UPTAKE AND BIOACCUMULATION OF PERFLUOROALKYL ACIDS IN EDIBLE CROPS VIA LAND-APPLIED BIOSOLIDS AND RECLAIMED WATER

by

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ABSTRACT

Perfluoroalkyl acids (PFAAs) are persistent, bioaccumulative, and toxic anthropogenic compounds that are both lipophobic and hydrophobic. This dual nature makes them both oil and water repellent, giving them a myriad of applications (e.g., non-stick packaging, stain-resistant textiles). Due to their widespread use, municipal wastewaters are a collection vehicle for these compounds; however, most conventional wastewater treatment plants (WWTPs) are ineffective at removing PFAAs. Due to their unique nature, these compounds reside in significant quantities in both the aqueous effluent and the treated sludge (i.e., biosolids) of WWTPs. Sustainability movements coupled with growing water scarcity encourage the land application of both biosolids and reclaimed water from WWTPs. However, concerns around these practices have recently arisen, particularly with respect to the potential uptake and subsequent bioaccumulation of PFAAs into food crops. The objective of this study was to understand the factors controlling the uptake of PFAAs via land-applied biosolids and reclaimed water by food crops under conditions representative of current agricultural practices.

In this study, greenhouse experiments were used to investigate various types of fresh food crops including lettuce, tomato, snap pea, radish, and celery grown in an industrially impacted biosolids-amended soil, a municipal biosolids-amended soil, and an unamended control soil. Concentrations of PFAAs in the edible portions of crops grown in soil amended with PFAA industrially impacted biosolids were high, whereas the edible compartments of crops grown in the municipal biosolids-amended soil and in the control soil were relatively low. Significant uptake factors and bioaccumulation trends of PFAAs were explored. Strong carbon chain length dependency trends were observed; short-chain PFAAs accumulated in much higher concentrations than long-chain PFAAs. In general, bioaccumulation factors decreased up to 0.5 log units for each additional carbon. To further explain the data, the roles

of crop anatomy and PFAA properties in controlling PFAA bioaccumulation were explored. Fruit crops accumulated high amounts of short-chain PFAAs but fewer long-chain PFAAs than did shoot or root crops, presumably due to an increasing number of biological barriers as the contaminant is transported throughout the plant (roots to shoots to fruits). These data were incorporated into a preliminary conceptual framework for PFAA accumulation in edible crops.

In addition, a limited-scale field study of biosolids-amended soils was conducted to verify greenhouse findings. The greatest accumulation was seen for short-chain PFAAs in both field-grown lettuce and tomato. PFAA levels measured in lettuce and tomato grown in field soil amended with only a single application of biosolids (at an agronomic rate for nitrogen) were predominantly below the limit of quantitation. In addition, corn stover, corn grains, and soil were collected from several full-scale biosolids-amended farm fields. At these fields, all PFAAs were below the limit of quantitation in the corn grains and only trace amounts were detected in the corn stover.

Finally, this study used authentic reclaimed water augmented with varying doses of PFAAs to investigate the potential uptake and dose-dependency trends in greenhouse grown lettuce and strawberry. Concentrations of PFAAs in lettuce leaves and strawberry fruit were measured for each incremental dose, while concentrations in strawberry shoot and root were measured for selected doses. Short-chain PFAAs showed the overall highest accumulation of any PFAAs in the edible parts of both lettuce and strawberry. Concentrations increased linearly with increasing dose for almost all PFAAs, with the exception of the long-chain PFAAs in strawberry fruit, which remained at a constant concentration regardless of dose. Chain length dependency trends were evident in both lettuce shoot and strawberry fruit, with decreasing concentrations occurring with increasing chain length. Lettuce grown in soils with varying organic carbon content was used to assess the impact of organic carbon sorption on PFAA bioaccumulation. In general, a higher fraction of organic carbon in the soil correlated to less bioaccumulation of PFAAs in lettuce. Bioaccumulation factors were

also correlated to carbon chain length of PFAAs showing approximately a 0.4 to 0.6 log decrease per CF_2 group.

This study confirms that PFAAs are able to be taken up and bioaccumulate in food crops via both biosolids and reclaimed water. PFAA bioaccumulation potential is highly dependent on analyte, crop, PFAA concentration in the uptake matrix, and the organic carbon content and quality of the soil. With industry trends shifting toward the use of short-chain PFAAs, it is important to recognize this increased potential of PFAA entry into the terrestrial food chain via plants. If the current use of land-applied biosolids and reclaimed water for food crops is to be sustained or increase in future years, these concerns about the potential contamination of food products must be fully addressed through careful scientific study, evaluation, and communication with the public.

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LIST OF SYMBOLS

Carbon-Fluorine-Fluorine Moiety . . . . . CF_2

Solid phase concentration . . . . . C_s

Aqueous phase concentration . . . . . C_w

Fraction of organic carbon . . . . . f_{oc}

Solid-water distribution coefficient . . . . . K_d

Organic carbon normalized distribution coefficient . . . . . K_{oc}

LIST OF ABBREVIATIONS

Bioaccumulation factor . . . . . BAF

Benchmark dose lower confidence limit for a 10% response . . . . . $BMDL_{10}$

Body weight . . . . . BW

Dichloromethane . . . . . DCM

United States Environmental Protection Agency . . . . . EPA

Fruit to soil concentration factor . . . . . FCF

Liquid chromatography tandem mass spectrometry . . . . . LC-MS/MS

Limit of quantitation . . . . . LOQ

Methanol . . . . . MeOH

Multiple reaction monitoring . . . . . MRM

No observed adverse affect level . . . . . NOAEL

Organic carbon . . . . . OC

Perfluoroalkyl acid . . . . . PFAA

Perfluorobutanoate . . . . . PFBA

Perfluorobutane sulfonate . . . . . PFBS

Perfluoroalkyl carboxylate . . . . . PFCA

Perfluorodecanoate . . . . . PFDA

Perfluorodecane sulfonate . . . . . PFDS

Perfluoroheptanoate . . . . . PFHpA

Perfluoroheptane sulfonate . . . . . PFHpS

Perfluorohexanoate . . . . . PFHxA

Perfluorohexane sulfonate . . . . . PFHxS

Perfluorononanoate . . . . . PFNA

Perfluorooctanoate . . . . . PFOA

Perfluorooctane sulfonate . . . . . PFOS

Perfluoropentanoate . . . . . PFPeA

Perfluoroalkane sulfonate . . . . . PFSA

Provisional health advisory . . . . . PHA

Root to soil concentration factor . . . . . RCF

Sub-chronic reference dose . . . . . RfD

Relative source contribution . . . . . RSC

Shoot to soil concentration factor . . . . . SCF

Transpiration stream concentration factor . . . . . TSCF

Uncertainty factor . . . . . UF

Waste water treatment plant . . . . . WWTP

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Ernest Hemingway wrote, "It is good to have an end to journey toward; but it is the journey that matters, in the end." For me, this PhD experience has most certainly been about the journey. I am excited to see what new opportunities may arise, but saddened as well to see such a meaningful chapter of my life close. This journey has been quite remarkable, and I have many, many to thank for traveling with me along the way. Sometimes our blessings come through rain, and for me, this PhD journey has been a mix of sunshine and rain. Were it not for my faith in God alone, the trials I faced over the last five years battling cancer along my PhD journey would have surely swallowed me whole.

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CHAPTER 1
INTRODUCTION AND BACKGROUND

Perfluoroalkyl acids (PFAAs) consist of a carbon backbone saturated with fluorine atoms in place of hydrogen and a terminal acid functional group such as a carboxylic acid or a sulfonic acid [1]. Their dual lipophobic and hydrophobic nature makes them both oil and water repellent. In addition, the strength of the carbon-fluorine bonds makes them resistant to degradation by heat and other environmental processes [2]. These unique properties give them a myriad of applications in both consumer and industrial settings. Common products that utilize PFAAs include stain repellents for textiles, non-stick food packaging, and fire-fighting foams [1, 3, 4].

PFAAs are ubiquitous and environmentally persistent; they have been detected in air, water, sediment, soil, wildlife, and human blood [2, 3, 5, 6]. In addition, some of these compounds are poorly eliminated by many higher level organisms, resulting in elimination half-lives ranging from a few days in rodents to more than five years in humans [2, 7]. Toxicity to wildlife and laboratory animals has also been well established; adverse effects include reduced survival rates, abnormal maturation and fertility [6, 7]. The persistence, bioaccumulation, and toxicity of PFAAs make them high priority contaminants of emerging concern.

Due to the widespread use of PFAAs in consumer products, municipal wastewaters are a collection vehicle for the compounds, post-consumer. In addition, the prominence of PFAAs in manufacturing processes can lead to high levels in industrial wastewaters [8]. However, most conventional wastewater treatment plants (WWTPs) are ineffective at removal of PFAAs [8, 9]. As a result, WWTPs represent a significant source for PFAA releases to the environment [8]. Unlike many organic contaminants, the dual hydrophobic/lipophobic nature of PFAAs enable these compounds to reside in significant quantities in both the aque-

ous and sludge effluent streams of WWTPs [9, 10]. The sludge from WWTPs is treated and termed "biosolids." The presence of PFAAs in biosolids has been well documented [10, 11]. Biosolids, like animal manures, are rich in both plant nutrients and organic matter and are commonly used as a fertilizer in crop production [12, 13]. As a consequence, concerns have arisen about the potential uptake and subsequent bioaccumulation of PFAAs into crops grown in biosolids-amended soils.

The aqueous effluent stream of a WWTP is, in general, returned to the surrounding aquatic environment; however, growing water scarcity is driving alternative uses of treated wastewater. In particular, interest in the use of recycled or reclaimed water (which typically consists of municipal wastewater treated to remove pathogens, organic matter, and nutrients) for agricultural purposes is growing and is likely to continue in the future [14]. Reclaimed water has been safely used for many years in several states for the irrigation of non-food crops [15] and on a more limited scale, food crops eaten fresh (e.g., in the Salinas Valley, CA). However, concerns have been raised regarding the presence of chemicals of emerging concern in reclaimed water Fatta-Kassinos et al. [16].

In particular, food crops consumed raw represent the greatest potential for unintentional human exposure, as cooking would likely lead to significant chemical transformation and/or volatilization. Unfortunately, human health risk assessments based on plant uptake models are limited to crop-specific data and very few organic chemicals of concern [17, 18]. If the current use of land-applied biosolids and reclaimed water for food crops is to be sustained or increase in future years, concerns about the potential contamination of food products must be fully addressed through careful scientific study, evaluation, and communication with the public. This dissertation seeks to understand the factors controlling the uptake of PFAAs via land-applied biosolids and reclaimed water by food crops under conditions representative of current agricultural practices.

1.1 Research Questions

Given that PFAAs are persistent, bioaccumulative, and toxic, that biosolids and reclaimed water are widely applied to agricultural fields, and that little data exist regarding the transfer of PFAAs from water and soil into plants, the questions arising are as follows:

1. Are edible crops a significant entryway for PFAAs into the terrestrial food chain via land-applied biosolids or reclaimed water?

2. If so, what are the trends in bioaccumulation of PFAAs in plants with respect to analyte, PFAA uptake matrix (soil or water), concentration, and crop?

1.2 Background

Relevant background information concerning PFAA risk and regulation, land-applied biosolids, reclaimed water, plant uptake and bioaccumulation of contaminants, and existing literature on plant uptake of PFAAs is discussed in this section.

1.2.1 Risk and Regulation of PFAAs

Risk and Toxicity of PFAAs. PFAAs are anthropogenic chemicals that consist of a carbon backbone saturated with fluorine atoms and a hydrophilic functional group at the end [1]. The two most common types are perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs). Within these groups, the two most well-known and widely studied PFAAs are perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA); however, new trends in manufacturing may increase the production of shorter chain PFAAs [19]. PFAA exposure to humans can occur through a variety of media, most significantly, through ingestion of contaminated food or water [7]. Sources of contamination continue to be a valuable area of research.

The toxicities of PFOS and PFOA have been established through both wildlife studies and laboratory experiments [2, 20, 21]; however, toxicity data on other PFAAs are much less prevalent [7]. In wildlife, PFAA exposure in the part-per-million range has been shown

to result in adverse effects such as reduction in survival of baby quail (10 ppm of PFOS in diet), delayed growth and metamorphosis in Northern Leopard frogs (3 ppm of PFOS), and reduced fertility in Fathead minnows (0.1 ppm of PFOS) [22]. Laboratory toxicity studies have focused primarily on mice, rats, and monkeys. Such studies have demonstrated that PFAAs are absorbed well orally, but not readily metabolized or eliminated [22]. Highest concentrations are primarily found in the serum, kidney, and liver. PFOS has an elimination half-life of 7 days in rats, 150 days in monkeys and 5.4 years in humans [22]. There are significant inter-gender and inter-species variations among elimination processes, indicating that data extrapolation across genders and species can potentially be skewed.

In rats, the effects of PFOA have been studied extensively. Dose-response experiments have demonstrated reduced body weight, hepatotoxicity, reduced cholesterol, and a steep curve for mortality [2, 22]. A reproductive study in rats showed significant reduction in pup body weight during post-weaning at an exposure level of 10 mg/kg-day. In addition, at 30 mg/kg-day, there was a significant increase in mortality of pups after weaning and a significant delay in sexual maturation [22]. Though fewer studies on laboratory animals have been done with other PFCAs, the available data suggest that the toxicological effects are similar to those of PFOA; however, more investigation into the cellular and molecular mechanisms is needed [7].

The toxicity of PFOS has also been examined extensively in rats and non-human primates. Dose-response experiments have demonstrated reduced body weight, hepatotoxicity, reduced cholesterol, and a steep curve for mortality [2, 22]. While significant effects occur in the rat at 2 mg/kg-day, the same effects occur in non-human primates at 0.75 mg/kg-day [22]. A reproductive study in rats resulted in neonatal mortality of all pups at a dose of 3.2 mg/kg-day within a day after birth [22]. Limited studies have been conducted with other PFSAs. Results indicate that developmental toxicity and hepatotoxicity are common effects within this class of chemicals [7].

Two specific studies, one sub-chronic study in mice and one in monkeys, are of particular interest as they were used by the U.S. Environmental Protection Agency (EPA) to determine quantitative values for the development of reference doses. The 95% lower bound on the benchmark dose ($BMDL_{10}$) for PFOA was calculated for the following toxicity endpoints in mice: neonatal eye opening, neonatal survival and body weight at weaning, reduced phalangeal ossification at term, live fetus weight at term, maternal liver weight at term, and maternal weight gains during pregnancy [21]. For PFOS, a no-observed-adverse-affect-level (NOAEL) was identified in a study on Cynomolgus monkeys. Effects on the monkeys dosed at the highest level included mortality (2 of 6), decreased body weights, increased liver weights, lowered serum total cholesterol, lowered triiodothyronin concentration, and lowered estradiol levels [23].

Current Standards for PFOA and PFOS. Based on the above laboratory studies, sub-chronic reference doses were calculated by the U.S. EPA for PFOA and PFOS [24]. In addition, provisional health advisories for drinking water and residential soil-screening levels were also calculated for PFOA and PFOS [24, 25]. These values are not regulations, and only account for two analytes out of more than twenty prominent PFAAs.

- Sub-Chronic Reference Dose: A sub-chronic reference dose (RfD) is defined by the EPA as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure for a subchronic duration (up to 10% of average lifespan) to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime" [26]. Currently, the EPA suggests RfD values for PFOA and PFOS of 0.2 µg/kg-day and 0.08 µg/kg-day respectively [24].

- Water – Provisional Health Advisories: The following equation was used to derive Provisional Health Advisories (PHA):

$$PHA\,(mg/L) = \frac{NOAEL\,or\,BMDL_{10}(mg/kg/day) \times BW(kg) \times RSC}{UF \times Extrapolation\,Factor \times Water\,Intake(L/day)} \tag{1.1}$$

where BW = body weight; RSC = relative source contribution; UF = uncertainty factors. In this case, the scenario was for a 10 kg child consuming 1 L/day of drinking water in order to be protective of sensitive populations [13, 24]. The RSC was estimated at 20% to account for exposure from other sources (i.e. food, dust, soil). A $BMDL_{10}$ of 0.46 mg/kg/day was used for PFOA, and a $NOAEL$ of 0.03 mg/kg/day was used for PFOS. An uncertainty factor of 10 was used to account for the intraspecies dose-response uncertainty within the $BMDL_{10}$ and NOAEL. Finally, since the $BMDL_{10}$ and $NOAEL$ were based on non-human studies, extrapolation factors were used to correct for interspecies variations. An extrapolation factor of (3 x 81), 3 for toxicodynamics and 81 for toxicokinetics, was used for PFOA; an extrapolation factor of (3 x 13), 3 for toxicodynamics and 13 for toxicokinetics, was used for PFOS [24]. Current drinking water PHAs issued by the EPA are 0.4 µg/L for PFOA and 0.2 µg/L for PFOS [24].

- Soil – Residential Soil Screening Levels: Using the above sub-chronic reference doses in the Superfund program's risk-based Regional Screening Level calculator, the following screening levels for surface soils were derived. The residential soil screening level for PFOA is 16 mg/kg and for PFOS is 6 mg/kg [25]. These guidelines were developed for children using a 6-year exposure period; this is the most protective screening level available for residential surface soil exposures [25]. At this time no soil screening levels have been set for any PFAAs other than PFOA or PFOS.

1.2.2 Land-Applied Biosolids

Many conventional WWTPs are ineffective at removal of PFAAs due to their resistance to degradation, and therefore, the presence of PFAAs in resulting municipal biosolids has been well documented [8, 10, 11]. Land application of biosolids has been practiced for decades around the world; in the U.S., approximately 60% of biosolids produced are distributed by land application [13]. Although biosolids are applied to a variety of sites including forest

and reclamation, nutrient-rich biosolids are also used as a fertilizer and soil conditioner in crop production.

Currently U.S. federal regulations limit the application of biosolids based on pathogen, metal, and nutrient constituents; however, synthetic organic contaminants concentrations are not restricted [13]. In Europe, a few countries have begun setting regulations for PFAAs in biosolids, for example, in Bavaria, sludge intended for use on agricultural land has been limited to 100 µg/kg for the sum of 11 perfluorinated chemicals [27]. Current studies indicate that levels of PFAAs in biosolids can easily exceed this limit [5]. PFAAs do not degrade in biosolids, and as end products, can actually increase in concentration with the degradation of precursors [28]. Some leaching, correlated with carbon chain-length, can occur; however, in general, once PFAAs are land-applied with biosolids, they can persist for decades [5, 28]. Furthermore, due to this persistence, repeated applications of biosolids to agricultural land present a potential exposure route for terrestrial food webs if PFAAs bioaccumulate from biosolids-amended soils to crops. A recent review conducted by Clarke and Smith [29] evaluated chemicals of potential concern for biosolids land application based on environmental persistence, human toxicity, evidence of bioaccumulation, evidence of eco-toxicity, and the quality of empirical data. According to this review, PFAAs were the number one priority for research and monitoring.

1.2.3 Reclaimed Water

Agricultural use of reclaimed water has a long history and represents a large percentage of the reclaimed water used in the U.S. Currently, the largest project in the U.S. that irrigates food crops eaten raw with reclaimed water, known as the Monterey County Water Recycling Projects, is located in the Salinas Valley, CA [30]. Tertiary treated reclaimed water has been used since 1998 to irrigate artichokes, lettuce, broccoli, cauliflower, strawberries, and celery. The system distributes reclaimed water to 222 parcels of farmland in the 12,000-acre service area [30]. Although the EPA has published guidelines for water reuse, no federal regulations govern water reclamation and reuse in the U.S. and thus regulations or guidelines are devel-

oped at the state level [15]. This has resulted in differing standards among states that have developed reuse criteria for various uses, including the irrigation of food crops with reclaimed water. Existing water reuse regulations for food crop irrigation are principally directed at health protection from microbial pathogens and do not typically include requirements addressing organic contaminants [31]. Several states have regulations pertaining to the use of reclaimed water for food crop irrigation. Requirements vary according to the type of crop and the method of irrigation. In addition, regulations often delineate the rules for direct and indirect contact between reclaimed water and edible crops [31].

Numerous past studies have reported the occurrence of organic contaminants, including PFAAs, in treated effluents of U.S. WWTPs [32, 33]. Even for treated effluents, concerns still exist about the potential for adverse human and eco-toxicological impacts of some contaminants through various water reuse applications [31]. However, the potential risks associated with organic contaminants may differ substantially when considering bioaccumulation in crops eaten fresh. Unfortunately, while data on the occurrence of many contaminants in reclaimed water are plentiful, limited data exist on the potential for uptake of these contaminants into edible plants.

1.2.4 Plant Uptake of PFAAs

Plant Uptake and Models. The natural ability of plants to take up nutrients, gases, and water also leave them susceptible to uptake of organic chemicals. Extensive research has been conducted on the potential for bioaccumulation of organic chemicals by plants, with a particular focus on priority pollutants, veterinary medicines, and pesticides [17, 34, 35]. Not surprisingly, bioaccumulation of organic chemicals in plants is a function of many factors stemming from environmental conditions, plant characteristics, and chemical properties. Primary environmental factors include temperature, humidity, and the soil matrix, while a few of the plant-specific factors are type and size of roots, xylem/phloem system, composition and pH of plant tissues, and the plant's metabolic capability [36]. In the traditional approaches for predicting and modeling plant uptake of organic chemicals, the dominant chemical-specific

factors affecting organic chemical bioaccumulation include molecular weight, water solubility, octanol-water partition coefficients (K_{ow}) [34], and vapor pressure [37].

Some predictions about plant uptake and transfer potential can be made based on classical plant physiology models [37–39]; however, they have limited applicability to PFAAs. Nevertheless, the general modeling approaches developed for predicting organic chemical uptake in plants may still provide significant insight into the potential processes and mechanisms responsible for bioaccumulation. In these models, chemical uptake from soil is usually driven by diffusion, as only natural or similar chemicals are actively transported into the roots of the plant [36].

Based on existing knowledge, small (molecular weight < 300), neutral, lipophilic ($\log K_{ow}$ $\sim$1 to 3) substances are most easily carried into the roots [40]. The initial transfer from the soil pore water and air pockets to the roots can be modeled using cylindrical diffusion models and the physicochemical properties of the chemical [34, 40]. Once a chemical enters the plant, the translocation and partitioning behavior of the chemical is highly varied and complex. Nonpolar molecules are mostly confined to the surface of the root due to lipid partitioning, whereas polar molecules can enter the transpiration stream and migrate throughout the plant [40, 41]. The contaminants can then travel through the apoplast (extracellular space) or symplast (intracellular space) until they reach the Casparian strip [42]. At this point they must cross through a cell membrane if they have not already done so. The Casparian strip acts somewhat as an ion trap allowing for higher concentrations of solutes in the xylem than in the pore water [42]. For those contaminants that enter the transpiration stream, the ratio of the concentration of the chemical in the transpiration stream to that in the soil pore water is defined as the transpiration stream concentration factor (TSCF) [37]. Original models commonly modeled TSCF using a K_{ow}-based equation with maximal TSCF values (gaussian distribution) for chemicals with $\log K_{ow}$ values $\sim$ 1.8. However, more recent models [39] suggest a sigmoidal shape indicating that hydrophilic compounds may actually be preferentially taken up into the vegetation.

This latter model is particularly relevant, as many shorter chain PFAAs are relatively hydrophilic and thus would be expected (using the new approach) to have high TSCF values. Once within the transpiration stream (i.e., in the xylem), the chemical can then be transported throughout the plant. If transported to the leaves, diffusive exchange with the atmosphere through the stomata is possible for volatile chemicals, though the relevance of this loss mechanism for PFAAs is minimal due to their generally low volatility. In fact, as ionisable contaminants that are soluble and non-volatile, PFAAs have the potential to accumulate very high concentrations in plants [43].

Bioaccumulation Metrics. To enable meaningful comparisons across soils and across crops, bioaccumulation factors (BAFs) can be calculated for each crop and PFAA. The BAF [44] is calculated by dividing the concentration of chemical in the plant tissue on a dry weight basis by the concentration of chemical in the exposure medium (e.g., soil) on a dry weight basis:

$$BAF = \frac{PFAA\,Concentration\,in\,Plant\,(ng\,g_{dw}^{-1})}{PFAA\,Concentration\,in\,Soil\,(ng\,g_{dw}^{-1})} \tag{1.2}$$

When calculating BAFs, several assumptions are made including the (1) absence of any chemical degradation in the plant and (2) negligible atmospheric exchange, thereby presuming the dominant uptake pathway for PFAAs is from the soil via the roots. As PFAAs are extremely stable and generally ionized at environmental pH values (and therefore nonvolatile) [45], these assumptions appear quite reasonable.

In addition, because PFAAs are known to sorb to organic matter [45], organic-carbon normalized BAFs (i.e., BAF_{oc}) can be calculated by normalizing the concentration of PFAAs in the soil to the fraction of organic carbon in soil to explore the impacts of soil organic carbon on bioaccumulation:

$$BAF_{oc} = BAF \times f_{oc} \tag{1.3}$$

TSCFs are also a widely used parameter in plant uptake studies and models. The values for TSCFs are obtained by dividing the concentration in the transpiration stream by the

concentration in the soil pore water:

$$TSCF = \frac{Concentration\,in\,Transpiration\,Stream\,(\frac{ng}{L})}{Concentration\,in\,Soil\,Porewater\,(\frac{ng}{L})} \tag{1.4}$$

1.2.5 Existing Studies on PFAA Uptake by Plants

A limited number of previous studies have documented the potential for bioaccumulation of PFAAs, particularly PFOA and PFOS, into food crops [27, 46]. Specifically, when growing crops in PFAA-spiked soils, Stahl et al. found carryover of PFOA and PFOS in maize, wheat, potato, and oats, with particularly high levels in the vegetative portions [46]. In a similar study using PFAA spiked soil, Lechner et al. found carryover of PFOA and PFOS in carrots, cucumbers, and potato, with the highest transfer factors for the vegetative portions [27]. Both studies found higher concentrations of PFOA than PFOS in the crops studied. However, both of these studies were based on spiked systems which are not representative of soils amended with biosolids containing PFAAs, as spiked systems are known to be problematic with respect to the aging effects on contaminant bioavailability [47, 48].

In a more relevant study, the transfer of PFAAs from industrially-contaminated biosolids-amended soils into grass was observed [49], with PFOA again bioaccumulating to a greater extent than PFOS. Although grass may be consumed by animals, thereby enabling PFAA entry into the terrestrial food chain, it does not represent a direct human consumption scenario. Additional literature has documented the transfer of PFAAs from spiked hydroponic systems into lettuce [50]. This study is the most relevant study for uptake of PFAAs by reclaimed water. However, a hydroponic system is vastly different than a soil scenario with respect to root structure of the plants and bioavailability of the contaminants [51, 52].

1.3 Research Objectives and Hypotheses

Objective 1: Determine if edible crops are a significant entryway for PFAAs into the terrestrial food chain via land-applied biosolids or reclaimed water.

- Hypothesis 1 – Under normal agricultural "field" conditions, PFAAs are more likely to be taken up by edible crops grown in biosolids-amended soils than in crops irrigated with reclaimed water.

- Rationale: Although accumulation in plants is expected from both biosolids-amended soils and reclaimed water, accumulation from the former is expected to be much greater due to the higher concentrations of PFAAs found in biosolids-amended soils versus reclaimed water. Biosolids-amended soils can have high concentrations of PFAAs both due to industrial impacts and, since they do not degrade, from multiple applications of biosolids. While some sorption is expected due to the presence of organic carbon [5, 45]; PFAAs will likely leach into the pore water due to their hydrophilic functional groups. PFAAs are relatively small, non-volatile, and somewhat water soluble compounds. These characteristics allow entry into the plant via diffusion into the roots and subsequently into the xylem advective system [40]. These same characteristics indicate that the TSCF should be very high for these compounds [39, 43]. PFAAs in reclaimed water will already be mobile, so although some sorption to soil is expected, depending on the organic carbon content of the soil, direct diffusion into plant roots will be possible. Since PFAAs are essentially non-volatile at environmental pH's they will not evaporate from the leaves, nor will they likely degrade or be metabolized in the plant. Once they have entered the plant, they will be prone to accumulate.

Objective 2: Characterize trends in bioaccumulation of PFAAs in plants with respect to analyte, PFAA uptake matrix (soil or water) concentration, and crop.

- Hypothesis 2A – Bioaccumulation will depend on both chain length and type of PFAA, with higher accumulation expected for the shorter chain length analytes. In addition, higher accumulation is expected for the perfluoroalkyl carboxylates versus the perfluoroalkane sulfonates.

