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Phytoextraction of per- and polyfluoroalkyl substances (PFAS) and the influence of supplements on the performance of short–rotation $crops^{\ddagger}$



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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic compounds threatening water quality and food safety worldwide. Phytoremediation is a nature-based, cost-effective, and scalable solution with high potential for treating PFAS-contaminated sites. However, there is a large knowledge gap regarding choice of plant species and methods to enhance performance. This study assessed the PFAS phytoextraction potential of sunflower (*Helianthus annuus*), mustard (*Brassica juncea*), and industrial hemp (*Cannabis sativa*) in a greenhouse experiment, using inorganic fertilizer and a microbial mixture as supplements. PFAS concentrations were measured using UPLC-MS/MS, and bioconcentration factors for different plant tissues and removal efficiency were determined. Perfluoroalkyl carboxylic acid (PFCA) accumulation was 0.4–360 times higher than that of perfluoroalkyl sulfonic acid (PFSA) homologues of similar perfluoroacroben chain length. Inorganic fertilizer significantly (p < 0.001) reduced PFAS concentration in all plant tissues, whereas the microbial mixture tested did not affect PFAS concentration. PFAS uptake ranged from 0.2 to 33% per crop cycle. Overall, the potential number of crop cycles required for removal of 90% of individual PFAS ranged from six (PFPeA) to 232 (PFOA) using sunflower, 15 (PFPeA) to 466 (PFOS) using mustard and nine (PFPeA) to 420 (PFOS) using Hemp. In this study, the percentage of PFAS removal by plants was determined, and an estimation of the time required for PFAS phytoextraction was determined for the first time. This information is important for practical phytoremediation applications.

1. Introduction

Mass contamination of land with per- and polyfluoroalkyl substances (PFAS) mainly occurs from use of contaminated biosolids, firefighting activities using PFAS-containing aqueous film-forming foams (AFFF), landfilling, and atmospheric deposition. The contaminated land becomes a hotspot and source of PFAS for other parts of terrestrial and marine ecosystems (Hamid et al., 2018; Bolan et al., 2021a). Thus remediation remains a vital measure for managing the fate of PFAS at newly and historically contaminated sites. A wide array of PFAS remediation techniques are being developed and assessed (Naidu et al., 2020).

Phytoremediation is the utilization of plants to accumulate (phytoextraction), immobilize (phytostabilization), or destroy (phytodegradation) pollutants in a target medium (EPA, 2000). This technique is potentially useful for managing PFAS-contaminated sites (Kavusi et al., 2023). PFAS in plants have received much attention, as they are a potential hazard to human health. Some studies have focused on the uptake and transportation of perfluoroalkylacids (PFAA) and the degradation and uptake of PFAS precursors and their metabolites in edible plants (Bizkarguenaga et al., 2016; Blaine et al., 2014; Wen et al., 2014). Other studies have examined the phytotoxicity of PFAS by investigating the effects on plant growth, biomass, and various enzymes and genes (Chen et al., 2019; Zhang et al., 2019a). However, few studies have examined the potential of plants as a PFAS remediation strategy, although various review articles on the topic have been published (Kavusi et al., 2023; Lesmeister et al., 2021; Mayakaduwage et al., 2022).

Plants differ in their ability to accumulate PFAS and the success of a phytoremediation program is strongly determined by the plant species used (Mench et al., 2010; Ghisi et al., 2019). The potential of phytoremediation was first highlighted in a study investigating the fate of PFAS in plant species at a former firefighting site, where removal of up to 1.4 g of 26 PFAS per year was estimated for both silver birch and pine

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(Gobelius et al., 2017). A few subsequent studies have investigated the phytoremediation potential of plant species such as reed grass in wetlands (30–50% removal), *Juncus sarophorus* (9–11% PFOS removal), and other woody and herbaceous species in a greenhouse experiment (Huff et al., 2020; Zhu et al., 2022; Ferrario et al., 2022). Thus, there is a need to identify plant species with good ability to accumulate PFAS. Sunflower, mustard, and hemp have been used previously in heavy metal phytoremediation programs, mainly for their high biomass production, tolerance to environmental stress, and ability to hyperaccumulate contaminants (Nehnevajova et al., 2005; Rathore et al., 2019; Todde et al., 2022). These promising plant species need to be assessed for their phytoextraction potential when exposed to a wide range of PFAS prior to field application.

