



# Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XL

## Perfluoroalkyl substances (PFASs), brominated flame retardants (BFRs) and heavy metals: exploring wild fauna contamination levels in different habitat of Central Italy

Ester Lucidi - Tutor: Prof.ssa Anna Annibaldi

Laboratorio Contaminanti Organici, Inorganici e Biotossine, IZSUM  
Laboratorio di Chimica Analitica per l'Ambiente e gli Alimenti, DISVA

### OVERVIEW

Persistent organic pollutants (POPs) and heavy metals, due to their bioaccumulation, biomagnification, and long-range transport properties, spread through air, water, soil, and food. Among them, perfluoroalkyl substances (PFASs), brominated flame retardants (BFRs: polybrominated diphenyl ethers - PBDEs, hexabromocyclododecanes - HBCDs and emerging BFR - eBFRs), regulated metals (Pb, Cd, Hg, As) and emerging elements are gaining increasing attention.

In these first six months of the project, the attention was focused on exploring PFASs and BFRs contamination levels in wild fauna of Marche and Umbria regions.

### METHODOLOGIES

#### SAMPLING



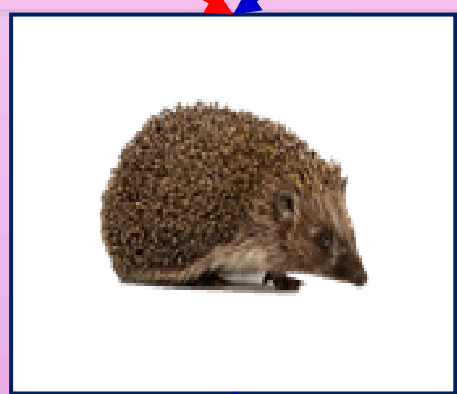
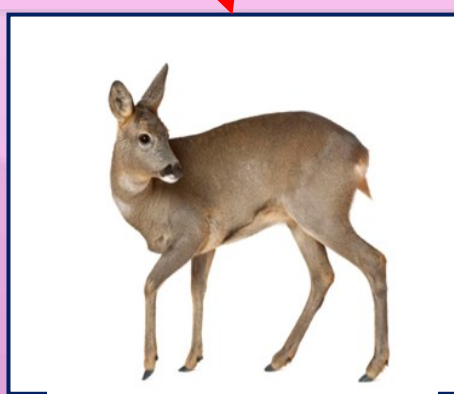
Samplig area: central Apennine Mountains, Marche and Umbria regions (Italy). All specimens were retrieved from Marche and Umbria Wildlife Rescue Centers.

ROE DEER - RD ( <i>Capreolus capreolus</i> )	10	TOT 49 SAMPLES
RED FOX - RF ( <i>Vulpes vulpes</i> )	10	
EUROPEAN HEDGEHOG – EH ( <i>Erinaceus europaeus</i> )	9	
EUROPEAN BADGER – EB ( <i>Meles meles</i> )	12	
WOLF ( <i>Canis lupus italicus</i> )	8	

Species were selected to represent different eating habits and trophic levels. Muscle and liver tissues were collected, homogenized and frozen until analysis.



- Primary consumers (herbivores)
- Secondary consumers (carnivores)
- Tertiary consumers (top-predators)



#### ANALYTICAL METHODS

##### PFASs

- Extraction of 2g sample (spiked with IS) with ACN in ultrasonic bath
- Purification on STRATA X-AW cartridges + d-SPE with Envicarb
- Detection of 20 PFASs by LC-MS/MS

20 PFASs: 11 perfluoroalkyl carboxylic acids PFCAs (PFBA, PFPeA, PFHxA, PFHpA, tot-PFOA, tot-PFNA, PFDA, PFUDa, PFDoA, PFTTrDA, PFTeDA) and 8 perfluoroalkane sulfonic acids (PFASs: PFBS, PFPeS, tot-PFHxS, PFHpS, tot-PFOS, PFNS, PFDS and PFDoS). PFOSA was also included.  
LOQs: 0,010 µg/kg (PFBA: 0,200 µg/kg )

