Phytoextraction of per- and polyfluoroalkyl substances (PFAS) by weeds: Effect of PFAS physicochemical properties and plant physiological traits

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HIGHLIGHTS

• Phytoextraction of PFAS by weeds is cost-effective and aesthetically pleasing.
• Up to 41.4%wt of PFAS can be removed from soil.
• The weeds showed a preference for extracting short-chain and hydrophilic PFAS.
• Mechanisms of plant uptake was explained via correlation analysis and FE-EPMA-EDS.

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ABSTRACT

Phytoextraction is a promising technology that uses plants to remediate contaminated soil. However, its feasibility for per- and polyfluoroalkyl substances (PFAS) and the impact of PFAS properties and plant traits on phytoextraction efficacy remains unknown. In this study, we conducted greenhouse experiment and evaluated the potential of weeds for phytoextraction of PFAS from soil and assessed the effects of PFAS properties and plant traits on PFAS uptake via systematic correlation analyses and electron probe microanalyzer with energy dispersive spectroscopy (FE-EPMA-EDS) imaging. The results showed that 1) phytoextraction can remove 0.04%–41.4%wt of PFAS from soil, with extracted PFAS primarily stored in plant shoots; 2) Weeds preferentially extracted short-chain and hydrophilic PFAS over long-chain homologues from soil. 3) PFAS molecular size and hydrophilicity determined plant uptake behavior, while plant morphological traits, particularly root protein and lipid content, influenced PFAS accumulation and translocation. Although plants with thin roots and small leaf areas exhibited greater PFAS uptake and storage ability, the impact of PFAS physicochemical properties was more significant. 4) Finally, short-chain PFAS were transported quickly upwards in the plant, while uptake of long-chain PFOS was restricted.

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Implication
The present study demonstrates the opportunity to remediate PFAS impacted sites via phytoextraction by weeds. Establishment of weeds cover on the surface of the site could remove diverse PFAS from soil through phytoextraction and bioaccumulated in the harvestable compartments. Greenhouse experiments together with electron probe microanalyzer with energy dispersive spectroscopy (FE-EPMA-EDS) suggested that legacy and alternative PFAS undergo divergent translocation pathways during plant uptake. Further, plants with thin roots and small leaf areas are desirable for PFAS uptake and storage. Phytoextraction by weeds could reduce the mobility of PFAS in the substrates and off-site leaching, and therefore the risk to the environment.

Data availability
Data will be made available on request.

1. Introduction
Per- and polyfluoroalkyl substances (PFAS) comprise an important class of chemicals with over 12,000 different species and complex physicochemical properties [1–5]. Among exposure routes, the intensive use of aqueous film forming foams (AFFFs), application of recycled water from wastewater treatment plants, landfill leachates, and applications of biosolids to agricultural land have led to the release of large volumes of PFAS in soils [6,7]. Previous studies have found that long-chain PFAS compounds (>7 fluorinated carbon atoms) are predominantly found in the topsoil, while short-chain homologues (<7 fluorinated carbon atoms) can migrate to deeper soil layers or leach to groundwater [6,8–11]. Recently, short-chain and other PFAS have been recognized as emerging contaminants in soil systems [12–18] and therefore may be targeted for removal in remediation efforts.

Additionally, phytoextraction is an emerging technology that utilizes plants, such as weeds, to clean up contaminated soils, water, and air pollutants [19–21]. It is gaining attention as an alternative technique for remediating contaminated soils [19–21]. PFAS-contaminated sites are characterized by multiple types of PFAS, low concentration, and high stability. Given these characteristics, using local weeds for phytoextraction to remediate PFAS-contaminated sites could be a cost-effective, environmentally friendly, aesthetically favorable, and appropriate approach, especially for developing countries [19–21]. Furthermore, urban spontaneous plants, also known as urban weeds have several advantages for environmental phytoremediation, such as adaptability to local conditions, low maintenance, and minimal resource requirements. They can also grow in contaminated soils and absorb and accumulate organic pollutants, thus improving soil quality and providing ecosystem services such as carbon sequestration and biodiversity conservation.

