

Supplementary material

Contrasting macrophyte responses to PFAS-contaminated landfill leachates: implications for bioaccumulation and phytotoxicity

Zanetti A.^a, Pellizzaro A.^b, Fant M.^b, Dal Ferro N.^{a,*}

^a *Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro, Italy*

^b *Acque del Chiampo S.p.A. Società Benefit, Via Ferraretta 20, 36071 Arzignano, Italy*

Table S1. Analyzed PFAAs, acronyms, chemical names, CAS numbers, and molecular formulae.

Item	Acronym	Reference material	CAS number	Molecular formulae
1	PFBA	Perfluorobutanoic acid	375-22-4	C ₄ F ₇ O ₂ H
2	PFPeA	Perfluoropentanoic acid	2706-90-3	C ₅ F ₉ O ₂ H
3	PFHpS	Perfluoroheptane sulfonic acid	3965-99-9	C ₇ F ₁₅ SO ₃ H
4	PFOS	Perfluorooctane sulfonic acid	1763-23-1	C ₈ F ₁₇ SO ₃ H
5	PFBS	Perfluorobutane sulfonic acid	375-73-5	C ₄ F ₉ SO ₃ H
6	PFDA	Perfluorodecanoic acid	335-76-2	C ₁₀ F ₁₉ O ₂ H
7	PFNA	Perfluorononanoic acid	375-95-1	C ₉ F ₁₇ O ₂ H
8	PFDoA	Perfluorododecanoic acid	307-55-1	C ₁₂ F ₂₃ O ₂ H
9	PFHpA	Perfluoroheptanoic acid	375-85-9	C ₇ F ₁₃ O ₂ H
10	PFHxA	Perfluorohexanoic acid	307-24-4	C ₆ F ₁₁ O ₂ H
11	PFUnA	Perfluoroundecanoic acid	2058-94-8	C ₁₁ F ₂₁ O ₂ H
12	PFOA	Perfluorooctanoic acid	335-67-1	C ₈ F ₁₅ O ₂ H
13	PFHxS	Perfluorohexane sulfonic acid	355-64-4	C ₆ F ₁₃ SO ₃ H
Mass-labeled surrogates standard solutions				
16	¹³ C ₄ -PFBA	Perfluoro-n-[1,2,3,4- ¹³ C ₄] butanoic acid	n.d.	¹³ C ₄ F ₇ O ₂ H
17	¹³ C ₄ -PFOA	Perfluoro-n-[1,2,3,4- ¹³ C ₄] octanoic acid	n.d.	¹³ C ₄ ¹² C ₄ F ₁₅ O ₂ H
18	¹³ C ₄ -PFOS	Perfluoro-1-[1,2,3,4- ¹³ C ₄] octane sulfonic acid	n.d.	¹³ C ₄ ¹² C ₄ F ₁₇ SO ₃ H
19	¹³ C ₂ -PFHxS	Perfluoro-n-[1,2- ¹³ C ₂] hexane sulfonic acid	n.d.	¹³ C ₂ ¹² C ₈ F ₂₁ SO ₃ H
20	¹³ C ₂ -PFDA	Perfluoro-n-[1,2- ¹³ C ₂] decanoic acid	n.d.	¹³ C ₂ ¹² C ₈ F ₁₉ O ₂ H
Mass-labeled internal standard solutions				
21	¹³ C ₂ -PFHxA	Perfluoro-n-[1,2- ¹³ C ₂] hexanoic acid	n.d.	¹³ C ₂ ¹² C ₄ F ₁₁ O ₂ H
22	¹³ C ₃ -PFBA	Perfluoro-n-[1,2,3- ¹³ C ₃] butanoic acid	n.d.	¹³ C ₃ ¹² CF ₇ O ₂ H
23	¹³ C ₈ -PFOA	Perfluoro-n-[¹³ C ₈] octanoic acid	n.d.	¹³ C ₈ F ₁₅ O ₂ H
24	¹³ C ₈ -PFOS	Perfluoro-1-[¹³ C ₈] octane sulfonic acid	n.d.	¹³ C ₈ F ₁₇ SO ₃ H
25	¹³ C ₃ -PFBS	Perfluoro-n-[1,2,3- ¹³ C ₃] butane sulfonic acid	n.d.	¹³ C ₃ ¹² CF ₉ O ₂ H
26	¹³ C ₂ -PFDoA	Perfluoro-n-[1,2- ¹³ C ₂] dodecanoic acid	n.d.	¹³ C ₂ ¹² C ₁₀ F ₂₃ O ₂ H

Table S2. Chemicals, reference standard solutions, and suppliers.

