

Un approccio integrato per la gestione
efficace dei rischi di inquinamento delle acque
da contaminanti emergenti



Perfluorinated compounds
HOlistic ENvironmental
Interinstitutional eXperience



Prevenire, Garantire, Promuovere

Progetto LIFE PHOENIX

lifephoenix.eu

ENTE COORDINATORE



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AZIENDA
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UNIVERSITÀ
DEGLI STUDI
DI PADOVA



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Metodiche analitiche di residui di PFAS in organismi animali e vegetali

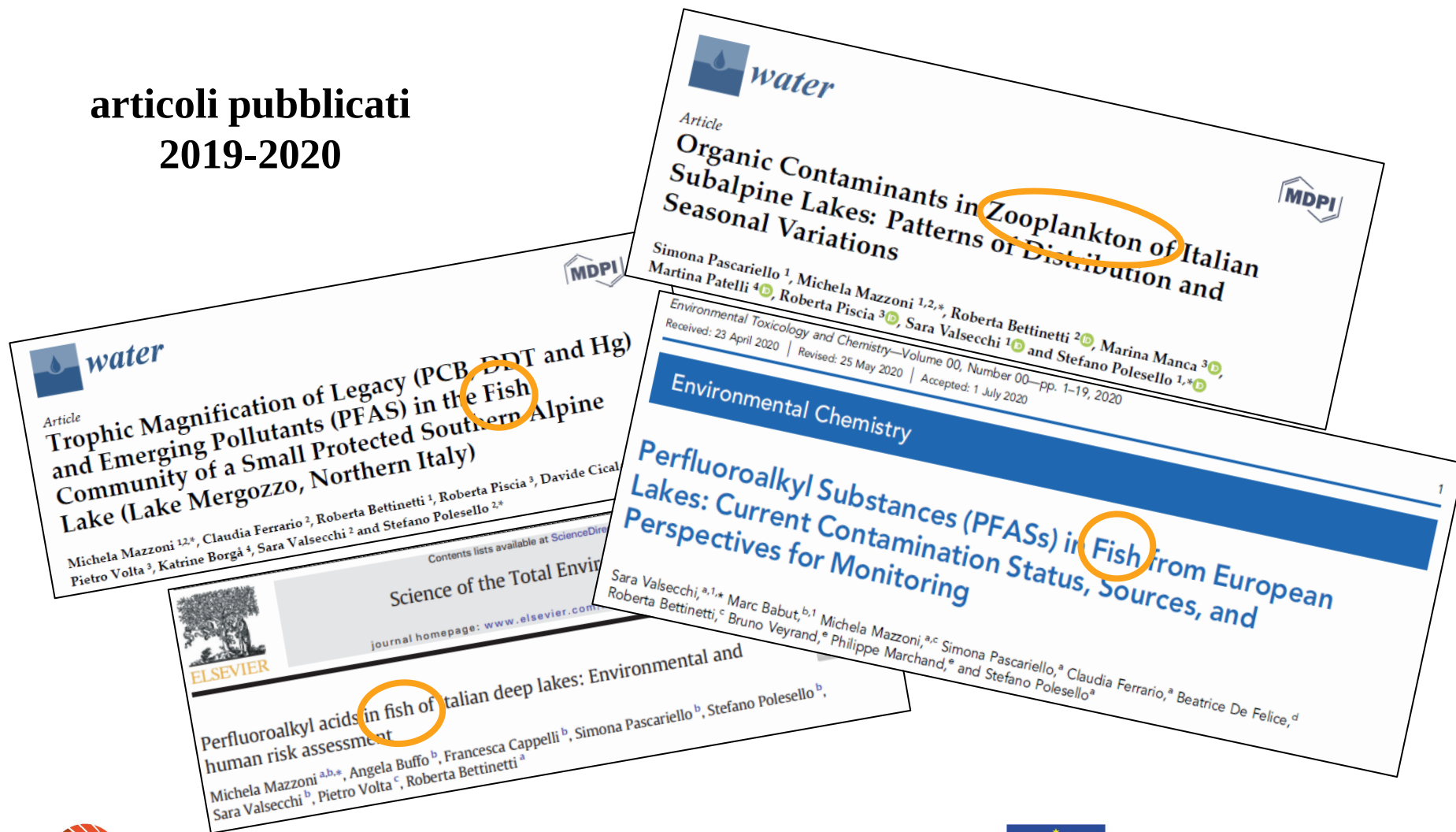
Claudia Ferrario, Stefano Polesello e Sara Valsecchi
IRSA-CNR Brugherio



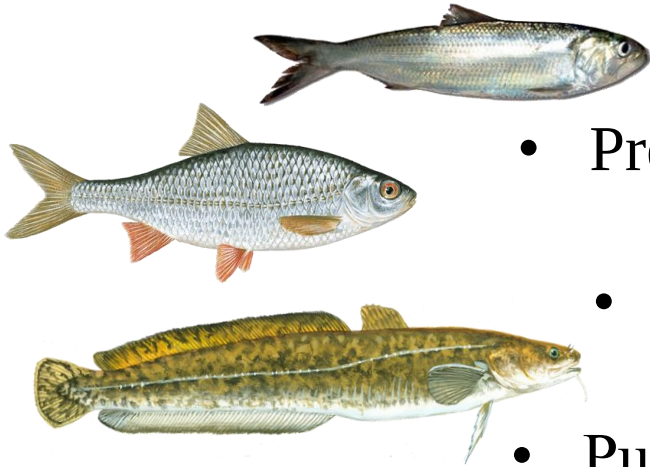
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Determinazione di PFAS in organismi animali

articoli pubblicati
2019-2020



Determinazione di PFAS negli animali



- Preparativa del campione
- Estrazione di PFAS
- Purificazione dell'estratto
- Quantificazione di PFAS (LC-MS)





SIL-IS +
10% H₂O: 90% CH₃CN
+ HCOOH



- Sfilettatura del pesce, separando le frazioni (filetto, fegato, viscere, carcassa)
- Omogeneizzazione del campione fresco
- Trasferimento del campione in falcon con aggiunta di standard interno marcato

Estrazione

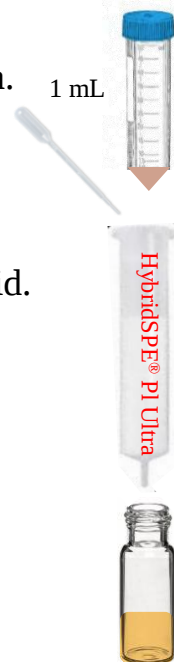
(metodo Lacina et al., 2011 modificato)



- Aggiunta di ACN acidificato e acqua in rapporto 90:10, vortex, ultrasuoni, centrifuga.
- Aggiunta di ACN acidificato vortex, ultrasuoni, centrifuga.
- Aggiunta di NaCl e MgSO₄
- Evaporazione sotto azoto fino ad 1 mL
- Eliminazione dei fosfolipidi: passaggio attraverso cartucce HybridSPE® Phospholipid.