The available data suggest that plants can extract certain PFAS from the environment through physical, chemical, or biological processes from soil, water, and air [22–30]. However, the effectiveness of this process varies greatly depending on the plant species and PFAS class. Roots are the primary means by which plants take up nutrients, water, and contaminants in the soil [19,31]. A larger root system with more surface area may increase the uptake of contaminants from the soil, but the specific effect of root length and biomass on phytoremediation effectiveness depends on the contaminants present and the plant species used [19,29,31,32]. Additionally, the physical and chemical properties of PFAS, such as molecular size and LogKow (logarithm of the octanol-water partition coefficient), greatly affect their behavior in plants [31–34]. Therefore, it is crucial to clarify accumulation patterns across different plant groups, screen high-accumulation species, and summarize the physiological characteristics of these species to identify suitable candidates for future phytoremediation work. Given the large number of PFAS substances, a better understanding of their molecular structures and physicochemical properties is needed to advance phytoremediation efforts.

In this study, we conducted a greenhouse study to further investigate the mechanisms of plant uptake of PFAS. Specifically, we examined the effects of PFAS physicochemical properties and plant physiological traits for PFAS phytoextraction. Moreover, we investigated the effects of PFAS molecular size [molecular weight (MW), van der Waals volume and surface area, maximum and minimum projection radius] and hydrophobicity [LogKow and LogD (pH-adjusted Kow to neutral species)] as well as plant physiological root and foliage traits on plants’ PFAS uptake and accumulation capacity. Seven local weed plant species were chosen for this study. In addition, we used the field-emission-type electron probe microanalyzer with energy dispersive spectroscopy (FE-EPMA-EDS) technique to investigate the partitioning behavior of different PFAS species (long and short chains, legacy and new ether-based) in plants by characterizing the distribution of PFAS in root cross-sections. The outcomes of this study may help identify PFAS properties and physiological plant traits that affect the translocation and accumulation of PFAS in plants, and shed light on the potential of local weed phytoextraction for remediating PFAS-contaminated sites in future studies.

2. Materials and methods
2.1. Chemical reagents and lab materials
Eleven representative PFAS were targeted for testing (Table S1 and Fig. S1). Analytes in this study include perfluoroalkyl ether acids (PFEA) [i.e., perfluoro-3-methoxypropionic acid (PFMOPA), perfluoro-4-methoxybutanoic acid (PFMOBA), hexafluoropropylene oxide dimer acid (HFPO-DA, parent acid of “GenX”), perfluoralkyl carboxylic and sulfonic acids [PFCA and PFSA, including perfluorobutanoic acid (PFBA), perfluoropentanoate (PFPeA), perfluoroheaxanoate (PFHxA), perfluoroheptanoate (PFHpA), PFPA, perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), and PFOS]. Analytical standards and isotopically labeled internal standards were obtained from Wellington Laboratories (Guelph, ON, Canada) and SynQuest Laboratories, Inc. (Alachua, FL). Upon receiving, a stock solution was prepared for each PFAS compound in methanol with a concentration of 2 g L−1. Ultrapure water (UPW) was obtained from a Milli-Q system (Millipore, Billerica, MA). Supelclean Envi-Carb 120/400 from Supelco (Sigma-Aldrich, Bellefonte, PA) was used in extract cleanup. All other chemicals and solvents were purchased from Sigma Aldrich (St. Louis, MO). The physicochemical properties of PFAS have been characterized in detail and listed in Table S1 in the Supplementary Material (SM). LogKow values of PFCA and PFSA were extracted from Gaglione et al., 2020 [35], while LogKow of PFEA were approximated using The EPI Suite™. Additional physicochemical properties of PFAS were computed using Chemicalize (http://www.chemicalize.com/).

2.2. Plant and soil preparation
All plants and soils used in this study were collected from the campus of Chongqing University (Chongqing, China). We confirmed that there was no source of industrial pollution (e.g., factories) within 10 kilometers of the study area. We measured the background concentration of PFAS in collected plants and soil but did not detect any peaks, indicating negligible background concentration. Our soil characterization results show that the soil contains 2.14% ± 0.24% organic carbon.

Seven weed species were used, including Phyllanthus urinaria (Pu), Aster indicus (Ai), Justicia procumbens (Up), Alternanthera philoxeroides (Ap), Imperata cylindrica (Ic), Juncus effuses (Je), and Setaria virens (Sv). Most of them belong to the Compositae and Poaceae families, which are widely distributed worldwide and have high similarity in physiological traits. Therefore, the research findings from these seven typical urban
spontaneous plants have significant international value and can serve as a reference for understanding the mechanism and application of plant uptake of PFAS. It is worth noting that these weeds belong to spontaneous vegetation, which is a typical component of any urban environment and comprises plants not intentionally planted by humans. Due to their strong vitality, spontaneous plants can quickly respond to the urban environment, exhibiting a strong ability to adapt to the natural environment and climate and can also be good candidates for phytoremediation. Moreover, these species differ significantly in their morphological and physiological traits (see photos in Figs. S2–4). As reported previously, some plant species have been shown to be effective in phytoremediation of heavy metals and dyes in contaminated water or soil [36–41]. Upon collection, the plants were carefully removed from the soil, and after which, the soil was washed off the roots with UPW.

