Chemosphere 370 (2025) 143859

Contents lists available at ScienceDirect

Chemosphere

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

Phytoremediation evaluation of forever chemicals using hemp (*Cannabis sativa* L.): Pollen bioaccumulation and the risk to bees

Timothy Wright [b], Marcus Crompton [a,b], Daniel Bishop [b], Glen Currell [b], Laxmi Suwal [b], Brett D. Turner [a,b,*]

[a] Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Ultimo, Sydney, NSW, Australia
[b] School of Engineering Engineering, Department of Civil Surveying and Environmental Engineering, The University of Newcastle, University Drive, Callaghan, NSW, 2308, Australia

HIGHLIGHTS

- Cannabis sativa L. (hemp) shows strong potential for PFAS phytoremediation based on its bioconcentration factors.
- Perfluorocarboxylates (PFCAs) accumulated more in pollen than perfluorosulfonates (PFAS) due to unknown mechanisms.
- Wind-dispersed hemp pollen poses a risk of spreading PFAS contamination during phytoremediation.
- A honey bee could consume as much as 124.5 and ~3.1 μg/kg.bw/day of total PFAS and PFOS, respectively, from hemp pollen.
- PFAS may alter the sex of hemp plants favouring male differentiation.

GRAPHICAL ABSTRACT

ARTICLE INFO

Handling Editor: T Cutright

ABSTRACT

Per- and polyfluoroalkyl substances (PFAS), often termed "forever chemicals," are a diverse group of persistent fluorinated compounds, including the well-known perfluorooctanesulfonic acid (PFOS), which has been identified as lethal to bee larvae. However, the risk of PFAS exposure through pollen, a bee's primary food source, has not been thoroughly investigated. In controlled greenhouse experiments, *Cannabis sativa* L. (hemp) plants were cultivated in soil contaminated with eight PFAS compounds. Phytoremediation potential was assessed by measuring bioconcentration factors (BCF) in both the total above-ground biomass and pollen. The study found that BCF for total PFAS in hemp pollen was significant (>20.8), with over 45% of the total PFAS uptake of around 3248 μg/kg concentrated in the pollen. Based on these figures, the estimated daily intake (EDI) of PFOS for western honeybees (*Apis mellifera*) was found to be about 124.5 μg/kg body weight per day. These findings

* Corresponding author. Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Ultimo, Sydney, NSW, Australia.
E-mail address: Brett.Turner@UTS.edu.au (B.D. Turner).

https://doi.org/10.1016/j.chemosphere.2024.143859
Received 16 August 2024; Received in revised form 14 November 2024; Accepted 28 November 2024
Available online 12 December 2024

underscore a critical global threat to pollinator health, with significant implications for agriculture and biodiversity.

1. Introduction

The media has dubbed the documented decline in the global insect pollinator population as a potential "ecological Armageddon" (Rhodes, 2018). The western honey bee (*Apis mellifera*), is the primary pollinator in the insect realm (Lima et al., 2022), and therefore holds critical significance for agricultural productivity, contributing to USD 15 billion worth of crop production annually in the United States alone (Dingha and Jackai, 2023). Consequently, the well-being and survival of honeybees are intrinsically linked to humanity's survival. Factors such as habitat loss, climate change, and exposure to environmental toxins like pesticides and herbicides have all been implicated in the decline of bee populations.

Recently, Sonter et al. (2021) demonstrated the detrimental effects of perfluorooctane sulfonate (PFOS), a member of the poly- or perfluoroalkyl substances family (PFAS), on bee colonies. They found that brood larvae development was completely inhibited at a PFOS concentration as low as 0.02 mg/L. At this level, pupae were observed to be removed by worker bees, indicating they were dead, with death hypothesized as being due to nurse bees feeding the larvae PFOS-contaminated food.

PFAS, comprising over 4700 synthetic, highly fluorinated aliphatic chemicals, possess beneficial properties that have led to their widespread incorporation into various products such as food wrappers, nonstick cookware, paints, carpets, and furniture protectants (Calvert et al., 2022). Additionally, PFAS are extensively utilized for suppressing hydrocarbon fuel fires, notably in aqueous film-forming foams (AFFFs) at airports and military installations worldwide (Turner et al., 2019). Recognizing their environmental persistence, tendency to accumulate in human and animal tissues, and associated health risks, efforts were initiated to phase out PFOS production and use in the early 2000s (USEPA, 2000). The global reduction of PFOS production and use occurred in 2009 when the Stockholm Convention on Persistent Organic Pollutants (POPs) listed PFOS as a persistent, bioaccumulative, and toxic substance (Secretariat of the Stockholm Convention, 2019). Subsequently, other commonly used PFAS chemicals like perfluorooctanoic acid (PFOA) and perfluorohexanesulfonic acid (PFHxS) were also listed as POPs in 2019 and 2022, respectively (Secretariat of the Stockholm Convention, 2019).

Due to their environmental persistence and potential toxicity PFAS are termed "forever chemicals" and are described as "one of the most seminal public health challenges for the next decades" (Knaus, 2017) particularly for soil contamination. Soil acts as a major reservoir (Rankin et al., 2016; Strynar et al., 2012; Washington et al., 2019) for these compounds, with an estimated PFOS global load of more than 7000 metric tons and documented concentrations as high as 5500 μg/kg (Strynar et al., 2012; Broussard et al., 2023). The extent of PFAS contaminated soil makes remediation a complex and costly endeavour, therefore making use of plants to remediate the soil is an attractive, relatively inexpensive option (Salt et al., 1998).

Hemp, with its rapid growth, high biomass production, and extensive root system, has proven effective in the phytoremediation contaminated soils, effectively removing heavy metals (Linger et al., 2002), radionuclides (Vandenhove and Van Hees, 2005), and Polycyclic aromatic hydrocarbons (PAHs)(Campbell et al., 2002). Although field tests for PFAS phytoremediation using hemp have shown promise (Nason et al., 2023; Tien-Chi, 2021; Nassazzi et al., 2023), the extent of PFAS contamination in hemp pollen remains unexplored despite concerns about their potential biomagnification and toxicity within the food chain. This knowledge gap necessitates further research to assess the efficacy and safety of hemp phytoremediation for PFAS-contaminated soils.

Bees and other pollinators may encounter environmental contaminants like PFAS through various pathways, including contaminated surface water and soil. Although hemp is primarily wind-pollinated, studies have confirmed that bees actively collect and utilize hemp pollen as a protein source for brood development (Dingha and Jackai, 2023; Saunders, 2018; Zhang et al., 2021; Rivernider et al., 2017; Flicker et al., 2020). Moreover, a study by Flicker et al.(ASTM, 2019) identified sixteen different bee species visiting hemp flowers, highlighting the importance of hemp pollen as a late-season resource when other floral sources dwindle. This raises concerns about bees' potential ingestion of PFAS through their primary food source, despite hemp not relying on insects for pollination. While the presence of PFAS in pollen and its subsequent impact on bees have been hypothesized (Sonter et al., 2021; Felizeter et al., 2014; Blaine et al., 2014) there is a lack of definitive confirmation.