##### BFRs

- QuEChERS-like extraction from 20g sample (spiked with IS)
- Purification on ExtrelutNT3/Si-SPE columns + Gel Permeation Chromatography (GPC)
- Detection of 9 PBDEs and 9 eBFRs by GC-MS/MS
- Detection of 3 HBCDs by LC-MS/MS

9 PBDEs: BDE-28, -47, -49, -99, -100, -138, -153, -183, -209; 9 eBFRs: pTBX, PBBz, PBT, PBEB, HBBz, EHTBB, HCDBCO, BTBPE, DBDPE;  
3 HBCDs: α, β, γ  
LOQs PBDEs: 0,005 (BDE 47: 0,015 and BDE 209: 0,100) µg/kg; LOQs HBCDs and eBFRs: 0,010 (BTBPE: 0,020 and DBDPE: 0,100) µg/kg

### RESULTS

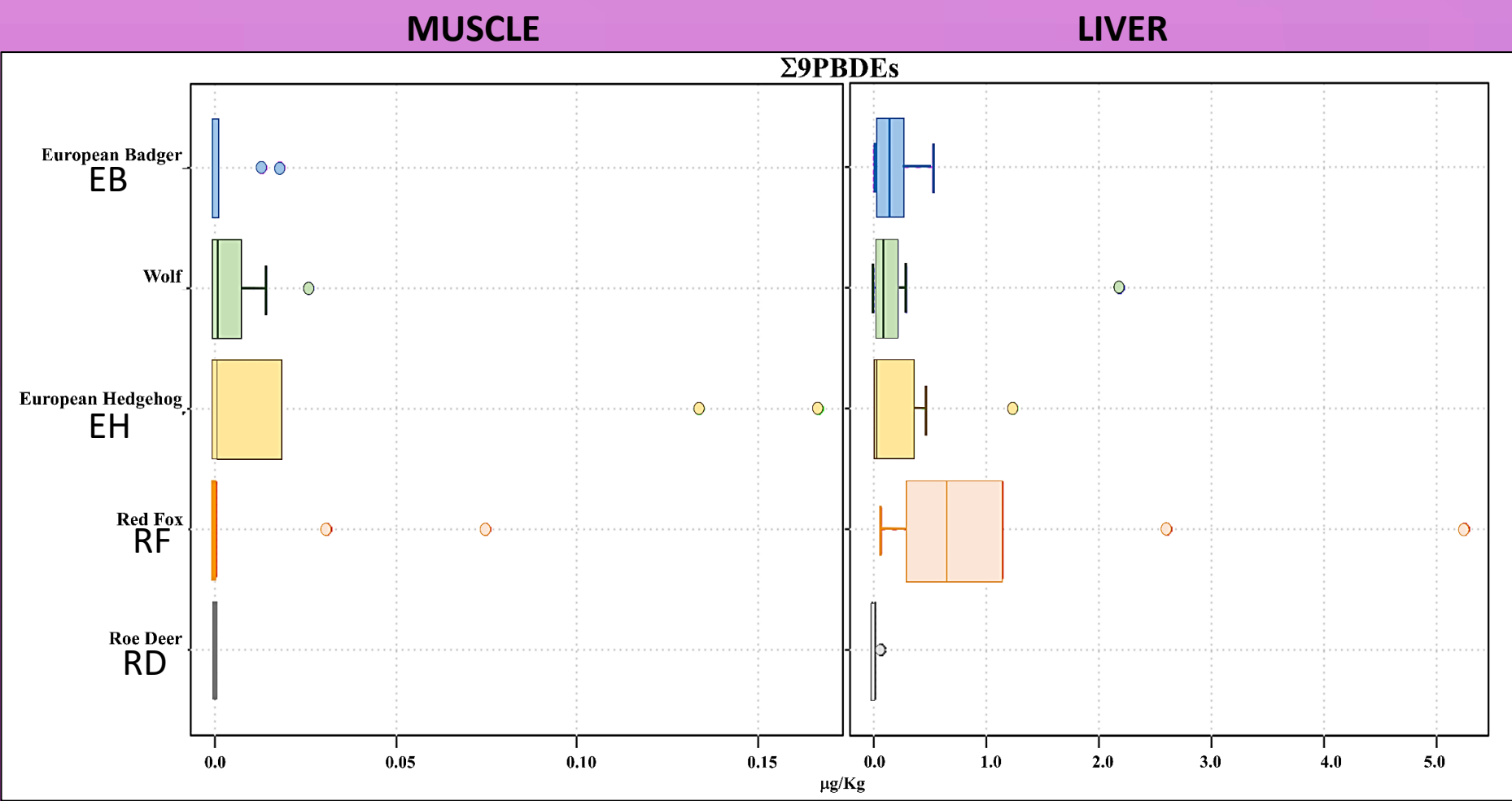
#### GLOBAL RESULTS

	MUSCLE						LIVER					
	N	Mean	sd	Median	Min.	Max.	N	Mean	sd	Median	Min.	Max.
Σ <sub>20</sub> PFASs l.b. (µg/kg)												
Roe Deer	10			n.c.			10	0,98	0,48	0,84	0,39	1,9
Red Fox	10	0,65	0,30	0,52	0,31	1,3	10	9,5	4,6	7,8	5,3	18
European Hedgehog	9	1,9	1,2	1,7	0,13	4,5	9	14	11	12	0,42	40
