3 and 10, respectively, and are reported in Table S5.

2.6. Health Risk Assessment

The assessment of non-carcinogenic health hazards associated with PFAS-contaminated lettuce consumption was conducted for both hydroponically-grown and field-cultivated

lettuce. In the first case, we referred to the guidelines established by EFSA, and the hazard index (HI) was calculated with the following equation [27]:

$$HI = \frac{\sum_i C_i \cdot DI}{TWI}$$

where C_i is the i-PFAS concentration (mg g^{-1} FW), DI is the average daily dietary intake per unit of body weight (BW) (g kg^{-1} BW day^{-1}), and TWI is the cumulative oral reference dose for PFHxS, PFOA, PFOS, and PFNA (mg kg^{-1} BW day^{-1}). The DI of lettuce for the Italian adult population (DI = 0.288 g kg^{-1} BW day^{-1}) was retrieved from the most recent version of the EFSA Comprehensive European Food Consumption Database [28].

Regarding the field-cultivated lettuce, since the only detected compound was PFBA and it is not taken into account by the EFSA regulation, the HI was calculated with the same formula using the chronic oral reference dose value for PFBA (0.001 mg kg^{-1} BW day^{-1}) retrieved from the US EPA's IRIS Toxicological Review for PFBA and related salts [29]. Values of HI < 1 indicate unlikely adverse effects due to PFAS exposure, while values of HI $\geq$ 1 indicate potential risk of chronic non-cancer adverse effects on human health.

2.7. Statistical Analysis

For the hydroponic experiment, statistical analyses were carried out using GraphPad Prism v.10.0 [30]. A three-way ANOVA was applied to assess the effects of the experimental factors and their interactions, followed by Sidak's post hoc test for multiple comparisons. Prior to the analysis, data were tested for normality and homoscedasticity to verify that ANOVA assumptions were met.

For the field experiment, left-censored data were analyzed with RStudio software v. 2022.12.0 [31] using the functions available in the NADA package for R, according to Helsel [32].

3. Results

3.1. Assessment of PFAS Bioaccumulation in the Hydroponic Experiment

3.1.1. PFAS Accumulation in Root Tissues

In the lettuce roots, all eleven PFAS were detected, with accumulation patterns related to PFAS doses, plant variety, and age (Figure 1; Table S6). All PFAS increased their concentrations to the highest exposure rate, with differences observed between the two varieties and across different developmental stages.

Among the short-chain PFAS compounds, PFBA and PFPeA showed moderate accumulation across all the treatments, with a distinct concentration-dependent pattern. At a PFAS concentration of 10 μg L^{-1}, PFBA levels were observed at around 3000 ng g^{-1} DW for both 28 and 56 DAS in Agila, and at around 3500 ng g^{-1} DW for Bonaly, whereas at 20 μg L^{-1} (Figure 1B), the levels increased up to 7990 ng g^{-1} DW. At a concentration of 10 μg L^{-1}, both varieties showed similar accumulation, whereas at 20 μg L^{-1}, Agila variety showed higher PFBA retention rates at both 28 and 56 DAS. PFBS exhibited relatively lower concentrations under all conditions.

The accumulation of mid-chain compounds, PFHxA and PFHpA, was more related to exposure levels and plant age. At a concentration of 20 μg L^{-1}, PFHxA levels in Agila increased from 1413 to 2436 ng g^{-1} DW over a period from 28 to 56 DAS, respectively, whereas in Bonaly the increase in the same period was from 1147 to 1904 ng g^{-1} DW (Figure 1B). For PFHpA, a more pronounced age-dependent increase was observed in Agila.