- Rationale: PFAAs are known to associate with soil and sediment organic carbon [5, 45], and therefore, reduction in the bioaccumulation potential associated with both carbon chain length and functional head group may be associated with increasing sorption to organic carbon in the soil. An increase in soil-water distribution coefficient of 0.5 to 0.6 log units per CF_2 group as well as a 0.23 log unit increase for PFSAs compared with PFCAs of the same chain length reported previously [45] could point to reduced bioavailability for plant uptake.

- Hypothesis 2B – PFAA uptake matrix (soil or water) concentrations will have a direct relationship on bioaccumulation within the plant, resulting in a linear relationship between accumulated concentration and dose.

- Rationale: Transport across plant cell membranes is driven by electrical-potential and concentration gradients; accordingly, solutes, or contaminants, travel down their free-energy gradient by passive diffusion [42]. With an increase in concentration of PFAAs in soil or water, a steeper gradient is created thus driving more contaminant into the plant. Linearity of the plant uptake response to soil and water concentration of PFAAs would therefore suggest that diffusive driven transport is the primary mechanism for uptake and translocation. Alternatively, a non-linear relationship (e.g., a plateau) might indicate either an active transport mechanism or another type of barrier in uptake.

- Hypothesis 2C – Bioaccumulation will vary with vegetative structure. Edible leaf and stem crops are expected to accumulate the highest amount of PFAAs followed by edible root crops and then edible fruit crops.

- Rationale: Various processes are responsible for uptake and translocation of PFAAs within a plant. While passive diffusive processes may account for entry into the root and accumulation in the vegetation; other mechanisms come into play if PFAAs are to continue on into a fruiting structure. To travel from the leaves to areas of growth and

storage, the PFAAs must travel via the phloem. The phloem is driven by an osmotic pressure gradient that moves from source to sink [42]. Due to this extra distance of translocation, high concentration in fruit or seeds is not expected. In addition, although the root system is the port of entry for PFAAs, and therefore by default may have a fairly high concentration of them, only a minimal amount is expected to sorb to the root since PFAAs are not very lipophilic and will thus likely remain in the transpiration stream and translocate further up the plant [53]. Overall, the vegetative portions are expected to accumulate the highest concentrations of PFAAs.

1.4 Dissertation Organization

This dissertation is organized into five chapters. Chapter 1 includes introductory material, background information, and hypotheses for this study. Chapter 5 concludes with a summary of findings, the significance of this research, and recommendations for future work. Chapters 2, 3, and 4 are papers that have either been published or are in preparation for publication. Supporting information for the papers can be found in the appendices. Brief descriptions of the papers are provided below.

- Chapter 2 is entitled "Uptake of Perfluoroalkyl Acids into Edible Crops via Land Applied Biosolids: Field and Greenhouse Studies" by Andrea C. Blaine, Courtney D. Rich, Lakhwinder S. Hundal, Christopher Lau, Marc A. Mills, Kimberly M. Harris, and Christopher P. Higgins and has been published in Environmental Science & Technology [54]. Andrea Blaine designed the study, coordinated field experiments, implemented and directed greenhouse experiments and lab protocols, conducted quality assurance and control measures, and drafted the manuscript. This paper describes uptake and bioaccumulation of PFAAs in lettuce and tomato from biosolids-amended soils in both greenhouse and field studies. Bioaccumulation factors were measured for edible compartments. Results were used to validate greenhouse experimental values, and establish baseline bioaccumulation factors and trends for edible crops.

- Chapter 3 is entitled "Perfluoroalkyl Acid Distribution in Various Plant Compartments of Edible Crops Grown in Biosolids-Amended Soils" by Andrea C. Blaine, Courtney D. Rich, Erin M. Sedlacko, Lakhwinder S. Hundal, Kuldip Kumar, Christopher Lau, Marc A. Mills, Kimberly M. Harris, and Christopher P. Higgins and has been submitted for publication. Andrea Blaine designed the study, implemented and directed greenhouse experiments and lab protocols, conducted quality assurance and control measures, and drafted the manuscript. This paper describes the uptake and distribution of PFAAs in greenhouse radish, celery, tomato, and snap pea grown in biosolids-amended soils. Bioaccumulation factors were calculated for root, shoot, and fruit compartments. These data were used to construct a preliminary conceptual framework for PFAA accumulation in edible crops.

- Chapter 4 is entitled "Perfluoroalkyl Acid Uptake in Lettuce (*Lactuca sativa*) and Strawberry (*Fragaria ananassa*) Irrigated with Reclaimed Water" by Andrea C. Blaine, Courtney D. Rich, Erin M. Sedlacko, Katherine C. Hyland, Cecil Stushnoff, Eric R.V. Dickenson, and Christopher P. Higgins and is being prepared for publication. Andrea Blaine designed the study, coordinated field experiments, implemented and directed greenhouse experiments and lab protocols, conducted quality assurance and control measures, and drafted the manuscript. This paper describes the uptake of PFAAs in greenhouse lettuce and strawberry via reclaimed water. Uptake factors including dose, PFAA chain-length, and organic carbon content of soil were investigated. These results were used to verify previous findings concerning PFAA uptake in edible crops and to compare the bioavailability and uptake of PFAA in edible crops from reclaimed water versus biosolids-amended soils.

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CHAPTER 2

UPTAKE OF PERFLUOROALKYL ACIDS INTO EDIBLE CROPS VIA LAND APPLIED BIOSOLIDS: FIELD AND GREENHOUSE STUDIES

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Abstract

The presence of perfluoroalkyl acids (PFAAs) in biosolids destined for use in agriculture has raised concerns about their potential to enter the terrestrial food chain via bioaccumulation in edible plants. Uptake of PFAAs by greenhouse lettuce (*Lactuca sativa*) and tomato (*Lycopersicon lycopersicum*) grown in an industrially impacted biosolids-amended soil, a municipal biosolids-amended soil, and a control soil was measured. Bioaccumulation factors (BAFs) were calculated for the edible portions of both lettuce and tomato. Dry weight concentrations observed in lettuce grown in a soil amended (biosolids:soil dry weight ratio of 1:10) with PFAA industrially contaminated biosolids were up to 266 and 236 ng/g for perfluorobutanoic acid (PFBA) and perfluoropentanoic acid (PFPeA), respectively, and

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reached 56 and 211 ng/g for PFBA and PFPeA in tomato, respectively. BAFs for many PFAAs were well above unity, with PFBA having the highest BAF in lettuce (56.8) and PFPeA the highest in tomato (17.1). In addition, the BAFs for PFAAs in greenhouse lettuce decreased approximately 0.3 log units per CF_2 group. A limited-scale field study was conducted to verify greenhouse findings. The greatest accumulation was seen for PFBA and PFPeA in both field-grown lettuce and tomato; BAFs for PFBA were highest in both crops. PFAA levels measured in lettuce and tomato grown in field soil amended with only a single application of biosolids (at an agronomic rate for nitrogen) were predominantly below the limit of quantitation (LOQ). In addition, corn (*Zea mays*) stover, corn grains, and soil were collected from several full-scale biosolids-amended farm fields. At these fields, all PFAAs were below the LOQ in the corn grains and only trace amounts of PFBA and PFPeA were detected in the corn stover. This study confirms that the bioaccumulation of PFAAs from biosolids-amended soils depends strongly on PFAA concentrations, soil properties, the type of crop, and analyte.

2.1 Introduction

Perfluoroalkyl acids (PFAAs), which have been used in a myriad of consumer and industrial products (e.g., stain repellents, nonstick food packaging, and fire-fighting foams) [1], are ubiquitous and persistent in the environment; they have been detected in air, house dust, water, sediment, soil, wildlife, and humans [2–4]. In addition, longer chain PFAAs are poorly eliminated by many higher trophic level organisms, with elimination half-lives of more than five years in humans for some PFAAs [5]. Toxicity to wildlife and laboratory animals is well established for perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), including adverse effects such as reduced survival rates, infertility, and abnormal maturation [3]. The toxicity of shorter-chain PFAAs is less well documented. The persistence, bioaccumulation, and potential toxicity of PFAAs make them high priority contaminants of emerging concern.

PFAAs entering conventional wastewater treatment plants (WWTPs) or produced from precursors during treatment can exit the plant in either the aqueous or sludge phase [6]. The

presence of PFAAs in municipal biosolids is well documented [7–9]. The land application of biosolids has been practiced for decades; in the United States, approximately 60% of biosolids are land applied [10]. Nutrient-rich biosolids are particularly attractive as a fertilizer for crop production. Currently, the United States Environmental Protection Agency (U.S. EPA) regulates the land application of biosolids based on pathogen, metal, and nutrient content under the U.S. 40 Code of Federal Regulations Part 503 [11]. However, PFAAs in biosolids are not currently regulated in the United States [10]. Furthermore, due to the persistence of PFAAs, repeated agricultural applications of PFAA-contaminated biosolids may present a potential exposure route for terrestrial food webs if PFAAs contaminate surface or groundwater destined for animal or human consumption [12] or are transferred to (i.e., bioaccumulate in) the edible portion of crops.

Previous studies have documented the potential for PFAA bioaccumulation into crops, particularly for PFOS and PFOA [13, 14]. While growing corn, wheat, potato, and oats in PFAA-spiked soils, Stahl et al. found PFOA and PFOS in the vegetative plant portions [13], a finding that was confirmed in follow-up studies [15]. In a similar study using PFAA-spiked soils, Lechner and Knapp found carryover of PFOA and PFOS in carrots, cucumbers, and potato, with the highest transfer factors for the vegetative portions [14]. Both studies found higher PFOA than PFOS levels; however, spiked soil systems are known to be problematic with respect to contaminant bioavailability [16, 17], and thus these studies may not adequately describe PFAA uptake from nonspiked, biosolids-amended soils. Wen et al. conducted hydroponic studies with corn, which revealed that there are potentially different uptake mechanisms for PFOA and PFOS [18]. In a more relevant study, the transfer of PFAAs from industrially contaminated biosolids-amended soils into grass was observed [19], with PFOA again bioaccumulating more than PFOS. Although grass may be consumed by animals, thereby enabling PFAA entry into the terrestrial food chain, it does not represent a direct human exposure scenario. PFAA uptake in hydroponically grown lettuce has also been observed [20], though again, this does not likely describe the bioavailability of PFAAs

to plants grown in biosolids-amended soils [21, 22].

Concerns about the potential bioaccumulation of PFAAs into crops grown in biosolids-amended soils are also supported by limited data on their plant uptake and transport behavior [13, 19, 20]. While some predictions about plant uptake and transfer potential can be made based on plant physiology models [23–25] and contaminant parameters such as octanol-water partition coefficients (K_{ow}) [26], a very limited number of plant uptake studies have focused specifically on PFAAs. Initial models correlating the transpiration stream concentration factor (TSCF) [25], or the concentration ratio of the compound in the xylem to the solution around the roots, to K_{ow} suggested maximal TSCFs for compounds with log K_{ow} values of 1.8. However, a more recent model [24] suggests hydrophilic compounds (e.g., sulfolane) may actually be preferentially accumulated. Moreover, ionized contaminants are very soluble and nonvolatile and thus have the potential to accumulate high concentrations in plants [27].

The objective of this study was to examine PFAA bioaccumulation in lettuce (*Lactuca sativa*) and tomato (*Lycopersicon lycopersicum*) grown in biosolids-amended soils using a combination of greenhouse and field-scale experiments. Plant bioaccumulation was studied with unspiked biosolidsamended soils known to contain residual PFAAs. In addition, corn (*Zea mays*) samples were also collected from several biosolids-amended farm fields. Lettuce and tomato were chosen because they represent common edible crops eaten fresh. This scenario represents the most direct route of human exposure from plants, thus avoiding complicating factors from processing and packaging. Although lettuce and tomato are not commonly grown in biosolids-amended soils, they represent crops from the scenario of a home gardener using commercial biosolids as fertilizer. Greenhouse studies were conducted to avoid confounding environmental factors, and pilot-scale field studies were performed to verify greenhouse results. Data from an existing full-scale system were also collected for comparison; however, the crop availability was limited to corn. To our knowledge, this study is the first to look at PFAA uptake in lettuce and tomato from biosolids-amended soils.

2.2 Materials and Methods

The following is a summary of the materials and methods used in this study.

2.2.1 Chemicals

Perfluorinated standards as well as stable isotope labeled standards (Table A.1) were obtained from Wellington Laboratories (Guelph, ON, Canada). Analytes in this study include perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), PFOA, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), PFOS, and perfluorodecane sulfonate (PFDS). All standards were prepared in a 70/30 (v/v) methanol/water with 0.01% ammonium hydroxide solution. HPLC-grade methanol and high purity dichloromethane (DCM) from Sigma Aldrich were used for extractions. All other solvents were reagent grade from Sigma Aldrich. Water used in extractions was obtained from a Milli-Q system, and HPLC-grade water was used for liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. For extraction cleanup, Chromabond diamino from Macherey-Nagel Inc. and Supelclean ENVI-Carb from Sigma-Aldrich were used.

2.2.2 Greenhouse Study

Accumulation was studied from three soils: industrially impacted soil (soil amended with PFAA contaminated biosolids), municipal soil (soil receiving a longterm field application of municipal biosolids), and an unamended control soil. The industrially impacted soil was created by mixing composted biosolids from a small municipal (but impacted by PFAA manufacturing) WWTP with the control soil on a 10% mass basis. Composted biosolids were prepared at the utility by mixing dewatered biosolids with woody material (e.g., wood chips, saw dust, etc.) to achieve a 30:1 carbon to nitrogen ratio. Although this application rate is 10 times higher than an average recommended agronomic rate (approximately 25 Mg/ha, on dry weight basis) of biosolids application, it was chosen to represent multiple

applications or industrially impacted PFAA-contaminated soil. The municipal soil came from a reclamation site in Illinois where municipal biosolids were applied at reclamation rates for 20 years, reaching the cumulative biosolids application rate of 1654 Mg/ha. This field was planted with rotations of cereal crops such as corn, wheat, and sorghum. The control soil was taken from a nearby field that had a similar cropping system to the reclamation site but only received commercial fertilizers. Both the amended and control soils were classified as Lenzburg silt loams. All three soils were sieved (6.3 mm), and pots were filled on a dry weight basis. The fraction of organic carbon (f_{oc}), determined by the Walkley-Black Method (Table A.2), and other soil characteristics (Table A.3) measured by Agvise Laboratories can be found in Appendix A.

Pots were seeded with either leaf lettuce (*Lactuca sativa* 'Multy') or tomato (*Lycopersicon lycopersicum* 'Stupice') to achieve a density of two lettuce plants/pot and one tomato plant/pot. Edible portions (lettuce leaves or tomato fruits) from each pot were combined as one experimental replicate. Each of the three soils was evaluated for each crop with five replicates. Pots were randomly arranged to account for any spatial variations in light and temperature within the greenhouse. Crops were harvested at maturation and frozen at -20^oC in sealed plastic bags until extraction. Detailed information about propagation, environmental conditions, and sampling are given in Appendix A.

2.2.3 Field Studies

A limited-scale field study was conducted in the Midwestern United States. Eighteen plots (3.0 m × 4.6 m) were established, and each was planted with lettuce (*Lactuca sativa* 'Black-Seeded Simpson') and tomato (*Lycopersicon lycopersicum* 'Burpee Big Boy Hybrid'). Fertilization via biosolids occurred at five application rates (plus control) with three replicate plots per application rate. The soil treatments included an unamended control (CTRL), one-half of the agronomic rate of biosolids application to meet nitrogen (N) requirements of the crop (0.5×), agronomic rate (1×), two times the agronomic rate (2×), and four times the agronomic rate (4×). Crops were grown and harvested following normal agricultural

practices. Lettuce and tomato were harvested at maturity (lettuce $\sim$ 45 days; tomato $\sim$ 100 days) using a sample collection protocol (detailed in Appendix A) developed to minimize cross-contamination. Duplicate soil samples as well as lettuce and tomato samples from each plot were collected, placed on ice, and shipped to the laboratory where they were frozen at -20^oC until extraction.

In addition, a full-scale field sampling campaign was conducted in the Midwestern United States. Because corn (*Zea mays*) is the most commonly grown crop in this region, several paired samples of corn grain, corn stover, and soil were collected from three agricultural fields amended (0.5×, 1×, 2×) with municipal biosolids (rural or urban). Rural biosolids (0.5× field) were from a WWTP receiving domestic waste only, and urban biosolids (1×, 2× fields) were from a WWTP receiving both domestic and industrial waste. In addition, control samples of corn plant tissues and soil were collected from two nonamended fields (each proximal to the rural and urban amended field sites). All samples were collected in triplicate using the above-mentioned protocol, placed on ice after collection, and shipped to the laboratory where they were frozen at -20^oC until extraction. A summary of both the greenhouse and field studies is shown in Table 2.1.

2.2.4 Extraction and PFAA Analysis

Plant material was homogenized prior to extraction using a food processor. An aliquot of the homogenized plant tissue (0.5 - 2 g) was transferred to a 50 mL polypropylene vial, to which a surrogate spiking solution containing 2 ng of each isotopically labeled surrogate standard was added. A solvent mixture of 50/50 DCM and 99:1 (v/v) methanol (MeOH) and ammonium hydroxide was chosen based on the exhaustive extraction results reported in a previous study [19]. The solvent mixture (7 mL) was added to the sample and heated (30^oC) in a sonication bath (Fisher Scientific FS110H) for 30 min followed by shaking (VWR 5000 STD 120 V) for 1 h. The sample was centrifuged (Eppendorf 5810) at 2700 rpm (1467 RCF) for 20 min, and the extract was decanted into a separate 50 mL tube. This procedure was repeated twice for a total of three extraction cycles. The combined extract

Table 2.1: Summary of experimental framework for each phase of study.

Study Phase	Soils and Amendment Rates	Plant Tissue Analyzed for Each Soil Condition
Greenhouse Experiments	Field-collected control (unamended) soil (5 replicate pots);	Lettuce leaves; Tomato fruit.
	Field-collected control + industrially-impacted biosolids (10%) (5 replicate pots);	
	Field-collected amended municipal soil ($\sum$1654 Mg/ha) (5 replicate pots).	
Field-Scale Trial Plots	Control (unamended) (3 replicate plots);	Lettuce leaves; Tomato fruit.
	0.5X agronomic rate for N (5 Mg/ha) (3 replicate plots);	
	1X agronomic rate for N (10 Mg/ha) (3 replicate plots);	
	2X agronomic rate for N (20 Mg/ha) (3 replicate plots);	
	4X agronomic rate for N (40 Mg/ha) (3 replicate plots).	
Full-Scale Field Study	Urban site (control) (3 replicate samples);	Corn stover; Corn grain.
	Urban site (1X agronomic rate for N) (3 replicate samples);	
	Urban site (2X agronomic rate for N) (3 replicate samples);	
	Rural site (control) (3 replicate samples);	
	Rural site (0.5X agronomic rate for N) (3 replicate samples).	

was evaporated at 50^oC under nitrogen (Organomation Associates Inc. N-EVAP 112) to dryness. To minimize matrix effects, the extract was cleaned up via oxidation with 1 mL of a basic hydrogen peroxide solution (20 µL ammonium hydroxide and 980 µL 30% hydrogen peroxide), vortexed, and sonicated in a heated (30^oC) bath for 2 h. An additional aliquot (7 mL) of the basic DCM/MeOH mixture was added to each oxidized extract, vortexed, and heated in a sonication bath for 30 min. The extract was centrifuged at 2700 rpm (1467 RCF) for 20 min and decanted into a glass 20 mL scintillation vial. This re-extraction procedure was repeated twice for a total of three cycles. The combined extract was evaporated at 50^oC under nitrogen to dryness and reconstituted with 1 mL of 99:1 (v/v) MeOH and acetic acid. The extract was run through a cleanup column packed with 100 mg of diamino and 100 mg of ENVICarb. To analyze, 105 µL of the cleaned extract was transferred to an autosampler vial, along with 1350 µL of water and 45 µL of dilution water consisting of 0.01% ammonium hydroxide. All results are reported on a dry weight basis, which was determined by drying separate aliquots of plant tissue at 70^oC overnight (at which time no additional change in mass was observed). Soil samples were extracted as per established protocols [28]. Additional details regarding the soil extraction procedure can be found in Appendix A.

All PFAAs were analyzed using isotope dilution LC-MS/MS under conditions similar to those previously described [28]. Briefly, chromatography was performed using an aqueous ammonium acetate (10 mM) and MeOH (10 mM) gradient delivered at a flow rate of 800 µL/min by a Shimadzu LC-20AD unit. Samples and standards were injected (1 mL) by a Shimadzu SIL-5000 auto injector onto a 50 mm × 4.6 mm Gemini C18 column with a 3 µm particle size also equipped with a C18 guard column and cartridge. Initial eluent conditions were 50% MeOH and 50% water. The percent MeOH was ramped to 95% over 4 min, held at 95% over 4 min, ramped back down to 50% over 1.5 min, and re-equilibrated at 50% until 13 min. An MDS Sciex Applied Biosystems API 3200 operating in negative electrospray ionization scheduled multiple reaction monitoring (MRM) mode was used to monitor two MRM transitions for all analytes.

2.2.5 Quality Control

Quantitation was performed using the software Analyst. A minimum of 20% of all samples in each matrix were extracted and analyzed in triplicate. In general, the relative standard deviation for analytical replicates was less than 25%. Values presented in this study are averages of experimental (greenhouse) or field (outdoor) replicates (n = 3 to 18). Limits of quantitation (LOQs) were derived from the lowest calibration standard calculated to be within 30% of its actual value and were analyte, matrix, and run-dependent. LOQs, in general, ranged from 0.01 to 1.5 ng/g_{dw}. Field, experimental, and analytical blanks were employed to monitor contamination. Sample values that were not at least twice the level of the highest concentration in a blank were reported as < LOQ. Internal surrogate standards were used for each analyte (Table A.1) to correct for any losses during extraction. Plant surrogate recovery varied with matrix and analyte but typically ranged from 10% to 60%, and samples with less than 8% were excluded from any calculations. These recoveries are low in comparison to soil recoveries [28], however, are somewhat typical in plant matrices [19, 20] due to matrix ion suppression. The results of additional spike-recovery experiments (accounting for surrogate losses) resulted in an average of 85% recovery for all analytes across all matrices (Figure A.1) with no clear chain length dependent trends among analytes.

2.2.6 Bioaccumulation Metrics

To enable meaningful comparisons across soils and crops, bioaccumulation factors (BAFs) were calculated for each crop and PFAA for which plant tissue concentrations were above the LOQ. The BAF [29] was calculated by dividing the concentration in the plant tissue on a dry weight basis by the concentration in the soil on a dry weight basis (2.1):

$$BAF = \frac{PFAA\,Concentration\,in\,Plant\,(ng\,g_{dw}^{-1})}{PFAA\,Concentration\,in\,Soil\,(ng\,g_{dw}^{-1})} \tag{2.1}$$

When calculating BAFs, several assumptions were made including (1) absence of any chemical transformation in the plant or plant extraction process and (2) negligible atmospheric exchange, thereby presuming the dominant uptake pathway for PFAAs was from the soil

via the roots. As PFAAs are extremely stable and generally ionized at environmental pH values [30], these assumptions appear quite reasonable. In addition, given the propensity of PFAAs to sorb to organic carbon [30], organic-carbon normalized BAFs (i.e., BAF_{oc}) were calculated by normalizing the PFAA soil concentrations to the soil f_{oc} to explore the impacts of soil organic carbon on bioaccumulation (2.2):

$$BAF_{oc} = BAF \times f_{oc} \tag{2.2}$$

Because TSCFs are a widely used plant uptake parameter, for comparative purposes, BAFs were also converted to TSCFs. Briefly, TSCFs were obtained by converting concentrations in plant tissues to concentrations in the xylem using an average rate of water transpired per mass of plant tissue and by converting the soil concentrations to pore water concentrations using soil-water partitioning coefficients and soil f_{oc} values. Detailed information concerning the TSCF calculations can be found in Appendix A.

2.2.7 Statistical Analysis

Data are presented as means with standard errors. Statistical analysis, including calculation of regression equations, was completed using OriginPro 8.6. Statistical difference was determined by using an analysis of variance (ANOVA) with Tukey's Test ($\alpha = 0.05$); homogeneity of variance was assessed by Levene's Test ($\alpha = 0.05$). Regression equation slopes were compared by first fitting a line across the difference of values for each analyte and then comparing the slope of the resulting line to zero at an α of 0.05.

2.3 Results and Discussion

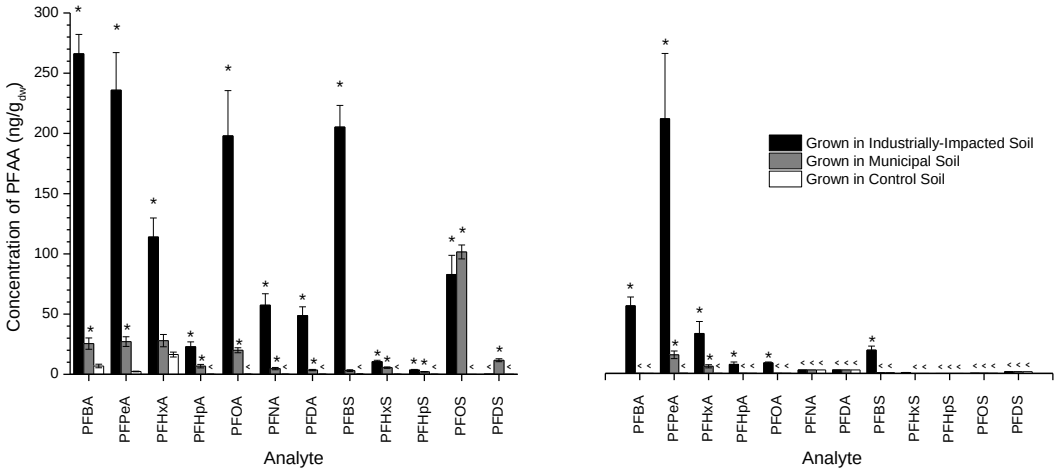
The results of this study are summarized and discussed below.

2.3.1 Greenhouse Study

Although the control soil was obtained from an unamended field, trace levels of PFAAs ($<$ 0.5 ng/g; Table A.5) were observed. Biosolids have long been applied in the surrounding area, and minor cross-contamination may have resulted from cultivation practices such as plowing

and planting or from atmospheric deposition [31]. In contrast, the industrially-impacted soil resulting from combining industrially-impacted biosolids with the control soil had a total of 335 ng/g PFAAs with the largest contributors being PFDA (93.5 ng/g), PFOA (78.5 ng/g), PFOS (49.7 ng/g), and PFBS (48.6 ng/g). The municipal biosolids-amended soil had a total of 434 ng/g PFAAs, consisting primarily of PFOS (319.5 ng/g) and PFDS (61.2 ng/g). Both biosolids-amended soils had comparatively low levels of the shorter chain carboxylates (PFBA, PFPeA, PFHxA, PFHpA): < 12 ng/g of each in the industrially-impacted soil and < 3 ng/g in the municipal biosolids-amended soil (Table A.5).

Despite the relatively low soil concentrations of the short chain PFAAs, elevated levels were observed in the greenhouse lettuce for all soil treatments. For lettuce grown in the industrially-impacted soil, concentrations were greatest for PFBA (266.1 ng/g), PFPeA (236.0 ng/g), and PFBS (205.2 ng/g), respectively (Figure 2.1). Lettuce grown in the municipal soil had the highest concentrations of PFOS (101.6 ng/g), PFHxA (28.0 ng/g), PFPeA (27.2 ng/g), and PFBA (25.5 ng/g), respectively (Figure 2.1). The preferential uptake of shorter chain PFAAs as has been previously observed [19, 20] was also exemplified in this study with the lettuce concentration of PFOS being only roughly 4-fold larger than the lettuce concentrations of the short chain perfluorocarboxylates (PFCAs) even though the initial soil concentration of PFOS was more than 100$\times$ greater than the soil concentrations of the short chain PFCAs. Even though control soil levels were below 0.5 ng/g for each PFAA, the lettuce grown in the control soil accumulated low levels of some PFAAs, notably PFHxA (16.4 ng/g) and PFBA (6.9 ng/g). The levels of all other PFAAs in the control lettuce were each less than 2.5 ng/g (Figure 2.1). An ANOVA test was used to compare concentrations of PFAAs in the different lettuce treatments. Concentrations of PFAAs in lettuce grown in the industrially-impacted soil were significantly different ($\alpha = 0.05$) than the control for all 11 analytes detected above the LOQ (Table A.5), and lettuce grown in the municipal soil was different than the control for 10 of the 12 analytes (Figure 2.1).