This paper, the first in a series derived from a comprehensive laboratory-controlled phytoremediation experiment, aims to address this knowledge gap by confirming the presence of PFAS chemicals in hemp pollen. By doing so, it seeks to validate the hypothesis that PFAS-contaminated pollen poses a risk to bee populations globally and underscores the need for further research into the environmental and ecological implications of PFAS contamination in plants.

2. Methods

PFAS analyses were performed with a Waters Xevo® TQ-XS quadrupole mass spectrometer in negative ion mode coupled with a Waters Acquity™ ultra-performance liquid chromatography (UPLC) I-class system (Waters Corporation, Sydney, Australia) fitted with the Waters PFAS analysis kit. The analytical column was a Waters ACQUITY UPLC I-class CSH Phenyl Hexyl (1.7 μm, 2.1 × 50 mm) at 35 °C with the eluent mobile phase A consisting of 95:5 deionised (DI) water: methanol + 2 mM ammonium acetate and mobile phase B methanol + 2 mM ammonium acetate. The isocratic program is summarised in Tables SI–1.

PFAS internal calibration consisted of a 9-point curve with triplicate injections using PFAC-30PAR standards (Wellington Labs) over the range of 2 to 2000 ng/L and analysed using Targetlynx 4.2 software (Waters Corporation) with a $1/x^2$ weighting for calibration curves. Internal calibration was achieved using respective mass-labelled internal standards (IS) (Wellington Labs; Tables SI–2). Mass-labelled surrogate recovery standards (RS) (Wellington Labs, Tables SI–3) were used to ensure that no PFAS source or sinks affected the analysis methods with recoveries of 70–130% required (ASTM, 2019). Quality control (QC) samples (with surrogates and standards only) were included in the analytical sequence to ensure MS performance.

2.1. PFAS liquid sample analysis

All water samples were prepared using ASTM D7979-17. Briefly, to a 15 mL centrifuge tube, 2.5 mL of methanol, 2.5 mL of the homogenised target solution and 25 μL of each isotopically labelled standards (MPFAC-C-IS and MPFAC-C-ES) were added. The sample was then vortex mixed for 1 min and filtered through a 0.22 μm regenerated cellulose (RC) filter into a new 15 mL centrifuge tube. A 2.5 mL aliquot of this filtrate was then transferred to another 15 mL centrifuge tube, followed by 10 μL of glacial acetic acid, 25 μL of isotopically labelled surrogates and further mixing. The samples were then analysed using LC-MS/MS as outlined above. A solvent and reagent blank was also prepared and analysed to determine the background PFAS concentrations.

The method Limit of Detection (LOD) and Limit of Qualification

(LOQ) were calculated (Shrivastava and Gupta, 2011) from the analysis of the nine-point (in triplicate) calibration curve where the LOD = 3.3σ/S and LOQ = 10σ/S (σ is the standard deviation of the response and S is the slope of the calibration curve). For the target PFAS in this study, the LOD ranged from 0.27 to 1.55 ng/L and LOQ from 0.82 to 4.71 ng/L and are reported in Tables SI–4.

2.2. PFAS soil analysis

The standard test method for the determination of PFAS compounds in soil, ASTM 7968, was used here. In summary, 2 g of dried and milled soil was added to 5 mL of methanol and 5 mL of deionised (DI) water and mixed. To this 20 μL of NH_4OH (30%) and mass labelled recovery standard (MPFAC-C-IS) was added to give a final concentration of 100 ng/L. The solution was then vortex mixed, and then tumbled end-over-end for 1 h. Samples were then centrifuged at 3000 g for 1 min, before being passed through a 0.22 μm RC syringe filter. To 5 mL of this filtered sample, 25 μL of isotopically labelled internal calibration standard (MPFAC-C-ES) and 50 μL of glacial Acetic acid was added and mixed. A solvent and reagent blank was also prepared and analysed to determine the background PFAS concentrations. ASTM 7968 defines the Lower Limit of Quantitation, LLOQ (ng/kg), to be the lowest concentration in the calibration curve (ng/L) normalised to the soil solid-to-liquid ratio i.e. 0.01 μg/kg for all PFAS analysed. However, herein, we adopt a more conservative approach and use the LOQ to determine the LLOQ if it exceeds the minimum standard concentration (2 ng/L (Tables SI–4);). Unless specified, all soil-based samples are presented in terms of dry weight.

2.3. PFAS biota analysis

The acetic acid extraction (ACE) method of Yoo et al. (Yoo et al., 2011) was used to extract PFAS from the biota samples. In summary, a maximum of 2.0 g of lyophilised and homogenised sample was weighed into a 50 mL vial 2.5 mL of 1% Acetic acid and an aliquot of mass-labelled recovery standard (MPFAC-C-ES) to achieve a concentration of 100 ng/L. This mixture was then placed in an ultrasonic bath for 30 min after which 7.5 mL of neat methanol was added, followed by end-over-end mixing for 60 min. The mixture was then centrifuged for 5 min at 3000 g and the supernatant was poured off into a separate vial. This process was repeated with the addition of the 1% Acetic acid using the remaining solid pellet and the resulting supernatant was added to the same vial used previously. The combined supernatant was dried under nitrogen, then reconstituted in 5 mL of neat methanol and mixed end-over-end for 60 min. A solvent and reagent blank was also prepared and analysed to determine the background PFAS concentrations. The sample was then analysed using the ASTM D7979 standard method for PFAS determination in a water matrix. As the recovery standards were already added during the ACE extraction, its addition during the ASTM 7979 method was omitted. All samples were injected twice, and the average concentration was used if the mass-labelled recovery standards were within 30% of the target value of 100 ng/L. Unless specified, all plant-based samples are presented in terms of dry weight.

2.4. Soil preparation

The trial utilized soil obtained from a local nursery which supplied Searles Platinum Potting Mix in 30 L bags (Searles, Queensland, Australia; see Tables SI–8 for complete chemistry or virgin soil). The uncontaminated (control) soil was mixed first to prevent cross-contamination. Details of the procedure are outlined in the SI.

The procedure was repeated for the PFAS contaminated soil, with the following deviations. In place of tap-water, PFAS-contaminated groundwater sourced from Williamtown Royal Air Force Base (RAAF), Newcastle Australia (with permission) was used to produce the PFAS soil. As the groundwater is predominately PFOS and PFHxS, aliquots of analytical grade PFBA, PFBS, PFPeA, and PFOA were added to observe the effect of the PFAS C–F chain length. Table 1 show the post-mix soil concentrations for the PFAS experimental and control soil. The initial PFAS chemistry of the spiked groundwater and the concentrations of an additional 210 inorganic and organic compounds analysed in the groundwater at Williamtown are shown in Tables SI–6 & SI-7 respectively. Apart from aluminium (830 μg/L) and iron (990 μg/L) no other compound was detected above the laboratory limits or reporting. Soil concentration and standard deviation were derived from the analysis of a single sample from each pot (n = 12) before hemp planting. The control samples feature detectable quantities of PFAS, possible sources of this contamination were the organic materials used to create the base commercial potting mix, and the plastic bags the potting mix was purchased in.