Wolf	8	2,2	1,4	2,0	0,63	4,2	8	39	26	33	12	89
European Badger	12	1,9	1,8	1,3	0,66	7,1	12	68	61	53	16	251
Σ <sub>9</sub> PBDEs l.b. (µg/kg)												
Roe Deer	10			n.c.			10	0,010	0,021	0,0	0,0	0,066
Red Fox	10	0,054	0,14	0,004	0,0	0,46	10	1,2	1,6	0,66	0,069	5,3
European Hedgehog	9	0,082	0,16	0,006	0,0	0,44	7	0,29	0,45	0,024	0,006	1,2
Wolf	8	0,081	0,22	0,0	0,0	0,62	8	0,35	0,75	0,075	0,0	2,2
European Badger	12	0,026	0,03	0,021	0,0	0,084	12	0,17	0,16	0,12	0,0	0,52
Σ <sub>3</sub> HBCDs l.b.(µg/kg)												
Roe Deer	10			n.c.			10			n.c.		
Red Fox	10	0,011	0,025	0,0	0,0	0,075	10	0,012	0,015	0,0	0,0	0,039
European Hedgehog	9	0,037	0,065	0,0	0,0	0,17	7	0,044	0,068	0,0	0,0	0,18
Wolf	8	0,005	0,010	0,0	0,0	0,026	8	0,002	0,005	0,0	0,0	0,015
European Badger	12	0,005	0,006	0,0	0,0	0,018	12	0,005	0,011	0,0	0,0	0,028
l.b. → lower bound: concentrations<LOQ=0; n.c.=not calculated (values<LOQ)												