(a) (b)

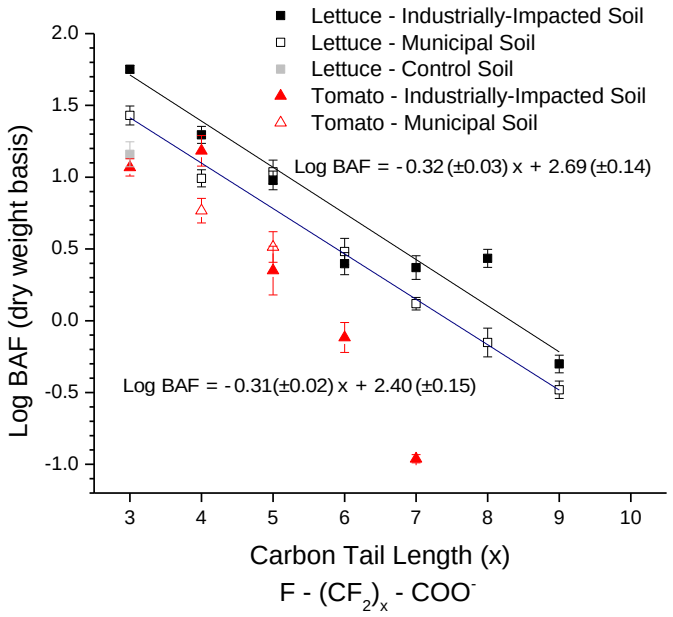
Figure 2.1: Concentrations of PFAAs in greenhouse lettuce (a) and tomato (b) grown in biosolids-amended soils. Mean and standard error are shown (n = 5). Values marked with an asterisk are significantly different ($\alpha = 0.05$) than the control. Values less than the LOQ are denoted by <; LOQs for respective matrix and analyte are listed in Table S5.

In contrast to the lettuce results, only seven and two PFAAs were detected above the LOQs for tomatoes grown in industrially-impacted soil and municipal soil, respectively. PFAAs in the control tomatoes were all less than LOQ (Figure 2.1). In the tomatoes grown in industrially-impacted soil, the highest levels were measured for PFPeA (211.4 ng/g), PFBA (56.1 ng/g), and PFHxA (33.2 ng/g). For tomatoes grown in the municipal soil, PFPeA (15.5 ng/g) and then PFHxA (5.9 ng/g) were present at the highest levels. Very little accumulation of any of the perfluoroalkyl sulfonates (PFSAs) was observed in tomatoes (only 19.4 ng/g PFBS and 0.8 ng/g PFHxS in the industrially-impacted soil), despite the fact that the soil concentration of PFOS in the municipal soil was 319 ng/g (Table A.5).

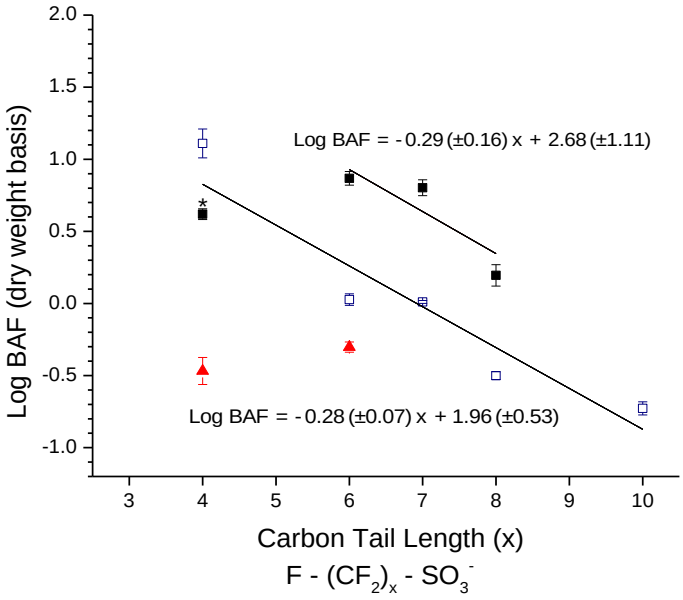
Bioaccumulation Factors. Average BAFs for lettuce grown in the industrially-impacted soil ranged from 56.8 for PFBA to 0.5 for PFDA, while values for the municipal soil lettuce ranged from 28.4 for PFBA to 0.2 for PFDS (Table 2.2). When log BAFs were plotted versus carbon chain length for PFCAs and PFSAs (Figure 2.2), a linear correlation was evident,

as was previously observed for PFCAs [19]. Within lettuce, the slopes of the regression equations are consistent in both biosolids-amended soils (Figure 2.2). The BAF decreases by approximately 0.3 log units per CF_2 group for PFCAs and PFSAs in both biosolids-amended soils, with no statistical differences between the slopes ($\alpha = 0.05$). However, the BAF for PFBS in lettuce grown in industrially-impacted soil was excluded from the regression calculation, as its value did not conform to the pattern displayed by the other data points. An increase in soil-water distribution coefficient of 0.5 to 0.6 log units per CF_2 group [30, 32] could point to reduced bioavailability for plant uptake as chain-length increases. The linearity of the plant uptake response to soil concentration of PFAAs suggests that passive transport may be the primary mechanism for uptake and translocation. However, the lower than expected BAF for PFBS of 4.2 (Table 2.2) versus the calculated one of 33.1 (Equation in Figure 2.2b) for lettuce grown in the industrially-impacted soil where PFBS concentrations were much higher than in the municipal soil indicates that bioaccumulation capacity for some PFAAs may be limited [27].

As is also apparent in Figure 2.2, BAFs for PFCAs and PFSAs in lettuce were, in general, slightly higher in the industrially-impacted soil than in the municipal soil (˜ 0.3 to 0.8 log units). Although the oxidation step in the plant extraction process could have potentially transformed precursors in one of the soils to several PFCAs [33], the consistency of the chain length trend among all of the PFCAs suggests this is not a significant contributing factor. Given that neither soil was spiked with PFAAs, differences in this apparent bioavailability to the lettuce was likely due to differences in soil properties and/or aging of the biosolids-soil mixture. In an effort to examine whether the f_{oc} of the soils could account for the differences, organic-carbon normalized BAFs were calculated. While for PFCAs, normalizing the BAFs more than compensated for the difference between the two soil treatments, for PFSAs, normalizing only accounted for about half the log difference (Figure A.2). It is possible that the difference in bioavailability of PFAAs may have also been due to the nature of the organic carbon, as the industrially-impacted soil contained carbon from fresh biosolids-based



(a)



(b)

Figure 2.2: Correlations between log BAF for PFCAs (a) and PFSAs (b) and carbon tail length in greenhouse lettuce and tomato grown in biosolids-amended and control soils. Means and standard errors are shown (n = 5). Linear regressions with slopes, intercepts, and associated error values are shown for lettuce in industrially-impacted and municipal soils; data point marked with an asterisk is excluded from regression calculation. Regressions for tomato BAFs were not performed.

Table 2.2: Summary of bioaccumulation factors (BAFs) for PFAAs in all 3 phases of this study and previous study (values not measured are designated as NM). BAFs were not calculated if analyte concentrations were below LOQ, and are denoted by $<$LOQ. Data are shown as means and standard errors (n = 3 to 5).

Analyte	Greenhouse Lettuce (Municipal Soil)		Greenhouse Lettuce (Industrially Impacted Soil)		Field Trial Lettuce (4× Soil)		Greenhouse Tomato (Industrially Impacted Soil)		Field Trial Tomato (4× Soil)		Field Corn Stover (2× Soil)		Previous Study [19]: Grass	
	Mean	(±)SE	Mean	(±)SE	Mean	(±)SE	Mean	(±)SE	Mean	(±)SE	Mean	(±)SE	Mean	(±)SE
PFBA	28.4	5.21	56.8	3.45	40.0	2.41	12.2	1.71	18.2	5.34	64.8	15.35	NM	
PFPeA	10.2	1.52	20.4	2.70	16.3	2.35	17.1	3.74	14.9	1.96	41.1	9.00	NM	
PFHxA	11.7	2.11	9.90	1.37	<LOQ		2.90	0.87	6.84	0.81	<LOQ		3.40	1.84
PFHpA	3.33	0.72	2.66	0.47	<LOQ		0.86	0.23	<LOQ		<LOQ		0.90	0.30
PFOA	1.34	0.14	2.52	0.48	<LOQ		0.11	0.01	<LOQ		<LOQ		.025	0.10
PFNA	0.77	0.15	2.85	0.47	<LOQ		<LOQ		<LOQ		<LOQ		.012	0.04
PFDA	0.34	0.05	0.52	0.08	<LOQ		<LOQ		<LOQ		<LOQ		0.10	0.04
PFBS	14.5	3.84	4.22	0.37	2.02	0.32	0.42	0.08	<LOQ		<LOQ		NM	
PFHxS	1.08	0.11	7.56	0.86	1.51	0.11	0.50	0.04	<LOQ		<LOQ		NM	
PFHpS	1.03	0.02	6.57	0.94	<LOQ		<LOQ		<LOQ		<LOQ		NM	
PFOS	0.32	0.02	1.67	0.32	0.10	0.01	<LOQ		<LOQ		<LOQ		0.07	0.02
PFDS	0.19	0.02	<LOQ		<LOQ		<LOQ		<LOQ		<LOQ		NM	

compost, whereas organic carbon in the municipal soil was derived primarily from aged soil organic matter rich in recalcitrant clay-humic complexes. While organic carbon is likely a contributing factor to differences in PFAA bioaccumulation, other geochemical factors may be important as well.

Tomato BAFs in the industrially-impacted soil ranged from 17.1 for PFPeA to 0.1 for PFOA (Figure 2.2). No other studies have measured the uptake of PFAAs in tomato. However, the BAF for PFOA in a fruit (cucumber) estimated at 0.75 using the value reported on a wet weight basis of 0.0314 and correcting for water content (assumed to be 96% for cucumber) [34] is on the same order of magnitude. Linear trends were not as apparent for PFAA log BAFs in tomato. However, for PFCAs in tomato grown in industrially-impacted soil, the BAF decreases approximately 0.5 to 0.9 log units if PFBA is excluded. Again, the shortest chain PFAAs (PFBA and PFBS) may be slightly less bioaccumulative than would be expected from trends in BAFs for their longer chain homologs, particularly if there is a concentration ceiling on the passive transport process or if there are other contributing transport process barriers. Furthermore, the difference in uptake patterns of lettuce and tomato suggest that the type of crop, or perhaps more importantly, the type of vegetative structure, may play an important role in PFAA bioaccumulation. Contaminants must be transported much further in the plant to reach a fruit crop (tomato) than a stem/leaf crop (lettuce).

Transpiration Stream Concentration Factors. As no other studies have reported PFAA BAFs for lettuce grown in biosolids-amended soil, comparable TSCFs were calculated to enable comparisons with results from a hydroponic lettuce study [20]. Calculated TSCFs are plotted in Figure 2.3 alongside literature values [20]. As organic-carbon derived partition coefficients were used to estimate soil pore water concentrations, the strong agreement between the TSCFs generated from the present study and those published previously reiterates the importance of f_{oc} in affecting the bioavailability of PFAAs in biosolids-amended soils. These results also support the passive transport mechanism as, in general, PFAAs are taken

up at a rate much lower than water (less than unity) [24].

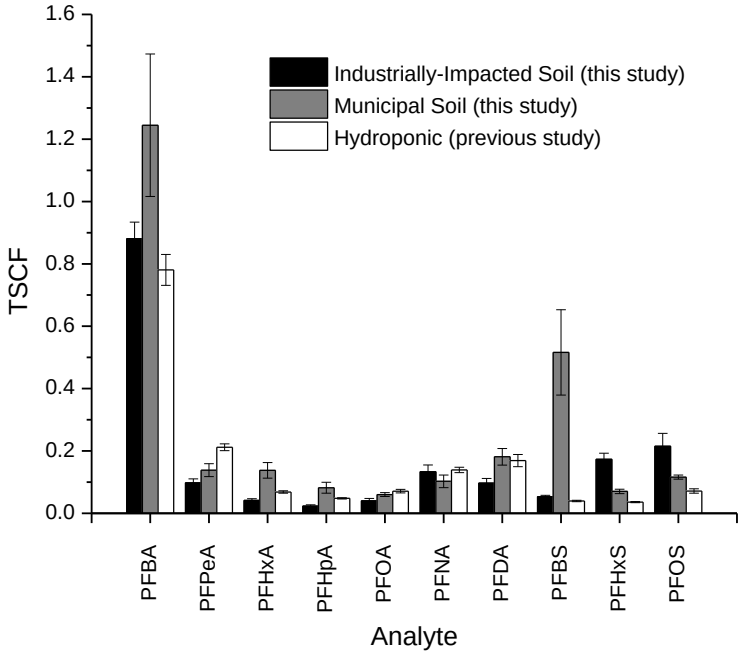


Figure 2.3: Comparison of transpiration stream concentration factors (TSCFs) for lettuce calculated from this study compared to TSCFs from a previous hydroponic lettuce study [20]. Means and standard errors (n = 5) are shown.

2.3.2 Pilot-Scale Field Trial

The five biosolids treatments used in the pilot-scale field trial plots were selected to represent increasing application rates; however, PFAA soil concentrations above background (i.e., >1.5 ng/g) were only observed for PFOA, PFNA, PFDA, PFOS, and PFDS (Table A.6). The two highest concentrations were for PFOS (13.9 ng/g) and PFOA (5.2 ng/g) in the 4× amended soil. Soil concentrations of shorter chain PFAAs did not significantly increase with increased biosolids amendment rate (Table A.6). These field soil values of PFAAs were significantly lower (3-20 times) than the levels found in the soils used in the greenhouse study. As a result of low initial soil concentrations, limited plant uptake data from the field trials were obtained, restricting the comparisons that could be made. PFAA concentrations

in field crops were averaged for the three replicate soil plots only if all three replicate values were above the LOQ (Table A.6). In the lettuce, the highest concentrations found were for PFBA (27.5 ng/g) and PFPeA (16.4 ng/g) in the 4× amended soil plot. For tomato, the highest concentrations were for PFBA (17.0 ng/g) in the 0.5× plot and PFPeA (15.0 ng/g) in the 4× plot. Minimal accumulation was found in crops grown in the 1× and 2× plots; all lettuce and tomato PFAA concentrations can be found in Table A.6. For the analytes that had concentrations above the LOQ in the 4× amended soil, lettuce and tomato BAFs were calculated. These values are shown alongside the respective greenhouse grown lettuce and tomato BAFs in Table 2.2.

A trend suggesting an inverse relationship between BAFs and chain length was seen for PFCAs in both the field trial lettuce and tomato (Figure A.3). Although the field data are limited, the difference between the log BAFs (1.6 for PFBA and 1.2 for PFPeA) for the field trial lettuce is a decrease of 0.40, which correlates well with the greenhouse grown lettuce decrease of 0.3 per CF_2 moiety. In addition, the field BAF values for tomato decrease approximately 0.1 to 0.3 log units per CF_2 moiety, similarly but less closely correlated to the greenhouse grown tomatoes (0.5 to 0.9 log units per moiety).

2.3.3 Full-Scale Field Study

Soil concentrations of PFAAs for the full-scale crop-soil system were similar to concentrations in the field trial plots. All PFAAs were individually less than 2 ng/g except for PFOA (4.4 ng/g), PFDA (2.6 ng/g), and PFOS (4.3 ng/g) from the Rural 0.5× field, and PFOS (2.8 ng/g) from the Urban 2× field (Table A.7). All PFAA corn grain concentrations were below the LOQ (Table A.7). In the corn stover, only PFBA (4.2 ng/g) and PFPeA (0.3 ng/g) were above the LOQ for the Urban 2× plot (Table A.7). This preferential accumulation in the vegetative compartment is consistent with the findings of Stahl et al. [13] in a previous study. In addition, the findings reiterate the consistent bioaccumulation of the short chain carboxylates as found in the greenhouse and field trial studies. From these limited data, BAFs for PFBA and PFPeA were calculated and are shown in Table 2.2 along

with grass-soil accumulation factors calculated by Yoo et al. [19] in a previous study. In the absence of other studies for comparison, the similarity of corn stover to grass is used to compare results. However, the longest PFCA detected in this study was PFPeA and the shortest PFCA that Yoo et al. reported was PFHxA, so no direct comparisons are possible. Trendwise, Yoo et al. reported a decrease of 0.2 log units per CF_2 group increase [19]; the limited log BAF data found for corn stover in the present study (1.8 for PFBA and 1.6 for PFPeA) also shows a decrease by 0.2 log units per CF_2 group. Stahl et al. [13] studied corn straw in spiked soil systems, and BAFs can be calculated from the data reported. BAFs for the only two PFAAs studied were 0.24 for PFOA and 0.16 for PFOS, which are in line with corn stover and grass trends provided in Table 2.2.

2.3.4 Implications

While some PFAA crop accumulation data are available from the literature, this is the first study examining PFAA accumulation in food crops grown in unspiked, biosolids-amended soils, though amendment rates were generally above typical agronomic application rates. From this study, it is clear that there is preferential uptake of PFCAs over PFSAs, and accumulation of shorter chain PFAAs over longer chain PFAAs. In addition, uptake differences in crops suggest that the vegetative structure of the crop may affect the amount of bioaccumulation. In both the field and greenhouse studies, BAFs for shorter chain PFAAs were greater than 1, indicating accumulation in the plant tissues. In the context of the U.S. EPA's risk assessment framework for potential contaminant accumulation in crops from biosolids-amended soils, the default "conservative" value for BAFs is 1 [**?**]; clearly, in light of these results, this estimate is not truly conservative for short chain PFAAs. This finding points to the need for more thorough research before full risk assessments can be completed for PFAAs. These results may also have important implications with respect to the potential routes of PFAA exposure in humans who might have repeatedly used biosolids to fertilize their home gardens, particularly if the biosolids were from a WWTP receiving industrially-impacted wastewater with elevated levels of PFAAs. More work is needed to verify the

trends observed in this study as plant accumulation of PFAAs varies with soil properties, crop type, biosolids application rate, and analyte.

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CHAPTER 3

PERFLUOROALKYL ACID DISTRIBUTION IN VARIOUS PLANT COMPARTMENTS OF EDIBLE CROPS GROWN IN BIOSOLIDS-AMENDED SOILS

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Abstract

The documentation of crop uptake of perfluoroalkyl acids (PFAAs) from biosolids-amended soil has exposed the potential of PFAAs to enter the terrestrial food chain. This study compared the uptake of PFAAs in greenhouse-grown radish (*Raphanus sativus*), celery (*Apium graveolens* var. *dulce*), tomato (*Lycopersicon lycopersicum*), and sugar snap pea (*Pisum sativum* var. *macrocarpon*) from an industrially impacted biosolids-amended soil, a municipal biosolids-amended soil, and a control soil. Concentrations of PFAAs in edible portions grown in soil amended with PFAA industrially impacted biosolids were highest for perfluorooctanoate (PFOA; 67 ng/g) in radish, perfluorobutanoate (PFBA; 232 ng/g) in celery, and PFBA (150 ng/g) in pea. Comparatively, PFAA concentrations in edible compartments

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of crops grown in the municipal biosolids-amended soil and in the control soil were minimal ($<$ 25 ng/g). Bioaccumulation factors (BAFs) were calculated for the root, shoot, and fruit compartments (as applicable) of all crops grown in the industrially impacted soil. BAFs were highest for PFBA in all crops for the shoot compartments, as well as in pea for the fruit compartment. Root-soil concentration factors (RCFs) for tomato and pea were independent of PFAA chain length, while radish and celery RCFs showed a slight decrease with increasing chain length. Shoot-soil concentration factors (SCFs) for all crops showed a decrease with increasing chain length (0.11 to 0.36 log decrease per CF_2 group). The biggest decrease (0.54 to 0.58 log decrease per CF_2 group) was seen in fruit-soil concentration factors (FCFs). Crop anatomy and PFAA properties were utilized to explain data trends. In general, fruit crops were found to accumulate fewer long-chain PFAAs than shoot or root crops presumably due to an increasing number of biological barriers as the contaminant is transported throughout the plant (roots to shoots to fruits). These data were incorporated into a preliminary conceptual framework for PFAA accumulation in edible crops. In addition, these data suggest that edible crops grown in soils conventionally amended for nutrients with biosolids (that are not impacted by PFAA industries) are unlikely a significant source of long-chain PFAA exposure to humans.

3.1 Introduction

Perfluoroalkyl acids (PFAAs) are used extensively both in industrial and consumer products [1], but resist degradation by conventional wastewater treatment plants (WWTPs) and persist in both aqueous effluent and treated biosolids [2, 3]. Land-application of biosolids on crops can then facilitate the entry of PFAAs into the terrestrial food web. Currently, there are no federal regulations in the U.S. that govern the use and application of biosolids based on PFAA concentrations [4]. Land-application of biosolids primarily occurs on grain crops; however, sustainability movements are encouraging more liberal use of biosolids on home gardens by consumers.

Blaine et al. [5] have shown that edible crops can uptake PFAAs from biosolids-amended soils. Both lettuce leaves and tomato fruit had bioaccumulation factors (BAFs) greater than one for short-chain perfluorocarboxylates (PFCAs) [5]. In addition, carbon chain length dependent trends were seen in lettuce leaves, resulting in an approximately 0.3 log decrease for each CF_2 group [5]. However, as only the edible portions were analyzed, more general correlations between plant compartment and PFAA accumulation were not made. In another recent greenhouse study, an inverse relationship between BAF and carbon chain length was also seen for PFCAs in alfalfa plants [6]. Felizeter et al. [7] studied accumulation of PFAAs in hydroponic lettuce and found that long-chain PFAAs accumulated more in the roots than in the foliage, whereas for short-chain compounds, there was more translocation from the roots to the foliage [7]. A more mechanistic study by Wen et al. [8] determined that PFOA and PFOS may have different uptake mechanisms in maize; potential active uptake and entry by anion channels were suggested for PFOA, while entry by aquaporins (water channels) or anion channels (different than the ones used by PFOA) were suggested for PFOS.

The translocation and partitioning behavior of a chemical in a plant is highly varied and complex. Various plant uptake models have been explored over the years with the majority focusing on uptake of neutral hydrophobic compounds based on the octanol-water partitioning coefficient (K_{ow}) [9, 10]. In these models, chemical uptake from soil is usually driven by passive diffusion, as only natural or structurally similar chemicals are actively transported [10], and small, neutral, lipophilic substances are most easily carried into the roots [11]. The compounds can travel across the root cortex through the apoplast (extracellular space) or symplast (intracellular space) until they reach the Casparian strip at the endodermis [12]. At this point, they must cross through a cell membrane (Figure 3.1). While neutral compounds may easily pass through a membrane, ionized compounds may pass through as neutral salts, through anion channels, or through water pores in the membrane [8, 13]. The Casparian strip acts as an ion trap, allowing for higher concentrations of solutes in the xylem than in the pore water [12].

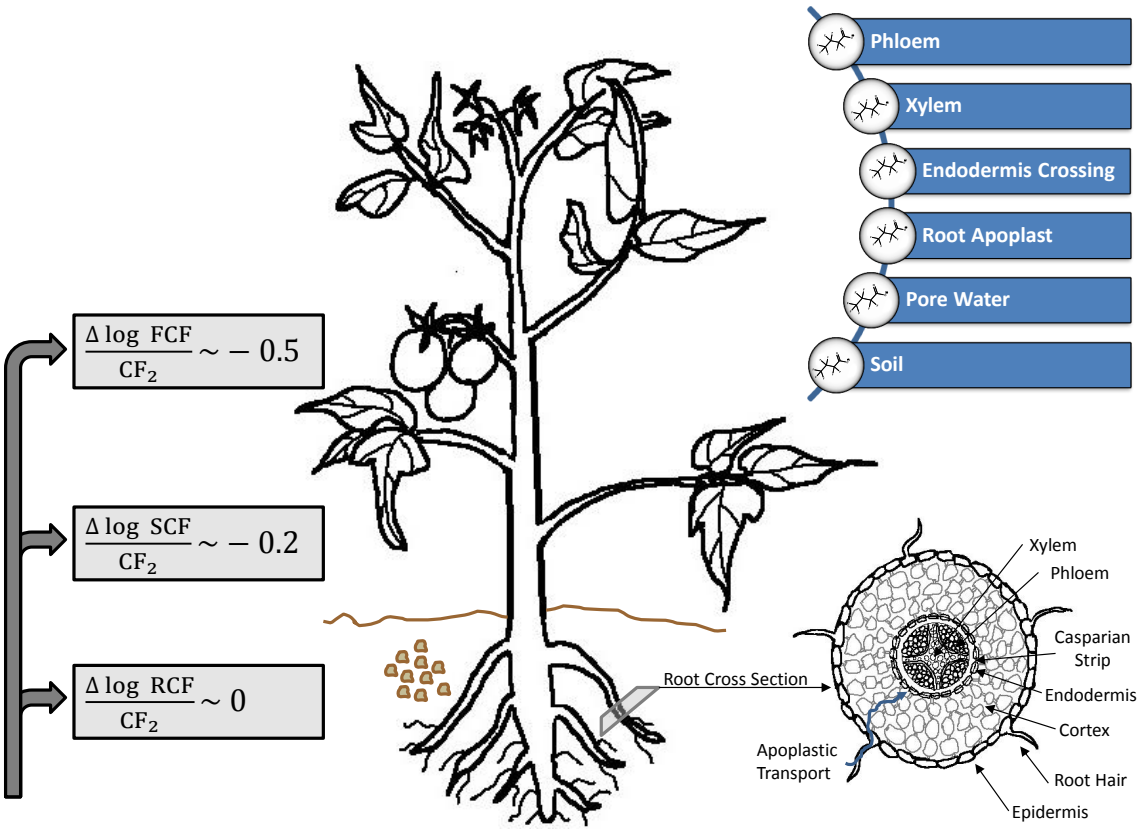


Figure 3.1: Conceptual model of PFCA uptake in tomato plant. Approximate values are shown for change in log bioaccumulation factor per CF_2 group. Uptake pathway is shown in the top right corner. Root cross-section modified from Taiz and Zeiger [12].

While nonpolar molecules are mostly confined to the surface of root membranes due to lipid partitioning, polar molecules can enter the transpiration stream and migrate throughout the plant [14, 15]. Once within the transpiration stream, a compound can be transported throughout the plant, first to the shoot (i.e., stem and leaves) via the xylem and then to storage organs (e.g., fruit) via the phloem. The xylem and phloem are separated by the vascular cambium, a single row of cells. Accumulation of solutes in plant cells near the leaves helps drive translocation from source (e.g., leaf) to sink (e.g., fruit) via a pressure-flow [12] model. As the concentration in a cell escalates, water is absorbed osmotically thus building up hydrostatic pressure. The subsequent movement of the water and solutes through the system of phloem sieve tubes equalizes the pressure. The sieve tubes are separated by sieve plates which allow flow through transport pores (plasmodesmata). Eventually, compounds may be stored in cell vacuoles or in inter-cellular spaces. Polar compounds with low lipophilicity, low volatility, and high persistence are particularly prone to accumulation in the leaves and other sinks by phloem transport [16]. PFAAs generally meet these criteria. In particular, PFAAs, being anionic at environmental pH values [17], are generally non-volatile thereby eliminating any potential release into the air from the leaf stomata.

This study evaluated the PFAA distribution in various plant structural compartments by examining both the edible and non-edible portions of radish (*Raphanus sativus*), celery (*Apium graveolens* var. *dulce*), tomato (*Lycopersicon lycopersicum*), and sugar snap pea (*Pisum sativum* var. *macrocarpon*) grown in biosolids-amended soils. Radish represents an edible root crop (i.e., below ground crop), although radish tops are also edible. Celery represents an edible shoot crop (i.e., stem and leaf crop) although certain varieties of celery are also harvested for the bulb and seeds. Tomato represents an edible fruit crop. Sugar snap pea, a legume, also represents a fruit and edible seed crop. Bioaccumulation factors for the root, shoot, and fruit portions were calculated. To our knowledge, this is the first study to examine PFAA uptake in celery, snap pea, and radish; in addition, it is one of the most detailed studies addressing inter-compartmental translocation of PFAAs in edible crops to

date.

3.2 Materials and Methods

The following is a summary of the materials and methods used in this study.

3.2.1 Chemicals

Native perfluorinated standards and stable isotopes were obtained from Wellington Laboratories and prepared as per established methods [5]. Analytes studied include perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS; Table B.1). HPLC-grade methanol (MeOH), high purity Chromasolv dichloromethane (DCM), and all other reagent grade solvents were obtained from Sigma Aldrich. A MilliQ system (Millipore) was used to provide water for extractions, and HPLC-grade water was used for liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. Chromabond diamino from Macherey-Nagel Inc. and Supelclean ENVI-Carb from Sigma-Aldrich were used in extraction clean-up.