2.5. Plant growth

This study used *Cannabis sativa* L. plants of the Yuma 1 cultivar, a low THC species with a high fibre yield. Three seeds were added to the centre of each pot with an equidistant spacing in a soil depth of approximately 1 cm. The overall dimensions of the 50 L pots used can be found in Figs. SI–6. At two days post-germination (~3 weeks), the smaller plants were removed, leaving the largest seedling to grow. Specific growing, lighting, and harvesting methods are outlined in the SI with Figs. SI–1 showing a schematic of the experimental setup. Control and PFAS plants were cultivated in separate greenhouses, completely isolated from each other.

Each plant was grown in its own pot and duplicated for each time-step viz 5, 7, 9, 11, 13 and 15-week timeframe post-planting. Figures SI-2 & 3 shows hemp plants at various stages of growth. At the beginning of every week, the height of all plants was measured, from a datum (top edge of pot) to the highest point of the plant. Once a plant had grown for its allotted time, it was harvested in the following order: Reproductive tissues (seeds on female plants or pollen sacks on male plants); see Figure SI-4 & 5), leaves, secondary stems, roots, and the primary stem.

Post-harvesting, all plant tissues were lyophilised using a freeze-dyer before processing. Stems and roots were then chopped into smaller pieces and then added individually to a commercial stainless steel food

Table 1
Initial PFAS soil and control soil dry weight concentrations (μg/kg.dw) and standard deviation (n = 13). *Total (∑) PFOS and PFHxS are the sum of the respective linear and branched (Br) isomers; Br-PFOS ~3.44 ± 0.48 μg/kg.dw; Br-PFHxS 1.50 ± 0.23 μg/kg.dw. Total (∑) PFAS is the sum of all PFAS presented.

PFAS Sub-class (Wang et al., 2017)	Compound	Abbreviation	PFAS Soil (μg/kg. dw)	Control Soil (μg/kg. dw)
Perfluoro-carboxylates (PFCAs)	Perfluorobutanoic acid	PFBA	11.64 ± 2.34	0.29 ± 0.042
	Perfluoropentanoic acid	PFPeA	12.06 ± 2.34	0.083 ± 0.0069
	Perfluorohexanoic acid	PFHxA	0.38 ± 0.05	0.13 ± 0.002
	Perfluorooctanoic acid	PFOA	6.53 ± 0.69	0.42 ± 0.015
Perfluoro-sulfonates (PFSAs)	Perfluorobutane sulfonic acid	PFBS	12.71 ± 1.96	0.021 ± 0.019
	Perfluoropentane sulfonic acid	PFPeS	0.06 ± 0.01	<LOQ
	Perfluorohexane sulfonic acid	ΣPFHxS*	9.45 ± 0.96	<LOQ
	Perfluorooctane sulfonic acid	ΣPFOS*	11.62 ± 1.52	0.086 ± 0.0 46
		TOTAL (ΣPFAS)	64.45 ± 4.30	1.45 ± 0.131

blender (Robocoupe). The blended stem and root material was then further pulverised with ceramic balls (approx. 1 cm diameter) inside a 120 mL polypropylene tube on a sample rotating apparatus at 30 RPM. Leaf tissue only required blending. Once these tissues were adequately pulverised (mimicking a coarse powder) they were then frozen at −40 °C before analysis. The above-ground tissue (AGT) concentration is defined as the sum of all PFAS compounds quantified (>LLOQ) in each above-ground plant component (i.e. AGT = Σ([stem], [leaf], [pollen]). Note: as the target here is pollen uptake of PFAS, only the male AGT will be discussed. Further publications will deal with the PFAS partitioning in below ground (roots and rhizosphere), and the individual above grant compartments (leaf, stem etc.)

2.6. Data analysis and bioconcentration factors

Using the online statistical software, Graphpad Prism (Graphpad, 2017), Grubbs' test was used to detect the presence of any outlier in a data set (Grubbs, 1950). The metric employed to gauge a plant's capacity to accumulate certain PFAS species was the bioconcentration factor (BCF). The BCF is defined as the ratio of the individual PFAS concentration, at any time *t*, found in the plant component studied (e.g. pollen) to its initial (*t=0*) soil concentration (Huff et al., 2020). Such that:

$$BCF = \frac{[Pollen]_t}{[Soil]_{t=0}} \quad (1)$$

Here, the BCF is unitless ([μg/kg.dw]/[μg/kg.dw); A BCF of above 1 indicates higher uptake of the contaminant in the plant than in the soil, while a BCF of less than 1 means more contaminant resides in the soil than taken up by plants (Hellen and Othman, 2016). To ascertain the uncertainty of BCFs, the general law of error propagation was applied (Schlechtriem et al., 2019). Where applicable, the one-way ANOVA or unpaired t-tests were used (Graphpad Prism) to determine the significance of the difference between the data at the $P < 0.05$ level.

The estimated daily intake (EDI; ng/kg.bw/day) of a chemical can be used to evaluate the exposure of a species to potentially harmful chemicals in the food and water consumed by the organism. For a particular PFAS chemical the EDI for a honeybee through pollen consumption (excluding water sources) be calculated from equation (2).

$$EDI = \frac{Daily\ intake\ \left(\frac{g}{d}.dw\right) x\ [PFAS]\left(\frac{ng}{g}.dw\right)}{Body\ weight\ (kg)} \quad (2)$$

3. Results & discussion

3.1. Plant growth

Fig. 1 displays the average plant height at each time point for both control and experimental groups. After 35, 42, and 49 days, the control plants were observed to be slightly taller (approximately 0.15 m) than those grown in PFAS-treated media. These differences were statistically significant ($P < 0.05$) at days 35 ($P = 0.036$), 42 ($P = 0.017$), and 49 ($P = 0.027$), as determined by a one-way ANOVA. However, from week 8 onwards, very little difference in the heights was observed. With sexual maturation beginning from ~10 weeks, it is not possible for any of these height-dependent differences to be due to differences in plant gender, as there are no height differences in immature *C.sativa* plants (Campbell et al., 2021). Growth tapered off with all plants reaching about 2.0–2.1 m in height due to sexual maturation and the limitations imposed by the height of the tents and the suspended lights (Figs. SI–3). These differences in plant height suggest that the presence of PFAS in the soil affected growth of the young plants, a finding supported by Ofoegbu et al. (2022) (Ofoegbu et al., 2022) in their study on the effect of PFAS on wheat.

