Underestimation of PFAS in Biosolids: Precursor Transformation During Conventional Treatment

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Section 1: Sample collection

A detailed sampling protocol and quality assurance project plan (QAPP).

1.0 Sample collection materials

Due to the nature of Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) all sampling equipment containing Teflon or Teflon parts will be avoided. Items with water and/or oil repellants will be avoided. Sample collection containers are to be made of high-density polyethylene (HDPE). Labels shall not be made from water resistant paper. Nitrile gloves are to be worn when handling the bottles. The individual collecting the samples is not to wear water resistant clothing, shoes, or rain gear to avoid contamination during sample collection.

Depending upon the nature of the sampling location, biosolid and compost material samples might be collected using a methanol rinsed stainless steel shovel and placed into a 5-gallon HDPE sample container or directly placed into a 5-gal HDPE bucket from a sampling port.

1.1 Sample collection for dewatered biosolids and compost material

For each biosolid sample, one 5-gallon HDPE sample container will be filled (approximately 15 kg) at the facility and transported to the University of Florida lab. A portion of each sample will be aliquoted into 2.5-quart HDPE buckets and frozen. The remainder of the sample will be stored for no longer than 14 days and tested for pH and moisture content using (EPA Method 9040C) and (ASTM D 2974-87), respectively.

In some scenarios, dewatered biosolids are transported to a separate facility for processing. The sample collection process is the same as a biosolid treatment facility except for compost. Composted biosolids are mixed with stabilization material such as mulch, animal bedding, or yard waste. Samples of stabilization materials shall be collected in one 5-gallon HDPE sample container that will be filled (approximately 12 kg) at the facility and transported to the University of Florida lab. A portion of each sample will be aliquoted into 2.5-quart HDPE buckets and frozen, based on the protocol for PFAS holding.

If PFAS analysis cannot be completed before the 28-day holding time limit, samples are to be frozen for long term storage at less than -10 °C for up to one year.

1.2 Sample and trip blanks

The inclusion of blanks is critical when collecting samples for PFAS analysis given the ubiquity of the chemical. The sample blanks serve to confirm the PFAS was in fact from the biosolids sample. If blanks are positive, sources can include the deionized water used to create the blank, contamination during the process used to transport the bottles, or contamination from the surrounding air at the facility. For each biosolid facility, a blank is prepared with deionized water. The deionized water is to come from a system that does not have Teflon parts. The sample

blank bottle with deionized water is then opened near the sample collection immediately prior to the biosolid sample collection. The bottle is then poured into an empty HDPE bottle and then closed immediately after the biosolid sample is collected.

In addition to sample blanks, a trip blank will be prepared. The trip blank will consist of a 0.25 L bottle with deionized water. It will be taken on the biosolid sampling trip but will not be opened during biosolid sampling.

Table S1 Information on the pathogen removal process utilized by each facility sampled

Sample name	Treatment description	Samples collected	Dry tonnes produced each year	Notes
Facility 1	1:2 Ratio of biosolids to yard waste/ animal bedding. Windrows are left outside to weather for 45 days where internal temperatures can reach 60^{o} C. Piles are inoculated with thermophilic *actinomycetes* during initial mixing. Piles are screened to ¾ inch for final processing.	Biosolids prior to mixing	2,700	Piles are exposed to rainfall and uncovered.
		Mixture of animal bedding and yard waste (composting material)		
		Post Treatment Compost (aged biosolids and composting material mixed)		
Facility 2	1:2 Ratio of biosolids to yard waste. Windrows are left outside to weather for 45 days where internal temperatures can reach 60^{o} C. Windrows are covered by an awning to shield the mixtures from rain. Piles are screened to ¾ inch when aging is completed.	Biosolids prior to mixing	4,000	Piles of raw and final product are left uncovered outside but after mixing the aging process is done on a covered surface.
		Mixture of animal bedding and yard waste (composting material)		
		Biosolids prior to mixing		
Facility 3	Biosolids are solar dried for 14 days going from ~12% solids to ~60%. Then biosolids go into a natural gas thermal chamber for pasteurization at 90 -120^{o} C for 2 hours and exit at a ~90% solids content.	Pre-heat treatment biosolids	2,700	Accepts biosolids from multiple wastewater treatment plants (WWTP) but samples were collected from the same WWTP pre and post - heat treatment.
		Post – heat treatment biosolids		

