Environmental Science & Technology

Article

pubs.acs.org/est

Uptake of Perfluoroalkyl Acids into Edible Crops via Land Applied Biosolids: Field and Greenhouse Studies

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Supporting Information

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 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.

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 U.S.[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

Received: July 12, 2013
Revised: October 29, 2013
Accepted: November 8, 2013
Published: November 8, 2013

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[25] (TSCF), 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 biosolids-amended 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.

MATERIALS AND METHODS

Chemicals. Perfluorinated standards as well as stable-isotope labeled standards (Supporting Information (SI) Table S1) 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 Chromasolv dichloromethane from Sigma Aldrich (St. Louis, MO) were used for extractions. All other solvents were reagent grade from Sigma Aldrich. Water used in extractions was obtained from a Milli-Q system (Millipore, Billerica, MA), 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. (Bethlehem, PA) and Supelclean ENVI-Carb from Sigma-Aldrich were used.

Greenhouse Study. Accumulation was studied from three soils: industrially impacted soil (soil amended with PFAA contaminated biosolids), municipal soil (soil receiving a long-term 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., woodchips, 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 (SI Table S2), and other soil characteristics (SI Table S3) measured by Agvise Laboratories (Northwood, ND) can be found in the SI.

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 °C in sealed plastic bags until extraction. Detailed information

about propagation, environmental conditions, and sampling are given in the SI.

Field Studies. A limited-scale field study was conducted in the Midwestern U.S. 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 ∼45 days; tomato ∼100 days) using a sample collection protocol (detailed in the SI) 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 °C until extraction.

In addition, a full-scale field sampling campaign was conducted in the Midwestern U.S. 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×, and 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 °C until extraction. A summary of both the greenhouse and field studies is shown in Table 1.

Table 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 (£1654 Mg/ha) (5 replicate pots)	
field-scale trial plots	control (unamended) (3 replicate plots)	lettuce leaves; tomato fruit
	0.5× agronomic rate for nitrogen (N) (12.5 Mg/ha) (3 replicate plots)	
	1× agronomic rate for N (25 Mg/ha) (3 replicate plots)	
	2× agronomic rate for N (50 Mg/ha) (3 replicate plots)	
	4× agronomic rate for N (100 Mg/ha) (3 replicate plots)	
full-scale field study	urban site (control) (3 replicate samples)	corn stover; corn grain
	urban site (1× agronomic rate for N) (3 replicate samples)	
	urban site (2× agronomic rate for N) (3 replicate samples)	
	rural site (control) (3 replicate samples)	
	rural site (0.5× agronomic rate for N) (3 replicate samples).	

Extraction and PFAA Analysis. *Sample Extractions.* 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 dichloromethane (DCM) and 99:1 (v/v) methanol (MeOH) and ammonium hydroxide was chosen based on the exhaustive extraction results of Yoo et al.[19] The solvent mixture (7 mL) was added to the sample and heated (30 °C) in a sonication bath (Fisher Scientific FS110H, Pittsburgh, PA) for 30 min followed by shaking (VWR 5000 STD 120 V, West Chester, PA) for 1 h. The sample was centrifuged (Eppendorf 5810, Hamburg, Germany) 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 was evaporated at 50 °C under nitrogen (Organomation Associates Inc. N-EVAP 112, Berlin, MA) 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 °C) 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 °C 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 ENVI-Carb. 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 °C overnight (at which time no additional change in mass was observed). Soil samples were extracted as per established protocols.[28] Additional details as to the soil extraction procedure can be found in the SI.

PFAA Analysis. 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 (Kyoto, Japan). 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 (Phenomenex, Torrance, CA) 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 (MDS Sciex, Ontario) operating in negative electrospray ionization scheduled multiple reaction monitoring (MRM)