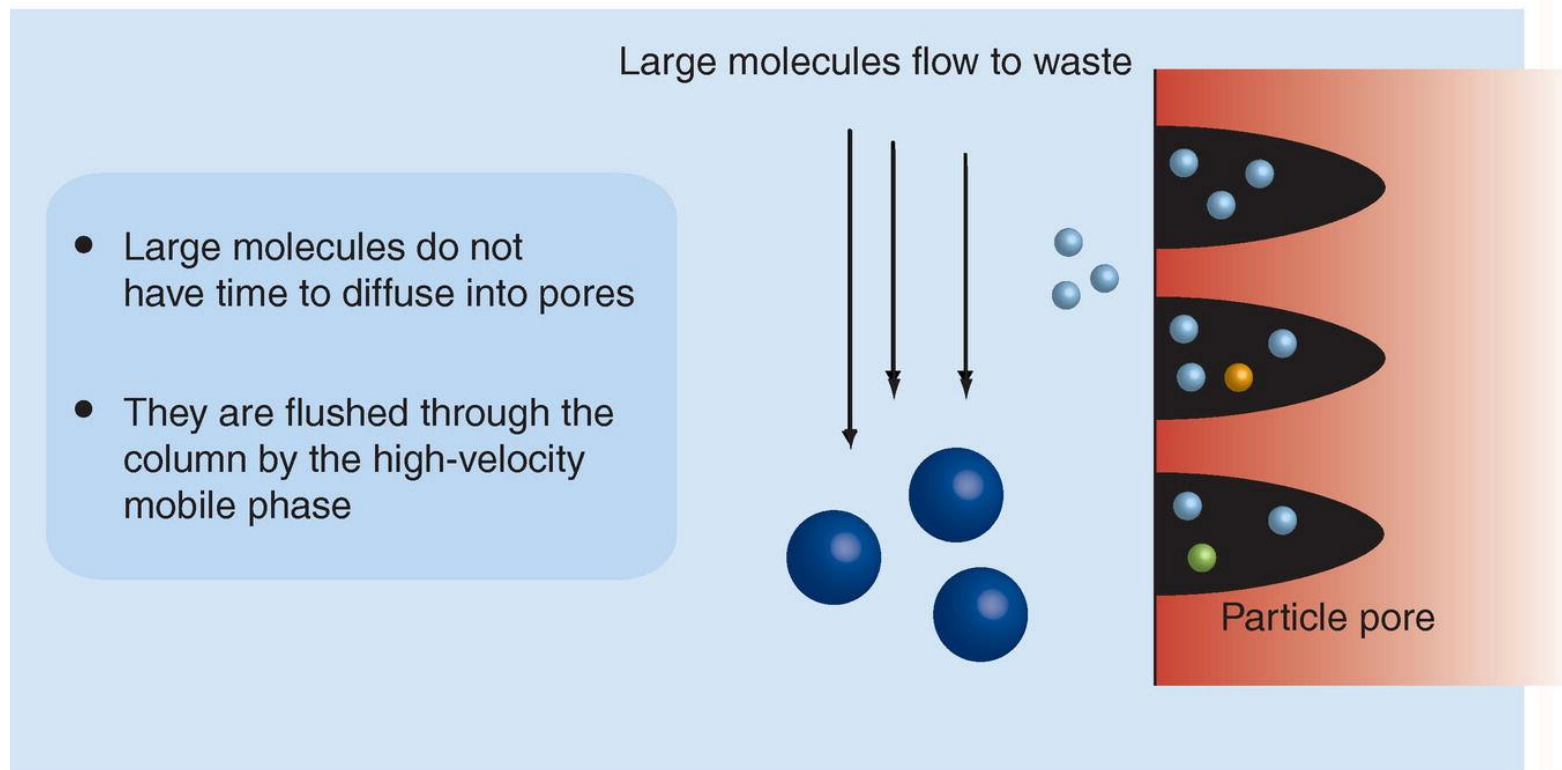


NaCl + MgSO₄



Purificazione on-line

Turbulent Flow Chromatography (TFC)



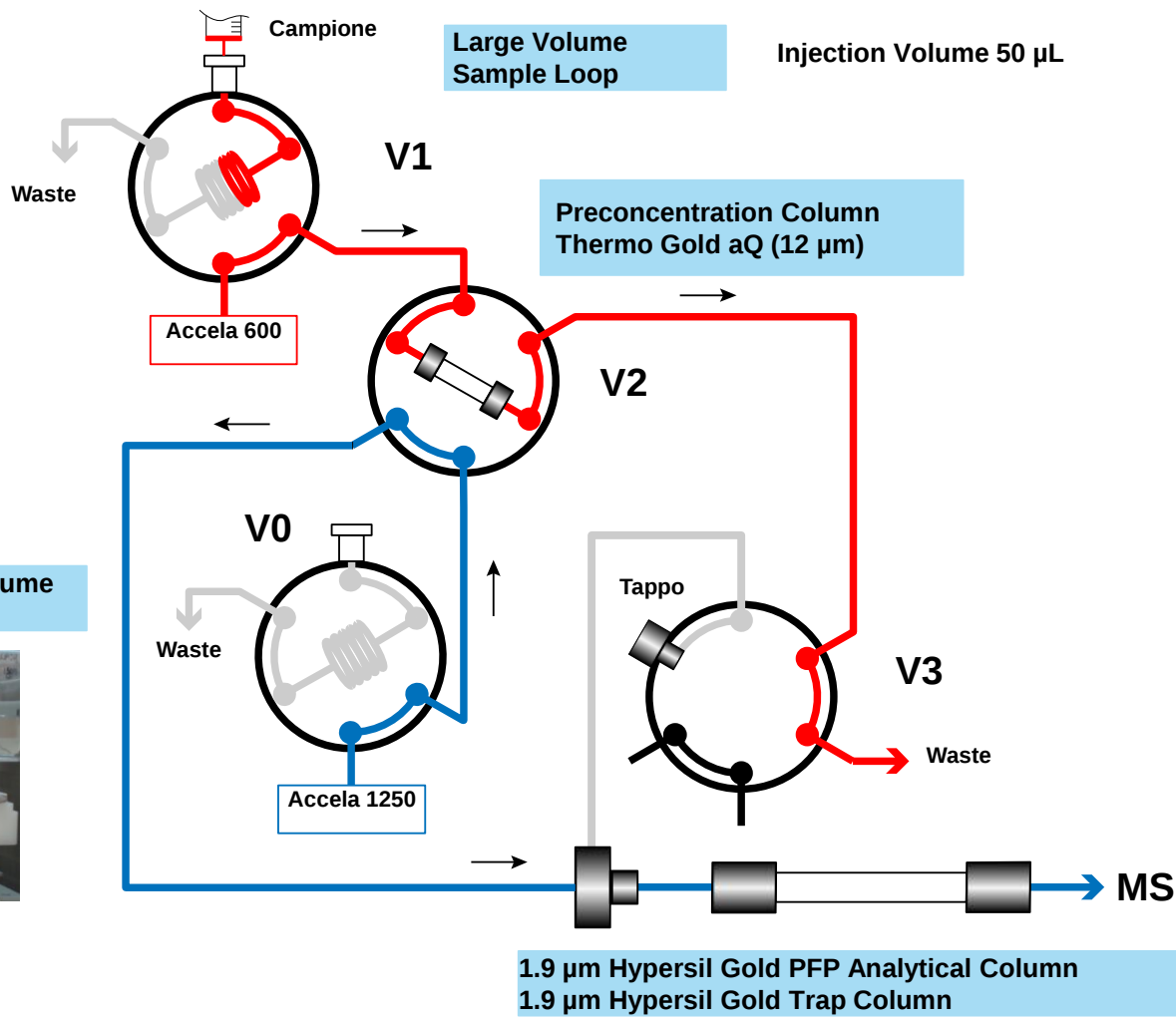
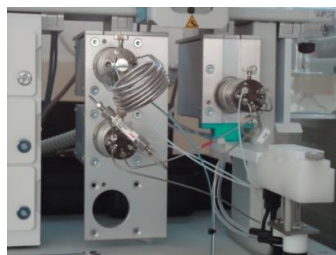
Mazzoni et al., 2019. Perfluoroalkyl acids in fish of Italian deep lakes: Environmental and human risk assessment. <https://doi.org/10.1016/j.scitotenv.2018.10.274>