Sample name	Treatment description	Samples collected	Dry tonnes produced each year	Notes
Facility 4	Wet cake is centrifuged before entering a natural gas fueled dryer drum. Hot air is passed over the biosolids (90 -120^{o} C) until they reach ~90% solids content and exit the drum via a cyclone separator.	Pre-heat treatment biosolids Post – heat treatment biosolids	20,000	Accepts biosolids from multiple WWTPs but samples collected were a blend of all biosolids processed for that day pre and post - heat treatment.
Facility 5	Dewatered sludge is mechanically mixed with CaO (quicklime) until a pH of 12 is reached. Sulphamic acid is added to the mix to increase pH and the temperature to 60^{o} C for a forty-minute retention time.	Pre-Lime treatment biosolids Post-Lime treatment biosolids	8,200	This is the only facility where the final product is a liquid biosolid.
Facility 6	Lime is added as a stabilizing agent (pH < 12) while biosolids are dried by a combination of solar and a natural gas burner to reach a 90% solids content.	Pre-Lime amended biosolids Post-Lime amended biosolids	4,500	Uses a natural gas burner to treat biosolids after lime addition.
Facility 7	Belt filter pressed biosolids are fed into a two stage digestor: mesophilic (35°C)/thermophilic(50°C) for a retention time of ~ 20 days. Post digestion biosolids are then dried by a belt filter press to reach 40% solids content.	Pre- Anaerobic Treatment Post-Anaerobic Treatment	6,300	Uses a belt press to dry the biosolids that have passed through the anaerobic digestor.
Facility 8	Belt filter pressed biosolids are fed into a two stage digestor: mesophilic(35°C)/thermophilic (50°C) for a retention time of ~ 20 days. Post digestion biosolids are then dried by natural gas burner (90 -120^{o} C) until 90% solids content.	Pre- Anaerobic Treatment Post-Anaerobic Treatment	11,000	Uses heat treatment to dry biosolids that have made it through the anaerobic digestor.

Section 2: Studies to characterize PFAS in biosolids

Table S2 Table summarizing information from previous studies regarding PFAS analysis and results compared to this study.

Author	# of biosolids	# of PFAA quantified	# of PFAA Precursors quantified	# of Species detected	Range of PFOS concentrations ng-g^{-1} dry	Range of PFOA concentrations ng-g^{-1} dry
This study	16	20	72	25	4.0 – 40	1.7 – 21
Venkatesan , 2001	94	13	0	10	308 – 618	12 – 70
Sinclair, 2006	6	11	3	14	< LOD – 65	18 – 241
Shultz, 2006	1	11	4	15	81 – 160	8 – 15
Loganathan, 2007	2	8	0	8	8 – 110	16 – 219
Yu, 2009	2	2	0	2	31 – 702	18 – 69
Yoo, 2009	3	10	0	10	32 – 418	20 – 128
D'eon, 2009	6	5	8	13	.1 – 460	.1 – 3.9
Navarro, 2016	16	17	3	9	< LOD – 84	<LOD – 14
Lazcano, 2019	4	17 30*	0	20	6 – 14	0.7 – 1.3
Gallen, 2016	14	9	0	9	10 – 370	.26 – 30
Erikson, 2015	3	24	20	40	.2 – 8.1	< LOD – 2.9
Sepulvado, 2011	6	14	2	16	80 – 219	8 - 68
(EGLE), 2020 *Lower impacted	35	18	6	22	3 – 90	< LOD – 18
(EGLE), 2020 *Higher impacted	7	18	6	22	1,060 – 2,150	< LOD – 5
Schaefer, 2022	7	18	34	39	0.8 – 8.1	0.4 – 150
Moodie, 2021	19	20	24	34	0.9 – 193	<0.3 – 45

*In the Lazcano study 30 additional PFAA-precursors underwent screening for identification.

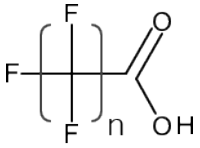
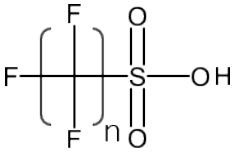
Section 3: Instrumentation analysis

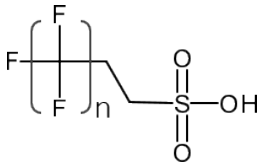
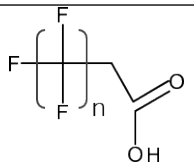
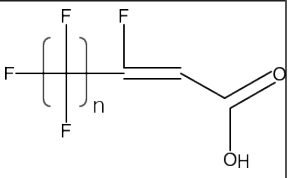
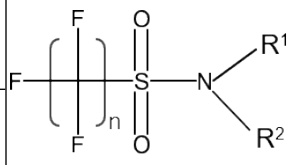
A Gemini C18 (100 x 2 mm; 3µm) column from Phenomenex was used for chromatographic separation. Water [A] and methanol [B] both containing 5 mM of ammonium acetate were used as the mobile phase. The gradient elution was set as 0-3 min 10% B, 3-4.5 min 10-35% B, 4.5-12.5 min 35-95% B, 12.5-12.51 min 95-99 % B, 12.51-19 min 99%, and then equilibrated to initial conditions in 30 min. The temperature of the autosampler was 4 °C, the flow rate was 0.5 mL min^{-1}, and injection volume was 10 µL. Water, methanol, and ammonium acetate used in the study were all Optimal grade purchased from Fisher Scientific. Scheduled selected reaction monitoring scan (SRM) mode (monitoring two transitions, if possible) was used to detect and quantify each PFAS compound (native and mass-labeled species are shown in Table S3.0). The most intense transition was used for quantification, while the second transition was used to confirm identification (if applicable). Table S3.1 displays additional LC/MS/MS parameters while Table S3.2 displays the mass spectrometric scan parameters (and transitions) for all targeted analytes.