3.2.2 Greenhouse Study

Two biosolids-amended soils as well as an unamended control soil were used in this study: a soil amended with industrially impacted biosolids (industrially impacted soil), a soil receiving a long-term field application of municipal biosolids (municipal soil), and an unamended control soil. Although the control soil was obtained from an unamended field, its proximity to biosolids-amended fields likely led to minor cross-contamination resulting in the detection of trace levels of PFAAs. Details on all three soils including PFAA concentrations can be found elsewhere [5]. In general, soils were sieved (6.3 mm) for homogeneity and pots were filled on a dry weight basis. Four edible crops including radish, celery, tomato and pea were grown from seed. Five pot replicates were grown for each crop in each soil. Pots were

randomly arranged to account for any spatial variations in light and temperature within the greenhouse. Additional information about propagation and greenhouse environmental conditions are given in Appendix B. Both edible and non-edible parts of all crops were harvested (Table B.2) at maturity and frozen at $-20°C$ in sealed plastic bags until extraction.

3.2.3 PFAA Extraction and Analysis

Prior to sample preparation, plant material was homogenized using a food processor. Aliquots (0.5-2 g) of soil or plant material were transferred to 50 mL polypropylene vials. To each vial, 2 ng of isotopically-labeled surrogate standard was added. Plant samples were then extracted with a 50/50 (v/v) solution of DCM and 99:1 (v/v) MeOH with ammonium hydroxide as detailed elsewhere [5]; soil samples were extracted based on the protocol from Sepulvado et al. [3] Results for both plants and soils are presented on a dry weight basis.

All PFAAs were analyzed with isotope dilution using LC-MS/MS under conditions outlined in previous work [5], though the method was validated for the wide variety of plant matrices included in the present study (Figure B.1). Chromatography was performed using a Shimadzu LC-20AD unit. Samples were injected onto a Gemini C18 Column with a 3-micron particle size (Phenomenex). Two transitions for each analyte were observed using an MDS Sciex Applied Biosystems API 3200 (MDS Sciex) with negative electrospray ionization operating in scheduled multiple reaction mode.

3.2.4 Data Analysis

Quality Control. The software Analyst was used for quantitation in this study. For each matrix, a minimum of twenty percent of the samples were extracted and analyzed in triplicate. The relative standard deviation for analytical replicates was less than 18%. Sample values are presented as the mean experimental replicate value (n = 3 to 5). One extraction blank with surrogate standard and one double blank without surrogate standard were prepared with each batch of samples. Limits of quantitation (LOQ) ranged from 0.03 to 0.71 ng/g; they were determined by the lowest calibration standard calculated to be

within 30% of its actual value and were analyte, matrix, and run-dependent. Sample values that were not at least twice as high as the highest concentration in a blank were reported as $<$LOQ. To account for any loss during the extraction process, each sample was fortified with isotopically labeled surrogate standards. Surrogate recoveries for the samples averaged 35% for root tissues, 36% for shoot tissues, and 40% for fruit tissues across all analytes. While lower than typical soil surrogate recoveries [3], this range is typical in plant matrices [7, 18] due to matrix ion suppression. Spike-recovery experiments that accounted for surrogate losses showed an average native recovery of 73% in root tissues, 80% in shoot tissues and 71% in fruit tissues for all analytes (Figure B.1).

Statistical Analysis. Data are shown as means with standard errors. Statistical analyses and regression lines were calculated using OriginPro 9.0. Statistical difference of means was established by an analysis of variance (ANOVA) with Tukey's Test ($\alpha = 0.05$); homogeneity of variance was assessed by Levene's Test ($\alpha = 0.05$).

3.2.5 Bioaccumulation Metrics

Bioaccumulation factors (BAFs), including root concentration factors (RCFs; eqn B.1), shoot concentration factors (SCFs; eqn B.2), and fruit concentration factors (FCFs; eqn. B.3) were calculated by dividing the concentration of chemical in the respective plant tissue on a dry weight basis by the concentration of chemical in the soil on dry weight basis (3.1):

$$BAF = \frac{PFAA\,Concentration\,in\,Plant\,Tissue\,(ng\,g_{dw}^{-1})}{PFAA\,Concentration\,in\,Soil\,(ng\,g_{dw}^{-1})} \tag{3.1}$$

Due to the stability and ionized nature of PFAAs at environmental pH values, plant entry via the stomata as well as formation by degradation of accumulated PFAA precursors are assumed to be insignificant compared with dominant uptake through the roots. BAFs were calculated using crops grown in the industrially impacted soil for each PFAA that had concentrations in the plant tissues above the LOQ.

RCFs were also calculated based on pore water concentrations (RCF_{pw}) derived in previous work [5]. Each RCF_{pw} was calculated by dividing the concentration of chemical in the

respective plant tissue on a dry weight basis by the concentration of chemical in the pore water (3.2):

$$RCF_{pw} = \frac{PFAA\,Concentration\,in\,Plant\,Tissue\,(ng\,g_{dw}^{-1})}{PFAA\,Concentration\,in\,Pore\,Water\,(ng\,ml^{-1})} \tag{3.2}$$

In addition, inter-compartmental concentration factors (i.e., shoot to root; 3.3 and fruit to shoot; eqn. B.4) were calculated:

$$Shoot - Root\,Concentration\,Factor = \frac{PFAAs\,in\,Shoot\,Tissue\,(ng\,g_{dw}^{-1})}{PFAAs\,in\,Root\,Tissue\,(ng\,g_{dw}^{-1})} \tag{3.3}$$

3.3 Results and Discussion

The results of this study are summarized and discussed below.

3.3.1 Edible Portions

For radish grown in the industrially impacted soil, PFAA concentrations in root were highest for PFOA (67 ng/g), PFBS (62 ng/g), PFDA (41 ng/g), and PFOS (35 ng/g) (Figure 3.2); these four analytes also had the highest concentration in the soil. In the municipal and control soil, PFBS showed the highest concentrations of 24 ng/g and 22 ng/g respectively (Table B.3). For celery grown in the industrially impacted soil, concentrations of PFAAs in the shoot were greatest for PFBA (232 ng/g), PFPeA (148 ng/g), PFHxA (137 ng/g) and PFBS (107 ng/g); unlike the radish root, accumulation in the celery shoot was higher for the short-chain compounds (PFHxA and below for PFCAs; Figure 3.2). Comparatively, lettuce grown in the same soil had similar concentrations of the short-chain compounds: PFBA (266 ng/g), PFPeA (236 ng/g) [5]. In the municipal soil, PFAA celery concentrations were all less than 8 ng/g with the exception of PFOS (17 ng/g), most likely due to the relatively high concentration of PFOS and low concentrations of short-chain PFAAs in the soil (Table B.3). All PFAA concentrations in the celery grown in control soil were less than 6 ng/g (Table B.3). Concentrations of PFAAs in the pea fruit grown in industrially impacted soil were highest for PFBA (150 ng/g) and PFPeA (46 ng/g); all PFAAs were below LOQ for pea fruit grown in municipal and control soils (Table B.3).

PFAA concentrations in the crops grown in the industrially impacted and municipal soils were compared to the control (unamended) treatments by an ANOVA test; statistical differences are shown in Figure B.2. Low PFAA concentrations in the municipal and control soils limited the ability to determine accumulation trends, and thus the remainder of the results and discussion focuses on the crops grown in the industrially impacted soil.

3.3.2 Plant Compartments

PFAA concentrations in non-edible plant compartments grown in the industrially impacted soil were also analyzed and plotted alongside edible compartment concentrations in Figure 3.2. The concentrations of PFAAs in the radish shoot follow the same trends as in the radish root (and the soil), but are approximately 5-10 times higher. Physiologically, radishes lack the typical barrier (Casparian strip) between the edible bulb and the above ground shoot [19]. The swollen edible portion of the radish is actually formed at the intersection of the hypocotyl (embryonic stem) and the fine roots below; as the fine roots below the bulb are not generally eaten, they were not analyzed as part of the edible root portion. Therefore, although the analytes accumulate in the same proportions, more accumulation is seen in the shoot, perhaps due to the unrestricted upward flow of PFAAs. For celery, the shoot and root portions do not have parallel concentration trends. The celery shoot has higher concentrations of short-chain PFCAs while the celery root has higher concentrations of long-chain PFCAs and perfluoroalkyl sulfonates (PFSAs). The tomato plant has three compartments: root, shoot, and fruit. The tomato root has the highest concentration of PFDA and PFOS, the longest chain compounds analyzed. The tomato shoot has the highest concentration of all the other PFAAs except PFPeA. The tomato fruit, as reported in Blaine et al. [5], has the most significant concentrations of the short-chain compounds. Pea roots and shoots exhibit similar results to the celery and tomato in that long-chain compounds are highest in the roots while short-chain compounds are highest in the shoots. Pea fruit is similar to tomato fruit in that it accumulates primarily the short-chain compounds.

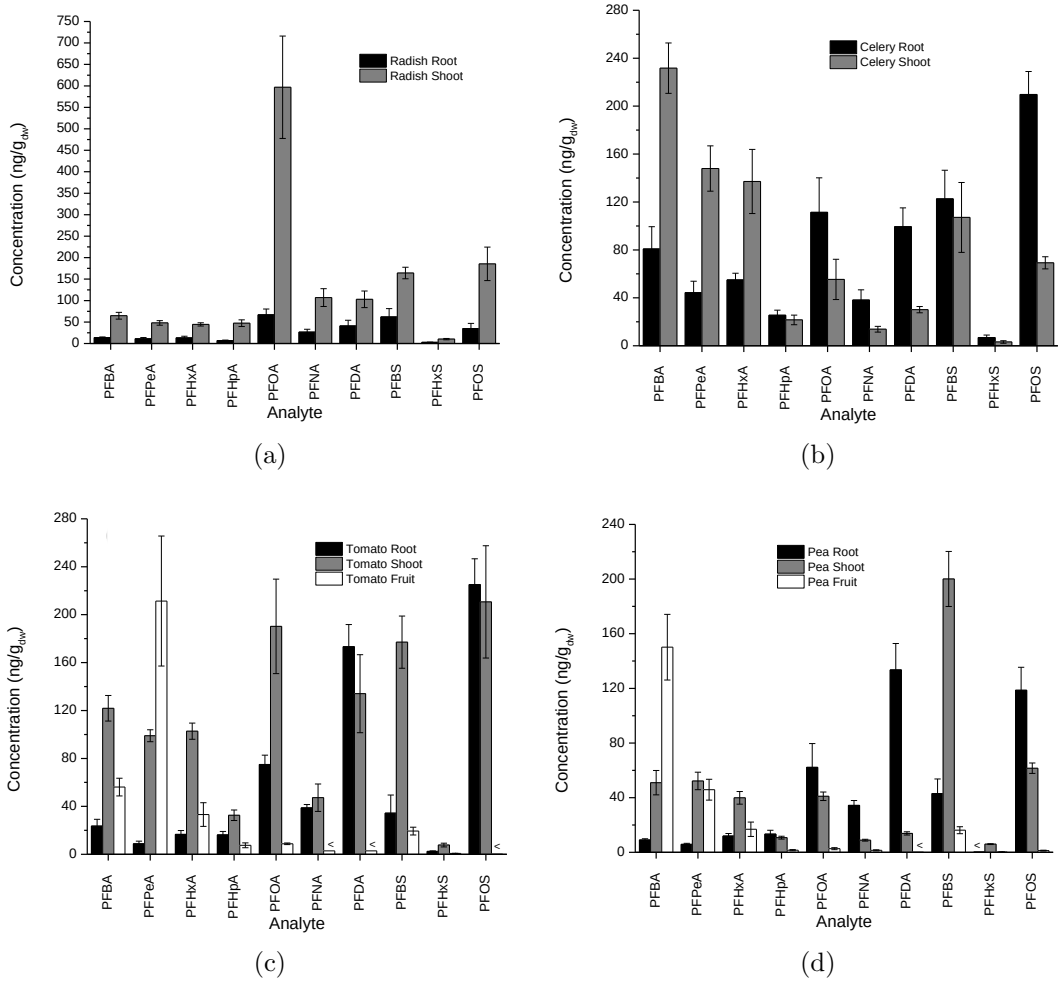
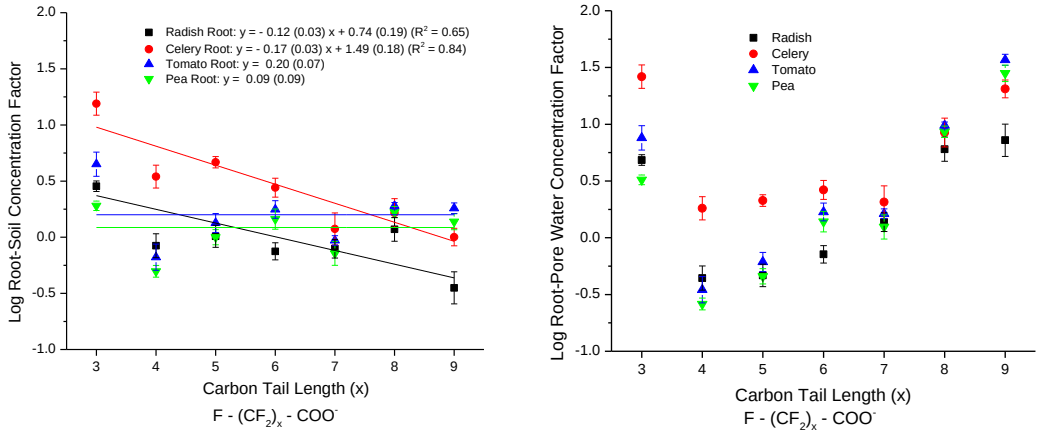


Figure 3.2: Concentrations of PFAAs in greenhouse radish (a), celery (b), tomato (c), and pea (d) grown in industrially impacted soil. Values for tomato fruit are from a previous study [5]. Bars represent means and standard errors of five determinations. Values less than the LOQ are denoted by <; LOQs for respective matrix and analyte are listed in Table B.3 and Table B.4.

3.3.3 Bioaccumulation

PFCAs. Root to soil concentration factors plotted versus carbon chain length of PFCAs for the four crops grown in the industrially impacted soil are shown in Figure 3.3; linear trend lines with equations and associated errors are shown. In general, the RCF values of celery are greater than the other three crops, indicating more overall accumulation in celery root. This could be due to the greater surface area of celery roots or could be correlated to the total water transpired during the duration of the crop. Tomato and pea have very similar RCF values, most likely resulting from similar root physiology and crop duration times. The slopes of the trend lines for tomato and pea root are not statistically different from zero ($\alpha = 0.05$), indicating no preferential accumulation of short- or long-chain PFCAs in the root tissues as compared to soil. Both of these crops have thicker tap root systems which may allow larger contaminants to cross the epidermis into the apoplast and yet be retained in the root tissue [13]. The trend line for radish shows a slope of -0.12, indicating a slight preference for uptake of the short-chain compounds. Taking into consideration that the edible portion of the radish root exhibits characteristics of both root and stem as a hypocotyl, this difference could reflect the prior impeded movement of long-chain compounds by the Casparian strip during translocation from the fine roots to the bulb. In this way, the radish data resemble more of a shoot trend than the expected root trend. However, other entryways into the hypocotyl may be possible (aquaporins or direct diffusion through hypocotyl endodermis) thus allowing more long-chain compounds than seen in the other crops [19]. The trend line for celery has a more obvious downward slope of -0.17 showing preferential entry for short-chain PFCAs. This could be due to the fact that celery has a very finely branched root system that is more likely to filter out larger contaminants by the Casparian strip at an early entry point. RCF_{pw} values were also calculated for PFCA accumulation in the four crops (Figure 3.3). When plotted versus chain length, all four crops exhibit a U-shape that is consistent with the trend reported by Felizeter et al. [7] for hydroponically grown lettuce and by Krippner et al. [20] for maize. PFBA as well as the long-chain PFCAs have higher

sorption tendencies to organic carbon [21], thus reducing their concentrations in the pore water and driving up the RCF_{pw}.



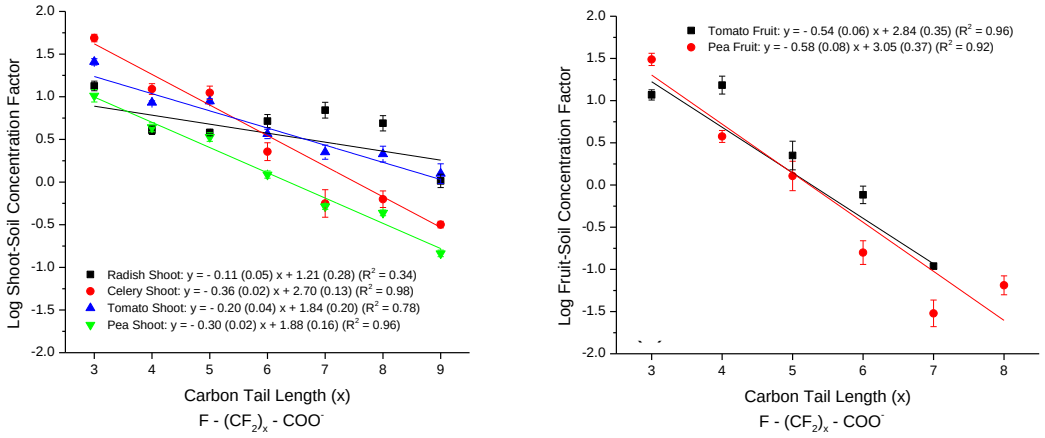
(a) (b)

Figure 3.3: Correlations between log RCF for PFCAs based on soil (a) and calculated pore water (b) concentrations and carbon tail length in greenhouse radish, celery, tomato, and pea grown in industrially impacted soil. Means and standard errors are shown (n = 3 to 5). Linear regressions with slopes (if significantly different than zero at $\alpha = 0.05$), intercepts, and associated error values are shown.

Shoot to soil concentration factors plotted versus PFCA chain length are shown in Figure 3.4 with corresponding linear trend lines, equations and associated errors. Comparing among crops, celery shoots have higher accumulation of the short-chain PFCAs, likely due to exclusion of long chain PFCAs by the roots, while radish and tomato shoots have higher accumulation of the long-chain PFCAs. Pea shoots have the least amount of accumulation; perhaps the woody, dry characteristics of its stem and its minimal leaves reduce the available accumulation area in the shoots. Celery, tomato and pea SCFs show a decrease of 0.36, 0.20, and 0.30 log units, respectively, per CF_2 moiety. As these SCFs encompass the movement of PFCAs traveling from soil through the root to the shoots, the slightly larger value for celery (0.36) may reflect the fact that the preferential accumulation of short-chain length compounds in the celery root is compounded by additional increased selectivity from the root to shoots. When shoot-to-root (inter-compartmental) factors are compared (Figure B.3), rela-

tive PFCA accumulation from roots to shoots are similar for celery and tomato; pea shows the greatest log decrease per CF_2 moiety. Overall, the preferential exclusion of long-chain PFCAs seen in celery, tomato, and pea shoots is consistent with the trend found in previous studies for lettuce shoots (decrease of 0.3 log units) [5] and for maize shoots [20]. Relative PFCA accumulation in radish shoots, however, is an exception: the trend of log SCF vs. chain length is significantly flatter and the slope is statistically equivalent ($\alpha = 0.05$) to the log RCF trend line (Figure 3.4), resulting in no preferential accumulation of long- or short-chain PFCAs in the radish shoot as compared to the root (Figure B.3). Considering that once PFCAs are in the radish root (hypocotyl), there is no Casparian strip to prevent upward translocation to the shoot; this lack of a trend is consistent with the Casparian strip serving as an important barrier to the inter-plant movement of long- chain PFCAs. Although, trend-wise, the radish root and shoot accumulation patterns correlate, more overall accumulation is seen in the shoot since after entry into the edible bulb, contaminants are subsequently transported upward with the flow of xylem and then accumulate in the leaves. There is potential for some of the smaller PFCAs to return to the bulb via the phloem as the plant stores nutrients for the winter in the bulb; however, this translocation is likely insignificant as radish is harvested before dormancy. In addition, small increases of PFAA concentration in the bulb may be obscured by growth dilution.

Fruit to soil concentration factor values for tomato and pea fruits for each PFCA are generally similar (i.e., on the same order of magnitude); however, variations in the values still exist due to the myriad of differences in the physiology of the roots and shoots encountered during translocation. In both tomato and pea plants, contaminants encounter additional membrane barriers (e.g., the cambium) in order to be loaded into the phloem and transported to their final destination (i.e., the fruit compartment). Additional chain length exclusion is evidenced by the decrease of 0.2 to 0.3 log units per carbon chain length for fruit to shoot concentration factors (Figure B.3) resulting in cumulative decreases of 0.54 and 0.58 log units per carbon chain length for fruit to soil accumulation factors (Figure 3.4).



(a) (b)

Figure 3.4: Correlations for PFCAs between log SCF (a) and log FCF (b) and carbon tail length in greenhouse radish, celery, tomato, and pea grown in industrially impacted soil. Means and standard errors are shown (n = 3 to 5). Linear regressions with slopes, intercepts, and associated error values are shown.

PFSAs. Bioaccumulation factors for PFSAs were also calculated (Table B.5); however, as only three analytes were studied, chain length trends were not calculated with linear regressions. Differences between PFCAs and PFSAs seem to magnify from the roots upward. In the roots, all analyte RCFs are below 5, with the exception of PFBA. Values for SCFs for PFSAs are all below 8, compared to the SCFs for the short-chain PFCAs which reach up to 50. In tomato and pea, values of FCFs for PFSAs are all below 1, while values for short-chain PFCAs are primarily greater than 1. A more direct comparison can be made by comparing similar chain length analytes (e.g., PFPeA to PFBS or PFNA to PFOS). PFPeA has significantly higher values than PFBS for the celery and tomato SCFs as well as for both tomato and pea FCFs; PFNA compares fairly well to PFOS with the only significant difference being slightly higher values of SCFs in celery, tomato, and pea for PFOS. As the core structure of PFCAs and PFSAs are almost identical, the larger size of the sulfonate head group may be a contributing factor to the accumulation differences in the shoots and fruits for short-chain analytes. For larger analytes that are already excluded based on size, the larger head group may not matter as much. Other differences in accumulation patterns

may be due to differing uptake mechanisms between PFCAs and PFSAs [8].

3.3.4 Conceptual Model and Implications

Figure 3.1 shows a conceptual model of PFAA accumulation in tomato, a typical three compartment crop. The primary translocation pathway for PFAAs is illustrated via an enlarged root cross section and an outline showing movement of PFAAs from the soil all the way to the phloem. In addition, approximate bioaccumulation factors are shown for a tomato plant indicating increasing discrepancy in PFAA accumulation per CF_2 moiety with acropetal movement.

In general, chain length dependent accumulation is seen as PFCAs translocate upward from the roots. However, each crop is anatomically different, presenting unique biological barriers in the translocation process; however, some common barriers do exist, namely the Casparian strip and in general, the permeation of membranes. To sufficiently model plant uptake of PFAAs, these various crop-specific factors as well as contaminant-specific factors must be considered. Plant factors examined in this paper were root structure and number of compartments, while the contaminant-specific factors examined included chain length and head group. Without plant-specific data, the best prediction that can be made consists of a generalization about plant compartment accumulation. In general, the data presented here suggest fruit crops accumulate fewer long-chain PFCAs than do shoot or root crops. For example, one would expect that 5 g of peas or tomatoes would contain roughly 5-25 times less PFOA than 5 g of celery or radish grown in the same soil. With a good understanding of plant physiology, it may be possible to extrapolate these generalizations to other crops; however, caution is warranted since visually similar crops can have anatomical or physiological differences that can significantly alter uptake potential. In terms of analytes, there is a much larger discrepancy; one could expect that shoot and fruit crops may have 1-3 orders of magnitude more PFBA than PFOA if these two analytes are present in equal concentrations in the soil. With industry trends shifting toward the use of short-chain PFAAs, it is important to recognize this increased potential of PFAA entry into the terrestrial

food chain via plants.

With respect to overall exposure, it is unlikely that edible crops grown in soils conventionally amended for nutrients with biosolids (that are not impacted by PFAA industries) are a primary source of long-chain PFAA exposure to humans; this has also been suggested from recent food basket studies [22]. However, in the absence of comprehensive toxicological data on short-chain PFAAs, precaution may be warranted for production of fruit or shoot crops grown in PFAA contaminated soils. More work is needed to discern all applicable factors needed to comprehensively model PFAA uptake in plants.

3.4 Acknowledgments

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CHAPTER 4

PERFLUOROALKYL ACID UPTAKE IN LETTUCE (*LACTUCA SATIVA*) AND STRAWBERRY (*FRAGARIA ANANASSA*) IRRIGATED WITH RECLAIMED WATER

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Abstract

The use of reclaimed water for irrigation of food crops presents an exposure route for persistent organic contaminants like perfluoroalkyl acids (PFAAs) to enter the human food chain. This greenhouse study used reclaimed water augmented with varying doses (0.2-40 µg/L) of PFAAs to investigate potential uptake and dose-dependency trends in lettuce (*Lactuca sativa*) and strawberry (*Fragaria ananassa*). Concentrations of PFAAs in lettuce leaves and strawberry fruit were measured for each incremental dose. Perfluorobutanoate (PFBA) and perfluoropentanoate (PFPeA), both short-chain PFAAs, showed the overall highest accumulation of any PFAAs in the edible parts of both lettuce and strawberry. Concentrations increased linearly with increasing dose for almost all PFAAs, with the exception of the long-chain PFAAs in strawberry fruit. Chain length-dependency trends were evident in both lettuce shoot and strawberry fruit, with decreasing concentrations occurring with increasing chain length. PFAA concentrations in strawberry root and shoot were also measured at selected doses (0.4, 4, and 40 µg/L). In strawberry plants, the distribution of PFAAs was mapped to illustrate that short-chain perfluorocarboxylates are the dominant fraction in the fruit and shoot compartments, while a more even distribution of all PFAAs appears in the

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root compartment. Lettuce grown in soils with varying organic carbon content (0.4%, 2%, 6%) was used to assess the impact of organic carbon sorption on PFAA bioaccumulation. The lettuce grown in soil with the highest organic carbon content had the lowest bioaccumulation of PFAAs. Bioaccumulation factors for lettuce were correlated to carbon tail length of PFAAs showing approximately a 0.4 to 0.6 log decrease per CF_2 group. This study confirms that PFAAs are able to enter and bioaccumulate in food crops via reclaimed water. PFAA bioaccumulation potential is highly dependent on analyte, concentration in the reclaimed water, and organic carbon content of the soil.

4.1 Introduction

Perfluoroalkyl acids (PFAAs) are ubiquitous synthetic chemicals with widespread uses in both consumer and industrial settings. They have drawn much attention in regard to their persistent, accumulative and toxic nature [1, 2]. Due to the extensive use of PFAAs in consumer products, municipal wastewaters have become a collection vehicle for these compounds. In addition, the prominence of PFAAs in manufacturing processes can lead to high levels in industrial wastewaters [3]. Most conventional wastewater treatment plants (WWTPs), however, are ineffective at removing PFAAs [3, 4]. As a result, WWTPs may represent a significant source for PFAA releases into the environment [5]. Unlike many organic contaminants, the dual hydrophobic/lipophobic nature of PFAAs enable these compounds to reside in significant quantities in both the aqueous and sludge effluent streams of WWTPs [4, 6].

The aqueous effluent stream of a WWTP is, in general, returned to the surrounding aquatic environment; however, growing water scarcity is driving alternative uses of treated wastewater. In particular, interest in the use of recycled or reclaimed water, which typically consists of municipal wastewater treated to remove pathogens, organic matter, and nutrients for agricultural purposes is growing and is likely to continue in the future [7]. Reclaimed water has been safely used for many years in the U.S. for the irrigation of non-food crops [8] and on a more limited scale, food crops eaten raw (e.g., in the Salinas Valley, CA). Recently,

however, concerns have been raised regarding the presence of chemicals of emerging concern in reclaimed water Fatta-Kassinos et al. [9].

Although the U.S. Environmental Protection Agency has published guidelines for water reuse, no federal regulations govern water reclamation and reuse in the U.S. and thus regulations or guidelines have been developed at the state level [8]. This non-unified approach has resulted in differing standards among states that have developed reuse criteria. Existing water reuse regulations for food crop irrigation in each state vary according to crop type and irrigation method, but are principally directed at health protection from microbial pathogens and do not typically include requirements addressing organic contaminants [10].

The potential risks associated with bioaccumulation of organic contaminants in edible crops are greatest for those eaten raw since processing and cooking could likely lead to significant chemical transformation and/or volatilization. Unfortunately, while data on the occurrence of many contaminants in reclaimed water are plentiful, limited data exist on the potential for uptake of PFAAs from reclaimed water into edible plants. To date, human health risk assessments based on plant uptake models are limited to crop-specific data and very few organic chemicals of concern [11, 12].