Furthermore, improving the plant growing environment increases accumulation of contaminants in plants (Vangronsveld et al., 2009; Bolan et al., 2021b), through increased bioavailability of the contaminants in the growing medium or increased plant survival and vigor (Mench et al., 2010; Vangronsveld et al., 2009; Mench et al., 2009). For example, aeration has been shown to increase the PFAS phytoextraction potential of duckweed grown on deionized water at pH 2.3 by up to 80% (Zhang and Liang, 2020). Soil additives such as chelating agents, fertilizers, and microbial supplements have been tested, especially at sites with heavy metal contamination (Radziemska et al., 2021; Wang et al., 2021; Haider et al., 2021). Application of supplements could increase plant biomass and water uptake which could in turn increase PFAS uptake, especially water-soluble PFAS. However, to our knowledge, no previous study has assessed the effect of soil supplements (i.e., inorganic fertilizers and microorganisms) on plant accumulation of PFAS.

This study evaluated the PFAS phytoextraction potential of three short rotation plants (sunflower (*Helianthus annuus*), mustard (*Brassica juncea*), and industrial hemp (*Cannabis sativa*)) in a pot experiment within a greenhouse set-up. Specific objectives were to: (i) determine PFAS concentrations and distribution in the different plants, (ii) evaluate the effect of inorganic fertilizer and a microbial supplement on PFAS uptake in the plants, (iii) estimate PFAS removal by the plants, and (iv) predict temporal changes in the concentrations of selected PFAS in soil hosting the different plant species.

2. Materials and methods

2.1. Chemicals

The target analytes comprised: 10 perfluoroalkyl carboxylic acids (PFCA), namely perfluorobutanoic acid (PFBA), perfluoropetanoic acid (PFPA), perfluorohexanoic acid (PFNA), perfluorohepatanoic acid (PFDA), perfluoroanoic acid (PFDDA), and perfluoroanoic acid (PFDDA); three perfluoroalkyl sulfonic acids (PFSA), namely perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS); and one perfluoroanoic (SI)). Nine mass-labelled internal standards (IS) were used $(^{13}C_4-PFBA, ^{13}C_2-PFHxA, ^{13}C_4-PFOA, ^{13}C_5-PFNA, ^{13}C_2-PFDA, ^{13}C_2-PFUnDA, ^{18}O_2-PFHxS, ^{13}C_4-PFOS, and ^{13}C_8-FOSA) (Table S2 in SI). All above-mentioned compounds (with purity <math display="inline">\geq$ 99% were obtained from Wellington Laboratories (ON, Canada).

Methanol, acetonitrile, ammonium acetonitrile, and formic acid of high analytical grade were obtained from Sigma Aldrich (USA). Ultrapure water was obtained from a Milli-Q Advantage Ultrapure water purification system coupled with a 0.22 μ m Millipak Express membrane and LC-Pak polishing unit from Merck Millipore (Billerica, USA).

2.2. Experimental design

The pot experiment was conducted in a greenhouse at the Swedish

University of Agricultural Sciences (SLU), Uppsala, Sweden, with temperature of 22 °C during the day and 18 °C at night, light/dark cycle set to 16/8 h, light intensity 150 µmol, and 50-60% relative humidity. The experiment had a 3 x 4 factorial design, with three plants (sunflower, mustard, hemp) and four soil supplements (a microbe mixture, fertilizer, fertilizer + microbes, and a control (no fertilizer or microbes)) (Fig. 1). The growing medium consisted of organic potting soil (S-jord garden soil, Hasselfors company, Sweden) spiked to achieve a theoretical concentration of 1 mg kg^{-1} for each PFAS (for details, see text in SI). The spiked concentration is environmentally relevant and has been reported at various contaminated sites worldwide (Brusseau et al., 2020). Measured PFAS concentrations in soil at time point 0 was 1.5 \pm 0.9 mg kg⁻¹ for each PFAS. Seeds of sunflower, mustard, and hemp were pre-germinated for six weeks, and then transplanted (one per pot) in plastic pots with dimensions 13.7 x 13.7 \times 23 cm (L x W x H) and 3 L volume, and containing 1 kg wet weight (ww) of PFAS-spiked soil. Each 3 x 4 experiment was performed in triplicate, resulting in a total of 36 pots (Fig. 1).