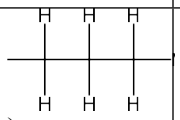
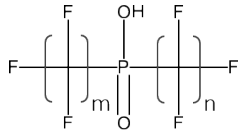
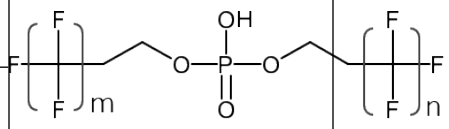
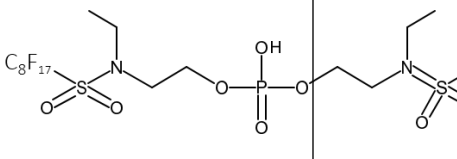
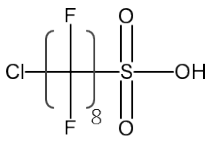
For quantification, a calibration curve that ranged from 10 ng L-1 to 100,000 ng L-1 (14 levels) was developed for all 51 PFAS through serial gravimetrically-weighed dilutions of primary stock solutions. Calibration information for each PFAS was derived and tailored using the prepared calibration levels. A mixture of 24 mass-labeled PFAS internal standards (see Table S3.0) at concentrations of ~ 800 ng L-1 was also added to each calibration level. All PFAS standards were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). The most intense transition was used for quantification.

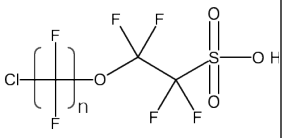
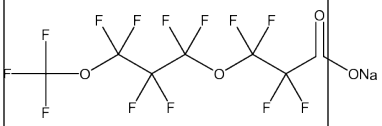
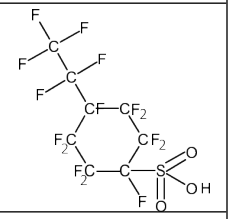
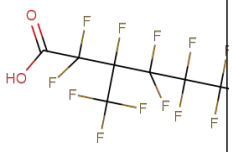
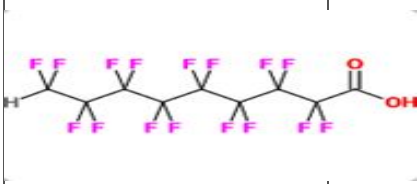
The methodological limit of detection (LOD) and limit of quantification (LOQ) were defined as the peak area of analyte that yielded a signal-to-noise (S/N) ratio of 3 and 10, respectively. Since the original 17 mL sample was extracted and evaporated to 5 mL for analysis, the LOD and LOQ of each original sample was determined using the LOD and LOQ of the instrument multiplied by the ratio between the final volume of extract being analyzed (5 mL) and the original volume of each leachate sample (17 mL). Table S4.8 shows the extraction efficiencies of each reported compound.

Table S3 Acronyms and structures of PFAS and the corresponding internal standard (IS) used for quantitation (concentration in the stock solution shown). When no mass-labeled compound is available, the mass-labeled compound with the closest retention time was selected.

Type	Group	Acronyms	Internal Standards	Internal Standards Concentrations (ng g^{-1})	Structure
Terminal PFAS	Perfluoroalkyl carboxylic acids (PFCA)	PFBA (n+1=4)	M4PFBA	22.1	
		PFPeA (n+1=5)	M5PFPeA	22.1	
		PFHxA (n+1=6)	M5PFHxA	22.1	
		PFHpA (n+1=7)	M4PFHpA	22.1	
		PFOA (n+1=8)	M8PFOA	22.1	
		PFNA (n+1=9)	M9PFNA	22.1	
		PFDA (n+1=10)	M6PFDA	22.1	
		PFUnDA (n+1=11)	M7PFUnDA	22.1	
		PFDoDA (n+1=12)	MPFDoDA	22.1	
		*PFTrDA (n+1=13)	d5-N-EtFOSA-M	111	
		PFTeDA (n+1=14)	M2PFTeDA	22.1	
		*PFHxDA (n+1=16)	M2PFTeDA	22.1	
		*PFODA (n+1=18)	M2PFTeDA	22.1	
	Perfluoroalkyl sulfonic acids (PFSA)	PFPrS (n=3)	M5PFPeA	22.1	
		PFBS (n=4)	M3PFBS	20.5	
		*PFPeS (n=5)	M3HFPO-DA	111	
		*PFHxS (n=6)	M3HFPO-DA	111	
		*PFHpS (n=7)	M2-6:2FTS	21.0	
		PFOS (n=8)	M8PFOS	21.2	
		*PFNS (n=9)	M2-8:2FTS	21.2	
		*PFDS (n=10)	d5-N-EtFOSAA	22.1	

