

Supplementary Information (SI) to the article:

Exploring the variability of PFAS in urban sewage: A comparison of emissions in commercial versus municipal urban areas

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1. Analytical Results

Table 1 : PFAS examined in the project, and their corresponding CAS Numbers

CAS	PFAS
375-22-4	PFBA
307-24-4	PFHxA
375-85-9	PFHpA
335-67-1	PFOA
375-95-1	PFNA
335-76-2	PFDA
2058-94-8	PFUnDA
307-55-1	PFDoDA
72629-94-8	PFTrDA
376-06-7	PFTeDA
375-73-5	PFBS
355-46-4	PFHxS
375-92-8	PFHpS
1763-23-1	PFOS
68259-12-1	PFNS
335-77-3	PFDS
757124-72-4	4:2 FTS
27619-97-2	6:2 FTS
39108-34-4	8:2 FTS
2355-31-9	N-MeFOSAA
2991-50-6	N-EtFOSAA
62037-80-3	GenX
958445-44-8	ADONA
73606-19-6	9Cl-PF3ONS / F53 B Major
83329-89-9	11Cl-PF3OUdS / F53 B Minor
31506-32-8	N-MeFOSA
4151-50-2	N-EtFOSA
24448-09-7	N-MeFOSE
1691-99-2	N-EtFOSE

Note: PFOSA (754-91-6), PFPeA (2706-90-3) and PFPeS (2706-91-4) were excluded from the monitoring campaign, given the poor recovery rates in initial stages of the project.

Table 5 Quantitative results of the ammonium-adjusted loads

Substance	Sewer M			Sewer C			Influent		
	25 th	50 th	75 th	25 th	50 th	75 th	25 th	50 th	75 th
PFBA	0.34	0.45	3.52	0.18	0.68	2.60	1.16	2.06	3.64
PFHxA	1.12	2.67	4.38	1.12	2.68	4.37	1.25	2.69	5.71
PFOA	0.26	0.47	0.95	0.47	0.77	1.36	0.63	0.85	1.65
PFBS	0.17	0.19	1.22	0.61	1.49	2.45	0.61	1.50	2.45
PFHxS	0.31	0.38	0.84	0.52	0.77	1.35	0.20	0.43	0.73
PFOS	0.27	0.57	0.74	1.03	1.23	1.59	1.49	2.16	3.16
6:2 FTS	0.25	0.41	0.59	0.72	1.00	1.86	3.60	4.63	5.55

2. Details regarding analytical methods – PFAS

Table 6 Instrument information

Instruments	Manufacturer	Description
LC system	PAL	PAL RTC
	Agilent	Agilent 1260 Infinity II
MS/MS system	Sciex	Qtrap 6500+
Analytical Column	Phenomenex	Phenomenex Luna Omega 3 µm PS C18 ; 150x5 , 100 Å
Delay Column	Phenomenex	phenomenex Luna C18 50x3 mm 110 Å
SPE Tubes	Phenomenex	Strata-X-AW 33µm Polymeric Weak Anion, 200 mg/6 mL

Table 7 Material information

Material	Manufacturer	Description
Polypropylene Vials, 1.5 mL, screw cap	Macherey&Nagel	Short thread vial
Polypropylene Sample Flasks	Azlon	Rinsed with Acid, Base and DW
Pipettes	Eppendorf SE	10 µL- 5000 µL

Table 8 Chemicals used

Chemical	Manufacturer	Description
Methanol	Merck	CAS 67-56-1 (Merck, 20864.290)
Ultra-Pure Water	MiliQ TUW	MiliQ TUW
Ammoniumhydroxyde	Merck	CAS 1335-21-6; (Merck1.05432.1000)

Table 9 Analytical standards

Material	Manufacturer	Description
PFAS-Standards (Mix and Single Substances)	Wellington Laboratories	PFAC30PAR 1 mg/L MXI 1mg/L,
Internal Standards (Mix and Single Substances)	Wellington Laboratories	MPFAC-HIF-IS 1 mg/L MPFAC-HIF-ES 1 mg/L

3. Analytical Process

For extraction and enrichment of samples, a solid phase extraction (SPE) of the samples should be performed. To enrich PFAS in samples an aliquot of 100 mL is spiked with internal standard solution (EIS) and extracted via manual SPE. The exact steps are presented in Table 10.