A few studies have demonstrated the potential for crop uptake of pharmaceuticals applied via real or simulated wastewater [13, 14]; however, the behavior of the contaminants studied is very different than that of PFAAs. Felizeter et al. [15] reported uptake of PFAAs in lettuce plants via hydroponic solution, with higher concentrations of the short-chain PFAAs accumulating in the leaves. However, fundamental differences between hydroponic and solid media experiments as well as differences of water quality between nutrient solutions and actual reclaimed water prevent direct applicability to crops irrigated with reclaimed water. Blaine et al. [16] examined lettuce uptake of PFAAs from biosolids-amended soils and found preferential short-chain accumulation in the lettuce leaves as well, although again, bioavailability of PFAAs for uptake may vary considerably depending on the uptake matrix.

This study was conducted to examine the uptake of PFAAs in lettuce (*Lactuca sativa* 'Multy') and strawberry (*Fragaria ananassa* 'Albion') via reclaimed water under conditions representative of current agricultural practices. Lettuce and strawberry crops were chosen to represent typical food crops grown in the U.S. using reclaimed water. Experiments were carried out using authentic reclaimed water augmented with varying doses of emerging contaminants. In addition, lettuce grown in soils with varying organic carbon (OC) content was used to assess the impact of OC sorption on PFAA bioaccumulation. To our knowledge this is the first study to examine PFAA uptake in lettuce and strawberry using reclaimed water.

4.2 Materials and Methods

The following is a summary of the materials and methods used in this study.

4.2.1 Chemicals

All calibration standards and stable isotopes were acquired from Wellington Laboratories (Guelph, ON, Canada) and prepared using established protocols [16]. Specific PFAAs used in this study include perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS; Table C.1). Spiking solutions for dosing experiments were prepared from individual standards purchased from Sigma Aldrich (St. Louis, MO). High purity Chromasolv dichloromethane, HPLC-grade methanol, and all other reagent grade solvents were acquired through Sigma Aldrich. Water for extractions was obtained from a MilliQ system (Millipore, Billerica, MA) whereas HPLC-grade water was utilized for liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. Extraction clean-up was facilitated with Chromabond diamino from Macherey-Nagel Inc. (Bethlehem, PA) and Supelclean ENVI-Carb from Sigma-Aldrich.

4.2.2 Greenhouse Study

Plant uptake experiments were conducted in a climate controlled greenhouse with two food crops, leaf lettuce (*L. sativa* 'Multy') and strawberry (*F. ananassa* 'Albion'). These selected cultivars are similar to cultivars currently grown in the U.S. using reclaimed water. Five replicate plants were grown for each set of experimental conditions. Pots (15 cm diameter) were randomly arranged to account for any spatial variations in light and temperature within the greenhouse. Day temperatures ranged from $18°C$ to $21°C$ and night temperatures ranged from $10°C$ to $13°C$. Full spectrum metal halide and high pressure sodium supplemental lighting was also supplied to achieve 16 hours of daylight to mimic field conditions. Additional information regarding plant propagation and cultivation can be found in Appendix C.

Reclaimed water was supplied by the Mines Park pilot-scale Sequencing Batch/Membrane Bioreactor. This test site at Colorado School of Mines treats raw sewage from a student-apartment complex (a graduate housing community of ∼400 individuals); a full description of the site can be found elsewhere [17]. The effluent from Mines Park provided a steady and realistic source of reclaimed water for the experiments. Dose-dependent accumulation was examined by fortifying the reclaimed water with eight differing levels of PFAAs (0.2, 0.4, 1, 2, 4, 10, 20, 40 µg/L) in addition to using ambient reclaimed water and tap water (control). The dosing scheme was constructed to give a range of values starting with ambient concentrations found in reclaimed water (∼0.02 µg/L), containing typical WWTP effluent concentrations (0.2 - 4 µg/L) [5], and reaching up to concentrations that could be representative of contaminated ground water (10 - 40 µg/L) [18]. To assess the accuracy of the dosing solutions, aliquots from each dosing solution were analyzed. Measured aqueous concentrations of the eight dosing solutions varied from the actual applied dose with average recoveries for each PFAA ranging from 99% to 23%. The lowest recoveries were for PFNA and PFOS, the strongest sorbing analytes, most likely due to losses on the glass container. The linearity of the doses remained fairly constant for each analyte; each successive dose

was 1.5 to 3 times the concentration of the previous dose. Both the tap water and ambient reclaimed water had background concentrations (< 25 ng/L) of PFHxA, PFOA, and PFOS. Measured concentrations of doses (Table C.2) were used in all calculations. Plants received doses via hand watering three times a week, 100 mL of solution per lettuce plant and 200 mL of solution per strawberry plant. Additional information on the reclaimed water quality is found in Table C.3.

Because soil organic matter can significantly impact the bioavailability of PFAAs [19], a sandy soil (3:1 sand:topsoil by mass) with only 0.4% OC was used to represent a "worst-case" scenario in terms of bioavailability. Plant essential nutrients were supplied by mixing slow release Osmocote (N-P-K: 19-6-12) at a rate of approximately 5 g/plant. To more specifically test the impacts of OC on PFAA uptake, lettuce was grown in two additional soils with varying OC content (2%, 6%) at a single PFAA dose (10 µg/L). Details on the soils utilized are provided in Appendix C.

Edible portions of lettuce and strawberry plants were harvested at maturity. In addition, after sufficient strawberry fruit biomass was harvested, whole strawberry plants were collected and separated into root and shoot portions. All plant material was frozen ($-20°C$) in PFAA-free plastic bags prior to analysis.

4.2.3 Sample Extraction and Data Analysis

Homogenized plant samples (0.5 - 2 g) were prepared and extracted using the protocol from previous work [16]. Lettuce shoots from all experimental replicate pots (n = 3 to 5) were extracted independently and concentrations were averaged. Ripe strawberry fruit from the replicate plants in each dose were composited to achieve adequate biomass for extraction resulting in composited averages of analytical triplicate measurements. Strawberry shoot and root experimental replicates for three doses (0.4, 4, 40 µg/L) were extracted separately to enable an estimation of inter-pot variability; concentrations in replicate plants (n = 3 to 5) were averaged to obtain sample values.

Soil and aqueous sample analyses were completed per established methods [20]. All results for plants and soils are presented in terms of a dry weight basis. Samples were analyzed with isotope dilution using LC-MS/MS under conditions outlined in previous work [16]. Briefly, chromatography was monitored using a Shimadzu LC-20AD unit (Kyoto, Japan) by injecting samples onto a Gemini C18 Column with a 3-micron particle size (Phenomenex, Torrance, CA). Additionally, two transitions for each PFAA were observed using an MDS Sciex Applied Biosystems API 3200 (MDS Sciex, Ontario) with negative electrospray ionization operating in scheduled multiple reaction mode. Quantitation of LC-MS/MS data was accomplished using Analyst software.

4.2.4 Quality Assurance and Control

All of the strawberry fruit, as well as approximately twenty percent of all other samples were extracted and analyzed in triplicate. The relative standard deviation for all analytical replicates averaged less than 25%. One laboratory blank with surrogate standard and one double blank without surrogate standard were prepared for each batch of samples. Limits of quantitation (LOQ) for plant material ranged from 0.07 to 1.43 ng/g; LOQs were determined by the lowest calibration standard calculated to be within 30% of its actual value and were analyte, matrix, and run-dependent. If a minimum of three pot replicates were above the LOQ, an average value was calculated for that treatment, otherwise the value was reported as $<$ LOQ. To account for any losses during the extraction process, each analyte contained an internal surrogate. In line with previous work analyzing PFAAs in plant tissues (Chapter 3), surrogate recovery for the samples averaged 43% for root tissues, 33% for shoot tissues, and 45% for fruit tissues across all analytes. Statistical analysis including all calculations of regression equations was completed using OriginPro 9.0.

4.2.5 Bioaccumulation Metrics

Bioaccumulation factors (BAFs) for lettuce leaves (at the 10 µg/L applied dose) and fruit to soil concentration factors (FCFs; Chapter 3) for strawberry fruit (at the 0.4, 10, and 40

µg/L applied doses) were calculated. To enable comparisons to previous studies examining PFAA bioaccumulation from soils, the aqueous dose (C_w) was first converted to an estimated soil concentration (C_s) using the respective solid-water partitioning coefficient (K_d) for each soil and analyte (4.1).

$$C_s\,(ng/kg) = C_w\,(ng/L) \times K_d\,(L/kg) \tag{4.1}$$

More information concerning the determination of K_d values can be found in Appendix C (Table C.4 and Table C.5). Concentration factors were then calculated as in previous work [16] by dividing the concentration of chemical in the respective plant tissue on a dry weight basis by the concentration of chemical in the soil. In addition, inter-compartmental concentration factors (fruit to shoot and shoot to root) were calculated as in previous work (3) for strawberry plants grown at the 0.4, 4, and 40 µg/L doses.

4.3 Results and Discussion

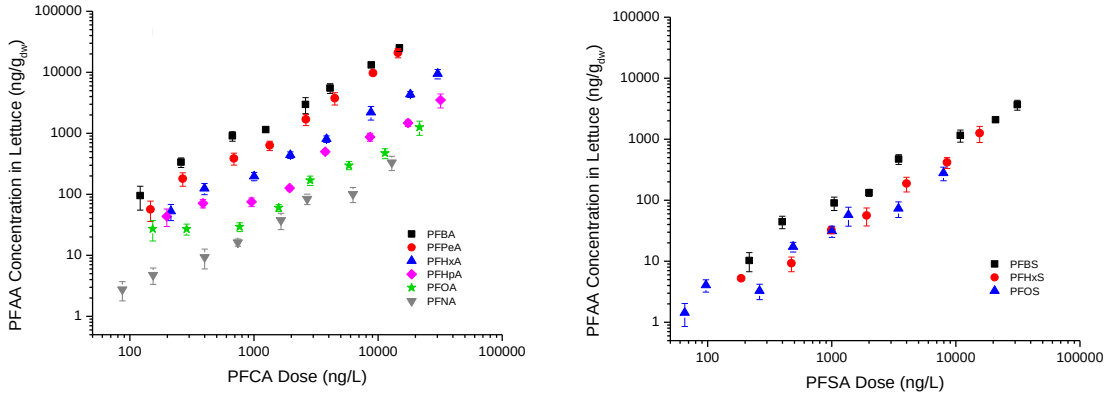
The results of this study are summarized and discussed below.

4.3.1 Dose-Dependency

All PFAA concentrations in lettuce leaves showed a linear dose-response relationship (Figure 4.1) indicating passive transport through the plant [21]. The slopes of the linear regressions for each analyte (Figure C.1) imply preferential uptake by the short-chain PFAAs. In general, PFCAs accumulated in much greater quantities than the perfluorosulfonates (PFSAs) with concentrations in lettuce leaves receiving the highest dose reaching 25 µg/g for PFBA. On the low end, some PFCA accumulation ($<$ 54 ng/g) was observed in the lettuce treated with control tap water and ambient reclaimed water, presumably due to the low background levels of PFAAs present despite the absence of dosing (Table C.6).

Short-chain PFCA concentrations in strawberry fruit exhibited linear behavior in response to dosing with concentrations in fruit receiving the highest dose reaching up to more than 10 µg/g for PFBA and PFPeA (Table C.7). However, long-chain PFCAs displayed

non-linear responses as evidenced by their flatter slopes in log-log space (Figure 4.2). When concentration versus dose was plotted in linear space, the slopes for PFOA and PFNA were not statistically different than zero ($\alpha = 0.05$; Figure C.2). This apparent constant response is suggestive of the saturation of sorption sites within the plant [21, 22]. Although the dose-dependency data collected for the long-chain PFCAs exhibit constant relationships with non-zero y-intercepts, this range of aqueous doses most likely represents the horizontal saturation asymptotes of a Langmuir, or limited sorption site, model [23]; additional dose-response data at lower aqueous concentrations would be necessary to show the complete curve. Alternatively, it is also possible that other specific transport mechanisms exist for long-chain PFAAs as reported by Wen et al. [24]; these mechanisms may contribute to the inhibition of accumulation of long-chain PFAAs. Accumulation of the PFSAs was minimal (< 56 ng/g) compared to the PFCAs (Table C.7). The inhibition of PFSA accumulation in the fruit compartment is consistent with previous findings in tomato and pea fruit (3).



(a) (b)

Figure 4.1: Concentrations of PFCAs (a) and PFSAs (b) in lettuce leaves versus measured aqueous dose of PFAAs. Means and standard errors (n = 5) are shown.

4.3.2 Chain Length Trends

As evidenced in Figure 4.1 and Figure 4.2, concentrations in both lettuce leaves and strawberry fruit decrease as PFAA chain length increases. To further illustrate this trend,

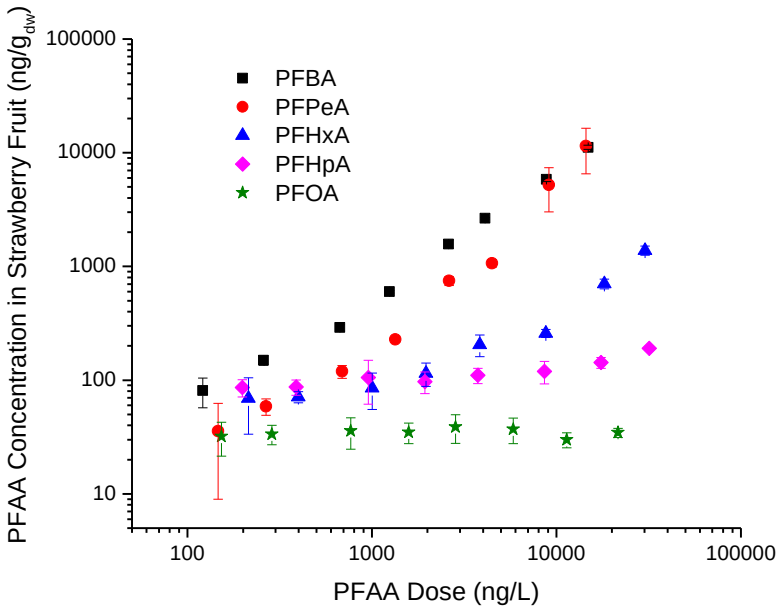


Figure 4.2: Concentrations of PFCAs in strawberry fruit versus measured aqueous dose of PFAAs. Means of composited berries are shown with analytical standard deviation (n = 3).

PFAA concentrations for a single dose (10 µg/L) in both lettuce and strawberry are provided in Figure 4.3. In lettuce leaves, PFAA concentrations span more than an order of magnitude from PFBA to PFNA, a gain of 5 carbons, and also more than an order of magnitude from PFBS to PFOS, a gain of 4 carbons. In strawberry fruit, PFAA concentrations span more than two orders of magnitude from PFBA to PFNA, further evidencing the disparity of accumulation potential between short- and long-chain PFCAs. This preferential accumulation of short chain carboxylates in plants is consistent with previous findings (Chapter 3).

4.3.3 Strawberry Plant Compartments

Non-edible portions of strawberry plants were analyzed to assess inter-pot variability (22%) and help elucidate bioaccumulation trends within the plant. At the highest dose applied (40 µg/L), strawberry root concentrations were greatest for PFHxA (5400 ng/g; Table C.8), strawberry shoot concentrations were greatest for PFBA (3900 ng/g; Table C.8), and strawberry fruit concentrations were greatest for PFPeA (11,500 ng/g; Table C.7).

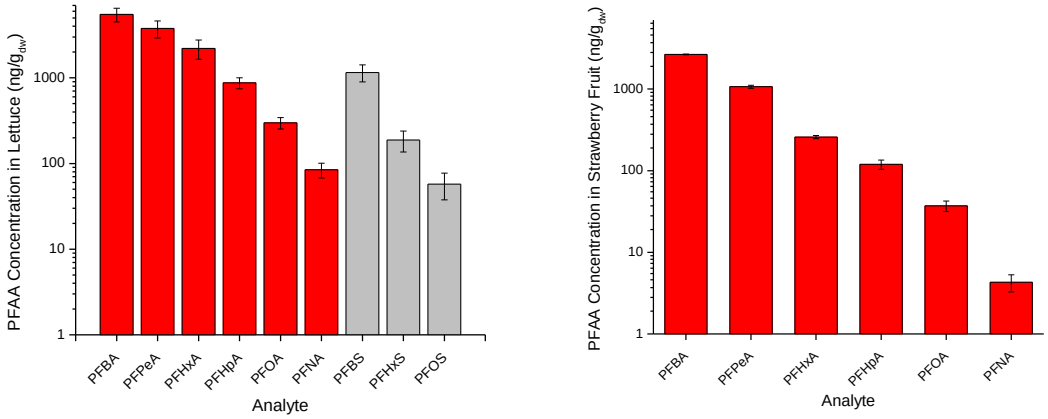


Figure 4.3: Concentrations of PFAAs in lettuce leaves (a) and strawberry fruit (b) for the applied PFAA dose of 10 µg/L. Mean and standard error for lettuce (n = 5) are shown. Means of composited berries are shown with analytical standard deviation (n = 3).

Moreover, the concentrations of both PFBA and PFPeA in the fruit were more than twice that of any analyte that accumulated in the root or shoot compartments. The distribution of PFAAs in each plant compartment (root, shoot, and fruit) for a representative dose (4 µg/L) are shown in Figure 4.4. Of the three compartments in the strawberry plant, the root compartment had the greatest accumulation of PFAAs, and the distribution of PFAAs in the root compartment was fairly evenly spread, confirming the lack of selectivity of analytes in the root compartment described in previous findings (Chapter 3). The shoot compartment had the lowest total accumulation of PFAAs of the three compartments, and its accumulation was dominated by the short-chain analytes, PFBA, PFBS, and PFPeA. The fruit compartment had almost as much total accumulation as the root compartment; however, the distribution of PFAAs was highly skewed toward the short-chain PFCAs with almost no PFSA presence at all. Looking at the data in a different way, the mass distribution between plant compartments for each analyte can be estimated by multiplying typical dry weights for each compartment (1.3 g for root, 4 g for shoot, 3 g for fruit) by the concentration of each analyte in the respective compartment. The dominant fractions of PFBA and PFPeA resided in the fruit compartment while the dominant fractions of the long-chain PFCAs as

well as all of the PFSAs accumulated in the root compartment (Figure C.3).

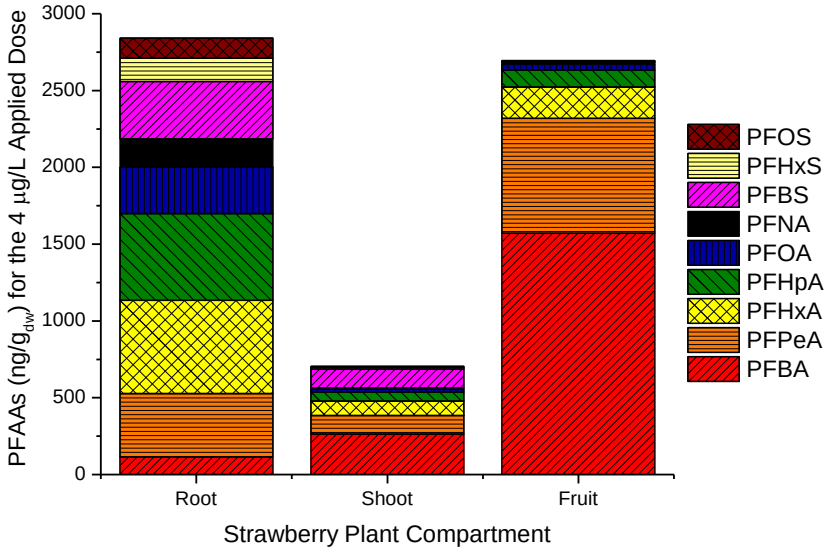


Figure 4.4: Distribution of PFAAs in strawberry root, shoot, and fruit compartments for the applied PFAA dose of 4 µg/L.

FCF values calculated on a soil basis for strawberry ranged from the 200's for PFBA and PFPeA to less than 0.2 for PFNA (Table C.9). When plotted versus carbon chain length, FCFs, calculated for the 10 µg/L dose, decrease approximately 0.6 log units per CF_2 group (Figure 4.5). These results agree well with the findings of Blaine et al. (3) for tomato and pea fruit, as shown alongside the strawberry fruit data in Figure 4.5. When FCF is plotted versus chain length for a low aqueous dose (0.4 µg/L), the trend line has a flatter slope indicating less of a chain length-dependent response (Figure C.4). This shift in trend is presumably due to the non-linear dose-response of the long-chain PFCAs that produces elevated accumulation at low doses. At the highest dose (40 µg/L), the FCF versus chain length trend is slightly steeper than in Figure 4.5, again most likely due to site-limited sorption or alternative transport mechanisms of the long-chain PFCAs that produce lowered accumulation at high aqueous doses (Figure C.4). Inter-compartmental factors for strawberry plotted versus PFAA chain length (Figure C.5) showed a decrease of 0.2 log units

from fruit to shoot per CF_2 group and 0.3 log units from shoot to root per CF_2 group. These factors also correspond well with the inter-compartmental factors calculated for tomato and pea fruits from previous work (Figure C.5; Chapter 3).

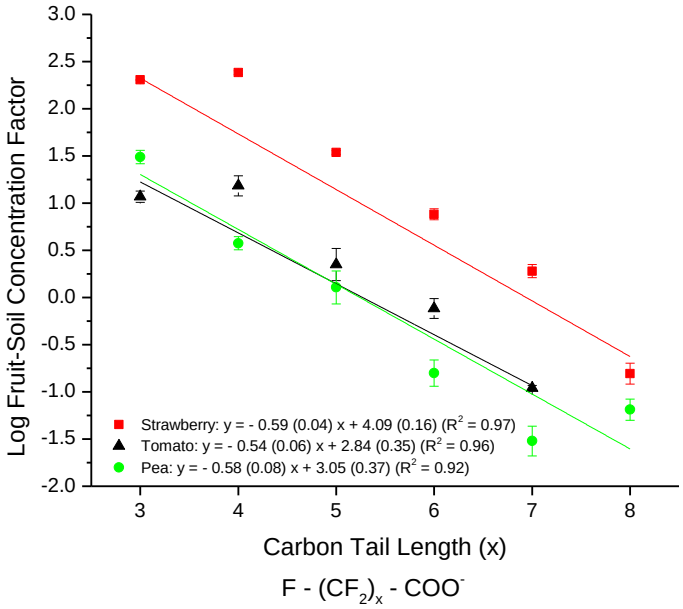


Figure 4.5: Correlations for PFCAs between log fruit-soil concentration factors and carbon tail length in strawberry, tomato, and pea. Strawberry values from this study were from the 10 µg/L applied dose; tomato and pea values were from a previous study (Chapter 3). Means and standard errors are shown (n = 3 to 5). Linear regressions with slopes, intercepts, and associated error values are shown.

4.3.4 Lettuce-Soil Organic Carbon Study

To assess the impact of sorption on plant uptake of PFAAs, lettuce was grown in soils with differing OC contents and compared to the lettuce grown in the sand-soil mix. Lettuce grown in the two additional soil treatments (2% and 6% OC content) at the 10 µg/L dose had similar values of PFAA concentrations to lettuce grown in the sand-soil mix (0.4% OC content). Lettuce in all three soils had the highest concentrations of PFBA and PFPeA. Concentrations ranged from approximately 15 µg/g of PFBA down to 47 ng/g of PFNA in the 2% OC soil, and from almost 5 µg/g of PFBA down to 21 ng/g of PFNA in the 6% OC soil (Table C.10).

Lettuce BAFs for all three soil treatments at the 10 µg/L dose varied widely, spanning more than two orders of magnitude within each treatment. The lettuce grown in the 6% OC soil had the smallest BAF values for all PFAAs, presumably due to sorption in the media (Table C.11). All BAFs, with the exception of PFNA and PFOS in the 6% OC soil, were greater than 1, indicating the accumulation of PFAAs in the lettuce. PFNA and PFOS have the highest K_d values of the PFAAs in this study (Table C.4), so it follows well that they would exhibit minimal bioavailability in the highest OC content soil.

A linear relationship between log BAF values and carbon chain length is shown in Figure 4.6 for lettuce grown in each soil treatment. For each increase in carbon tail length, the BAF decreases approximately 0.4-0.6 log units. In addition, lettuce grown in two different PFAA contaminated biosolids-amended soils (2.2% and 6.3% OC) from a previous study [16] are plotted alongside the values from the present study for comparison. The slopes of all lines are fairly similar, with the slopes of biosolids-grown lettuce being slightly flatter than aqueous-dosed lettuce. This difference could indicate that the mobility of the PFAAs supplied by aqueous dosing allows immediate plant uptake prior to significant sorption in the soil. Figure 4.6 assumes that the dosing solution is representative of pore water, and that the water-soil system has reached equilibrium prior to plant uptake. In reality, however, equilibrium may not have been reached, thus increasing the bioavailability of the PFAAs applied in the reclaimed water. The aqueous-dosed lettuce slopes represented in Figure 4.6 may therefore be artificially steep compared to equilibrium conditions; however, they may be more representative of field conditions. Regardless, greater bioaccumulation overall is seen in the aqueous dosed plants, suggesting that the mobility and bioavailability of PFAAs is greater when delivered via irrigation water as compared to biosolids-amended soil.

4.3.5 Implications

The results of this study are novel and important as it is the first of its kind to examine PFAA accumulation in food crops simulating a real-world field scenario by using reclaimed water as the delivery medium. The data presented in this paper show clearly that PFAAs

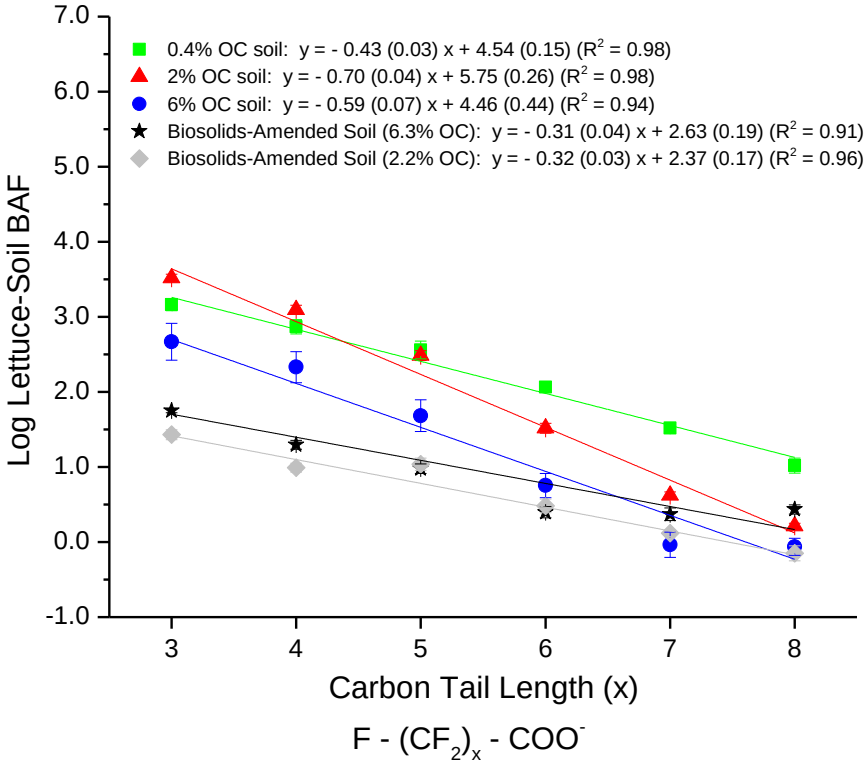


Figure 4.6: Correlations for PFCAs between log BAFs and carbon tail length in lettuce. Log BAFs from lettuce grown in soils with varying OC content (0.4%, 2%, 6%) at the 10 µg/L applied dose are shown alongside values from lettuce grown in biosolids-amended soils in a previous study [16]. Means and standard errors are shown (n = 3 to 5). Linear regressions with slopes, intercepts, and associated error values are shown.

can be taken up and accumulated from reclaimed water into food crops indicating the potential for human exposure if irrigation water contains PFAAs. At typical WWTP effluent concentrations of PFAAs (0.02-4 µg/L) [5], values reached up to 0.2 µg/g for PFOA and 3 µg/g for PFBA in lettuce and up to 0.05 µg/g for PFOA and 2 µg/g for PFBA in strawberry fruit. In addition, at higher aqueous concentrations, more representative of contaminated surface or ground waters (10-40 µg/L) [18], concentrations of PFAAs soared up to 1 µg/g for PFOA and 25 µg/g for PFBA in lettuce and up to and 11 µg/g for PFBA in strawberry fruit. The sub-chronic reference dose for PFOA according to the U.S. EPA is 0.2 µg/kg-day [25]; for an average 70 kg adult the maximum daily allowance of PFOA would then be 14 µg/day. If a person were to consume lettuce irrigated with contaminated water (40 µg/L of PFOA), then presumably, less than half of a small head of lettuce (126 g on a wet weight basis) would be enough to reach the daily maximum for PFOA. Concentrations of short-chain PFAAs in the lettuce would be even higher; however, substantial toxicological data are lacking for short-chain PFAAs.