Type	Group	Acronyms	Internal Standards	Internal Standards Concentrations (ng g^{-1})	Structure
		*PFDoDS (n=12)	MPFDoDA	22.1	
PFAA-precursors	Fluorotelomer sulfonic acids	4:2 FTS (n=4)	M2-4:2FTS	20.7	
		6:2 FTS (n=6)	M2-6:2FTS	21.0	
		8:2 FTS (n=8)	M2-8:2FTS	21.2	
		*10:2 FTS (n=10)	MPFDoDA	22.1	
	Saturated fluorotelomer carboxylic acids (FTCA)	*FHEA or 6:2 FTCA (n=6)	M4PFHpA	22.1	
		FPePA or 5:3 FTCA (n = 5)	MFOEA	23.0	
		FHpPA or 7:3 FTCA (n = 7)			
		FOEA or 8:2 FTCA (n=8)			
		*FDEA or 10:2 FTCA (n=10)	M7PFUnDA	22.1	
	Unsaturated fluorotelomer carboxylic acids (FTUCA)	*FOUEA or 8:2 FTUCA (n=7)	MFOEA	23.0	
		*FDUEA or 10:2 FTUCA (n=9)	M7PFUnDA	22.1	
	Perfluoroalkane sulfonamido substances	*FBSA (n=4, R^1=H, R^2=H)	M3PFBS	20.5	
		*FHxSA (n=6, R^1=H, R^2=H)	M3PFHxS	20.9	
		*AP-FHxSA (n=6, R^1=H, R^2=	M2-6:2FTS	21.0	

Type	Group	Acronyms	Internal Standards	Internal Standards Concentrations (ng g^{-1})	Structure
		)			
		FOSA (n=8, R^1=H, R^2=H)	M8FOSA	22.1	
		MeFOSA (n=8, R^1=H, R^2= CH_3)	d3-N-MeFOSA-M	118	
		EtFOSA (n=8, R^1=H, R^2= C_2H_5)	d5-N-EtFOSA-M	111	
		*FOSAA (n=8, R^1=CH_2COOH, R^2= H)	M2-8:2FTS	21.2	
		MeFOSAA (n=8, R^1=CH_2COOH, R^2= CH_3)	d3-N-MeFOSAA	22.1	
		EtFOSAA (n=8, R^1=COOH, R^2= C_2H_5)	d5-N-EtFOSAA	22.1	
	Perfluoroalkyl phosphinic acids (PFPi)	*6:6 PFPi (m=6, n=6)	MPFDoDA	22.1	
		*6:8 PFPi (m=6, n=8)	M2PFTeDA	22.1	
	Polyfluoroalkyl phosphate diester (diPAP)	*6:2 diPAP (m=6, n=6)	M2PFTeDA	22.1	
		*8:2 diPAP (m=8, n=8)	M2PFTeDA	22.1	
		*6:2/8:2 diPAP (m=6, n=8)	M2PFTeDA	22.1	
	Polyfluorooctane sulfonamide phosphate diester	*diSAmPAP	M2PFTeDA	22.1	
Other PFAS	Chloro-perfluorooctane sulfonic acids	*8Cl-PFOS	M8PFOS	21.2	

Type	Group	Acronyms	Internal Standards	Internal Standards Concentrations (ng g^{-1})	Structure
	Polyfluoroether sulfonic acids (PFESAs)	*9Cl-PF3ONS or 6:2 Cl-PFESA (n=6, trade name F-53B major)	M2-8:2FTS	21.2	
		*11Cl-PF3OUdS or 8:2 Cl-PFESA (n=8, trade name F-53B minor)	d5-N-EtFOSAA	22.1	
	Perfluoroether carboxylic acids (PFECAs)	*NaDONA	M4PFHpA	22.1	
	Cyclic perfluorinated acid	*PFECHS	M2-6:2FTS	21.0	
	Perfluorodimethyloctanoic acid	Syn 35	MFOEA	23.0	
	Hexadecafluorononanoic acid	Oak 10	M4PFHpA	22.1	

*- the isotopically labeled standard is either not available or had poor response. The isotopically labeled standard that was similar by structure or retention time (or provided the most stable response within the batch) was selected.

Table S4 LC/MS/MS operation conditions

Mass Spectrometry Analysis – Thermo Quantis	
Ion Source Parameters	Electrospray – Negative Mode
	Ion Spray: 1500 V
	Sheath Gas: 50 Arb
	Auxiliary Gas: 10 Arb
	Ion Transfer Tube Temperature: 250 °C
	Vaporizer Temperature: 550 °C
Chromatography Conditions – Thermo Scientific Vanquish UHPLC	
LC Conditions	Column: Gemini Phenomenex 100 x 2 mm 3µm
	Gradient Elution using Water and Methanol both containing 5 mM of ammonium acetate
	Oven Temperature 40 °C
	Flow Rate: 0.5 mL min^{-1}
	Injection Volume: 10 µL

Table S5 LC/MS/MS parameters for targeted analysis.