Turbulent Flow Chromatography (TFC)

riempimento del *loop*



Conventional Volume Sample Loop

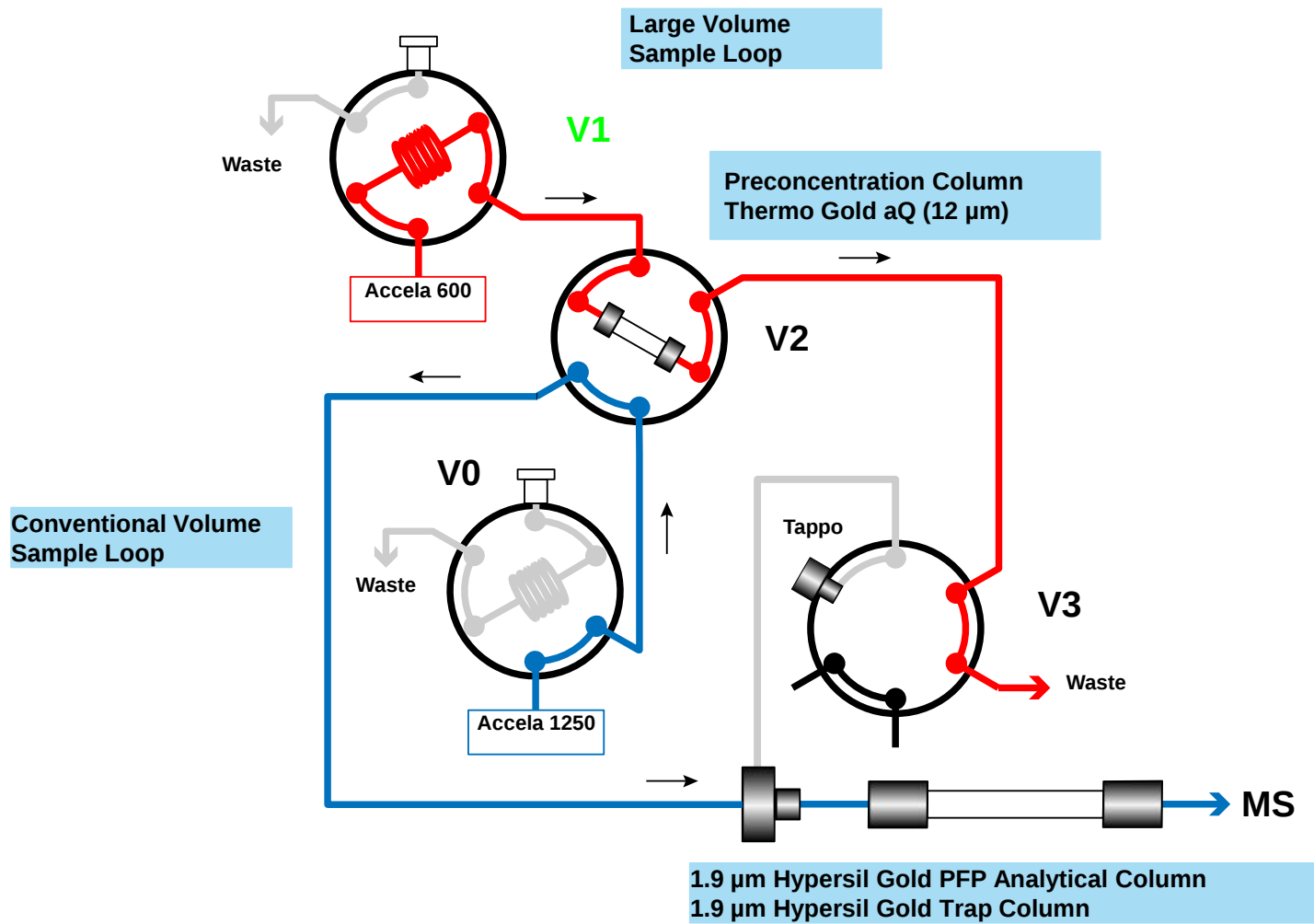


SRM
Negative Ion.
Mode



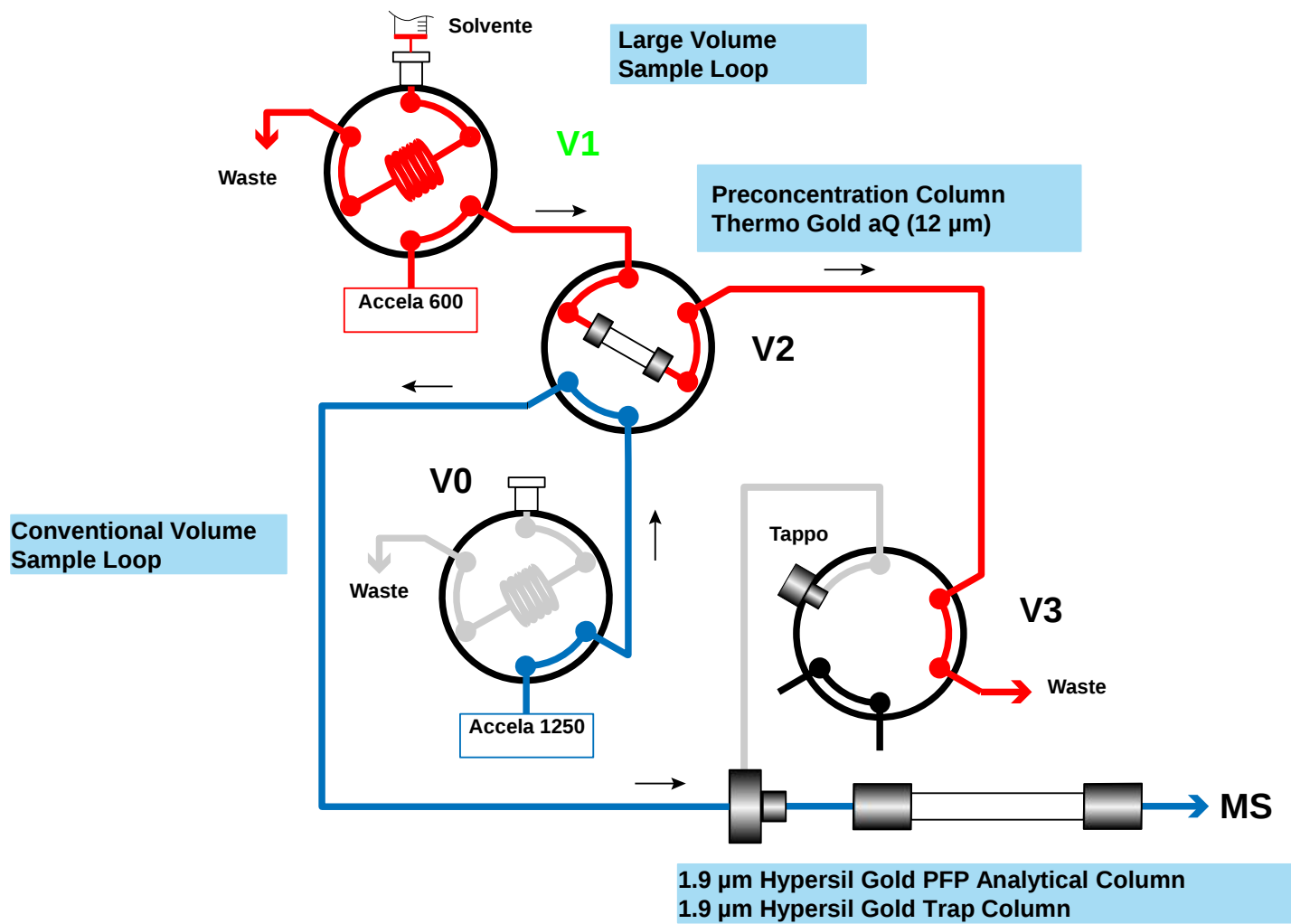
Turbulent Flow Chromatography (TFC)