Table 10 Preparation steps for PFAS analysis

Step	Details
1. Conditioning	<ol style="list-style-type: none">2 mL MeOH2 mL Ultra-Pure Water
2. Loading	<ol style="list-style-type: none">Loading Volume: 100 mL, influent WWTPLoading Speed: 10 mL/min
3. Washing	<ol style="list-style-type: none">1 mL 25 mM Ammonium acetate in Ultra-Pure-Water
4. Drying	<ol style="list-style-type: none">Put cartridges in falcon tubes and centrifuge at 4000 rpm for 6 min.
5. Elution	<ol style="list-style-type: none">2x1 mL MeOH (+1% NH₄OH), each into appropriate glass vessel using pressurized airElution Speed: 0.8 mL/min
6. Transfer	<ol style="list-style-type: none">1 mL of the eluate into a HPLC vial

After application of SPE 2 mL of the eluate with MeOH (+1% NH₄OH) is collected. 1 mL of the eluate was transferred to polypropylene vials (1.5 mL) and spiked with 50 µL of the internal standard solution (NIS).

For direct injection, an external calibration was used for quantification. At least five different concentrations were diluted with methanol from the standard solution. The concentrations in the vial ranged from 50 ng L⁻¹ to 1000 ng L⁻¹. Additionally, the non-extracted internal standards (NIS) and extracted internal standards (EIS) were also added to each calibration solution. EIS was used in order to establish recovery (Figure 2), and NIS was used to establish the initial calibration (Figure 1). The exact assignment of internal standards to native substances is seen in Table 11.

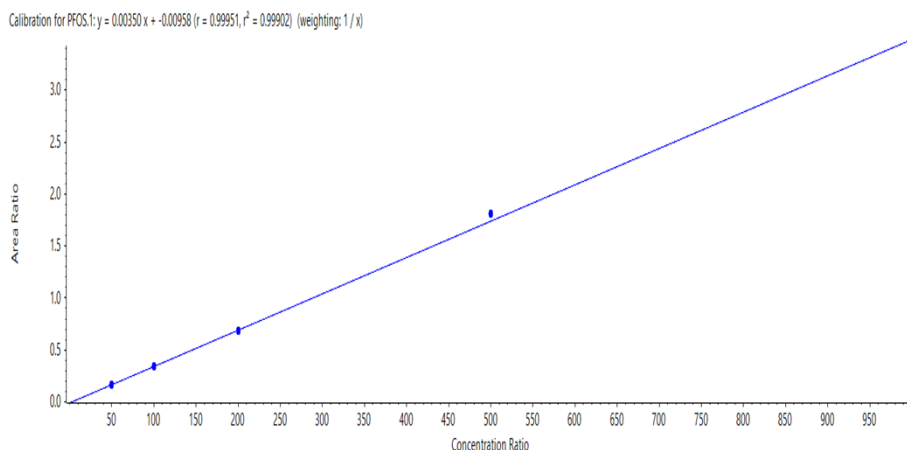


Figure 1 : Example of an external calibration curve using internal standards, per the EPA 1633 norm. The compound, in this case, is PFOS in a wastewater matrix.

Table 11 : Assignment of internal standards (EIS and NIS) to native substance

	Native	EIS	NIS	
Perfluoroalkyl carboxylic acids (PFCAs)	PFBA	M3PFBA_13C4	M3PFBA_13C3	
	PFHxA	M5PFHxA_13C5	MPFHxA_13C2	
	PFHpA	M4PFHpA_13C4		
	PFOA	M8PFOA_13C8	MPFOA_13C4	
	PFNA	M9PFNA_13C9	MPFNA_13C5	
	PFDA	M6PFDA_13C6	MPFDA_13C2	
	PFUdA	M7PFUdA_13C7		
	PFDoA	MPFDoA_13C2		
	PFTeDA	M2PFTeDA_13C2		
	Perfluoroalkyl sulfonic acid (PFSA)	PFBS	M3PFBS_13C3	MPFHxS_18O2
PFHxS		M3PFHxS_13C3		
PFHpS				
PFOS		M8PFOS_13C8	MPFOS_13C4	
PFNS				
PFDS				
Fluorotelomer sulfonic acid (n:2 FTCA)	4:2 FTS	M2-4:2 FTS_13C2		MPFOS_13C4
	6:2 FTS	M2-6:2 FTS_13C2		
	8:2 FTS	M2-8:2 FTS_13C2		
	N-MeFOSA	d-N-MeFOSA_d3		
	N-EtFOSA	d-N-EtFOSA_d5		
Perfluoroalkane sulfonamido acetic acid (FASAA)	N-MeFOSAA	d3-N-MeFOSAA	MPFHxA_13C2	
	N-EtFOSAA	d5-N-EtFOSAA		
	HFPO-DA	M3HPFO-DA_13C3		

Perfluoroether acid (PFECA)	carboxylic	ADONA	MPFDoA_13C2	MPFOA_13C4
		9Cl-PF3ONS	M3PFHxS_13C3	MPFOS_13C4
		11Cl-PF3OUdS	M3PFHxS_13C3	
		N-MeFOSE	d7-N-MeFOSE	MPFOS_13C4
		N-EtFOSE	d9-N-EtFOSE	

After 12 injections, a calibration standard series was made, in order to test the stability of the system and determine the limit of quantification.