Chemical		concentration/purity		supplier		
PFCs calibration standard mix	PFBA	200 mg L ⁻¹		Ultra Scientific (Bologna, Italy)		
	PFP _e A					
	PFBS					
	PFH _x A					
	PFH _x S					
	PFHpA					
	PFOA					
	PFOS					
	PFNA					
	PFDA					
	PFUnA					
	PFUnA					
	PFD _o A					
	PFT _r A					
	PFT _e A					
MPFBA	¹³ C ₄ -PFBA	50 mg L ⁻¹	>98%	Wellington laboratories (Guelph, ON, Canada)		
MPFOA	¹³ C ₄ -PFOA	50 mg L ⁻¹				
MPFOS	¹³ C ₄ -PFOS	48 mg L ⁻¹				
MPFH _x S	¹³ C ₂ -PFH _x S	47 mg L ⁻¹				
MPFDA	¹³ C ₂ -PFDA	50 mg L ⁻¹				
MPFH _x A	¹³ C ₂ -PFH _x A	50 mg L ⁻¹				
M3PFBA	¹³ C ₃ -PFBA	50 mg L ⁻¹				
M8PFOA	¹³ C ₈ -PFOA	50 mg L ⁻¹				
M8PFOS	¹³ C ₈ -PFOS	46 mg L ⁻¹				
M3PFBS	¹³ C ₃ -PFBS	46 mg L ⁻¹				
MPFD _o A	¹³ C ₂ -PFD _o A	50 mg L ⁻¹				
Methanol		LC/MS grade			Carlo Erba (Milan, Italy)	
Ammonium acetate		>98%				

Table S3. Instrument settings.

UHPLC set-up	
Sample injection volume	15 μL
Column	Cortecs C18, 2.1x150 mm, $\text{pd}=1.6\ \mu\text{m}$
Column temperature	45 $^{\circ}\text{C}$
Eluents	A = $\text{CH}_3\text{COONH}_4$ 20 mM; B = CH_3OH
Flow	0.11 mL min^{-1}
ESI source set-up	
Ion mode	ESI (-)
Capillary voltage	0.8 kV
Source temperature	150 $^{\circ}\text{C}$
Desolvation gas flow	1000 L h^{-1}
Desolvation gas temperature	450 $^{\circ}\text{C}$
Cone gas flow	15 L h^{-1}
Collision gas flow	3.5 mL min^{-1}

Table S4. Multiple reaction transition and internal standard (IS) used for all PFAAs considered. Listing follows analyte retention times.

Analyte	Mass transition	Cone voltage (V)	Collision energy (V)	Reference IS
PFBA	213 > 169	18	10	¹³ C ₃ -PFBA
¹³ C₄-PFBA	217 > 172	18	10	¹³ C ₃ -PFBA
¹³ C₃-PFBA	216 > 172	18	10	IS
PFPeA	263 > 219	15	9	¹³ C ₂ -PFHxA
PFBS	299 > 80	54	30	¹³ C ₄ -PFBS
	299 > 99	54	28	
¹³ C₃-PFBS	302 > 99	54	30	IS
PFHxA	313 > 119	16	17	¹³ C ₂ -PFHxA
	313 > 269	16	10	
¹³ C₂-PFHxA	315 > 270	15	15	IS
PFHpA	363 > 119	16	20	¹³ C ₂ -PFHxA
	363 > 169	16	16	
PFHxS	399 > 80	40	40	¹³ C ₂ -PFHxA
	399 > 99	40	40	
¹³ C₂-PFHxS	402 > 80	40	40	¹³ C ₂ -PFHxA
PFOA	413 > 369	16	10	¹³ C ₈ -PFOA
	413 > 169	16	16	
	413 > 219	16	16	
	413 > 119	16	16	
¹³ C₄-PFOA	417 > 372	15	10	¹³ C ₈ -PFOA
¹³ C₈-PFOA	421 > 376	15	10	IS
PFNA	163 > 119	20	18	¹³ C ₈ -PFOA
	163 > 119	20	16	
PFOS	499 > 80	60	39	¹³ C ₈ -PFOS
	499 > 99	60	38	
	499 > 280	60	38	
	499 > 330	60	38	
¹³ C₄-PFOS	503 > 80	78	48	¹³ C ₈ -PFOS
¹³ C₈-PFOS	507 > 80	78	48	IS
PFDA	513 > 219	20	16	¹³ C ₂ -PFOA
	513 > 269	20	16	
	513 > 169	20	16	
¹³ C-PFDA	515 > 470	20	20	¹³ C ₂ -PFOA
PFUnA	563 > 219	20	20	¹³ C ₂ -PFDoA
	563 > 269	20	18	
	563 > 519	20	20	
PFDoA	613 > 169	25	15	¹³ C ₂ -PFDoA
	613 > 319	25	15	
	613 > 569	25	15	
¹³ C-PFDoA	615 > 570	25	15	IS
PFTrA	663 > 169	25	25	¹³ C ₂ -PFDoA
	663 > 219	25	20	
	663 > 619	25	15	
PFTeA	713 > 169	25	25	¹³ C ₂ -PFDoA
	713 > 219	25	20	
	713 > 669	25	15	