A list of plant foliar (area and mass) and root traits (i.e., total length, project area, surface area, surface area-to-length ratio, average diameter and length per volume) were measured after collection (Table S2, methods are consistent with ref [29]). They are regarded as important functional facets for water and nutrient uptake. Lipid and protein content of root were also measured (see Text S1 for details). Microstructure of root hair zone, such as thickness of root epidermis (ep), vascular column (vc), endothelial layer (en) and xylem (xy) (Fig. S5 and Table S3) were determined to indicate the proportion of root diameter available for resource transport.

In addition, soil was sampled at 0–20 cm depth and then air-dried and sieved (<2-mm mesh) prior to use. An aliquot of 2 kg soil was spiked with 500 mL of methanol solution containing a mixture of 11 PFAS. The soil was mixed thoroughly in a clean motorized copper concrete mixer for 30 min. Then PFAS-spiked soil was spread in a polypropylene (PP) dish to volatilize methanol on the soil surface. Afterwards, the spiked soil was combined with 30 kg of clean soil in the concrete mixer for 60 min to ensure a homogeneous distribution. The PFAS-containing soil was stored in the laboratory for an additional week to stabilize its composition. PFAS were spiked into the soils at a nominal concentration of 200 ng g⁻¹ (see Table S4 for concentrations of individual compounds). The concentration is comparable to that in the natural environment, 2–10 ng g⁻¹, 5–100 ng g⁻¹ and 10–30 ng g⁻¹ for PFOS, PFBA, and PFDA, respectively [42].

2.3. Plant culture and exposure experiments

A plant uptake experiment was conducted in this study using triplicates in a climate-controlled greenhouse (day: 25 ± 5 °C; night: 21 ± 5 °C, 14 h light) including PFAS-free and plant-free controls. 700 g of soil was transferred to each individual high-density polyethylene pot (12 cm in diameter at top and 12 cm in height). Depending on the individual density in the natural environment, 2–9 plants were placed in each pot (Table S5). Soil in the pots were maintained at 60% of maximum water holding capacity by daily irrigation with tap water. No additional nutrient was added to the system, because the weeds are spontaneous plants that are capable of growing under very harsh conditions. Pots were randomly arranged to account for any spatial variation in temperature and light.

The plants were harvested 30 days after being exposed. The duration of exposure was determined based on previous studies that ranged from 10 to 30 days [26,43–45]. The plants were carefully rinsed with UPW to remove the attached soil particles, carefully dried with Kimwipes, and divided into roots and shoots. The plant tissues were then weighed, placed in Ziploc plastic bags, freeze-dried at –80 °C in a freeze-dryer, and ground into powders. Upon harvest, soil samples were also collected from the individual pots to measure the PFAS in soil.

2.4. Imaging the distribution of PFAS in roots by FE-EPMA-EDS

Out of the seven weed plants used in the study, four plants were chosen to explore the tissue distribution of PFAS, i.e., Phyllanthus urinaria (Pu), Aster indicus (Ai), Imperata cylindrica (Ic), and Setaria viridis (Sv). The plant selection process was based on availability and intentionally included two dicots and two monocots. Additionally, Pu was chosen due to its strong bioaccumulation for PFAS. The selected species are dominant native plants from globally distributed families, such as Compositae (Phytolacca acinosa), and Poaceae (Setaria viridis, Imperata cylindrica). Differences in tissue-level distribution between emerging PFAS (GenX), short-chain compound (PFBA), and legacy PFOS were explored by using field emission electron probe microanalyzer with energy dispersive spectroscopy (FE-EPMA-EDS). The plants were cultured in PFBA, GenX, or PFOS aqueous solution. Upon harvest, plants were gently rinsed with UPW three times and air dried. Longitudinal and cross-sectional root slices were cut from fresh primary roots by using a stainless steel blade, after which samples were freeze-dried. After then, the root sections, which were cut into appropriate orientations, were fixed onto standard glass slides via double-sided tape. The dried samples were then evenly coated with gold (MSP-mini magnetron sputter, Vacuum Device, Japan) before being loaded onto the FE-EPMA (JXA-8530 F Plus, Hyper Probe, JEOL Ltd., Japan). The distributions of F in the roots were mapped with an accelerating voltage of 10 kV and a beam current of 7.27 nA. The probe diameter was 4.0 μm and retention time was 20 ms.