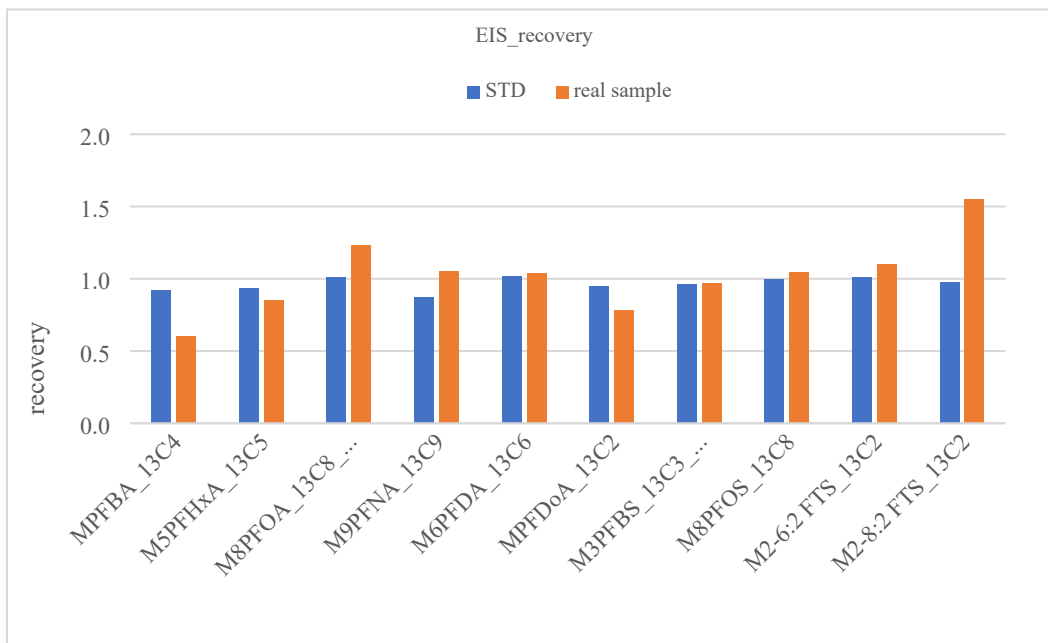


Figure 2: Example of recovery determination for PFAS analysis for standard solutions, versus real samples, according to the EPA 1633 norm.

A PAL RTC sampler was used for injection, an Agilent 1290 Infinity II HPLC (Agilent 2021) pump was used for separation, whereas the Sciex Qtrap 6500+ (SCIEX 2021) with electrospray ionization source was used for measurement. A Phenomenex Luna Omega 3 μm PS C18 100 x 3.0 mm 100 \AA was used at a temperature of 40 $^{\circ}\text{C}$ and an injection volume of 40 μL , for the analytical column. Furthermore, the Phenomenex Luna C18 50 x 3 mm 110 \AA delay column (Phenomenex 2023) was installed. The test and optimisation of injection volume for LCMS system was made (Figure 3).