Analytes	**Precursor Ion (*m/z*)**	**Fragment Ion (*m/z*)**	**Collision Energy (V)**	**RF Lens (V)**
PFBA	213	169	11	75
M3PFBA	216	172	11	75
M4PFBA	217	172	11	75
L-PFPrS-1	249	80	29	138
L-PFPrS-2	249	99	26	138
PFPeA	263	219	11	64
M5PFPeA	268	223	11	64
FBSA-1	298	78	25	144
FBSA-2	298	119	18	144
L-PFBS-1	299	80	32	152
L-PFBS-2	299	99	28	152
M3PFBS	302	99	29	152
PFHxA	313	269	10	73
M5PFHxA	318	273	10	73
4:2FTS-2	327	81	29	138
4:2FTS-1	327	307	18	138
M2-4:2FTS	329	81	18	138
HFPO-DA	329	285	7	65
M3HFPO-DA	332	287	7	65
FPePA	341	217	23	122
PFPeS-1	349	80	34	174
PFPeS-2	349	99	31	174

Analytes	**Precursor Ion (*m/z*)**	**Fragment Ion (*m/z*)**	**Collision Energy (V)**	**RF Lens (V)**
PFHpA-1	363	169	17	81
PFHpA-2	363	319	11	81
M4PFHpA	367	322	11	81
FHEA	377	293	20	100
NaDONA	377	251	10	280
FHxSA-1	398	78	28	185
FHxSA-2	398	378	20	185
L-PFHxS-2	399	80	37	174
L-PFHxS-1	399	99	35	174
M3PFHxS	402	99	35	151
PFOA-1	413	169	19	94
PFOA-2	413	369	11	94
M2PFOA	415	370	11	94
M8PFOA	421	376	11	94
6:2FTS-2	427	81	30	166
6:2FTS-1	427	407	21	166
M2-6:2FTS-2	429	81	21	166
M2-6:2FTS-1	429	376	21	166
FHpPA	441	316	20	148
Oak 10	445	381	11	120
PFHpS-1	449	80	39	223
PFHpS-2	449	99	38	223

Analytes	**Precursor Ion (*m/z*)**	**Fragment Ion (*m/z*)**	**Collision Energy (V)**	**RF Lens (V)**
FOUEA	457	393	12	100
PFECHS-2	461	99	29	171
PFECHS-1	461	381	26	171
PFNA-1	463	219	16	101
PFNA-2	463	419	12	101
M9PFNA	472	427	12	101
FOEA	477	393	18	100
MFOEA-1	479	394	11	107
N-AP-FHxSA-1	483	169	27	244
N-AP-FHxSA-2	483	319	22	244
FOSA-I-1	498	78	30	206
FOSA-I-2	498	478	23	206
L-PFOS-1	499	80	40	214
L-PFOS-2	499	99	41	214
MPFOS-2	503	80	42	266
MPFOS-1	503	99	42	266
M8FOSA-I-2	503	80	30	196
M8FOSA-I-1	503	486	23	196
M8PFOS-1	507	80	44	259
M8PFOS-2	507	99	44	259
N-MeFOSA-M-2	512	169	26	187
N-MeFOSA-M-1	512	219	24	187
PFDA	513	469	11	94

Analytes	**Precursor Ion (*m/z*)**	**Fragment Ion (*m/z*)**	**Collision Energy (V)**	**RF Lens (V)**
Syn 35	513	469	8	113
8Cl-PFOS-1	515	80	41	273
8Cl-PFOS-2	515	99	40	273
d3-N-MeFOSA-M-2	515	169	26	194
d3-N-MeFOSA-M-1	515	219	24	194
MPFDA	515	470	11	94
M6PFDA	519	474	11	94
N-EtFOSA-M-2	526	169	27	209
N-EtFOSA-M-1	526	219	24	209
8:2 FTS-2	527	487	31	179
8:2 FTS-1	527	507	25	179
M2-8:2 FTS	529	81	25	179
9Cl-PF3ONS-1	531	351	25	155
d5-N-EtFOSA-M-3	531	169	27	203
d5-N-EtFOSA-M-2	531	219	25	203
L-PFNS-1	549	80	43	280
L-PFNS-2	549	99	43	280
FOSAA-2	556	419	23	210
FOSAA-1	556	498	26	210
FDUEA	557	493	21	280
PFUnDA	563	519	11	116

Analytes	**Precursor Ion (*m/z*)**	**Fragment Ion (*m/z*)**	**Collision Energy (V)**	**RF Lens (V)**
N-MeFOSAA-1	570	419	18	178
N-MeFOSAA-2	570	483	14	178
M7PFUnDA-2	570	525	11	116
d3-N-MeFOSAA-1	573	419	19	174
d3-N-MeFOSAA-2	573	483	14	174
FDEA	577	493	8	100
N-EtFOSAA-2	584	419	19	179
N-EtFOSAA-1	584	526	18	179
d5-N-EtFOSAA-2	589	419	19	176
d5-N-EtFOSAA-1	589	531	19	176
L-PFDS-1	599	80	45	280
L-PFDS-2	599	99	47	280
PFDoDA-2	613	319	18	129
PFDoDA-1	613	569	10	129
MPFDoDA	615	570	10	129
10:2 FTS-2	627	81	34	280
10:2 FTS-1	627	607	29	280
11Cl-PF3OUdS	631	451	27	225
SAmPAP-1	650	526	25	280
PFTrDA-2	663	319	19	136
PFTrDA-1	663	619	11	136
L-PFDoDS-1	699	80	55	280
L-PFDoDS-3	699	99	55	280