2.5. Sample pretreatment, PFAS quantification, and quality assurance and control

Methods of sample pretreatment were detailed in Text S2. Quantitative analyses of PFAS were performed using ultra-performance liquid chromatography coupled to tandem mass spectrometry (LCMS-8060, Shimadzu, Japan) via a negative electrospray ionization source. Calibration standards were prepared from 0.05 μg L⁻¹ to 50 μg L⁻¹, with eight calibration points in the regression line. The limit of quantification (LOQ) was defined as the lowest point of the standard curve, provided that the regression equation yielded a calculated value within less than ±30% error. Additional details on the quantitative analytical method are provided in Table S6 in the SI.

Matrix blanks were examined from the collected plants, soils, and irrigation water. PFAS concentrations were below the level of detection (LOD) in laboratory extraction blanks (n = 3). To ensure the determination quality, solvent blank, spiked blank, spiked matrix, and sample duplicate were determined every 10 samples. Matrix effects were evaluated in triplicate at two spike levels (2 μg L⁻¹ and 20 μg L⁻¹) using Ai and Jp, and root and shoot tissues were tested separately. The PFAS response in plant extracts was within ±30% of the nominal concentrations (Tables S7). Spike-recovery experiments were performed in triplicate at three concentration levels (concentration in the final extract = 1 μg L⁻¹, 5 μg L⁻¹, and 50 μg L⁻¹) in Ai and Jp tissues, and both roots and shoots were included for testing. PFAS recoveries were within 70–130% for most analytes (Tables S8).

2.6. Data analysis

Bioconcentration factors of the root (BCFroot) and shoot (BCFshoot) as well as the translocation factor (TF) were calculated using Eqs. 1–3.

\[
\text{BCF}_{\text{root}} = \frac{C_{\text{root}}}{C_s} \tag{1}
\]

\[
\text{BCF}_{\text{shoot}} = \frac{C_{\text{shoot}}}{C_s} \tag{2}
\]

\[
\text{TF} = \frac{C_{\text{shoot}}}{C_{\text{root}}} \tag{3}
\]

where \(C_s\) is the PFAS concentration in the soil at harvest time (ng g⁻¹), \(C_{\text{root}}\) and \(C_{\text{shoot}}\) are the PFAS concentration in plant roots and shoots (ng g⁻¹) at harvest, respectively. Spearman correlation analysis was used to examine possible correlations among BCFroot, BCFshoot, Croot and Cshoot, TF, PFAS physicochemical properties, and plant physiological traits. \(\rho\) is the Spearman’s correlation coefficient, a significance value (p) < 0.05 is
considered statistically significant (95% confidence level). Correlation analysis was done using SPSS (Version 25, IBM).

3. Results and discussion

3.1. Species specific accumulation of PFAS in weeds

Fig. 1 presents the mass distribution of PFAS in soil and plant tissues after 30-day exposure. Seven plant species were investigated, i.e., Phyllanthus urinaria (Pu), Aster indicus(Ai), Justicia procumbens(Jp), Alternanthera philoxeroides(Ap), Imperata cylindrica(Ic), Juncus effusus(Je), and Setaria viridis(Sv).

Fig. 1. Mass percentage of PFAS in soil and plant root and shoot after 30-day exposure. Seven plant species were investigated, i.e., Phyllanthus urinaria (Pu), Aster indicus(Ai), Justicia procumbens(Jp), Alternanthera philoxeroides(Ap), Imperata cylindrica(Ic), Juncus effusus(Je), and Setaria viridis(Sv).

The ability of plants to take up PFAS varies greatly among plant and PFAS species. Across these results, Pu exhibited the highest uptake of all PFAS, which is consistent with the previous hydroponic study [29]. As shown in Fig. 2, the total mass loading of PFAS in an individual plant ranged between 1.59 and 16.8 μg in roots and 5.8–39.2 μg in shoots. Across the seven weeds, Je shows the highest overall PFAS mass-loading (39.2 μg in shoot and 16.8 μg in root). This is mainly due to its high biomass (3.85 gdw of shoot and 1.63 gdw of root) (Fig. 2). These findings may indicate that greater plant biomass is desirable for phytoextraction efforts. These data also demonstrate the feasibility of using local, native weeds for phytoremediation of PFAS-contaminated sites in some areas.