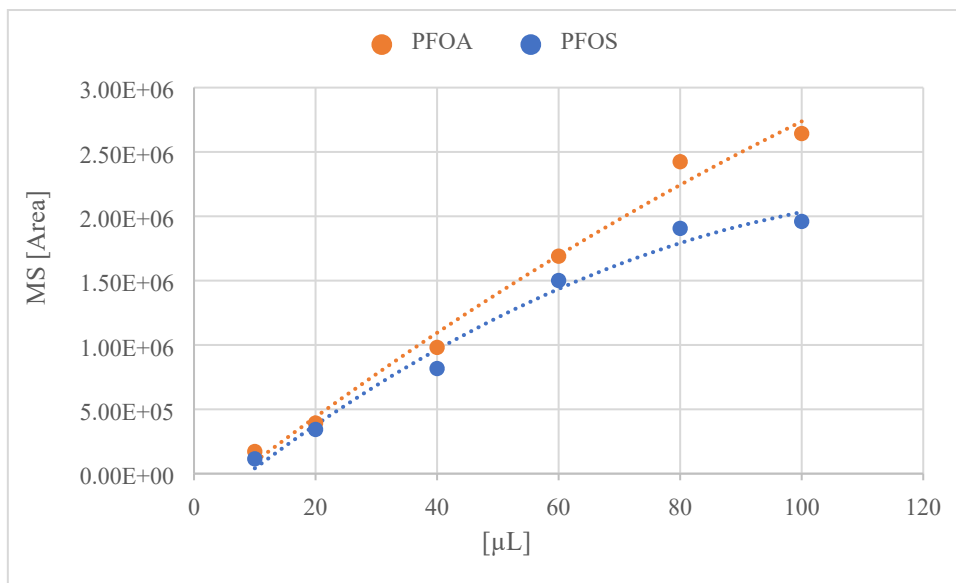


Figure 3 : An example of optimization injection volume for LCMS PFAS analysis

The separation is achieved by using a binary gradient mobile phase consisting of ultra-pure water with 20 mM Ammonium acetate buffer (A) and HPLC-MS grade methanol (B) at a constant flow of 0.6 mL min⁻¹. The conditions of the gradient are shown in Table 12.

Table 12 Gradient conditions for the analysis in this study

Time	Mobile phase A	Mobile phase B	Flow
min	%		mL/min
0.00	90	10	0.600
1.50	35	65	0.600
8.00	5	95	0.600
8.10	1	99	0.600
12.00	1	99	0.600
12.10	90	10	0.600
16.00	90	10	0.600

The mass spectrometer ESI was operating in negative ion mode using multiple reaction monitoring (MRM) for the components, as shown in Table 13. The capillary voltage was set to -3000 V. The sheath gas temperature was 350 °C, and the drying gas temperature was 80 °C.

Table 13 : details regarding mass spectrometry

Name	Q1 mass	Q3 mass		dwell time	DP	EP	CP	CXP
		Fragment Mass 1	Fragment Mass 2					
		(m/z)		ms	V			
PFBA	213	169		10	-5	-10	-12	-9
PFHxA	313	269	119	10	-30	-10	-13	-10
PFHpA	363	319	169	10	-30	-10	-15	-15
PFOA	413	369	219	10	-30	-10	-16	-15
PFNA	463	419	218.9	10	-30	-10	-16	-20
PFDA	513	469	269	10	-30	-10	-18	-20
PFUdA	563	519	269	10	-30	-10	-19	-20
PFDoA	613	569	319	10	-30	-10	-19	-20
PFTTrDA	663	619	169	10	-30	-10	-20	-20
PFTeDA	713	669	169	10	-30	-10	-21	-20
PFBS	299	80	99	10	-30	-10	-64	-4
PFHxS	399	80	99	10	-30	-10	-95	-10
PFHpS	449	80	99	10	-3	-10	-94	-10
PFOS	499	80	99	10	-30	-10	-80	-10
PFNS	549	80	99	10	-30	-10	-107	-15
PFDS	599	80	99	10	-30	-10	-118	-15
4:2 FTS	327	307	81	10	-30	-10	-29	-15
6:2 FTS	427	407	81	10	-30	-10	-35	-15
8:2 FTS	527	507	81	10	-30	-10	-18	-15
N-MeFOSAA	570	419		10	-30	-10	-29	-15
N-EtFOSAA	584	419	526	10	-30	-10	-30	-15
HFPO-DA	285,1	169,1	329	10	-30	-10	-11	-15
ADONA	377	251	85	10	-30	-10	-17	-20
9Cl-PF3ONS	531	351		10	-30	-10	-38	-20
11Cl-PF3OUdS	631	451	199	10	-30	-10	-43	-20
N-MeFOSE	616	59		10	-30	-10	-83	-20
N-EtFOSE	630	59		10	-30	-10	-70	-20

The analysis of the measurements is performed with Sciex Analyst 1.8 and OS Software .