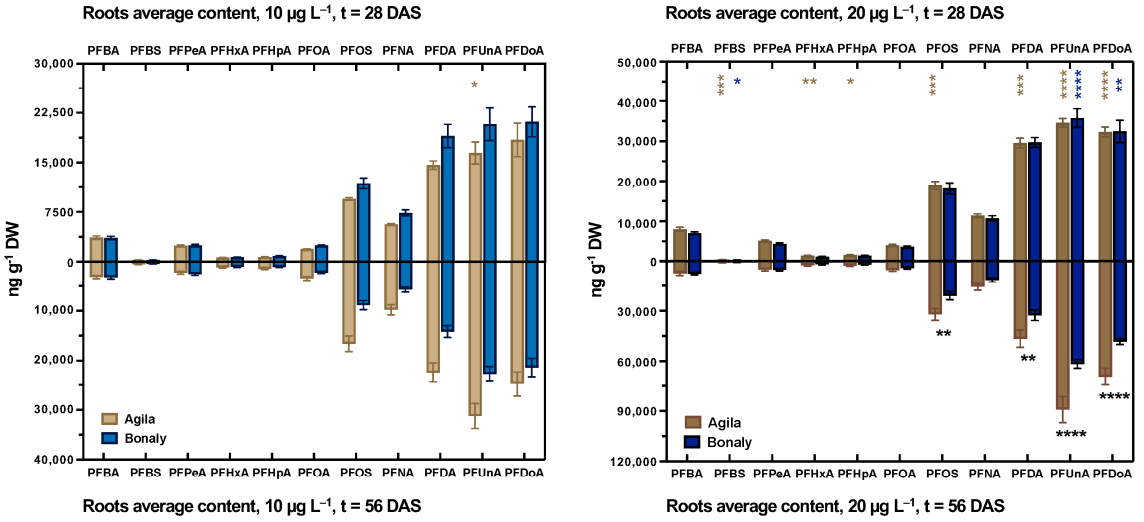

Figure 1. PFAS concentrations quantified in Agila and Bonaly lettuce roots at two plant ages (28 and 56 DAS) exposed to two PFAS concentrations of 10 (**A**) and 20 (**B**) µg L^{-1}. Values are reported as mean ± standard error (n = 5). Black asterisks (*) indicate statistically significant differences between varieties, while colored asterisks denote significant differences between plant ages within the same variety. Symbols *, **, ***, and **** indicate significant difference at *p* levels of 0.05, 0.01, 0.001, and 0.0001, respectively.

Concerning long-chain compounds, namely, PFOA, PFOS, PFNA, PFDA, PFUnA, and PFDoA, the accumulation was strongly dependent on treatments and varieties. At a concentration of 20 µg L^{-1}, roots of Agila after 56 DAS accumulated PFUnA and PFDoA up to 69,200 and 89,200 ng g^{-1} DW, respectively, the highest observed concentrations (Figure 1B). Bonaly variety also exhibited increased accumulation with increasing age and dose, but at lower levels. Different accumulation between the two varieties was observed consistently across other long-chain PFAS.

The bioconcentration factor (BCF) values for the roots confirmed the tendency of accumulation for short-chain PFAS (Figure 2; Table S7). At the exposure of 10 µg L^{-1} (Figure 1A), the BCF value for PFBA exceeded 340 µg g^{-1} DW/µg L^{-1} in both varieties at 28 DAS, with further increments observed at a concentration of 20 µg L^{-1}, especially in Agila (Figure 2B). PFBS showed lower BCF values compared to PFBA and PFPeA, parallel to its reduced accumulation levels.

PFHxA and PFHpA exhibited moderate BCFs, with Agila showing higher values than Bonaly, particularly under 20 µg L^{-1} exposure. The BCF value for PFHxA increased over time in Agila, which is consistent with its total concentration, whereas Bonaly exhibited lower and more stable values, indicative of decreased retention of these two compounds.

Long-chain PFAS exhibited the highest BCFs, frequently surpassing 1000 µg g^{-1} DW/µg L^{-1}, with peaks of 4421 µg g^{-1} DW/µg L^{-1} for PFDoA in Agila under 20 µg L^{-1} exposure at 56 DAS. Agila consistently demonstrated higher bioconcentration of long-chain PFAS in comparison to Bonaly, particularly after prolonged exposure.