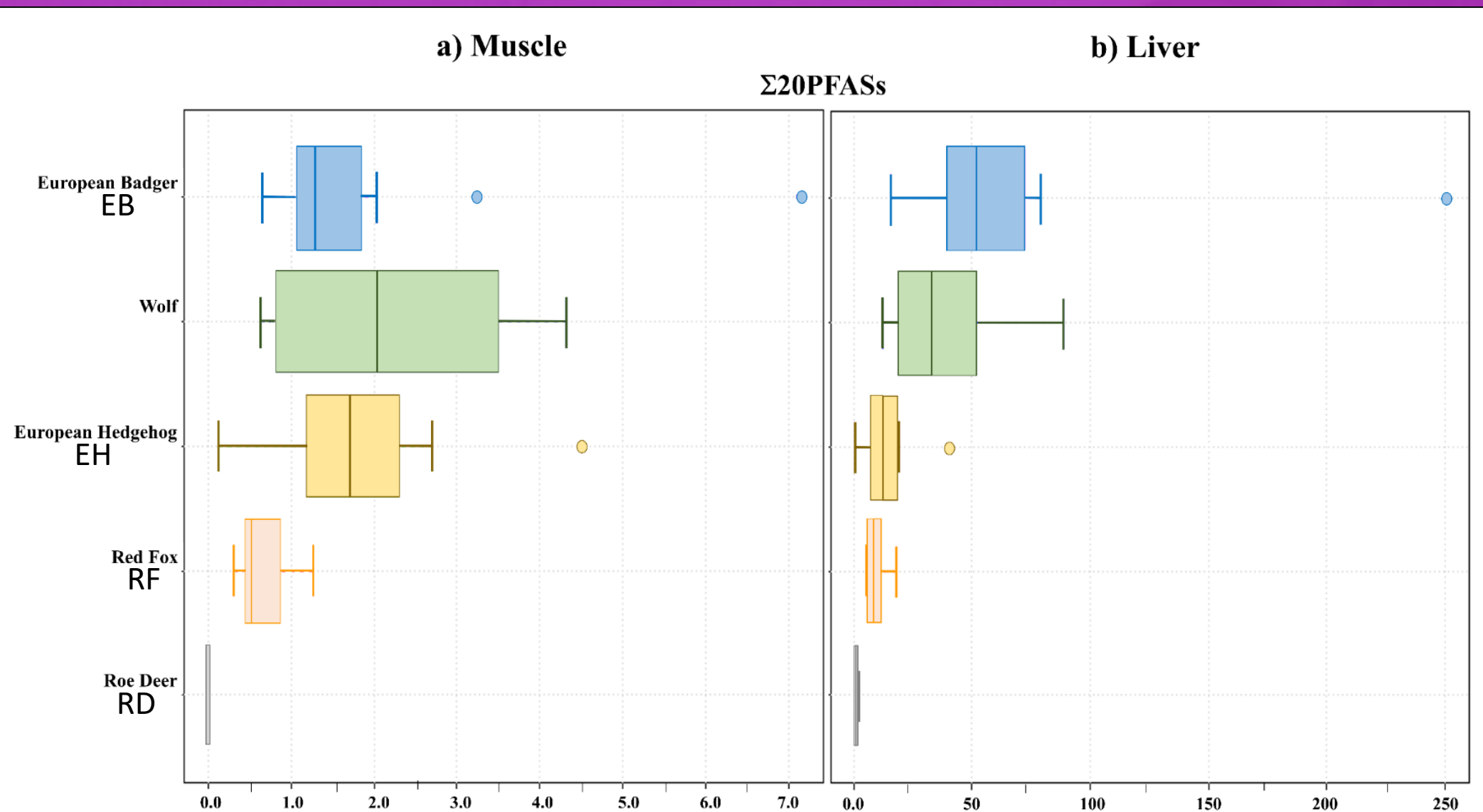
(l.b. → lower bound: concentrations<LOQ=0; n.c.=not calculated (values<LOQ))

#### BFRs RESULTS

e-BFRs < LOQs in almost all muscle and liver samples.  
Σ<sub>3</sub>HBCDs was negligible in muscle and liver of all the species.  
Σ<sub>9</sub>PBDEs was very low in muscle samples but not in liver's (except for RD), with the highest levels in RF, followed by EB, wolf and EH.  
BDE-209 was the predominant compound in RF and wolf, while in RD, EB and EH BDE-47 was the most abundant one.