4. Water quality parameters – NH₄⁺ and COD

Table 14 represents the monthly average values of ammonium and chemical oxygen demand for each observed area. Data were obtained from the responsible city authorities, and no analytical method was conducted within the scope of this work, with respect to these parameters. The ammonium-adjusted PFAS per-person loads are presented in Table 15

Table 14 : NH₄-N and COD measurements during the monitoring campaign

Month	Ammonium-Nitrogen [mg L ⁻¹]			COD [mg L ⁻¹]		
	Sewer M	Sewer C	Influent	Sewer M	Sewer C	Influent
Jan	61,1	39,1	52,5	1574	711	762
Feb	61,6	38,5	43,9	1122	949	791
Mar	65,2	45,6	47,7	1195	973	779
Apr	55,8	32,3	39,6	981	509	727
May	63,6	33,2	38,0	1112	637	754
Jun	48,1	27,7	37,2	1582	566	700
Jul	45,3	32,2	36,1	1390	642	666
Sep	34,0	36,6	35,5	1096	818	691
Oct	44,5	36,8	37,7	944	814	684
Nov	49,3	32,6	41,9	1131	999	850
Dec	48,0	34,4	41,8	1334	726	801

Table 15 : Ammonium-adjusted per-person loads

Substance	Sewer	25 th Percentile	50 th Percentile	75 th Percentile
PFBA	C	0.18	0.68	2.60
PFHxA	C	1.12	2.68	4.37
PFOA	C	0.47	0.77	1.36
PFNA	C	0.15	0.17	0.46
PFDA	C	0.09	0.11	0.26
PFBS	C	0.61	1.49	2.45
PFHxS	C	0.52	0.77	1.35
PFOS	C	1.03	1.23	1.59
PFDS	C	0.048	0.053	0.31
6:2 FTS	C	0.72	1.00	1.86
8:2 FTS	C	-	-	-
ADONA	C	-	-	-
PFBA	M	0.34	0.45	3.52
PFHxA	M	1.12	2.67	4.38
PFOA	M	0.26	0.47	0.95
PFNA	M	-	-	-
PFDA	M	0.038	0.046	0.21
PFBS	M	0.17	0.19	1.22
PFHxS	M	0.31	0.38	0.84
PFOS	M	0.27	0.57	0.74
PFDS	M	0.08	0.34	0.77
6:2 FTS	M	0.25	0.41	0.59
8:2 FTS	M	0.07	0.09	0.15
ADONA	M	0.01	0.05	0.12
PFBA	Influent	1.16	2.06	3.64
PFHxA	Influent	1.25	2.69	5.71
PFOA	Influent	0.63	0.85	1.65
PFNA	Influent	0.17	0.18	0.81
PFDA	Influent	0.12	0.18	0.33
PFBS	Influent	0.61	1.50	2.45
PFHxS	Influent	0.20	0.43	0.73
PFOS	Influent	1.49	2.16	3.16
PFDS	Influent	-	-	-
6:2 FTS	Influent	3.60	4.63	5.55
8:2 FTS	Influent	0.17	0.18	0.24
ADONA	Influent	0.03	0.13	0.16

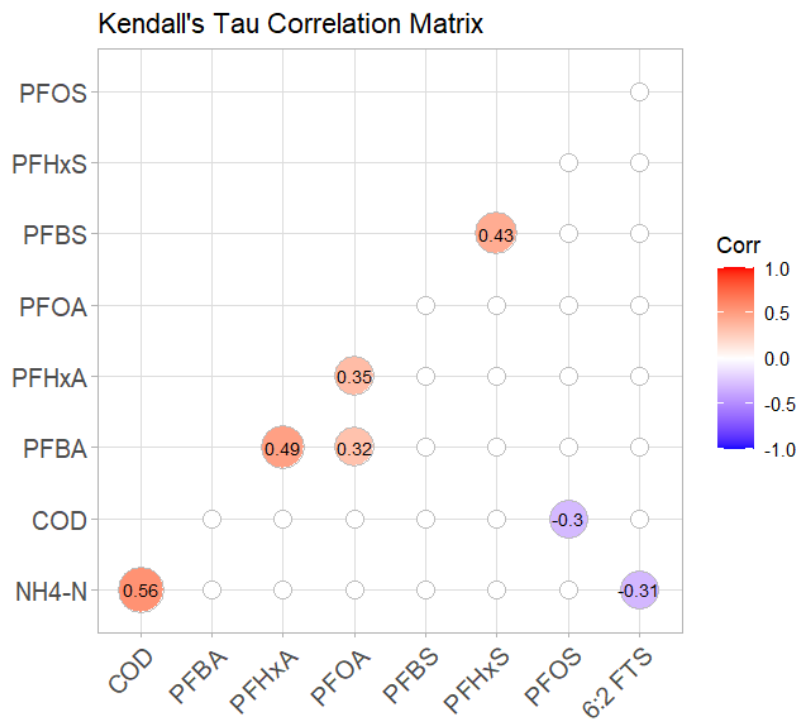


Figure 4 : Kendall's Tau Correlation Matrix, used for the quantification of the PCA results. Only significant correlations ($p < 0.05$) are presented.

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