purificazione del campione



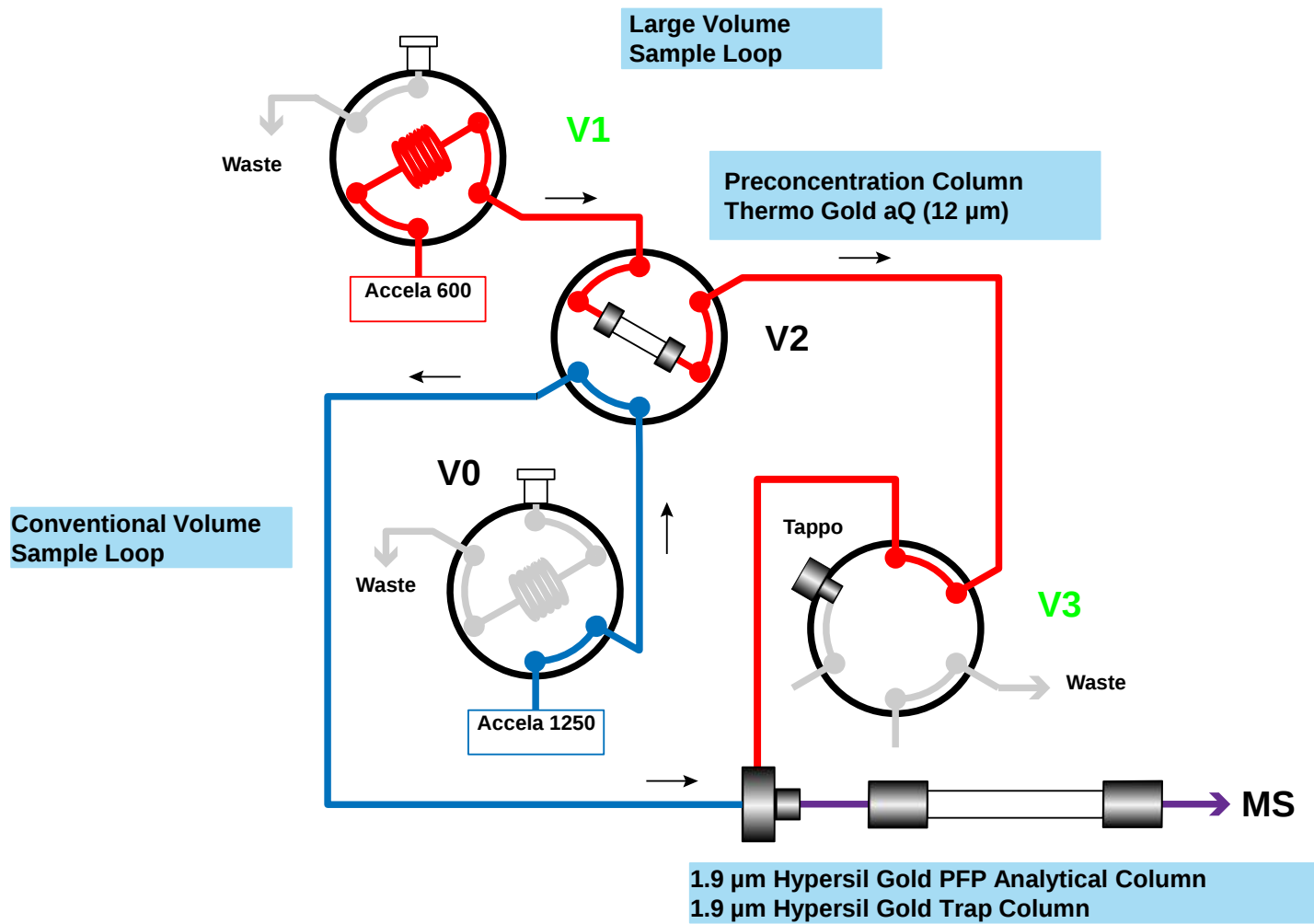
Turbulent Flow Chromatography (TFC)

caricamento del *loop* con solvente



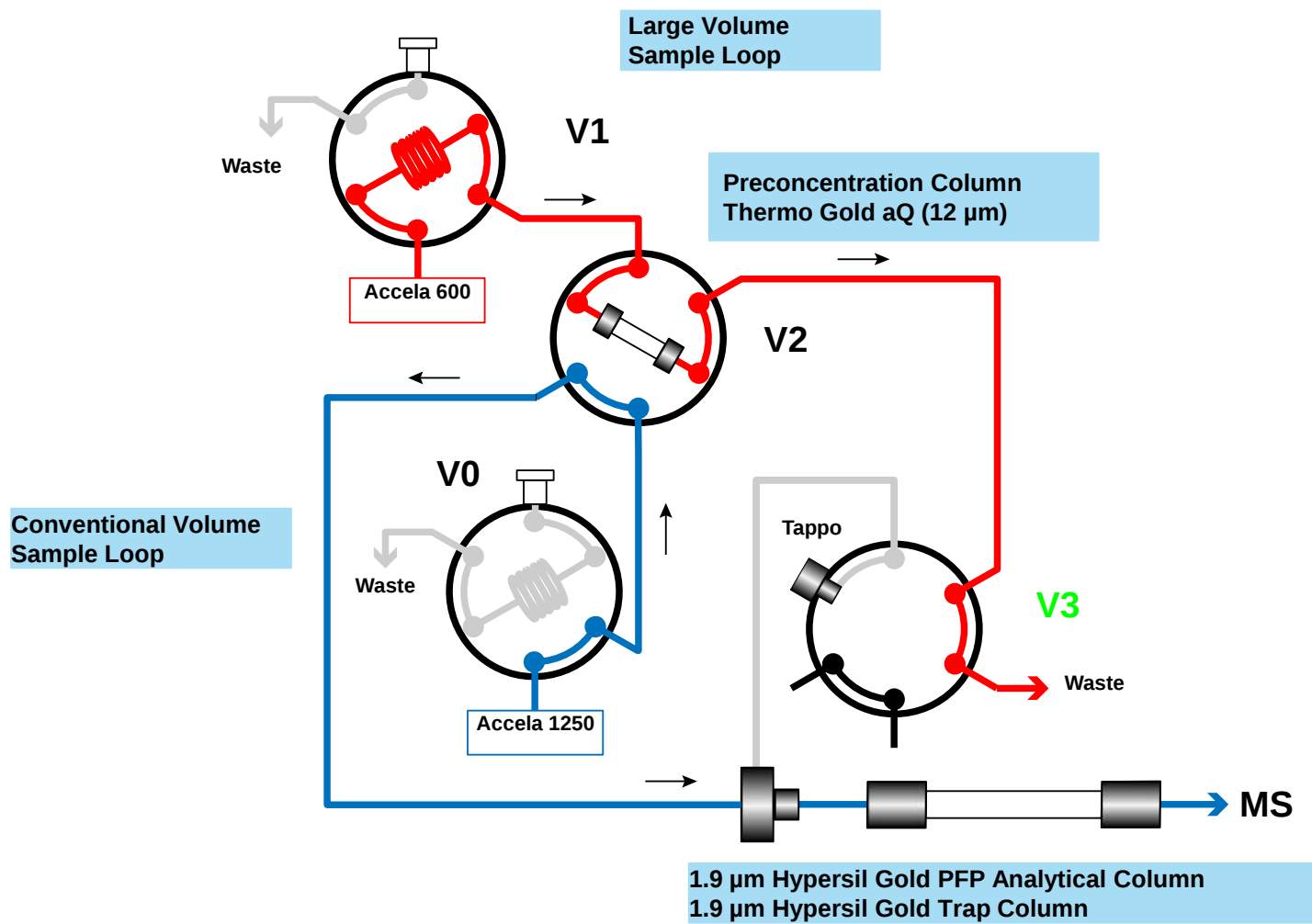
Turbulent Flow Chromatography (TFC)

trasferimento alla colonna analitica



Turbulent Flow Chromatography (TFC)

separazione cromatografica



Validazione matrice animale



Matrice di prova

Stable Isotopic Labelled-Internal Standard

Acido perfluoro-n-[$^{13}\text{C}_5$] pentanoico	$^{13}\text{C}_5$ -PFPeA
Acido perfluoro-n-[$^{13}\text{C}_2$] hexanoico	$^{13}\text{C}_2$ -PFHxA
Acido perfluoro-n-[$^{13}\text{C}_4$] octanoico	$^{13}\text{C}_4$ -PFOA
Acido perfluoro-n-[$^{13}\text{C}_5$] nonanoico	$^{13}\text{C}_5$ -PFNA
Acido perfluoro-n-[$^{13}\text{C}_2$] decanoico	$^{13}\text{C}_2$ -PFDA
Acido perfluoro-n-[$^{13}\text{C}_2$] undecanoico	$^{13}\text{C}_2$ -PFUnDA
Acido perfluoro-n-[$^{13}\text{C}_2$] dodecanoico	$^{13}\text{C}_2$ -PFDoDA
Acido perfluoro-n-esan [$^{18}\text{O}_2$] solfonico	$^{18}\text{O}_2$ -PFHxS
Perfluoro-n-octane [$^{13}\text{C}_4$] sulfonate	$^{13}\text{C}_4$ -PFOS