Moreover, Fig. 1 reveals that the accumulated PFAS is mainly stored in the aboveground compartment. In comparison, plant roots showed slight retention of all PFAS, with the mass percentage ranging from 0.41%–4.4%. In addition, results show that plant uptake of short-chain substances from soil is greater than that of long-chain homologues (Fig. 1). The accumulation of PFOS by plants is very limited, more than 98% of the mass of PFOS was still preserved in the soil (Fig. 1B). This is different from the observed phenomenon in the hydroponic experiment where considerable amount of PFOS can be accumulated in the roots [24,26,29,32,48]. In our previous hydroponic study [32] we demonstrated that long-chain PFOS in the liquid media was present in plant roots through adsorption and precipitation; a substantial amount of associated PFOS could be rinsed off by methanol washing [32]. In contrast, the current soil culture study found that the amount of PFOS detected in plants is negligible (Figs. 1–2), the presence of soil would seriously restrict the root adsorption and absorption of PFOS.

As indicated in Figs. 1 and 2, short-chain compounds were taken up to a greater extent by the native weeds compared to their long-chain homologues. For instance, mass percentage of PFBA in shoot (8.2%–37.8%) is significantly higher than that of PFOA (0.8%–7.9%, Fig. 1A). Preferential plant uptake of short-chain PFAS has also been observed in previous studies [28,48–50]. In contrast, the long-chain compounds that possess greater molecular size and hydrophobicity tend to be retained by soil particles, particularly soil organic matter (SOM) [10,51,52]. In the soil-plant system, PFAS that are available for root uptake are typically those that are dissolved in the soil solution. However, the presence of sorption to soil particles can decrease the dissolved fraction of PFAS and limit their availability to plant roots [53]. The sorption and desorption of PFAS in soil are mainly influenced by hydrophobic interactions between the SOM and the hydrophobic fluorinated carbon tail, and electrostatic interactions between clay particles and the polar head group [10,51,52,54]. SOM plays a critical role as a sorbent in the sorption of PFAS compared to other soil components, and the uptake of PFAS by plant roots is inversely proportional to their sorption to SOM [22,55,56]. In this regard, PFAS with long-chain structures and high hydrophobicity,
such as PFOS, tend to have strong adsorption to SOM in the soil, making them less available for uptake by plant roots. Consequently, such PFAS may not be suitable for phytoremediation.

Given that there has been a global trend to replace long-chain PFAS and their precursors (mainly C6-C10 products) with corresponding shorter-chain homologues since 2000, and the widespread occurrence of new PFAS alternatives in the environment, flora and fauna [15,42,47], shorter-chain and new alternatives should be the focus of remediation. Our results demonstrated that phytoextraction could offer a new form of remediation to separate and eliminate short-chain (e.g., PFBA) and ether-based PFAS alternatives (e.g., GenX) from contaminated sites. Moreover, the combination of plant and soil amendments could be used to minimize both short and long-chain PFAS leaching from soil into receiving waters [57].

3.2. Assessment of phytoremediation potential by bioconcentration and translocation factors

Because PFAS tend to be taken up by plants in a concentration-dependent manner, higher concentrations of PFAS in culture media consistently lead to elevated accumulations in plants [49,58]. Further, $\text{BCF}_{\text{root}}$ and $\text{BCF}_{\text{shoot}}$ are key parameters to consider when assessing the suitability of plant species for their phytoremediation potential. As shown in Fig. 3A, the median value of $\text{BCF}_{\text{root}}$ across the seven weed species investigated was between 0.87 and 6.12. Pu exhibited the highest accumulating potential for PFAS; except for PFOS (2.08) and PFOA (9.86), and the $\text{BCF}_{\text{root}}$ values of Pu for other PFAS species were all $>10$, with the highest value as 19.6 (PFHxA). Moreover, Pu’s $\text{BCF}_{\text{shoot}}$ value was also the highest across the seven species, ranging from 0.06 (PFOS) to 94.7 (PFMOPrA), indicating its hyperaccumulation potential (Fig. 3B). These values may be compared to the standard criterion of $\text{BCF}_{\text{root}}$ values $>10$ to indicate the plant is a hyperaccumulator, although this standard was developed mainly for uptake of metals or metalloids rather than PFAS compounds [59,60]. It is interesting to note that Pu is an annual spontaneous perennial herbal species distributed in various parts of the world [61].