The *C.sativa* grown in this study is a dioecious cultivar, meaning

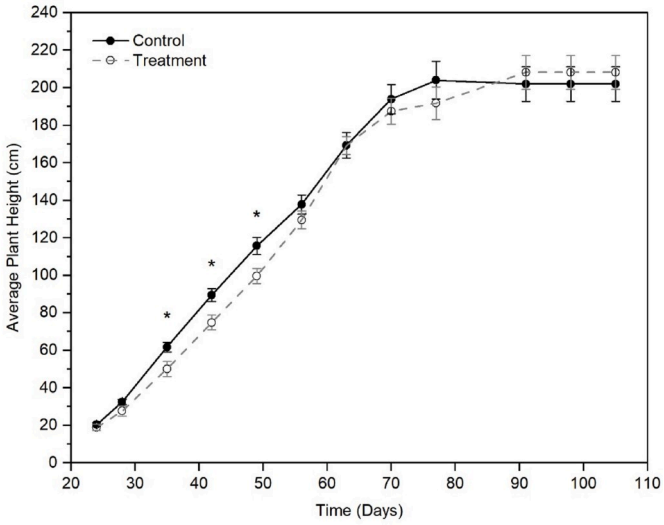

Fig. 1. Plant height of control and experimental (PFAS-exposed) hemp plants after planting. Asterisks (*) represent weeks where the height of treatment and control plants were significant different ($P < 0.05$).

individual plants are either male (pollen producing) or female (seed producing). Research suggests that female plants predominate, especially in fertilised soil (Matros et al., 2023) with no well-established methods for differentiating between male or female individuals until sexual maturation (Campbell et al., 2021). By the time maturation occurred (~10 weeks of growth), six (three time-steps in duplicate) of the initial twelve plants (six time-steps all in duplicate; Figs. SI–1) in each tent had already been harvested. In the experimental (PFAS--treated) soil, four of the remaining six plants were male, and two were female. Conversely, in the control group five of the remaining plants were female and one male. Of note, in a pre-trial experiment (results not shown) plants grown in PFAS-contaminated soil were also predominately male. Applying the binomial theorem, there is only a 0.88% chance that the plant sex distribution observed here could occur. This suggests that the presence of PFAS may influence plant sex, potentially through changes in hormone signalling (Malabadi et al., 2023). This is significant because male hemp plants produce pollen, with a single hemp male flower producing up to 350,000 pollen grains, with each plant bearing hundreds of flowers (Small and Antle, 2003). A skew toward male plants would increase the amount available to pollinators and enhance the wind-borne spread of PFAS contamination. Figs. SI–5 shows the extent to which large amounts of yellow, sticky pollen was produced. This covered all surfaces of the lights and tent fixtures.

3.2. PFAS partitioning

Fig. 2 shows the total PFAS concentration in the combined above-ground tissues (AGT) and pollen for the experimental and control plants. PFAS was found to have accumulated within the pollen, with Grubbs' Test (Graphpad, 2017; Grubbs, 1950) showing that there was only one statistical outlier (PFPeA) in all data points at the $P < 0.05$ significance level ($n = 4$, weeks 11–15). Therefore, all data points from weeks 11–15 were combined to increase statistical reliability.

In the experimental plants, approximately 3247 μg/kg dw of PFAS was found in the AGT, with about 1345 μg/kg dw in the pollen, indicating that pollen is a significant PFAS sink, containing around 41% of the total PFAS by week 15. In comparison, the control group pollen contained approximately 43 μg/kg of total PFAS, with about 114 μg/kg dw in the AGT. In the experimental group (Fig. 2) the total PFAS concentration between the AGT and pollen was significantly different, as determined by the unpaired *t*-test ($P = 0.0016$; <0.05, 0.039). Due to the presence of only one male (pollen producing) plant in the control group,

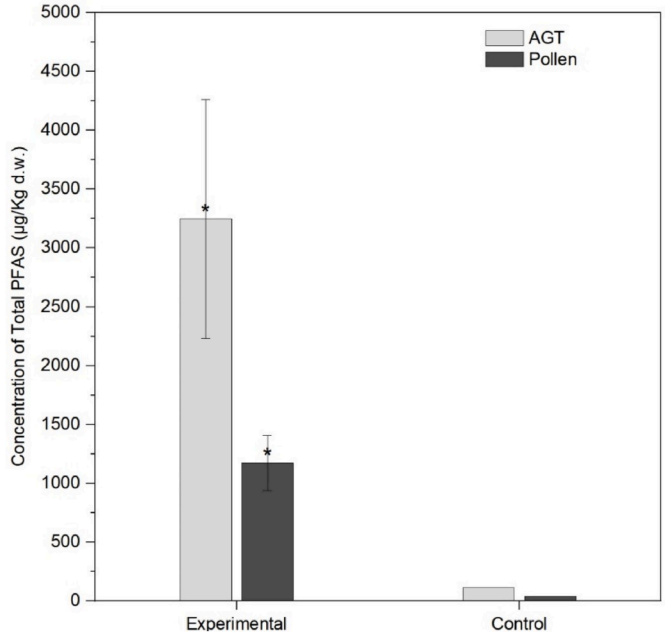

Fig. 2. Total PFAS concentration in above-ground tissue (AGT = ∑stem, leaf, pollen)), and pollen from experimental and control hemp plants. Note: For control, n = 1 (only one male plant was present in this group). Asterisks (*) represent results that are significantly (P < 0.05) different from each other.

there is no statistical analysis of this data.

3.3. Bioconcentration factor (BCF)

Fig. 3 shows the BCF for AGT and pollen as a function of the PFCAs, PFSAs and total PFAS. The Bioconcentration Factor (BCF) normalises the PFAS removal relative to the soil concentration, making it a better indicator of PFAS uptake in specific plant components, compared to absolute concentrations (Tables SI–5). A BCF value higher than one indicates that a plant is a hyperaccumulator with good phytoremediation potential, whereas a value less than one is indicative of an excluder (Usman et al., 2019; Mocek-Płóciniak et al., 2023).

Overall, the total PFAS results indicate that hemp plants are an effective accumulator for PFAS contamination from the soil, with the total PFAS removal having BCFs of ~45.6 and ~20.8 for the AGT and pollen, respectively. The PFCAs appear to be translocated to the pollen

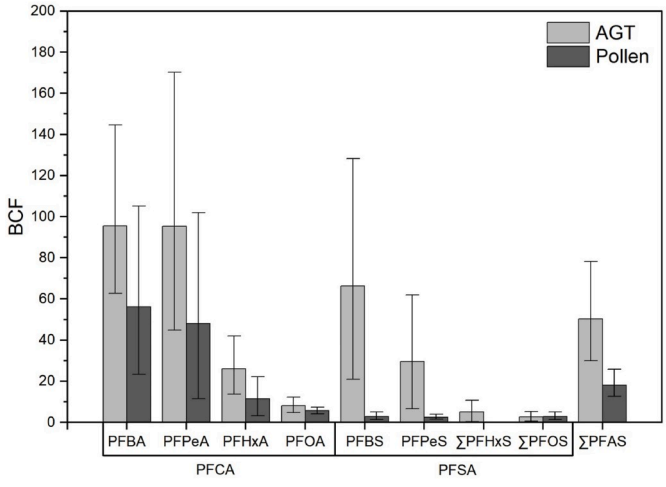

Fig. 3. Hemp experimental BCF for AGT and pollen. The concentration of each PFAS for each data point can be viewed in Tables SI–5.

more effectively than the PFSAs with the BCF decreasing with increasing C–F chain length in the order PFBA (BCF~56) > PFPeA (BCF~28) > PFHxA (BCF~11) > PFOA (BCF ~5). This trend is not apparent with the PFSAs with PFHxS being transported into the AGT (BCF ~4.7) whilst having little affinity for the pollen (BCF <1) and PFBS, PFPeS and PFOS all having around the same BCF (~2–4). These observations align with previous research by Felizeter et al. (2014) who observed similar trends when growing cabbage, tomato and zucchini hydroponically in PFAS solutions.