Irrigation water containing supplements was applied ad libitum to all pots throughout the experiment. For the treatment with fertilizer, a fertilizer solution containing (g L⁻¹): 51 N, 10 P, 43 K, 4 S, 3 Ca, 4 Mg, 0.17 Fe, 0.20 Mn, 0.10 B, 0.03 Zn, 0.015 Cu and 0.004 Mo obtained from Wallco Plant Nutrition (Cederroth International, Sweden) was used. For the treatment with microbes, a commercial microbial supplement (Tarantula Beneficial Bacterial Liquid fertilizer) containing *Arthrobacter globiformis, Bacillus brevis, Bacillus coagulans, Bacillus licheniformis, Bacillus megaterium, Bacillus polymyxa, Bacillus pumilus, Bacillus subtilis, Bacillus thuringiensis, Bacillus thuringiensis, and Paenibacillus polymyxa was used. It was mixed with irrigation water in a ratio of 1:2 before application. For the fertilizer + microbes treatment, the microbial supplement was mixed with the fertilizer solution. Tap water was used to irrigate all control pots.*

2.3. Sample preparation and analysis

All plants were harvested after three months of PFAS exposure and samples of each plant were divided into seeds, leaves, stem, and root. Water and soil samples were also collected. Preparation and extraction of plant and soil samples for PFAS was done using validated methods published elsewhere (Nassazzi et al., 2022) (details available in SI). Samples of irrigation water were extracted by solid phase extraction (SPE) using Oasis WAX cartridges (Waters, 150 mg, 6 mL, 30 μ m) and the method can be found elsewhere (Gobelius et al., 2017). Branched isomers of PFOS and FOSA were quantified using the corresponding linear standards.

All samples were analyzed using an ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) (Thermo Scientific Dionex Ultimate 3000 Pumps; TSQ Quantiva, Thermo Fisher Scientific, San Jose, CA USA). An Acquity UPLC BEH-C18 (2.1×50 mm, 1.7μ m particle size; Waters Corporation, Manchester, UK) analytical column was used for chromatographic separation. The data were evaluated using TraceFinder software (version 4.1, Thermo Fisher, USA) (details available in SI).

2.4. Quality control and assurance

Laboratory blanks, replicates, method detection limits (MDLs), linearity, and recovery were assessed. MDLs for plants were determined using a signal to noise ratio of 3 in matrix-spiked samples with a concentration of 5 ng g⁻¹ dry weight (dw). The MDLs for water and soil samples were calculated based on average blank + 3xstandard deviation. A calibration curve with concentration ranging from 0.01 to 200 ng mL⁻¹ for each PFAS was used for quantification. Correlation coeffients (R²) of the calibration curve were used to determine the linearity. The relative recovery of the method was assessed using reference composite plant samples (pre-spike n = 3 and post-spike n = 3). The



Fig. 1. Schematic diagram of the triplicate greenhouse experiment set-up of mustard, hemp, and sunflower pots, with and without fertilizer and microbe supplements.



Fig. 2. Average PFAS concentration (μ g g⁻¹ dw) and composition profile (%) in different tissues (n = 3) of sunflower, mustard, and hemp grown in PFAS-spiked soil with different supplements: A) Untreated control, and supplementation with B) only microbes, C) both fertilizer and microbes, and D) only fertilizer.

composition of the reference samples can be found elsewhere (Nassazzi et al., 2022). Recoveries of the internal standards were also determined. Details on the MDLs, relative recovery values, and blank levels are available in Tables S3–S5 in SI.

2.5. Calculations

Plant concentration factors, representing the ability of different tissues (leaf, stem and root) to accumulate contaminants from soil, were calculated using the following equations:

Leaf concentration factor (LCF) =
$$C_{leaf}/C_s$$
 (1)

Stem concentration factor (SCF) = C_{stem}/C_s (2)

Root concentration factor (RCF) =
$$C_{root}/C_s$$
 (3)

Bioconcentration factor (BCF) =
$$C_p/C_s$$
 (4)

where Cs is the PFAS concentration in soil (ng g^{-1} dw), C_{leaf}, C_{stem} and C_{root} is the PFAS concentration in the leaves, stem and root, respectively (ng g^{-1} dw), and C_p is the PFAS concentration in the whole plant (ng g^{-1} dw) at time of harvest.

Removal efficiency (r) was calculated as:

$$r = \frac{C_p M_p}{C_{si} M_s} \times 100$$
(5)

where M_p is plant biomass (g dw), C_{si} is initial soil concentration (ng g^{-1} dw), and M_s is soil mass (g dw).