Furthermore, Ap is a commonly used plant in phytoremediation worldwide [40] given its creeping and layering root system that forms interwoven, dense mats. In our study, the $\text{BCF}_{\text{root}}$ and $\text{BCF}_{\text{shoot}}$ values of Ap were relatively low (0.08–1.23 and 0.07–52.5); while Ap’s TF value was the highest among the seven species (0.11–173.9) (Fig. 3C). This...
suggests that Ap could transfer PFAS from root to shoot compartments comparatively faster than other plant species investigated in this study.

In addition, the BCF\textsubscript{root}-chain length dependency was absent for various PFAS, which is consistent with previous studies in which BCF\textsubscript{root} values were derived from soil concentrations (Fig. 3A) [49,62,63]. Unlike the soil culture system, BCF\textsubscript{root} derived from hydroponic culture or based on pore water either increased with greater PFAS chain length (C4–C11) [48,64] or showed an U-shaped dependency with minima for C6 or C7 PFAS [24,49,65]. Further, there are a variety of processes involved for root uptake of PFAS, and the adsorption of soil can compete with the process of PFAS uptake by plant roots and thereby confounds the results. Other than these phenomena, both BCF\textsubscript{shoot} and TF values show chain-length dependence (Fig. 3B-C). Overall, these findings are consistent with findings from other studies that suggest long-chain PFAS may be more limited in their ability to be taken up, accumulated, and transported upwards in plant tissues [28,48–50].

Our results demonstrated that phytoextraction has the added benefit of immobilizing certain PFAS within the rhizosphere and storing it in the plant tissues that can be harvested once weeds are established on contaminated sites. This method can effectively reduce the off-site leaching of PFAS, while also separating short-chain PFAS from the soil. However, there is currently no definitive solution for the disposal of plants used in phytomediation, particularly those that contain PFAS. If the plants have accumulated high levels of pollutants, they should be treated as hazardous waste and disposed of properly, such as through incineration or burial in a designated hazardous waste landfill.

### 3.3. Physicochemical properties of PFAS affecting phytoextraction

The physicochemical properties of PFAS as well as the physiological structures of plants will affect the uptake and distribution of PFAS. Given that there are > 12,000 PFAS species identified [5], recognizing and

| Table 1 | Results of Spearman correlation among bioconcentrations in root and shoot (C\textsubscript{root} and C\textsubscript{shoot}), BCF\textsubscript{root}, BCF\textsubscript{shoot}, TF, PFAS physicochemical properties and plant physiological traits. See Tables S1-S3 for parameter values and explanations. \( p \) is the Spearman’s correlation coefficient. The single and double asterisks are marked to indicate significant differences at \( p < 0.05 \) and \( p < 0.01 \), respectively (95% confidence level). Correlation analysis was done using SPSS (Version 25, IBM). |
| --- | --- | --- | --- | --- | --- | --- |
| | \( C_{\text{root}} \) | \( C_{\text{shoot}} \) | \( \text{BCF}_{\text{root}} \) | \( \text{BCF}_{\text{shoot}} \) | TF | \( \rho \) |
| Root physiological characteristics |
| Protein content of root | 0.38** | 0.21 | 0.50** | 0.33** | -0.07 |
| Lipid content of root | 0.29* | 0.29* | 0.51** | 0.46** | 0.06 |
| Root length | 0.16 | -0.03 | 0.28* | 0.05 | -0.13 | -0.80 |
| Project area | 0.01 | -0.16 | 0.04 | -0.17 | -0.16 | -0.60 |
| Surface area | 0.01 | -0.16 | 0.04 | -0.17 | -0.16 | -0.30 |
| Surface area per unit length | 0.07 | -0.22 | -0.01 | -0.28** | -0.25* | 0.00 |
| Average diameter | 0.09 | -0.22 | -0.03 | -0.31** | -0.26* | 0.30 |
| Length per volume | 0.16 | -0.03 | 0.28 | 0.05 | -0.13 | 0.50 |
| Thickness of epidermis | 0.02 | 0.09 | -0.04 | 0.03 | 0.09 |
| Thickness of endothelial layer | 0.21 | -0.04 | 0.19 | 0.08 | 0.18 |
| Thickness of xylem | 0.13 | -0.25 | 0.10 | 0.27 | 0.19 |
| Thickness of vascular column | 0.00 | 0.01 | 0.04 | 0.02 | 0.04 |
| Foliage |
| Specific leaf area | -0.18 | -0.30** | -0.31** | -0.39** | -0.09 |
| Avg. leaf area | -0.48** | -0.20 | -0.51** | -0.30** | 0.18 |
| Avg. leaf mass | 0.15 | -0.23 | 0.06 | -0.23 | -0.26 |
| Physicochemical properties of PFAS |
| LogK\textsubscript{ow} | -0.17 | -0.61** | -0.10 | -0.56** | -0.53** |
| LogD | -0.13 | -0.78** | -0.06 | -0.71** | -0.71** |
| MW | -0.18 | -0.80** | -0.11 | -0.73** | -0.69** |
| vDW volume | -0.18 | -0.80** | -0.11 | -0.73** | -0.69** |
| vDW surface area | -0.18 | -0.80** | -0.11 | -0.73** | -0.68** |
| Mini. projection radius | -0.24* | -0.59** | -0.17 | -0.54** | -0.46** |
| Maxi. projection radius | -0.25* | -0.77** | -0.16 | -0.68** | -0.62** |
understanding the most significant parameters that contribute to PFAS uptake and accumulation by plants is therefore essential to processes of phytoremediation. As can be seen in Table 1, the correlation analysis showed that the physicochemical properties of PFAS showed the most significant correlation with $C_{\text{shoot}}$, $BCF_{\text{shoot}}$, and TF compared to plant physiological characteristics. They are significantly negatively correlated with molecular size (MW, van der Waals volume and surface area, maximum and minimum projection radius), the correlation coefficient $\rho$ is between $0.46 - 0.80$ (Table 1). By contrast, $C_{\text{root}}$ and $BCF_{\text{root}}$ have relatively weak correlations with physicochemical properties of PFAS (Table 1). The correlation diagrams plotted in Fig. 4 A-F illustrated that both $BCF_{\text{shoot}}$ and TF show a downward trend with the increase of LogK\textsubscript{ow} or LogD when the MW of PFAS closes to ca. 400 mol g\textsuperscript{-1}, the values of $BCF_{\text{shoot}}$ and TF approach 1, indicating that the transport in the plant becomes hindered. PFAS in the soil solution can take apoplastic or symplastic pathways to reach the xylem, along which it is transported to aboveground plant compartments via the transpiration stream [34, 66, 67]. Therefore, PFAS with smaller MW or size may pass through the plant cell membranes relatively easily, resulting in greater translocation and bioaccumulation.