PFAS ricercati

Acido perfluoropentanoico	PFPeA
Acido perfluoroesanoico	PFHxA
Acido perfluoroeptanoico	PFHpA
Acido perfluorooctanoico	PFOA
Acido perfluorononanoico	PFNA
Acido perfluorodecanoico	PFDA
Acido perfluoroundecanoico	PFUnDA
Acido perfluorododecanoico	PFDoDA
Acido perfluorobutansolfonico	PFBS
Acido perfluoroesansolfonico	PFHxS
Acido perfluorooctansolfonico	PFOS

Effetto matrice

$$ME (\%) = (Area_{\text{molluschi}} \times Area_{\text{std}}) \times 100; (N = 3)$$

Composti	Effetto matrice (%)		Effetto matrice (%)	
	Nativo	SIL-IS	Nativo	SIL-IS
	2 µg/L	4 µg/L	15 µg/L	4 µg/L
PFPeA	n.d.	n.a.	41	n.a.
PFHxA	40	42	40	46
PFHpA	43	n.a.	46	n.a.
PFOA	75	109	98	94
PFNA	66	51	57	55
PFDA	57	52	57	57
PFUnDA	66	54	55	54
PFDoDA	99	87	83	84
PFBS	n.d.	n.a.	49	n.a.
PFHxS	85	81	81	93
PFOS	n.d.	135	99	149

Linearità e sensibilità

Composti	Linearità		Sensibilità (ng/g p.f.)			
	(µg/L)	R ²	ILOD	ILOQ	MLOD	MLOQ
PFPeA*	1-100	0,997	0,2	0,7	0,3	0,9
PFHxA	1-100	0,994	0,1	0,2	0,06	0,2
PFHpA*	1-100	0,998	0,04	0,1	0,1	0,4
PFOA	1-100	0,997	0,03	0,1	0,1	0,4
PFNA	1-100	0,992	0,04	0,1	0,07	0,2
PFDA	1-100	0,995	0,03	0,07	0,07	0,2
PFUnDA	0,5-50	0,997	0,02	0,04	0,04	0,1
PFDoDA	0,5-50	0,998	0,03	0,08	0,03	0,1
PFBS*	1-100	0,999	0,2	0,4	0,3	0,8
PFHxS	1-100	0,989	0,03	0,07	0,1	0,2
PFOS	1-100	0,987	0,04	0,1	0,2	0,5

* Valori corretti con 13C2-PFHxA

Accuratezza

Composti	Precisione sol. std (%RSD)				Precisione in estratti (RSD%)				Recupero (%)		
	Intra-diem	Inter-dies	Intra-diem	Inter-dies	Intra-diem	Inter-dies	Intra-diem	Inter-dies	Bianco	Biota	Biota
	2 µg/L	2 µg/L	10 µg/L	10 µg/L	4 µg/L	4 µg/L	30 µg/L	30 µg/L	1,5 ng/g p.f.	0,4 ng/g p.f.	3 ng/g p.f.
PFPeA*	7	9	6	14	19	36	5	11	96	<LOQ	120
PFHxA	9	28	8	17	8	21	4	13	104	65	98
PFHpA*	12	9	8	7	10	6	8	4	135	<LOQ	132
PFOA	16	14	3	12	13	6	5	13	121	134	133
PFNA	7	16	4	19	4	6	3	9	108	101	109
PFDA	12	1	5	10	8	6	2	9	111	106	109
PFUnDA	13	8	5	10	12	10	4	7	112	97	111
PFDoDA	3	8	4	11	6	13	4	5	107	95	109
PFBS*	34	40	8	15	15	26	10	19	51	86	60
PFHxS	21	13	13	20	7	26	6	12	100	49	53
PFOS	47	38	7	13	18	9	5	27	71	29	40

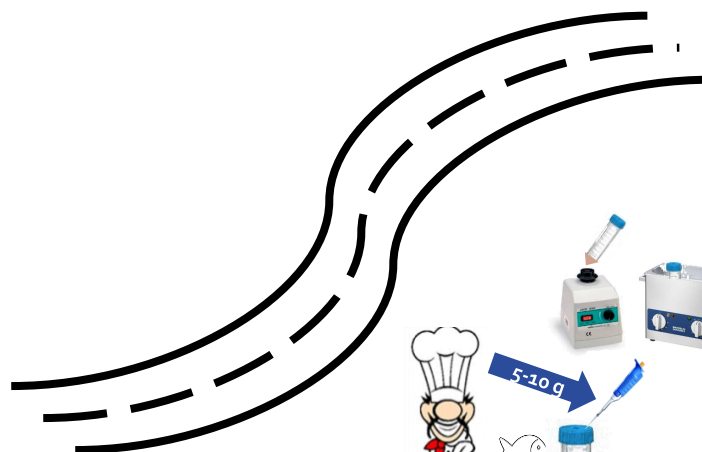
Le concentrazioni sono state calcolate con il metodo della diluizione isotopica. * Valori corretti con ¹³C2-PFHxA

