

Supplementary materials (Figure S1 and Tables)

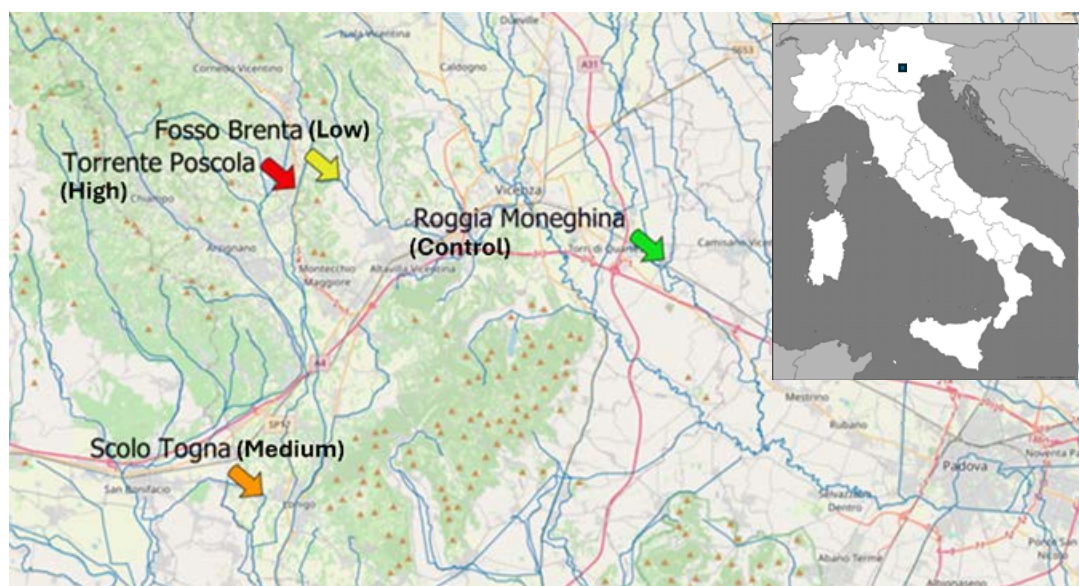


Figure S1. Map of the study area in the Veneto region (Vicenza province), showing Roggia Moneghina as control site, Fosso Brenta as low-polluted site, Scolo Togna as medium-polluted site, and Torrente Poscola as highly-polluted site, indicated by green, yellow, orange, and red arrows, respectively. Gauss-Boaga coordinates of the sites are reported in Table S1.

Table S1. Total PFAS average concentration in freshwater from the four sampling sites, measured by ARPAV in 2022 and 2023. The Gauss-Boaga coordinates, obtained with qGIS (version 3.22) software, are reported for the geo-localisation of the sites.

Sampling sites	Gauss-Boaga coordinates	Total PFAS average concentration (ng/l)
Control site (Roggia Moneghina)	X: 1707494.771; Y: 5043242.570	< 10
Low-polluted site (Fosso Brenta)	X: 1688840.326; Y: 5047241.734	185.263
Medium-polluted site (Scolo Togna)	X: 1685003,474; Y: 5029108,091	855.704
Highly-polluted site (Torrente Poscola)	X: 1686344.594; Y: 5046652.211	1116.14

Table S2. Primer sequences used for qRT-PCR. For all the primers, 60°C was used as the annealing temperature.

Primers	Sequences 5'-3'
CAT_RT_forward	GCGTCCTGAATCGTTGCACCA
CAT_RT_reverse	TGACCCTCAGCGTTGACCAGT
GPx1_RT_forward	TCCATTCCCATTTCGATGACCCAAT
GPx1_RT_reverse	GGCACTCCATCAGAACCGATAAGA
1_FW_GPx4_CAV_RT	AGCGGACATAAAGGAGTTTGCT
1_RV_GPx4_CAV_RT	CCTCTTCACGACCCGACCTT
sq_GAPDH_b_fw	ATCACAGCCACACAGAAGAC
sq_GAPDH_b_rv	AGGAATGACTTTGCCACAG

Table S3. Optimized mass transitions for PFAS and their internal standards, with corresponding cone voltage and collision energy. The first transition was used for quantification and the second for confirmation.

Analyte	MRM transitions (<i>m/z</i>)	Cone voltage (V)	Collision energy (eV)
PFBA	212.0 > 169.0	8	7
	212.0 > 64.7	8	22
PFBS	299.0 > 79.9	22	28
	299.0 > 98.9	22	28
PFPeA	263.0 > 219.0	6	7
	263.0 > 69.0	6	32
PFHxA	313.0 > 269.0	10	7
	313.0 > 118.9	10	18
PFHxS	399.0 > 79.9	20	36
	399.0 > 98.9	20	34
PFHpA	363.0 > 319.0	15	8
	363.0 > 169.0	15	16
PFOA	412.9 > 369.0	15	9
	412.9 > 169.0	15	18
PFOS	498.0 > 98.9	30	40
	498.0 > 79.9	30	45
PFNA	463.0 > 418.9	20	9
	463.0 > 218.9	20	15
PFDA	512.8 > 469.0	20	10
	512.8 > 218.9	20	17
GenX	285.0 > 169.0	12	7
	285.0 > 118.9	12	28
¹³ C ₂ -PFOA	415.0 > 370.0	20	9
¹³ C ₄ -PFOS	503.0 > 80.0	20	9
¹³ C ₃ -GenX	286.0 > 169.0	12	9

Table S4. Concentrations of different PFAS compounds were detected in the organs of *S. cephalus* from the four sampling sites (the total environmental PFAS average concentration, as referenced in Table S1, has been reported). Due to insufficient tissue availability, samples from 8 specimens were pooled. Dashes (-) indicate PFAS not detected.

SITES AND TOTAL PFAS	ORGANS	PFOS (µg/l)	PFOA (µg/l)	PFDA (µg/l)	PFNA (µg/l)	PFHxS (µg/l)	PFBS (µg/l)
CONTROL (< 10 ng/l)	Liver	7.58	-	4.00	-	-	-
	Caudal kidney	14.29	-	5.38	-	-	-
LOW-POLLUTED (185 ng/l)	Liver	-	0.43	2.24	-	-	-
	Caudal kidney	-	1.33	4.11	-	-	-
MEDIUM-POLLUTED (856 ng/l)	Liver	55.40	0.36	3.31	0.062	0.95	0.067
	Caudal kidney	41.87	1.84	10.12	0.25	0.52	0.030
HIGHLY-POLLUTED (1116 ng/l)	Liver	72.78	0.64	21.67	-	0.55	0.15
	Caudal kidney	70.54	0.96	32.90	-	-	-