2.6. Statistics

Descriptive statistics (mean, standard deviation, and range), regression, correlation analyses, and data visualization were performed using GraphPad Prism (version 9.2.0 (332)). Statistical differences between means were evaluated using analysis of variance (ANOVA) at significance level $\alpha = 0.05$, using the R software.

3. Results and discussion

3.1. PFAS concentration in plants of the different species

Of the 14 target PFAS, 12 were detected in different tissues of sunflower, mustard, and hemp (Fig. 2, Tables S7-S8 in SI). ΣPFAS concentration was significantly higher (ANOVA, p < 0.05) in mustard than in sunflower and hemp in all treatments (Table S6 in SI). Without any supplement (control), mustard plants were observed to contain 2-7 times higher concentrations of some PFAS than sunflower (PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFOS, FOSA) or hemp (PFOA, PFDoDA, PFOS, FOSA). A previous study investigating PFAS accumulation in various plants, including sunflower and mustard, found that the concentration of six PFAS was 3-6 times higher in mustard than in sunflower. Studies on other plants have also reported differences in PFAS uptake with plant species and genotypes (Blaine et al., 2014; Gobelius et al., 2017; Xiang et al., 2018). Variations in PFAS uptake are caused by plant anatomy and physiological traits such as biomass, transpiration rate, growth rate, root composition, and exudates (Sheoran et al., 2016).

 $\Sigma PFAS$ concentrations were also significantly different (p < 0.001) between the plant tissue groups and generally decreased in the order: leaf > stem > root \approx seed (sunflower only). In the control, $\Sigma PFAS$ concentrations in sunflower were within the range 0.018–11 $\mu g \ g^{-1} \ dw$ in leaves, 0.003–3.3 $\mu g \ g^{-1} \ dw$ in stems, 0.029–0.41 $\mu g \ g^{-1} \ dw$ in roots, and 0.004–1.3 $\mu g \ g^{-1} \ dw$ in seeds. The $\Sigma PFAS$ concentration range in mustard was 0.015–14 $\mu g \ g^{-1} \ dw$ in leaves, 0.005–2.4 $\mu g \ g^{-1} \ dw$ in stems, and 0.069–0.54 $\mu g \ g^{-1} \ dw$ in roots. In hemp, the $\Sigma PFAS$

concentration range was 0.0008–7.4 μ g g⁻¹ dw in leaves, 0.007–0.48 μ g g⁻¹ dw in stems, and 0.07–0.96 μ g g⁻¹ dw in roots.

In general, the concentration of individual PFAS in the three plant species decreased in the order: PFBA > PFPeA > PFBS > PFHxA > PFHpA > PFHxS > PFOA > PFNA > PFOS > PFDA > PFUnDA > FOSA > PFDoDA. FOSA was only detected in the roots. Short-chain PFCA (i.e., PFBA, PFPeA, PFHxA, PFHpA) were the predominant PFAS accumulated in sunflower seeds (95% of Σ PFAS), and leaf (57–62%) and stem (52-95%) tissues in all three plant species. Sunflower stems had a lower proportion of PFSA (3.7% of Σ PFAS) than stems of mustard (14%) and hemp (21%). In contrast, the composition profile of roots was dominated by PFSA (27-31% of **Σ**PFAS) and long-chain PFCA (32-52%) homologues in all three plant species. This is consistent with previous findings for other plant species (Gredelj et al., 2020; Krippner et al., 2015). Some studies have also reported presence of PFAS in seeds of various cereals such as maize, wheat, rye, and canola in different experimental set-ups (Krippner et al., 2015; Stahl et al., 2013; Stahl et al., 2009). The variation in PFAS composition of different plant parts suggests that water-soluble and mobile short-chain PFAS are transported in the plant during water uptake and transpiration, and accumulate in upper plant parts. Hence, short-chain PFAS dominated in leaves and stems.

Use of a supplement (fertilizers, microbes, or fertilizer + microbes) significantly (p < 0.001) affected $\Sigma PFAS$ concentrations in plants. Fertilizer application (with or without microbes) significantly (p < 0.001) reduced the $\Sigma PFAS$ concentration in all plant tissues, by on average 19% (roots) to 49% (foliage) (Fig. 2C and D). A previous study involving supplementation of lettuce, tomato, and maize with biosolids to meet their nitrogen requirement observed increased concentrations of PFBA and PFPeA at high biosolid application rates to the soil (4 times the agronomic nitrogen requirement) (Blaine et al., 2013). However, this could have been due to more PFAS being applied with increasing biosolids application, rather than an effect of nitrogen on PFAS uptake.