Determinazione di PFAS

Vegetali



Animali



5-10 g

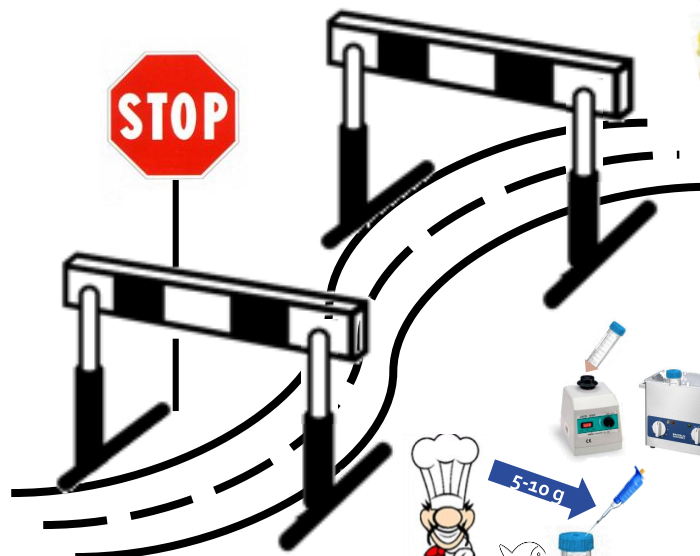


Determinazione di PFAS

Vegetali



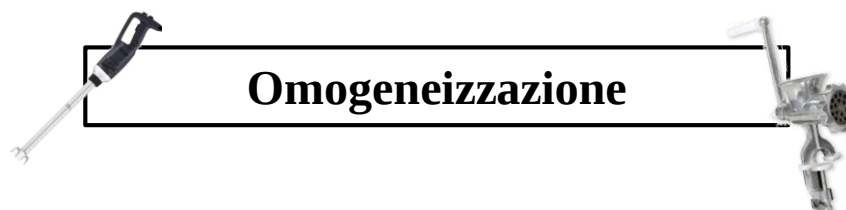
Animali



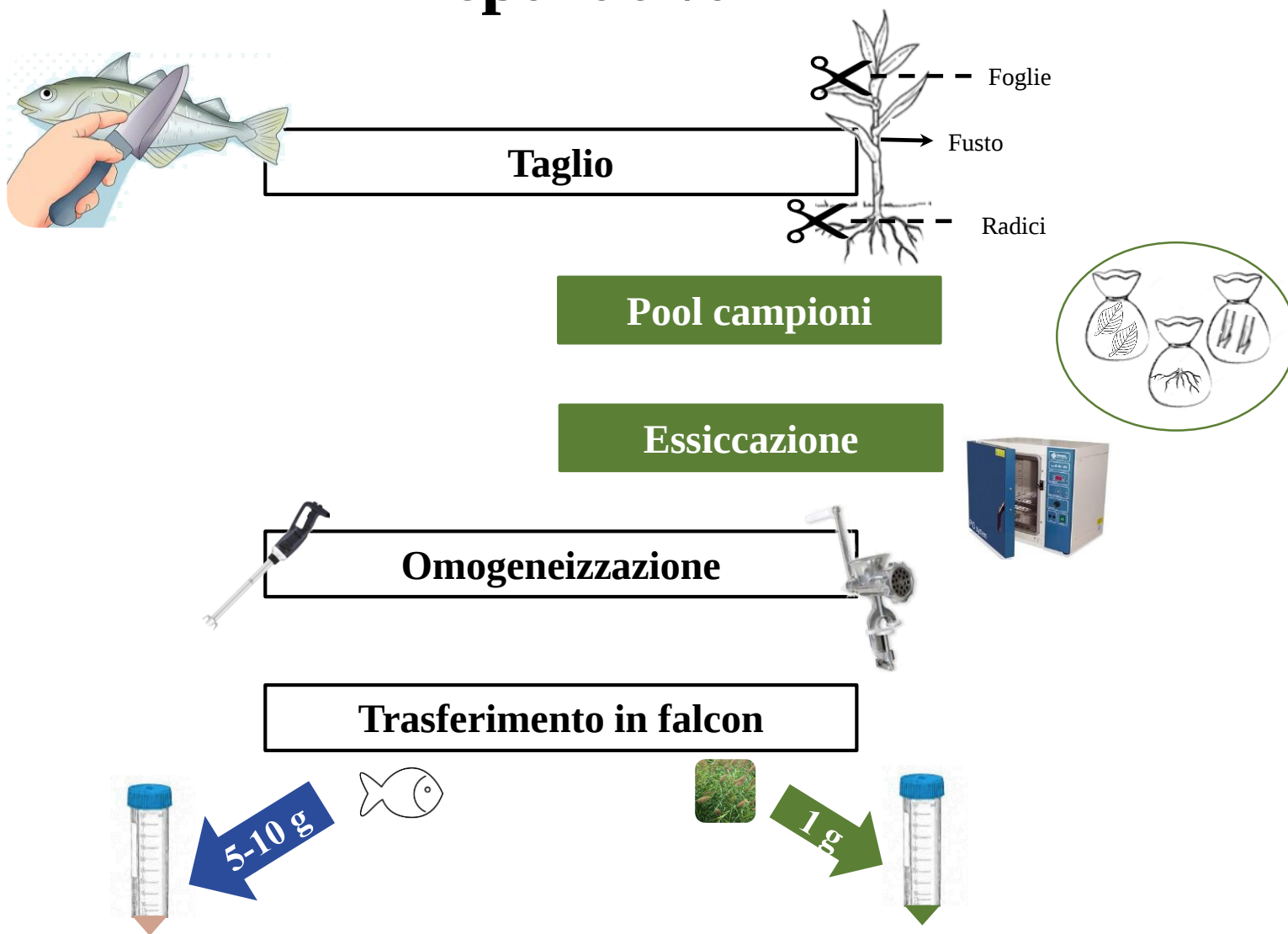
5-10 g



Preparativa

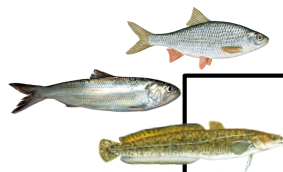


Preparativa



Estrazione

(metodo Lacina et al., 2011 modificato)