#### PFASs RESULTS



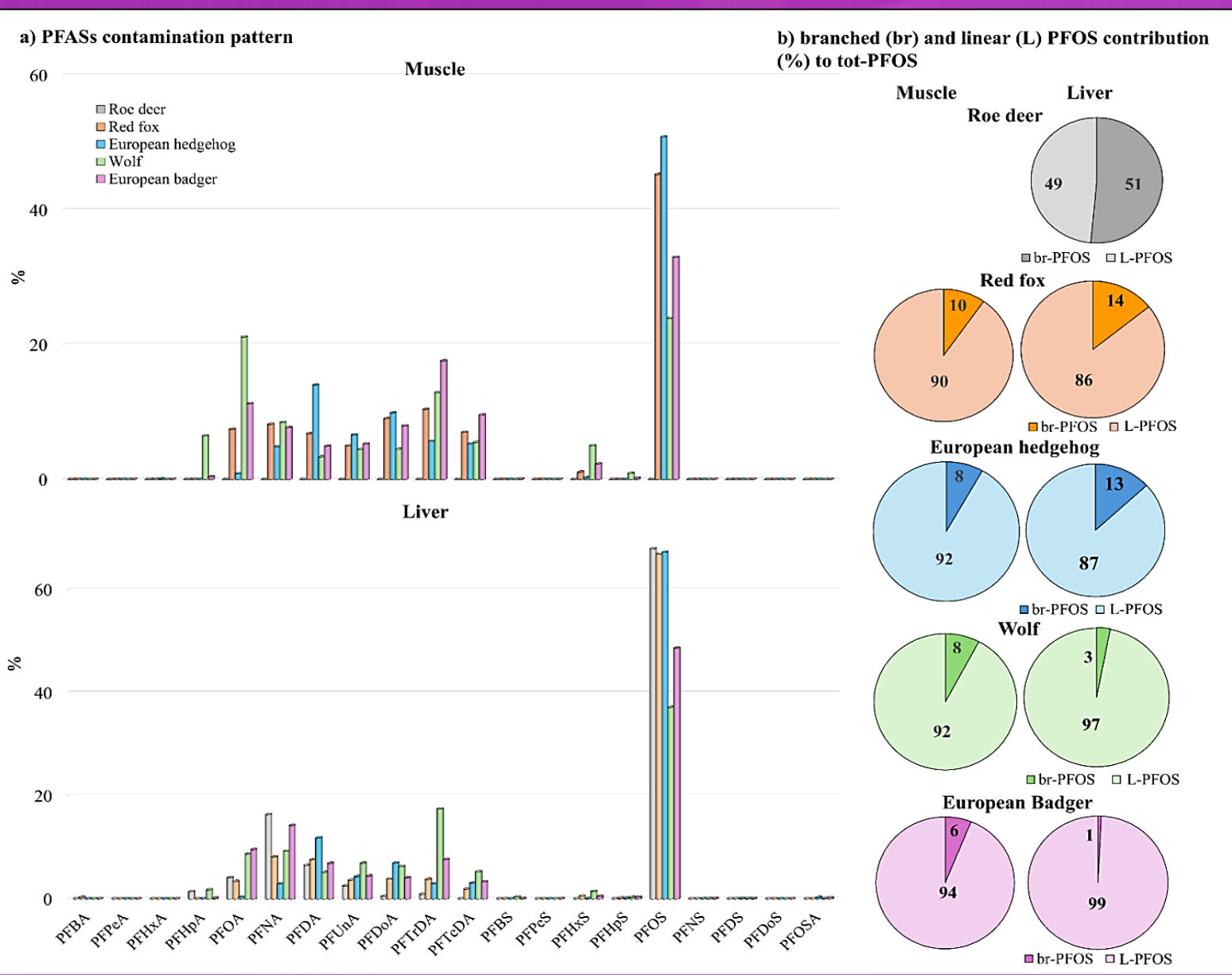
In muscle, Σ<sub>20</sub>PFASs < LOQs in all RD samples, while the highest levels were measured in wolf, followed by EH and EB.

In liver, levels were significantly higher, with the highest concentrations in wolf and EB. Higher concentrations in liver reflect results of previous studies [a,b].