PFAS translocation as a function of tail hydrophobicity (i.e. C–F chain length rather than number of carbons), can be seen in Fig. 4A (AGT BCF) and Fig. 4B (pollen BCF). Again, the translocation of PFAS appears to be negatively correlated with C–F chain length suggesting that hydrophobic transport pathways, such as protein transport play a role (Liu et al., 2019; Wen et al., 2016). Fig. 4A shows there was no statistical difference (P < 0.05) in uptake from soil to the AGT between PFCAs and PFSAs with the same number of fluorinated carbons. For example, PFPeA and PFBS both have a tail with four fluorinated carbons and similar initial soil concentrations (12.0 and 12.7 µg/kg dw respectively; Table 1), with PFPeA having a BCF ~80.2 compared to ~58.9 for PFBS, however there is no statistically significant difference (P = 0.3943). Similarly, the 5C–F (PFHxA and PFPeS; P = 0.7569) showed no statistically significant difference nor was there any between PFOA (7C–F) and the more hydrophobic PFOS (8C–F) (P = 0.9612) indicating that the head group does not appear to play a significant role in PFAS uptake to the AGT. This suggests that longer chain PFSAs (and PFCAs) are inhibited by plant accumulation compared to shorter chain PFAS. This is supported by research (Felizeter et al., 2014; Krippner et al., 2015; Stahl et al., 2009; Xu et al., 2022) where it was shown that accumulation in the tested plants decreases with increasing C–F chain length and as a consequence, short-chain molecules have the highest accumulation.

Fig. 4B shows that both a hydrophobic and head group effect on transport to pollen. Comparing the pollen BCF there is a significant difference (P = 0.0441) between the 4C–F (PFPeA vs PFBS), P = 0.0441), a "not quite" statically significant (P = 0.0641) between the 5C–F species (PFHxA and PFPeS) species and a significant (P = 0.031) difference between PFOA (7C–F) and PFOS (8C–F). These results suggest that the translocation pathway to pollen differs from that to AGT, with Felizeter et al. suggesting that the transfer of PFAS from above-ground plant components to the fruit (i.e. sex organs including pollen) occurs primarily through phloem sap. The biochemical composition of phloem sap is not well known but contains numerous metabolites (Broussard et al., 2023), such as sugars, amino acids and hormones. Amino acids such as asparagine (Asp) and aspartic acid have been shown to be prominent in plant sap (Broussard et al., 2023) and plant leaves (Tegeder and Masclaux-Daubresse, 2018). Turner et al. (2019) reported that the aspartic acid present in hemp seed protein powder likely plays a role in PFAS removal from solution. This binding is thought to occur through hydrogen bonding with its positively charged amino group at physiological pH. This has been supported by Mei et al. (2021) whereby PFASs may form an electrostatic attraction between the PFAS anion and positively charge amide groups of proteins indicating that, PFAS/protein (hydrophobic) interactions appear to be a dominant pathway of PFAS uptake in plants.

Results presented here, albeit with limited replicates, suggest that in addition to the hydrophobic effect another mechanism(s) is involved in the translocation of PFSAs to pollen. The elucidation of the nature of this mechanism(s) is outside the scope of this paper with many plant scientists examining various possible avenues. For example, Wang et al. (2020) (Wang et al., 2020) indicated that PFOS and PFOA could be adsorbed by plant cell walls and organelles with PFOS affected more hence reducing the translocation of PFOS to other plant components. More recently, aquaporins (AQPs), which are membrane proteins found in all cells and integral to water and solute transport, have gained attention in the plant sciences (Wang et al., 2020; Ortiz-Delvasto et al.,

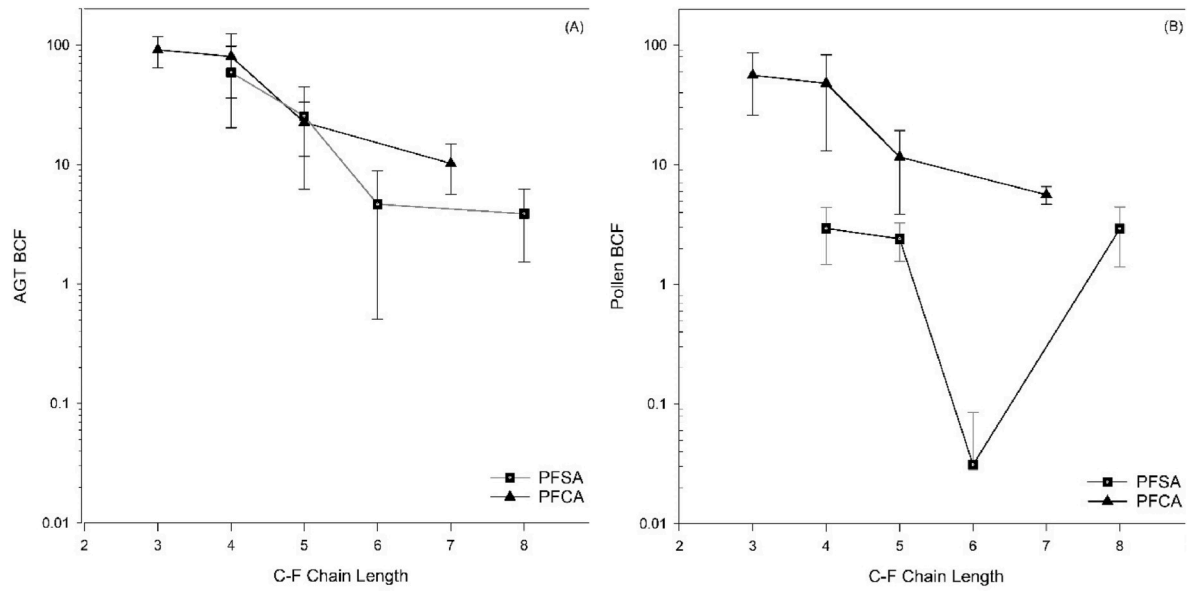

Fig. 4. Perfluorosulfonic acids (PFSA) and perfluorocarboxylic acids (PFCA) C–F chain length versus Above Ground Tissue (AGT) BCF (A); and Pollen BCF (B).