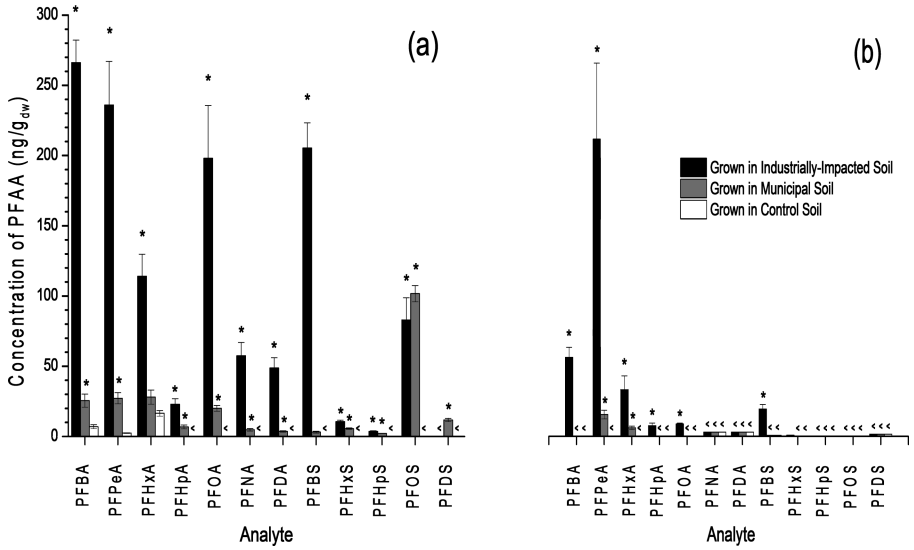

Figure 1. Concentrations of PFAAs in greenhouse lettuce (a) and tomato (b) grown in biosolids-amended soils. Mean and standard error are shown (n = 5). Values marked with an asterisk are significantly different (α = 0.05) than the control. Values less than the LOQ are denoted by <; LOQs for respective matrix and analyte are listed in SI Table S5.

mode was used to monitor two MRM transitions for all analytes.

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 = 3−18). 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 (SI Table S1) 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 (SI Figure S1) with no clear chain length dependent trends among analytes.

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:

$$\mathrm{BAF} = \frac{\text{PFAA concentration in plant } (\text{ng g}_{\text{dw}}^{-1})}{\text{PFAA concentration in soil } (\text{ng g}_{\text{dw}}^{-1})} \quad (1)$$

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:

$$\mathrm{BAF}_{oc} = \mathrm{BAF} \times f_{oc} \quad (2)$$

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 the SI.

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 (α = 0.05); homogeneity of variance was assessed by Levene's Test (α = 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.

RESULTS AND DISCUSSION

Greenhouse Study. Although the control soil was obtained from an unamended field, trace levels of PFAAs (<0.5 ng/g; SI Table S5) 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

Table 2. Summary of Bioaccumulation Factors (BAFs) for PFAAs in All Three Phases of This Study and Previous Study (Values Not Measured Are Designated as NM)[a]

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
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	0.25 ± 0.10
PFNA	0.77 ± 0.15	2.85 ± 0.47	<LOQ	<LOQ	<LOQ	<LOQ	0.12 ± 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

[a]BAFs were not calculated if analyte concentrations were below LOQ and are denoted by < LOQ. Data are shown as means and standard errors (n = 3–5).

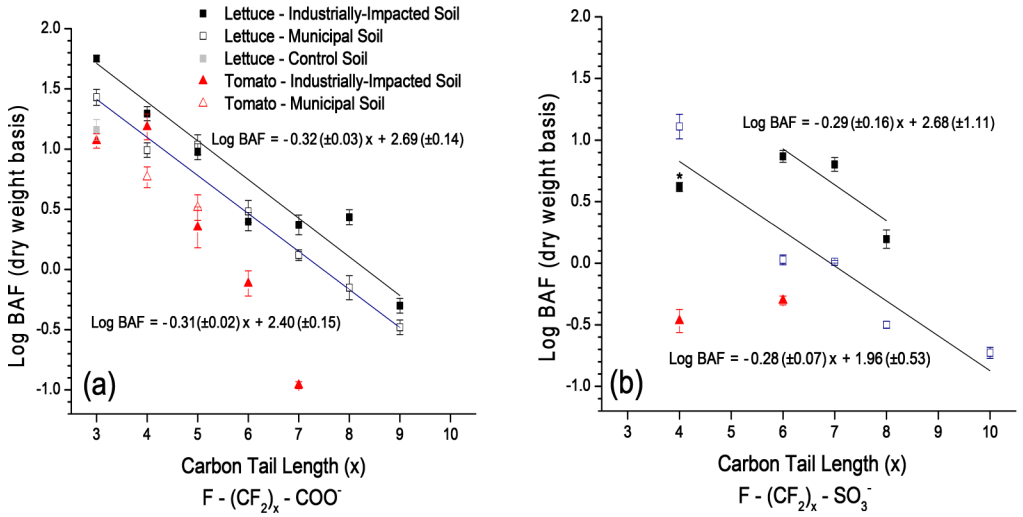

Figure 2. Correlations between log BAF for PFCAs (a) and PFSAs (b) and carbon tail length in greenhouse lettuce and tomato grown in biosolids-amended and control soils. Means and standard errors are shown (n = 5). Linear regressions with slopes, intercepts, and associated error values are shown for lettuce in industrially impacted and municipal soils; the data point marked with an asterisk is excluded from the regression calculation. Regressions for tomato BAFs were not performed.