Supplementation with the microbial mixture did not significantly affect PFAS concentration in any of the plant species studied (p > 0.05) (Fig. 2B). The microorganisms applied possess pesticidal effects, and also the ability to increase soil fertility and plant tolerance to stress (Hashem et al., 2019; Dobrzyński et al., 2022). These traits can enhance plant growth and survival, and could potentially increase PFAS concentration in the plant. However, this was not observed under the experimental conditions in the present study. The effect of PFAS on soil microbial communities and microbial PFAS remediation in the presence and absence of plants has been discussed in previous studies (Zhang et al., 2019b; Arslan and Gamal El-Din, 2021), but no published data are currently available on the effect of plant-microbial interactions on PFAS accumulation. Inoculation of plants with microorganisms has been shown to enhance the concentration of heavy metals in plants (Jankong et al., 2007; Alves et al., 2022), but more research is needed on PFAS uptake in plants.

3.2. Plant tissue-specific concentration factors

The bioaccumulation factors for leaf (LCF), stem (SCF) and root (RCF) of the different species were evaluated. Σ PFAS accumulation was generally highest in the order leaves > stem > roots. Observed LCF values for individual PFAS ranged between 0.6 (PFUnDA) and 2092 (PFBA) for sunflower, 0.13 (FOSA) and 1816 (PFBA) for mustard, and 0.033 (PFDoDA) and 2671 (PFBA) for hemp. Observed SCF for individual PFAS ranged between 0.1 (PFUnDA) and 656 (PFBA) for sunflower, 0.17 (PFDoDA) and 365 (PFBA) for mustard, and 0.2 (PFDoDA) and 197 (PFPeA) for hemp. Observed RCF for individual PFAS ranged between 0.37 (PFHxS) and 42 (PFPeA) for sunflower, 0.27 (PFHxS) and 12 (PFBA) for mustard, and 0.97 (PFBS) and 11 (PFUnDA) for hemp PFAS (for details, see Tables S9–S11 in SI). Thus LCF was higher than SCF or RCF, which is similar to previous findings (Navarro et al., 2017; Lechner and Knapp, 2011). This study is the first to report LCF and SCF

for 12 different PFAS in sunflower, mustard, and hemp.

The actual plant tissue concentration factors were generally higher than those previously reported for various edible plants (Ghisi et al., 2019), grass (Yoo et al., 2011), and forest trees (Gobelius et al., 2017). This could indicate that sunflower, mustard, and hemp have higher PFAS accumulation and uptake efficiency than previously studied plant species at similar PFAS concentration. However, plant concentration factors are influenced by PFAS bioavailability which is controlled by the physicochemical properties of PFAS, soil and plant factors (Lesmeister et al., 2021). Our results also revealed that PFAS uptake is dominated by roots, in which dissolved contaminants together with nutrients and

and accumulated in the leaves (Collins et al., 2006). Linear regression plots of log-transformed data showed a significant decrease in LCF and SCF for PFCA with increasing perfluorocarbon chain length for all plant species studied (p < 0.05) (Fig. 3). Each addition of a perfluorocarbon moiety (CF₂) led to a decrease of 0.3–0.5 log units in both LCF or SCF. This is consistent with trends reported for vegetables and grass (Blaine et al., 2013; Yoo et al., 2011; Felizeter et al., 2012), and demonstrates the reliance of PFAS uptake and transport on their physiochemical properties. PFAS bioavailability in the soil is predominantly influenced by compound mobility, which can be predicted using

water can be acropetally transported through the transpiration stream

the soil sorption coefficient (K_d) (Nguyen et al., 2020). A higher K_d value results in increased sorption, due to increases in both hydrophobicity and lipophilicity. Thus with each CF₂ added, both the absorption and transport of PFAS are reduced (Collins et al., 2006; Felizeter et al., 2014).