Analytes	**Precursor Ion (*m/z*)**	**Fragment Ion (*m/z*)**	**Collision Energy (V)**	**RF Lens (V)**
6:6 PFPi	701	401	55	188
PFTeDA	713	669	12	107
M2PFTeDA	715	670	12	107
6:2 diPAP-2	789	97	29	216
6:2 diPAP-1	789	443	17	216
6:8 PFPi-2	801	401	55	188
6:8 PFPi-1	801	50	55	188
PFHxDA-2	813	419	20	167
PFHxDA-1	813	769	12	167
6:2/8:2 diPAP	889	443	20	280
PFODA-2	913	319	25	188
PFODA-1	913	869	13	188
8:2 diPAP-2	989	523	27	280
8:2 diPAP	989	543	20	280
diSAmPAP	1203	526	25	280

*-1 indicates the first transition of the analyte, and -2 indicates the second transition of the analyte; the first transition and the second transition were used for quantitation and confirmation, respectively.

Section 4: Results and Extraction Efficiency

Table S6 Summary results of PFAS concentrations in biosolid processing Facility 1

Compound	Facility 1 Before Treatment		Facility 1 After Treatment	
	Mean ng/g dry	Standard deviation	Mean ng/g dry	Standard deviation
PFPeA	3.7	0.121	17	0.883
PFBS	0.0	N/A	3.3	0.997
PFHxA	6.9	0.107	15	0.519
PFHpA	0.0	N/A	0.0	N/A
Oak 10	0.0	N/A	0.0	N/A
FPePA	17	0.158	2.0	0.181
6:2FTS	0.0	N/A	2.7	0.298
PFOA	20	0.203	4.7	0.104
PFOS	41	0.509	2.1	0.193
PFNA	4.5	0.078	0.60	0.016
Syn35	0.0	N/A	0.0	N/A
8:2FTS	0.0	N/A	0.30	0.028
PFUdA	3.3	0.071	0.47	0.064
6:2diPAP	57	1.227	4.2	0.582
6:2/8:2diPAP	58	0.954	5.9	1.169
8:2 diPAP	83	1.220	6.0	0.497
diSamPAP	0.47	0.019	0.08	0.005
FHEA	0.0	N/A	0.0	N/A
FHpPA	0.24	0.002	0.038	0.003
FOSAA	64	0.544	6.5	1.145
PFDA	30	0.310	4.1	0.120
N-MeFOSAA	11	0.120	1.6	0.119
N-EtFOSAA	4.0	0.084	1.0	0.048
PFDoA	4.1	0.034	1.0	0.090
PFTeDA	1.5	0.011	0.28	0.008
Total PFAS	408.9		78.8	

Table S7 Summary results of PFAS concentrations in biosolid processing Facility 2

Compound	Facility 2 Before Treatment		Facility 2 compost After Treatment	
	Mean ng/g dry	Standard deviation	Mean ng/g dry	Standard deviation
PFPeA	2.7	0.057	26	7.088
PFBS	2.9	0.484	1.3	0.456
PFHxA	3.9	0.131	13	0.823
PFHpA	0.0	N/A	0.68	0.175
Oak 10	0.32	0.036	0.0	N/A
FPePA	25	1.110	2.7	0.302
6:2FTS	0.15	0.012	2.6	0.279
PFOA	4.9	0.039	6.8	0.128
PFOS	4.2	0.428	2.6	0.011
PFNA	0.92	0.026	1.0	0.043
Syn35	0.0	N/A	4.9	0.612
8:2FTS	1.0	0.090	1.1	0.082
PFUdA	0.49	0.008	0.76	0.026
6:2diPAP	48	1.954	28	1.621
6:2/8:2diPAP	52	3.124	27	3.598
8:2 diPAP	52	4.106	23	2.919
diSamPAP	0.24	0.029	0.094	0.016
FHEA	0.0	N/A	0.0	N/A
FHpPA	0.43	0.007	0.039	0.002
FOSAA	9.3	1.439	20	3.764
PFDA	13	0.488	10	0.607
N-MeFOSAA	1.8	0.078	3.1	0.232
N-EtFOSAA	1.2	0.034	1.2	0.116
PFDoA	1.5	0.167	1.8	0.199
PFTeDA	0.33	0.007	0.40	0.049
Total PFAS	227.3		177.8	

Table S8 Summary results of PFAS concentrations in biosolid processing Facility 3