The dose-dependent response for all PFAAs in lettuce and for short-chain PFAAs in strawberry fruit implies that PFAA accumulation in this range of aqueous doses does not necessarily have a ceiling and thus the uptake potential for crops grown with contaminated water (e.g., surface water or groundwater near industry) is high. Long-chain PFCAs and PFSAs, however, do not readily accumulate in high quantities in strawberry fruit regardless of increased dose, and therefore indicate that fruit may not necessarily be a major route of exposure for long-chain PFAAs. In general, bioaccumulation patterns observed in this study serve to validate previous literature [15, 16] showing greater uptake and accumulation for PFCAs over PFSAs and for short-chain PFAAs over long-chain PFAAs. These plant compartment accumulation trends are important with respect to assessing potential human exposure through consumption. As industry trends shift toward the manufacture of short-chain PFAAs, increased concentrations of shorter PFAAs in WWTP effluents can be expected.

If the current use of reclaimed water for food crops is to be sustained or increase in future years, concerns about the potential contamination of food products must be fully addressed through careful scientific study, evaluation, and communication with the public. Future research is warranted by this potential exposure route to humans. More work is needed to understand PFAA transport mechanisms in additional crops, and in particular, fruit crops which seem to exhibit saturable transport. Investigations of crop uptake using a broader suite of PFAAs present in reclaimed water, including potential precursors of PFAAs, are also needed to expand the body of knowledge on this emerging topic of concern.

4.4 Acknowledgments

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CHAPTER 5
CONCLUSIONS

Understanding the uptake factors and bioaccumulation potential of perfluoroalkyl acids (PFAAs) into food crops is critical given the persistent, bioaccumulative, and toxic nature of this class of chemicals and the increasing movement toward the sustainability practices of land-applying biosolids and irrigating with reclaimed water. The objectives of this research were (1) to determine if edible crops are a significant entryway for PFAAs into the terrestrial food chain via land-applied biosolids or reclaimed water and (2) to characterize trends in bioaccumulation of PFAAs in plants with respect to analyte, PFAA uptake matrix (soil or water) concentration, and crop.

5.1 Summary of Findings

Three studies were done to investigate the objectives above. A summary of findings by each study is presented below.

1. In the first study, uptake of PFAAs by greenhouse lettuce (*Lactuca sativa*) and tomato (*Lycopersicon lycopersicum*) grown in three different soils (an industrially impacted biosolids-amended soil, a municipal biosolids-amended soil, and a control soil) was measured. Bioaccumulation factors (BAFs) were calculated for the edible portions of both crops. Dry weight concentrations in lettuce grown in industrially impacted soil were as high as 266 and 236 ng/g for perfluorobutanoate (PFBA) and perfluoropentanoate (PFPeA), respectively, and reached 56 and 211 ng/g for PFBA and PFPeA in tomato, respectively. BAFs for many PFAAs were greater than one; the highest BAFs were for PFBA (56.8) in lettuce and for PFPeA (17.1) in tomato. Trendwise, the BAFs for PFAAs in greenhouse lettuce decreased approximately 0.3 log units per CF_2 group. A limited-scale field study was conducted to verify greenhouse findings. The greatest

accumulation was again seen for PFBA and PFPeA in both lettuce and tomato; BAFs for PFBA were highest in both crops. However, PFAA levels measured in lettuce and tomato grown in field soil amended with only a single application of biosolids (at an agronomic rate for nitrogen) were predominantly below the limit of quantitation (LOQ). In addition, corn (*Zea mays*) stover, corn grains, and soil were collected from several full-scale biosolids-amended farm fields. At these fields, all PFAAs were below the LOQ in the corn grains and only trace amounts of PFBA and PFPeA were detected in the corn stover. This study confirms that some edible crops can take up PFAAs and that the extent of bioaccumulation from biosolids-amended soils depends strongly on PFAA concentrations, soil properties, the type of crop, and analyte.

2. The second study compared the uptake of PFAAs in greenhouse-grown radish (*Raphanus sativus*), celery (*Apium graveolens* var. *dulce*), tomato (*Lycopersicon lycopersicum*), and sugar snap pea (*Pisum sativum* var. *macrocarpon*) from three different soils (an industrially impacted biosolids-amended soil, a municipal biosolids-amended soil, and a control soil). Concentrations of PFAAs in edible portions grown in industrially impacted soil were highest while PFAA concentrations in edible compartments of crops grown in the municipal and control soils were minimal. BAFs were calculated for the root, shoot, and fruit compartments (as applicable) of all crops grown in the industrially impacted soil. BAFs were highest for PFBA in the shoot compartment for all crops as well as in the fruit compartment for pea. Root-soil concentration factors (RCFs) for tomato and pea were independent of PFAA chain length, while radish and celery RCFs showed a slight decrease with increasing chain length. Shoot-soil concentration factors (SCFs) for all crops showed a decrease with increasing chain length (0.11 to 0.36 log decrease per CF_2 group). The biggest decrease (0.54 to 0.58 log decrease per CF_2 group) was seen in fruit-soil concentration factors (FCFs). In general, fruit crops were found to accumulate fewer long-chain PFAAs than shoot or root crops, most likely because of additional encounters with biological barriers as PFAAs are transported throughout

the plant (roots to shoots to fruits). Finally, a preliminary conceptual framework was developed for PFAA accumulation in edible crops.

3. The third study used reclaimed water fortified with varying doses (0.2-40 µg/L) of PFAAs to investigate the potential uptake and dose-dependency trends in lettuce (*Lactuca sativa*) and strawberry (*Fragaria ananassa*). Concentrations of PFAAs in lettuce leaves and strawberry root, shoot, and fruit were measured for each incremental dose. PFBA and PFPeA had the overall highest accumulation in the edible parts of both lettuce and strawberry. Concentrations increased linearly with increasing dose for almost all PFAAs, with the exception of the long-chain PFAAs in strawberry fruit, which remained at a constant concentration regardless of dose. Site limited sorption, or alternative transport mechanisms are suspected for long-chain PFAA accumulation in the fruit. Chain length trends were seen in both lettuce shoot and strawberry fruit; as chain length increased, accumulation decreased. In strawberry plants, the distribution of PFAAs was mapped to illustrate that short-chain perfluorocarboxylates constitute the dominant fraction of PFAAs in the fruit and shoot compartments, while a more even fraction of all PFAAs are found in the root compartment. In addition, lettuce grown in soils with varying organic carbon content was used to assess the impact of organic carbon sorption on PFAA bioaccumulation. BAFs for lettuce were calculated for each soil treatment. In general, the higher the fraction of organic carbon, the less bioaccumulation of PFAAs was seen in lettuce. BAFs were also correlated to carbon chain-length of PFAAs showing approximately a 0.4 to 0.6 log decrease per CF_2 group. This study confirmed that PFAAs are able to enter and bioaccumulate in food crops via reclaimed water and helped to illustrate both dose-dependent and chain length-dependent bioaccumulation trends.

5.2 Research Contributions and Significance

The research presented in this dissertation is expected to fill gaps in the body of knowledge concerning the entry of PFAAs into the terrestrial food chain via plant uptake. The results of this work have the potential to impact both agricultural land-application of biosolids and agricultural use of reclaimed water. This research is not specifically intended to provide data for regulatory development; it is, however, intended to justify the need for additional research and monitoring of biosolids and reclaimed water applications.

The results of this dissertation are novel and important as this investigation is the first of its kind to examine PFAA accumulation in food crops by simulating real-world field scenarios using authentic biosolids-amended soils and reclaimed water as the delivery media. The data presented in this study show clearly that PFAAs can be taken up and bioaccumulated from both biosolids-amended soils and reclaimed water into food crops. Overall, chain length dependent bioaccumulation was seen as PFAAs translocate upward from the roots. These bioaccumulation patterns agree with previous literature [1] showing greater uptake and accumulation for PFCAs over PFSAs and for short-chain PFAAs over long-chain PFAAs. With industry trends shifting toward the use of short-chain PFAAs, it is important to consider this increased potential of PFAA entry into the terrestrial food chain via plants.

In both the field and greenhouse studies involving biosolids-amended soils (Chapters 2 and 3), BAFs for shorter chain PFAAs were greater than one, indicating bioaccumulation in the plant tissues. In terms of the U.S. EPA's risk assessment framework for potential contaminant bioaccumulation in crops from biosolids-amended soils, the default "conservative" value for BAFs is one [**?**]; however, based on this research, this estimate is not truly conservative for short-chain PFAAs. This finding supports the need for more thorough research before more risk assessments can be completed for this class of compounds.

Each food crop is physiologically different, presenting unique aspects to the translocation process; however, some commonalities do exist, namely the Casparian strip barrier. Without plant-specific data, only generalizations can be made about plant compartment ac-

cumulation. In general, the data (Chapter 2) suggest fruit crops accumulate fewer long-chain PFCAs than do shoot or root crops. For example, one would expect that peas or tomatoes would contain much less PFOA than celery or radish grown in the same soil. In terms of analytes, there is also a large discrepancy; shoot and fruit crops may have 1-3 orders of magnitude more PFBA than PFOA if both analytes are present in equal concentrations in the soil. Again, with the industry moving toward production and use of the short-chain PFAAs, a fundamental understanding of plant uptake is essential to accurately predict the ramifications on the terrestrial food chain.

The implications from the dose-dependent study (Chapter 4) suggest that although PFAA uptake is predominantly passive for all PFAAs in lettuce and for short-chain PFAAs in strawberry fruit, site-limited sorption [2] and/or unique mechanisms for uptake [3] may be at play for long-chain compounds in fruit. Comparison of the reclaimed water study (Chapter 4) to the biosolids-amended soil studies (Chapters 2 and 3) implies that bioavailability of PFAAs from reclaimed water may be greater than from biosolids-amended soils. This increased bioavailability translates to higher concentrations in food crops. Recognizing dose-dependency trends in terms of media, analytes, and plant compartments is essential for establishing eventual regulations. Knowing that it is possible for food crops to take up concentrations of PFAAs in the parts-per-million range from PFAA-contaminated sources suggests that media concentrations must be monitored and or controlled if edible crops are to be grown in potentially polluted areas.

With respect to overall exposure, it is unlikely that edible crops grown in soils conventionally amended for nutrients with biosolids (that are not impacted by PFAA industries) are a primary source of long-chain PFAA exposure to humans. However, for edible crops unknowingly grown in PFAA contaminated biosolids-amended soils, as are becoming more prevalent, the exposure could be substantial. For example, given the sub-chronic reference dose for PFOA (200 ng/kg-day) [4], a typical 70 kg person could safely consume up to 14,000 ng of PFOA per day. If that person were to eat lettuce grown in PFAA contami-

nated biosolids (˜200 ng PFOA/gdw) [5], the reference dose would translate to a maximum of 70 g_{dw} or 630 g_{ww} of lettuce per day. Consuming a relatively small head of lettuce in a day would therefore exceed the PFOA limit. Moreover, PFOA, being a long-chain PFAA, does not bioaccumulate in food crops nearly to the extent of its short-chain analogues. Consequently, in the absence of comprehensive toxicological data on short-chain PFAAs, even more precaution may be warranted for production of fruit or shoot crops grown in PFAA contaminated soils.

These results may also have important implications with respect to the potential routes of PFAA exposure in humans who use or have used biosolids in their home gardens, particularly if the biosolids were from a wastewater treatment plant receiving industrially impacted wastewater with elevated levels of PFAAs. As sustainability becomes more and more attractive, the recycling and reuse of biosolids is inevitable, and thus it is critical that this increased use be guided by knowledge. Moreover, if the current use of reclaimed water for food crops is also to be sustained or increase in future years, concerns about the potential human exposure to PFAAs from the terrestrial food chain must be more fully addressed through additional research and subsequently communicated to the public.

5.3 Recommendations for Future Work

- Results from this work showed that high levels of human exposure to PFAAs are possible when food crops are grown in contaminated biosolids-amended soils. With ever increasing sites around the world reporting previously unidentified PFAA contaminated soils, a more comprehensive characterization of biosolids destined for land-application is needed. Perhaps rapid, economically viable screening methods for contaminants of concern could be developed to routinely monitor biosolids to proactively address issues. In addition, with current sustainability trends encouraging the use of biosolids as home garden amendments, it is imperative that future work be aimed at monitoring biosolids available to the public so that PFAA-contaminated biosolids are not inadvertently used on food crops.

- Likewise, reclaimed water used for irrigation may require additional screening to ensure that industrial or non-point source pollution has not contaminated the water source with PFAAs. While monitoring of pathogens and metals is routinely performed, additional testing is not always available or cost effective. Future research is needed to develop additional efficient screening methods for emerging contaminants.

- This study examined a few of the important parameters surrounding plant uptake of PFAAs including plant compartments and soil organic carbon. To potentially develop a comprehensive model that could extend to multiple crops in various soils, more extensive research is needed to elucidate other pertinent crop and soil factors. Specifically, the accumulation patterns of PFAAs in differing root structures and fruit compartments examined in this research suggest that there may yet be unidentified mechanisms that impact contaminant accumulation. Mechanistic studies with PFAAs are needed to completely understand and model their accumulation behavior in plants.

- While this study focused on two specific classes of fluorochemical end-products, numerous other types of fluorochemicals with varying structures have not been adequately investigated with respect to plant uptake. More work is needed to examine plant uptake of this broader suite of fluorochemicals present in biosolids and reclaimed water. In addition, many other hydrophilic and/or surfactant chemicals may potentially behave similarly to PFAAs. Mechanistic studies should be expanded to these similar classes of compounds to predict their fate and potential exposure to humans.

- With current data pointing to plant uptake as a potential PFAA exposure route both to humans and in general to the terrestrial food chain, more substantial toxicological data are needed to assess actual risk. While very preliminary data exist on PFOA and PFOS in terms of sub-chronic reference doses, short-chain PFAAs are minimally studied with respect to human effects and safe exposure limits. Given the propensity of short-chain PFAAs to bioaccumulate in food crops and the shift in manufacturing

trends to the production of short-chain PFAAs, filling this gap in toxicological data is critical.

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APPENDIX A - SUPPORTING INFORMATION FOR UPTAKE OF PERFLUOROALKYL ACIDS INTO EDIBLE CROPS VIA LAND APPLIED BIOSOLIDS: FIELD AND GREENHOUSE

A.1 Materials and Methods

The following information provides additional details concerning the materials and methods used in this study.

Table A.1: PFAAs and surrogate standards used in this study.

Analyte	Surrogate Standard	
PFBA	[$^{13}C_4$]	PFBA
PFPeA	[$^{13}C_3$]	PFPeA
PFHxA	[$^{13}C_2$]	PFHxA
PFHpA	[$^{13}C_4$]	PFHpA
PFOA	[$^{13}C_4$]	PFOA
PFNA	[$^{13}C_5$]	PFNA
PFDA	[$^{13}C_2$]	PFDA
PFBS	[$^{18}O_2$]	PFHxS
PFHxS	[$^{18}O_2$]	PFHxS
PFHpS	[$^{18}O_2$]	PFHxS
PFOS	[$^{13}C_4$]	PFOS
PFDS	[$^{13}C_4$]	PFOS

A.1.1 Greenhouse Experiment Details

Lettuce seeds were obtained from Paramount Seeds Inc. (Stuart, FL) and were seeded at a density of 2 seeds per pot in 6 inch squat pots filled with 1 kg_{dw} of soil. Tomato seeds, obtained from Lake Valley Seed (Boulder, CO), were directly sown 5 per pot in 2 gallon pots filled with 5 kg_{dw} of soil. All soils were sieved (6.3 mm) for homogeneity and to eliminate rocks and other large debris. Tomato seeds were thinned early at the cotyledon stage to achieve 1 plant per pot in order to minimize uptake by the extraneous plants. Environmental conditions were set to accommodate cool season crops. Although tomato

Table A.2: Percent Soil Organic Carbon (OC) for All Phases of Study.

Phase	Soil	% OC
Greenhouse	Industrially-Impacted	2.24
	Municipal	6.34
	Control	1.51
Field Trial Plots	0.5×	1.84
	1×	2.11
	2×	2.34
	4×	3.51
	Control	1.45
Full-Scale Field Study	Rural 0.5×	3.42
	Rural Control	3.28
	Urban 1×	0.49
	Urban 2×	0.57
	Urban Control	0.76

Table A.3: Solid phase characteristics for greenhouse study measured by Agvise Laboratories (Northwood, ND).

Characteristic	Compost Used for Industrially-Impacted Soil	Municipal Soil	Control Soil
pH	6.1	6.4	7.6
Cation Exchange Capacity (meq/100g)	19.0	16.1	14.8
Nitrate Nitrogen (ppm)	390	47	14
Olsen Phosphorus (ppm)	231	181	2
Calcium (ppm)	3133	2387	1946
Magnesium (ppm)	312	440	580
Potassium (ppm)	227	142	75
Sodium (ppm)	44	27	16
Soluble Salts (dS/m)	2.38	0.49	0.28

typically prefers warmer temperatures, 'Stupice' tomato is a cooler climate variety. Day temperatures ranged from $18°C$ to $21°C$ and night temperatures ranged from $10°C$ to $13°C$. Full spectrum supplemental lighting was supplied to achieve 16 hours of daylight. Drip irrigation was used to prevent splashing and was supplied at various rates (2-3 times per day for 1-2 minutes each; approximately 100-600 mL per day) based on crop needs and seasonal demand. Irrigation water was tested for PFAA contamination; trace levels ($\leq$ 30 ng/L) were found for PFHxA, PFHpA, and PFOA with all other PFAAs less than LOQ (typically 5-25 ng/L). All soil treatments received the same water source to eliminate variability from irrigation. Lettuce leaves and tomato fruit were harvested upon maturation, for lettuce, 56 days after sprouting, and for tomato, 84-112 days after sprouting.

A.1.2 Soil and Produce Sampling

Clean nitrile gloves were worn during each sampling event. Prior to use, equipment (e.g., stainless steel shovel) was decontaminated by physically wiping with a clean paper towel to remove any attached soil/debris, rinsed twice with methanol and then rinsed once with de-ionized water. PFAA contamination was minimized before and during sampling events by avoiding potential consumer product sources (e.g., clothing with stain- or water-repellents, self-sticky memos). All soil and plant samples were stored in polypropylene or polyethylene containers to avoid PFAA contamination.

A.1.3 Soil Extraction Procedure

Soil samples were extracted by placing a 1 g aliquot into a 50 mL polypropylene vial to which a solution containing isotopically labeled surrogate standard was added prior to sequential extraction via sonication with basic methanol, as per established protocols [6]. All extracts were combined, evaporated to dryness, reconstituted in acidic methanol, subjected to a dispersed ENVI-Carb™ clean-up, and analyzed by LC-MS/MS. Further details for the soil extraction method are published elsewhere [6]. All results are reported on a dry weight basis, which was determined by drying separate aliquots of soil overnight at $105°C$.

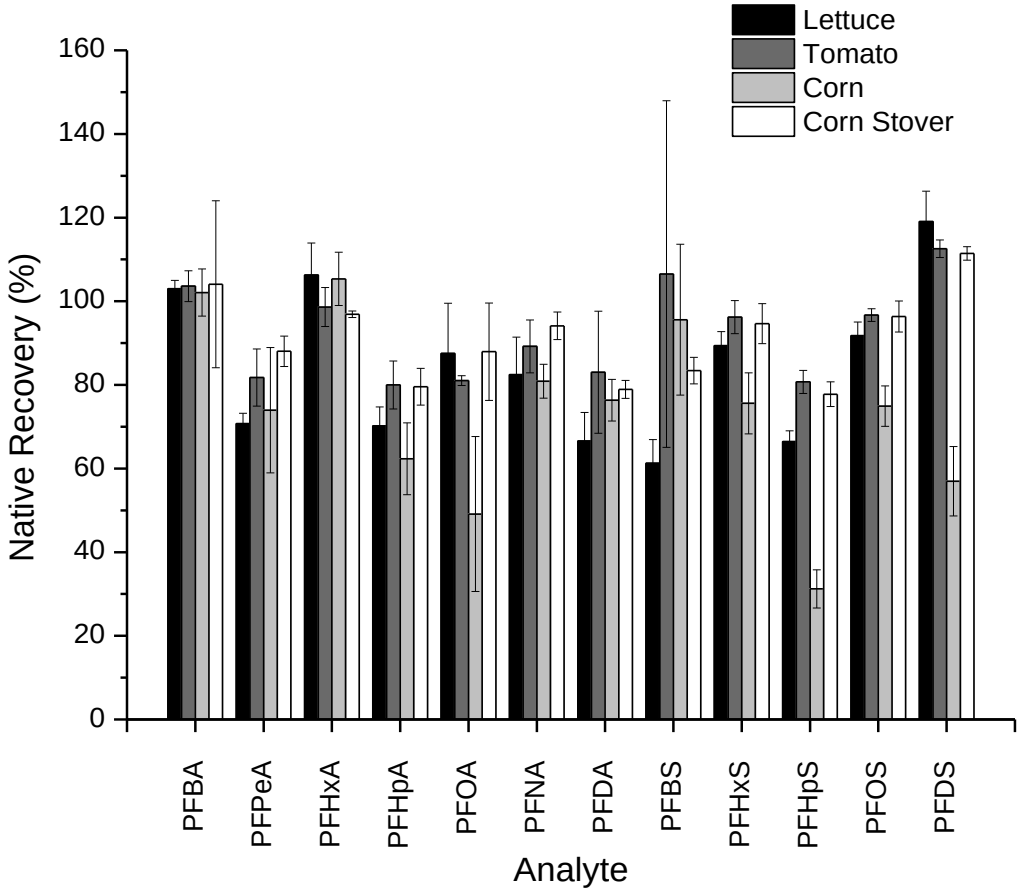


Figure A.1: Surrogate-corrected native spike recovery percentages for all analytes in plant matrices. For each matrix, a 10 ng native spike for each compound was added prior to extraction.

A.1.4 Transpiration Stream Concentration Factor

Transpiration stream concentration factor (TSCF) values converted from BAFs were obtained by first estimating the concentration of PFAAs in the transpiration stream:

$$Conc.\,in\,Transpir.\,Stream\,(\frac{ng}{L}) = \frac{concentration\,in\,foliage\,(\frac{ng}{g})}{avg\,H_2O\,transpired\,per\,g\,of\,biomass\,(\frac{L}{g})} \tag{A.1}$$

The average water transpired per gram of biomass depends on many factors (e.g., temperature, humidity, and crop variety). Due to the experimental setup in this study, the amount of water transpired was not directly measured. In the absence of a measured value, a value of 10.96×10^{-3} L/g was used from the previous study [1] to which the resulting TSCF values are being compared. The next step in converting BAFs into TSCFs was to estimate the concentration of PFAAs in the soil pore water from the concentration in the soil:

$$Concentration\,in\,Soil\,Porewater\,(\frac{ng}{L}) = \frac{concentration\,in\,soil\,(\frac{ng}{kg})}{f_{oc} \times K_{oc}\,(\frac{L}{kg})} \tag{A.2}$$

The fraction of organic carbon for each soil is listed in Table A.2. Organic carbon water partitioning coefficients for PFAAs were taken from a previous study [7] and are shown in Table A.4.

Table A.4: Log K_{oc} values for PFAAs from previous study [7].

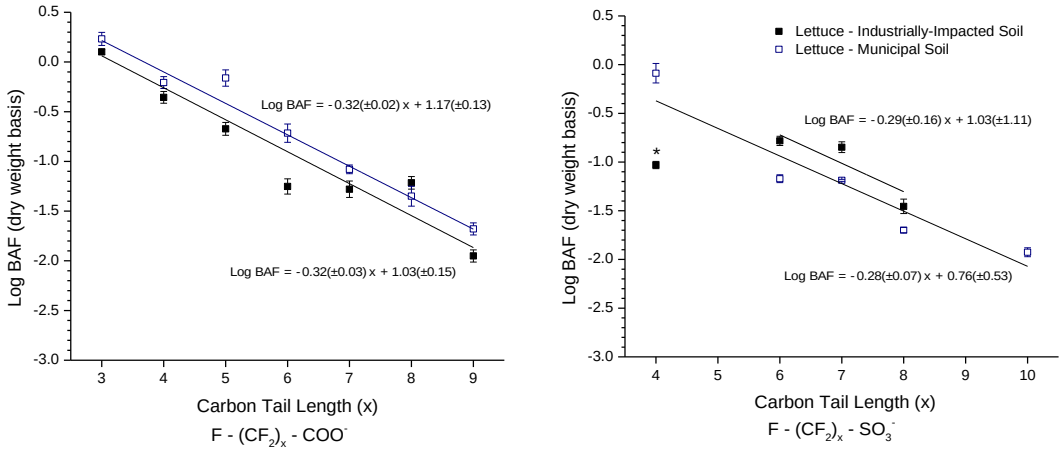
Analyte	log K_{oc}
PFBA	1.88
PFPeA	1.37
PFHxA	1.31
PFHpA	1.63
PFOA	1.89
PFNA	2.36
PFDA	2.96
PFBS	1.79
PFHxS	2.05
PFOS	2.80

The final values for TSCFs were obtained by combining the two equations above (i.e., dividing the concentration in the transpiration stream by the concentration in the soil pore water):

$$TSCF = \frac{Concentration\,in\,Transpiration\,Stream\,(\frac{ng}{L})}{Concentration\,in\,Soil\,Porewater\,(\frac{ng}{L})} \tag{A.3}$$

A.2 Results and Discussion

The following information provides additional details concerning the results of this study.



(a) (b)

Figure A.2: Correlations between organic carbon normalized log BAF for PFCAs (a) and PFSAs (b) and carbon tail length in greenhouse lettuce grown in biosolids-amended soils. Mean and standard error are shown (n = 5). Linear regressions with slopes, intercepts, and associated error values are shown for lettuce; data point marked with an asterisk is excluded from regression calculation.

Table A.5: Average concentrations of PFAAs in Greenhouse Study. For samples where the response was below the LOQ, the LOQ is listed preceded by a less than sign. Standard error (n = 3 to 5) is shown.

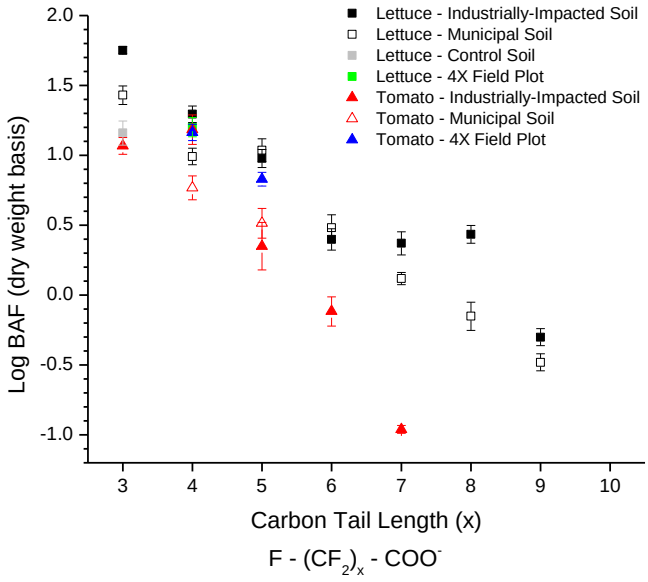
Soil	Analyte	Soil (ng/g)	(±)SE	Lettuce (ng/g)	(±)SE	Tomato (ng/g)	(±)SE
Control	PFBA	0.44	0.09	6.89	1.48	< 0.07	
	PFPeA	< 0.20		2.38	0.28	< 0.29	
	PFHxA	< 0.20		16.44	2.03	< 0.14	
	PFHpA	0.11	0.03	< 0.04		< 0.14	
	PFOA	0.15	0.004	< 0.01		< 0.14	
	PFNA	0.30	0.03	< 0.04		< 2.86	
	PFDA	< 0.20		< 0.04		< 2.86	
	PFBS	< 0.10		< 0.01		< 0.65	
	PFHxS	0.44	0.02	< 0.01		< 0.03	
	PFHpS	< 0.01		< 0.01		< 0.01	
	PFOS	0.40	0.02	< 0.04		< 0.14	
	PFDS	< 0.50		< 0.07		< 1.43	
Industrially Impacted Soil	PFBA	4.68	0.45	266.08	16.15	56.11	7.38
	PFPeA	11.55	0.55	235.96	31.14	211.39	54.26
	PFHxA	11.51	1.21	114.02	15.77	33.17	9.91
	PFHpA	8.61	0.85	22.91	4.05	7.48	2.02
	PFOA	78.52	10.65	197.91	37.66	8.81	0.67
	PFNA	20.15	4.07	57.39	9.48	< 2.86	
	PFDA	93.45	14.69	48.70	7.33	< 2.86	
	PFBS	48.58	2.49	205.24	18.06	19.38	3.26
	PFHxS	1.38	0.15	10.44	1.19	0.76	0.03
	PFHpS	0.54	0.11	3.53	0.50	< 0.01	
	PFOS	49.66	11.02	82.90	15.93	< 0.14	
	PFDS	< 0.50		< 0.07		< 1.43	
Municipal Soil	PFBA	0.90	0.15	25.50	4.68	< 0.07	
	PFPeA	2.66	0.07	27.15	4.03	15.46	3.14
	PFHxA	2.40	0.12	27.97	5.07	5.93	1.28
	PFHpA	2.03	0.05	6.76	1.46	< 0.14	
	PFOA	14.91	0.29	20.01	2.06	< 0.14	
	PFNA	6.11	0.19	4.73	0.93	< 2.86	
	PFDA	10.29	0.35	3.54	0.52	< 2.86	
	PFBS	0.21	0.01	3.03	0.80	< 0.65	
	PFHxS	5.11	0.10	5.54	0.56	< 0.03	
	PFHpS	2.05	0.13	2.10	0.05	< 0.01	
	PFOS	319.49	5.11	101.62	5.80	< 0.14	
	PFDS	61.20	5.59	11.70	1.18	< 1.42	

Table A.6: Average concentrations of PFAAs in Field Trial Plot Phase. For samples where the response was below the LOQ, the LOQ is listed preceded by a less than sign. Standard error (n = 3 to 18) is shown.