2024). AQP's function to determine the uptake selectivity of chemicals into the plant as well as playing a key role in the flowering (sexual maturity) stage of plant development through pollen hydration as well as fruit and seed formation. AQPs have a gating mechanism through which the channels can be opened and closed and actively interact with each other leading to the regulation of specific physiological plant prcoesses (Deshmukh et al., 2017). Although thirty aquaporins (AQPs) have been identified in various Cannabis strains, their roles in *Cannabis sativa* physiology, and particularly in relation to PFAS interactions, remain unstudied to date (Ortiz-Delvasto et al., 2024).

Compared to other studies with similar initial soil PFAS concentrations, the BCF values observed here suggest that hemp has a higher potential for PFAS phytoremediation than many other studied plants. For example with an initial PFBA soil concentration of ~14 μg/kg, Stahl et al. (2009) found the wheat AGT (sum straw, husks and grains) was ~2.45 compared to ~91 for hemp (this study); using PFOS, at an initial soil concentration of ~41 μg/kg (~3.5 times higher than this study), Stahl et al. found the AGT to have a BCF <0.4 compared to ~3.9 in this study. At a PFAS soil concentration >20 times used in this study, Krippner et al. (2015) reported BCF factors in the straw component of the *Zea mays* plant as ~64 for PFBA and decreasing with increasing C–F chain length to ~0.32 for PFOS (note the AGT BCF was not calculated in the Krippner et al. study due to the very small values observed in the maize kernels). The efficacy and financial efficiency of using hemp to phytoremediate PFAS contaminated soil requires further study.

3.4. Estimated daily intake (EDI) of bees

The average body weight of honeybee adults has been reported at 100 mg with nurse bees consuming about 9.25 mg of pollen daily (Yang et al., 2023). Assuming that a beehive relies exclusively on hemp pollen with a mean PFOS concentration of 34 μg/kg (ng/g) (Tables SI–5) then the EDI (equation (2)) for *Apis mellifera* would be approximately 3150 ng/kg.bw/day and ~3390 ng/kg.bw/day for PFOA. The acute oral toxicity of PFOS to *A. mellifera* has been determined to be 0.4 μg per bee (4 mg/kg bodyweight)(Wilkins, 2001). Therefore, if a honeybee subsisted only on Hemp pollen it would receive a total PFOS oral dose of ~0.3 ng per day. Assuming no elimination it would take over 1200 days to consume a lethal dose, which is far greater than the insect's lifespan (Remolina and Hughes, 2008). This does not consider the other PFAS species present within the pollen though.

Recent studies have provided evidence that PFAS mixtures have a synergistic effect on toxicity to humans (Ríos-Bonilla et al., 2024; Ojo et al., 2021), and invertebrates (McCarthy et al., 2021; Labine et al., 2022) with a combination of PFHxS and PFOS shown (McCarthy et al., 2021) to cause "more severe toxicity" in the insect *Chironomus dilutus* than either compound alone. For a synergistic EDI, the results presented here indicate that there was a total of 1344 (±526) ng/g.dw of total PFAS in hemp pollen (Tables SI–5), which would equate to ~124.5 μg/kg.bw/day of total PFAS or an oral dose of ~12.5 ng per day. It is interesting note that the Tolerable Daily Intake (TDI), an estimate of the amount of a substance that can be ingested daily over a lifetime without an appreciable health risk (chemsec.org, 2019), is 20 ng/kg.bw/day and 160 ng/kg.bw/day respectively for PFOS and PFOA in rats (FSANZ, 2024). For humans the European Food and Safety Authority (EFSA) recommended that the maximum PFOS and PFOA TDI be set at 13 and 6 ng/kg.bw/week (i.e. <2 and < 1 ng/kg.bw/day) respectively (chemsec.org, 2019).

TDI values across species are not directly comparable due to differences in metabolism and physiology. However, the stark contrast between the known human TDI for PFOS (<2 ng/kg body weight/day) and the estimated daily intake (EDI) for bees calculated in this study (3150 ng/kg body weight/day) strongly indicates the need for further research. This discrepancy raises questions about the safety of such high PFOS exposure levels for bees, especially given the limited existing research on PFAS impacts on pollinators, either individually or in mixtures (synergistically). Since pollinators are crucial for agriculture, and PFAS contamination is widespread in the environment, additional studies are essential to understand the risks and develop strategies to manage PFAS exposure in ecosystems critical for food production.

4. Conclusions

The bioconcentration factor (BCF) findings from this study underscore the potential of hemp (*Cannabis sativa* L.) as an effective candidate for PFAS phytoremediation. Hemp not only extracts and accumulates PFAS from contaminated soils, but it also stores these chemicals prominently in pollen, highlighting a potentially impactful reservoir. The preferential accumulation of perfluoroalkyl carboxylic acids (PFCAs) in pollen, inversely correlated with the length of the C–F chain, illustrates the intricate dynamics driving PFAS uptake and transport within plant tissues.

This pattern of PFAS accumulation raises serious concerns for pollinators, as hemp pollen serves as a significant food source for various bee

species. Existing research has demonstrated both lethal and sublethal effects of PFOS on bees, affecting colony behaviors, activity levels, and overall health. Emerging evidence on mixed PFAS exposures also points to potential synergistic toxicological responses that may exacerbate harm to pollinators and other wildlife. Moreover, wind-dispersed hemp pollen carrying PFAS could spread contaminants up to 5 km^{39}, posing risks to surrounding ecosystems and non-target species. This potential for secondary environmental exposure underscores the need for careful management of PFAS phytoremediation with hemp to limit unintended dispersal and exposure risks.

While this study highlights hemp's substantial phytoaccumulation potential and associated ecological risks, it also emphasizes the need for deeper research into PFAS transport mechanisms within plants. Future work should aim to clarify the roles of phloem transport, ion channels, aquaporins, hormonal signaling, and metabolic pathways in PFAS movement. Such insights, particularly into PFAS binding sites and possible synergistic effects, will be essential for optimizing remediation strategies that mitigate PFAS contamination while protecting critical pollinator health and ecosystem integrity.

CRediT authorship contribution statement

Timothy Wright: Writing – original draft, Investigation, Formal analysis, Data curation. **Marcus Crompton:** Supervision, Investigation, Formal analysis, Data curation. **Daniel Bishop:** Supervision, Methodology. **Glen Currell:** Investigation, Data curation. **Laxmi Suwal:** Supervision, Investigation. **Brett D. Turner:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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.

Acknowledgments

This research was funded by an Australian Government Research Training Program (RTP) Scholarship and the Australian Government's Department of Industry, Innovation and Science under Commonwealth Standard Grant Agreement "PFAS000001". The authors gratefully acknowledge the significant support of Former Senator Brian Burston in securing this research funding.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143859.

Data availability

Data will be made available on request.