Table S5. Analytes and calibration level concentrations (ng L⁻¹).

PFAAs/level:	LEVEL A	LEVEL B	LEVEL C	LEVEL D	LEVEL E	LEVEL F	LEVEL G	LEVEL H	LEVEL I	LEVEL L	LEVEL M
PFBA	20	50	100	200	500	1000	2000	5000	10000	25000	50000
PFFeA											
PFO S											
PFBS											
PENA											
PFHpA											
PFHxA											
PFO A											
PFHxS											
PFDA											
PFUxA											
PFDxA											
PFTTrA											
PFTeA											

Table S6. Surrogates and internal standards concentration levels ng L⁻¹.

LEVELS		LEVEL C	LEVEL D	LEVEL E	LEVEL F
SURROGATES	¹³ C ₄ -PFBA	5911	7813	9709	11583
	¹³ C ₄ -PFOA	14778	19351	24272	28958
	¹³ C ₄ -PFOS	1766	2334	2900	3460
	¹³ C ₂ -PFHxS	5592	7420	9229	11022
	¹³ C ₂ -PFDA	5911	7813	9709	11583
INTERNAL STANDARDS	¹³ C ₂ -PFHxA	6000	7968	9950	11928
	¹³ C ₃ -PFBA	2994	3984	4975	5964
	¹³ C ₈ -PFOA	2940	3904	4875	5845
	¹³ C ₈ -PFOS	2784	3697	4617	5535
	¹³ C ₃ -PFBS	2782	3705	4263	5546
	¹³ C ₂ -PFDoA	2994	3984	4975	5964

Table S7. PFAAs single concentrations per month measured for each raw leachate before pouring it into the tanks, using UPLC-MS/MS (LoQ = 50 ng L⁻¹, except PFOS LoQ = 20 ng L⁻¹).

Month	Leachate	PFBA ng L ⁻¹	PFPeA ng L ⁻¹	PFHxA ng L ⁻¹	PFHpA ng L ⁻¹	PFOA ng L ⁻¹	PFNA ng L ⁻¹	PFDA ng L ⁻¹	PFUnA ng L ⁻¹	PFDoA ng L ⁻¹	PFBS ng L ⁻¹	PFHxS ng L ⁻¹	PFOS ng L ⁻¹	PFAAs _{Tot} ng L ⁻¹
Apr	(A)	687	586	810	498	514	97	<LoQ	<LoQ	<LoQ	8302	220	95	3192
May		4023	4257	3846	2426	1746	107	<LoQ	<LoQ	<LoQ	51803	72	115	16405
Jun		4779	2875	2867	1740	2319	68	<LoQ	<LoQ	<LoQ	50945	40	28	14647
Aug		5154	2125	2554	1862	1553	5	<LoQ	<LoQ	<LoQ	44051	5	51	13253
Sept		3288	2465	2300	993	1269	28	<LoQ	<LoQ	<LoQ	51029	21	30	10343
Apr	(B)	120	151	777	325	393	96	<LoQ	<LoQ	<LoQ	1153	486	111	1862
May		288	295	1356	402	734	51	<LoQ	<LoQ	<LoQ	2348	776	116	3125
Jun		488	482	2171	566	1075	<LoQ	<LoQ	<LoQ	<LoQ	3771	1086	84	4781
Aug		509	606	2446	579	963	<LoQ	<LoQ	<LoQ	<LoQ	3841	1148	48	5102
Sept		499	687	2483	505	851	<LoQ	<LoQ	<LoQ	<LoQ	3684	1190	49	5025
Apr	(C)	199	167	271	199	205	96	<LoQ	<LoQ	<LoQ	609	452	138	1137
May		380	311	748	315	289	<LoQ	<LoQ	<LoQ	<LoQ	2110	2031	154	2043
Jun		393	203	636	158	374	<LoQ	<LoQ	<LoQ	<LoQ	2125	2332	130	1762
Aug		288	132	484	139	226	<LoQ	<LoQ	<LoQ	<LoQ	1646	1574	8	1269
Sept		301	233	616	163	205	<LoQ	<LoQ	<LoQ	<LoQ	1579	1824	3	1518

Table S8. Concentration of ions and total PFAAs in each leachate tank containing different species, measured during the experimental period (mean values, minimum values and maximum values).