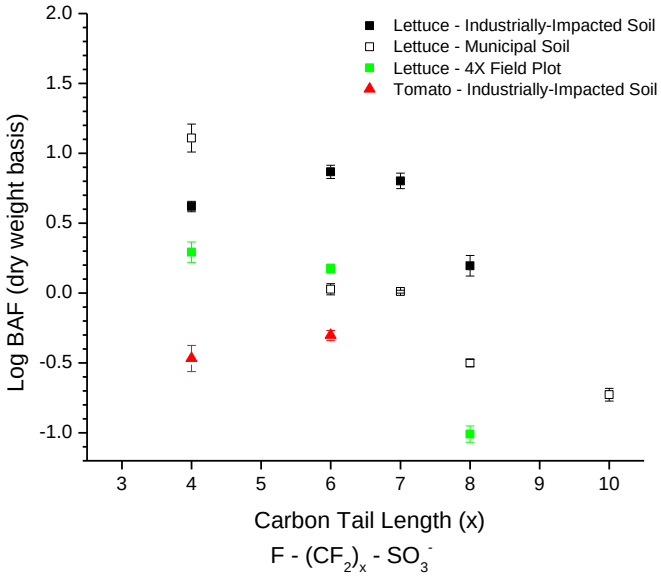
	Analyte	Soil (ng/g)	(±)SE	Lettuce (ng/g)	(±)SE	Tomato (ng/g)	(±)SE
Control	PFBA	0.72	0.11	< 0.07		< 0.07	
	PFPeA	0.39	0.10	< 0.14		< 0.29	
	PFHxA	0.34	0.09	< 0.07		< 0.71	
	PFHpA	0.19	0.04	< 0.07		< 0.29	
	PFOA	0.42	0.07	< 0.14		< 0.29	
	PFNA	0.14	0.01	< 0.01		< 0.14	
	PFDA	< 0.50		< 0.07		< 0.14	
	PFBS	0.20	0.02	< 0.04		< 0.07	
	PFHxS	0.07	0.01	< 0.04		< 0.07	
	PFHpS	< 0.10		< 0.01		< 0.07	
	PFOS	1.22	0.16	< 0.04		< 0.14	
	PFDS	< 0.10		< 0.04		< 0.29	
0.5×	PFBA	0.42	0.07	< 0.07		17.04	4.41
	PFPeA	0.24	0.02	< 0.14		< 0.29	
	PFHxA	0.22	0.02	< 0.07		< 0.71	
	PFHpA	0.21	0.02	< 0.07		< 0.29	
	PFOA	0.74	0.08	< 0.14		< 0.29	
	PFNA	0.32	0.02	< 0.01		< 0.14	
	PFDA	0.51	0.07	< 0.07		< 0.14	
	PFBS	0.20	0.01	< 0.04		< 0.07	
	PFHxS	0.08	0.01	< 0.04		< 0.07	
	PFHpS	< 0.10		< 0.01		< 0.07	
	PFOS	2.20	0.23	< 0.04		< 0.14	
	PFDS	0.17	0.02	< 0.04		< 0.29	
1×	PFBA	0.48	0.05	< 0.07		< 0.07	
	PFPeA	0.32	0.02	< 0.14		6.44	0.49
	PFHxA	0.36	0.04	< 0.07		< 0.71	
	PFHpA	0.35	0.02	< 0.07		< 0.29	
	PFOA	1.48	0.09	< 0.14		< 0.29	
	PFNA	0.73	0.05	< 0.01		< 0.14	
	PFDA	1.13	0.08	< 0.07		< 0.14	
	PFBS	0.30	0.04	< 0.04		< 0.07	
	PFHxS	0.14	0.02	< 0.04		< 0.07	
	PFHpS	< 0.10		< 0.01		< 0.07	
	PFOS	4.31	0.34	< 0.04		< 0.14	
	PFDS	0.61	0.07	< 0.04		< 0.29	

Table A.6: Continued.

	Analyte	Soil (ng/g)	(±)SE	Lettuce (ng/g)	(±)SE	Tomato (ng/g)	(±)SE
2×	PFBA	0.43	0.03	< 0.07		< 0.07	
	PFPeA	0.58	0.06	8.52	5.84	< 0.29	
	PFHxA	0.76	0.08	< 0.07		< 0.71	
	PFHpA	0.51	0.04	< 0.07		< 0.29	
	PFOA	2.11	0.07	< 0.14		< 0.29	
	PFNA	1.12	0.04	< 0.01		< 0.14	
	PFDA	1.75	0.05	< 0.07		< 0.14	
	PFBS	0.31	0.04	< 0.04		< 0.07	
	PFHxS	0.13	0.01	< 0.04		< 0.07	
	PFHpS	< 0.10		< 0.01		< 0.07	
	PFOS	6.12	0.25	2.14	1.27	< 0.14	
	PFDS	1.19	0.11	< 0.04		< 0.29	
4×	PFBA	0.69	0.04	27.50	1.66	12.56	3.68
	PFPeA	1.01	0.08	16.42	2.37	14.97	1.98
	PFHxA	1.49	0.12	< 0.07		10.17	1.20
	PFHpA	1.03	0.09	< 0.07		< 0.29	
	PFOA	5.17	0.28	< 0.14		< 0.29	
	PFNA	3.01	0.18	< 0.01		< 0.14	
	PFDA	4.00	0.20	< 0.07		< 0.14	
	PFBS	0.80	0.09	1.62	0.26	< 0.07	
	PFHxS	0.33	0.03	0.50	0.04	< 0.07	
	PFHpS	0.16	0.02	< 0.01		< 0.07	
	PFOS	13.91	0.91	1.39	0.17	< 0.14	
	PFDS	3.17	0.21	< 0.04		< 0.29	



(a)



(b)

Figure A.3: Correlations between log BAF for PFCAs (Panel a) and PFSAs (Panel b) and carbon tail length for both greenhouse and field studies. Means and standard errors are shown (n = 3 to 5).

Table A.7: Average concentrations of PFAAs in Field Trial Plot Phase. For samples where the response was below the LOQ, the LOQ is listed preceded by a less than sign. Standard error (n = 3 to 18) is shown.

	Analyte	Soil (ng/g)	(±)SE	Corn Grain (ng/g)	(±)SE	Corn Stover (ng/g)	
Rural Control	PFBA	0.12	0.02	< 0.20		< 0.29	
	PFPeA	0.04	0.01	< 0.10		< 0.57	
	PFHxA	< 0.02		< 0.20		< 0.57	
	PFHpA	< 0.10		< 0.20		< 0.57	
	PFOA	0.33	0.08	< 0.20		< 0.57	
	PFNA	0.06	0.01	< 0.10		< 0.29	
	PFDA	< 0.10		< 0.10		< 1.43	
	PFBS	< 0.01		< 0.10		< 0.57	
	PFHxS	< 0.01		< 0.04		< 0.29	
	PFHpS	< 0.02		< 0.04		< 0.14	
	PFOS	0.96	0.09	< 0.10		< 0.14	
	PFDS	< 0.02		< 0.10		< 0.14	
Rural 0.5×	PFBA	0.28	0.04	< 0.20		< 0.29	
	PFPeA	0.89	0.11	< 0.10		< 0.57	
	PFHxA	0.71	0.08	< 0.20		< 0.57	
	PFHpA	0.59	0.09	< 0.20		< 0.57	
	PFOA	4.41	0.54	< 0.20		< 0.57	
	PFNA	0.75	0.12	< 0.10		< 0.29	
	PFDA	2.57	0.46	< 0.10		< 1.43	
	PFBS	1.43	0.14	< 0.10		< 0.57	
	PFHxS	0.16	0.02	< 0.04		< 0.29	
	PFHpS	< 0.02		< 0.04		< 0.14	
	PFOS	4.33	0.64	< 0.10		< 0.14	
	PFDS	0.28	0.04	< 0.10		< 0.14	
Urban Control	PFBA	< 0.02		< 0.20		< 0.29	
	PFPeA	0.13	0.05	< 0.10		< 0.57	
	PFHxA	0.10	0.04	< 0.20		< 0.57	
	PFHpA	< 0.10		< 0.20		< 0.57	
	PFOA	0.55	0.25	< 0.20		< 0.57	
	PFNA	0.20	0.07	< 0.10		< 0.29	
	PFDA	0.69	0.23	< 0.10		< 1.43	
	PFBS	0.10	0.04	< 0.10		< 0.57	
	PFHxS	< 0.01		< 0.04		< 0.29	
	PFHpS	< 0.02		< 0.04		< 0.14	
	PFOS	1.79	0.66	< 0.10		< 0.14	
	PFDS	< 0.02		< 0.10		< 0.14	

Table A.7: Continued.

	Analyte	Soil (ng/g)	(±)SE	Corn Grain (ng/g)	(±)SE	Corn Stover (ng/g)	
Urban 1×	PFBA	0.07	0.01	< 0.20		< 0.29	
	PFPeA	0.13	0.01	< 0.10		< 0.57	
	PFHxA	0.09	0.01	< 0.20		< 0.57	
	PFHpA	< 0.10		< 0.20		< 0.57	
	PFOA	0.56	0.06	< 0.20		< 0.57	
	PFNA	0.28	0.04	< 0.10		< 0.29	
	PFDA	1.01	0.22	< 0.10		< 1.43	
	PFBS	0.17	0.03	< 0.10		< 0.57	
	PFHxS	< 0.01		< 0.04		< 0.29	
	PFHpS	< 0.02		< 0.04		< 0.14	
	PFOS	1.50	0.35	< 0.10		< 0.14	
	PFDS	0.13	0.04	< 0.10		< 0.14	
Urban 2×	PFBA	0.10	0.02	< 0.20		4.21	1.26
	PFPeA	0.24	0.05	< 0.10		0.28	0.06
	PFHxA	0.16	0.02	< 0.20		< 0.57	
	PFHpA	0.19	0.03	< 0.20		< 0.57	
	PFOA	1.28	0.17	< 0.20		< 0.57	
	PFNA	0.40	0.03	< 0.10		< 0.29	
	PFDA	1.36	0.45	< 0.10		< 1.43	
	PFBS	0.39	0.06	< 0.10		< 0.57	
	PFHxS	< 0.01		< 0.04		< 0.29	
	PFHpS	< 0.02		< 0.04		< 0.14	
	PFOS	2.82	0.64	< 0.10		< 0.14	
	PFDS	0.22	0.07	< 0.10		< 0.14	

APPENDIX B - SUPPORTING INFORMATION FOR PERFLUOROALKYL ACID DISTRIBUTION IN VARIOUS PLANT COMPARTMENTS OF EDIBLE MATERIALS AND METHODS

B.1 Materials and Methods

The following information provides additional details concerning the materials and methods used in this study.

Table B.1: PFAAs and surrogate standards used in this study.

Analyte	Surrogate Standard	
PFBA	[$^{13}C_4$]	PFBA
PFPeA	[$^{13}C_3$]	PFPeA
PFHxA	[$^{13}C_2$]	PFHxA
PFHpA	[$^{13}C_4$]	PFHpA
PFOA	[$^{13}C_4$]	PFOA
PFNA	[$^{13}C_5$]	PFNA
PFDA	[$^{13}C_2$]	PFDA
PFBS	[$^{18}O_2$]	PFHxS
PFHxS	[$^{18}O_2$]	PFHxS
PFOS	[$^{13}C_4$]	PFOS

B.1.1 Greenhouse Experiment Details

Radish seeds ('Ricardo') were obtained from Paramount Seeds Inc. (Stuart, FL) and were seeded at a density of 3 seeds per pot in 6 inch pots filled with 2.5 kg of soil. Celery seeds (Tall Utah 52/70 Improved) were obtained from Botanical Interests (Broomfield, CO) and were seeded at a density of 5 seeds per pot in 6 inch pots filled with 2.5 kg of soil. Tomato seeds ('Stupice'), obtained from Lake Valley Seed (Boulder, CO), were directly sown 5 per pot (thinned to 1 per pot) in 2 gallon pots filled with 5 kg of soil. Pea seeds (Sugar Snap) were obtained from Botanical Interests (Broomfield, CO) and were seeded at a density of 2 seeds per pot (thinned to 1 per pot) in 6 inch pots filled with 2.5 kg of

soil. Greenhouse daytime temperatures ranged from $18°C$ to $21°C$ and night temperatures ranged from $10°C$ to $13°C$. Crops were exposed to 16 hours of daylight via supplemental full spectrum lighting. Drip irrigation, selected to prevent splashing, was supplied at various rates (2-3 times/day for 1-2 minutes each; ∼100-600 mL/day) based on crop needs and seasonal demand. PFAA levels were tested in irrigation water; trace levels ($\leq$ 30 ng/L) were found for PFHxA, PFHpA, and PFOA with all other PFAAs less than LOQ (typically 5-25 ng/L). All soil treatments for all crops received the same water source to eliminate variability from irrigation. Crops were harvested upon maturation and subsequently separated into root, shoot and fruit compartments. Crop duration was 67 days for radish, 224 days for celery, 162 days for tomato, and 129 days for pea. All soil and plant samples were stored in polypropylene or polyethylene containers to avoid PFAA contamination.

Table B.2: Edible and non-edible compartments analyzed for each crop. All edible compartments marked with an asterisk were analyzed in industrially-impacted, municipal, and control soils; all other compartments were analyzed solely in industrially-impacted soil.

Crop	Root	Shoot	Fruit
Radish	Edible* – Below ground bulb (hypocotyl)	Above ground stem and leaves	N/A
Celery	Below ground roots	Edible* - Above ground stem and leaves	N/A
Tomato	Below ground roots	Above ground stem and leaves	Edible – reported in Blaine et al. [5]
Pea	Below ground roots	Above ground stem and leaves	Edible*

B.1.2 Bioaccumulation Metrics

Root Concentration Factor:

$$RCF = \frac{PFAA\,Concentration\,in\,Root\,Tissue\,(ng\,g_{dw}^{-1})}{PFAA\,Concentration\,in\,Soil\,(ng\,g_{dw}^{-1})} \tag{B.1}$$

Shoot Concentration Factor:

$$SCF = \frac{PFAA\,Concentration\,in\,Shoot\,Tissue\,(ng\,g_{dw}^{-1})}{PFAA\,Concentration\,in\,Soil\,(ng\,g_{dw}^{-1})} \tag{B.2}$$

Fruit Concentration Factor:

$$FCF = \frac{PFAA\,Concentration\,in\,Fruit\,Tissue\,(ng\,g_{dw}^{-1})}{PFAA\,Concentration\,in\,Soil\,(ng\,g_{dw}^{-1})} \tag{B.3}$$

Fruit-Shoot Concentration Factor:

$$Fruit - Shoot\,Concentration\,Factor = \frac{PFAAs\,in\,Fruit\,Tissue\,(ng\,g_{dw}^{-1})}{PFAAs\,in\,Shoot\,Tissue\,(ng\,g_{dw}^{-1})} \tag{B.4}$$

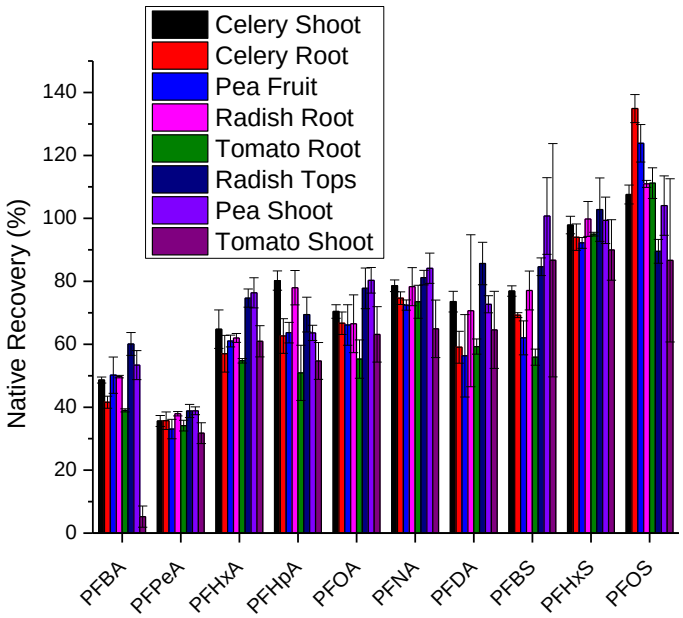


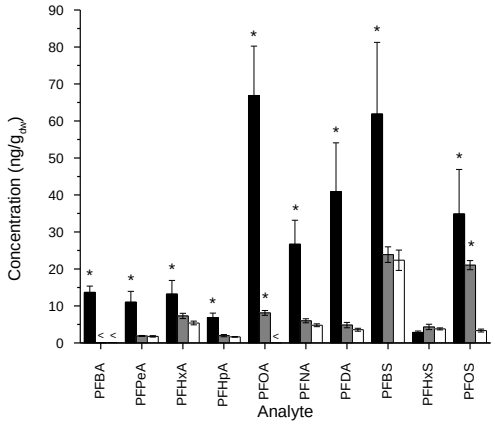
Figure B.1: Surrogate-corrected native spike recovery percentages for all analytes in plant matrices.

B.2 Results and Discussion

The following information provides additional details concerning the results of this study.

Table B.3: Average concentrations of PFAAs in three soils for edible portions of crops. Detections below LOQ are listed. Standard error (n = 3 to 5) is shown.

Soil	Analyte	Celery (ng/g)	(±)SE	Pea (ng/g)	(±)SE	Radish (ng/g)	(±)SE
Control	PFBA	< 0.29		< 0.29		< 0.07	
	PFPeA	5.93	0.28	< 0.07		1.79	0.19
	PFHxA	< 0.71		< 0.71		5.40	0.51
	PFHpA	< 0.07		< 0.07		1.64	0.08
	PFOA	2.58	0.27	< 0.07		< 0.01	
	PFNA	1.89	0.23	< 0.07		4.79	0.39
	PFDA	< 0.14		< 0.14		3.57	0.39
	PFBS	< 0.07		< 0.07		22.36	2.74
	PFHxS	< 0.03		< 0.03		3.81	0.27
	PFOS	< 0.14		< 0.14		3.35	0.39
Industrially Impacted Soil	PFBA	231.69	21.05	150.14	± 24.03	13.67	± 1.68
	PFPeA	147.94	18.95	45.84	7.66	11.02	2.93
	PFHxA	137.14	26.74	16.91	5.26	13.20	3.68
	PFHpA	21.58	4.05	1.57	0.43	6.86	1.20
	PFOA	55.40	16.79	2.65	0.76	66.89	13.36
	PFNA	13.81	2.44	1.45	0.42	26.68	6.49
	PFDA	30.14	2.58	< 0.14		40.91	13.19
	PFBS	107.13	29.18	16.18	2.55	61.89	19.35
	PFHxS	3.19	1.04	0.24	0.01	2.84	0.40
	PFOS	69.27	5.07	1.28	0.30	34.86	12.05
Municipal Soil	PFBA	< 0.29		< 0.29		< 0.07	
	PFPeA	2.98	0.49	< 0.07		1.90	0.13
	PFHxA	7.19	1.21	< 0.71		7.30	0.72
	PFHpA	1.33	0.29	< 0.07		1.96	0.31
	PFOA	1.99	0.30	< 0.07		8.11	0.66
	PFNA	1.62	0.37	< 0.07		5.99	0.55
	PFDA	2.10	1.00	< 0.14		4.83	0.73
	PFBS	4.49	2.80	< 0.07		23.88	2.10
	PFHxS	0.38	0.09	< 0.03		4.33	0.73
	PFOS	17.21	1.76	< 0.14		21.03	1.23



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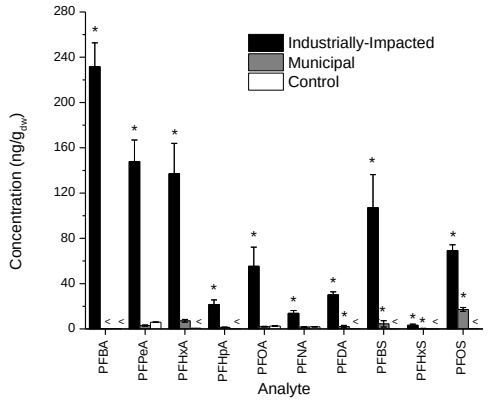
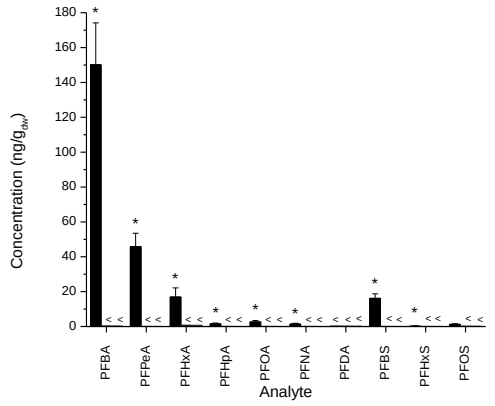


(b)



(c)

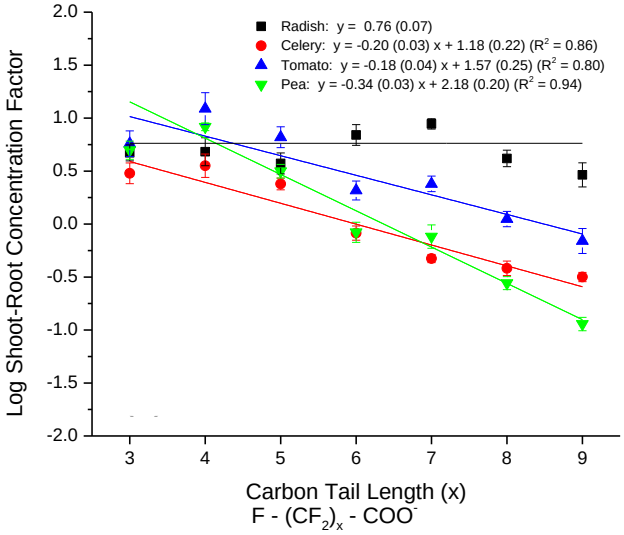
Figure B.2: Concentrations of PFAAs in greenhouse radish (a), celery (b), and pea (c) grown in biosolids-amended soil. Mean and standard error are shown (n = 3 to 5). Values marked with an asterisk are significantly different ($\alpha = 0.05$) than the control. Values less than the LOQ are denoted by <; LOQs for respective matrix and analyte are listed in Table B.3.

Table B.4: Average concentrations of PFAAs for non-edible portions of crops. Detections below LOQ are listed. Standard error (n = 3 to 5) is shown.

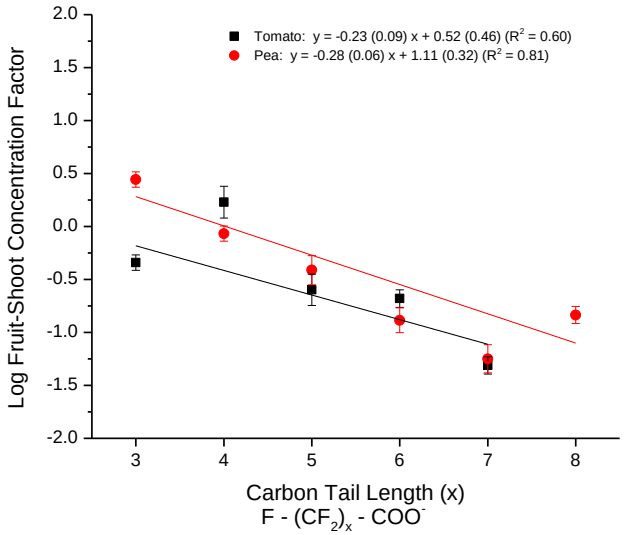
Analyte	Celery Root (ng/g)	(±)SE	Radish Shoot (ng/g)	(±)SE	Tomato Root (ng/g)	(±)SE	Tomato Shoot (ng/g)	(±)SE	Pea Root (ng/g)	(±)SE	Pea Shoot (ng/g)	(±)SE
PFBA	80.83	18.54	64.69	7.80	23.60	5.63	121.86	10.69	9.05	0.89	50.98	8.93
PFPeA	44.37	9.52	48.00	5.27	8.77	2.18	98.96	4.96	5.87	0.70	52.23	6.42
PFHxA	54.93	5.62	44.46	3.81	16.64	3.21	102.75	6.74	11.94	1.80	39.88	4.66
PFHpA	25.47	4.24	47.38	7.70	16.22	2.84	32.62	4.40	13.34	2.80	10.73	1.01
PFOA	111.35	28.79	596.76	119.26	75.00	7.72	190.24	39.40	62.23	17.40	41.03	3.09
PFNA	38.21	8.52	107.00	20.70	38.77	2.76	47.25	11.43	34.37	3.57	8.87	0.66
PFDA	99.41	15.69	103.01	19.30	173.30	18.46	134.05	32.53	133.53	19.30	13.84	1.19
PFBS	122.62	23.86	164.23	13.36	34.40	15.08	177.10	21.78	43.02	10.63	200.09	20.13
PFHxS	6.87	2.05	10.31	1.48	2.42	0.42	7.73	1.54	< 0.14		5.98	0.28
PFOS	209.77	19.14	185.52	38.93	225.14	21.53	210.65	46.87	118.65	16.77	61.57	3.84

Table B.5: Bioaccumulation factors for PFAAs in all crops. Factors for analytes below the LOQ were not calculated and are listed as LOQ. Standard error (n = 3 to 5) is shown.