References

ASTM, 2019. Standard Test Method for Determination of Per- and Polyfluoroalkyl Substances in Water, Sludge, Influent, Effluent and Wastewater by Liquid Chromatography Tandem Mass Spectrometry. LC/MS/MS.

Blaine, A.C., et al., 2014. Perfluoroalkyl acid distribution in various plant compartments of edible crops grown in biosolids-amended soils. Environ. Sci. Technol. 48, 7858–7865. https://doi.org/10.1021/es500016s.

Broussard, L., et al., 2023. Phloem sap composition: what have we learnt from metabolomics? Int. J. Mol. Sci. 24. https://doi.org/10.3390/ijms24086917.

Calvert, L., et al., 2022. Assessment of the emerging threat posed by perfluoroalkyl and polyfluoroalkyl substances to male reproduction in humans. Front. Endocrinol. 12. https://doi.org/10.3389/fendo.2021.799043.

Campbell, S., Paquin, D., Awaya, J.D., Li, Q.X., 2002. Remediation of benzo [a] pyrene and chrysene-contaminated soil with industrial hemp (Cannabis sativa). Int. J. Phytoremediation 4, 157–168.

Campbell, L.G., Peach, K., Wizenberg, S.B., 2021. Dioecious hemp (Cannabis sativa L.) plants do not express significant sexually dimorphic morphology in the seedling stage. Sci. Rep. 11, 16825.

chemsec.org. Scientists just cut the tolerable intake of PFAS by 99,9%, 2019. https://chemsec.org/scientists-just-cut-the-tolerable-intake-of-pfas-by-999/.

Deshmukh, R.K., Nguyen, H.T., Belanger, R.R., 2017. Editorial: aquaporins: dynamic role and regulation. Front. Plant Sci. 8.

Dingha, B.N., Jackai, L.E., 2023. Chemical composition of four industrial hemp (cannabis sativa L.) pollen and bee preference. Insects 14. https://doi.org/10.3390/insects14080668.

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

Flicker, N.R., Poveda, K., Grab, H., 2020. The bee community of cannabis sativa and corresponding effects of landscape composition. Environ. Entomol. 49, 197–202. https://doi.org/10.1093/ee/nvz141.

FSANZ, 2024. Hazard Assessment Report- Perfluorooctane Sulfonate (PFOS), Perfluorooctanoic Acid (PFOA), Perfluorohexane Sulfonate (PFHxS), vol. 164. Food Standards Australia, New Zealand.

Graphpad, 2017. Detecting outliers with Grubbs' test. https://www.graphpad.com/quickcalcs/Grubbs1.cfm.

Grubbs, F.E., 1950. Sample criteria for testing outlying observations. Ann. Math. Stat. 21, 27–58. https://doi.org/10.1214/aoms/1177729885.

Hellen, L.E., Othman, O.C., 2016. Heavy metal levels in soil, tomatoes and selected vegetables from Morogoro region, Tanzania. Int. J. Environ. Monit. Anal. 4, 82.

Huff, D.K., Morris, L.A., Sutter, L., Costanza, J., Pennell, K.D., 2020. Accumulation of six PFAS compounds by woody and herbaceous plants: potential for phytoextraction. Int. J. Phytoremediation 22, 1538–1550. https://doi.org/10.1080/15226514.2020.1786004.

Knaus, C., 2017. Toxic firefighting chemicals "the most seminal public health challenge". The Guardian. https://www.theguardian.com/australia-news/2017/oct/18/toxic-firefighting-chemicals-the-most-seminal-public-health-challenge'.

Krippner, J., et al., 2015. Accumulation potentials of perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs) in maize (Zea mays). J. Agric. Food Chem. 63, 3646–3653. https://doi.org/10.1021/acs.jafc.5b00012.

Labine, L.M., et al., 2022. Comparison of sub-lethal metabolic perturbations of select legacy and novel perfluorinated alkyl substances (PFAS) in Daphnia magna. Environ. Res. 212, 113582.

Lima, M.A.P., Cutler, G.C., Mazzeo, G., Hrncir, M., 2022. Editorial: the decline of wild bees: causes and consequences. Frontiers in Ecology and Evolution 10. https://doi.org/10.3389/fevo.2022.1027169.

Linger, P., Müssig, J., Fischer, H., Kobert, J., 2002. Industrial hemp (Cannabis sativa L.) growing on heavy metal contaminated soil: fibre quality and phytoremediation potential. Ind. Crop. Prod. 16, 33–42.

Liu, Z., et al., 2019. Multiple crop bioaccumulation and human exposure of perfluoroalkyl substances around a mega fluorochemical industrial park, China: implication for planting optimization and food safety. Environ. Int. 127, 671–684. https://doi.org/10.1016/j.envint.2019.04.008.

Malabadi, R.B., et al., 2023. Cannabis sativa: dioecious into Monoecious plants influencing sex determination. International Journal of Research and Innovations in Applied Science (IJRIAS) 8, 82–91.

Matros, A., et al., 2023. Non-invasive assessment of cultivar and sex of Cannabis sativa L. by means of hyperspectral measurement. Plant Environ Interact 4, 258–274. https://doi.org/10.1002/pei3.10116.

McCarthy, C.J., et al., 2021. Toxicological response of Chironomus dilutus in single-chemical and binary mixture exposure experiments with 6 perfluoralkyl substances. Environ. Toxicol. Chem. 40, 2319–2333. https://doi.org/10.1002/etc.5066.

Mei, W., et al., 2021. Per- and polyfluoroalkyl substances (PFASs) in the soil–plant system: sorption, root uptake, and translocation. Environ. Int. 156, 106642. https://doi.org/10.1016/j.envint.2021.106642.

Mocek-Płóciniak, A., Mencel, J., Zakrzewski, W., Roszkowski, S., 2023. Phytoremediation as an effective remedy for removing trace elements from ecosystems. Plants 12. https://mdpi-res.com/d_attachment/plants/plants-12-01653/article_deploy/plants-12-01653-v2.pdf?version=1681709611.

Nason, S., et al., 2023. A Comprehensive Trial on PFAS Remediation: Hemp Phytoextraction and PFAS Degradation in Harvested Plants. https://doi.org/10.26434/chemrxiv-2023-prt38.

Nassazzi, W., Wu, T.-C., Jass, J., Lai, F.Y., Ahrens, L., 2023. Phytoextraction of per-and poly-fluoroalkyl substances (PFAS) and the influence of supplements on the performance of short–rotation crops. Environ. Pollut. 122038.

Ofoegbu, P.C., et al., 2022. Impacts of perfluorooctanesulfonic acid on plant biometrics and grain metabolomics of wheat (Triticum aestivum L.). Journal of Hazardous Materials Advances 7, 100131. https://doi.org/10.1016/j.hazadv.2022.100131.

Ojo, A.F., Peng, C., Ng, J.C., 2021. Assessing the human health risks of per- and polyfluoroalkyl substances: a need for greater focus on their interactions as mixtures. J. Hazard Mater. 407, 124863. https://doi.org/10.1016/j.jhazmat.2020.124863.