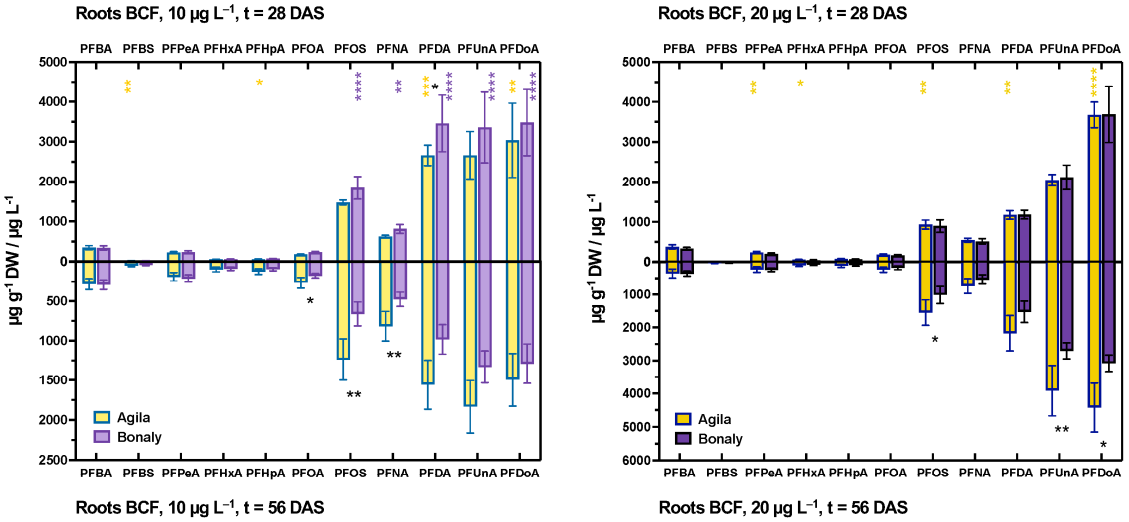

Figure 2. Bioconcentration factor (BCF) values of PFAS calculated for Agila and Bonaly lettuce roots at two plant ages (28 and 56 DAS) exposed to two PFAS concentrations of 10 (**A**) and 20 (**B**) µg L^{-1}. Values are reported as mean ± standard error (n = 5). Black asterisks (*) indicate statistically significant differences between varieties, while colored asterisks denote significant differences between plant ages within the same variety. Symbols *, **, ***, and **** indicate significant difference at *p* levels of 0.05, 0.01, 0.001, and 0.0001, respectively.

3.1.2. PFAS Accumulation in Leaf Tissues

Compared to the roots, leaves demonstrated higher concentrations of short-chain PFAS, particularly after exposure at 20 µg L^{-1} (Figure 3; Table S6). PFBA and PFPeA were the predominant compounds identified across all samples, and at concentrations of 10 and 20 µg L^{-1}, PFBA reached approximately 800 and 1700 ng g^{-1} DW, respectively, in both varieties after 28 DAS, whereas after 56 DAS, its concentration decreased, particularly in Bonaly variety. PFPeA showed similar trends, suggesting an age-related 'dilution' effect in the translocated short-chain PFAS.

As for the roots, PFBS was generally undetectable under most conditions (<LOQ), except at 20 µg L^{-1} after 56 DAS, where its concentrations were 47 ng g^{-1} DW in Agila and 26.7 ng g^{-1} DW in Bonaly (Figure 3B).

Agila also accumulated PFHxA and PFHpA markedly at higher exposure levels. At 10 µg L^{-1}, PFHxA concentrations increased slightly from 77.5 ng g^{-1} at 28 DAS to 79.5 ng g^{-1} DW at 56 DAS in Agila, while Bonaly showed a notable drop from 73.3 to 28.5 ng g^{-1} DW. PFHpA also decreased over time in Bonaly (from 25 to 14.9 ng g^{-1} DW) but increased in Agila (from 36.9 to 55.5 ng g^{-1} DW). At 20 µg L^{-1}, the age-dependent accumulation was even more marked.

PFOA and PFOS showed exposure- and age-dependent accumulation trends in lettuce leaves. At 10 µg L^{-1}, PFOA in Agila increased from 44.7 to 75 ng g^{-1} DW from 28 to 56 DAS, whereas in Bonaly variety, the concentrations declined from 29.2 to 15.9 ng g^{-1} DW. At 20 µg L^{-1}, Agila variety accumulated PFOA at a concentration of 114.5 ng g^{-1} DW and PFOS at 140.5 ng g^{-1} DW (Figure 3B).

Other long-chain PFAS, like PFDA and PFUnA, followed a similar pattern, with Agila accumulating higher concentrations at both tested exposure rates. PFDoA was detected only after 56 DAS at the highest exposure (18.2 ng g^{-1} DW in Agila and 12.8 ng g^{-1} DW in Bonaly), showing limited translocation to the aerial parts of the plant.