Furthermore, hydrophobicity (expressed by LogK\textsubscript{ow} or LogD) is another important parameter influencing PFAS’s root uptake (Table 1 and Fig. 4). When a PFAS’s LogK\textsubscript{ow} > 4 or the LogD > 1.5, and its upward translocation and bioaccumulation is diminished (Fig. 4A,B,D and E). Previous studies have shown that highly lipophilic compounds may readily be retained in the lipid materials present in the endodermis and therefore have difficulty crossing the endodermis [68,69]. Furthermore, given that most of the PFAS of interest are anions under environmentally relevant pH, LogD could be a better indicator than LogK\textsubscript{ow} to describe the equilibrium distribution of ionic PFAS in soil-water-plant systems.

Currently, the effects of PFAS chain length and functional groups have been related to plant uptake [31]. As shown in Fig. 4G-I and S7–9, similar behaviors were observed between compounds of similar structures. For instance, most short-chain PFAS showed strong pairwise correlations of root uptake (measured by $BCF_{\text{root}}$) with that of PFBA (Fig. 4G and S7) regardless of the type of head group. Fig. 4H-I also

Fig. 4. Panels A-F present the correlations between average bioconcentration factor of root ($BCF_{\text{root}}$), translocation factor (TF) and molecular weight (MW), octanol-water partition coefficient (LogK\textsubscript{ow}) and LogD (pH6.5) of PFAS. The dotted lines in Panel A-F represent the y-axis values equal to 1. Panels G-I present correlations of $BCF_{\text{root}}$ and $BCF_{\text{shoot}}$, and TF of PFBA and corresponding parameters of other ten PFAS. The dotted line represents 1:1. See Table S1 for parameter values and explanations.
Moreover, there was a significant correlation between the BCF of PFOS and PFOA always deviated from the 1:1 regression line. This indicates that species with shorter chain lengths behave closer to PFBA, as shown in Fig. 5. In addition, in terms of BCF values, PFAS, correlation analysis revealed that the plant morphological traits were strongly correlated with PFBA and PFPeA, indicating similar translocation pathways (Figs. S9). Overall, these results indicate that short- and long-chain PFAS undergo divergent plant uptake paths. These results also suggest that PFAS size and hydrophilicity predominantly determine its overall plant uptake behavior, and chain length is a more important than functional group when predicting its potential for phytoextraction.