Compound	Facility 3 Treatment		Facility 3 Treatment	
	Mean ng/g dry	Standard deviation	Mean ng/g dry	Standard deviation
PFPeA	3.1	0.128	2.4	0.344
PFBS	4.2	0.432	2.3	0.438
PFHxA	5.1	0.132	4.5	0.244
PFHpA	1.1	0.146	0.0	N/A
Oak 10	0.0	N/A	0.0	N/A
FPePA	130	10.866	52	2.679
6:2FTS	0.17	0.019	0.84	0.448
PFOA	21	0.823	7.7	0.363
PFOS	4.0	1.398	1.4	0.237
PFNA	1.2	0.118	1.1	0.086
Syn35	14	0.410	10	1.308
8:2FTS	0.34	0.064	0.57	0.062
PFUdA	1.3	0.013	1.0	0.068
6:2diPAP	105	0.343	200	17.471
6:2/8:2diPAP	50	3.266	74	22.531
8:2 diPAP	105	5.262	86	5.992
diSamPAP	0.17	0.011	0.20	0.010
FHEA	0.0	N/A	0.0	N/A
FHpPA	1.0	0.063	0.48	0.008
FOSAA	29	1.471	27	5.632
PFDA	28	1.358	18	1.060
N-MeFOSAA	4.6	0.409	4.7	0.289
N-EtFOSAA	2.3	0.109	2.9	0.482
PFDoA	7.3	0.244	3.9	0.351
PFTeDA	0.85	0.036	0.49	0.043
Total PFAS	519.1		501.1	

Table S9 Summary results of PFAS concentrations in biosolid processing Facility 4

Compound	Facility 4 Before Treatment		Facility 4 After Treatment	
	Mean ng/g dry	Standard deviation	Mean ng/g dry	Standard deviation
PFPeA	1.3	0.084	4.6	0.712
PFBS	11	0.604	1.0	0.139
PFHxA	10	0.182	2.2	0.123
PFHpA	0.0	N/A	0.0	N/A
Oak 10	0.0	N/A	0.0	N/A
FPePA	81	1.415	18.6	1.799
6:2FTS	0.46	0.031	0.0	0.025
PFOA	4.3	0.064	2.3	0.121
PFOS	33	0.640	3.3	1.076
PFNA	2.0	0.054	1.5	0.055
Syn35	0.0	N/A	5.1	0.647
8:2FTS	1.4	0.040	0.8	0.075
PFUdA	2.2	0.051	1.3	0.023
6:2diPAP	53	0.461	261.2	38.993
6:2/8:2diPAP	81	1.786	28	N/A
8:2 diPAP	73	0.936	75.4	2.970
diSamPAP	0.32	0.011	0.2	0.008
FHEA	0.0	N/A	0.0	N/A
FHpPA	0.7	0.011	0.17	0.003
FOSAA	40	1.034	25	0.793
PFDA	9.1	0.240	7.6	0.364
N-MeFOSAA	8.9	0.261	6.0	0.805
N-EtFOSAA	5.0	0.184	3.4	0.213
PFDoA	2.1	0.087	2.4	0.258
PFTeDA	0.70	0.028	0.5	0.016
Total PFAS	421.7		450.9	

Table S10 Summary results of PFAS concentrations in biosolid processing Facility 5

Compound	Facility 5 Before Treatment		Facility 5 After Treatment	
	Mean ng/g dry	Standard deviation	Mean ng/g dry	Standard deviation
PFPeA	2.0	0.186	0.0	N/A
PFBS	1.7	0.362	2.1	0.201
PFHxA	1.7	0.136	2.1	0.144
PFHpA	0.0	N/A	0.0	N/A
Oak 10	0.0	N/A	0.0	N/A
FPePA	47	0.920	29	1.845
6:2FTS	0.20	0.017	4.3	0.198
PFOA	1.7	0.047	1.4	0.008
PFOS	4.4	0.523	2.3	0.201
PFNA	0.49	0.046	0.21	0.007
Syn35	0.0	N/A	0.0	N/A
8:2FTS	0.10	0.011	0.0	N/A
PFUdA	0.46	0.014	0.33	0.016
6:2diPAP	52	1.280	28	0.575
6:2/8:2diPAP	24	1.736	7.8	0.135
8:2 diPAP	24	1.502	5.9	0.675
diSamPAP	0.20	0.018	0.14	0.009
FHEA	0.0	N/A	0.0	N/A
FHpPA	0.45	0.008	0.09	0.002
FOSAA	11	0.453	22	2.153
PFDA	5.4	0.169	3.2	0.072
N-MeFOSAA	2.9	0.112	2.2	0.122
N-EtFOSAA	1.4	0.115	0.0	N/A
PFDoA	0.85	0.062	0.38	0.036
PFTeDA	0.29	0.016	0.26	0.009
Total PFAS	182.3		112	

Table S11 Summary results of PFAS concentrations in biosolid processing Facility 6