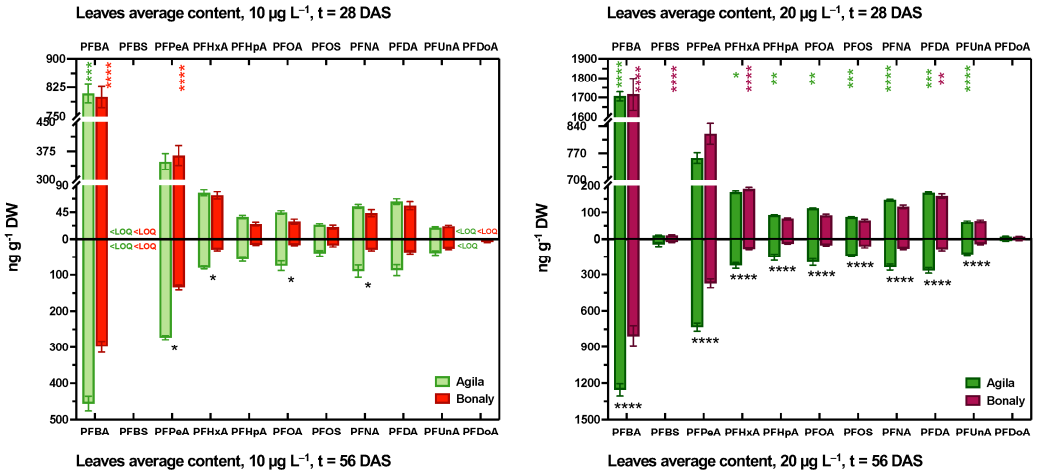

Figure 3. PFAS concentrations quantified in Agila and Bonaly lettuce leaves at two plant ages (28 and 56 DAS) exposed to two PFAS concentrations of 10 (**A**) and 20 (**B**) µg L^{-1}. Values are reported as mean ± standard error (n = 5). Black asterisks (*) indicate statistically significant differences between varieties, while colored asterisks denote significant differences between plant ages within the same variety. Symbols *, **, ***, and **** indicate significant difference at *p* levels of 0.05, 0.01, 0.001, and 0.0001, respectively. '<LOQ' indicates values below the limit of quantification.

The BCF values confirmed the variety-specific differences in PFAS accumulation between Agila and Bonaly (Figure 4; Table S7). Short-chain PFAS showed the highest values across both varieties at 10 µg L^{-1} exposure. After 28 DAS, BCF values for PFBA were similar between Agila and Bonaly, but with plant aging, BCFs declined more markedly in Bonaly, while Agila maintained higher values, and a similar pattern was observed for PFPeA, suggesting a stronger ability of Agila to retain short-chain compounds over time. PFHxA and PFHpA showed moderate BCF values that increased with exposure rate concentration, and were consistently higher in Agila than in Bonaly variety.