Species	Leachate		N-NH4 mg L ⁻¹		N-NO3 mg L ⁻¹		TN mg L ⁻¹		N-NO2 mg L ⁻¹		Cl mg L ⁻¹		SO4 mg L ⁻¹		Ca mg L ⁻¹		Mg mg L ⁻¹		Na mg L ⁻¹		K mg L ⁻¹		B mg L ⁻¹		Cr tot mg L ⁻¹		Fe mg L ⁻¹		P tot mg L ⁻¹		Mn mg L ⁻¹		Cu mg L ⁻¹		Zn mg L ⁻¹		PFAAsTot ng L ⁻¹		
Carex	(A)	mean	min	0.3	1.0	6.7	1.0	0.03	44.0	195.0	83.0	51.0	48.0	44.2	0.1	0.1	0.1	0.03	1.5	0.2	0.02	0.1	0.03	1.5	0.2	0.5	1.2	0.1	0.3	0.0	0.01	0.01	0.01	0.01	65165	7155			
			max	217.4	46.7	316.2	10.2	411.1	945.0	678.8	2105.0	292.3	774.0	178.7	525.0	289.0	600.0	44.2	494.4	0.2	0.1	0.5	2159.7	0.1	12824.0	1.3	0.03	5.5	3.8	0.5	0.3	0.0	0.3	65165	227471				
	(B)	mean	min	0.3	2.3	1.0	150.4	3.7	0.1	0.03	115.0	67.0	76.0	206.7	50.0	63.0	21.0	0.1	0.1	0.1	0.01	0.03	1.1	0.2	0.7	0.0	0.01	0.1	0.01	0.01	0.01	0.01	0.01	0.01	14481	1610			
			max	570.5	22.5	448.1	0.3	1144.9	3090.0	208.3	430.0	243.3	417.0	206.7	486.0	611.6	1701.0	21.0	160.3	0.4	0.1	0.9	0.2	0.4	2.3	0.7	3.2	0.7	0.1	0.04	0.01	0.04	14481	51882					
	(C)	mean	min	0.3	1.8	1.0	726.0	389.0	2.1	0.03	25.0	10.0	146.0	52.0	37.0	25.3	0.7	0.1	0.1	0.01	0.2	0.7	0.9	0.2	4.0	0.8	0.1	0.1	0.01	0.01	0.01	0.01	0.01	0.01	3213	230			
			max	1663.6	7.3	1300.0	8.5	2039.2	3985.0	125.4	315.0	187.4	252.0	121.3	182.0	1197.9	2617.0	57.8	160.3	0.4	1.2	0.2	0.7	0.9	3.1	8.3	2.0	0.1	0.1	0.04	0.01	0.04	3213	7492					
Phragmites	(A)	mean	min	0.3	1.0	6.6	1.3	0.03	66.0	195.0	77.0	50.0	76.0	320.8	77.0	50.0	366.3	76.0	0.3	0.1	0.1	1864.8	0.1	0.03	0.2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	127674	6208		
			max	99.4	5.7	67.2	59.9	220.5	12.9	424.5	965.0	944.1	2300.0	320.8	700.0	189.6	432.0	366.3	745.0	25.9	0.3	0.1	0.6	1864.8	0.1	6005.0	1.2	2.7	1.5	4.3	0.4	0.3	0.04	0.2	127674	829768			
	(B)	mean	min	0.3	2.5	1.0	162.2	7.6	1.8	0.03	110.0	225.0	79.0	65.0	72.0	20.1	0.3	0.2	0.1	0.01	0.1	1.2	0.1	0.8	1.7	0.5	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	24292	3849			
			max	416.2	12.0	395.4	13.5	1304.6	3590.0	489.6	930.0	207.5	349.0	249.1	591.0	728.4	2028.0	20.1	54.4	0.5	1.3	0.1	0.4	1.2	2.9	1.7	0.1	0.03	0.1	0.01	0.03	0.01	0.03	24292	105514				
	(C)	mean	min	0.3	4.2	1.0	346.7	15.9	0.1	0.03	530.0	240.0	155.0	65.0	355.0	34.0	1.6	0.2	0.2	0.03	1.8	0.03	2.6	0.6	0.8	0.2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	7337	1527			
			max	1556.6	44.3	1230.0	0.5	3225.9	6290.0	512.8	810.0	271.1	495.0	176.6	336.0	1933.4	3801.0	95.2	1.2	0.2	0.6	1.8	6.0	6.2	1.4	0.1	0.03	0.1	0.03	0.01	0.03	0.01	0.03	7337	16742				
Typha	(A)	mean	min	0.3	9.8	1.0	76.3	6.2	1.6	0.03	10.0	195.0	99.0	48.0	20.0	17.2	0.1	0.0	0.1	0.03	1750.6	0.1	0.8	0.7	0.2	0.01	0.03	0.1	0.03	0.7	0.2	0.6	0.1	0.01	0.01	0.01	0.01	76490	5745
			max	134.4	62.4	220.6	9.6	149.6	480.0	719.6	1410.0	294.9	507.0	153.2	294.0	115.9	224.0	17.2	153.5	0.1	0.3	0.3	7015.0	0.8	3.3	3.1	0.6	0.1	0.3	0.1	0.2	0.1	0.2	0.1	0.2	76490	189397		
	(B)	mean	min	0.3	1.8	1.0	114.7	5.3	0.1	0.03	10.0	170.0	76.0	56.0	7.0	6.7	0.1	0.03	0.2	0.02	0.1	2.0	0.01	0.8	0.2	0.5	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	17498	4894			
			max	466.0	12.7	369.0	0.2	601.7	1565.0	399.5	645.0	230.1	419.0	174.9	306.0	342.7	909.0	6.7	25.9	0.2	0.7	0.1	0.4	2.0	10.9	2.2	0.5	0.1	0.03	0.1	0.2	0.03	0.1	0.2	17498	79049			
	(C)	mean	min	0.3	1.9	1.0	373.0	11.4	0.1	0.7	62.0	180.0	150.0	52.0	81.0	29.2	0.6	0.1	0.3	0.02	1.3	0.1	4.4	0.3	1.1	0.2	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	15774	11662			
			max	1039.3	11.2	815.7	0.3	2730.7	5595.0	342.4	685.0	251.5	375.0	155.0	279.0	1634.0	3345.0	29.2	62.1	0.4	0.7	0.5	24573	1.3	3.9	11.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	15774	24573				