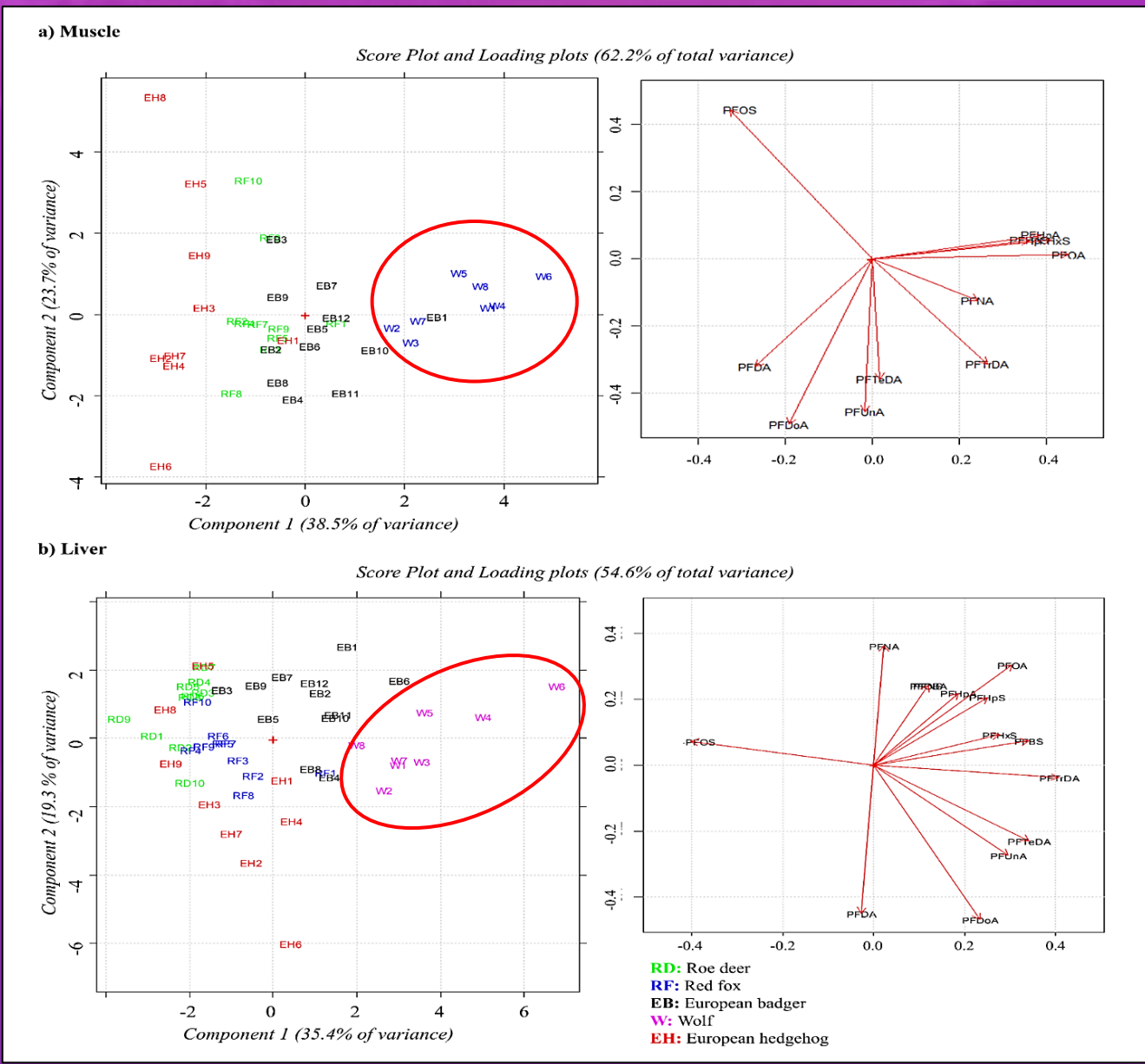
[a] Stecconi, T. et al. *Toxics*, 2024, 12(3), 196. <https://doi.org/10.3390/toxics12030196>

[b] Tavaloni, T., et al. *Sci. Total Environ.*, 2023, 858, 159745 <http://dx.doi.org/10.1016/j.scitotenv.2022.159745>

Some of the analyzed wild animals are considered game species, in particular RD and, in some countries, EB. PFASs levels were compliant with maximum limits (MRLs) set by EU Regulation 915/2023 in meat and offal of game animals. Only one sample of EB liver exceeded limits for PFOS and PFOA. No MRLs are yet defined for BFRs in food.



The PFASs pattern in muscle and liver of all species was comparable, with PFOS being the dominant compound followed by long chain PFCAs (C8-C14). Among branched (br) isomers, only br-PFOS was quantified in all the samples (except RD muscle), with levels close to the linear in RD liver.



PCA analysis revealed that wolf exhibits a distinctive profile in both muscle and liver, consistently with its feeding habits.

#### FUTURE PERSPECTIVES

Exploring contamination levels in fauna of other Central Italy's habitat, assessing the safety of matrices consumed as food and investigating anthropogenic pressures in the study areas.