The LCF and SCF values for PFSA showed similar dependence on perfluorocarbon chain length as seen for PFCA. However, plant tissue accumulation of PFCA was 0.4–360 fold higher than for PFSA homologues of similar perfluorocarbon chain length. Although PFOA and PFOS uptake was observed to be a non-competitive process, a previous study found higher accumulation of PFOA compared with PFOS in wheat straw grown on biosolids-amended soil (Wen et al., 2014), which is in agreement with the results in this study. This can be explained by the physicochemical properties of PFSA molecules, which have a larger structure and stronger sorption to surfaces than PFCA molecules of similar perfluorocarbon chain length (Higgins and Luthy, 2006). In this study, FOSA, which has been shown to have higher K_d than PFOS and PFNA (Nguyen et al., 2020), was mainly found in the roots of all plants investigated. This implies that FOSA was strongly sorbed to the roots, which limited its transportation to the upper parts of the plant.

With regard to RCF, a different relationship with perfluorocarbon chain length was found for PFCA (Fig. 4). A significant decrease in RCF



Fig. 3. Relationship between leaf concentration factor (LCF), stem concentration factor (SCF), and root concentration factor (RCF) and perfluorocarbon chain length for sunflower, mustard, and hemp plants grown on PFAS-spiked soil.



Fig. 4. Estimated number of crop cycles (1 crop cycle = 90 days) required to phytoextract the PFAS A) PFCA and B) PFSA from a contaminated site using sunflower.

with an increase in perfluorocarbon chain length was found for C₃ (PFBA) to C_6 (PFHpA) compounds (p < 0.05), but a significant increase for C₇ (PFOA) to C₁₁ (PFDoDA) compounds (p < 0.05) in all plants. Thus RCF was lowest at C₆ (PFHpA) for all plants. A similar trend has been observed previously for hydroponically cultivated lettuce (Lactuca sativa) and for wheat (Triticum aestivum) in field experiments (Wen et al., 2014; Felizeter et al., 2012). The RCF values were also generally lower than both the LCF and SCF values. The low RCF observed for shorter-chain PFAS was probably due to their high mobility and continuous transportation to other plant tissues. Long-chain PFAS are structurally larger and more lipophilic than their short-chain counterparts (Buck et al., 2011), so limited amounts of long-chain PFAS are absorbed into the roots and there is limited transportation to other plant tissues (Costello and Lee, 2020). It should also be noted that all plant tissues in this study were thoroughly washed with water and MeOH (50:50) before analysis. Therefore, the results obtained mainly represent PFAS taken up by the roots, but it is possible that some PFAS were still sorbed onto root surfaces before washing and analysis. Studies using soil as the planting medium have generally found no relationship between RCF and chain length, especially at high PFAS concentrations (Blaine et al., 2014; Wen et al., 2014).

3.3. Species-specific accumulation

Bioconcentration factor, determined as the ratio of **SPFAS** concentration in the plant to Σ PFAS concentration in the soil at harvest, was used to assess and compare the overall PFAS accumulation and phytoextraction potential of the three plant species studied. Hemp had the highest BCF for Σ PFAS (0.05–1170), followed by sunflower (0.03–957) and mustard (0.19-590) (Tables S12-S13). BCF values >1 signify plant ability to accumulate a contaminant, while BCF values >10 indicate that the plant is a hyperaccumulator (Huff et al., 2020). Based on these thresholds, all three plant species tested were classified as hyperaccumulators of at least five compounds (PFBA, PFPeA, PFHxA, PFHpA, and PFDoDA). In addition, hemp was a hyperaccumulator of PFOA, PFNA, PFDA, PFBS, and PFHxS. A previous study assessing PFAS accumulation in both woody and herbaceous plants observed similar results, but found that sunflower only hyperaccumulated PFPeA among six compounds analyzed (Huff et al., 2020), in contrast to our results. In the same study, mustard was observed to have higher BCF values for all compounds except PFPeA than the BCF values found in our study. Other studies on vegetables and forest plants also report variations in plant BCFs, which they attribute to plant chemical composition (lipid:protein content) and the PFAS fingerprint of the growing medium (Gobelius et al., 2017; Xiang et al., 2018; Blaine et al., 2013; Wen et al., 2013).