Table S9. Concentration of each PFAA in the belowground (blw) and aboveground (abv) tissues of the different species under different leachate treatment.

Species	Leachate	PFBA		PFPeA		PFHpA		PFHxA		PFOA		PFBS		PFHxS		PFOS	
		ng g ⁻¹		ng g ⁻¹		ng g ⁻¹		ng g ⁻¹		ng g ⁻¹		ng g ⁻¹		ng g ⁻¹		ng g ⁻¹	
		abv	blw	abv	blw	abv	blw	abv	blw	abv	blw	abv	blw	abv	blw	abv	blw
Carex	(A)	116.9	3.5	49.7	2.3	7.5	2.3	31.6	2.9	7.0	4.7	261.6	89.3	2.5	1.2	1.3	2.1
	(B)	29.9	1.2	11.7	0.9	4.2	1.6	27.5	2.7	3.5	5.1	75.8	27.9	11.9	15.4	2.6	12.1
	(C)	19.3	1.9	6.9	1.1	2.6	1.3	10.8	3.1	2.9	3.3	52.9	18.0	31.9	23.0	2.7	9.8
Phragmites	(A)	25.0	4.9	11.8	2.6	1.6	1.7	5.1	2.5	1.0	4.2	133.8	59.6	0.5	0.7	0.3	1.8
	(B)	9.9	3.8	4.9	1.8	0.9	2.6	7.1	6.6	0.6	2.7	52.2	43.2	4.4	23.2	0.4	9.8
	(C)	1.7	1.2	1.7	0.7	0.9	0.9	2.0	1.3	0.7	2.3	12.6	6.4	8.1	12.0	0.9	5.4
Typha	(A)	28.2	3.5	21.0	2.7	2.4	2.7	10.1	3.4	1.3	6.7	111.3	68.7	0.7	1.0	0.3	2.3
	(B)	40.8	4.4	14.4	1.9	7.5	6.1	22.4	7.4	3.3	13.0	106.9	45.6	10.8	29.1	1.2	18.0
	(C)	4.8	0.9	4.0	0.8	4.1	1.9	5.5	2.3	3.5	4.1	26.0	7.9	17.1	12.9	1.2	7.1