**ACN + ACQUA
90:10**

ACN



ACN

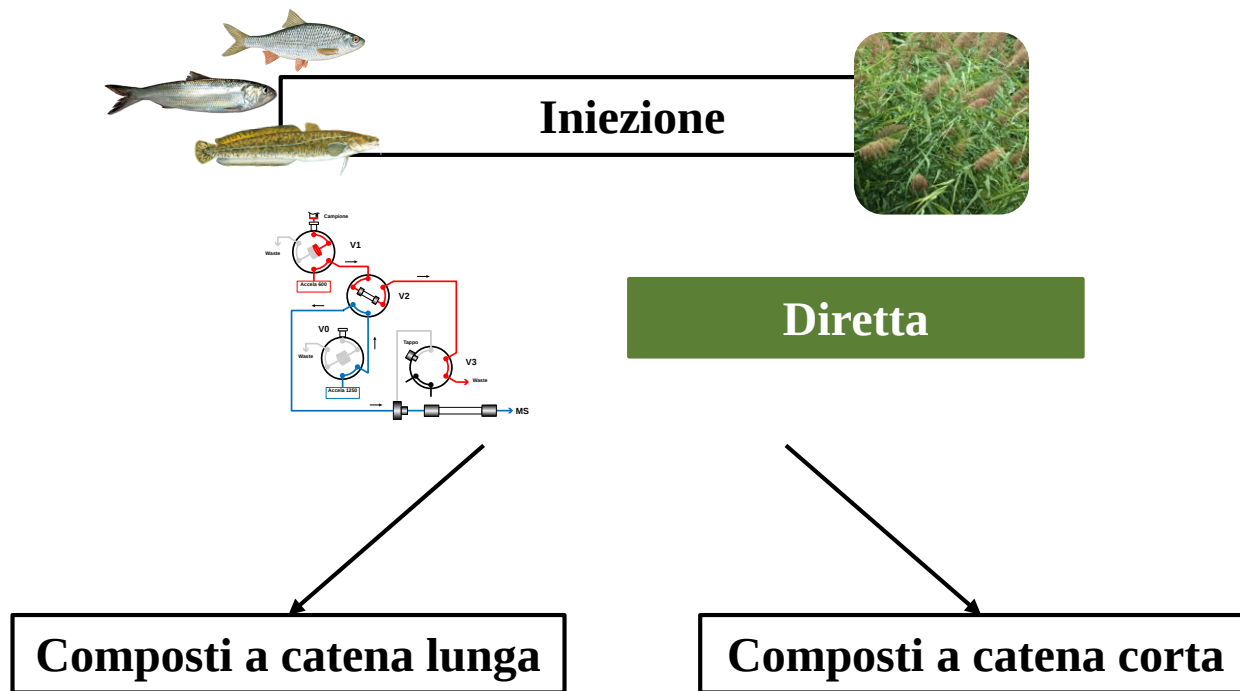
Concentrazione fino a 1 mL

Aggiunta sali

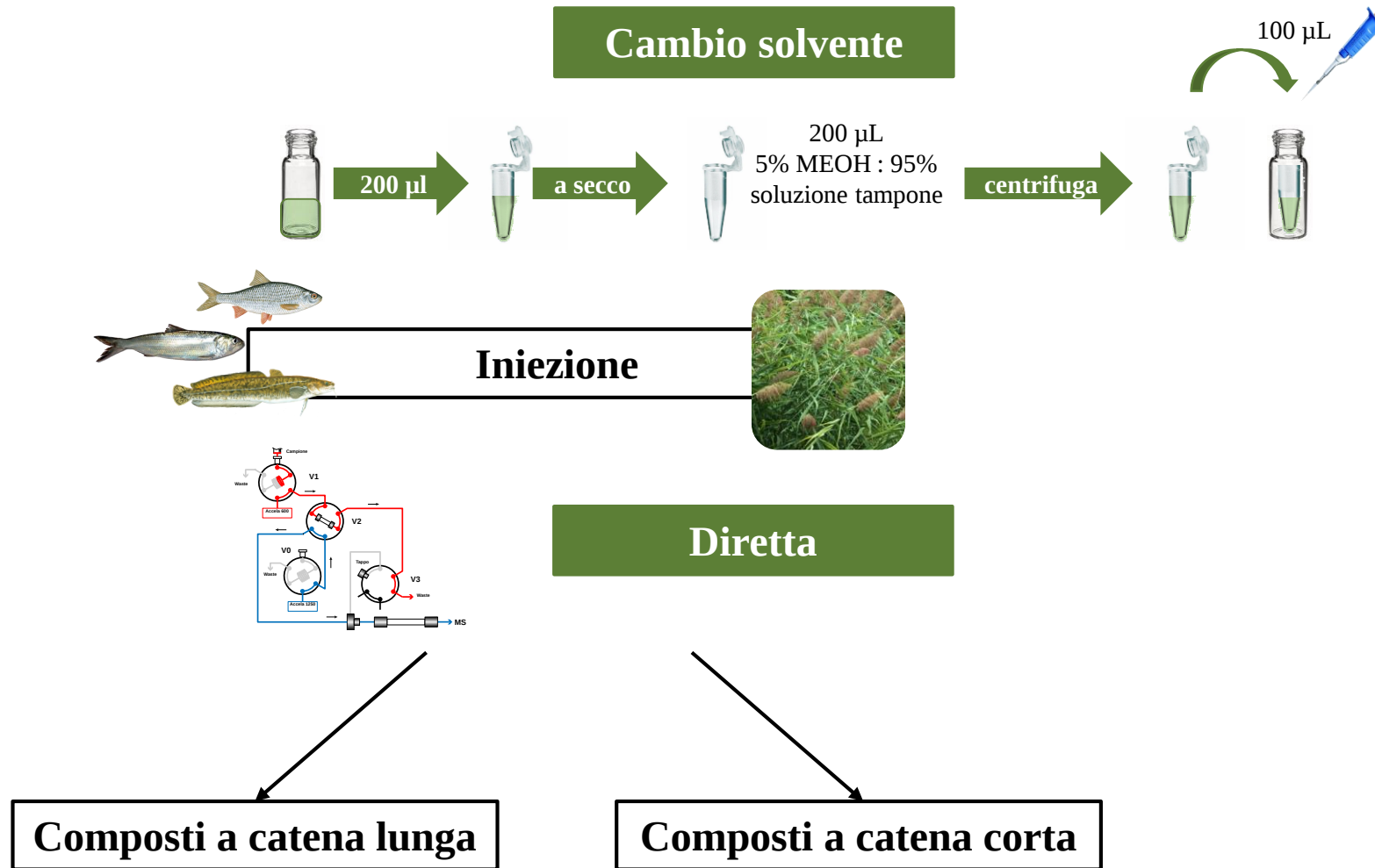


Eliminazione fosfolipidi

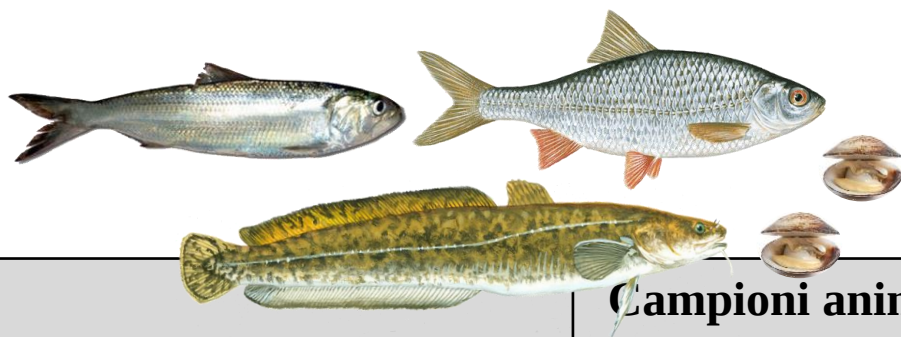
Determinazione in LC-MS



Determinazione in LC-MS



Determinazione di PFAS nel biota



	Campioni animali	Campioni vegetali
Preparativa	Campione fresco	Campione secco
	5-10 g	1 g
Numero di estrazioni con solvente	2	3
Determinazione in LC-MS	TFC	Iniezione diretta
Solvente	ACN	Cambio solvente

Validazione matrice vegetale



Matrice di prova

Stable Isotopic Labelled-Internal Standard

Acido perfluoro-n-[$^{13}\text{C}_4$] butanoico	$^{13}\text{C}_4$ -PFBA
Acido perfluoro-n-[$^{13}\text{C}_5$] pentanoico	$^{13}\text{C}_5$ -PFPeA
Acido perfluoro-n-[$^{13}\text{C}_2$] hexanoico	$^{13}\text{C}_2$ -PFHxA
Acido perfluoro-n-[$^{13}\text{C}_4$] octanoico	$^{13}\text{C}_4$ -PFOA
Acido perfluoro-n-[$^{13}\text{C}_5$] nonanoico	$^{13}\text{C}_5$ -PFNA
Acido perfluoro-n-[$^{13}\text{C}_2$] decanoico	$^{13}\text{C}_2$ -PFDA
Acido perfluoro-n-[$^{13}\text{C}_2$] undecanoico	$^{13}\text{C}_2$ -PFUnDA
Acido perfluoro-n-[$^{13}\text{C}_2$] dodecanoico	$^{13}\text{C}_2$ -PFDoDA
Acido perfluoro-n-esan [$^{18}\text{O}_2$] solfonico	$^{18}\text{O}_2$ -PFHxS
Perfluoro-n-octane [$^{13}\text{C}_4$] sulfonate	$^{13}\text{C}_4$ -PFOS

PFAS ricercati

Acido perfluorobutanoico	PFBA
Acido perfluoropentanoico	PFPeA
Acido perfluoroesanoico	PFHxA
Acido perfluoroeptanoico	PFHpA
Acido perfluorooctanoico	PFOA
Acido perfluorononanoico	PFNA
Acido perfluorodecanoico	PFDA
Acido perfluoroundecanoico	PFUnDA
Acido perfluorododecanoico	PFDoDA
Acido perfluorobutansolfonico	PFBS
Acido perfluoroesansolfonico	PFHxS
Acido perfluorooctansolfonico	PFOS

Recupero percentuale

	Composti											
Frazione della pianta	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	PFOS
Radice	84.7	93.2	75.2	51.9	68.0	76.9	92.0	89.9	92.5	162.0	92.9	54.0
Fusto	84.4	86.8	77.7	82.7	59.4	65.3	76.6	83.3	69.0	154.4	61.7	46.1
Foglia	80.1	70.7	93.0	196.1	66.7	90.4	nd	nd	nd	125.4	70.6	57.5

LOD e LOQ

	Composti											
	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	PFOS
LOD (ng/g pf)	0.4	0.3	0.3	0.2	0.2	0.4	0.6	0.7	0.7	0.3	0.1	0.5
LOQ (ng/g pf)	1.3	1.0	1.0	0.6	0.8	1.2	2.0	2.3	2.3	0.8	0.5	1.6

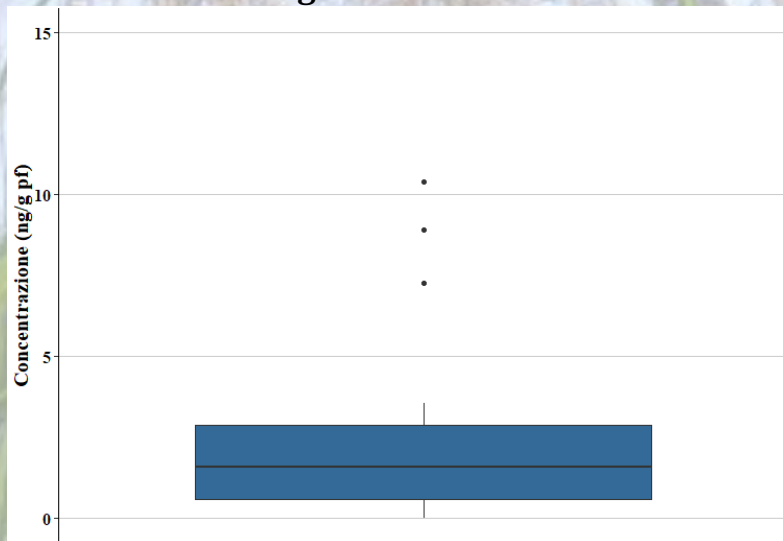
Ripetibilità

Coefficiente di Variazione (%)