Compound	Facilty 6 Before Treatment		Facility 6 After Treatment	
	Mean ng/g dry	Standard deviation	Mean ng/g dry	Standard deviation
PFPeA	1.7	0.165	3.8	0.523
PFBS	1.7	0.128	0.0	N/A
PFHxA	6.9	0.543	4.5	0.248
PFHpA	0.61	0.130	0.0	N/A
Oak 10	0.0	N/A	0.0	N/A
FPePA	83	4.587	9.8	0.211
6:2FTS	1.1	0.226	2.4	0.867
PFOA	3.8	0.183	3.2	0.141
PFOS	15	4.224	19	3.403
PFNA	2.2	0.100	0.44	0.043
Syn35	6.3	0.587	4.7	0.751
8:2FTS	1.4	0.117	0.86	0.199
PFUdA	2.3	0.085	0.65	0.019
6:2diPAP	35	2.449	245	11.482
6:2/8:2diPAP	62	2.553	34	2.068
8:2 diPAP	44	1.075	19	1.201
diSamPAP	0.27	0.033	0.22	0.006
FHEA	0.0	N/A	0.0	N/A
FHpPA	0.61	0.021	0.036	0.002
FOSAA	57	7.544	26.0	3.525
PFDA	13	0.671	5.9	0.417
N-MeFOSAA	12	0.346	1.6	0.078
N-EtFOSAA	6.4	0.247	1.9	0.092
PFDoA	3.9	0.163	1.9	0.056
PFTeDA	0.87	0.044	0.50	0.032
Total PFAS	362		385	

Table S12 Summary results of PFAS concentrations in biosolid processing Facility 7

Compound	Facility 7 Before Treatment		Facility 7 After Treatment	
	Mean ng/g dry	Standard deviation	Mean ng/g dry	Standard deviation
PFPeA	0.00	N/A	0.0	N/A
PFBS	1.0	0.190	0.0	N/A
PFHxA	2.8	0.510	1.6	0.038
PFHpA	1.5	0.233	0.0	N/A
Oak 10	0.00	N/A	0.0	N/A
FPePA	105	16.884	65	0.426
6:2FTS	0.00	N/A	0.22	0.023
PFOA	3.5	0.642	1.1	0.018
PFOS	11	0.739	9.1	0.238
PFNA	1.8	0.251	0.90	0.018
Syn35	4.9	0.723	1.3	0.022
8:2FTS	0.00	N/A	0.0	N/A
PFUdA	0.00	N/A	1.2	0.064
6:2diPAP	750	123.348	240	3.073
6:2/8:2diPAP	392	63.456	140	1.455
8:2 diPAP	300	47.377	100	0.494
diSamPAP	2.0	0.364	0.38	0.030
FHEA	0.00	N/A	0.0	N/A
FHpPA	0.94	0.092	0.59	0.005
FOSAA	40	8.087	9.1	0.382
PFDA	7.4	1.371	2.7	0.050
N-MeFOSAA	6.0	0.264	4.4	0.033
N-EtFOSAA	7.6	1.003	3.9	0.062
PFDoA	8.2	1.707	2.0	0.017
PFTeDA	1.8	0.267	0.72	0.018
Total PFAS	1650		584	

Table S13 Summary results of PFAS concentrations in biosolid processing Facility 8

Compound	Facility 8 Before Treatment		Facility 8 After Treatment	
	Mean ng/g dry	Standard deviation	Mean ng/g dry	Standard deviation
PFPeA	13.9	0.425	2.2	0.139
PFBS	0.0	0.645	0.0	N/A
PFHxA	6.7	0.339	1.4	0.042
PFHpA	1.2	0.132	0.32	0.012
Oak 10	0.0	N/A	0.21	0.017
FPePA	24	1.353	21	1.669
6:2FTS	0.0	N/A	0.82	0.150
PFOA	6.3	0.230	4.9	0.361
PFOS	30	2.673	14	6.990
PFNA	2.0	0.037	2.7	0.206
Syn35	0.0	N/A	11	0.544
8:2FTS	0.0	N/A	0.87	0.143
PFUdA	1.7	0.135	1.5	0.044
6:2diPAP	27	1.233	160	34.129
6:2/8:2diPAP	24	1.663	61.9	10.547
8:2 diPAP	21	0.787	59.7	4.503
diSamPAP	0.2	0.006	0.2	0.020
FHEA	0.0	N/A	0.0	N/A
FHpPA	0.5	0.052	0.3	0.023
FOSAA	12	0.639	8.4	1.514
PFDA	12	0.631	24.2	1.893
N-MeFOSAA	5.4	0.200	3.5	0.263
N-EtFOSAA	0.0	N/A	2.5	0.094
PFDoA	2.9	0.189	5.2	0.430
PFTeDA	0.85	0.035	0.87	0.040
Total PFAS	192		388	

Table S14 Extraction efficiencies for pooled samples of untreated and treated biosolids

Compound	Extraction Efficiency (Treated) %	Extraction Efficiency (Untreated) %
PFPeA	80	117
PFBS	93	103
PFHxA	92	76
PFHpA	82	86
Oak 10	57	55
FPePA	99	103
6:2FTS	110	83
PFOA	99	94
PFOS	103	106
PFNA	94	82
Syn35	70	69
8:2FTS	71	84
PFUdA	70	74
6:2diPAP	107	105
6:2/8:2diPAP	93	117
8:2 diPAP	104	147
diSamPAP	53	52
FHEA	107	103
FHpPA	105	101
FOSAA	88	83
PFDA	105	101
N-MeFOSAA	103	94
N-EtFOSAA	70	68
PFDoA	18	18
PFTeDA	79	51