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 (SI Table S5).

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 1a). 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 1a). 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× 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 1a). 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 (α = 0.05) than the control for all 11 analytes detected above the LOQ (SI Table S5), and lettuce grown in the municipal soil was different than the control for 10 of the 12 analytes (Figure 1a).

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 1b). In the tomatoes grown in industrially impacted soil, the highest levels were measured for PFPeA (211.4 ng/g), PFBA

dx.doi.org/10.1021/es403094q | Environ. Sci. Technol. 2013, 47, 14062–14069

(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, respectively), despite the fact that the soil concentration of PFOS in the municipal soil was 319 ng/g (SI Table S5).

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). When log BAFs were plotted versus carbon chain length for PFCAs (Figure 2a) and PFSAs (Figure 2b), 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). 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 (α = 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 0.5–0.6 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) versus the calculated one of 33.1 (equation in Figure 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, BAFs for PFCAs and PFSAs in lettuce were, in general, slightly higher in the industrially impacted soil than in the municipal soil (~0.3–0.8 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 (SI Figure S2). 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 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 2a). 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 0.03[14] 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 0.5–0.9 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 homologues, particularly if there is a concentration ceiling on the passive transport process or if there are other contributing barriers to transport. 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 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]

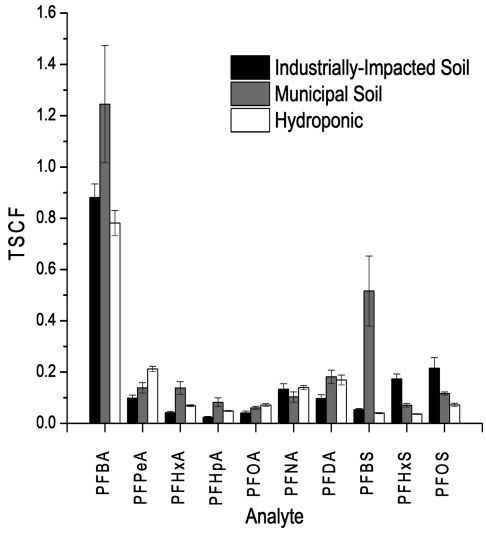

Figure 3. Comparison of transpiration stream concentration factors (TSCFs) for lettuce calculated from this study compared to TSCFs from a previous hydroponic lettuce study.[20] Mean and standard error (n = 5) are shown.

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 (SI Table S6). 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 (SI Table S6). 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 (SI Table S6). 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 SI Table S6. 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.

A trend suggesting an inverse relationship between BAFs and chain length was seen for PFCAs in both the field trial lettuce and tomato (SI Figure S3). 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 0.4, 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 0.1−0.3 log units per CF_2 moiety, similarly but less closely correlated to the greenhouse grown tomatoes (0.5−0.9 log units per moiety).

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 (SI Table S7). All PFAA corn grain concentrations were below the LOQ (SI Table S7). 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 (SI Table S7). This preferential accumulation in the vegetative compartment is consistent with the findings of Stahl et al.[13] In addition, the findings reiterate the consistent bioaccumulation of the short chain PFCAs 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 along with grass-soil accumulation factors from Yoo et al.[19] In the absence of other studies for comparison, the similarity of corn stover to grass was 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.

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, although 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 than unity (i.e., 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;[35] 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.

■ ASSOCIATED CONTENT

Supporting Information

Additional details are available regarding analytical methods, soil characteristics, greenhouse experiment details, sampling details, the soil extraction procedure, PFAA concentrations in soils and crops, log and normalized plots of BAFs, and TSCF calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The information in this document has been funded by the U.S. Environmental Protection Agency. It has been subjected to review by the Region 5 Office and the Office of Research and Development (ORD) and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was funded by a RARE grant from the U.S. EPA and was supported by efforts from the Metropolitan Water Reclamation District of Greater Chicago and the New Lenox Wastewater Department. We appreciate the help of various U.S. EPA staff and, in particular, Lee Thomas (Region 4), Carole Braverman, Bradley Grams, Gerald Golubski, Kenneth Gunter, Erin Newman, Thomas Poy, David Schroeder (Region 5), Andy Lindstrom, Mark Strynar, and John Washington (ORD). We also acknowledge the help of Erin Sedlacko, Lisa Kudryk, Amanda Hering, and Karen Kazor from CSM.

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