3.4. Total plant burden

Total plant burden was determined as the absolute weight (in µg) of PFAS in plant biomass. Sunflower had the highest Σ PFAS burden (819 ± 262 µg per plant), followed by hemp (732 ± 111 µg). Despite mustard

having high PFAS concentrations in plant tissues, it had the lowest Σ PFAS burden (417 \pm 97 μ g), which can be explained by the lower biomass of mustard plants compared with sunflower and hemp. At the time of harvest, hemp had not reached flowering, whereas sunflower and mustard had flowered. Therefore, the phytoextraction potential of hemp may not have been fully exploited in this study as plants probably did not attain full maturity. Mass PFAS distribution in different tissues was relatively similar between the plants (Table 1). Of the total PFAS mass (µg) found in the plants, C3-C9 perfluorocarbon PFAS were dominant in the shoot system (leaves and stem), while C10-C11 perfluorocarbon PFAS were dominant in the root system, as also indicated by the LCF, SCF, and RCF values. The PFAS dominance in the shoot system could have positive implications for phytoremediation, as shoots are easier to harvest and complete root harvest can be difficult to achieve. Mustard and sunflower had 4-6 times more short-chain PFAS in their stems than hemp, which accumulated >90% of this group of compounds in the leaves. Only a small fraction (<6%) of C₃-C₆ perfluorocarbon PFAS accumulated in seeds in sunflower.

3.5. Effect of fertilizer and microbial supplements

Plant response to the different supplements was examined using plant biomass, PFAS concentration in plant tissues, and effect on total plant burden. Sunflower (281 g ww, 54 g dw) and hemp (140 g ww, 47 g dw) produced more average biomass per plant than mustard (12 g ww, 7.9 g dw). Mustard had a much higher proportion of dry matter (up to 68%) than sunflower (19%) and hemp (34%). Addition of fertilizer was observed to increase plant biomass by 2- to 3-fold in sunflower and hemp, but slightly reduced the dry mass proportion for both species (from 34 to 18% for hemp, and from 19 to 15% for sunflower). There were no observable changes in biomass and dry matter content for mustard. The increase in biomass in sunflower and hemp did not result in an increase in PFAS accumulation. As previously noted (section 3.1), fertilizer application led to reduced PFAS concentration in plants. However, the greater biomass obtained for plants treated with fertilizer led to no significant difference in absolute PFAS mass in plants (ANOVA, p < 0.05) (Table S14 in SI). The mechanism for reduction of PFAS concentration in plants due to addition of inorganic fertilizers is not fully understood. However, possible reasons include (i) increased cation concentration that could reduce PFAS bioavailability (Cai et al., 2022), or (ii) increased water uptake, which led to dilution of contaminants in the plant. In the present study, use of the microbial supplement had no observable effects on biomass, dry matter content, or plant burden of PFAS. This is consistent with previous findings of increased plant biomass, but reduced heavy metal concentration, in rye (Secale cereale) supplemented with both inorganic fertilizers and microbes at a contaminated site in China (Chen et al., 2023). Further studies using metagenomics and root microscopy are needed to identify potential synergistic effects between specific organisms and plants, and their effect on PFAS accumulation.

Table 1

Distribution of individual PFAS based on the total burden in tissues of sunflower, mustard, and hemp, expressed as a percentage of their total PFAS uptake. A color gradient from green (highest) to red (lowest) represents the mass distribution.

	Sunflower (%)				Mustard (%)			Hemp (%)		
Compound	Seed	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
PFBA	4	61	35	0	64	36	0	94	6	0
PFPeA	6	46	47	1	56	43	1	91	9	0
PFHxA	3	65	30	2	67	32	1	94	6	0
PFHpA	2	76	21	1	73	26	1	94	5	1
PFOA	0	91	6	2	77	21	2	93	6	1
PFNA	0	88	5	7	71	24	5	84	13	3
PFDA	0	63	0	37	55	23	22	54	33	14
PFUnDA	0	18	2	81	28	19	53	16	41	43
PFDoDA	0	0	0	100	6	7	87	2	21	78
PFBS	0	94	5	1	72	27	2	95	5	1
L-PFHxS	0	95	4	2	79	19	3	95	3	1
Br-PFHxS	3	92	2	3	87	7	7	95	2	3
L-PFOS	0	86	1	13	71	19	10	75	15	9
Br-PFOS	0	89	0	11	75	17	8	88	0	12
FOSA	0	0	0	100	12	0	88	0	0	100

3.6. Phytoremediation potential

All plants grew without any visible abnormalities (such as chlorosis, stunting, or reduction in weight) despite the presence of PFAS, with or without supplements. This indicates that the species studied had high tolerance to PFAS contamination and could grow at PFAS-contaminated sites. Other studies have also observed no negative impact of PFAS on plant growth (Gobelius et al., 2017; Zhao et al., 2016), except at very high concentrations (e.g., 5–20 mg L⁻¹) (Chen et al., 2019; Wen et al., 2013). This is ≥ 10 times higher than the concentration used in this study and not realistic for PFAS-contaminated sites (Gobelius et al., 2017).