Ortiz-Delvasto, N., Garcia-Gomez, P., Carvajal, M., Bárzana, G., 2024. Aquaporins-mediated water availability in substrates for cannabis cultivation in relation to CBD yield. Plant Soil 495, 469–485. https://doi.org/10.1007/s11104-023-06341-8.

Rankin, K., Mabury, S.A., Jenkins, T.M., Washington, J.W., 2016. A North American and global survey of perfluoroalkyl substances in surface soils: distribution patterns and mode of occurrence. Chemosphere 161, 333–341. https://doi.org/10.1016/j.chemosphere.2016.06.109.

Remolina, S.C., Hughes, K.A., 2008. Evolution and mechanisms of long life and high fertility in queen honey bees. Age (Dordr) 30, 177–185. https://doi.org/10.1007/s11357-008-9061-4.

Rhodes, C.J., 2018. Pollinator decline – an ecological calamity in the making? Sci. Prog. 101, 121–160. https://doi.org/10.3184/003685018X15202512854527.

Ríos-Bonilla, K.M., et al., 2024. Neurotoxic effects of mixtures of perfluoroalkyl substances (PFAS) at environmental and human blood concentrations. Environ. Sci. Technol. 58, 16774–16784. https://doi.org/10.1021/acs.est.4c06017.

Rivernider, R., Venturini, E., Drummond, F.A., 2017. Phleum pratense (poales: poaceae), a pollen forage for native bumble bees bombus latrielle (Hymenoptera: Apidae). J. Kans. Entomol. Soc. 90, 63–68.

Salt, D.E., Smith, R., Raskin, I., 1998. Phytoremediation. Annu. Rev. Plant Biol. 49, 643–668.

Saunders, M.E., 2018. Insect pollinators collect pollen from wind-pollinated plants: implications for pollination ecology and sustainable agriculture. Insect conservation and diversity 11, 13–31.

Schlechtriem, C., et al., 2019. Bioconcentration studies with the freshwater amphipod Hyalella azteca: are the results predictive of bioconcentration in fish? Environ. Sci. Pollut. Res. Int. 26, 1628–1641. https://doi.org/10.1007/s11356-018-3677-4.

Secretariat of the Stockholm Convention, 2019. PFASs Listed under the Stockholm Convention.

Shrivastava, A., Gupta, V.B., 2011. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chronicles Young Sci. 2, 21–25.

Small, E., Antle, T., 2003. A preliminary study of pollen dispersal in cannabis sativa in relation to wind direction. J. Ind. Hemp 8, 37–50. https://doi.org/10.1300/J237v08n02_03.

Sonter, C.A., Rader, R., Stevenson, G., Stavert, J.R., Wilson, S.C., 2021. Biological and behavioral responses of European honey bee (Apis mellifera) colonies to perfluorooctane sulfonate exposure. Integrated Environ. Assess. Manag. 17, 673–683. https://doi.org/10.1002/ieam.4421.

Stahl, T., et al., 2009. Carryover of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) from soil to plants. Arch. Environ. Contam. Toxicol. 57, 289–298. https://doi.org/10.1007/s00244-008-9272-9.

Strynar, M.J., Lindstrom, A.B., Nakayama, S.F., Egeghy, P.P., Helfant, L.J., 2012. Pilot scale application of a method for the analysis of perfluorinated compounds in surface soils. Chemosphere 86, 252–257.

Tegeder, M., Masclaux-Daubresse, C., 2018. Source and sink mechanisms of nitrogen transport and use. New Phytol. 217, 35–53. https://doi.org/10.1111/nph.14876.

Tien-Chi, W., 2021. Phytoremediation Potential for Polyand Perfluoroalkyl Substances (PFASs) Using Various Plant Species. Swedish University of Agricultural Sciences.

Turner, B.D., Sloan, S.W., Currell, G.R., 2019. Novel remediation of per- and polyfluoroalkyl substances (PFASs) from contaminated groundwater using Cannabis Sativa L. (hemp) protein powder. Chemosphere 229, 22–31. https://doi.org/10.1016/j.chemosphere.2019.04.139.

USEPA, 2000. EPA Newsroom.

Usman, K., Al-Ghouti, M.A., Abu-Dieyeh, M.H., 2019. The assessment of cadmium, chromium, copper, and nickel tolerance and bioaccumulation by shrub plant Tetraena qataranse. Sci. Rep. 9, 5658. https://doi.org/10.1038/s41598-019-42029-9.

Vandenhove, H., Van Hees, M., 2005. Fibre crops as alternative land use for radioactively contaminated arable land. J. Environ. Radioact. 81, 131–141. https://doi.org/10.1016/j.jenvrad.2005.01.002.

Wang, Z., DeWitt, J.C., Higgins, C.P., Cousins, I.T., 2017. A never-ending story of per- and polyfluoroalkyl substances (PFASs)? Environ. Sci. Technol. 51, 2508–2518.

Wang, Y., Zhao, Z., Liu, F., Sun, L., Hao, F., 2020. Versatile roles of aquaporins in plant growth and development. Int. J. Mol. Sci. 21. https://doi.org/10.3390/ijms21249485.

Washington, J.W., Rankin, K., Libelo, E.L., Lynch, D.G., Cyterski, M., 2019. Determining global background soil PFAS loads and the fluorotelomer-based polymer degradation rates that can account for these loads. Sci. Total Environ. 651, 2444–2449. https://doi.org/10.1016/j.scitotenv.2018.10.071.

Wen, B., et al., 2016. The roles of protein and lipid in the accumulation and distribution of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in plants grown in biosolids-amended soils. Environ. Pollut. 216, 682–688. https://doi.org/10.1016/j.envpol.2016.06.032.

Wilkins, P., 2001. Perfluorooctanesulfonate, potassium salt (PFOS): an acute contact toxicity study with the honey bee. Study number HT5601. (Environmental Biology Group, Central Science Laboratory, Sand Hutton, York, UK.

Xu, B., et al., 2022. Translocation, bioaccumulation, and distribution of perfluoroalkyl and polyfluoroalkyl substances (PFASs) in plants. iScience 25, 104061. https://doi.org/10.1016/j.isci.2022.104061.

Yang, Y., et al., 2023. Global honeybee health decline factors and potential conservation techniques. Food Secur. 15, 855–875. https://doi.org/10.1007/s12571-023-01346-8.

Yoo, H., Washington, J.W., Jenkins, T.M., Ellington, J.J., 2011. Quantitative determination of perfluorochemicals and fluorotelomer alcohols in plants from biosolid-amended fields using LC/MS/MS and GC/MS. Environ. Sci. Technol. 45, 7985–7990. https://doi.org/10.1021/es102972m.

Zhang, G., St Clair, A.L., Dolezal, A.G., Toth, A.L., O'Neal, M.E., 2021. North American prairie is a source of pollen for managed honey bees (Hymenoptera: apidae). J. Insect Sci. 21, 17.