### 3.4. Effect of plant-specific characteristics

Although not as significant as the physicochemical properties of PFAS, correlation analysis revealed that the plant morphological traits influenced the magnitude of PFAS accumulation and translocation (Table 1). As indicated in Table 1, $C_{\text{root}}$, BCF$_{\text{root}}$, and BCF$_{\text{shoot}}$ are significantly positively correlated with the protein and lipid content of roots. Similar phenomenon has been found previously [26]. Lipid and protein content are key determinants of plant uptake and bioaccumulation of PFAS [70–72]. Root proteins and lipids may engage hydrophobic interactions and electrostatic interactions with anionic PFAS, moreover, specific proteins may mediate the transport of PFAS in plants [44,45]. At present, there are few articles on the influence of protein/lipids on the transmembrane and bioaccumulation of PFAS. Animal based studies suggested that these impact depends on the specific types of proteins, lipids and PFAS species [73]. In this context, more data is needed to uncover functions of specific types of proteins (e.g., albumin and structural proteins) or lipids (e.g., neutral triglycerides and carbohydrates and polar phospholipids) on plant uptake of PFAS.

Previous studies have shown that the morphological structure of plants can significantly impact the effectiveness of phytoremediation. However, the correlation between root microstructure and PFAS bioaccumulation was missing from Table 1. Regarding root macrostructure, Table 1 illustrates that BCF$_{\text{root}}$ is positively correlated with root length, while BCF$_{\text{shoot}}$ and TF were negatively correlated with surface area per unit length and average diameter. These correlations suggest that a larger root system with thinner, more fibrous roots can increase the uptake of PFAS from the soil, as roots are the primary means by which plants absorb water (and PFAS) from the soil. Therefore, increasing root length can enhance a plant’s ability to remove PFAS from the soil. Conversely, thicker roots may be less effective at PFAS uptake and mass transfer, consistent with previous research [29]. This is because the fine roots are more efficient at absorbing PFAS, and ion uptake by roots mainly occurs in the root hair zone [74]. Additionally, specific leaf area was negatively correlated with $C_{\text{shoot}}$, BCF$_{\text{root}}$ and BCF$_{\text{shoot}}$ ($r$ of −0.30 to −0.51, Table 1). These results are consistent with previous studies, $Pu$ with small and dense leaves (Fig. S1A), densely distributed stomata, and higher relative growth rates [75], which may result in a faster and higher level of PFAS accumulation in weeds via transport in the vascular system.

To gain further insight into plant uptake pathways, we investigated the primary root cross-sections of Phyllanthus urinaria ($Pu$), Aster indicus (Ai), Setaria viridis (Sv), and Imperata cylindrica (Ic) exposed to PFBA, GenX, and PFOs using FE-EPMA-EDS. Although we faced methodological challenges, such as collapsed fine root sections during preparation or low F response that hindered imaging. As shown in Fig. 5, short-chain PFAS, such as PFBA and GenX, displayed noticeable differences in spatial distribution and had an overall higher signal intensity than PFOs. The higher F response in the PFBA and GenX group suggests a stronger...
tendency for root uptake. In the GenX treatment group, the F signal was observed near the vascular column, with sporadic signal responses in the cortex (Fig. 5E-F). In contrast, PFOS typically had a signal strength of around 1%, resulting in a distribution area similar to background noise. It indicates negligible absorption of PFOS.

4. Conclusions

Currently, the remediation of PFAS-impacted sites is hampered by the diversity of compounds of complex structures, their trace-level concentrations, high stability, and persistence. In this study, we evaluated the feasibility of PFAS phytoextraction by weeds and examined the effect of PFAS physicochemical properties and plant physiological traits by systematic correlation analyses and FE-EPMA-EDS imaging. We found that:

- Up to 41.4%wt of PFAS can be removed from soil through phytoextraction and PFAS taken up through plant roots are accumulated in aboveground plant compartments. Short-chain PFAS compounds were preferentially extracted by weeds compared to their long-chain homologues.
- Physicochemical properties of PFAS showed stronger correlations with their plant uptake potential than plant physiological traits.
- Size and hydrophilicity of PFAS determine its overall plant uptake behaviors, short- and long-chain PFAS undergo divergent plant uptake paths.
- Root lipid and protein content are key determinants of plant uptake and bioaccumulation of PFAS; Plants with thin roots and small leaf areas are desirable for PFAS uptake and storage.

The results of this study emphasize the potential of using local weeds for phytoextraction to remediate PFAS-contaminated sites in future studies.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.131492.

References


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