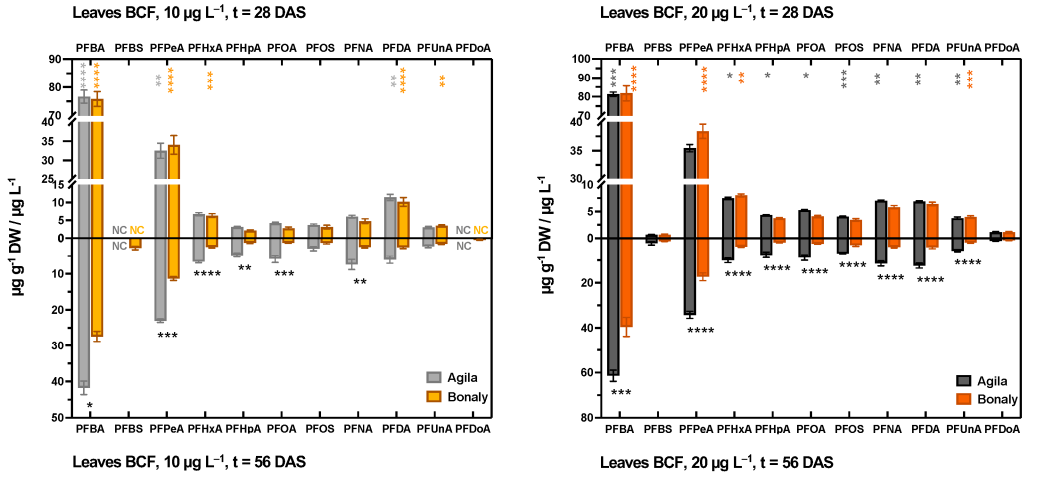

Figure 4. Bioconcentration factor (BCF) values of PFAS calculated for Agila and Bonaly lettuce leaves at two plant ages (28 and 56 DAS) exposed to two PFAS concentrations of 10 (**A**) and 20 (**B**) µg L^{-1}. Values are reported as mean ± standard error (n = 5). Black asterisks (*) indicate statistically significant differences between varieties, while colored asterisks denote significant differences between plant ages within the same variety. Symbols *, **, ***, and **** indicate significant difference at *p* levels of 0.05, 0.01, 0.001, and 0.0001, respectively. 'NC' indicates not computable values.

For long-chain PFAS, BCF values were lower but showed differences between the two varieties, with Agila showing consistently higher BCF values for PFOA, PFOS, PFNA, and PFUnA than Bonaly, particularly at the highest exposure level and after 56 DAS (Figure 4B). Notably, the BCF value for PFNA in Agila increased significantly at 56 DAS, in line with the greater PFNA accumulation, whereas Bonaly maintained lower values throughout the growth period. PFOS and PFUnA followed similar trends, whereas PFDoA showed the lowest BCF values for both varieties (Figure 4A).

3.2. PFAS Accumulation in Lettuce Cultivated in the Field

Despite relatively high PFAS concentrations in the used irrigation water [23], particularly for PFBA, the most mobile and short-chained compound, which was consistently present in the irrigation water at moderate concentrations (1630–1769 ng L^{-1}), PFOA (9867–7036 ng L^{-1}) and PFBS (3366–2205 ng L^{-1}), Agila lettuce variety cultivated in the open field trial, accumulated only PFBA at a concentration of 29.2 ng g^{-1} DW, whereas all other PFAS were either below the limit of detection (PFPeA, PFHpA, PFNA, PFDA, PFUnA, and PFDoA), or below the limit of quantification (PFBS, PFOA, and PFOS). Moreover, though the PFHxA concentration increased in the soil during the experiment (from <LOD to 2542 ng kg^{-1} DW), it was not detected in the leaves.

3.3. Dietary Exposure Assessment

The dietary risk assessment based on the average daily consumption of lettuce by the Italian adult population (18–64 years) was conducted to calculate the associated non-carcinogenic chronic risk. In the case of plants grown in hydroponics, the HI values were calculated considering the cumulative exposure to PFOA, PFOS, and PFNA, whereas for the open field-cultivated lettuce, only PFBA was considered, since PFOA and PFOS were <LOQ, and PFNA was <LOD. The HI values indicated that the risk associated with the consumption of all hydroponically-grown plants was significant, ranging from values of >500 to values up to >4500, whereas for the consumption of the lettuce cultivated in the field trial, the HI value was <1, indicating no risk (Table 1).

Table 1. Hazard Index (HI) calculated for the consumption of lettuce grown both under hydroponic and open field conditions.

Variety	Age	Treatment	HI
Agila	28 DAS	10 µg L^{-1}	1029
		20 µg L^{-1}	740
	56 DAS	10 µg L^{-1}	1699
		20 µg L^{-1}	4585
Bonaly	28 DAS	10 µg L^{-1}	3022
		20 µg L^{-1}	2389
	56 DAS	10 µg L^{-1}	545
		20 µg L^{-1}	1674
Agila	/	Open field	0.15

4. Discussion

4.1. Influences of the Plant Age and Variety on PFAS Uptake and Accumulation

Previous hydroponic studies carried out on lettuce and other leafy vegetables with comparable experimental designs and PFAS treatments have pointed out that PFAS accumulation strongly depends on the carbon chain length, dose, and plant species [33,34].