Individual PFAS uptake efficiency from soil was 0.2–33% for sunflower, 0.2–14% for mustard, and 0.2–24% for hemp, based on the PFAS concentrations in soil and all plant tissues (Table S15 in SI). For all plants, PFUnDA and PFDoDA had the lowest PFAS removal by plants, while the highest PFAS uptake efficiency was observed for PFPeA (14–33%), followed by PFBA (12–30%), PFBS (3.2–12%), PFHxA (3.7–6.8%), PFOA (1–1.1%), PFHxS (1.5–3.3%) and PFOS (0.4–0.5%).

The phytoremediation potential of the three plant species for individual PFAS was predicted based on crop cycles (1 crop cycle = 90 days of PFAS exposure), assuming constant PFAS uptake for subsequent crop cycles. Previous studies have reported an effect of PFAS concentration in the growing medium (e.g., water and soil) and PFAS concentration in plant tissues (Gobelius et al., 2017; Wen et al., 2013). However, there is no consensus on the influence of PFAS concentration on PFAS removal efficiency (Lesmeister et al., 2021; Wen et al., 2013). We, therefore, estimated the number of crop cycles required to phytoremediate soil with a similar PFAS concentration as tested in this experiment using sunflower (highest PFAS removal) and mustard (lowest PFAS removal). PFAS concentration after a cycle of phytoextraction was determined as $C_i - rC_i$, where C_i is initial PFAS concentration and number of cycles required were determined using an iterative approach.

For sunflower, shorter-chain PFAS required fewer crop cycles to reach 90% PFAS removal from soil. PFBA and PFPeA were estimated to require 6–7 crop cycles, PFHxA 34, PFHpA 96, PFOA 232, PFBS 20, PFHxS 70, and PFOS 458 crop cycles (Fig. 4 and Table S16 in SI). Estimated crop cycles required when using hemp increased as follows: PFPeA required 9 crop cycles, PFBA 14, PFBS 16, PFHxA 32, PFHxS 66, PFHpA 68, PFOA 165, PFOS 420. Similar findings were made for mustard, but this species generally was estimated to require more cycles than sunflower, i.e., PFBA required 19 crop cycles, PFPeA 15, PFHxA 60, PFHpA 100, PFOA 192, PFBS 20, PFHxS 70, and PFOS 466 crop cycles. The results highlighted the suitability of the method for media dominantly contaminated with short-chained PFAS. Furthermore, the results suggest that sunflower is a more suitable plant than mustard for phytoremediation of PFAS-contaminated sites, however, field experiments are required to verify these findings.

4. Conclusions

This study investigated the PFAS phytoextraction potential of sunflower, mustard, and hemp in greenhouse experiments. The results showed differences between the plant species in phytoremediation and PFAS-specific accumulation in different tissue types. All three species hyperaccumulated at least five of the target PFAS, and are thus potentially suitable for phytoremediation in the field. Treatments to optimize the phytoextraction potential of the species by using inorganic and microbial supplements gave only a limited improvement in PFAS uptake for all species. The estimated number of crop cycles required to remove individual PFAS from contaminated soil was lowest, i.e., removal efficiency was highest, for short-chain PFAS. This new information can be used in risk management and practical application of phytoremediation in the field. Harvested plant biomass can be used for energy production through which extracted PFAS can be degraded. However, life cycle analysis to determine and prevent potential negative environmental impacts of this process is needed for the future. Future studies should also examine other microbial species and the effects of microbial interactions with plants.

Credit author statement

Winnie Nassazzi: Conceptualization, Methodology, Data curation, Formal analysis, Writing-original draft, and editing. Tien-Chi Wu: Investigation, Data curation and Writing-review and editing, Jana Jass: Methodology, Writing-review and editing, Supervision, Foon Yin Lai: Conceptualization, Validation, Formal analysis, Writing-review and editing, Supervision. Lutz Ahrens: Conceptualization, Writing-review and editing, Supervision, Funding acquisition, and Project administration.

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.122038.

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