Composti	Campioni fortificati			Campioni tal quali		
	Radice	Fusto	Foglia	Radice	Fusto	Foglia
PFBA	7.6	12.3	13.7		30.7	9.4
PFPeA	19.0	10.1	10.6	7.5	125.3	15.6
PFHxA	12.4	7.1	12.9	9.0		41.3
PFHpA	23.6	9.3	23.8	7.8	59.9	58.1
PFOA	10.3	13.7	16.2			37.6
PFNA	9.7	16.2	27.3	19.2	70.5	74.2
PFDA	22.4	12.3	12.6	124.5	64.9	60.1
PFUnA	12.2	17.3			104.5	68.6
PFDoDA	18.3	8.2		2.3	142.6	21.2
PFBS	12.2	11.9	14.4			21.3
PFHxS	9.9	11.4	8.8			18.0
PFOS	38.8	31.2	65.6			87.0

Risultati preliminari

Σ PFAS
Phragmites 2019-2020



Pool di piante analizzate: 31

Frequenza: 97%

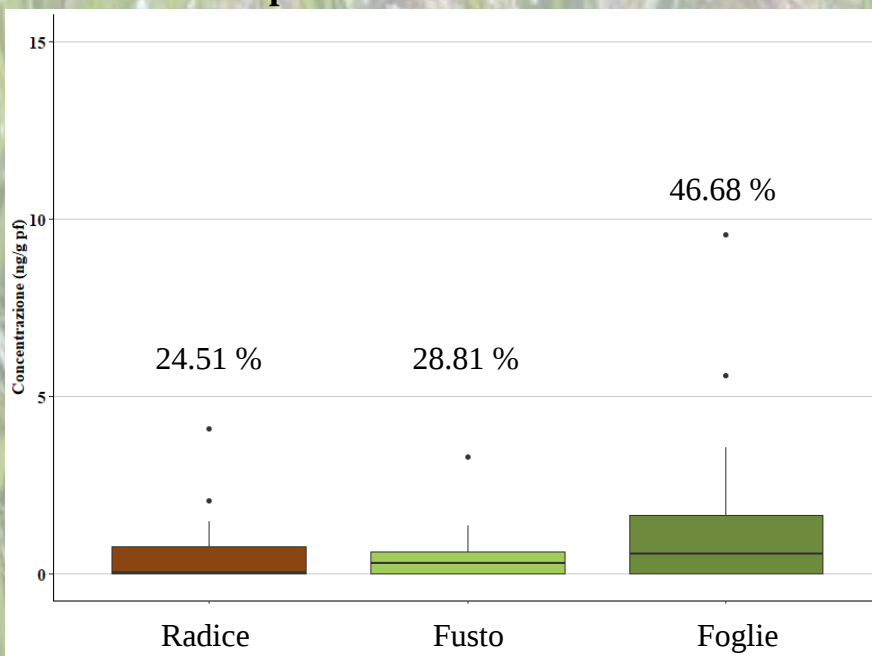
Media: 5.21 ng/g pf

Mediana: 1.60 ng/g pf

Minimo: <LOD

Massimo: 10.41 ng/g pf

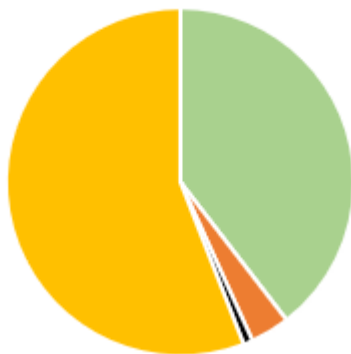
Ripartizione tra le frazioni



Ripartizione dei composti

Phragmites 2019-2020

Radice



Fusto

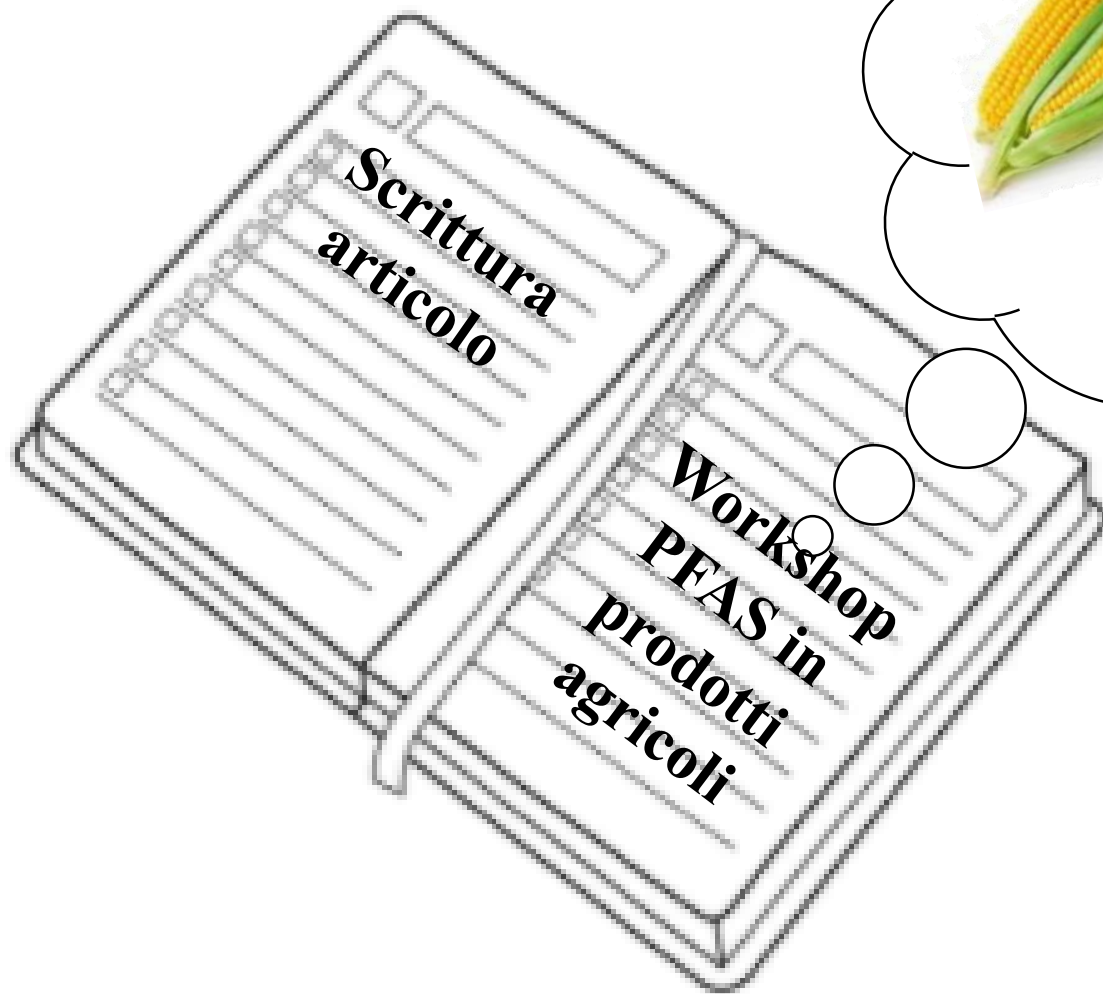


Foglie



■ PFAS C4-7 ■ PFHxS + PFOS ■ PFOA ■ PFAS C9-12

Risultati definitivi



Grazie per l'attenzione

Claudia Ferrario, Stefano Polesello e Sara Valsecchi
IRSA-CNR Brugherio