Our results indicated that the stage of plant development was pivotal in the uptake, translocation, and retention of PFAS in the two studied lettuce varieties. This could be related to different nutrient demand and shifts throughout growth stages, influencing the overall plant health and yield [35]. Older Agila plants exhibited consistently higher PFAS accumulation of long-chain PFAS (>C8) in roots at both exposure levels, with a continuous increase in PFOA, PFOS, PFUnA, and PFDoA over time, compared to Bonaly variety, which showed only moderate increases. The increase over time could be attributed to the development of the root systems and the relevant greater exploratory capacity [36]. From a physiological point of view, root aging increases the deposition of secondary cell wall components such as suberin, lignin, and structural proteins, especially for the production of the Casparian strip [37,38]. Variations in the Casparian strip development or permeability depending on the lettuce variety may therefore contribute to the observed differences in PFAS distribution between Agila and Bonaly [20,39–42]. These root anatomical changes could create a physical and biochemical endodermal barrier to the accumulation of PFAS [20,39,40], which could explain the age-dependent reduction in PFAS uptake observed for both lettuce species. Reduced root translocation along with leaf expansion could explain the age-dependent 'dilution' effect observed in the lettuce leaves from 28 to 56 DAS, particularly of the long carbon chain PFAS (Figure 3). An explanation for the difference in PFAS uptake and translocation between the two lettuce varieties is that Bonaly is an early-medium maturing lettuce cultivar characterized by rapid developmental progression and a shorter vegetative phase. This precocity may accelerate the onset of anatomical and physiological arrangements such as increased tissue thickness, reduced root plasticity, nutrients and water demand and allocation, contributing to the reduced PFAS uptake observed in older plants compared to Agila variety.

In lettuce and other crops, short-chain PFAS are transported via the xylem stream to the aerial tissues [26,43], and in our work, a similar trend was also observed for PFHxA and PFHpA. Notably, while both varieties responded to age and concentration, Agila variety was consistently more efficient in accumulating PFAS, particularly under high-concentration treatments, also towards longer-chain molecules. Observed differences in PFAS accumulation between the two lettuce varieties could be due to inherent physiological traits that enhance PFAS retention in Agila variety, resulting in varietal differences in the BCF values, particularly for long-chain PFAS. Wen and Huang [44] reported a promotion effect of proteins for root uptake and a positive correlation between BCFs and leaf-to-root protein content ratios, and that more lipophilic long-chain PFAS have strong interactions with biological macromolecules in plant roots, causing their limited upward translocation. The peculiar PFAS accumulation capacity of Agila variety shows that more research is needed to elucidate the main anatomical, physiological, and biochemical factors responsible for such differences. Globally, PFAS accumulation in lettuce leaves was driven predominantly by their carbon chain length and the exposure level, with age and variety determining the accumulation patterns.

The BCF values paralleled the total concentration data, confirming that Agila has a higher and more sustained capacity to accumulate mid- and long-chain PFAS, whereas Bonaly exhibited a more pronounced decline in PFAS uptake with plant aging.

4.2. Accumulation in Lettuce Cultivated in the Field

Many experiments on PFAS accumulation in plants have been carried out in hydroponics and under controlled conditions. However, the possibility of extending the findings to open field conditions is hampered by the complexity of the soil matrix and interactions. There is extensive literature on the soil-water-plant relationship, which describes how PFAS

can move across the soil matrix through water, reaching the root surface and entering the plant (e.g., [40,45,46]).

In this study, we observed that, despite its relatively high accumulative potential when grown in hydroponics, Agila lettuce variety cultivated in the field trial exhibited a very limited PFAS translocation to leaves, with only PFBA being detected in leaves. Although this outcome could potentially be attributed to the plants being exposed to reduced PFAS concentrations, it was surprising since the plants were subjected to a range of PFAS that were absorbed during hydroponic growth, and over a similar duration. This result could be explained by the PFAS retention by the soil solid phases, especially for the long-chain compounds such as PFOS and PFDA, present in the irrigation water. In lettuce plants grown in soil spiked with PFBA, PFBS, PFOA, and PFOS in equal amounts, Scearce et al. found that PFBA showed the highest bioconcentration factor [46], in agreement with our results. PFBA has a lower sorption affinity and higher water solubility, allowing it to remain mobile and more readily enter plant roots and translocate to leaves. Moreover, PFBA may also arise as a terminal degradation product of precursors as long-chain PFAS, through biotic or abiotic transformation processes in soil or water. However, these results were different from those of tomato plants cultivated on the same soil, which absorbed various PFAS present in the irrigation water [23]. Felizeter et al. reported that PFAS root bioconcentration factors were from one to two orders of magnitude higher in hydroponics than in soil, especially for long-chain compounds ($\geq$C6) due to their sorption by the soil organic matter [47]. However, despite the low BCFs calculated for leaves were found to be similar between hydroponic- and soil-grown lettuce. These findings suggest that PFAS translocation to leaves is influenced more by the plant physiology, including the transpiration rate, than by the presence or absence of soil sorption processes. In other words, while hydroponics can significantly overestimate root accumulation due to a lack of soil sorption, it remains a valid model for studying foliar translocation [21,22,34,48].