	Analyte	RCF	(±)SE	RCF_{pw}	(±)SE	SCF	(±)SE	FCF	(±)SE
Radish	PFBA	2.92	0.36	4.96	0.61	13.82	1.67		
	PFPeA	0.95	0.25	0.50	0.13	4.16	0.46		
	PFHxA	1.15	0.32	0.52	0.15	3.86	0.33		
	PFHpA	0.80	0.14	0.76	0.13	5.50	0.89		
	PFOA	0.85	0.17	1.48	0.30	7.60	1.52		
	PFNA	1.32	0.32	6.79	1.65	5.31	1.03		
	PFDA	0.44	0.14	8.94	2.88	1.10	0.21		
	PFBS	1.27	0.40	1.76	0.55	3.38	0.27		
	PFHxS	2.05	0.29	5.16	0.73	7.46	1.07		
	PFOS	0.70	0.24	9.91	3.43	3.74	0.78		
Celery	PFBA	17.27	3.96	29.32	6.72	49.49	4.50		
	PFPeA	3.84	0.82	2.02	0.43	12.81	1.64		
	PFHxA	4.77	0.49	2.18	0.22	11.91	2.32		
	PFHpA	2.96	0.49	2.82	0.47	2.51	0.47		
	PFOA	1.42	0.37	2.46	0.64	0.71	0.21		
	PFNA	1.90	0.42	9.72	2.17	0.69	0.12		
	PFDA	1.06	0.17	21.72	3.43	0.32	0.03		
	PFBS	2.52	0.49	3.48	0.68	2.21	0.60		
	PFHxS	4.98	1.48	12.50	3.72	2.31	0.76		
	PFOS	4.22	0.39	59.65	5.44	1.39	0.10		
Tomato	PFBA	5.04	1.20	8.56	2.04	26.03	2.28	12.16	1.71
	PFPeA	0.76	0.19	0.40	0.10	8.57	0.43	17.06	3.74
	PFHxA	1.45	0.28	0.66	0.13	8.93	0.59	2.90	0.87
	PFHpA	1.88	0.33	1.80	0.32	3.79	0.51	0.86	0.23
	PFOA	0.96	0.10	1.66	0.17	2.42	0.50	0.11	0.01
	PFNA	1.92	0.14	9.87	0.70	2.35	0.57	LOQ	
	PFDA	1.85	0.20	37.86	4.03	1.43	0.35	LOQ	
	PFBS	0.71	0.31	0.98	0.43	3.65	0.45	0.42	0.08
	PFHxS	1.75	0.31	4.40	0.77	5.60	1.11	0.50	0.04
	PFOS	4.53	0.43	64.02	6.12	4.24	0.94	LOQ	
Pea	PFBA	1.93	0.19	3.28	0.32	10.89	1.91	32.07	5.13
	PFPeA	0.51	0.06	0.27	0.03	4.52	0.56	3.97	0.66
	PFHxA	1.04	0.16	0.47	0.07	3.46	0.40	1.47	0.46
	PFHpA	1.55	0.32	1.48	0.31	1.25	0.12	0.18	0.05
	PFOA	0.79	0.22	1.38	0.39	0.52	0.04	0.03	0.01
	PFNA	1.71	0.18	8.75	0.91	0.44	0.03	0.07	0.02
	PFDA	1.43	0.21	29.17	4.22	0.15	0.01	LOQ	
	PFBS	0.89	0.22	1.22	0.30	4.12	0.41	0.33	0.05
	PFHxS	LOQ		LOQ		4.33	0.20	0.17	0.01
	PFOS	2.39	0.34	33.74	4.77	1.24	0.08	0.03	0.01



(a)



(b)

Figure B.3: Inter-compartmental concentration factors for PFCAs between Shoot-Root (a) and Fruit-Shoot (b) and carbon tail length in greenhouse radish, celery, tomato, and pea grown in industrially-impacted soil. Means and standard errors are shown (n = 3 to 5). Linear regressions with slopes (if significantly different than zero at $\alpha = 0.05$), intercepts, and associated error values are shown.

APPENDIX C - SUPPORTING INFORMATION FOR PERFLUOROALKYL ACID UPTAKE IN LETTUCE (*LACTUCA SATIVA*) AND STRAWBERRY (*FRAGARIA ANANASSA*) IRRIGATED WITH RECLAIMED WATER

C.1 Materials and Methods

The following information provides additional details concerning the materials and methods used in this study.

Table C.1: PFAAs and surrogate standards used in this study.

Analyte	Surrogate Standard	
PFBA	[$^{13}C_4$]	PFBA
PFPeA	[$^{13}C_3$]	PFPeA
PFHxA	[$^{13}C_2$]	PFHxA
PFHpA	[$^{13}C_4$]	PFHpA
PFOA	[$^{13}C_4$]	PFOA
PFNA	[$^{13}C_5$]	PFNA
PFDA	[$^{13}C_2$]	PFDA
PFBS	[$^{18}O_2$]	PFHxS
PFHxS	[$^{18}O_2$]	PFHxS
PFOS	[$^{13}C_4$]	PFOS

C.1.1 Greenhouse Experiment Details

Lettuce seeds were obtained from Paramount Seeds Inc. (Stuart, FL) and were started in seed starter plugs using tap water to maintain moisture. When lettuce plants reached the four-true-leaves stage, they were transplanted bare root by gently submerging the roots in tap water to remove soil debris. Lettuce plants were then planted in 15 cm squat pots at which point they started receiving their respective doses via 100 mL of water 3 times a week. They were grown to maturity (just before bolting) and harvested at that time. Because not all of the lettuce plants matured at the same rate, the lettuce plants were transplanted and harvested based on maturity instead of a standard time period.

Strawberry plants were obtained as bare root plants from Sakuma Bros. Farm (Burlington, WA). The roots were planted directly in standard 15 cm pots and watered with tap water until new leaves started to emerge. When all of the plants had at least one leaf, they started receiving their respective doses via 200 mL of water 3 times a week. Strawberry fruits were harvested upon maturation. When sufficient fruit biomass had been collected, strawberry roots and shoots were harvested.

Table C.2: Measured PFAAs in all aqueous treatments.

Applied Dose	Measured Dose								
All PFAAs	(ng/L)								
(µg/L)	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFBS	PFHxS	PFOS
Tap	< 6	< 12	30	27	13	< 12	< 25	< 2	4
Ambient	< 6	19	33	< 12	9	< 12	< 25	< 2	3
0.2	121	146	214	198	152	87	216	92	65
0.4	259	267	399	388	287	154	399	186	97
1	670	688	1003	955	766	400	1045	473	262
2	1244	1340	1963	1938	1580	741	1994	991	488
4	2599	2617	3840	3747	2833	1648	3463	1907	1001
10	4105	4469	8747	8599	5833	2685	10864	4006	1364
20	8821	9086	18210	17469	11383	6265	20864	8475	3451
40	14877	14444	30247	31790	21543	12877	31296	15556	7944

Table C.3: Reclaimed water quality data. Average parameters for Mines Park effluent from March to July in 2013 are shown. Standard deviation is shown in parentheses.

pH	Conductivity (µS/cm)	Alkalinity (mg/L $CaCO_3$)	Chemical Oxygen Demand (mg/L)	Total Nitrogen (mg/L)	NO_3 (mg/L)	Total Phosphorus (mg/L PO_4)
6.6 (0.4)	752 (130)	54 (21)	17.5 (4.7)	9.1 (7.5)	5.9 (6.6)	9.2 (6.5)

C.1.2 Soil Characteristics for Organic Carbon Study

The sand-soil mix (0.4% organic carbon content) was prepared by mixing washed playsand with topsoil (both obtained from a local nursery) at a mass ratio of 3:1. The 2.0% organic carbon content soil was obtained from Agvise Labs (Northwood, ND) and had a sandy loam texture. It was 62%, 19%, and 19% sand, silt, and clay respectively. The 6.0% organic carbon content soil was also obtained from Agvise Labs (Northwood, ND) and had a loam texture. It was 45%, 36%, and 19% sand, silt, and clay respectively.

C.1.3 Determination of Solid-Water Partitioning Coefficient (K_d)Values

Single-point K_d values were measured in the 0.4%, 2% and 6% OC soils. Triplicate batch reactors were prepared in 50 mL polypropylene centrifuge tubes containing 1 g of soil and 50 mL of reclaimed water from Mines Park. Prior to adding the water to the reactors, soils were spiked with 10 µg/L of each PFAA, and sodium azide (1 g/L) was added to inhibit microbial growth. Reactors were equilibrated for 14 days (adequate time to reach equilibrium [7]) on a shaker table. Reactors were then centrifuged and the aqueous phase was decanted. Both the soil and aqueous phases were extracted and analyzed for PFAAs. K_d values were calculated by dividing the concentration of PFAA in the soil (C_s) by the concentration of PFAA in the water (C_w):

$$K_d\,(L/kg) = \frac{C_s\,(ng/kg)}{C_w\,(ng/L)} \tag{C.1}$$

Calculated K_d values are shown in Table C.4. In addition, in order to assure the validity of the values, they were converted to K_{oc} values by dividing by the f_{oc} in each soil and compared with values from previous studies (Table C.5).

C.2 Results and Discussion

The following information provides additional details concerning the results of this study.

Table C.4: Calculated K_d values with standard deviation (n = 3) for all three soils in this study.

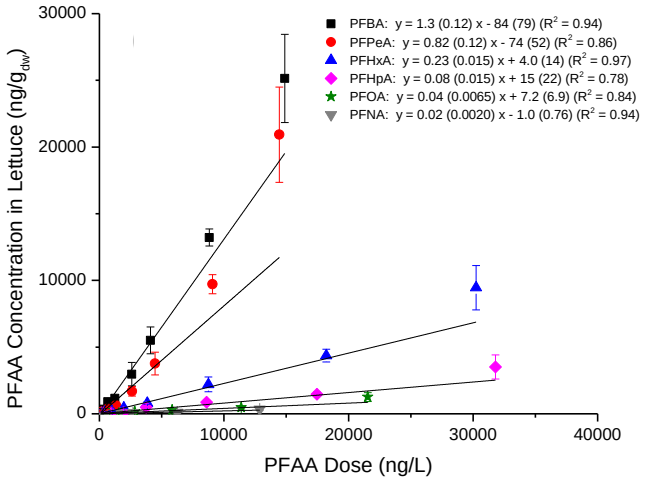
	0.4% OC Soil		2% OC Soil		6% OC Soil	
PFBA	0.86	± 0.06	1.11	± 0.07	1.54	± 0.12
PFPeA	1.03	± 0.04	1.27	± 0.06	1.55	± 0.08
PFHxA	0.61	± 0.03	0.72	± 0.07	1.08	± 0.06
PFHpA	0.84	± 0.01	1.08	± 0.08	1.62	± 0.13
PFOA	1.46	± 0.11	3.82	± 0.79	5.53	± 0.12
PFNA	2.74	± 0.62	10.65	± 1.06	8.40	± 0.35
PFBS	1.04	± 0.08	1.42	± 0.02	1.88	± 0.23
PFHxS	1.01	± 0.06	1.40	± 0.05	3.61	± 0.09
PFOS	6.48	± 1.18	10.82	± 0.34	32.81	± 3.54

Table C.5: Calculated log K_{oc} values for this study compared to previous studies.

	0.4% OC Soil	2% OC Soil	6% OC Soil	Average for this Study	Previous Work [7]
PFBA	2.33	1.74	1.41	1.83	1.88
PFPeA	2.41	1.80	1.41	1.88	1.37
PFHxA	2.18	1.56	1.26	1.66	1.31
PFHpA	2.32	1.73	1.43	1.83	1.63
PFOA	2.56	2.28	1.96	2.27	1.89
PFNA	2.84	2.73	2.15	2.57	2.36
PFBS	2.42	1.85	1.50	1.92	1.79
PFHxS	2.40	1.85	1.78	2.01	2.05
PFOS	3.21	2.73	2.74	2.89	2.8

Table C.6: Average concentrations of PFAAs in lettuce leaves for all aqueous treatments. Detections below LOQ are listed. Standard error (n = 5) is shown.

Applied Dose All PFAAs (µg/L)	PFBA (ng/g)	(±)SE	PFPeA (ng/g)	(±)SE	PFHxA (ng/g)	(±)SE
Tap	25.76	11.31	< 0.07		32.76	3.44
Reclaimed	53.61	13.62	20.58	6.94	37.18	4.20
0.2	95.58	40.60	56.55	20.79	52.77	15.76
0.4	335.52	59.94	180.43	44.73	124.93	26.13
1	905.35	160.61	387.09	85.34	197.26	30.29
2	1151.95	97.02	631.18	108.51	440.85	55.09
4	2972.82	875.66	1688.94	352.39	808.43	100.88
10	5500.19	1005.26	3767.31	853.13	2201.40	554.73
20	13214.96	639.02	9718.43	721.17	4358.30	478.79
40	25144.68	3309.11	20926.44	3572.33	9454.79	1660.64
	PFHpA (ng/g)	(±)SE	PFOA (ng/g)	(±)SE	PFNA (ng/g)	(±)SE
Tap	46.66	10.87	16.01	1.89	2.48	0.38
Reclaimed	40.14	3.87	16.36	0.78	2.79	0.27
0.2	43.44	13.80	27.16	9.92	2.75	0.95
0.4	71.04	11.06	26.94	5.50	4.78	1.44
1	75.06	12.29	29.33	4.91	9.35	3.34
2	126.24	11.84	60.22	7.66	16.30	2.19
4	498.38	41.71	169.17	29.65	37.65	11.16
10	873.20	129.79	298.13	45.65	84.39	16.57
20	1469.79	173.93	471.59	88.20	101.02	28.01
40	3509.71	908.26	1255.75	330.14	332.43	88.05
	PFBS (ng/g)	(±)SE	PFHxS (ng/g)	(±)SE	PFOS (ng/g)	
Tap	< 0.07		< 0.29		< 0.07	
Reclaimed	< 0.07		< 0.29		< 0.07	
0.2	10.29	3.56	< 0.29		1.44	0.59
0.4	44.40	10.24	5.24	0.32	4.05	1.04
1	89.89	22.28	9.25	2.52	3.28	0.93
2	133.16	17.42	32.63	4.29	17.28	3.11
4	473.87	85.92	56.25	18.29	31.13	6.41
10	1154.08	259.12	187.55	51.25	57.39	19.71
20	2082.76	174.54	416.88	84.17	73.11	20.97
40	3671.19	677.58	1253.44	370.58	279.01	69.90



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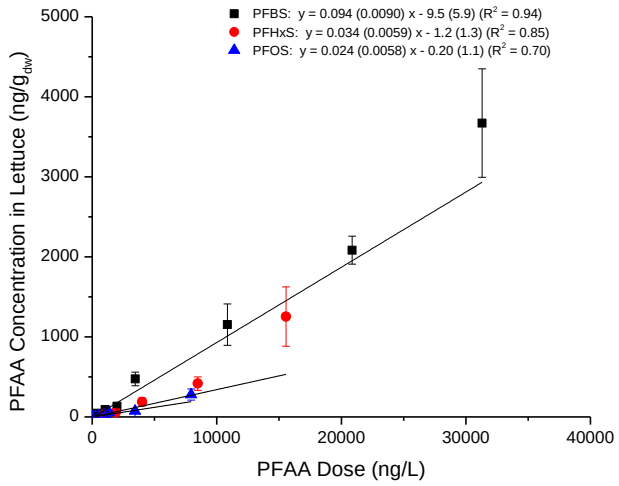
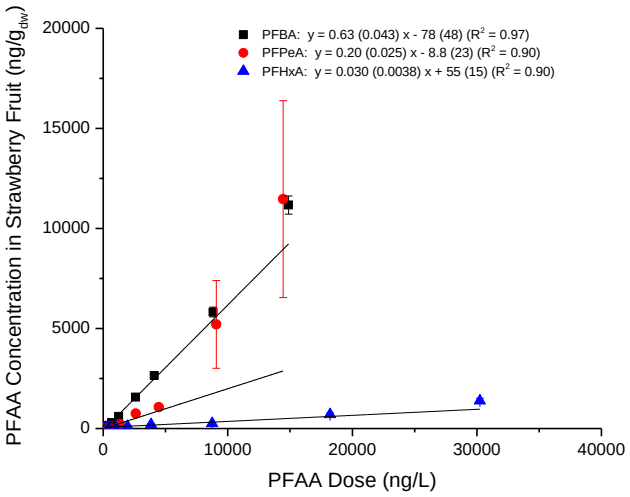


(b)

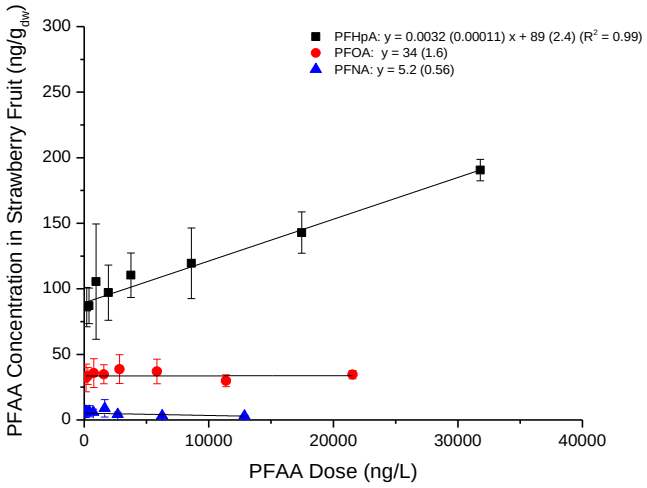
Figure C.1: Concentrations of PFCAs (a) and PFSAs (b) in lettuce leaves versus measured aqueous dose of PFAAs. Means and standard errors (n = 5) are shown. Linear regressions with slopes, intercepts, and associated error values are shown.

Table C.7: Concentrations of PFAAs in strawberry fruit composited for all aqueous treatments. Detections below LOQ are listed. Analytical standard deviation (n = 3) is shown.

Applied Dose All PFAAs (µg/L)	PFBA (ng/g)	(±)SE	PFPeA (ng/g)	(±)SE	PFHxA (ng/g)	(±)SE
Tap	38.43	27.97	< 1.43		109.53	73.75
Reclaimed	64.92	65.51	< 1.43		127.31	76.46
0.2	80.91	23.75	35.70	26.74	69.18	35.64
0.4	149.40	14.29	58.78	9.72	71.36	7.95
1	290.18	16.89	119.19	15.35	85.32	30.02
2	599.17	16.43	228.28	12.10	114.93	26.61
4	1571.71	121.34	747.23	68.53	204.99	44.17
10	2649.23	47.75	1067.12	80.77	258.49	20.58
20	5820.38	245.24	5202.40	2189.85	703.66	69.78
40	11171.34	457.40	11465.47	4923.02	1383.05	126.32
	PFHpA (ng/g)	(±)SE	PFOA (ng/g)	(±)SE	PFNA (ng/g)	(±)SE
Tap	110.03	41.17	38.48	19.16	11.26	7.24
Reclaimed	131.27	63.22	51.85	24.37	12.75	6.43
0.2	86.11	15.01	32.04	10.55	5.45	3.50
0.4	87.05	13.53	33.52	6.47	6.66	3.69
1	105.52	43.92	35.75	11.01	6.68	4.29
2	97.11	21.08	34.81	7.23	6.03	2.09
4	110.38	17.02	38.77	10.92	8.81	6.66
10	119.44	26.92	37.04	9.38	4.28	1.76
20	142.86	15.78	29.91	4.46	3.23	1.09
40	190.65	8.18	34.56	3.19	2.99	0.49
	PFBS (ng/g)	(±)SE	PFHxS (ng/g)	(±)SE	PFOS (ng/g)	
Tap	< 0.71		< 0.07		11.38	6.39
Reclaimed	< 0.71		8.28	5.65	7.38	3.59
0.2	< 0.71		6.30	5.23	5.15	1.83
0.4	< 0.71		0.99	0.11	< 0.29	
1	< 0.71		< 0.07		< 0.29	
2	< 0.71		3.09	2.03	7.19	3.42
4	< 0.71		6.12	4.97	7.46	4.13
10	18.22	6.00	2.91	1.53	< 0.29	
20	27.04	2.70	1.99	0.18	< 0.29	
40	55.06	7.18	2.73	0.29	< 0.29	



(a)

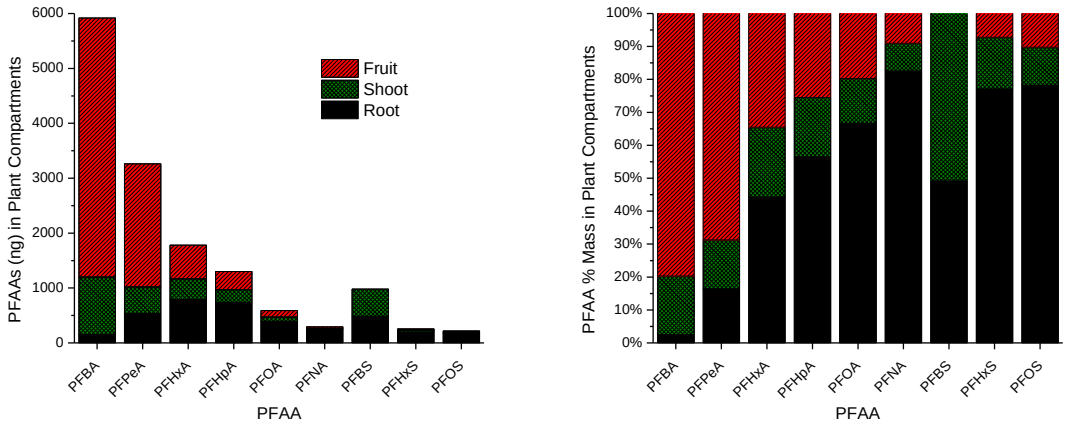


(b)

Figure C.2: Concentrations of PFCAs in strawberry fruit versus measured aqueous dose of short-chain PFAAs (a) and long-chain PFAAs (b). Means of composited berries are shown with analytical standard deviation (n = 3). Linear regressions with slopes (if significantly different than zero at $\alpha = 0.05$), intercepts, and associated error values are shown.

Table C.8: Concentrations of PFAAs in strawberry root and shoot for 3 applied doses. Means and standard error (n = 5) are shown.

	0.4 µg/L Applied Dose				4 µg/L Applied Dose				40 µg/L Applied Dose			
	Root		Shoot		Root		Shoot		Root		Shoot	
	(ng/g)	(±)SE	(ng/g)	(±)SE	(ng/g)	(±)SE	(ng/g)	(±)SE	(ng/g)	(±)SE	(ng/g)	(±)SE
PFBA	24.84	6.68	55.25	6.94	116.14	48.45	263.10	23.04	538.87	71.03	3898.50	863.15
PFPeA	36.14	8.05	24.57	5.90	411.04	181.32	121.45	7.76	3927.35	422.08	3449.30	837.90
PFHxA	98.84	20.26	64.55	14.34	606.75	75.05	94.51	7.91	5447.99	284.40	1159.52	166.20
PFHpA	80.92	12.04	81.67	26.70	565.09	169.21	58.66	11.93	4329.95	531.41	326.31	73.24
PFOA	28.91	5.10	36.87	15.20	302.17	64.90	20.11	4.42	768.76	127.90	147.43	37.83
PFNA	9.71	1.83	24.92	17.77	185.44	42.69	6.17	1.50	501.40	87.81	68.77	17.23
PFBS	41.61	10.75	23.13	5.03	372.68	127.10	124.24	29.89	2833.31	203.34	1473.02	192.14
PFHxS	9.30	2.81	4.96	0.94	150.48	51.07	9.98	2.50	527.62	112.65	123.34	26.65
PFOS	7.11	1.80	6.93	1.40	131.55	32.23	6.33	0.77	527.47	87.97	66.43	15.05



(a) (b)

Figure C.3: Mass of PFAAs (a) and % mass (mass of PFAA in each compartment normalized to total mass of PFAA in whole plant; b) in strawberry root, shoot, and fruit compartments for the applied PFAA dose of 4 µg/L.

Table C.9: Fruit-soil concentration factors for strawberry at the applied PFAA dose of 10 µg/L.

	FCF	(±)SE
PFBA	202.56	2.11
PFPeA	242.52	10.60
PFHxA	34.46	1.58
PFHpA	7.75	1.01
PFOA	1.95	0.28
PFNA	0.17	0.04

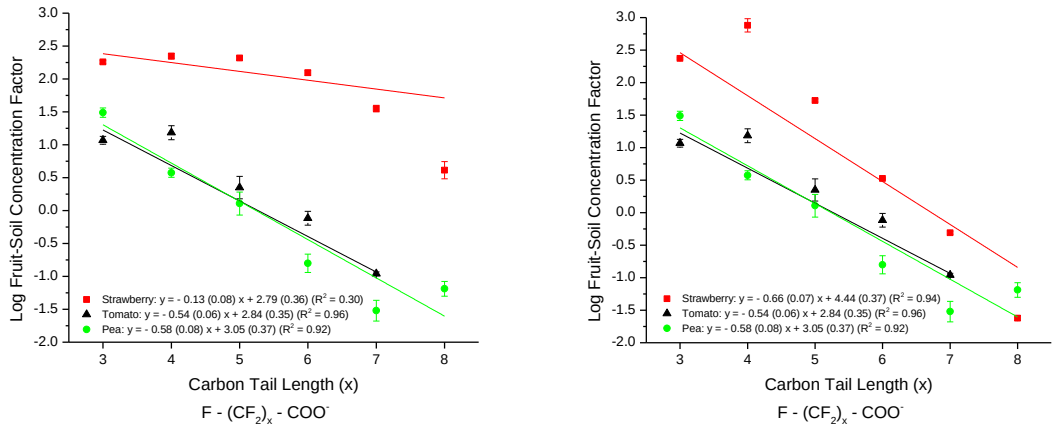


Figure C.4: Correlations for PFCAs between log fruit-soil concentration factors and carbon tail length in strawberry, tomato, and pea. Strawberry values from this study were from the 0.4 µg/L (a) and 40 µg/L (b) applied doses; tomato and pea values were from a previous study (Chapter 3). Means and standard errors are shown (n = 3 to 5). Linear regressions with slopes, intercepts, and associated error values are shown.

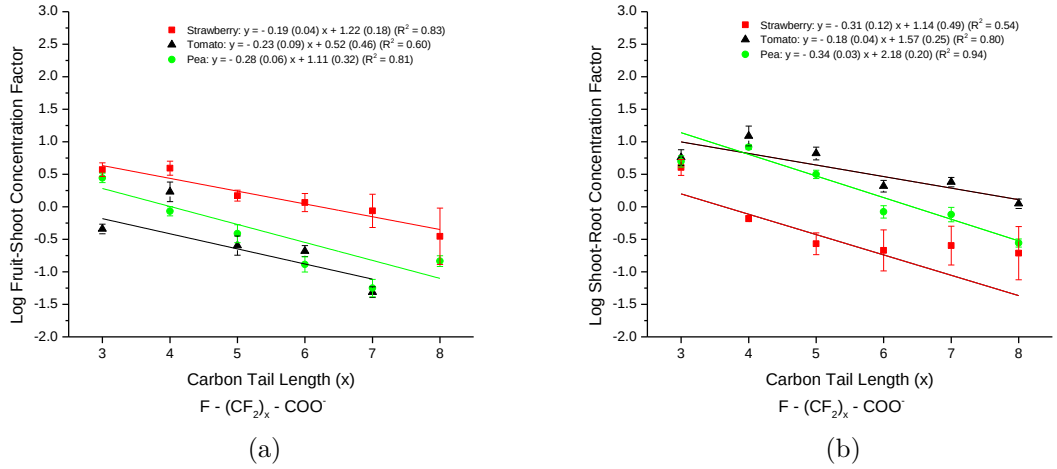


Figure C.5: Correlation of inter-compartmental concentration factors for PFCAs between Fruit-Shoot (a) and Shoot-Root (b) and carbon tail length in strawberry, tomato, and pea. Strawberry values from this study were averaged from the 0.4, 4, and 40 µg/L applied doses; tomato and pea values were from a previous study (Chapter 3). Means and standard errors are shown (n = 3 to 5). Linear regressions with slopes, intercepts, and associated error values are shown.

Table C.10: PFAA concentrations in lettuce grown in soils with varying organic carbon content (2%, 6%) at the applied dose of 10 µg/L.

	2% OC Soil		6% OC Soil	
	(ng/g)	(±)SE	(ng/g)	(±)SE
PFBA	15392.64	2009.30	4862.07	1845.41
PFPeA	7392.02	1192.64	2176.89	789.59
PFHxA	2026.27	363.11	677.72	251.58
PFHpA	320.45	59.16	100.97	31.55
PFOA	95.67	9.89	38.00	10.83
PFNA	47.27	4.22	21.16	3.66
PFBS	4872.85	679.47	1191.62	359.37
PFHxS	99.49	11.06	50.93	2.51
PFOS	70.99	4.89	33.95	2.81

Table C.11: Bioaccumulation factors for lettuce leaves grown in soils with varying organic carbon content (0.4%, 2%, 6%) at the applied dose of 10 µg/L.

	0.4% OC Soil	(±)SE	2% OC Soil	(±)SE	6% OC Soil	(±)SE
PFBA	1555.82	284.35	3389.97	442.51	767.33	291.24
PFPeA	818.69	185.40	1297.85	209.40	313.61	113.75
PFHxA	415.11	104.60	320.85	57.50	71.75	26.64
PFHpA	121.60	18.08	34.67	6.40	7.26	2.27
PFOA	34.91	5.34	4.30	0.44	1.18	0.34
PFNA	11.45	2.25	1.65	0.15	0.94	0.18
PFBS	101.84	22.87	316.31	44.11	58.34	17.59
PFHxS	46.15	12.61	17.74	1.97	3.52	0.22
PFOS	6.49	2.23	4.81	0.37	0.76	0.08

APPENDIX D - REFERENCES FOR SUPPORTING INFORMATION

The following references are cited in the supporting information provided in Appendices A, B, and C.

D.1 References Cited

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