4.3. Health Risk

Lettuce is consumed primarily as fresh leaf biomass [17,19], which was shown to accumulate huge amounts of PFAS, leading to potential human exposure [34], when produced in contaminated environments like the Veneto Region. Our work shows that carboxylic short-chain PFAS (i.e., PFBA and PFPeA) are the most abundant in edible leaves. These compounds, although less bio-accumulative in humans than their long-chain counterparts, are more mobile and persistent in the environment [49,50]. Although short-chain PFAS are currently underregulated, there is toxicological evidence showing that their intake is associated with a wide range of adverse effects on human health, including oxidative stress, immunotoxicity, and developmental issues [51–53], warranting greater attention to their presence in all edible crops [54,55]. Hydroponics is seen as a sustainable agricultural farming practice, especially for lettuce and herbs in general, with increasing amounts of generated value owing to the possibility of providing fresh products year-round regardless of weather conditions, full automation of temperature, water, and nutrient supplies even in arid and semi-arid zones and reduced cultivated surface using vertical hydroponics [56]. However, our work shows that this cultivation mode can raise risks of PFAS accumulation in lettuce, and potentially in other hydroponically produced crops, in particular if the water used is contaminated, like that in the Veneto Region, where prolonged industrial discharge has resulted in widespread PFAS contamination of the groundwater. Similar considerations on the risk derived from the consumption of PFAS-contaminated lettuce were made by Jin et al., underlining the need to further study the human health exposure to these pollutants through the diet [34].

5. Conclusions

The present study demonstrated that lettuce plants can take up, translocate, and accumulate a wide range of PFAS compounds when grown hydroponically in contaminated nutrient solution, with accumulation patterns strongly influenced by plant age, variety, and cultivation system. In comparing two varieties, Agila accumulated significantly higher concentrations of long-chain PFAS in both roots and leaves than Bonaly, with the first showing a more efficient translocation of all PFAS, particularly at later growth stages, and the second showing more translocation of short-chain PFAS during early development. These findings highlight the physiological and morphological traits that modulate PFAS uptake and distribution in leafy vegetables, as well as the role of environmental complexity in influencing PFAS bioavailability, possibly due to interactions with soil components and dynamic plant-environment processes. In contrast, Agila variety cultivated in open field and irrigated with contaminated water showed negligible PFAS accumulation in edible tissues. Only PFBA was detected at low levels in field-grown lettuce. This suggests either a filtering effect by the soil or the activation of protective plant mechanisms under natural conditions.

From a food safety perspective, hydroponically grown lettuce can accumulate high concentrations of both short- and long-chain compounds that exceed the EFSA tolerable weekly intake threshold and can result in unacceptable hazard index values. Conversely, the lower PFAS content in field-grown lettuce suggests a reduced immediate risk, though the presence of even low levels of mobile compounds like PFBA cannot be ignored, particularly in high-consumption populations. These results support the need for varietal selection, cultivation strategy optimization, and strict monitoring in contaminated agricultural areas.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11070775/s1, Table S1: Soil physical and chemical properties. Values are expressed as mean ± standard error (n = 3); Table S2: PFAS concentrations measured in soil and irrigation water used for the open field trial, as reported by Battisti et al. (2024) [23]. Values are expressed as ng kg^{-1} DW for soil and ng L^{-1} for water; Table S3: Recovery rates (%) of PFAS extraction by ASE and coefficient of variation (CV %). Recovery values indicate mean ± standard deviation (n = 3); Table S4: LC-MS/MS optimized parameters for PFAS compound detection; Table S5: Limit of detection (LOD), limit of quantification (LOQ), and correlation coefficients (R^2) of matrix-matched PFAS compounds standard calibration curves; Table S6: PFAS content in roots and leaves; Table S7: PFAS bioconcentration factors (BCF) calculated for roots and leaves.

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